PRECLINICAL AND CLINICAL EVALUATION OF SAFETY AND EFFICACY OF SIDDHA HERBO-MINERAL FORMULATION NANDHI MEZHUGU IN THE TREATMENT OF UTHIRA VATHA SURONITHAM (*RHEUMATOID ARTHRITIS)*

Thesis submitted to

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In partial fulfillment for the award of the degree of

Doctor of Philosophy

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ABBREVATION

ACPA- Anti-citrullinated protein antibodies

ALB- Albumin

ALT- Alanine transaminase

ANA- Anti nuclear antibody

ANOVA- Analysis of variance

Anti CCP- Anti Cyclic citrullinated protein antibodies

AOAC-

AST-Aspartate Transaminase

AZA-Azathioprine

B- Basophils

BLQ: Below limit of Quantification /LOQ- Limit of quantification

CIA- Collagen Induced Arthritis

CPCSEA- Committee for the purpose of control and supervision on experimental animal

CPDD- Calcium phosphate deposition disease

CRP- C-Reactive Protein

DC- Differential Count of WBC

DEPC

DIP – Distal inter-phalangeal joints

DMARD's- Disease modifying anti-rheumatic drugs

DMSO- Dimethyl sulfoxide

DNA- Deoxy ribonucleic acid

D-PBS-

EDTA- Ethylene diamine

E-Erythrocytes

ELISA-

ESR- erythrocyte sedimentation rate

FCA- Freund’s complete adjuvant arthritis

FP-Forward primer

HCl- Hydrochloric acid

HCQ- Hydroxychloroquine

HCT- Haematocrit value

HDL- High density lipoprotein

HLA- Human leucocyte antigen

HPTLC- High performance Thin layered chromatography

IAEC- Institution Animal Ethical Committee

ICP-OES- Inductively coupled plasma optic emission spectroscopy

IMPCOPS- Indian medical practitioner’s co-operative society

LDL- Low density lipoprotein

LFT- Liver function test

L-Lymphocytes

LPS- Lipo polysaccharide

M- Monocytes

MCH- Mean corpuscular Haemoglobin

MCHC- Mean corpuscular Haemoglobin concentration

MCP- Meta-carpo phalangeal Joint

MCV- Mean corpuscular volume

MHAQ - Modified health assessment questionnaire

MPV- Mean Platelet volume

MRI- Magnetic resonance imaging

MTP- Meta tarso phalangeal joint

MTT- 3(4,5-Dimethylthiazol-2-Y1)-2,5-Diphenyltetrazolium bromide

MTX- Methotrexate

NAD- No abnormal defect

NaOH- Sodium hydroxide

NBF- Neutral buffer fluid

NM-NandhiMezhugu

NSAID's- Non steroidal anti-inflammatory drugs

OA- Osteo Arthritis

OECD- Organization for Economic Co-operation and Development

PAD-Peptidyl arginine deaminase

PDW- Platelet distribution width

PIP- Proximal inter-phalangeal joints

PJ-Palm Jaggery

PLT- Platelet count

P-Polymorph

Q.S- Quantity sufficient

RA- Rheumatoid Factor

RBC- Red blood cell

RDW- Red cell distribution width

RF- Rheumatoid factor

RFT- Renal function test

RNA- Ribonucleic acid

RO-Research officer

RP- Reverse primer

RT-PCR- Real Time Polymerase chain reaction

S.Bil- Serum bilirubin

SAP-Serum Alanine Phosphatase

SD-Standard Deviation

SEM-Standard Error mean

SGOT- Serum glutamate oxalo-phosphate transaminase

SGPT- Serum glutamate phosphate transaminase

SLE- Systemic lupus erythematosis

SSZ-Sulfasalazine

TC- Total count of WBC

TGL-Triglycerides

TLC- Thin layered chromatography

TRP- Total protein

UA-Undifferentiated arthritis

UV- Ultra violet Rays

VLDL- Very low density lipoprotein

WBC- White blood cell

WHO- World Health Organization

YLDS- Years lived with disabilities

1. INTRODUCTION

In Siddha system of medicine, siddhars mentioned 4448 diseases.[1] *Vatha* disease is one of them. According to Saint *Yugi*, the Vatha diseases are classified into 80 types and one of them is *Uthira vatha suronitham*.[2] The term '*Uthiram*' means blood and '*Vatham*' is a constructive humour responsible for all kinds of movements in our body. *Suronitham* is one of the physical constituents (U*dal thathukkal*) responsible for reproduction in females. The incidence of the disease has always been much higher in female population and hence the term *suronitham*.[3]

As mentioned by *Yugi Munivar* in his verses aetiology of *Vatha* diseases,[4] the socio-economical (starvation and doing hard work like lifting heavy weights), psychological (disrespecting elders and neglecting parents and teachers and defying *vedic* scriptures), environmental (seasonal changes), and lifestyle factors (consumption of bitter astringent, rancid and salty food, alcohol, day time sleep and night-time overwork) cause immunological modification thereby leading to infection, which is highly correlated to the aetiology of Rheumatoid Arthritis (RA) as mentioned in modern science.

Uthira vatha suronitham indicates a systemic illness caused by the derangement of *vatham* which manifests as pain, swelling, tenderness and limitation of movements in major and minor joints, low grade fever, tiredness, mental depression. The signs and symptom of *Uthira vatha suronitham* is correlated to Rheumatoid Arthritis in modern science.

One of the major causes of disabilities at global level, accounting for 0.8% of total years lived with disabilities (YLDS), is RA.[5] The prevalence and incidence of RA are higher in women when compared to men. [6,7]The prevalence and incidence increase with age and peaks at the age 70 then declines. As per WHO statistics 2016, the total prevalence of RA and OA in India is about 5.5%.[8]

Rheumatoid arthritis is a chronic multi-system disease of unknown cause. Characteristically, RA is a chronic polyarthritis.[9] Gradually, it begins with fatigue, anorexia, generalized weakness, weight loss, increase in body temperature and vague musculoskeletal symptoms followed by specific symptoms such as pain, swelling and tenderness of several joints especially those of hands, wrist, knees and feet in a symmetric fashion. The pain is aggravated by the movement of the affected joints, which is a well-

established common sign of RA. Generalized stiffness is frequent and is usually greatest after a period of inactivity. Morning stiffness of more than one hour duration is an almost invariable feature of RA. Swelling, tenderness and limitation of motion are clinically caused by synovial inflammation. Initially, pain and inflammation impair physical function and disability owing to this is an early feature of aggressive RA.

RA is associated with reduced life expectancy. More than 50% of RA patients are incapable of doing full-time job within 10 years of onset of the diseases.[10]

The siddha science has successfully contributed many anti-*vatha sastric* herbo mineral formulations for the treatment of arthritis from time immemorial. One such formulation is *Nandhi Mezhugu*, a *sastric* Siddha herbo mineral preparation indicated for treating 80 types of *Vatha* diseases. The long term treatment of RA with medicines of other systems has its own limitations. *Nandhi Mezhugu* is a cost effective and time tested Siddha herbo mineral formulation and it is well-known for its unique action against *Uthira Vatha Suronitham* (Rheumatoid Arthritis) as evidenced by several decades of its use in clinical practice. For global acceptance, scientific validation of *Nandhi Mezhugu* by safety and efficacy studies is the need of the hour.

2. AIM AND OBJECTIVE

AIM:

To evaluate the safety and efficacy of Siddha herbomineral formulation *Nandhi Mezhugu* for the treatment of *Uthira Vatha Suronitham* (Rheumatoid Arthritis).

OBJECTIVE:

Qualitative and Quantitative analysis:

- To analyze the physico chemical parameters of *Nandhi Mezhugu* as per Ayush Guidelines.

Safety study:

- To establish the safety profile of *Nandhi Mezhugu* in Rat model by doing Acute, Sub acute (28-day Repeated oral toxicity studies), Sub chronic (90-day Repeated oral toxicity studies) studies as per OECD guidelines.

Pharmacological Study:

In-vivo:

- To evaluate the Analgesic effect and Vascular permeability of *Nandhi Mezhugu* in Acetic acid-induced writhing in mice and Evans blue vascular permeability assay model.
- To evaluate the Acute and Chronic Anti-inflammatory activity of the *Nandhi Mezhugu* in Carrageenan induced rat paw oedema and Cotton pellet granuloma method respectively.
- To evaluate the Anti-rheumatoid activity of the *Nandhi Mezhugu* in Freund's complete adjuvant (FCA) Arthritis Model.
- To evaluate the Gene expression effect of the Nandhi Mezhugu in RT-PCR.

In-vitro:

- To evaluate the cyto-toxicity and cyto-protective effect of the *Nandhi Mezhugu* in MTT Assay using RAW 264.7–Mouse macrophage cell line.
- To evaluate the Gene expression effect of the Nandhi Mezhugu in RT-PCR.

Clinical study:

- To validate the safety and efficacy of *Nandhi Mezhugu* for the treatment of *Uthira Vatha Suronitham* by doing open labelled clinical study.

3. REVIEW OF LITERATURE

3a. DISEASE REVIEW:

3a.1.SIDDHA ASPECTS:

3a.1.1.Definitions and descriptions of *Vatham*:

Vatham is one of the 3 humours and it consists of wind and space, two of the five elements, i.e. pancha boothas. It is said to occupy the region below the naval in our body. It is responsible for all movements in the body. It spreads throughout the body and causes respiration, hunger, thirst, etc., without itself undergoing any change.[2]

Vatham mainly refers to the *Vayu*(wind) and its functions. The *Vayu* (Wind) forms the vital forces of the human body and is present everywhere in the system.

Although it is invisible, its presence is manifest everywhere. It is known by its two attributes, namely sound and touch. It passes through the whole system in a rapid current. It is the energy or power that prevails all over the body keeping the various tissues in good condition.

Vatham(wind) is of 10 types- *Praanan, Abaanan, Uthaanan, Vyaanan, Samaanan, Naagan, Koorman, Kirukaran, Devathathan* and *Thananjayan*. Of these, the first five play an important role in the physical functions necessary for the preservation of the physical body.[11]

1. *Praanan*: It regulates the respiratory system and helps the digestive system.

Unneyae melezhundhu paniraendangulam
Uyarnthavaet tangkulanthaan meela vaangi
Nanninalan gulanthaan paazhir paayum
Namaana kunamaellaam piraana vaayu

Pappalavaam posippaellam seernamaagum

2. *Abaanan*:

It helps in the excretions from lower organs, evacuation and generation. It controls the sphincteric action of anus.

Marukkavae keezhnokki malasalan thllum;

Arukkavae yaasanththais surukki vaikkum;
Annasaarath thaiyaellan saera vaikkum;

Abaana Vatha(Vayu) is one of the 14 physiological reflex actions (vegas) of the body. When its expulsion is partially or completely controlled or obstructed it leads to diseases of the chest *Vayu gunmam*(characterized by dryness of the liver and chest, burning sensation in abdomen, gnawing pain on the sides, heaviness and aching of head, dizziness, acidic vomiting and intestinal colic), *Uthara vatham* (characterized by uni-lateral in-drawing of the testis, scrotal swelling due to accumulation of gases, decent of the drawn- in testis in to scrotum due to the displacement of gases in to abdomen, and resultant pain), stabbing and gnawing pain all over the body, *Vallai vatham* (enlargement of spleen), obstruction of daefecation and micturiation, leading to indigestion and loss of appetite.

3. *Vyaanan:*

It helps in the circulation of energy throughout the entire nervous system and helps in the movements of various part of the body. It is responsible for the tactile sensation.

"Sirappana vyaananthu thooli ninru
Thigazhaezhupath theeraayira narambir senru
Tharuppaana saravasaranth thanilae ninru
Thaaneetal mudakk pannip parisa mariyum
Aruppaana vannasaaranthan naiththaan
Aagaangae niraippithaak kaiyaik kaakkum"

4. *Uthaanan*:

It regulates the higher functions of the brain like speech. It is responsible for the physiological reflex action like vomiting, hiccup, cough, etc.

"Ezhilaana utharavak kiniye zhuppip
Poomanaa mannasaarantha naiththaan
Porunthavae samiipaththu niruththi vaikkum
Kamanaa yaeduppiththum kalakki vaiththum
Kalakkiyae vruththuvikkum valappa maagum"

5. Samaanan:

When any one of the other vayus is affected, this samaanan, also is affected. It helps in the proper digestion, etc. by balancing the other vayus, the six tastes, water and food.

"Vaamaenra vaayuvinai minjottaamal
Madakkiyae samanseithu naruvap pannum
Thmaenra arusuvainth thannee rannam
Samanseithu sareeramaelaaj saarap pannum"

6. *Naagan:*

It is responcible for the intelligence of an individual. It helps learning different arts, singing of good songs, etc. It is responsible for winking, opening of eyes and hair-raising.

"Seyalaana sakalakalai yaakku viththup
Payvamaayp paaduvikkum kanvizhikkum;
Paangkaaga vaesimizhkkum roma masaikkum;"

7. *Koorman:*

This is responsible for yawning, closure of mouth (movement of lower jaw), yielding strength and also winking. It helps in closing and opening of the eyes and shedding of tears. It is responsible for the vision.

" Kodiyamananh thanininru nimaikot tummae;
Nimaikotutung kottaavi thaanung kollum;
Naeraaga vaaymoodum pala nundaakkum;
Kamai kottumng kanvizhkkum ndap pannum;
Kaatciyaelaang kaanpikkum vizhinee rodum
Emai kottum;"

8. *Kirukaran:*

It is responsible for the salivation in the oral cavity and mucous secretion in the nasal cavities. It is responsible for good appetite. It helps in meditation. It produces cough and sneeze.

"Kasinaavu naasithanir kasivundaamae;
Kasivundaang kadumpasiyir kanmaj saellum
Kanniyae yiruththalodu poethlaagum;
Thusivudaayth thummalo diruma lundaam;"

9. *Devathathan:*

It is responsible for the laziness and also lassitude while waking up. It helps movements of the eyeball in various directions. It is responsible for begging, quarrelling, arguing, etc., and also for much anger.

“ Kudilamaays soembi murith thidutha laamae;
Muriththiduthal vizhikkumpo thuyarchi yaagi;
Mukulithamaayk kannaiyot tulaavu viththuth;
Thariththiduthal sandai koelal tharkkam paesal;
Sandaala kopaththai yundu pannal”

10. *Thananjeyan:*

It is responsible for the swelling all over the body. It produces sensation of roaring like the sea in the ears. It leaves the body by blowing up the cranium only on the 3rd day after death.

“Thadiththumae yudampaellaam veengap pannum
Thanthiramaaik kannaththir samuththiram poel
Thudiththumae sundaramaaik koesa maagith
Thirandumae thujsukaa lanthaniraan;
Vadithumae vaayuvaellaam poena pinbu;
Valamaana thanalavaedikkum moonra naalil
Adiththmae thalaivaedithaa lappaar paegum;”

3a.1.2.Aetiology:

In *yugi*, we cannot find any specific aetiological factors for Uthira Vatham,[4] but causes for all types of vatha diseases in general have been described and they are as follows:-

“Ennavae vaathnthaa naenpathaagum
Egaththilae manitharkaluk kaeyyumvaaru
Pinnavae ponthanaiyae soranjseithu
Paeriyorkal piraamanaraithudaniththum
Vannathevas soththil soranj seithu
Maathapithaa guruvai maranhthaperkkum
Kannavae veththai ninthai seithaal
Kaayaththir kalanthidumae vaathanhthaanae”

-Yugi Chinthamani- verse 243

i.e. Breach of trust, abusing the pious elderly people, the priests and also the holy scripts, exploitation of charitable properties, ingratitude towards mother, father and teacher:-

“Thanaenra kasappoedu thuvarppu raippu
Saathagamaay minjsugilum samaiththa vannam
Aanaentra vaarinathu pusiththa laalum
Aagaayath thaeralathu kudiththa laalum
Paanaenra pagalurarakka miraavizhippu
Pattiniyae migavuruthal paara meithal
Thaenaenra moezhiyaar maer sinthaiyaathal
Seekkiramaay vaathamathu senikkun thaanae”

-Yugi Chinthamani- verse 244

i.e. Excessive eating of food items of bitter, astringent and pungent taste, intake of dry and old cooked rice, drinking raw rain water, sleeping during day and keeping awake during night, undue starving, lifting or carrying of heavy loads and sexual pre-occupations.

“Vaathavarth thanaikaala maethoe vennil
Maruvukinra vaanigark kadaga maagum
Aathavaip pasiyodu kaarththigai thannil
Adarumae mattramaa thanggal thannil
Poethavae samikkugintra kaala maagum”

-Yugi Chinthamani- verse 245

i.e The disease will be precipitated in the months from Aani to Karthigai(from June to December).

“Aanaana varantranaiyae mathiyaamaanhthar
Agathi parathaesiyarkatkanna meeyaar
Koenaana gurumoezhiyai maranhthapaergal
Koelai kalavupoi kaamanhkuriththaperkku
Oonaana sadanhthannil vaatham vanhthu
Urpavikkum vaethatthin unmai thaanae”.

-Yugi Chinthamani- verse 253

i.e. Disrespectful attitude towards god, refusing food for destitutes and sanyasins, disregarding the advice of preceptors, engaging in murdering, stealing and lustful activities and lying.

"Pagaravae vaathamathu kopith thappoe
Panpaagap paenpoega mathuthaan seiyil
Nagaravae vaeguthoora vazhina dakkil
Naliraana kaatrumae pani maerpattaal
Migaravae kaaykal kani kizhangu thannai
Migavarunthi meeriyae thayirthaan koendaal
Mukaravae muthukaelumpai murukki noenhthu
Muzhankaalum kanaikaalum kaduppundaamae"
-Yugi Chinthamani- verse 285

i.e. Indulging in sexual act during the abnormally increased condition of vatha, walking for a long distance, exposure to chillness, excessive intake of curd immediately after excessive intake of vegetables, fruits, and tubers will lead to twisting pain in the back and burning pain in the ankle and knee joints.

Kanma as a cause:

In Siddha system, many diseases are said to be precipitated by Kanma which means the deeds good or bad committed by an individual in his previous and the present births. The genetic disposition of certain diseases are probably the result of kanma. Vatha disease, according to Agasthiyar Kanma Kandam-300, verse-56, may also be precipitated by Kanma.[12]

"Noo lentra vaatham vanhtha vagaithaanaethu
Thunmaiyaayk kanmaththin vagaiyaik kaelu
Kaalilae thoentriyathu kaduppa thaethu
Kaikalil mudakkiyathu veekkamaethu
Koelilae padugintra virutsamaana
Kuzhanthaimaranhthanai vaetta mael thoel seeval
Naalilae seeva saenhthu kaal murithithal
Nalla koembu thazhai murithithal nalithithal kaanae".

i.e. cutting or denuding of green young living trees, breaking the legs of living-beings, cutting the branches of a living tree, etc. lead to vatha disease. These deeds are detrimental to fellow-beings and such psychosocial aspects of an individual implies psychogenesis of the vatha disease.

3a.1.3.Mukkutra Pathology:

The primary humour affected is *Vatham*. Vitiation of *Vatham* accompanied by vitiation of *pitham* and *kapham* lead to the clinical condition and this is clearly indicated by *Theraiyar* as[13]

"Vathamalaathu meinikaedathu"-Theraiyar saegarappa

Vitiation of *vatham* leads to the disorders of function of certain types of *vayus* like *Pranan, Abaanan, Uthaanan, Vyaanan, Samaanan, Naagan, Koorman, Kirukaran* and *Devathathan*.Involvement of Pranan results in anorexia and dyspnoea on exertion in mild and moderate anaemic patients of uthira vatha suronitham.Involvement of *Vyaanan* causes local heat, tenderness,pain in the affected joints, joint stiffness ,restricted movements of the joints of upper and lower limbs. Involvement of *Abaanan* leads to constipation. Involvement of *Samaanan* leads to indigestion, anorexia and imbalance of functions of other *Vayus*. Involvement of *Naagan* leads to sluggishness and mental depression and of *Koorman* leads to diminished vision and *Devathathan* affects the normal sleep rhythm.

In uthiravathasuronitham patients, involvement of Anal pitham causes anorexia and involvement of Ranjaga pitham affects senneer thathu. Involvement of Saathaga pitham causes difficulty in doing routine day to day activities like walking,holding, gripping,fisting of hands and flexion,extension and rotation of the joints and Prasaka pitham accounts for the dryness and loss of lustre of skin.Alosaka pitham accounts for defective vision

Vitiation of five types of kapam in uthira vatha suronitham patients are as follows;

Kilethakam affected(anorexia),Bothakam affected(tastelessness,bitter taste sense),Tharpagam affected(burning sensation of eyes),Santhigam affected(difficulty in flexion,extension movements of the joints,crepitation),Avalambagam affected(since it supports other four kapams).

In Seven *Udal thadhus, Saaram* and *Senneer* are affected leading to anorexia, lethargic and depressed conditions. *Senneer* when affected leads to nervousness, dryness of skin, diminution of bodily luster. *Oon* is affected leading to muscle spasm, musclewasting. *Kozhuppu* is the main *thathu* affected and vitiation of this *thathu* leads to difficulty in movements, crepitations as a result of diminution of the pulpy semifluid material. *Enbu thathu* is affected leading to bone erosion,cartilage destruction, osteophytic changes.

3a.1.4.Classification of *Vatham*:

Based on *Yugi Chinthamani* and *Siddha Maruthuvam* text book, *Vatha* diseases are classified here. In the classification, we can find contradictory views regarding the number.[1,14]

Yugi says *"Ennavae Vathamathu enbhathaagum"*

i.e. there are 80 types of Vatham but 'Siddha Maruthuvam' mentions 85 types while describing the names and symptoms. The same Yugi while ending a verse describing the names of the types of Vatham reaffirms that the number of types Vatham is 80:-

"Thakkaana vathanthaan enbhathaagum"

In the concluding section of the yugi vaithiya chinthamani, the number of vatha diseases has been given as 84.

"Aamappa vaatham aen baththu naalu
Athinudaiya gunaagungaladangalaaga"

In some books, vatham has been classified as 80 types based on the symptomatology and involvement of different parts of the body. e.g. Astanga Sangiraham and Noi Nadal Noi Mudhal Naadal- Part II.[14]

In Theraiyar Vagadam, 81 types of Vatha diseases[15] have been described and in Thanvanthri Vaithiyam and Jeeva Rakshamirtham the number has been given as 80.

In Agasthiyar 2000[11]

"Enpathu vaatha maagu miruvagaip paduththuk kaanil
Nanpuru araikku maelae naarpathu vaathamagum
Pan saeraraikkumk keelae paththunaankkagum maentru
Vandu saer guzhalinaalae vaathaththin kooruthaanae"

i.e. 40 types of Vatha are in the upper half and 40 in the lower half of the body and the total number is 80.

3a.1.5.Clinical features of Vatha Diseases:

The signs and symptoms of *Vatha* diseases have been given in many Siddha literatures. In *Sathaka Naadi*, the following signs and symptoms have been given:[2]

Vatham

"Vaathamaenum naadiyathu thoentril
Seetha manthamodu vayiru poerumal thiratchivaayu
Seethamurung kiraani magotharam neeramai
Thiralvaayu soolaivali kadupputh thirai"

Vathapitham:

"Porulaana vaathathil pithanj saernthu

Karuvaana thaegamathil ulaitchal sombal
Kaikaaltharippu naakkasakkumannam"

In *Agathiyar naadi*:

"*Sollavae vaathamathu meerittraanal*
Sorvadainthu vaayuvinaal thaegamaengum
Maella kaikaalkalasathi yundaagum
Maeimudangum nimira vonnaath thimirundaagum"

"*Vaatidum saeththumaththil vanhthidum vaathamaagil*
Naatiya kaalgal poel narambellam valiththu nirkum"

Vatha kapham:

"*Paangaana vaathathil saeththumanaadip*
Parisiththaal thimirmaevu mulaichalaagum"
--- Sathaka Naadi

Kapha Vatham:

"*Kandaayoe silaerpanaththil vaathanaadi*

Uruthiratchai vaayuvali sannithodam"

In the above verses, symptoms like gnawing and stabbing pain, stiffness, numbness, sluggishness, weakness of limbs, constipation have been described

Theraiyar Vagadam, describes the following features:

"*Vaathaveeru annameeranggaathu kaduppundaam vannamundaam*
Moothu kattu roegam suramundaamirumalumaa muranggaa thaentrum

Oothusooriya vaathamanalaagum nadukkamundaam poerulkalaaynh Theethaenavae narampisiththu santhugal thorungkadukkanh thinamunhthaanae"

i.e. when *Vatham* is increased,bodily pain, diminution of the body lustre, fever, cough and sleeplessness occur.

In *Agasthiyar* 2000, the following features are given: [11]

"*Vaathaththin gunamae thennil mayangunhthiyangum malar sivakkum*
Paathangulirnthu saruvaangam patri nadakkumugangkadukkunj
Seethaththudanae vayirupunnaaj sirippith thathunhtaeri mootchaam
Poethath thanneer thaanvaangum pugazhum panja gunamaamae"

"Vaathathin gunaththaik kaenmin vayiroothum porumikkollum
Thaathuttra udambu kaikaal santhugal kadupputh thoentrum
Theethutra siruneer thaanunj sivanhthudal gaduththu veezhum
Poethutrra uppusamaay poethavum pasiththidaathae"

"Kaalkai kadukkunnh thimirundaang kannunh thuunggi soebikkum
Koelanjseriyum manhgamaellaang kulirnhtha sanhthuganang kolluj
Seelamigunhthu seerkaanil siruneer vatrri varumigavae
Maalath thadangan maananaiyaai maathae vaatha rogamithae"

i.e. giddiness, redness of eyes, stabbing pain in the face, burning pain in body and limbs, numbness in limbs, etc., are found when *vatham* is increased.

3a.1.6.Vatha suronitham:

Vatham is generated below the abdomen and spreads all over the body and is responsible for the movements of the body. According to *Yugi Vaidhiya Chinthamani vatha,* diseases are classified into 80 types, which include *Vatha Suronitham*. *Yugi* classified *Vatha Suronitham* into seven types

- Vatha suronitham
- Uthira Vatha suronitham
- Sithu Vatha suronitham
- Vaigitha Vatha suronitham
- Paithiya Vatha suronitham

- Slethuma Vatha suronitham
- Uthara Vatha suronitham

3a.1.7.Uthira Vatha Suronitham: (pothu maruthuvam p.no: 608)

Clinical Features of Utira Vatha suronitham:[1,4,14]

"*Vaigithamaaik kanaikkaalu muzhangal thaanu*

Markadanth sandthu puravadiyum veengich

Saeigithamaanj siruviralgal migavum nondhu

Sinthai thadumaariyae salippundaagum

Paigithamaam paithiyaththil vathamminjip

Baaramaai urpaviththu azhalundaagum

Uyikithamaai asanamathu thaanumvaenda

Uthiravatha suronithathin unaarchchi yaamae"

- Yugi Vaithiya Chinthamani

Table no: 3a.1.7.1.Correlation of Uthira vatha suronitham and Rheumatiod arthritis:[18,19]

S.No	Symptoms for Uthira Vathasuronitham	Symptoms for Rheumatoid Arthritis
1.	"*Vaigithamaaikkanaikkaalu muzhangal thaanu Markadanth sandthu puravadiyum veengich*	Swelling of Ankle, Knee, and smaller joints of the Hand (flexion of Distal inter phalangeal joints and Extension of Proximal inter phalangeal joints looks like Apes Hand-Swan neck Deformity.
2.	*Saeigithamaanj siruviralgal migavum nondhu*	Pain and tenderness of minor joints especially phalanges.

3.	*Sinthai thadumaariyae salippundaagum*	Depression and Apathetic mood.
4.	*Paigithamaam paithiyaththil vathamminjip Baaramaai urpaviththu*	Signs of Inflammation (Elevation of Pitham) pain and restricted movements of the joints (Elevation of vatham).
5.	*Azhalundaagum*	Fever
6	*Uyikithamaai asanamathu thaanumvaenda*	Loss of appetite

3a.1.8.Diagnosis:[2]

Diagnosis can be arrived at using the following *Siddha* methods:-

1) *Poriyalarithal*, *Pulanalarithal* and *Vinathal*(and some of these are included in the "*Envagai Thervu*" also). i.e. inspection, palpation, percussion and interrogation.

2) *Envagai Thervu*:

"*Naadip parisam naa niram moezhi vizhi*

Malam moothiram evai mruththuvaraayutham"

a) *Naadi:*

By examining the pulse, the condition of humour and the features are understood.

b) *Sparism:*

By palpation, the conditions of the disease are understood.

c) *Naa:*

Examination of tongue

d) *Niram:*

Colour of the skin and mucous membrane is examined.

e) *Mozhi:*

Nature of Speaking

f) Vizhi:

Vision and other aspects are tested.

g) Malam:

Examination of stools.

h) Moothiram:

Examination of urine which includes Neerkuri and Neikuri

Examination of urine- *Neikkuri*[2]

Neikuri is an important test to assess the predominantly affected humour. A drop of gingelly oil is allowed to fall on the surface of the urine kept in a kidney tray, exposed to bright sunlight.

"Aravaena neendidin agthae vaatham"
"Aazhi poerparavin agthae pitham"
"Muthoththu nikkin moezhivathaena kabhamae"

If the drop of oil spreads like a snake, it indicates *Vatha* disease, if it spreads like a ring, it indicates *Pitha* disease, and if it appears like a pearl it indicates *Kapha* disease.

The final diagnosis (here as *Uthara Vathasuronitham*) is arrived at by summarizing all the clinical findings observed by the methods described above which includes examination of pulse and *Neikkuri*.

3a.1.9.Differential diagnosis:[4,14]

Uthira Vathasuronitham is differentiated from other types of *Vatha Suronitham* as follows:

According to the text '*Yugi Vaithiya chinthamani'*

Vaatha Suronitham:

"Arinthitta angamaellaa maelivu maagi
Asaivaana thavvidangal veekka maagi
Narinthitta nadaikoedaa thaani ruththal
Naliyaagi moezhimozhiya veekka maagatch
Sorinthitta thaegamaengu masaivu kaanal
Sotrrinmae ninaivinrith thookka maathal
Varinhthitta vvayathani neerthaa nooral
Vathasuro nithanhthaanum vaguththa vaarae"

In *Vatha Suronitham,* gradual weight loss, weakness, pain and swelling in the joints, loss of appetite, somnolence, excessive salivation are the symptoms described.

Sithuvatha surunitham:

"Vaarana sareeramaellaa nuzhainthu oothum
Maasatra thoelthaanunh thirainthu poogum
Naarana naarupoel narampu sukkum
Naakkuththaan vazhavazhaththuk koezhai yaagum
Thooraana naeruppuththaan pattaar poela
Noenhthumae sadamaellaang koeppa likkum
Veerana vurinhthupinnai vaethumpi veenggum
Mikkasiththu vaathasuroe nithamaamae"

In *Sithuvatha surunitham* symptoms like generalized body swelling, neuralgia, blisters all over the body, excessive salivation have been listed.

Paithiyavaatha suronitham:

"Unarchchiyaay suronithanhthaan migavae thumpi
Ookkamaayth thaekamaengu migavae noenthu
Unarchchiyaay muzhangaalgal muzhangai yoekka
Munaiyaana siruvralgal kannam naetri
Thanarchchiyaaych sanhthusaru vaanga maenhgum
Thaattiga maaykkudainthu suramu mundam
Panarchchiyaayp paandathupoen maeni yaagum
Payiththiyava thasuroenithathin panpu thaanae"

In *Paithiyavaatha suronitham* symptoms like weakness of the body, pain in the knee, elbow, fingers, feverishness and paleness of the body have been listed.

Sethumavaatha suronitham*:*

"Panpaaga udalkulirnthu vaeru veengip
Pathaipaana vidanhthottaar pola noevaam
Thinpaana sirasunaetri noekkaa dundaam
Silaettumamaayk koezhaiyoedu suvaasa maagum
Manpaaga mayakkamoedu kanavu mundaam
Vaayvarandu rusiyillaa varuththa maagum
Nanpaaga naadiyumae padapa dakkum
Narselaetma suronithamaam naadung kaalae"

In *Sethumavaatha suronitham* symptoms like chillness of the body, swelling and pain on touch, headache, and shortness of breath, giddiness, dryness of mouth and loss of taste have been listed.

***Uthara Vathasuronitham*:**

"*Naadumae suaramvanthu nadukka lundaam*
Naavarandu thalainoenthu udam pazhundhi
Vaadumae thaegamaellaa manichcham pooppoel
Magaavaruththa mundaagi mayakka maagum
Saadumae yadikkadithaan paethi thaanum
Thavikkumae thanneerthaa naatta maagith
Thaedumae soetrinmael ninaivu thaanum
Saeya uthara vaathasuro nithanhthaan naennae"

In *Uthara Vathasuronitham*, symptoms like fever with rigor, dryness of tongue, inflammation and redness all over the body like Anicham flower, generalized tiredness due to pain, diarrhea, excessive thirst and hunger have been described.

Vaigithavaatham:

"Aamaentra veenhginathor vidaththil raththam
Azhuththamaaith thirandumae engum paaynhthu
Omaentru oottiyae thirandi rukkum
Uruthiyaayth thottudanae maeththaen raagum
Thaemaentra thaegamaen kaanuki sikkum

Paamaentra sadanhthanilae thimirun daagum
Paaramaay vaigithamaam vaathanhthaanae"

In *Vaigithavaatham* presence of swelling, pain, tenderness over the joints, cough, fever are the symptoms described.[4,14]

3a.1.10.Treatment of Uthira vatha suronitham [1]

The line of treatment consists of:-

1. Oil bath, a pre-requisite to alleviate vatha kuttram.
2. Purgation,a pre-requiste to alleviate vatha kuttram.
3. Internal medicine to relieve pain and stiffness of the minor and major joints to inhibit the progression of the disease process.
4. Pathiyam i.e., dietary regimen to suit the drug and the disease.
5. Kanma neekam

Treatment

On day -1, patients were instructed to have oil bath with Chittra mutti thylum.

On day-2 patients were given a purgative like Agasthiyar kulambu 100mg with hot water in the early morning.

On day-3 patients were advised to take rest and instructed not to take any medicines.

On day-4 patients were advised to take internal medicine Nandhi mezhugu in the dose of 500mg along with palm jaggery twice a day after food intake. Medicine was given for 7 days followed by 7 days drug holiday likewise for a period of 60 days.

Pathiyam

Pathiyam forms an important part of treatment.Certain dietary and other restrictions are imposed in relation to the disease or the drug.While on treatment with Nandhi mezhugu,the patients were advised to avoid tamarind and salt and take plenty of cow's butter milk,cow's milk,cow's ghee(these measures will probably neutralize the toxicities if any,and irritability of the internal drug).

Siddhars' view on Pathiyam

Kadum pathiyam:

"*Kadumai entridu pathiya muvar varuththundal*
Adaivilaa maru paththiyath thuvar varuth tharunthal
Kodumai sei pulithanei suttu cootida lantri
Padiyil katthari chickkurup pinjineip parugal
--Sundharananthar Ayulvedha Pothu Lakshanam.

In strict (diet) pathiyam, a small quantity of fried salt is added to cooked rice which is eaten after adding hot water.This has to be observed during the entire period of taking medicine and then re-dieting is prescribed in which burnt tamarind,unripe brinjal and drumstick are taken in the form of soup along with fried salt.

'*Poerunthidathu pen poegamore marunthukkum puviyil'*

Refraining from sexual intercourse during drug treatment is insisted.

According to Therer:

"*Uppinaa lappaana loottuppun pinpoema*

Vappinaa dappaanaa laampiramaet- truppinaa

Doppiraap baratha mee thutrathai laathikalukk

Kuppiraap pathiya meethoor"

A strict (diet pathiyam is observed when taking mercurial drugs .i.e., salt is completely avoided during the days of pathiyam (during drug treatment) and also during redieting(maru pathiyam) for the same number of days.Fried salt is taken after redieting.A bath ,with application of milk, boiled with adjowan seeds over scalp, is taken 2 days after redieting and then tamarind and cow's ghee can be added in the diet.[20]

Pathiyam for vatha diseases in "Agasthiyar Vallathi 600"

"*Kaelunj sirupairru pasuvinpal thayirneiyaagum vasamoththuththu*

Varaiyuda paruppumaagum mainhthanae pudalangkaaya yavaraikkaayam, naesa

Motrra murungkakaay sirukeeraiyaagum naellikkaai thoothuvalai paruppaampaarae"

"Paararaana vaellaadu suraameenkaadai parum viraaludumpudanae

Muyalumaagum, vaaraana karappanaera vasthvaaga manggaiyaraik

Koodaathae milakaaymaaththu paeraana soolaipathinaettum pogum

---

Aamaera vaathmaellaamaganru poga mappanae:"

In *Pathartha Guna Chinthaamani*:

Vaatharogaari vasthukkal:

"*Saengazhu neer kottanh then milagu nallaennai*

Thanggu perunggaayanh thazhuthaalai- yaengngaenggum

Kootu siru muththunaei kothilunhthivaigal

Vaattu manilaththai mathi"

i.e. Root of water lily (Pontederia vaginalis), Costus root(Costus speciosus), Honey collected on branches of trees, black pepper(Piper nigram) gingelly oil, asafoetida, leaves of clerodendron phlomides, castor oil, black gram, etc., cure Vatha diseases.[21]

3a.1.11.Siddha Medicines for Rheumatoid Arthritis:

3a.1.11.1.Herbo-Mineral Formulations for RA:

In Siddha system, there are several herbo mineral formulations mentioned for *Vatha* Disease. Some major formulations are given below

Chooranam:

1. Attathi Chooranam - Thanjai Vaidhiyaraja chinthamani Part 1 P.No:21-22[22]
2. Elathi Chooranam - Agathiyar Vaithiya Rathina surukkam Song No:149-150.[23]
3. Thalisathi Chooranam - Siddha Vaithiya Thirattu P.No:228-229.[24]
4. Seenthil Chooranam - Agathiyar Paripooranam-400 Song No:324-325[25]

Chendooram:

1. Gantha Chendooram - Gunapaadam Part 2 and 3 P.No:80[26]
2. Velli Chendooram - Gunapaadam Part 2 and 3 P.No:129-130[26]
3. Ulogamandoora Chendooram - Therayar Yamaga Venbha [27]
4. Linga Chendooram - Gunapaadam Part 2 and 3 P.No:159 [26]
5. Aya Chendooram - Agathiyar Paripooranam-400 Song No:261-264[25]
6. Aya veera Chendooram - Gunapaadam Part 2 and 3 P.No:58[26]
7. Kaalamega Naarayana Chendooram – Vaidiya saarasangiragam P.No:496-497.[40]

Parpam:

1. Muthu Chippi Parpam - Siddha Vaithiya Thirattu P.No:128
2. MuthuParpam - Therayar Maha Karisal P.No:132- Song No:50 134[28]
3. Thalaga Parpam - Agathiyar Paripooranam-400 Song No:195-201
4. Thanga Parpam - Gunapaadam Part 2 and 3 P.No:109-110

Pathangam:

1. Linga Pathangam - Therayar Karisal 300 Song No:2 P.No:8[29]

Thylam:

1. Gandhaga Sudar Thylam - Agathiyar Paripooranam-400 SongNo:351-352 P.No:63
2. Gundhiriga Thylam - Hospital Pharmacoepia P.No:133[30]
3. Laguvisamushiti Thylam - Therayar Thyla Vargasurukkam Song No:79 P.No:101[31]
4. Mayana Thylam - Therayar Thyla Vargasurukkam Song No:79 P.No:130
5. Merugulli Thylam - Therayar Thyla Vargasurukkam P.No:75
6. Vaathakesari Thylam - Therayar Thyla Vargasurukkam Song No:9, P.No:48

Leghium:

1. Maha vallathi Leghiyam - Boogar Vaithiyam 700 Song No:175-187[32]
2. Thettrankottai Leghiyam - Agathiyar Paripooranam-400 Song No:281-282

Manappagu:

1. Aadathodai Manappagu - Siddha Vaidhiya Thirattu P.No:257

Rasayanam:

1. Gandhaga Rasayanam - Pulipaani-500 Song No:324-330[33]
2. Parangipattai Rasayanam - Agathiyar Vaithiya Rathina surukkam Song No:114-118[23]

Mezhugu:

1. Mahaveera Mezhugu - Siddha Vaithiya Thirattu P.No:203-204
2. Navauppu Mezhugu - Siddha Vaithiya Thirattu P.No:193-194
3. Nandhi Mezhugu - Siddha Vaithiya Thirattu P.No:187-193
4. Panchasootha Mezhugu - Yugi Karisal-151 Song No:16-24 [34]
5. Rasa Mezhugu - Agathiyar Paripooranam-400 Song No:126-129

Mathirai, Kuligai, Vadagam:

1. Korosanai Mathirai - Agathiyar Vaithiya Rathina surukkam Song No:149-150
2. Mehanaatha Kuligai - Siddha Vaithiya Thirattu P.No:41
3. Pachaikarpoora Mathirai - Siddha Vaithiya Thirattu P.No:30-31
4. Thalisathi Vadagam - Therayar Paadal Thirattu P.No:28[35]
5. Vatha Rakshanan - Agathiyar Vaithiya Rathina surukkam Song No:36-38
6. Viresana Poobathi - Bala Vaagadam P.No:79-80[36]

Kulambu:

1. Koushigar Kulambu - Siddha vaidhiya Thirattu P.No:204-213

Pattru:

1. Moosambra Pattru - Siddha Vaithiya Thirattu P.No:305

Karuppu:

1. Sivanar Amirtham - Siddha Vaithiya Thirattu P.No:165-166

Kattu:

1. Poorak Kattu - Gunapaadam Part 2 and 3 P.No:163

Chunnam:

1. Velvanga Chunnam - Gunapaadam Part 2 and 3 P.No:122

3a.1.11.2.Anti-*Vatha* Herbs:

Drugs which are prevent the *Vatha* Diseases in the body mentioned in *Gunapadam* Part I *Mooligaivaguppu*. [37]Some important herbs which prevent *Vatha* diseases are mentioned in following table

Table no: 3a.1.11.2.1.Herbs Prevent Vatha Diseases.

S.No	Herbs	Botanical Name	Part Used
1.	*Aamanakku*	*Ricinus communis,* Linn.	Leaves,roots
2.	*Aanaipuli*	*Adansonia digitata,* Linn.	Leaves
3.	*Kadambu*	*Anthocephalus cadamba* Roxb	Seed
4.	*Chinni*	*Acalypha fruticosa,* Forsk	Root
5.	*Kattaamanakku*	*Jatropha curcus,* Linn.	Leaves,root

6.	*Chevvamanakku*	*Ricinus tanarius,* Linn.	Leaves,root
7.	*Thakkolam*	*Illicium veram,* Hook.f.	Seed
8.	*PirappanKizhangu*	*Calamus rotang* Linn.	Rhizome
9.	*Mizhagu*	*Piper nigram* Linn.	Seed
10.	*Musuttai*	*Rivea ornate* (Roxb)W.& A	WholePlant
11.	*Merugu*	*Alocasia indica*, Schott.	Rhizome
12.	*Maikonrai*	*Poinciana pulcherrima*, Linn.	Flower,Bark
13.	*VathaNaarayanan*	*Delonix elata,* (L) Gamble	Leaves
14.	*Vizhuthi*	*Cadaba trifoliate* (Roxb)W.& A.	Leaves and Fruit

3a.2. RHEUMATOID ARTHRITIS (RA) MODERN ASPECT

3a.2.1.Definition:

Rheumatoid arthritis (RA) is a chronic inflammatory disorder that typically affects small and medium-sized joints in a symmetric fashion.The acellular synovium is invaded by immune cells causing the primary lesion called synovitis, leading to the formation of inflammatory 'pannus'. This hyperplastic, invasive tissue causes cartilage breakdown, bony erosion and ultimately, loss of function of the affected joints. Systemic involvement- for example of the respiratory, cardiovascular and haematopoietic system- may happen. The risk of developing lymphoma and atherosclerosis is high in RA, with 7 years reduction in life expectancy. [38]

3a.2.2.Epidemiology:

RA affects approximately 0.5-1% of European and North American adults, with considerable regional variation. Prevalence estimates for Southern European countries (median of 3.3 cases per 10^3) are lower than for Northen Europe(5.0 per 10^3), and highest rates are found in America (10.7 per 10^3). Asian studies report between 2.8 and 3.5 cases per 10^3.

Women are about three times more frequently affected than men. It has long been documented that RA cluster in Families: the likelihood that a first-degree relative of the patients will share the diagnosis is 2-10 times the population prevalence of the disease, and recurrence risks are highest for relatives of the most severely affected index cases.

3a.2.3.Pathogenesis:

Auto antibodies: RA as an autoimmune disease.

Witebsky postulated in 1957 that a disease must fulfil three criteria to be considered autoimmune nature. A contemporary understanding of these postulates requires: 1) the presence of autoantibodies or a cell mediated immune response against an auto antigen: 2) that the respective auto antigen is known, and 3) that a similar disease can be initiated in animals based on an analogous immune response. The status of RA as an autoimmune entity, while generally accepted, remains somewhat controversial based on these requirements. For example, although an array of anti-bodies targeting 'Self' antigen

including collagen type II, calreticulin, cathepsin, BiP, CH65, and human collagen glycoprotein has been described, the identity of a dominant arthrito genic auto antigen has remained elusive. Moreover, while inflammatory arthritis phenotypically similar to human RA with autoimmune aetiology, may be artificially induced in animals (as in the example of collagen induced arthritis (CIA), the direct relevance of such models to human disease has been difficult to demonstrate.

Rheumatoid factor (RF):

The Fc part of human Ig G is targeted by the auto antibodies (called as RF) in the affected patients' blood. Ig M – RF is the common form of RFs present in the patient blood. But Ig G- RF and Ig A- RF which form immune complexes, activating complement in the joints are detectable in the sub groups of patients.

The mere presence of RF, however, is insufficient to initiate arthritis development, as RF is also found in other autoimmune diseases, infectious diseases and in15% of healthy elderly individuals. Thus, the sensitivity and specificity of RF are, depending on the population studied, 60-70% and 50-90% respectively, and their role in disease aetiology remains unclear. Despite this lack of specificity, presence of RF is one of seven diagnostic criteria for RA put forward by the American College of Rheumatology in 1987.

Anti-citrullinated peptide antibody:

An enzyme called peptidyl arginine deiminase(PAD) post-translationally modifies the arginine residues in a given protein in the presence of high calcium concentrations in a process called Citrullination . Citrullination plays a major role during apoptosis for degradation of intracellular proteins.

Citrulline-specific reactivities against a number of additional citrullinated proteins (eg fibrinogen, vimentin, and α-enolase) have since been identified. Using novel assays that employ synthetic cyclic citrillinated peptides(CCP) as antigens, anticitrullinated protein antibodies(ACPA) are found in 60-70% of RA patients, but hardly ever in other diseases or healthy subjects.

Circumstantial evidence for their role in disease induction comes from observations that ACPAs can be detected in sera several years before clinical onset of

arthritis, that their presence predicts progression from undifferentiated arthritis (UA) to RA, and that they are associated with a more severe course of disease.

Interestingly, IgM-ACPA are detectable in samples from patients with early as well as longstanding RA, indicating that new antibody secreting cells are continuously generated as a reflection of an ongoing immune response.

Genetic risk factors:

HLA association

The strongest and most relevant genetic risk factor for the development of RA, contributing around 30% to the total genetic effect, is found at the HLA(Human leucocyte antigen) class II molecule-encoding locus(chromosomal position 6p21.3) and relates to experiments first performed in 1969.It is notable that a distinct amino acid sequence containing the aspartine (D) residue(DERAA), and encoded by several HLA-DRB1 alleles(*0103,*0402,*1102, *1103, *1302, *1304), has been found to independently protect its carriers from the development of RA when present at the same DRB1 position. Its protective effect seemingly remains even in the presence of coexisting SE alleles, and it is associated with less erosive disease in both ACPA-positive and ACPA-negative patients. The protective mechanism of DERAA has remained elusive, however and remains at present no more than a tantalising hypothesis.

The first robust non-HLA genetic association with RA was found during investigation of the PTPN22 candidate gene, which encodes a lymphoid specific protein tyrosine phosphate, Lyp. A single nucleotide polymorphism(SNP) at position 1858(C->T) leads to gain-of-function mutation in Caucasian populations, whereby enhanced regulation of T cell receptor(TCR) signalling during thymic selection is thought to permit autoantigen-specific T cells to escape clonal deletion. This appears to predispose to autoimmunity in general, and to ACPA-positive (but not ACPA-negative) RA in particular.

Several whole genome association scans have recently been performed on large RA cohorts and healthy controls originating from different countries. Such genome-wide scans test up to 500 000 SNPs distributed over the entire genome for their association with disease.In addition to confirmation association at HLA-DRB1 and PTPN22 loci, these

scans have identified polymorphism in genetic regions encoding a large number of protein products that may have pathophysiological relevance in RA. Initially, polymorphism at signal transducer and activator of transcription 4 (STAT4), 6q23 and 9q33.2 loci were identified as novel risk factors for ACPA-positive disease(Wellcome Trust Case Control Consortium 2007). STAT4(chromosome 2q32) encodes a transcription factor (STAT4) involved in the differentiation of both type 1 helper T cells (Th 1) and Th 17 cells (discussed later). Although direct evidence for specific gene disruption by risk alleles at the other two loci has not yet been forthcoming, the most biologically compelling candidate genes remain TNFAIP3 and TRAF1, respectively. TNFAIP3(tumour necrosis factor-α induced protein protein 3) codes for a protein(A20) that is a negative regulator of NFκB and as such involved in the regulation of TNFα signalling. TRAF-1(tumour necrosis factor receptor associated factor-1) is involved in signalling of TNFα via TRAF-1 and, interestingly, downstream NFκB. A potential implication of RA association with TNFAIP and TRAF1 is therefore the importance of the NFκB pathway in disease induction, and several additional risk loci containing NFκB-related genes have now been identified: CD40, PRKQC, and TNFRSF14. Functional studies are needed to confirm and further explore the mechanism of such associations.

Additional RA-associated genetic variants have been found in regions or genes encoding CTLA4, KIF%A- PIP4K2C, CCL21, CDK6, CD28, PRDM!, CD2/CD58, and IL2RA. It is important to note that the majority of association studies performed to date have been in Caucasian populations, and some important racial differences exist with regards to risk profiles. For example, the PTPN22 risk allele is not seen in Asian populations, where convincing evidence conversely exists for an association of RA with an SNP in the PADI4 locus(chromosome 1p36.13), which encodes the enzyme PAD. Moreover, the non-HLA genetic variants described here invariably confer only very modest independent disease risk, often displaying individual odds ratios of little more than 1. The additive effects of such minor genetic determinants are unlikely to ever describe all of the unaccounted heritability of RA, and it seems likely that distinct genetic risk factors may provide multiplicative, rather than merely additive, combined risk because of their compound molecular consequences, with gene-environment interactions further completing the picture.

Smoking, genes, autoantibodies, and the problem of ACPA-negative RA:

Cigarette smoking is the most important environmental risk factor for RA. Importantly, and analogous to the case with SE and PTPN22 risk alleles described `above, smoking is seen to represent a risk factor for ACPA-positive, but not ACPA- negative, RA. Indeed, this risk is further increased in the presence of SE alleles(up to an estimated 21 fold as compared to SE-negative non-smokers), illustrating the multiplicative effect of combined risk factors(Klareskog et al 2006, Kallberg et al 2007). PTPN22 risk alleles also appear to interact with SE alleles to confer multiplicative risk(Kallberg et al 2007). In the light of these observations, it has been proposed that cigarette smoke could be involved in the induction of protein citrullination. Supporting this hypothesis, citrullinated proteins have been detected in bronchoalveolar lavage fluids from smokers but not from non-smokers. How and why this response eventually targets the joints, however, remains unknown and could require additional environmental factors. A possible genetic risk factor is the MCH class II gene HLA-DR3,which is part of a conserved haplotype(A1;B8;DRB1*03) that includes the MCH class III region, itself comprising many immune-related genes. In addition, polymorphisms defining several haplotypes in the promoter region of the gene coding for IRF-5 (interferon regulatory factor 5) were found to associate with ACPA-negative RA. IRF-5 is involved in signalling via Toll-like receptors(TLRs) 7 and 9, and the polymorphisms might influence the induction of IRF-5 dependent type 1 interferons.

Additional environmental risk factors:

In a Danish cohort, however, coffee consumption did show an association with ACPA-positive RA after adjustment for smoking.Occupational exposure to mineral oils(eg, motor oils, hydraulic oils, etc) was found to be risk factor for ACPA-positive RA in males in a Swedish cohort, independent of the presence of shared epitope alleles. This finding is of interest as mineral oils are arthritogenic in certain rat strains due to an as yet unknown mechanism.

Female predominance in various autoimmune diseases including RA suggests that sex hormones and reproductive factors influence both RA development and severity. Women with lower age at menarche have a comparatively low risk for the development of RA. In the Danish cohort, for example, women with older age at menarche had an almost

twofold risk of developing RA. Pregnancy is in itself a risk factor for the development of RA, as around 12% of women with RA experience disease onset within 1 year after pregnancy. During pregnancy, most women with RA(in older studies up to 90%) experience a significant reduction in disease activity(including complete remissions), but almost all patients relapse within 6 months after delivery. Multiparity(>3 children) favours a more severe course of disease, but does not additionally increase the risk for developing RA.

Disturbance of Immune system:

The heritability of RA, together with the presence of autoantibodies that precede clinical onset in a subset of patients, support a now widely-held pathogenetic model in which autoimmune propensity is long established by the time joint inflammation is 'triggered'. The precise nature of the trigger(s), and the tendency for ensuing inflammatory pathways to converge in peripheral synovial joints, remain tantalisingly unexplained, however, though innate immune mechanisms undoubtedly play an important role. Thereafter rheumatoid synovitis is characterised by the infiltration of various immune effector cells, typically via chemo-attractant gradients. Over-time, this process creates a cytokine milieu in the joint that activates synovial fibroblast(SF) and osteoclast, which in turn degrade cartilage and bone. Key cellular mediators ae well as important cytokines supporting this process are discussed here.

T cells:

T cells are one of the most abuntant cell types in RA synovium, comprising 30 - 50% of synovial tissue mononuclear cells. The majority are CD4+, 'T helper'(Th) cells, matured from naïve precursors, which have traditionally been divided into Th1 and Th2 subtypes.

In health, Th1 cells secrete interferon-ɣ (IFNɣ) and defend against intracellular bacteria; IL4-secreting Th2 cells combact extracellular parasites. Only recently have two novel CD4+ subsets begun to be properly characterised: Th 17 cells appear to defend against extracellular bacteria such as Klebsiella pneumoniae, while regulatory T cells(T-regs) plays a vital role in immune tolerance and the modulation of established immune response.It now seems likely that newly described Th17 cells are significant players in autoimmune inflammation. The differentiation of this cell type from naïve precursors

requires the presence of TGFß,IL23 and pro-inflammatory cytokines such as IL6 and IL1ß.Lineage commitment of human TH17 cells appears to be flexible, with subpopulation producing IFNɣ or IL10 in addition to IL17. Apart from these cytokines, Th17 cells can produce TNFα,IL6,IL22 and granulocyte- macrophage colony-stimulating factor (GM-CSF). Th17 cells are critical orchestrators of CIA in mice, and evidence for their importance in human RA comes from the observation that both IL17 and IL23 are found in sera, synovial fluid and synovial biopsies of patients, but are mostly absent from the same compartment in osteoarthritis(OA). IL17 itself is highly pleiotropic, acting on a variety of cell types to perpetuate local inflammation while promoting angiogenesis, osteoclastogenesis and, ultimately, destruction of cartilage and bone. It has therefore, become an interesting novel therapeutic target in RA.

T-regs appear to be overrepresented in the synovium of RA patients, but controversy remains as to whether this might simply reflect ongoing methodological challenges in distinguishing cells of this phenotype from activated T cells in human. If the suggested overpresentation is real, it may suggest the functional impairment of T-regs within the auto immune microenvironment they are supposed to supress. A possible explanation is that T-reg cells express TNFR-II, which makes them susceptible to the deleterious effects of TNFα.Indeed, functional T-reg defects may be reversed following treatment with TNF antagonist, apparently through the generation of a distinct T-reg cell population that secretes TGFß and IL10.

B Cells

During inflammation ,B cells infiltrate RA synovium although the degree of infiltration and local structural organisation varies significantly between patients.Fibroblasts- like synoviocytes and dendritic cells in synovium secrete factors attracting B cells and influencing their differentiation and survival,as well as orchestrating the formation of tertiary lymphoid structures in some patients which perpetuate autoimmunity.These include BAFF,CXCL12,and APRIL,some of which are currently being evaluated as therapeutic targets.B cells can also take up antigen via surface Ig and are very efficient APCs.Moreover, recent evidence suggests that Ig- class switching and somatic hyper mutation,which are classically dependent on CD-40-CD40L interactions with CD4+T cells, may also occur independently of T cells.This process again requires

BAFF,which is present in elevated amount in RA synovium and additionally prolongs B cell survival.

B cells are prolific producers of cytokines.By secreting TNFα and IL6, they contribute to the activation of macrophages and directly participate in inflammation .IL6 is also an important autocrine differentiation factor for B cells themselves.RF from B cells form immune complexes that can induce tissue damage and efficiently activate the complement. System they can also be taken up by macrophages, thereby perpetuating inflammatory cytokine secretion. Finally, as with CD4+T cells,regulatory B cell phenotype has recently been described,which producesIL10 in abundance and may downregulate immune responses by tolerising T cells.

Mast cells

Mast cells are highly granular cells best known for their role in allergy and anaphylaxis, and as components of the innate immune system.They may be stimulated to degranulate in response to direct injury,Fcε receptors cross-linking,TLR ligation or activated complement,releasing cytokines,proteases and biogenic amines including histamine.TGFβ ,complement components C3a and C5a,serum amyloid A and platelet activating factor are important for mast cell recruitment,and their differentiation is supported by stem cell factor secreted from fibroblasts,stromal cells,and epithelial cells.Mast cells are the producers of IL17.Indeed ,within the rheumatoid synovium they may be more important in this regard than Th17 cells(Huber et al 2010).In addition,a prominent role for mast cells in the pathogenesis of ACPA-positive RA Iin particular is suggested by the presence of IgE ACPA,and the observation that synovial fluid histamine levels are highest in sero positive disease,possibly as a result of ACPA IgE-mediated mast cell degranulation.

Monocytes/macrophages

The role of activated macrophages in RA synovium is central to driving and maintaining chronic inflammation.Macrophages are multipotent effector cells that very efficiently integrate innate and adaptive immune responses. They are abundantly present in the rheumatoid synovial membrane and at the cartilage/pannus junction(Fig 3a.2.3.1).Important function include strong phagocytic activity,antigen processing and presentation,expression of Fc receptors responsive to (auto-) antibodies and immune

complexes,complement activation and regulation,TLR expression,and tissue degradation and remodelling.They are important producers of pro inflammatory cytokines(eg,TNFα,IL1,IL6),cartilage degrading enzymes(MMP9 and 12) and growth factors(GM-CSF), among other mediators of pathology.

As macrophages are tissue-resident in most organs,they are ,along with dendritic cells,likely to be the first to encounter pathogen-derived antigen,and are well- placed to present it to autoreactive T cells,providing the initial trigger necessary to start an immune response on the basis of genetic predisposition.In this regard,synovial macrophages have been shown to respond to direct cell contact with Tcells and fibroblasts.Co-culture with fibroblsts not only induces secretion of IL6,IL8 and GM-CSF,but also enhances cartilage degradation in vitro.Bacterial cellwall[eg,lipopolysaccharide(LPS)] and nuclear(eg,DNA) components, but also cartilage degradation products such as hyaluronic acid,are strong macrophage activators.Macrophages are also responsive to oestrogens,a finding that could explain changes in disease activity during pregnancy.Interestingly,physiological oestrogen levels induce IL1 expressions,where as higher levels(as occur in pregnancy) inhibit IL1 secretion.Macrophage function is in itself regulated by various cytokines that, in some cases, have autocrine effects.IL4, for example,downregulates macrophage function by reducing the expression of TNFα,IL1β,and PGE2.IL10 lowers the expression of HLA-DR and reduces antigen processing and the expression of Fc receptors.Both IL4 and IL10 have strong anti arthritic properties in murine models of arthritis,and some studies have linked polymorphisms in theIL10 gene to disease susceptibility.

Inflammatory cytokines

Proinflammatory cytokines represent mediators of active disease,encouraging the recruitment and activation of the adaptive immune system,and inducing SFs to secrete cartilage degrading matrix metalloproteases-but they remain non specific targets which may be less important in early disease.With this in mind, it is notable that patients with early arthritis(symptom duration less than 3 months) who progressed to RA exhibited a different synovial cytokine profile than patients who went into remission or developed other arthritides.Patients prone to develop RA showed elevated levels of T cell-derived cytokines IL2,IL4,IL13, and IL17 and of stromal and macrophage related cytokines EGF,bFGF,IL1 and IL15.IFNγ was not detected in these samples,and IL6 seemed to be associated with inflammation independent of underlying disease phenotype.Interestingly,

this profile was absent from patients with established RA and seemed to be present only transiently.The absence ofTh1-cytokine IFNγ and the presence of Th2-cytokines IL4 and IL13 together with (presumably) Th17-derived IL17 in early RA favour an important role for T cells- in the initiation phase of RA.

T cells,macrophages,and stromal cells are also the main source of cytokines in the established RA.Macrophage derived cytokines,however,predominate, and T cell derived cytokines become less abundant.Examinations of synovial biopsies indicate the disease subtype specific patterns might exist.Distinct cytokine profiles were found to correlate with subtypes of lymphocyte infiltration in active RA.Hence ,diffuse lymphocytic infiltrates were associated with RF-negative RA and displayed low levels of IFNγ,IL4,IL1β,and TNFα.Germinal centre formation ,on the other hand, correlated with high levels of IFNγ and IL10,and absence of IL4.Patients with extra- articular disease manifestations showed synovial granuloma formation associated with high levels of IFNγ,IL4,IL1βand TNFα.

IL18 needs consideration as a proinflammatory cytokine, driving macrophage activation together with IL12and IL15.IL18 is over expressed in RA synovium,it induces RANKAL expression in T cells, and strongly aggravates experimental arthritis. The existence of a natural inhibitor,IL18 binding protein,makes IL18 an interesting therapeutic target.IL15 attracts T cells and IL15 activated Tcells in turn activate macrophages.It is expressed in the synovial membrane and by macrophages themselves.Finally ,TGFβ is an important regulator of tissue degradation and remodelling and displays pro- and anti-inflammatory properties.It is produced by macrophages in RA synovium, and low expression of TGFβ due to genetic polymorphisms is associated with disease severity.In addition,TGFβ drives development of regulatory T cells,but can also enhance Th17 development in the presence of IL6.

Cartilage and bone degradation: the role of fibroblasts and osteoclasts

While immune effector cells discussed above are responsible for initiating and maintaining inflammation, two cell types are of prime importance for the destruction of cartilage and bone. SFs adhere to cartilage and degrade extracellular matrix.Osteoclasts ,on the other hand ,are mainly involved in bone destruction.Both cell types closely interact with cells of the immune system and by secreting large amounts of

cytokines,participate in the maintenance of inflammation.SFs in RA are characteristically found in the sub-lining layer of the synovium.Expression of various transcription factors indicates that they proliferate locally in disease,contributing to synovial hyperplasia.SF in RA patients show prolonged survival,and resistance to apoptosis.Intriguingly,they have been shown experimentally to have the potential to migrate between joints in experimental models,suggesting a possible route to disease progression in humans.Functionally, they adhere to cartilage via attachment to fibronectin,collagen type VI, and cartilage oligomeric matrix protein(COMP),and display an aggressive invasive behaviour.SFs are an abundant source of IL15,IL16,and IL17.They aiso secrete CXCL12,CXCL13, and members of the IL6 family(eg,IL11).These cytokines activate T cells and influence B cell migration and survival.Large amounts of secreted PGE2 additionally support inflammation.In addition, degradation of cartilage by SFs is due to the secretion of matrix metalloproteinases(MMPs) and cathepsins.Specifically ,SF produce MMP-1,-3,-13,-14 and -15 as well as cathepsins B,K and L.These enzymes degrade extracellular matrix providing a rich source of potential neo- antigens for T and B cell polyclonal proliferation.

Bone degradation in RA is mainly mediated by osteoclasts.Osteoclastogenesis—that is, the differentiation of osteoclasts from precursor cells –requires M-CSF and the presence of an osteoclast differentiation,as it was found that ODF and osteoprotegrin ligand(OPG-L) are identical to RANKL(receptor activator of NFkB ligand) and TRANCE(TNF-related activation induced cytokine) molecules first identified in activated T cells.Meanwhile,synovium,and RANKAL –deficient mice are protected from bone destruction in experimental arthritis despite active infiammation.RANKAL is expressed not only by T cells,which thereby directly drive osteoclastogenesis,but also by neutrophils and in large amounts by SFs.TNFα accelerates this process by inducing RANKAL expression and enhancing RANKAL signalling.Interestingly,Th1- and Th2-cytokines(IFNγ,IL4,IL10) as well as IL12 and IL18 inhibit osteoclastogenesis.IL 17 however induces RANKAL expression in osteoblasts and might significantly contribute to osteoclast formation.

Figure No:3a.2.3.1 Development of rheumatoid arthritis(RA)

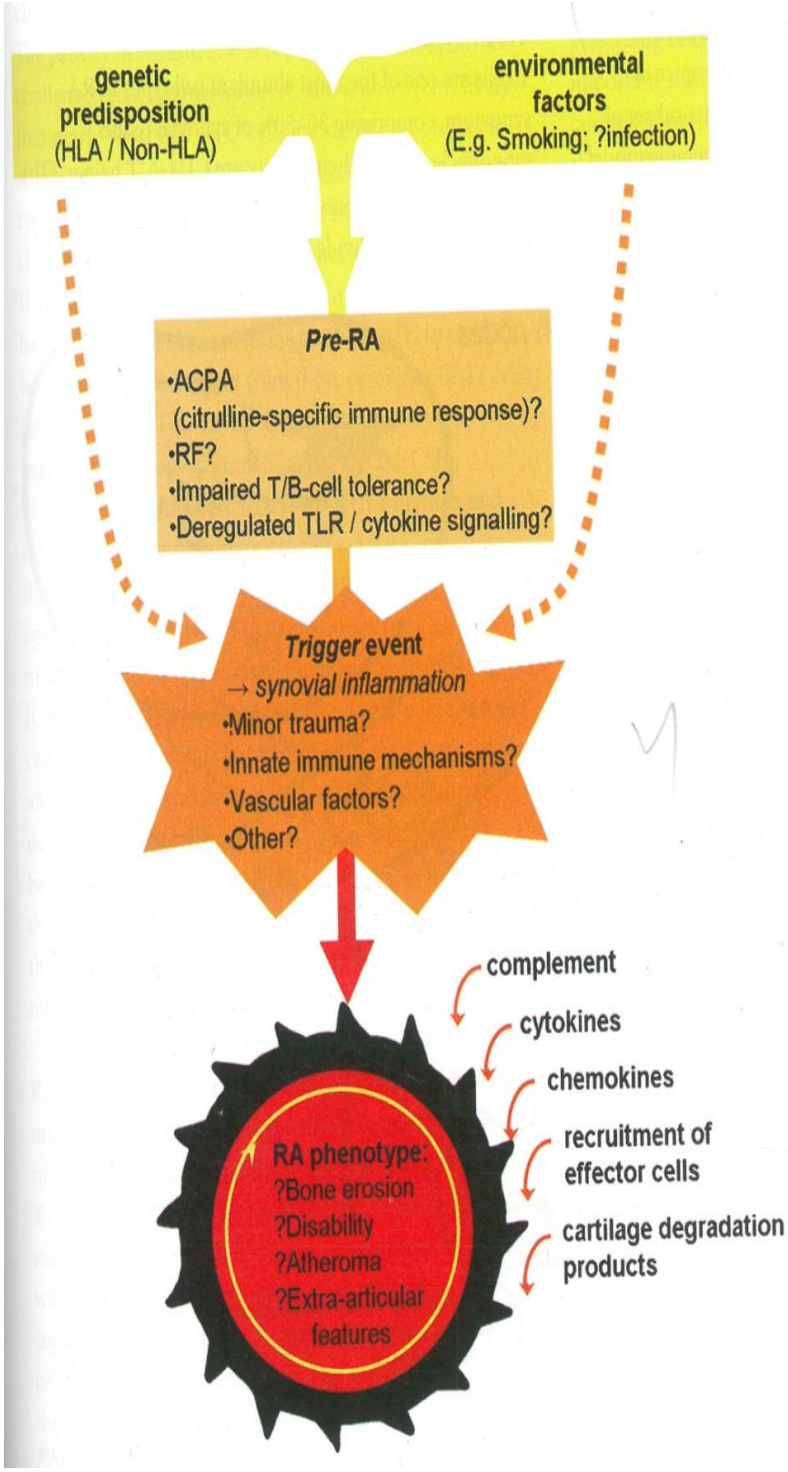


Figure 1 Schematic representation of factors relevant for the development of rheumatoid arthritis (RA). Genetic and environmental determinants interact to create an adverse immune state in the predisposed individual, which may include the generation of circulating anticitrullinated protein antibodies (ACPA). When, how and why a 'trigger event' subsequently causes pathology to become focused in the synovium is unknown, but it likely involves the innate immune system, and further interaction of genetic and environmental factors. A self-perpetuating inflammatory cascade follows, producing the clinically recognisable, albeit heterogeneous, RA phenotype. HLA, human leucocyte antigen; RF, rheumatoid factor; TLR, Toll-like receptor.

Figure No: 3a.2.3.2 Mechanism of the development of the immune response

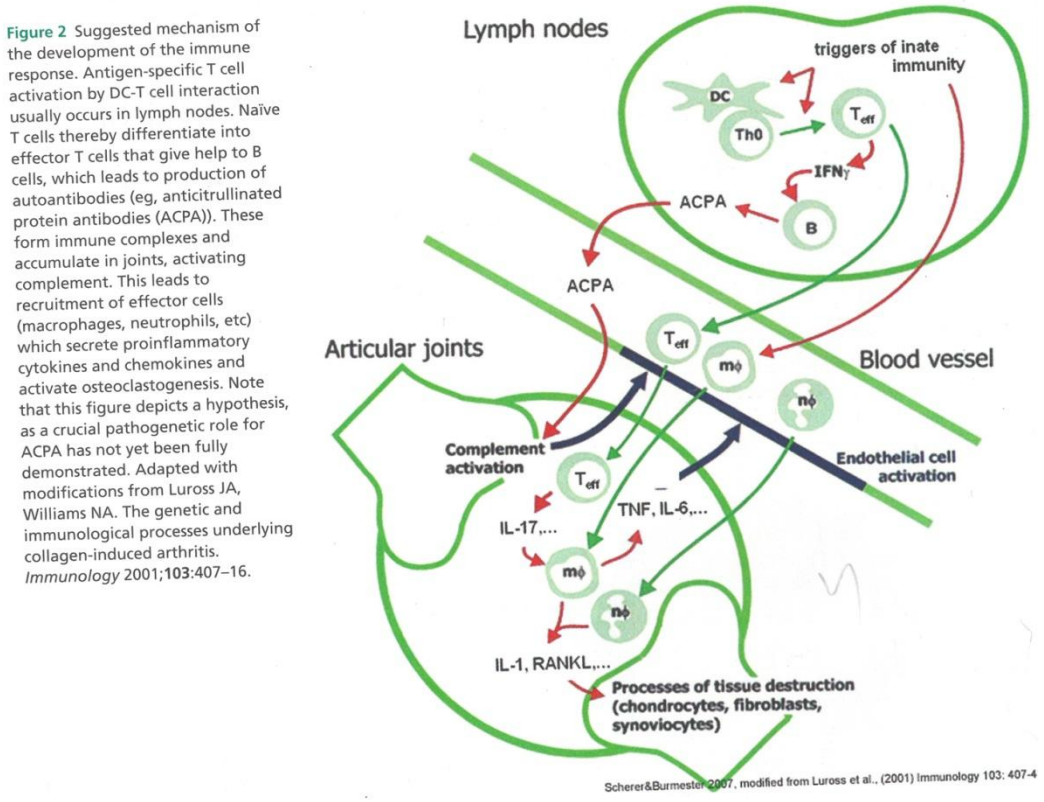


Figure 2 Suggested mechanism of the development of the immune response. Antigen-specific T cell activation by DC-T cell interaction usually occurs in lymph nodes. Naïve T cells thereby differentiate into effector T cells that give help to B cells, which leads to production of autoantibodies (eg, anticitrullinated protein antibodies (ACPA)). These form immune complexes and accumulate in joints, activating complement. This leads to recruitment of effector cells (macrophages, neutrophils, etc) which secrete proinflammatory cytokines and chemokines and activate osteoclastogenesis. Note that this figure depicts a hypothesis, as a crucial pathogenetic role for ACPA has not yet been fully demonstrated. Adapted with modifications from Luross JA, Williams NA. The genetic and immunological processes underlying collagen-induced arthritis. *Immunology* 2001;**103**:407–16.

Figure No: 3a.2.3.3 Factors contributing to osteoclastogenesis.

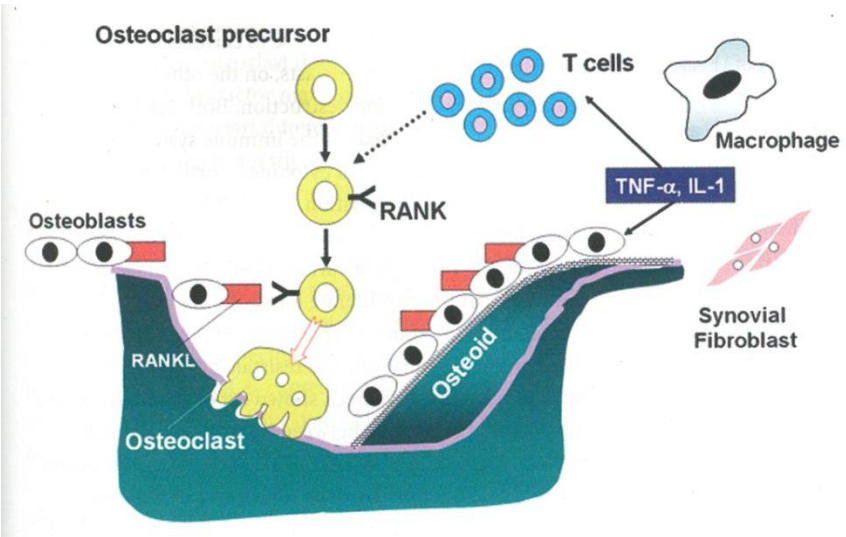


Figure 3 Factors contributing to osteoclastogenesis. Macrophages, synovial fibroblasts and T cells express IL1, TNFα and RANKL, factors important for differentiation of osteoblasts to osteoclasts. Multinucleated osteoclasts degrade bone, thereby creating radiographically detectable erosions

3a.2.4.Clinical aspect of rheumatoid arthritis:

Introduction

RA is the most prevalent chronic inflammatory joint disease.RA is a heterogeneous condition with a variable mode of disease onset and disease course.Some patients have very acute disease onset with fever,polyarthritis and extra-articular manifestations, whereas other patients have a more gradual and insidious onset.The latter presentation is more common.Typical articular signs and symptoms include pain,stiffness and swelling.Redness and warmth of involved joints are less common findings.Concomitant tenosynovitis,bursitis,and even carpal tunnel syndrome may be present.RA is a systemic disease as manifested by generalised weakness,weight loss or low grade fever and extra-articular features such as sicca syndrome,nodules,and interstitial lung fibrosis.

A special type of RA has been called palindromic rheumatism.This clinical picture includes variable episodes of poly arthritis which suddenly may affect one or more large or peripheral joints.The duration may be hours or a few days and then spontaneous improvement with complete disappearance of all rheumatic signs between attacks.About one third of these palindromic cases will eventually evolve into typical RA.Both prevalence and incidence rates are about two-to fourfold higher in women and symptoms are more severe than in men.The female:male ratio decreases with age.The reasons for the greater prevalence of RA among women have not been firmly established.A decline in the incidence has been especially apparent in women possibly as a consequence of some environmental factor such as the introduction of oral contraceptives in the1960s.A shift in the incidence towards a higher age of disease onset has been observed across several cohorts. The incidence rate seems to increase with age up to a plateau of around 60 years.

Disease onset

Articular manifestations:

The typical joint involvement at disease onset is swelling of the proximal interphalangeal (PIP) joints, the Metacarpophalangeal(MCP) joints, the wrists and the Metatarsophalangeal(MTP) joints. However, the disease starts gradually with involvement of one or a few joints and then over time, develops from undifferentiated oligo-or polyarthritis into a more polyarticular, and classically symmetrical, disease.Sometimes,

the disease also starts with monoarthritis, for example,of the knee.The differential diagnosis depends on the pattern of joint involvement at disease onset.Sometimes, the disease onset may be similar to reactive arthritis, but polyarticular inflammatory OA is a more common differential diagnostic consideration,especially in patients aged 50 or over.

Extra-articular manifestation:

The dominating feature at disease onset is usually joint involvement. However, the disease may start much more dramatically with fever and inflammatory manifestation of internal organs, for eg, with pericarditis and pleuritis. In such cases, other systemic diseases may be the most important differential diagnosis, for eg, Systemic lupus erythematoses. Other extra-articular manifestations such as Sicca syndrome, nodules and interstitial lung fibrosis are more commonly seen in established RA.

Symptoms:

1. Tenderness and pain in the inflammed joints.
2. Joint stiffness while moving the joints.
3. Swelling of the joints.
4. Morning stiffness is a typical and common symptom.
5. Fatigue and loss of energy.

Clinical findings:

The typical clinical finding of inflammed joints is soft tissue swelling and tenderness, and frequently, limited motion.Detection of synovitis is essential for the diagnosis.

Symmetrical joint swelling is noted in finger joints, wrist joints, ankle joints and in the forefeet.

As extra–articular manifestations are more commonly seen in established RA general physical examination is included in the clinical examination. Bibasilar inspiratory crepitations can point to underlying interstitial lung fibrosis.

A general organ examination is needed to find out concomitant diseases, since the intake of the related medications may result in an undesired harmful effect and worsen existing concurrent conditions.

3a.2.5.Laboratory investigation:

Typical findings are elevated erythrocyte sedimentation rate (ESR) and C reactive protein (CRP) concentration. Thrombocytosis and leucocytosis can be seen in active inflammatory disease. Reduced haemoglobin is also common. Serum iron mey be lowered whereas ferritin concentration may be elevated as a reflection of the acute phase reaction.

Liver and kidney function tests as well as routine urine examination are included in the laboratory screening to rule out liver or kidney related abnormalities.

Most importantly, tests for RF and anti-citrullinated peptide/protein antibodies (ACPA such as anti-CCP antibodies) are important both for the diagnosis and for the staging of the disease, since the presence of RF and ACPA are associated with a more severe disease course. Other immunological examinations may be important for differential diagnosis. It is common to test for the presence of antinuclear anti body (ANA), but the value of this test can be questioned if there is no clinical evidence of any systemic connective tissue disease. The presence of ANA has not been shown to have any prognostic value. However, secondary Sjogren's syndrome (anti-SSA and anti-SSB) may also be present in RA

Imaging procedures:

Ultrasonography may be helpful at disease onset to demonstrate synovitis of involved joints. Subclinical synovitis can be detected with ultrasonography and Doppler, but the clinical utility of this for clinical decision making remains to be established. At an early stage of the disease, it may also be useful to detect erosions of minor joints. X-ray is the gold standard conventional diagnostic to assess the joint damage, but the x-ray report of absence of erosions does not rule out RA.

Scoring system have been developed and validated to assess the extent of and changes in radiographic damage, such as the modified Sharp-van der Heijde score which is commonly used in clinical trials.

Magnetic resonance imaging (MRI) of wrist and finger joints may be an important tool for early diagnosis and staging of the disease. imaging procedure with gadolinium is performed to detect Synovitis.

3a.2.6.Diagnosis:

RA Clinical diagnosis based on the signs and symptoms that are considered typical of RA, and by excluding other diseases. The new EULAR/ACR classification criteria for patients with early arthritis to tailor treatment ,taking these prognostic markers in to account.(table) (Aletaha et al 2010)

The ACR/EULAR 2010 classification criteria for RA[39]

To be applied to patients: (1) who have ≥ 1 joint with definite synovitis, excluding the DIP joints, first MTP joints, and first CMC joints, and (2) in whom the synovitis cannot be explained by another disease.

Table No: 3a.2.6.1 The ACR/EULAR 2010 classification criteria for RA

Criteria	Score
A. Joint involvement:	
1 large joint	**0**
2- 10 large joints[a]	**1**
1-3 small joints(With or without involvement of large joints)	**2**
4-10 small joints[b](With or without involvement of large joints)	**3**
>10 joints(at least 1 small joints)	**5**
B. Serology(at least 1 test result is needed for classification): Negative RF and negative anti-CCP antibodies	**0**
Low-positive RF or low-positive anti-CCP antibodies[c]	**2**
High-positive RF or high-positive anti-CCP antibodies[d]	**3**

C. Acute phase reactants: **Normal CRP level and normal ESR**	**0**
Abnormal CRP level or abnormal ESR	**1**
D. Duration of symptoms: **<6 weeks**	**0**
≥6 weeks	**1**

BT Score: AT Score:

ACR, American College for Rheumatology; EULAR, European League Against Rheumatism; RA, rheumatoid arthritis; DIP, distal interphalangeal; MTP, metatarsophalangeal; CMC, Carpometacarpal; MCP, metacrpophalangeal; PIP, proximal interphalangeal; RF, Rheumatoid factor; anti-CCP, anti-cyclic citrullinated protein; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; BT-Before Treatment; AT-After Treatment.

[a]Large joints= shoulder, elbow, hips, knees, ankles.

[b]Small joints= MCPs, PIPs, Second-fifth MTPs, thumb IPs, wrists.

[c]Low-positive is ≤ 3 times the upper limit of normal

[d]High-positive is > 3 times the upper limit of normal.

3a.2.7.Differential diagnoses:[38]

The DD to be considered, depends on the clinical feature while onset of the disease. RA incidence increases with age and reaches a level around 60 years of age. This means that hand OA/generalised OA often will be the most frequent differential diagnosis to be considered in patient with a typical polyarticular disease with involvement of finger joints. The typical clinical feature which differentiates RA from hand OA is involvement of the MCP and wrist joints, which are very frequently involved in hand OA(except in cases with haemochromatosis).

Polymyalgia rheumatic and calcium pyrophosphate deposition disease (CPDD) are also important differential diagnoses in elderly patients. RA in the elderly may start with symptoms of polymyalgia rheumatic and it is difficult to diagnose RA of elderly onset and polymyalgica rheumatic with existing arthritis. RF is often negative in these cases and large joints are most frequently involved than the small finger joints.

A differentiation between inflammatory rheumatic disease and OA of large joints with secondary inflammation of synovial membrane can also be considered in these cases.

RA should be differentiated from other inflammatory rheumatic diseases in younger patients. Reactive arthritis frequently involving knees, ankles and MTP joints and infrequently involving finger joints should be differentiated. Among the connective tissue diseases, SLE is the most frequent differential diagnosis.

Psoriatic arthritis and RF negative RA can also be difficult to differentiate. It is common to classify polyarthritis combined with the presence of RF as RA even if the patient has psoriasis, whereas RF negative arthritis combined with psoriasis is classified as psoriatic arthritis.

The incidence of various inflammatory arthropathies has been examined in Southern Sweden (Soderlin et al 2002) and in Finland, and these results highlight that many inflammatory arthropathies have to be considered in patients with recent onset joint swelling.

Table no: 3a.2.7.1.Differential diagnosis of RA

Psoriatic arthritis
Spondyloarthropathies
Polymyalgia rheumatic with peripheral arthritis
Reactive arthritis
Acute sacoid arthropathy(Lofgren’s syndrome)
Systemic lupus erythematosus
Other connective tissue diseases
Calcium pyrophosphate deposition disease
Lyme arthritis

In Table no 3a.1.7.1 the most common inflammatory joint diseases which are to be considered as differential diagnoses to rheumatoid arthritis are shown.

3a.2.8.Disease assessment

The EULAR disease activity and response criteria are based on the disease activity score, but several other scores mentioned below have been validated and are used in clinical practice.Core measures of disease activity include tender and swollen joint counts,patient and investigator global assessments of disease activity on a visual analogue scale(VAS), intensity of joint pain on a VAS, a patient- reported measure of physical disability(usually Health Assessment Questionnaire(HAQ)), and acute phase reactants(ESR and CRP)

Functional disability is most commonly measured by the Stanford HAQ, which is the most widely used instrument measuring disability in RA. The shortened version, the Modified Health Assessment Questionnaire(MHAQ),reduces the number of items from 20 to eight,one for each of the eight components,and does not allow upgrading of scores by use of technical devices or help by another person.

Joint damage is usually assessed by imaging modalities,using one of the accepted scoring systems to assess erosions and joint space narrowing.The most widely used systems are the Larsen score and the Sharp score(with modifications by Genant or van der Heijde).Clinical scores of deformities are also available.

3a.2.9.Identification of prognostic markers

Since RA can have a variable disease course, it is of particular importance and relevance to identify markers that may predict the disease course.In the ideal scenario, one would be able to tailor treatment according to such indicators – that is,to treat patients with predictors of severe disease course aggressively,and to protect the patients with a potentially mild disease from drugs that are associated with severe adverse reactions.

Three different outcomes can be considered to be of particular relevance; radiograhic damage, functional disability, and mortality. Table no 3a.9.1 shows known predictors for these long term outcomes

Table no: 3a.2.9.1.Prediction of outcome in rheumatoid arthritis[38]

Prediction of future physical disability(HAQ score)	Prediction of structural damage	Prediction of increased mortality
HAQ score Age Acutephasereactants(?) Female gender(?) Level of education(?) Jointcounts(?) Rheumatoid factor(?) Erosive disease(?)	Erosive disease Acutephasereactants Rheumatoid factor ACPA Jointcounts(?) Geneticmarkers(shared epitope) Female gender(?) Present HAQ score(?)	Age Levelofeducation/coping Physical status Rheumatoid factor Comorbidities Extra-articular manifestations Gender(?) Jointcounts(?) Acute phase reactants(?) Structural damage(?) Use of corticosteroids(?)

ACPA,anticitrullinated protein antibodies;HAQ,Health Assessment Questionnaire.

3a.2.10.Recent onset arthritis

Patient should be monitored frequently,especially during the first year of the disease.Tight control of disease activity in this period is important to induce remission or achieve a low disease activity and to prevent future damage and disability,because of the relationship between level of inflammatory activity and future damage.This concept has been supported by several studies –for example,the TICORA study(Grigor et al 2004).Patients who were followed by tight control of disease activity had less radiographic progression than patients followed by a regular follow-up regimen.

3a.2.11.Established disease

The clinical challenge in patients with established disease is different from recent onset or early RA. The joint involvement can be dominated by deformities as a consequence of longstanding inflammation and radiographic damage. It is also particularly important to be aware of the possibility of involvement of internal organs due to RA.

3a.2.12. Involvement of hands:

MCP and PIP joints are frequently involved. Deformities of the PIP joints usually result from lack of ligament support.

Boutonnier's deformity (Fig 3a.2.12.1): It describes a finger with flexion of the PIP joint and hyperextension of the DIP joint.

Z-deformity (Fig 3a.2.12.3): The similar deformity of the thumb is also called the 90-90 thumb because both the flexion of the MCP and the hyperextension of the IP joint may be 90˚ which is otherwise called as Z deformity.

Swan-neck deformity (Fig 3a.2.12.2): of the finger describes a hyperextension of PIP joint and a flexion of the DIP joints.

Deformities of the MCP joints include volar sub luxation, which may be seen as a step, ulnar drift (often in combination with radial deviation of the wrist), and flexion deformities.

Wrist deformities include volar subluxation with a visible step opposite the radiocarpal joint and radial deviation of the carpus from the axis of the wrist and hand.

Arthritis of the distal radioulnar joint results in instability and dorsal subluxation of the ulnar head with a ‘piano key’ movement on downward pressure. A dorsally sub-luxated ulnar head with erosive abnormalities will often mechanically lead to rupture of neighbouring tendons.

Figure no: 3a.2.12.1.Boutonnier’s deformity

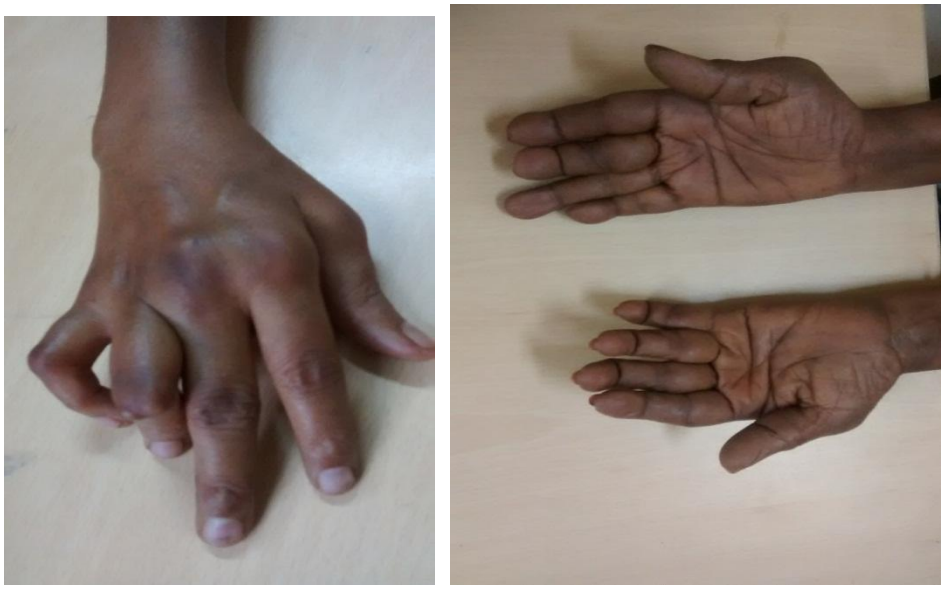
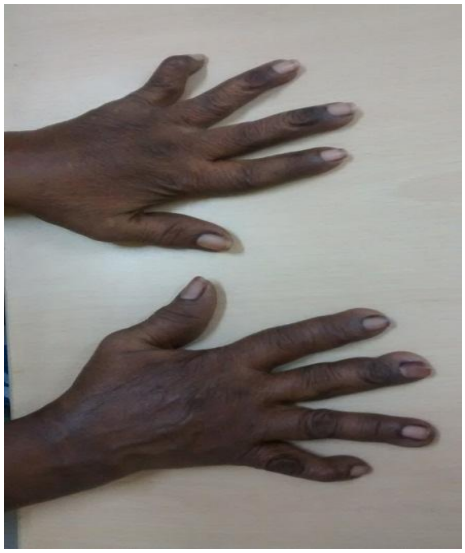


Figure No 3a.2.12.2.Swan neck deformity



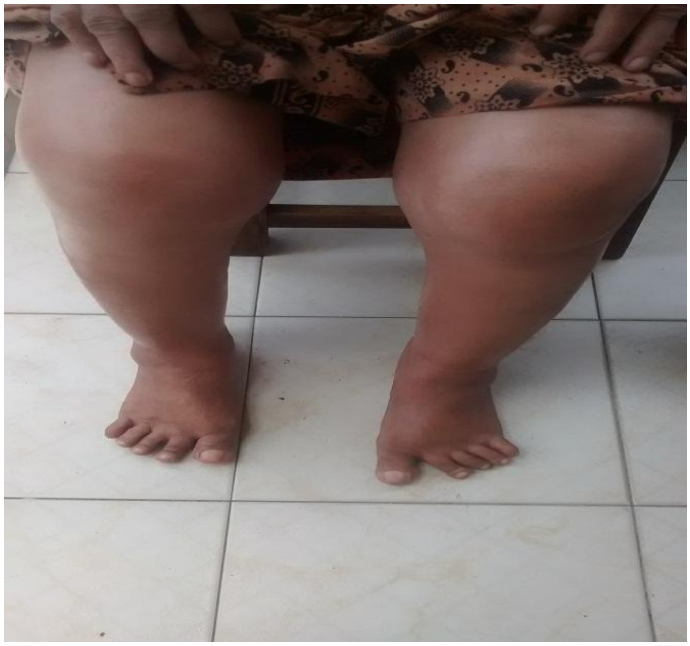


Figure No: 3a.2.12.3 Z deformity

Elbow and shoulder

The elbow joint is frequently involved in established RA.Loss of extension is a typical early sign.Functionally, loss of supination is disabling when eating and performing daily activities.The other disabling limited motion is reduced flexion. This may lead to inability to reach hair, face, etc.

Shoulder involvement in RA is not very frequent, but most patient with severe disabling RA also have shoulder involvement.Shoulder symptoms will usually appear when joint destruction has become advanced and will limit daily self-care activities.Synovitis leads to erosion and damage of both the humeral head and glenoid fossa.

The long head of the biceps muscle may rupture.This can be detected by biceps bulge when the patient flexes the elbow against resistance.The rotator cuff can also frequently be involved with inflammation and destruction.Involvement of the acromioclavicular joint is infrequent.The shoulder pain is infrequent.The shoulder pain is typically localised in the C4 region,whereas pain developing from the humerogleoidal joint or from the rotator cuff is typically perceived in the C5 region,including upper arm.

3a.2.13.Cervical spine

Involment of the cervical spine is relatively rare but potentially life- threatening.The space between the odontoid process from C2 and the arch of the atlas normally measures 3mmor less.If this space exceeds 5mm the condition is defined as atlantoaxial subluxation.

Synovial involvement may also affect the apophyseal joints in the occiput atlantal area.The consequence of the synovitis in this area may be an atlanto-axial subluxation with neurological symptoms due to myelomyopathy.

This deformity is important and should be detected before general anaesthesia. When a patient with RA requires surgery the presence of cervical subluxation presents a general anaesthetic risk.Routine cervical radiographs with the head in flexed positions is recommended before surgical procedures.Special precautions are required preoperatively to prevent cervical cord damage when atlantoaxial subluxation is detected.

3a.2.14.Joints of the lower limbs

Knee involvement is much more common in RA (3a.2.12.3). Synovial effusion is the typical sign of inflammatory activity of the knee.Patients with knee swelling have a tendency to assume a more comfortable position with the knee flexed, and if this becomes a habit ,a loss of full knee extension eventually results.

Reduction of knee extension may be associated with a cyst of the popliteal region called Baker's cyst.This cyst can extend from its location in the gastrocnemius bursa down into the medial aspects of the calf.This is best seen when patients are standing. The baker's popliteal cyst arises as an extension from the joint cavity.Rupture of this cyst can give a general swelling of the calf and a clinical picture which mimics deep vein thrombosis.Destruction of the knee may lead to instability due to laxity of the collateral and cruciate ligaments.This laxity is detected by stressing the knee joint for lateral and medial collateral stability.A special problem arises in women with physiological valgus.This valgus increases the load on the lateral compartment and as the valgus increases due to arthritis the loading will also increase so that a vicious cycle is produced.Varus position is more common in OA than RA.

Within the foot ,the subtalar and mid-tarsal joints are more frequently involved than the ankle joint.The ankle is usually quite stable,but reduced dorsal flexion may interfere

with walking ability.The subtalar and talonavicular joints are commonly affected in RA.Synovitis causes pain and stiffness and will sometimes lead to subtalar dislocation.As cartilage loss and bone erosion develop,valgus deformity increases with progressive flattening of the longitudinal arch.Involvement of the MTP joints is common in RA causing pain and disability.Forefoot deformity starts with synovitis of the MTP joints and involvement of the flexor tendons,which can result in clawing of the toes and dorsal dislocation of the MTP joints.The metatarsal heads can be destroyed due to the synovitis and can lead to extreme pain.

3a.2.15.Other joint areas

The temporomandibular joint may be involved with tenderness and painful limitation of mouth opening.Occasionally the cricoarytenoid joint may be involved and associated with hoarseness. The ossicles of the ear may also be involved with hearing loss independent of medication-induced effects.

3a.2.16.Nodules, tenosynovitis, and bursitis:

Subcutaneous nodules overlying the extensor aspects of the proximal ulna are present in about one quarter of the patients with RA.Subcutaneous nodules may form over other pressure locations such as the occiput, palms of the hands,and Achilles tendon.

Flexor tenosynovitis can lead to loss of active flexion.A trigger finger is a frequent feature of flexor tenosynovitis.The pathology is a thickening of the tendon or may be stenosis of the tendon sheet.Flexor tenosynovitis is often also accompanied by a tenosynovitis of the carpal tunnel, which may lead to entrapment of median nerve and development of carpal tunnel syndrome.

The synovial membrane lining the bursa secretes synovial fluid and will develop rheumatoid synovitis. Typical example of bursitis by RA is olecranon bursitis.

3a.2.17.Extra-articular manifestations and RA-related comorbidities

Because of the systemic and chronic nature of the disease, patients with established RA are at risk of developing extra-articular manifestations such as subcutaeneous nodules, vasculitis, pericarditis, pulmonary nodules or interstitial fibrosis, mononeuritis multiplex, episcleritis or scleritis, and specific comorbidities including osteoporosis, premature atherosclerosis, muscle weakness, infections and cancer.

3a.2.18.Secondary osteoporosis

RA is associated with an increased generalised bone loss, as diagnosed by a reduced bone mineral density(BMD).RA in itself is directly associated with the increased occurrence of vertebral fractures.There is two-fold increase in the occurrence of osteoporosis both in RA males and females.It is generally assumed that osteoporosis is due to inflammation(eg,cytokine release with osteoclast activation),but use of glucocorticoids and immobility can also be risk factors in some patients.

3a.2.19.Muscles

Muscle weakness and atrophy is common in RA, often due to either neuropathy, use of steroids or less use due to joint involvement.

3a.2.20.Secondary Sjogren’s syndrome

Secondary Sjogren’s syndrome is a typical manifestation of RA with sicca symptoms from eyes and also dryness of the mouth.

3a.2.21.Other eye complications

Scleritis, episcleritis or both occur in some patients with RA,but are rather infrequent.In episcleritis the eye becomes red within minutes.Unlike other inflammatory conditions of the eye,episcleritis results in no discharge other than tearing in response to great discomfort.Loss of vision does not occur as a direct result of episcleritis,but cataracts may develop secondarily and cause visual loss.Scleritis causes severe ocular pain and a dark red discolouration

3a.2.22.Infections

The incidence of bacterial infections is increased in RA patients partly due to the disease itself, and partly due to the use of glucocorticoids and other drugs with immunosuppressive actions; in particular, the new anti-TNF drugs.It is important to be aware that RA in itself also increases the risk for septic arthritis.It can be extremely challenging to differentiate between an acute flare of arthritis or a septic arthritis.The most important examination is to culture the synovial fluid.

3a.2.23.Cancer

There is a slight increased risk of malignancy in RA patients, especially lymphoma in certain patient subsets.

3a.2.24.Haematological abnormalities:

Mild normocytic hypochromic anaemia is associated with raised ESR and the disease activity.A few of these patients respond to iron therapy.Folate and B12 deficiency may also be a cause of anaemia in RA. Similarly thrombocytosis is also frequent in RA and is related to disease activity.

3a.2.25.Vascular complications

Initial pathological changes in RA includes inflammatory changes in the small blood vessels.However, when talking about vasculitis as a complication, the focus is more on the arteries or arterioles that lead to cutaneous ulceration or peripheral neuropathy or involvement of internal organs.Vasculitis is fortunately uncommon, but the presence of vasculitis is associated with disease severity and activity.

3a.2.26.Renal complication:

The kidney is rarely directly involved in RA but may be compromised by therapy. Both analgesics and non-steroidal anti-inflammatory drug(NSAIDs), as well as some DMARDs(eg,gold,ciclosporin), can affect renal function,resulting in elevated serum creatinine and/or proteinuria.

3a.2.27.Lung disease:

There are several forms of lung disease in RA. Pleuritis can be seen both at disease onset and during the disease course and may be seen together with pericarditis.Interstitial pneumonitis and fibrosis is rather common in longstanding RA.Nodular lung disease can be seen but usually in patients who also have rheumatic nodules in other parts of the body and in smokers.Pulmonary hypertension is rare.

3a.2.28.Heart disease

As part of the inflammatory activity, pericarditis can be seen with RA and often Co-existing with pleuritis.Myocardial and endocardial inflammations may also be present.

3a.2.29.Arteriosclerosis

Several studies have shown that patients with RA have an increased risk of clinical coronary heart disease(CHD) compared with age-and sex-matched non-RA subjects,and that this risk is maintained after accounting for traditional CHD risk factors(diabetes mellitus,hypertension,dyslipidaemia,body mass index,smoking).

Some studies support the view that atherosclerosis is correlated to the level of systemic inflammatory activity over time and that the inflammatory process impacts on cardiovascular morbidity and mortality and accelerated coronary and extra-coronary atherosclerosis.The therapeutic consequence is that anti-inflammatory therapy would therefore reduce the risk, and some studies have shown that therapy with methotrexate and anti-TNF drugs may reduce cardiovascular morbidity and mortality.

3a.2.30.Gastrointestinal complication

RA does not affect the gastrointestinal tract directly.Gastroduodenal ulcers are,however,rather frequent due to use of NSAIDs,which is why proton pump inhibitors are routinely prescribed to RA patients on chronic NSAID therapy.Liver disease may also be secondary to drugs commonly used in RA—for example,methotrexate,diclofenac,and other NSAIDs as well as paracetamol.

3a.2.31.Secondary amyloidosis

Secondary amyloidosis is an increasingly uncommon complication of RA.This is thought to be related to the implementation of more effective treatment strategies in RA.Amyloidosis results from the deposition in various organs of autologous protein in an insoluble fibrillar form,especially in kidneys.In patients with RA,excess serum amyloid A protein production is stimulated by the cytokines of the inflammatory response.Previously, renal amyloidosis was a rather frequent complication leading to Renal failure,dialysis and renal transplantation,and this should now primarily be regarded as treatment failure of RA itself.

3a.2.32.Functional disability and its consequences

Important correlates of functional disability in RA patients include female sex, age, disease duration,and disease activity measures such as the Ritchie articular index and ESR,which can be considered predictors of a poorer functional status.

In addition to indicating a requirement for more aggressive treatment,high levels of disability have a negative impact on the psychological and social functioning of the patient,the consequences of which include mental distress,depression,and fatigue.

Fatigue is also a significant and frequently debilitating problem for people with RA,as it contributes to work difficulties,personal injury,reduced ability to participate in rehabilitation programmes,and strained relationships.

3a.2.33.Mortality

Disease severity,activity and disability are strongly linked to mortality in RA sufferers, and other independent predictors include age,level of education,male sex,comorbidity,and usage of prednisone.The major contributing causes of death among RA patients are cardiovascular and cerebrovascular diseases.Some studies have indicated that RA currently is a milder disease than some years ago and also that the disease on average starts later than before.It is also assumed that the advances in treatment strategies and access to new therapies improves the health of patients with RA.However,it is still important to consider RA as a severe disease with a potential reduction in life expectancy.

3a.2.34.Rheumatoid Arthritis: Treatment

DMARDs : Disease Modifying Anti-Rheumatoid Drugs:

DMARDs constitute the backbone of RA pharmacological treatment and all patients with RA are candidates for DMARD therapy. The qualification, disease-modifying, is given to any antirheumatic drug that has a positive impact on radiological outcome of joint damage (erosion and joint space narrowing), which is considered the final common pathway of the different pathogenic mechanism that underlie RA.

The synthetic DMARDs most commonly used include methotrexate (MTX), sulfasalazine (SSZ), leflunomide, hydroxylchloroquine(HCQ), and glucocorticoids. Those now rarely used, due to their unfavourable risk/benefit ratios, include azathioprine(AZA), gold, minocycline, ciclosporin and D-penicillamine.

Table No: 3a.2.34.1.Rheumatoid Arthritis: Treatment

Common Name	Drugs	Adverse reaction
Synthetic DMARDs	Methotrexate	Probably safe to continue at least in low dose. Adverse reaction to MTX occur in half of treated patients, mainly comprise gastrointestinal complaints and mild transaminase elevation.
	Sulfasalazine	Patients withdrew because of intolerable side effect of gastro intestinal symptoms or central nervous system toxicity(headache, dizziness) ,4-5% of rash. Contra-indication with impaired renal or hepatic functions or blood dyscrasias.
	Leflunomide	Teratogenic effect. Contra-indication to the use of leflunomide include liver disease, severe renal impairment, severe immune deficiency, and rifampicin therapy. Elevated liver enzymes, diarrhea, alopecia, high blood pressure, rash , penumonitis.

	Glucocorticoids	Osteoporosis, hyper- glycaemia, hypertension, skin fragility, peptic ulcer disease, premature atherosclerosis, cataracts, myopathy, osteonecrosis, infection, mood changes, sleep disturbances and weight gain.
	Hydroxychloroquine/ chloroquine	Retinopathy, impaired renal and hepatic functions, rash, abdominal cramps and diarrhea.
Biological DMARDs	Infliximab	Contra-indication with systemic tuberculosis, allergic granulomatosis of the lung, or mild leucopenia.
	Adalimumab	Upper respiratorytract infection, headache, sinusitis flu-like symptoms, pain and reaction at injection site, skin rash, antibody development urinarytractinfection abdominal pain.
	Etanercept	Redness, itching, pain, or swelling at the injection site may occur.
	Certolizumab	Shortness of breath, swelling ankles/feet, severe/unusual tiredness, easy bruising/bleeding,swollen joints, numbness/tingling of arms/legs, rash on nose and cheeks, eye pain, vision changes, dizziness, seizures.
	Golimumab	Cold symptoms like sore throat or redness, sneezing, stuffy nose, itching, pain or swelling at the injection site, dizziness.
	Tocilizumab	Runny or stuffy nose,sinus pain, sore throat, headache, dizziness, itching, mild stomach cramps, or urinary tract infection (UTI).
	Abatacept	Nausea, diarrhea, stomach pain, indigestion; or headache, dizziness; cold symptoms such as stuffy nose, sneezing, sore throat, cough; back pain.
	Rituximab	Headache, fever, chills, stomach pain, nausea, diarrhea, heartburn, flushing.

3b. DRUG REVIEW:

1. ***Seraankottai*:** [41]

Botanical Name: *Semecarpus anacardium* L.f

Taxonomical classification:

Kingdom: Plantae

Subkingdom: Tracheobionta

Super division: Spermatophyta

Division: Magnoliophyta

Class: Magnoliopsida

Sub class: Rosidae

Order: Sapindales

Family: Anacardiaceae

Genus: Semecarpus

Species: Anacardium

Vernacular Names:

Sans: Bhallaka ; Hindi&Beng: Bhela ;Tam: Shenkottai; Eng:Marking-nut tree[42,43]

It is katu, tikta, kashaya& ushna, anthelmintic,helpful in deranged kapha vata, intestinal affections, epistasis, polyuria &piles.

Occurrence & Distribution:

It occurs throughout the hotter parts of India including tropical outer Himalayas.

Description:

A Moderate-sized, dioecious, deciduous tree, exuding a dark juice, young branches, inflorescence, petioles and underside a leaves clothed with a fine pale pubescence, Leaves oblong-obovate, large, rounded at apex, cartilaginous at margin, very coriaceous, Flowers fasiculate, arranged in erect compound, terminal panicles, greenish yellow, Drupes obliquely oval or oblong, smoth, shining, purplish-black when ripe cup orange –red.

Flowering at different times of the year, mostly during May-June. The Fruit ripens from November to February.

Part used: Fruits and bark

Therapeutic uses:

Fruits: in asthma, ascites, epilepsy, neuralgia, psoriasis and rheumatism, as abortifacient and vermifuge, decoction mixed with milk and butter, asthma, gout, hemiplegia, neuritis, piles, rheumatism, sciatica and syphilitic complaints, kernel is anthelmintic.Cardiotonic, carminative and digestive, oil used externally in gout, leprosy and leucoderma.

Bark:

Brownish gum exudates found useful in nervous debility and in leprous, scrofulous and venereal affections.

Chemical constituents:

Nicotinic acid, riboflavin,thiamine and the essential amino acids,arginine, histidine,isoleucine, leucine,lysine,methionine, phenylalanine,threonine,tryptophan and valine (fruits);nacardic acid ,aromatic amines,bhilawanols(1-pentadeca-Δ^{8}-enyl-2,3-dihydroxy benzene) and 1-pentadeca- $\Delta^{7,10}$ –dienyl-2,3-di dihydroxy benzene,being the major components of more than sevenclosely related aromatic carboxylic acids(also from nut shell);biflavanoids A,B, and C,latter two characterized as 3,8-binaringenin and 3-8-biliquisitigenin;tetrahydrobustaflavanone, tetrahydroamentoflavanone, nallaflavone (nuts); galluflavanone, jeediflavanone(nutshell);linolenic,myristic,oleic palmitic and stearic acids(kernel oil); amentoflavone (leaves)biflavones A&A also reported.

Semecarpus anacardium Linn. (*Family:* Anacardiaceae), commonly known ‘Ballataka’ or ‘Bhilwa’, has been used in various traditional system of medicines for various ailments since ancient times. Its nuts contain a variety of biologically active compounds such as biflavonoids, phenolic compounds, bhilawanols, minerals, vitamins and amino acids, which show various medicinal properties. The fruit and nut extract shows various activities like antiatherogenic, antinflammatory, antioxidant, antimicrobial, anti-reproductive, CNS stimulant, hypoglycemic, anticarcinogenic and hair growth promoter. The article reviews the various activities of the plant.[42]

Semecarpus anacardium is used for various medicinal properties. The fruit and nut extract shows various activities like antiatherogenic, antiinflammatory, antioxidant, antimicrobial, anti-reproductive, CNS stimulant, hypoglycemic, anticarcinogenic and hair growth promoter.

Bhallataka should be used with caution since it is extremely hot and sharp in its attributes,. Individuals showing allergic reactions to it should stop and avoid the usage of Bhallataka. It should not be used in small children, very old persons, pregnant women and individuals of predominant pitta constitution. The use of the same should be restricted in summer season. For its allergic reactions like itching rash and swelling, the antidotes used externally are coconut oil, rala ointment, ghee, coriander leaves pulp or butter mixed with musta (Cyperus rotundus). The salt and spices should be strictly restricted and during Bhallataka treatment, exposure to sun, heat and excessive sex is to be avoided. The oily part of the nut is toxic and its degree of removal is proportional to its safety margin.

Ramprasathet *et al*. investigated the antiinflammatory effects of SA nut extract on developing and developed adjuvant arthritis. *Semecarpus anacardium* significantly decreased the carrageenan-induced paw edema and cotton pellet granuloma. These results indicate the potent antiinflammatory effect and therapeutic efficacy of SA Linn. Nut extract against all phases of inflammation is comparable to that of indomethacin.

Salvem *et al*. investigated that ethyl acetate extract of SA led to the isolation of the major active principle, tetrahydroamentoflavone (THA), a biflavonoid. The *in vitro* cyclooxygenase (COX-1)-catalyzed prostaglandin biosynthesis assay of THA gave an IC50 value of 29.5 μM (COX-1) and 40.5% inhibition at 100 g/mL (COX-2). The *in vivo* carrageenan-induced paw edema assay resulted in dose-dependent antiinflammatory effect of THA and the activity was comparable to that of ibuprofen.

Bhitre *et al*. prepared the methanolic, ethanolic, chloroform, ethyl acetate and petroleum ether extracts of fruits of SA and tested to study the anti-inflammatory activity using the technique of carrageenan-induced paw edema in albino rats. The extract showed significant antiinflammatory activity comparable to the reference standard aspirin.Satayavati *et al*. and Bajpai *et al*, reported the antiinflammatory activity of SA for both immunological and non-immunological origin.

Singh *et al*. evaluated that SA extract can inhibit proinflammatory cytokine production. Crude ethanolic extract of SA nuts was studied for its antiinflammatory activities *in vitro* using peripheral blood and synovial fluid mononuclear cells of healthy individuals and rheumatoid arthritis (RA) patients. *Semecarpus anacardium* extract

inhibited the spontaneous and LPS-induced production of proinflammatory cytokines IL-1beta and IL-12p40 but had no effect on TNF-alpha and IL-6 production, both at protein and mRNA level. The crude fruit extract ,also suppressed LPS-induced nuclear translocation of transcription factors, AP-1 and NF-kappaB; through the inhibition of I kappa Bα phosphorylation, the inhibition of NF-kappa B was attained. In mouse macrophage cell line, RAW 264.7, the extract also suppressed LPS-activated nitric oxide production .Premlatha *et al*. have been reported for immunomodulatory potency, antioxidative, membrane stabilizing, tumors marker regulative, glucose level restoring and mineral regulation properties of the nut extract in hepatocellular carcinoma and found to detoxify a potent hepatocarcinogen aflatoxin B_1 and causes its metabolites to be excreted in urine.

In the other case, they explained the therapeutic effects of extract on the changes associated with collagen and glycosaminoglycan metabolism in adjuvant arthritic Wistar rats. Decreased levels of glycosaminoglycans (GAGS) and collagen components (hyaluronic acid ,chondroitin sulfate, heparan sulfate,) and increase in the levels of connective tissue degrading lysosomal glycohydrolases such as beta-*N*-acetyl glucosaminidase, beta-glucuronidase, acid phosphatase and cathepsin-D observed in arthritic animals were reverted back to near normal levels upon treatment with SA.

Ramprasath *et al*. found that nut milk extract modulates reactive oxygen/nitrogen species levels and antioxidative system in adjuvant arthritic rats. A significant increase in the levels of lipid peroxides (LPOs), ROS (superoxide radical, hydroxyl radical, H_2O_2 and myeloperoxidase) and RNS (nitrate + nitrite) observed in adjuvant arthritic animals were found to be significantly decreased on administration of the drug at 150 mg/kg body weight/day. Treatment with *Semecarpus anacardium* recovered the reformed antioxidant defense components to near normal levels. These evidences suggest that the SA preparations are mainly used for abnormalities produced during arthritis and to cure arthritis.

Kalphaamrutha is a siddha medicine which has been reported for its potent antioxidant, antipyretic, analgesic properties. Mythilypriya *et al*. studied in adjuvant-induced arthritic rat (AIA) model, the antiinflammatory activity of SA with reference to mediators of inflammation (lysosomal enzymes) and its effect on proteoglycans. The levels of plasma protein bound carbohydrate components of glycoproteins and activities of various enzymes were measured and their elevation in arthritic rats when compared to control animals was observed.

Krishnamurthy *et al*. developed Kalpaamruthaa (KA), a modified Siddha preparation, which contains SA Linn., EO and honey, and studied for the variations in lipids, lipid-metabolizing enzymes and lipoproteins in cancerous animals and the effect of KA on lipid metabolism. The increased levels of total cholesterol, free cholesterol, phospholipids, triglycerides and free fatty acids and decreased levels of ester cholesterol in plasma, liver and kidney found in cancer-suffering animals were reverted back to near normal levels on treatment with KA and SA. The effects of KA were found to be more effective than SA.

Immunomodulatory effects[43] of Semecarpus anacardium LINN. nut milk extract (SA) were investigated in adjuvant induced arthritis by studying the alterations in humoral and cell mediated immune responses and also the anti-inflammatory effects by evaluating the changes in paw edema, tumour necrosis factor (TNF-alpha), nitric oxide and myeloperoxidase activities. Pharmacological studies were also conducted with SA and indomethacin on experimental animals for evaluating the anti-inflammatory, analgesic, antipyretic and ulcerogenic activities. The alterations in humoral and cell mediated immunity were significantly reverted back to near normal levels on treatment with SA. The drug significantly reduced the elevation in the paw edema, TNF-alpha, nitric oxide and myeloperoxidase levels when compared with adjuvant induced arthritic animals, which shows the anti-inflammatory activity of the drug. SA showed strong anti-inflammatory effects in xylene-induced ear edema and formalin-induced inflammation. In analgesic test, the extract elicited a potential activity on both acetic acid-induced writhing response as well as hot plate test showing its central and peripheral mediated action. The drug also elicited antipyretic action in yeast-induced hyperemia in rats. In addition, the extract did not produce any ulceration on gastric mucosa during ulcerogenic test and did not produce any serious adverse effects. All these effects are nearly similar to the activities of indomethacin except the ulceration where indomethacin produced significant ulceration. From this study, the protective immunological and pharmacological role of SA is demonstrated.

Confirmation of Structure of Semecarpus biflavanone B by chemical studies ,a new biflavonoid Jeediflavanone-isolated from nut shells and characterized; galluflavanone isolated from nut shells and its structure determined; isolation and structure elucidation of semecarpus flavanone from nut shells.[43]

Plant extract showed direct depressant effect on isolated frog heart & rabbit intestine and antagonized spasmogenic effects of carbachol, histamine, barium chloride and pitocin, Trypan blue capillary permeability test was found to be positive. It produced delayed hypotension in dogs which remained unaltered after prior atropinisation.

Bhilawanol from fruits was shown to be a mixture of cis and trans isomers of ursubenol.[44]

2. Etti : [45,46]

Botanical name: *Strychnos nux-vomica*

Taxonomical classification:
Kingdom: Plantae
Sub kingdom: Tracheobionta
Super division: Spermatophyta
Division: Magnoliophyta
Class: Magnoliopsida
Sub class: Asteridae
Order: Gentianales
Family: Loganiaceae
Genus: Strychnos
Species: nux-vomica

It is used in bronchitis, diabetes, intermittent fever, dyspepsia, chronic constipation from atony of the bowels, chronic dysentery, atonic diarrhea, prolapses of the rectum, gouty, rheumatic, paralytic and neuralgic affections, worms, tobacco-amaurosis, insomnia from over-fatigue, hydrophobia, bronchitis, emphysema, phthisis, impotency, spasmodic diseases, spermatorrhoea, excessive venery, alcoholism, opium and lead poisioning, nocturnal incontinence, retention of urine and externally headaches, swollen glands, odema of the hands feet and abdomen, rat bites and bites of venomous reptiles, muscular and chronic rheumatism, palsy and hypodermically in narcotic poisioning, chronic alcoholism and snakes-bites.

Vernacular names:

Sans: Kupilu, Kulaka, Vishamushti, Vishtindu: Eng: Nux-vomica or strychnine tree; Poison-Nut; Quaker button. Fr: - Noix Vomique. Ger: Gemeiner Brechnussbaum, Hindi: - Jahar; Kuchla, Bom& Mah: Kajra, Kuchala, Tel: Mushti-vittulu Tam: Yetti; Yetti kottai Mal: Kanjiram, Can: Kasarkana mara, Kon: Karya-ruku Burm: Khaboung

Habitat: This tree is wild and plentiful throughout tropical India, commonly in the jungles about Manbhoom, in the Madras presidency, Malabar and Coromandal coast, Cochin, Travancore, Southern India, Orissa and Ceylon.

Parts used: Stem-bark, dried ripe seeds called nux-vomica.

Constituents: Indian nux-vomica seeds contain 2.6 to 3 % total alkaloids approximately, of which 1.25 to 1.5 p.c is strychnine. (Chopra); brucine is 1.7% and vomicine; Igasurine or impure brucine in combination with igasuric or strychnic acid; loganin, a glucoside (which is present also in pulp of the fruit); proteids 11 p.c., yellow colouring matter, a concrete oil or fat, gum, starch, sugar 6 p.c., wax, earthly phosphate and ash 2 p.c. Wood, bark and leaves contain the largest percentage of brucine, i.e. 3.1 p.c. Leaves contain 1/3 rd p.c. Though the alkaloids occur in numerous species of Strychnos, they are not present in suffient amounts to serve as commercial sources.

(Chopra). N.B:- Investigation shows that the alkaloidal content is not altered by long storage in a moist condition. Adulteration of the seeds with S.blanda, a non-strychnine bearing seed, appears to be the real cause of the reported variation.

3. *Chukku*

Botanical name: *Zingiber officinale,* Roscoe

Family: Scitaminae

Part used: Scraped and Dried Rhizome

Chemical constituents:

Aromatic volatile oil having a characteristic odour and containing camphene, phellandrene, zingiberine, cineol and borneol; gingerol a yellow pungent body; an oleo-resin-gingerin the active principle, other resins and starch; K-oxalate.

Actions:

Aromatic, carminative, stimulant to the gastro-intestinal tract and stomachic also sialagogue and digestive. Externally it acts as a local stimulant and rubefacient.

Uses:

Ginger is extremely valuable in dyspepsia, flatulence, colic, vomiting, spasms and other painful affections of the stomach and the bowels unattended by fever; for cold, cough, asthma, dyspepsia and indigestion.

4. *Milagu*: P.no:969[45]

Botanical name: *Piper nigrum*, Linn

Family: Piperaceae

Part used: Dried unripe fruit

Chemical constituents:

A volatile alkaloid Piperine or pipirine ,Piperidine or piperidin, a balsamic volatile essential oil, fat, mesocarp contains chavicin, a balsamic volatile oil, starch, lignin, gum, fat ,proteids and ash containing organic matter .Chavicin is a soluble pungent concrete resin; It contains very little piperine and no volatile oil. Piperine crystallizes in flat, four-sided glassy prisms insoluble in water.

Actions:

Acrid, pungent, hot, carminative also used as antiperiodic. Externally it is rubefacient and stimulant to the skin and resolvent. On the mucous membrane of the urethra it acts like cubebs; Piperine is a mild antipyretic and antiperiodic

Uses:

It is used in cholera, dyspepsia, flatulence, diarrhoea and various gastric ailments. It is used in constipation, piles, colic, gastric troubles, ascites, anaemia, worms, asthma, etc.

5. *Thippili*: [45]

Botanical name: *Piper longum*, Linn.

Family: Piperaceae

Part used: Immature berries dried in sun.(dried unripe fruits or fruiting spikes)

Chemical constituents: Resin, volatile oil, Starch, Gum, Fatty acid, In organic matter and an alkaloid, Piperine

Actions: Stimulant, carminative, alterative, tonic, aphrodisiac, diuretic, vermifuge and emmenagogue.

Uses: It relieves cough cold, asthma, hoarseness and hiccup.

6. ***Ellam*:** [45]

Botanical name: *Elettaria cardamomum*, Maton

Family: Scitaminaceae

Part used: Dried riped seed

Chemical constituents: Fixed oil, essential oil, volatile oil of the seeds. It contains considerable amount of terpinyl acetate, cineole, free terpineol, limonene , potassium salts, starch, nitrogenous mucilage, yellow colouring matter, Ligneous fibre and ash containing manganese.

Actions: Powerful aromatic, stimulant, carminative, stomachic and diuretic.

Uses: It is used to treat nausea, vomiting, head ache, refrigerant, liquefies matter, resolvent, cardiac stimulant, absorbs moisture, expels wind, helps digestion, relieves hepatic colic.

7. ***Cittrathai:*** [45]

Botanical name: *Alpinia officinaram*, Hance

Family: Scitaminaceae

Part used: Dried Rhizome

Chemical constituents: Flavonoids Galangin, 3-O-methyl galangin, essential oil, 1, 8-cineole, methyl cinnamate, ∞-cadinene, kaempferide, alpinin, galangol.

Actions: Stomachic, stimulant, carminative.

Uses: It is used to cure indigestion, vomiting sensation and gastric problems (sankarnarayanan 2009)

8. ***Sadamanjil*:**

Botanical name: *Nardostachys jatamansi*, DC

Family: Valeriancaceae

Part used: Rhizome

Chemical constituents: A volatile essential oil, resin, sugar, starch, bitter extractive matter and gum.

Actions: Bitter taste, aromatic, anti-spasmodic, diuretic, emmenagogue, nerve sedative, nerve stimulant, tonic, carminative, deobstruent, sedative to the spinal cord, promotes appetite and digestion.

Uses: Tonic for heart, liver, brain. It removes obstruction, diuretic and emmenagogue, jaundice and to treat stone in kidney.

9. *Akkiragaram*:

Botanical name: *Anacylus pyrethrum*, DC

Family: Compositae

Part used: Root

Chemical constituents: It contains essential volatile oil and an alkaloid pellitorin or pyrethrin.

Actions: Cordial, stimulant and sialagogue.

Uses: Root is a valuable sialagogue and is regarded as a tonic to the nervous system. It is powerful irritant. A decoction of the root is useful as a gargle in sore throat, tonsillitis, carious teeth and toothache,. It has been given in paralysis, hemiplegia, epilepsy, chorea and rheumatism and a host of other diseases. An infusion of this drug is useful in cases of rheumatism.

10. *Amukkura*:

Botanical name: *Withania somnifera*, Dunal.

Family: Solanaceae

Part used: Roots

Chemical constituents: Plant growing in southern Europe is found to contain a bitter alkaloid somniferin having hypnotic property, also has resin, fat and colouring matters.

Actions: tonic, alterative, astringent, aphrodisiac and nervine sedative. Root is also diuretic and deobstruent.

Uses: Root is used as an application in obstinate ulcers and rheumatic swellings. Root is used to treat all cases of general debility, senile debility, emaciation of children, rheumatism, brain-fag, loss of memory, nervous exhaustion, loss of muscular energy and spermatorrhoea. It infuses fresh energy and vigour in a system worn out owing to any constitutional disease like rheumatic fever etc.

11. Avuri: [47]

Botanical name: *Indigofera tinctoria*, Linn.

Family: Papilionaceae

Part used: Leave

Chemical constituents: Indican(a glucoside) the oxidized form of Indigo-white, the product obtained by the fermentation of the fresh green plant.

Actions: The plant is stimulant, alterative, deobstruent and purgative. Indigo is anti-septic and astringent.

Uses: It is used for enlargement of liver and spleen, epilepsy and other nervous infection. It is used for haemostatic sedative, piles, healers of ulcers, diuretic, dropsy.

12. *Thalisapathri*: [45]

Botanical name: *Taxus baccata*, Linn

Family: Coniferae

Part used: Leaves

Chemical constituents: Alkaloid (taxine), Volatile oil, tannic acid, gallic acid

Actions: Carminative, expectorant, stomachic, tonic, anti –spasmodic.

Uses: Asthma, haemoptysis, epilepsy and other spasmodic affections.

13. *Siruthekku*:

Botanical name: *Premna herbacea*, Roxb

Family: Verbenaceae

Part used: Root

Chemical constituents: Root contains an orange brown acid resin, trace of alkaloid, starch.

Actions: Stimulant, alterative, bitter stomachic

Uses: Cough, asthma, fever, dropsy and rheumatism.

14. *Kurosani omam*:

Botanical name: *Hyoscyamus niger* Linn.

Family: Solanaceaea

Part used: Seeds

Chemical constituents: Hyoscyamine, empyreumatic oil.

Actions: Narcotic, anodyne, digestive, astringent and anthelmintic

Uses: A paste of seed in brandy or in wine is applied to gouty enlargements, inflamed breasts and swollen testicles. Internally is used for cough, asthma, gout along with henbane seeds and poppy seeds.

15. *Vaaividangam:*

Botanical name: *Embelia ribes* Burm.

Family: Myrsinaceae

Part used: Fruits or dried berries

Chemical constituents: Embelic acid, volatile and fixed oil, colouring matter, tannin, a resinoid body and alkaloid called Christembine.

Actions: Carminative, anthelmintic, stimulant, and alterative.

Uses: It is used to expel intestinal worms especially tape-worms.

16. *Athividayam:*

Botanical name: *Aconitum heterophyllum*, Wall

Family: Ranunculaceae

Part used: Dried tuberous roots

Chemical constituents: Amorphous intensely bitter alkaloid, atisine which is non-toxic, aconitinic acid, tannic acid, starch, fat, mixture of oleic, palmitic, stearic glycerides.

Actions: Bitter, tonic, astringent, stomachic, antiperiodic and aphrodisiac.

Uses: Very efficacious in diarrhoea, dysentery, acute inflammatory affections.

17. *Jathikkai*:

Botanical name: *Myristica fragrans*, Houtt

Family: Myristicaceae

Part used: Dried seed nut meg

Chemical constituents: Kernel(nut meg) contains a volatile oil, fixed oil(myristin,myristic acid), proteoids, fat, starch, mucilage, and ash

Actions: aromatic, stimulant, carminative in large dose narcotic. anti-inflammation

Uses: Concrete oil of nut meg is used as a good liniment for chronic rheumatism,paralysis and sprains.

18. *Jathipathiri*:

Botanical name: *Myristica fragrans*, Houtt

Family: Myristicaceae

Part used: Arill

Chemical constituents: It contains Volatile oil, fixed oil, essential oil(myristicene,myristicol), resin, fat, sugar, destrin and mucilage.

Actions: Rubefacient, stimulant, aperient and carminative.

Uses: The medicated oil of this drug is useful in relieving painful cramps in cholera.

19. *Karunjeeragam*:

Botanical name: *Nigella sativa*, Linn.

Family: Ranunculaceae

Part used: seeds

Chemical constituents: Seeds contains yellowish volatile oil and fixed oil, essential oil, albumen, sugar, mucilage, organic acids, metarbin, toxic glucoside, carvone, terpene or d-limonene (carvene), Cymene.

Actions: aromatic, diuretic, diaphoretic, anti-bilious, stomachic, stimulant, carminative, digestive, anthelmintic and emmenagogue. Oil is locally anaesthetic.

Uses: Seeds form a very useful remedy in worms, external application for skin diseases.

20. *Jeerakam*:

Botanical name: *Cuminum cyminum* Linn

Family: Umbelliferae

Part used: seeds

Chemical constituents: It contains fatty oil, resin, mucilage, gum, protein compound malates, essential oil (thymine, carvone, cuminol, cumic aldehyde,cymene, cymol,terpene etc

Actions: Carminative, aromatic, stomachic, stimulant and astringent

Uses: It is used for hoarseness of voice, dyspepsia, chronic diarrhoea.

21. *Ilavangam*:

Botanical name: *Eugenia caryophyllata*.

Family: Myrtaceae

Part used: Flower bud

Chemical constituents: Eugenol, acetyl eugenol, beta-caryophylline, vanillin, crategolic acid, tannins, gallotannic acid, metyl salicylate, flavonoids eugenin, kaempferol, rhamnetin, eugenitin, triterpenoids, oleanolic acid, stigmasterol, campesterol, sesquiterpenes and metyl- amyl-ketone.

Actions: Local anesthetic, anti-spasmodic. It also has carminative, stomachic, anti-septic.

Uses: It is used to cure head ache, tooth ache and gum problems. It cures flatulence and improves digestion.

22. *Chevviyam*:[45]

Botanical name: *Piper nigram*, Linn

Family: Piperaceae

Part used: root

Chemical constituents:

It contains volatile alkaloid piperine, piperidine, piperidin, volatile oil, chavicin, starch, lignin.

Actions: Acrid, pungent, hot, carminative, anti-periodic. Piperine is a mild anti-pyretic and anti-periodic.

Uses: cholera, flatulence, diarrhoea and various gastric ailments.

23. *Kattu milagu*: [48]

Botanical name: *Piper attenuatum* Buch.-Ham

Family: Piperaceae

Part used: Dried fruit berries

Chemical constituents: Pipoxide chlorohydrin, galbelgin, 8-hentriacontanol, several aristolactams and 4, 5-dioxaporphines.

Actions: Anti-cancer, Antioxidant, aromatic, stimulant, stomachic, carminative.

Uses: It is effective for head ache and muscular pains.

Sunil kumar, Vinod Gauttam, Mukunda Sudhakar Rao Bande, Kunal Nepali, Ankit Tyagi and Kanaya Lal Dhar. Estimation of crotepoxide in the fruit of Piper attenuatum Buch.-Ham. Using HPTLC and APLC. Current Pharmaceutical Analysis, 12(4):343-348.

24. *Chithramoolam:*

Botanical name: *Plumbago zeylanica*, Linn.

Family: Plumbaginaceae

Part used: root

Chemical constituents:

Roots contains an acrid crystalline Plumbagin

Actions: Alterative, gastric stimulant, appetiser in large doses, it is acro-narcotic poison. It has a specific action on the uterus.

Uses: It is used as a rubefacient, application in rheumatism, paralytic affection and in enlarged glands.

25. *Thippli Kattai*:

Botanical name: *Piper longum*, Linn

Family: Piperaceae

Part used: stem

Chemical constituents: Roots contains resin, volatile oil, starch, gum, fatty oil, inorganic matter and an alkaloid piperine.

Actions: Stimulant, carminative, alterative, tonic, aphrodisiac, diuretic, vermifuge and emmenagogue.

Uses: Immunomodulatory and antitumor activity

26. *Kasakasa*:

Botanical name: *Papaver somniferum*, Linn

Family: Papaveraceae

Part used: seeds

Chemical constituents: linoleic acid, oleic, and palmitic acid 1-Pentanol, 1-hexanal, 1-hexanol 2-pentylfuran and caproic acid could be identified as the main volatile compounds in all examined poppy seed oil.

Actions: Hypnotic, tonic to brain.

Uses: It is used to treat urinary diseases.

27. Shombu:

Botanical name: *Foeniculum vulgare*, Gaertn.

Family: Umbelliferae

Part used: seeds

Chemical constituents: Essential oil.

Actions: Stimulant, aromatic, carminative, diuretic, emmenagogue and purgative.

Uses: It is used to cure amenorrhoea, vomiting and indigestion.

28. *Naervaalam*:

Botanical name: *Croton tiglium*, Linn

Family: Euphorbiaceae

Part used: Seeds

Chemical constituents: Fatty fixed oil, tiglinic acid, crotonic or quartenylic acid and croton oil; fats present in croton oil are glycerides of stearic,palmitic,myristic,lauric acids.

Actions: purgative, vermifuge, stimulant.

Uses: It is used in dropsy, cerebral affections like apoplexy, convulsions.

29. *Palm jaggery*: [49]

Botanical name: *Borassus flabellifer* L.

Family: Arecaceae

Part used: Palm jaggery

Chemical constituents: good source of vitamin B complex and contains ascorbic acid

Actions: Liver tonic, Digestive, Blood purifier, Anti-oxidant, Muscle relaxant, Aphrodisiac

Uses: It prevents diseases like anaemia. It contains essential nutrients like magnesium and potassium which are vital for proper functioning of the nervous system and regulate blood pressure and heart functions respectively.

30. *Kungumapoo*: [50]

Botanical name: *Crocus sativus* L.

Family: Iridaceae

Part used: Dried styles and stigma

Chemical constituents: Saffron contains more than 150 volatile and aroma-yielding compounds mainly terpenes, terpene alcohol, and their esters, crocin, picrocrocin and safranal.

Actions: Anti-hypertensive, anti-convulsant, anti-tussive, anti-genototoxic, anti-oxidant, anti-depressant, anti-nociceptive, anti-inflammatory, relaxant.

Uses: It is used for various kinds of mental illnesses. The crude extract of pistils of saffron increases regaining in ischemia, reperfusion injury, memory and learning in rats.

***31. Padikaram:*[51]**

Common Name: Alumen

Source**:** Chiefly found with peroxide of iron in silajit or in alum earths of Nepal or prepared from the alum shales in the Punjab, Rajputana, Bihar. Alum is a common term for a class of double sulphates holding aluminium and such metals as ammonium, potassium , iron etc..

Characters: Colourless, transparent crystals, with acid, sweetish astringent taste.

Action: astringent, caustic, haemostatic, anti-spasmodic and anti septic, irritant and purgative in large doses.

Uses: It is useful in leucorrhoea, haematuria, haemoptysis, menorrhagia, gastric and intestinal catarrh and other haemarrhages.

32. Vellaiphasanam:

Common Name: Arsenum; Acidum arseniosum

Source**:** Found in arsenical ores such as arsenates of iron, nickel or cobalt; commercial arsenious acid is obtained by roasting the native ores in the form of a sublimate. The metal, arsenic is widely distributed in nature but in small quantities.

Characters: Solid, heavy white powder or stratified masses or minute transparent and glass-like crystals, tasteless, soluble in water, in boiling water, in glycerine, very slightly in alcohol, in alkalies and their carbonates and in hydrochloric acid.

Action: It is stomachic, nervine tonic, alterative and anti-periodic and cardiac, respiratory, intestinal and sexual stimulant.

Uses: It is used in variety of disease; but chiefly in fever, either alone or combined with other substances.

33. Gowriphasanam: [52]

Common Name: Arsenic penta sulphide

Source**:** vaippu sarraku ; obtained by isolation from yellow arsenic trisulphide

Characters: It is classified into three types –red, yellow and white arsenic penta sulphide

Action: Anti-pyretic, anti-periodic and anti-vata

Uses: The golden coloured padaana cures Kapha, delirum, vatha diseases, scabies, poisonous bites, disorders of three humors, various toxicity, haemorrhage and eight types of throbbing pains.

34. *Kuthiraipal pashanam*:[51]

Common Name: Orthoclase feldspar; Potassium aluminium silicate

Source**:** It is natural padaana. In zoological department it is categorized under silicate group.

Characters: Greenish, greyish, yellowish, white pink colour. It can be an hedral and euhedral. Grains are commonly elongated with a tubular appearance. Hardness 6-6.5; vitreous;diaphenity- Transparent to translucent.

35. Kalmatham:

Common Name: Erythrite; Hydrous cobalt arsenate

Source**:** It is a natural ore categorised under uprasam. Erythrite is a mineral of secondary origin, found commonly in the upper portion of cobalt mineral deposits.

Characters: In zoological department it is categorised under Arsenate group. crimson and peach red sometimes grey in colour. hardness-1.5-2.5: crystals prismatic and vertically striated, also in globular and reniform shapes having a drusy surface and a columnar structure.

Uses: It is used to cure diseases in neck region, urogenital diseases, venereal diseases and urolisthisis.

36. Thalagam: [52]

Common Name: Orpiment; Arsenii trisulphidum

Source: It is native to China and Persia.

Characters: It occurs in two forms viz., in smooth shining, gold-coloured scales called Vansapatri haritala and in fine lemon yellow opaque masses called pinda haritala.

Action: Alterative, febrifuge, emmenagogue, anti-periodic.

Uses: It is given as a cure for fevers and skin diseases. It is used for asthma, paraplegia, hemiplegia, monoplegia and facial paralysis, in cough, chronic fever, gonorrhoea, epilepsy, dropsy etc.

37. Manoislai:

Common Name: Red orpiment; Arsenic Disulphidum-Bisulphuret of arsenic realgar

Source: vaippu sarakku

Characters: Short prismatic small from druses also found is granular and earthy aggregates and powdery coatings; Aurora red to orange red; Resinous; varying from Orange-red to aurora red Transparent-translucent.

Uses: It is effective in the treatment of skin leprosy, fever with chills, asthma, eye diseases, urinary tract infection, kapha diseases, cervical adenitis etc.

38. Rasam:

Source: Mercury is sometimes found in nature in its free form of small, shining, silvery globules called as quick silver. But it is mostly found as sulphide or native cinnabar. It is scattered through different kinds of stones, clay or ores.

Characters: It is a shining ,sliver-white metal liquid at ordinary temperature divisible in to spherical globules, mobile, without any odour and taste, slowly volatilizing at ordinary temperature; insoluble in water, hydrochloric acid and hot sulphuric acid.

Action: It is used as a tonic, alterative, purgative, indirect cholagogue, anti-phlogistic,anti-septic and sialagogue.

Uses: Syphilis, small amount of mercury diminish the amount of oxidation of the tissue.

39. *Rasa sindooram*:

Common name: Red sulphide of mercury

Source: Red sulphide of mercury is commercially prepared by combination of mercury and sulphur.

Characters: Its properties and actions are more or less similar to cinnabar

Uses: It is used to cure scabies.

40. Lingam:[52]

Common name: Cinnabar; Red sulphide of mercury,

Formula: Hgs; Sulphur -13.8, Mercury-86.2

Source: It occurs in nature as a mineral ore, in many parts of the world, particularly in California, China and Spain. Cinnabar is the only common mineral of mercury. It is usually associated with antimony, pyrite, the sulphide of copper, stibnite, realgar and gold

Characters: Bright dark red; hardness-2-2.5;

Action: Tonic. It has the properties of curing diseases caused by the earth and the water elements.

Uses: It is used to cure skin diseases, arthritic pain, peptic ulcer, fever, anaemia and bronchial asthma.

41. *Pooram*:

Common name: Rasa karpooram; Calomel; Hydrargyrum subchloride;

Source: It is prepared by the combination of rasam and salt.

Characters: It has hot potency and salty taste.

Action: Laxative, tonic, anti-septic, diuretic properties.

Uses: It cures throbbing pain in the lumbar region, burning sensation, ulcer due to disorder of vatha humour, hepatomegaly, pyrexia, jaundice, basillary dysentery, dropsy, chronic ulcers, venereal diseases, indigestion, vomiting, diarrhoea, worm infestation, rheumatism, itching, constipation, scabies etc.

42. Gandhagam:

Common name: Sulphur

Source: A non-metallic element found free in the bed of gypsum and in state of sublimation in region of extinct volcanoes; also in combination with several ores called pyrites, as sulphates and sulphides of iron, copper, lead, zinc, mercury etc .In India it occurs naturally in some parts, in Nepal, Kashmir, Afghanistan and in Burma.

Characters: It is bitter and has an astringent taste. It is yellow, straw and honey yellow, yellowish brown, greenish, reddish to yellowish grey in colour. Its hardness is 1.5-2.5.

Action: Laxative, tonic, anti-septic. It increases various secretions of the body including that of the skin.

Uses: It is used to treat 18 types of skin diseases, liver enlargement, abdominal distension, eye diseases, chronic venereal diseases, chronic diarrhoeas, gastric ulcers, poisonous bites, fever due to vatha, chronic dysentery etc.

43. Pooneeru:

Common name: Fuller's earth -Impure sodium carbonate-Hydrated carbonate of sodium and calcium.

Formula: $Na_2CaCo_32H_2O$

Source: It is present in brackish soil. It is collected from brackish soil during winter season and in early the morning before sunrise. It is collected from Sivaganga, Kalasthiri and Mosur from the earth in the dew season, before sunrise.

Characters: orthorhombic, White colourless, Conchoidal, Easily fusible

Uses: It is used to purify the tortoise shell, egg shell, pearl oyster, Asbestos, fosil of crab and conch shell. Arsenic compound may be purified with solution prepared from pooneeru. Chunnam form of pooneeru promotes the action of the medicine. It is used to cure arthritis.

It is the one of the main ingredients in Muppu and Saint Bogar suggested that purified pooneeru considered as muppu as per text Bogar muthallayiram.

44. Mayilthutham:

Common name: Blue vitriol: Copper sulphate

Source: Copper sulphate is available in nature and is also synthesized chemically.

Action: Powerful astringent, emetic and anti-septic.

Uses: It is used to cures ulcers, eye diseases, disorders of three humors,fever and mouth diseases .

45. Paalthutham:

Common name: White vitriol, Zinc sulphate

Action: It has got body strengthening, astringent and emetic properties.

Uses: It is used for douching white discharge, eye diseases, piles.To induce vomiting in case of poisoning.

46. Pachaikarpooram:

Common name: Borneo camphor

Source: Natural source.

Characters: It is soluble in atmospheric air. It has pleasant odour. Cooling agent.

Action: This has astringent and salty taste. Its potency is cold, and has expectorant tonic, and demulcent properties.

Uses: It is effective in eight types of gastric ulcer, vatha diseases and joint pains and kapha.

47. Kalnar:

Common name: Asbestos

Source: It is available in nature. In India, it is available in plenty in Karnataka state. It is also available in plenty in Japan and is taken out from the earth.

Characters: Its look like a stone and when crushed it becomes a fibre like material.

Action: Diuretic and bitter properties.

Uses: It is used to treat rheumatism, disease of teeth, gingivitis, urethritis, urinary retention, bilious vomiting, dysuria, spermatorrhoea, indigestion, hernia, thirst due to bilious etc.

48. Ponnimilai:

Common name: Gold Bismuth; Chalcopyrite

Source: Bismath is formed as kalimbu paste when the metals like gold, silver, copper and lead are melted. Chalcopyrite, the most important Cu ore mineral, is widespread and common. It is present in most sulphide deposits. Hardness is 3.5

Characters: Brass yellow, Honey yellow

Action: Astringent, tonic, sedative and alterative properties.

Uses: It improves spermatogenesis and controls excess pitha and apoplexy. It is given internally for gunmam, diarrhoea and dysentery. It is applied externally for skin diseases and on the fissures of lips and nipples.

49. Nandukkal:

Common name: Fossil Stone Crab; Fossilo ferrus limestone

Source: It is present in nature in sea shores. Limestones are mainly of the biogenic origin. They are formed at the expense of calcareous skeletons and remnants of shell of organisms. Shell and skeletons of organisms may either be fully preserved or may appear to have been crushed, disintegrated and recrystallized.

Characters: Crinoidal limestone consist of particles of calcareous skeleton of marine crinoids.

Action: diuretic

Uses: It is used in the treatment of stranguary, urinary calculus, chronic ascites, gonorrhoea, leucorrhoea and phlegm.

50. *Gorosanam:*

Common name: Ox-Bile

Source: The bile of Cow.

Action: The bile has got bitter, laxative, mucolytic and hypothermic properties.

Uses: It is used for the treatment of Reddish syphilis with the breast milk or leaf juice of anisochilus carnosus(Karpooravalli). It is given in cow’s milk, twice a day for small pox.

51. *Then*:

Common name: Honey

Source: Honeybees absorb the sweet substance from flower and store in the honey- sac present in their body. The sweet substance undergoes some changes in the honeybees to form the honey.

Characters: Sweet and appears as a clear yellow liquid.

Action: Anti-inflammatory, Laxative, bitter, Mucolytic, Appetite stimulant, nutritious and sedative properties.

When given to children, it acts as a mild diuretic and reduces flatulence.

Uses: It is useful for preparation of various medicaments such as leghium, syrup, mezhugu, kattu and collyrium.

It is also the best adjuvant for parpam, chendooram, chooranam, pills and decoction. As an ambrosia it maintain the body health, besides curing the diseases caused by vatha, pitha, Kapha

52. *Pasunei:*

Common name: Cow's ghee

Source: Milk product from cow's milk.

Action: It controls thirst, vomiting, excessive pitha, burning sensation of the stomach, pitha hiccup, abdominal pain, dryness, prickly heat, cough, hyper motility of the gut, weakness of bones, piles ect.

Uses: If ghee is added to the usual diet, it helps in proper digestion and utilisation of the diet and gives strength and vigour to the body.

53. *Pasunchanam:*

Common name: Cow's dung

Source: Excretory product of the cow.

Characters: greenish paste consistency material.

Action: It reduces the swelling controls haemorrhage, worm infestations, Kapha associated fever and excessive thrist. It keeps the body purified and it is equivalent to coconut milk.

Uses: The liquefied cow dung is used as an antidote for paadanas and the dung of young cow, which has not given birth to a calf, can be given internally to counteract poisioning due to scorpion bite. It is also used for the purification of croton tiglium and semecarpus anacardium and is also useful for making kavasam and dung puda.

- **Prepared Rasa Chendooram: Impcops Vaidhiya yoga ratnavali P.no: 402-403**

Drug Required: [53]

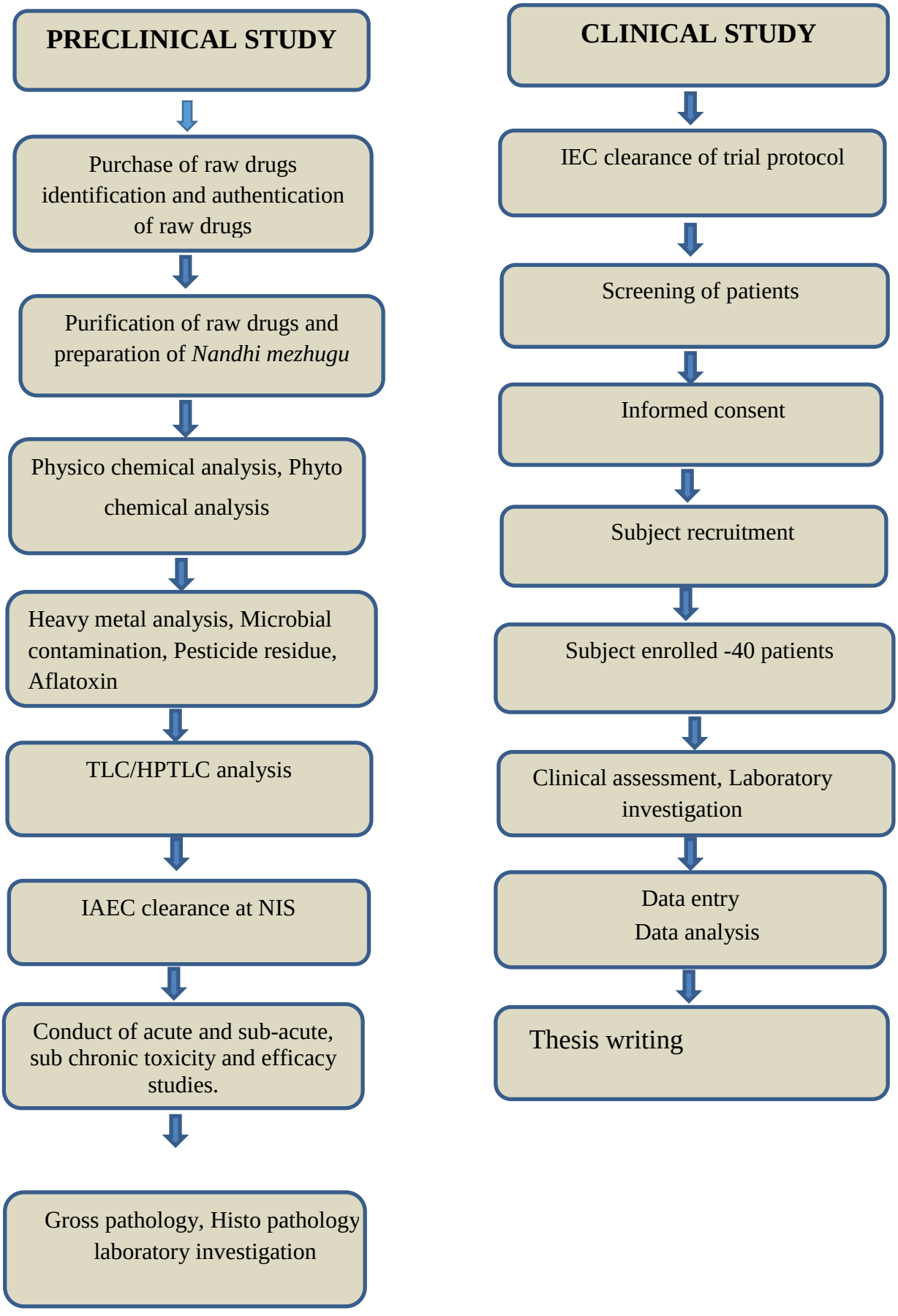
1. Rasa Sunddhi -Mercury purified - Part 1
2. Gandhaka suddhi - Sulphur Purified - Part 1
3. Karpasa puspa Rasa - Red Cotton plant flower juice - Q.S
4. Kumari svarasa - Aloe juice -Q.S

Preparation:

Mercury and sulphur were ground to prepare the black sulphide of fine consistency. To this, drug 3 was added and ground with drug 4. The product was dried and powdered. The process of sublimation had been spread over 3 days.

Action and uses: Vitaliser and appetiser; Gives for fevers. Used in weakness and cough in children with the powder of three pungents and withania roots with milk.

4. PLAN OF WORK



MATERIALS AND METHODS

STANDARD OPERATING PROCEDURE FOR *NANDHI MEZHUGU*:[54,55]

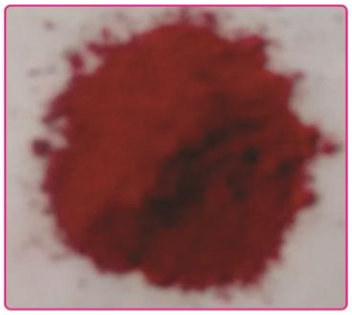
Preparation of the drug *Nandhi Mezhugu*:

5.1 Collection of Raw Drugs:

The required raw drugs for the preparation of ***Nandhi Mezhugu*** were procured from the "Indian Medical Practitioner's Co-operative Society Pharmacy and Stores" (IMPCOPS) Chennai 41

PAALTHUTHAM

RASAM



RASA CHENDURAM (PREPARED)

LINGAM

MANOSILAI

GANDHAGAM

THALAGAM

GUTHIRAIPAL PADANAM

VELLAIPADANAM

GOWRI PADANAM

HERBAL RAW DRUGS IN NANDHI MEZHUGU

SERANKOTTAI

ETTIKOTTAI

THIRIKADUGU (CHUKKU,MILAGU,THIPII

ELAM

SIRUTHEKKU

THALISAPATHIRI

KUROSANI OMAM

VAIVIDAMKAM

ATHIVIDAYAM

JATHIKKAI

JATHIPATTRI

KARUNJEERAGAM

CIRAKAM

ILAVANKAM

CHEVVIYAM

KATTUMILAGU

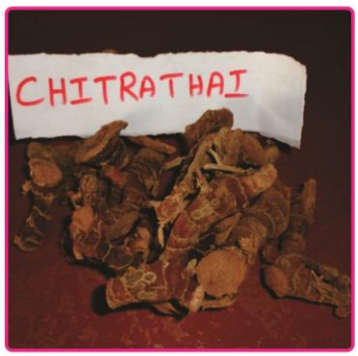
CHITHIRAMOOLAM

THIPHILIKATTAI

KACHAKASA

SOMBU

NERVALAM

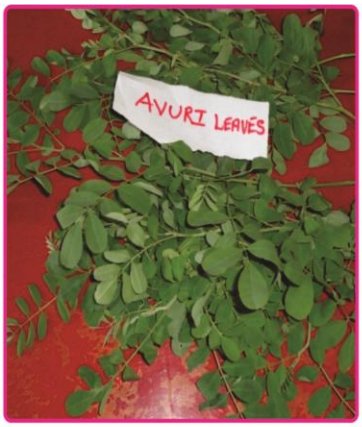


CHITTARATHAI

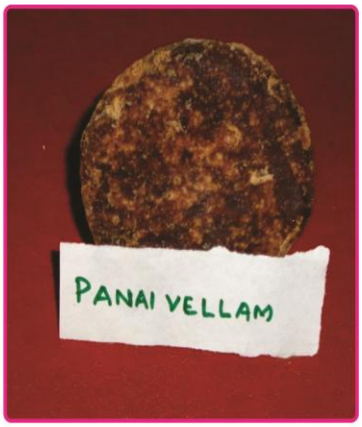
CATAMANCIL

AKKIRAKARAM

NATTUAMUKKARA



AVURIELAI



PANAIVELLAM

THEN

PASUNEI

RAW DRUGS USED FOR PURIFICATION

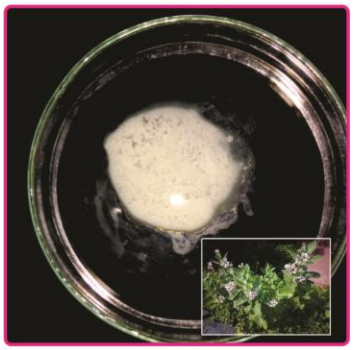
KARIUPPU

VEDIUPPU

NAVAL PATTAI

MURUNGAIPATTAI

KUPPAIMENI



ERUKKAMPAL

PASU SAANAM

PASU MOR

SIRU KEERAI

KARCHUNNAM

PASUPALL

PASUNEER

VETRILAI

ELUMICHAM PAZHAM

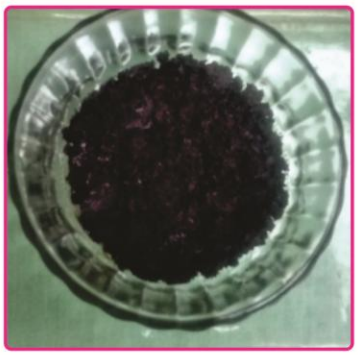
KOPPARAI THENKAAI

SENKAZHUNEER

KAADI NEER

SENGAL THOOL

MANJAL THOOL



NANDHI MEZHUGU
(PREPARED MEDICINE)

5.2 Raw drugs Identification and authentication:

Herbal raw drugs were authenticated by Pharmacognosist, the metals and mineral raw drugs were identified and authenticated by Chemist, Siddha Central research Institute, Arumbakkam, Chennai-106.

5.3 Purification processes of ingredients of NM: [56,57,58]

The ingredients were purified as per the technique stated in the Siddha literature. The raw drugs were purified at the Indian Medical Practitioner's Cooperative Pharmacy.

1. Purification of Pooram:

The poultice made of betel leaf and pepper i.e betel leaf -4 parts, pepper-4 parts were dissolved in 1.3 litre of water. Pooram(Calomel) -1 part was tied in a cloth and immersed in the liquid from the cross bar and heated. After the water was reduced to ¾ of its volume, the calomel was taken out, washed with water and dried to get it its purified form.

2. Purification of Seemai Kalnar:

Asbestos was soaked in cow's urine for 10 days and then washed and dried

3. Purification of Gandhagam :

Sulphur was placed in an iron spoon. A small quantity of cow's butter was added and the spoon was treated to heat till the butter melted; this mixture was immersed in cow's milk. This procedure was repeated 30 times to get purified sulphur. Each time, fresh milk was used.

4. Purification of Gowri pashanam:

It was kept soaked for 3 days each in leaf juice of Indian indigo plant and bitter gourd plant juice individually.

5. Purification of Vellai pashanam:

Vellai pashanam was powdered and triturated with lemon juice(1:3 Ratio) and made into small cakes and dried. This process was repeated 7 times.

Ref:Siddha Materia Medica(Mineral and Animal Kingdom) Page No:295

5. Purification of Padikaram:

Padikaaram was dissolved in water, filtered, boiled till it attained the consistency of jelly and then it was cooled to get the purified form.

6. Purification of Thalagam:

Small pieces of Thalagam were bundled in a double layer cloth. They were kept immersed in cow‘s urine and heated for 3 days. The same process was repeated with rice water and vinegar individually to get purified form.

7. Purification of Ligam:

Lime juice, cow’s milk and the Indian acalypha juice were mixed in equal proportions and allowed to fuse with cinnabar so as to get it in a consolidated potency state.

8. Purification of Rasa chendooram:

It was kept soaked in lemon juice for 24 hours. It was then washed and dried to get purified form.

9. Purification of Ponnimilai:

Nimilai was soaked in Cow’s milk and washed.

10. Purification of Pooneeru:

Fuller’s earth was dissolved in lemon juice and filtered. The filtrate was boiled till the water completely evaporated.

11. Purification of Manosilai:

Manosilai was triturated with lemon juice for 3 hours and then allowed to dry.

12. Purification of Palthutham:

The copper sulphate was fried, till it turned to whitish.

13. Purification of Mayilthutham:

Copper sulphate was fried, till it turned whitish.

14. Purification of Karpoora silajath:

The Silasaththu was boiled in cow’s milk and washed.

15. Purification of Pachai karpooram:

It was soaked in red Indian water Lily flower juice(Nymphae odorata) for 24 minutes and isolated.

16. Purification of Nandukkal:

Limestone and Fuller's earth were mixed in water and allowed to settle for sometime. The clear solution was then taken out. Fossil stone crab was then placed in this solution and heated for three hours. The fossil stone crab was taken out and washed in water to get its purified form.

17. Purification of Rasam:

Rasam was ground with red Indian water Lily plant juice.

18. Purification of Akkirakaram:

The outer skin of the rhizome was removed and dried in the shade.

19. Purification of Nattu Amukkura:

The drug was powdered and boiled in milk for 3 hours and then dried well.

20. Purification of Ativitayam:

The outer skin of the rhizome was removed and dried in shade.

21. Purification of Avuri elai:

The leaf was taken and washed with water

22. Purification of Catamancil:

The drug was cleaned and dried in shade.

Purification of Catikkai:

The drug was cleaned and dried

23. Purification of Catipattiri:

The drug was cleaned and dried

24. Purification of Cerankottai:

The nose like projection of the marking nuts were removed and nuts were tied in the white cloth and immersed in earthen pot containing cow dung solution and boiled till the cow dung solution was completely reduced.

25. Purification of Chevviyam:

The outer skin of pepper root was scraped and cut into small pieces and dried.

26. Purification of Chukku:

The outer skin of dry Ginger was scraped and soaked in lime stone water.

27. Purification of Cirakam:

The drug was cleaned and dried in sunlight for 6 hours and fried.

28. Purification of Cirutekku:

The outer skin was removed, sliced and then dried.

29. Purification of Cittarathai:

The outer skin was removed, and sliced and then dried.

30. Purification of Elam:

The drug was cleaned and fried till it attained brown colour.

31. Purification of Kumkumappoo:

The drug was spread over the paper and subjected to indirect heat till the drug attained fragile quality.

32. Purification of Etti vittu:

The drug was steamed with paddy and soaked in Amaranthus tricolor juice for 1 hour and cleaned.

Purification of Ilavankam:

The drug was cleaned and dried in the shade.

33. Purification of Kacakaca vitai:

The drug was cleaned and dried in the shade

34. Purification of Kattu milaaku:

The drug was soaked in betel leaf juice for 12 minutes and dried in the shade.

35. Purification of Kodiveli ver:

Root bark was powdered and steamed in milk for three hours.

36. Purification of Kurocani omam:

The omam was mildly roasted until the aroma rose.

37. Purification of Milaku:

The fruits were soaked in sour butter milk for three hours then dried in the sunlight.

38. Purification of Nervalam:

The seed was opened and the cotyledons were collected discarding the embryonic plant. The cotyledons were suspended in cloth bag and immersed in earthen pot containing cow dung solution and boiled till the complete reduction of cow dung solution.

39. Purification of Perumcirakam:

The drug was cleaned and dried in the shade.

40. Purification of Talica pattiri:

Long stalks of the drug were removed and dried in the shade.

41. Purification of Tippili:

The drug was soaked in plumbago decoction for 3 hours

42. Purification of Thipilik kattai:

The nodes of the roots were removed and dried.

43. Purification of Vaividankam:

The drug was cleaned and dried in the shade.

44. Purification of Korojanam:

A needle was heated and inserted into the ox- bile and taken out, appearance of yellow colour on the needle and yellow colour smoke confirmed its purity.

45. Purification of Karunjeeragam:

The drug was cleaned and dried in sunlight for 6 hours and fried.

4.4 Preparation of *Nandhi Mezhugu*: (Siddha Vaidhiya Thirattu)[54,55,59]

The medicine *Nandhi Mezhugu* was prepared at The Indian Medical Practitioner's Cooperative Pharmacy (IMPCOPS).

INGREDIENTS:

1. Purified Marking Nut (*Serankottai*) - 1Kg
2. Purified Nux Vomica (*Ettikkottai*) seeds - 315 gms
3. Cow's Ghee *(Nei*) -1400 gms
4. Common Alum (*Padikaram*) -1120 gms
5. Palm Jaggery (*Panai Vellam*) -2240 gms
6. Honey (*Then*) -1400 gms

The following drugs were added in equal ratio as 53 gms each:

7. Calx of Copper pyrites (*Ponnimilai parpam*)
8. Calx of Asbestos (*Kalnar Parpam*)
9. Calx of white asphaltum (*Kalmatha Parpam*)
10. Calx of *Nandukkal* (Crab's fossil)-*Nandukkal Parpam*
11. Borneol (*Pachai karpooram*)
12. Gorochan (*Korochanam*)

13. Prepared *Rasa Chenduram*(IMPCOPS) -140 gms

The following drugs were added in equal ratio as 35 gms each:

14. Three Pungents - (*Thirikadugu*) each
15. Cardamom Seeds (*Elarisi*)
16. Beetle killer roots (*Siruthekku*)
17. Yew leaves (*Thalisa Pathiri*)
18. Henbane niger (*Kurosani Omam*)
19. Embelia (*Vaividangam*)
20. Atis (*Athividayam*)
21. Nutmeg (*Jathikkai*)
22. Mace (*Jathipathiri*)
23. Cumin seeds (*Seeragam*)
24. Cloves (*Lavangam*)
25. Black pepper root (*Sevviyam*)
26. Wild black pepper (*Kattu Milagu*)
27. Lead wort root (*Kodiveli ver*)
28. Long pepper stem (*Thippili Kattai*)
29. Poppy seeds (*Kasa Kasa vidhai*)
30. Fennel (*Perunjeeragam*)
31. Purified Croton cotyledon (*Nervalam*)

32. Lesser galangal (Sitrathai)

33. Indian spike nard (*Jadamanchil*)

34. Pelllitory roots (*Akrakaram*)

35. Withania roots (*Nattu Amukkara*)

The following drugs were added in equal ratio as 25 gms each:

36. *Rasa Sinduram* (Market quality)

37. *Pooneeru Uppu* (alkaline earth salt)

38. Purified *Mayil thutham* (Blue vitreol)

39. Purified *Pal thutham* (White vitriol)

40. Purified *Rasam* (Mercury)

41. Purified *Kandhagam* (Sulphur)

42. Purified *Pooram* (Calomel)

43. Purified *Lingam* (Cinnabar)

44. Purified *Manosilai* (Red Orpiment)

45. Purified *thalagam* (Yellow Orpiment)

46. Purified *Gowri Pashanam* (Arsenic pentasulphide)

47. Purified *Vellai Pashanam* (White Arsenic)

48. *Neeli Ilai charu* and *karkam*(Indigo Juice and Paste) (**Q.S)** Quantity sufficient

Preparation:

(a) 1,2,3 ingredients were mixed and subjected to heat and fried. Ghee and seeds were separated. The seeds were ground into a fine paste.

(b) 14-36 were ground to fine powder and the drug number-4 was also powdered.

(c) 37-47 were ground in to a paste with juices of drug no 48 for 6 hours and were made into cakes and dried. The cakes were covered with the paste of drug no 48 and also covered by cow dung and subjected to calcination process, then the product was taken and powdered.

(d) Syrup of palm jaggery was prepared and the ingredients a, b and c and the calxes were added followed by ghee and honey. To this mixture powders of camphor and Korochanam were added and thoroughly mixed.

5.5 Physico chemical characterization of *Nandhi Mezhugu*:[60,61]

Physico chemical analysis as per AYUSH Guidelines:[60]

Qualitative analysis of *Nandhi Mezhugu*:

5.5.1 Organoleptic properties

The organoleptic characters such as colour, odour, taste and consistency were recorded.

5.5.2 Physico Chemical Parameters:

Loss on drying at 105°C, total ash, water soluble ash, acid-insoluble ash, water soluble extractive, alcohol soluble extractive, rancidity, acid value, saponification value, iodine value, pH, total solid, fat content , reducing sugar and total sugar were carried out as per the procedures mentioned in standard references (Protocol for testing(AYUSH))

1 .Determination of Loss on Drying of drug sample

10 g of the drug (without preliminary drying) was placed in a tarred evaporating dish after having been weighed accurately. It was then dried at 105˚C for 5 hours. The procedure was repeated till the weight difference between two consecutive readings were less than 0.25 percentages. When two consecutive weighing after drying and cooling each for 30 minutes in a desiccator showed not more than 0.01 g difference, the constant weight was reached

2. Total Ash

2g of the drug was taken in a tarred silica dish and was incinerated at 450ºC until free from carbon, cooled and weighed**.** The percentage of ash was determined with respect to the air-dried drug.

3. Water Soluble Ash determination

For 5 minutes, the ash which was obtained from the above test was boiled with 25 ml of distilled water repeatedly; collected the insoluble matter on an ashless filter paper and ignited to constant weight.Calculated the percentage of water soluble ash with reference to the air dried drug.

4. Acid Insoluble Ash

For 5 minutes the ash, obtained(from the above test) was boiled with 25 ml of dilute hydrochloric acid repeatedly; collected the insoluble matter on an ash-less filter paper, washed with hot water and ignited to constant weight. Calculated the percentage of acid-insoluble ash with reference to the air dried drug.

5. Alcohol Soluble Extractive of drug sample

5 gms of the study drug was dissolved in 100 ml of alcohol of the specified strength (90%) in a closed flask for twenty-four hours, shaken frequently during six hours and allowed to stand for eighteen hours. Filtered rapidly, taking safety measures against loss of solvent, evaporated 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish and dried up at 105º, to constant weight and weighed. Calculated the percentage of alcohol-soluble extractive with reference to the air-dried drug.

6. Water Soluble Extractive determination

The procedure for the determination of alcohol soluble extractive was followed using distilled water instead of ethanol.

7. Determination of pH

Taken 10g of sample, added 100 ml of distilled water, stirred well and filtered. Used the filtrate for the experiment. Switched on the instrument.Given 30 minutes time for warming the pH meter. Introduced the pH 4 solution first and adjusted the pH meter by using the knob to 4.00 for room temperature 20°C, 4.01 for room temperature 25°C, 4.02 for room temperature 30°C. Introduced the pH 7 solution and adjusted the pH meter to 7 by using the knob.After Introducing the pH 9.2 solution, without adjusting the knob, the pH reading was checked . Then introduced the sample solution and noted the reading. Repeated the test four times and took the average reading as result.

8. Acid Value of drug sample

10 g of drug sample was placed in a 250ml flask and mixed with 50 ml of a mixture of equal volumes of alcohol and solvent ether, which was neutralized after the addition of 1 ml of solution of phenolphthalein. Gently heated on a water-bath, after which titration was done with 0.1 N potassium hydroxide,was shaken constantly until a pink colour was obtained which lasted for 15 seconds.. Noted the number of ml required. Calculated the acid value from the following formula:

$$\text{Acid Value} = \frac{a \times 0.00561 \times 1000}{W}$$

Where ‘a’ is the number of ml. of 0.1 N potassium hydroxide required and ‘w’ is the weight in g of the substance taken.

9. Determination of Saponification value

The fatty acids, produced from the complete hydrolysis of 1 g of the oil or fat is neutralised by number of mg of potassium hydroxide this number was called saponification value.

35 to 40 g of potassium hydroxide was dissolved in 20 ml of water and added sufficient alcohol to make 1,000 ml. Allowed it to stand overnight and poured off the clear liquor.

Weighed accurately about 2 g of the substance in a tared 250 ml flask, added 25 ml of the alcoholic solution of potassium hydroxide, attached a reflux condenser and boiled on a water-bath for one hour, frequently rotating the contents of the flask cool and added 1 ml of solution of phenolphthalein and titrated the excess of alkali with 0.5 N Hydrochloric acid. Noted the number of ml required (a) Repeated the experiment with the same quantities of the same reagents in the manner omitting the substance. Noted the number of ml required (b).

Calculated the saponification value from the following formula:—

$$\text{Saponification Value} = \frac{(b-a) \times 0.02805 \times 1.000}{W}$$

Where ‘W’ is the weight in g of the substance taken.

10. Determination of Iodine Value

Iodine value:

Iodine value is usually expressed as the number of parts by weight of Iodine absorbed by 100 parts by weight of an oil or fat.

Procedure

Preparation of Iodine monobromide solution (Hanus method)

Dissolved 13.2 g of Iodine in 1 litre of Glacial acetic acid using a Mortar and pestle. Filtered through cotton and transfered all the iodine with Glacial acetic acid. Added 3 ml of liquid bromine and shaken well.

Preparation of 0.1M Sodium thiosulphate solution

Dissolved 25g of Sodium thiosulphate AR in 1000ml of water in a volumetric flask

Preparation of 0.1N Potassium dichromate solution

Dissolved 4.904g of Potassium dichromate AR in 1 litre volumetric flask and made up to the mark with distilled water

Preparation of Starch solution

Dissolved 1g of Starch in 100 ml of distilled water. Heated till it becomes colourless.

Standardization of Sodium thiosulphate solution

Taken 10ml of 0.1N Potassium dichromate solution, added 150 ml of distilled water, 2g of potassium iodide and 6 ml of Conc. Hydrochloric acid. Stirred well. Allowed to stand for 10 minutes. Titrated with sodium thiosulphate solution using starch as indicator. End point was the disappearance of blue colour.

Determination of Iodine Value

Weighed about 0.2 gm of oil/fat in an Iodine flask. Added 15 ml of Chloroform and dissolve. Added 25 ml of Iodine monobromide from a burette. Moistened the stopper with a few drops of potassium iodide solution. Kept the flask in dark for half an hour. Added 10 ml of potassium Iodide and 100 ml of distilled water. Titrated the mixture with standard 0.1M Sodium thiosulphate with starch as indicator. Added starch solution only when the solution in the flask was pale yellow in colour. The end point was disappearance of blue colour. Noted the number of ml required (a). Did the blank determination without the oil using exactly the same quantity of chloroform and the same burette for delivering the Hanus reagent (b).Repeated the experiment twice for concordant values.

Calculation:

$$\text{Iodine value} = \frac{1.269 \times \text{Strength of thiosulphatex Difference in titre (b-a)}}{0.1 \times \text{Weight of oil}}$$

11. Rancidity test (Kreis Test)

The test depends upon the formation of a red colour when oxidized fat is treated with conc. HCl and a phloroglucinol in ether solution. Epihydrin aldehyde is the compound present in rancid fats is account able for the colour reaction. The response obtained from all oxidized fats to the kries test and the quality of the colour.

Procedure

Mixed 1 ml of melted fat and 1 ml of conc. HCl in a test tube. Added 1 ml of a 1 % solution of phloroglucinol in diethyl ether and mixed thoroughly with the fat-acid mixture. Formation of pink colour was the indication of slight oxidation of fat; red colour was the indication definite oxidation of the fat.

12. Determination of the Fat content

Weighed 3-4g of the sample in a thimble and placed it in a soxhlet fitted with a condenser. Taken 100 ml of petroleum ether (B.P. 40-60°C) in the RB flask and boiled for 4 hours. Taken the extract in a pre-weighed conical flask and evaporated petroleum ether on a water bath. Removed the traces of petroleum ether in vacuum pump. Taken the weight of fat to constant weight.

Calculation

$$\text{Percentage of Fat content} = \frac{\text{Weight of petroleumether extract} \times 100}{\text{Weight of the sample taken}}$$

13. Determination of Sugar content (Lane&Eynon's method)

Preparation of Fehling's solution:

Solution A :

34.639 g of pure crystallized $CuSO_4 .5\ H_2O$ in 500 ml water.

Solution B :

173 g of Rochelle salt (Potassium Sodium tartrate and 50 g of Sodium hydroxide in 500 ml distilled water.

Mixed equal volumes of solutions A and B.

Methyleneblue indicator: 1g of Methylene blue in 100 ml of distilled water.

Sample preparation

Taken 10 g of sample in a 250 ml volumetric flask.Added 200 ml water.Added slightly excess solid basic lead acetate to remove tannins.Made up to the mark without

disturbing the solution. Shaken and filtered. Added slightly excess of solid Sodium oxalate to remove excess of basic lead acetate.Shaken and filtered. Used this filtrate for the estimation of reducing sugar.

Reducing sugar: Take the sugar solution in a 50 ml burette

Preliminary titration

Pipetted 10 ml of Fehling's solution into a 250 ml conical flask, added from the burette, 15 ml of the sugar solution. Boiled the liquid on asbestos-covered gauze and added further quantities of the sugar solution (One ml at a time) at 10 to 15 second intervals to the boiling liquid until the blue colour is nearly discharged. Added 3-5 drops of aqueous methylene blue solution (1%) and continued the titration until the indicator was completely decolorized.

Accurate titration

The titration was repeated, adding before heating, almost all of the sugar solution required to effect reduction of copper. Boiled gently for two minutes.Added 3-5 drops of Methylene blue indicator and completed the titration within a total boiling time of three minutes. At the end point all the blue colour has discharged and the liquid was red.The proportions of the sugars, equivalent to 10 ml of Fehling's solution was taken from the table.

Total Sugar

Taken 20ml of reducing sugar solution, and added 10ml of Concentrated Hydrochloric acid and kept it aside overnight. Neutralised with approximately 1M Sodium hydroxide solution or with solid sodium carbonate and made up to 100 ml in a volumetric flask. The total sugar content was determined by the titrimetric method as described above.The experiment was repeated twice and the average value was taken.

Calculation

$$\text{mg of sugar in 100 ml} = \frac{\text{Total reducing sugar from table} \times 100}{\text{Titre value}}$$

$$\text{Reducing sugar \%} = \frac{\text{mg of sugar in 100 ml} \times 250 \times 100}{1000 \times 100 \times 10}$$

$$\text{Total sugar \%} = \frac{\text{mg of sugar in 100 ml} \times 250 \times 100 \times 100}{1000 \times 100 \times 20 \times 10}$$

Qualitative Phytochemical Analysis: [62]

Various tests for different types of secondary metabolites, viz., Steroids, terpinoids, alkaloids, flavonoids etc. were carried out as per the procedures quoted in standard organic book.

1. Test for Steroids (Lieberman Burchard Test)

To few mg of the extract 2 ml of chloroform was added in a dry test tube. Few drops of acetic acid were added, heated and few drops of acetic anhydride and 2 drops of concentrated sulphuric acid were added. The green colour indicated the presence of steroid.

2. **Triterpenoids (Noller's Test)**

To few mg of extract, added tin and thionyl chloride and heated in a water bath. Purple colour indicated the presence of tritepenoids.

3. Test for Flavonoids (Shinoda test):

Magnesium bits and Conc. HCl were added to the substance which was dissolved in alcohol. The presence of flavonoid was assessed by the appearance of magenta colour on heating over water bath confirmed the presence of flavonoid.

4. Test for Alkaloids (Dragendorff'sTest):

Few mg of extract in a separate test tube were warmed with 2% Sulphuric acid for 2 minutes; it was filtered in a separate test tube and a few drops of Dragendorff's reagent were added. The presence of orange red precipitate indicated the presence of alkaloids.

5.Test for Phenol:

Substance in water was added to 5 % alcoholic ferric chloride. Dark blue or green colour showed presence of phenol.

6. Test for Tannin:

Substance was shaken with water and added to lead acetate solution. White precipitate showed the presence of tannin.

7. Test for presence of Saponins:

Foam formation after shaking the mixture of distilled water with few mg of extract confirmed the presence of Saponins.

8. Test for presence of Coumarin:

Extract was shaken with 10% sodium hydroxide. Yellow colour showed the presence of coumarin.Upon adding concentrated sulphuric acid, original extract colour was regenerated.

9. Test for presence of Glycosides:

Concentrated sulphuric acid and anthrone were treated with the substance and heated in the water bath; showed green colour confirmed the presence of glycoside.

10. Test for Quinones:

To asss few mg of extract, added few drops of concentrated sulphuric acid. Appearance of red colour showed the presence of quinone.

5.5.4 Qualitative Inorganic Analysis: [63]

1. Test for Aluminium:

To the sample solution, ammonium chloride and aqueous ammonia were added. White precipitate was obtained. This precipitate was dissolved in drops of 1N hydrochloric acid and added 2 drops of aluminon reagent (0.1% aqueous solution of ammonium salt of aurinetricarboxylic acid), shaken, allowed to stand for 5 minutes and then added excess of ammoniacal ammonium carbonate solution. Formation of bright red precipitate indicated the presence of aluminium.

2. Test for Calcium:

To a drop of the neutral sample solution, added a drop of saturated aqueous solution of picrolonic acid. Rectangular crystallization showed the presence of calcium.

3. Test for Cobalt:

To a drop of the sample solution, added two drops of the saturated solution of ammonium thiocyanate. Blue colour development showed the presence of cobalt.

4. Test for Copper:

To a drop of sample solution, added a drop of ammonium mercuri thiocyanate solution. A violet precipitate indicated the presence of copper.

5. Test for Iron:

To a drop of sample solution, added a drop of 5% solution of potassiumferrocyanide. A blue colouration indicated the presence of iron.

6. Test for Potassium:

To a drop of sample solution, added a drop of dipicrylamine (1 % in 0.1 N sodium carbonate)

Orange-red coloration showed the presence of potassium.

7. Test for Magnesium:

To a drop of sample solution, added a drop of magneson reagent ((0.01% paranitrobenzeazoresinol in 1 N NaOH). A blue precipitate formation inferred the presence of magnesium.

8. Test for Mercury:

To a drop of sample solution, added crystals of ammonium thiocyanate and a little of solid cobalt acetate. Blue colour development indicated the presence of mercury.

9. Test for Zinc:

To a drop of sample solution, added a drop of 0.0 2% cobalt sulphate and a drop of the ammonium mercurithiocyanate solution. Blue precipitate was obtained indicating the presence of zinc.

10. Test for Sodium:

To a drop of sample solution, added eight drops of the zinc uranyl acetate. An yellow precipitate showed the presence of sodium.

11. **Test for Arsenate:**

The sample gave a white precipitate with magnesia mixture showing the presence of arsenate. Further it was confirmed by the liberation of iodine on addition of ammonium iodide and dil.HCl.

12. Test for Chloride:

The sample solution gave white precipitate when added with silver nitrate.

13. Test for Silicate:

The sample was heated with microcosmic salt ($NaNH_4\ HPO_4.\ 4H_2O$). A white speck was floating on the bead without dissolving in it which showed the presence of silicate.

14. Test for Nitrate:

The sample was mixed with saturated solution of ferrous sulphate and shaken con. H_2SO_4 was added along the side of the test tube. A brown ring was formed showing the presence of nitrate.

15. Test for Acetate:

When heated with ethanol and H_2SO_4 gave fruity odour.

16. Test for Carbonate:

The sample was mixed well with potassium dichromate and few drops of dil.HCl were added. Effervescence showed the presence of carbonate.

17. Test for Sulphide:

To the solution freshly prepared cadmium carbonate was added and shaken well. Yellow precipitate showed the presence of sulphide.

18. Test for Sulphate:

The above precipitate was removed by centrifugation and to the supernatant dil. acetic acid was added to remove excess carbonate, barium chloride solution was added white precipitate showed the presence of sulphate.

Quantitative assay

5.5.5 Assays:

Quantitative assays for Calcium, Magnesium**,** Potassium**,** Aluminium**,** Copper**,** Iron and Zinc**,** were observed in ICP-OES using standards. Sulphur (as SO_2) was estimated by following AOAC 990.28 method and Chloride (as NaCl) was calculated by following AOAC 950.52 method.

Calcium (as Ca)	SO-CHML-CTS-C-01 -QU-063-by ICPOES
Magnesium(as Mg)	SO-CHML-CTS-C-01 -QU-063-by ICPOES
Potassium (as K)	SO-CHML-CTS-C-01 -QU-063-by ICPOES
Aluminum (as Al)	SO-CHML-CTS-C-01 -QU-063-by ICPOES
Copper (as Cu)	SO-CHML-CTS-C-01 -QU-063-by ICPOES
Iron (as Fe)	SO-CHML-CTS-C-01 -QU-063-by ICPOES
Zinc (as Zn)	SO-CHML-CTS-C-01 -QU-063-by ICPOES
Sulphite (as S02)	AOAC 990.28
Chloride(as Nacl)	AOAC 950.52

5.5.6 Heavy metal Analysis:

Tests for heavy metals, viz., lead, cadmium, arsenic and mercury were carried out in ICP-OES instrument (Perkin Elmer Optima 3000 DV).

Lead (as Pb)	SO-IN-MUL-TE-063 by ICP-MS
Cadmium (as Cd) Arsenic (as As)	SO-IN-MUL-TE-063 by ICP-MS SO-IN-MUL-TE-063 by ICP-MS
Mercury (as Hg)	SO-IN-MUL-TE-063 by ICP-MS

5.5.7 Microbial contamination:

Tests for total bacterial /fungal counts *E. coli*, *Salmonella* spp., *Staphylococcus aureus* and *Entero bacteriacea* were done.

5.5.8 Pesticide residues:

Various pesticide residues of organo chlorine and organo phosphorous viz., alphaBHC, betaBHC**,** gam BHC(Lindane)**,** deltaBHC**,** Aldrin, Dieldrin, trans Chlordane, cis Chlordane, Endrin**,** Endrinaldehyde**,** Endrinketone**,** Endosulfan-I**,** Endosulfan-II**,** Endosulfansulfate**,** Heptachlor**,** Heptachlorepoxide**,** Dicofol**,** Chlorthalonil**,** Hexachlorobenzene**,** o,p"DDT**,** P,P"DDT**,** o,p"DDD**,** p,p"DDD**,** o,p"DDE**,** P,P"DDE, 4-Bromo,2-Chlorophenol**,** Acephate**,** Chlorfenvinphos**,** Chlorpyrifos**,** Phorate sulphone, Chlorpyrifos methyl**,** Malathion, Diazinon**,** Dichlorvos**,** Dimethoate**,** Ethion**,** Monocrotophos, Etrimfos**,** Fenitrothion**,** Iprobenphos**,** Methamidophos, , Omethoate, Oxydemeton-methyl, Quinalphos, Parathion ethyl, Parathion methyl, Phorate, Phosalone, Phosphamidon, Profenophos, , Triazophos, , Phorate sulphoxide were tested by following AOAC 2007.01 methods.

5.5.9 Tests for Aflatoxins:

Aflatoxins such as B1, B2, G1 and G2 were checked using AOAC 2008.02 methods.

5.5.10 TLC Photodocumentation/HPTLC Finger prints profiling:[64,65]

Sample preparation

Four gm of the drug was extracted successively by hexane, chloroform and ethanol using Soxhlet apparatus. The extracts were filtered freed from solvents and made upto 10 ml in standard flasks using the respective solvents.

TLC plate

Aluminium plate precoated with silica gel $60F_{254}$ of 0.2 mm thickness (Merck) was used for the TLC/HPTLC analysis.

Developing chamber

Camag's twin trough chamber was used for the development.

Solvent system

Many solvent systems were tried for a better separation and the same was achieved in Toluene: Ethyl acetate (10 : 0.5, v/v) for hexane extract; Toluene : Ethyl acetate (5:1.5, v/v) for chloroform extract and ethanol extract.

Derivatization reagent

For derivatization vanillin-sulphuric acid reagent was used (1 gram vanillin dissolved in a mixture of ethanol and sulphuric acid with the composition 95 ml : 5 ml).

Instrument

Linomat 5 automatic applicator, CAMAG's visualizer, CAMAG's scanner 030618 attached with WINCATS software were the instruments used for photo documentation and HPTLC finger printing. CAMAG's plate heater was used for derivatization.

Procedure

5 µl, 10 µl and 15 µl of the hexane, chloroform and ethanol extracts were applied on three different plates as 10 mm bands with 8 mm distance in between and developed up to 8 cm in the above mentioned solvent systems. The air dried developed plates were visualized under UV 254 and 366 nm for documenting TLC chromatograms. The plates were scanned in UV 254 nm (all extracts) & 366 nm (hexane and chloroform) and the finger print profiles were recorded. Until the development of coloured spots the vanillin-sulphuric acid reagent plates were heated in an oven at 105˚C. After derivatization the TLC photo documentation in white light and 575 nm (hexane and chloroform) finger print profiles were recorded.

5.6 Toxicity studies of *Nandhi Mezhugu* in Animal model:

5.6.1 Animal Care and Husbandry:

The study protocol involving animals was reviewed and approved by Institutional Animal Ethical Committee (IAEC), National Institute of Siddha, Chennai-600047, with the experimental protocol number IAEC approved Number: **1248/AC/09/CPCSEA-4/June2011/10** for Acute toxicity study and IAEC approved Number: **1248/AC/09/CPCSEA-9/Dec2013/5** for Sub-acute and Sub-chronic toxicity studies. Experiments were performed as per the guidelines prescribed by the CPCSEA, Ministry of E & F, Govt of India. Male and female *Wistar* albino rats, (160–200 g) obtained from LAM, CAHS, Madhavaram, Chennai-600051 and housed in Animal house of National Institute of Siddha. Each group of rats was separately housed in polypropylene cages in a well-ventilated room under an ambient temperature of 22±3°C and 30-70% relative humidity, with a 12-h light/dark artificial light cycle. They were provided with rodent chow from VRK Nutritional Solutions, Sangli, Maharastra and Reverse Osmosis purified water *ad libitum*. All the animals were acclimatized to the laboratory conditions at least for 7 days prior to experimentation.

5.6.2 Acute toxicity studies:[66]

The acute oral toxicity study was performed in accordance with Organization for Economic Cooperation and Development (OECD) test guideline 423 [Newbould 1963]. The limit test dose of 2000 mg/kg was used as stipulated in Organization for Economic Cooperation Development (OECD) guidelines.

Dose Preparation:

Palm jaggery 400mg per dose was added in 7.5 ml of distilled water and dissolved well. Administratered through oral route to Group I Animals.

NM 50mgs per dose and Palm jaggery 400mg were added in 7.5ml of distilled water and dissolved well. Administratered through oral route to Group II Animals.

NM 300mgs per dose and Palm jaggery 2.4gms were added in 7.5ml of distilled water and dissolved well. Administratered through oral route to Group III Animals..

NM 2000mgs per dose and Palm jaggery 16gms were added in 7.5ml of distilled water and dissolved well Administratered through oral route. to Group IV Animals.

The dose was determined for a required concentration before administration by dissolving *Nandhi Mezhugu* and palmjaggery in distilled water. It was mixed well. The preparations for different doses vary in concentrations to allow a constant dosage volume.

In the experiments the commonly observed parameters were body weight, clinical signs and gross pathology and were as follows;

a. Mortality or morbidity was noted.

b. Following test item administration weekly body weight was recorded.

c. Clinical signs such as lethality, convulsion, tremor, straub tail, sedation, excitation, abnormal gait (rolling), abnormal gait (tiptoe), jumps, motor coordination, loss of balance, fore paw treading, writhes, piloerection, stereotypies (chewing), stereotypies (Head movements), loss of grasping, akinesia, head twitches, scratching, respiration, aggressiveness, fear, reactivity to touch, muscle tone, loss of righting reflex, analgesia, ptosis, exophthalmos, catalepsy, loss of traction, loss of corneal reflex, defecation, salivation, lacrimation, others were observed at approximately 30 mins, 1hr, 2hr and 4hr on day 1 and daily thereafter for 14 days.

At the end of 14 days, the experimental animals were necropsied and investigated for gross pathological examination.

5.6.3 Sub-acute Toxicity Study of Nandhi mezhugu[67]

On the basis of these results i.e., acute toxicity study of Nandhi mezhugu and the therapeutic dose level as mentioned in Siddha literature , extrapolated to rat body surface area, the following doses of 9 mg/kg, 45 mg/kg and 90 mg/kg body weight were selected for the sub-acute toxicity study and administered orally twice daily (7 days drug dosing followed by 7 days drug holidays).

Preparation and administration of dose: Repeated-dose (28 days) oral toxicity study was carried out according to the OECD guideline 407.

Dose Preparation:

Palm Jaggery 72 mg per dose was added to distilled water and dissolved well. Administered through oral route to Group I(Vehicle control) .

Nandhi Mezhugu 9mg per dose and 72mg palm jaggery were dissolved well in distilled water and administered through oral route to Group II(Low Dose) .

Nandhi Mezhugu 45mg per dose and 360mg palm jaggery were added to distilled water and dissolved well. Administered through oral route to Group III(Mid Dose) .

Nandhi Mezhugu 90mg per dose and 720mg palm jaggery were added to distilled water and dissolved well. Administered through oral route to Group IV(High Dose) .

Palm Jaggery 72 mg per dose was added to distilled water and dissolved well. Administered through oral route to Group V(Recovery control) .

Nandhi Mezhugu 90mg per dose and 720mg palm jaggery were added to distilled water and dissolved well. . Administered through oral route to Group VI(High Dose Recovery).

The NM was administered by oral gavage for 28 days (4 weeks) twice daily at regular intervals as per the protocol mentioned in the Siddha literature where 7 days of test drug administration followed by 7 days of drug holiday alternatively for four cycles of dosing to match 28 days of dosing period. Recovery groups were scheduled for follow-up observations for the next 14 days without NM administration. High dose recovery and Recovery control were included in the study to determine the delayed occurrence, or persistence of, or recovery from toxic effects, if any. All groups were observed twice daily for morbidity and mortality, clinical signs once daily. Bodyweight and food intake of the animals were evaluated at weekly intervals. Any change in the water consumption was observed and recorded.

On the 57^{th} day, after an overnight fast, the rats were anaesthetized with Thiopentone sodium and blood samples were collected from retro-orbital plexus under light ether anaesthesia by the trained personnel. The blood samples for haematological and biochemical analyses were collected in tubes with and without EDTA, respectively. Total WBC, Differential Counts, RBC, Haemoglobin, HCT, MCV, MCH, MCHC, RDW, PLT, MPV, PDW and PCT. Biochemical analysis was performed on serum obtained after centrifugation of total blood (without anticoagulant) at 2500 rpm for 15 min. Blood Glucose, Urea, Creatinine, Cholesterol, Triglyceride, HDL, LDL, Total serum protein , ALT or SGPT, AST or SGOT were analysed.

Pathology

Necropsy was done in all animals in Group I to Group IV on 57^{th} day and Group V and Group VI (recovery groups) on 70^{th} day. Blood samples were collected from abdominal aorta following which all the animals were euthanized by excess dose of Thiopentone Sodium administered by intra-peritoneal route. The carcasses were observed for gross lesions in all major internal organs. The organs such as brain, heart, lungs, liver, kidneys, and spleen were weighed and relative organ weights were calculated. The relative organ weight of each animal was then calculated as follows: [71]

$$\text{Relative Organ Weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight on the day of euthanasia}} \textbf{ X 100}$$

The organs were fixed in 10% neutral buffered formalin, trimmed and a 5µ thickness of tissue sections were stained with hematoxylin and eosin for histopathological investigation.

5.6.4 Sub-chronic Toxicity Study:[68]

Justification for Dose Selection: As stated in results of acute toxicity studies in *Wistar* rats, NM was nontoxic up to the maximum dose level of 2000 mg/kg body weight. On the basis of these results and the therapeutic dose level as mentioned in Siddha literature extrapolated to rat body surface area, the following doses of 9 mg/kg, 45 mg/kg and 110 mg/kg body weight were selected for the study and administered orally twice daily. 90 days Repeated-dose oral toxicity study was carried out according to OECD test guideline 408.

Dose Preparation:

Group I: Palm Jaggery 72 mg per dose was added to distilled water and dissolved well. Administered through oral route to Group I (Vehicle control).

Group II: *Nandhi Mezhugu* 9mg per dose and 72mg palm jaggery were added to distilled water and dissolved well. Administered through oral route to Group II (Low Dose).

Group III: *Nandhi Mezhugu* 45mg per dose and 360mg palm jaggery were added to distilled water and dissolved well. Administered through oral route to Group III (Mid Dose).

Group IV: *Nandhi Mezhugu* 110mg per dose and 880mg palm jaggery were added to distilled water and dissolved well. Administered through oral route to Group IV (High Dose) .Since 90mg dose level did not show any toxic signs the sub chronic toxicity high dose level was increased from 90mg to 110mg.

Group V: Palm Jaggery 72 mg per dose was added to distilled water and dissolved well. Administered through oral route to Group V (Recovery control) .

Group VI: *Nandhi Mezhugu* 110mg per dose and 880mg palm jaggery were added to distilled water and dissolved well. Administered through oral route to Group VI (High Dose Recovery).

NM was administered twice daily by oral gavage for 7 days, followed by 7 days drug holiday, alternatively for 180 days (90 days drug dosing and 90 days drug holiday).

This protocol was followed to mimic the treatment protocol in humans as mentioned in the traditional Siddha literature.

Recovery groups were scheduled for follow-up observations for the next 14 days without NM administration. High dose recovery and recovery control were included in the recovery study to define the late occurrence, or persistence of, or recovery from toxic effects. All group of animals were observed, twice daily, for morbidity and mortality; and clinical signs daily.The bodyweights and food intake of the animals were evaluated on a weekly basis. Any change in the water consumption was observed and recorded.

On the 181st day, after an overnight fast, the rats were anaesthetized with Thiopentone sodium and blood sample for haematological and biochemical analysis were collected into tubes with and without EDTA, respectively. Total WBC, differential Counts, RBC, haemoglobin, HCT, MCV, MCH, MCHC, RDW, PLT, MPV, PDW and PCT,Blood Glucose, Urea, Creatinine, Cholesterol, Triglyceride, HDL, LDL, Total serum protein, ALT or SGPT, AST or SGOT were screened.

Histopathology

Necropsy was done on all animals in Group I through IV on the181st day and for Group V and Group VI (recovery groups) on the 195th day. Blood samples were collected from abdominal aorta following which all the animals were euthanized by excess dose of Thiopentone sodium administered by intra-peritoneal route. The gross pathological examination of all major internal organs performed. The organs such as brain, heart, lungs, liver, kidneys, and spleen were weighed and relative organ weights were calculated as mentioned in the sub-acute toxicity study. The cropped organs were secured in 10% neutral buffered formalin,and the 5µ size tissue sections were stained with haematoxylin and eosin for histopathological observation.

Statistical Analysis

All the data were expressed as Mean ± Standard Error Mean. The data were statistically analysed by using one-way ANOVA if the number of groups to be compared is more than two followed by Post-hoc test and Dunnet-t test. If only two groups are to be compared as in the case of recovery groups, students't' test was used. It was assumed that the data were normally distributed and there was homogeneity of variance.Statistically significant valueof P is less than 0.05

5.6.5 ICP-OES – Detection of Heavy metal traces in animal tissue samples (Brain, Liver, Kidney) in Sub-chronic toxicity studies (High dose group) **of *Nandhi Mezhugu*:**

In sub chronic repeated oral 90 days (7 days drug dosing and 7 days drug holidays) toxicity study of *Nandhi Mezhugu* in *Wistar* rats (OECD 408) the tissues of brain, kidney, liver of high dose group were subjected to ICP-OES study to screen heavy metal content and it was found that there was absence of traces of heavy metal such as lead, cadmium, mercury and arsenic.

5.7 Pharmacological studies of *Nandhi Mezhugu* in Animal model:

5.7.1 Analgesic activity:[72,73,74]

Experiment animal's husbandry:

The study protocol involving animals was reviewed and approved by Institutional Animal Ethical Committee (IAEC), National Institute of Siddha, Chennai-600047, with the experimental protocol number IAEC approved Number: **1248/AC/09/CPCSEA-4/June2011/10** for Analgesic and Acute Anti–inflammatory study. Experiments were performed as per the guidelines prescribed by the Committee for the Purpose of conduct and Supervisions of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India. Male and female *Wistar* albino rats(160–200 g) and Swiss albino mice(25-35g) obtained from Laboratory Animal Medicine, Centre for Animal Health Studies, Madhavaram Milk Colony, Chennai-600051 were housed in Animal House of National Institute of Siddha. Each group of rats was separately housed in polypropylene cages in a well-ventilated, with room temperature of 22±3°C under an ambient temperature and 30-70% relative humidity, with a 12-h light/dark artificial light cycle maintained room.. The rodents were fed with chow from VRKNS(VRK Nutritional Solutions), Sangli, Maharastra and Reverse Osmosis purified water *ad libitum*. All the animals were acclimatized to the laboratory conditions for at least for 7 days prior to experimentation.

Acetic acid induced writhing in mice:

This study was done by the method of Koster et al., 1959. There was no mortality in the acute oral toxicity test up to a dose of 2000mg/Kg. One tenth of the maximum tolerable was considered for further pharmacological activities. Acetic acid induced writhing method was preferred to evaluate the analgesic efficacy of the drugs/compound

in mice. Abdominal constractions in mice were caused by the intra-peritoneal injection of acetic acid. Animals were divided into five groups such as Vehicle control (0.1 ml glacial acetic acid + Palm jaggery solution), Positive control(0.1 ml glacial acetic acid + Disprin (50mg), Low Dose(0.1 ml glacial acetic acid +NM 25mg +PJ solution), Mid Dose(0.1 ml glacial acetic acid +NM 40mg +PJ solution), High Dose(0.1 ml glacial acetic acid +NM 50mg + PJ solution).

Wistar albino mice of either sex were divided into five different groups each containing six animals and the animals were separately marked. Food was withdrawn 12 hours prior to drug administration till the finishing point of experiment. The animals were weighed and numbered correctly. The test and standard drugs were given orally. After 60 minutes writhing was induced by intra-peritoneal injection of 1% acetic acid in volume of 0.1 ml/10g body weight. The writhing episodes were recorded for 30 minutes; stretching movements consisting of elongation of body, arching of the back and extension of hind limbs were counted. Percentage of inhibition was estimated using following formula. The results of acetic acid induced writhing method in mice were tabulated.

$$\% \text{ Inhibition} = \frac{\text{Mean of writhing test (control)} - \text{Mean writhing test (test)} \times 100}{\text{Mean number of writhing test (control)}}$$

5.7.2 Acute anti-inflammatory activity:

Carrageenan induced rat paw oedema:[75,76,77]

The Method of Winter *et al* was used to evaluate the Anti-inflammatory activity with some minor modifications .The rats were divided in to four groups containing six rats in each group. In each group the left paw volume at 0 hr was treated as control. In each group (GII, GIII, GIV) the test drug (*Nandhi Mezhugu*) was administered with palm jaggery in1:8 ratio (1 hr prior to carrageenan injection) at three dose levels which were as follows: Group II (low dose group) -90mg/kg b.w, Group III (Mid dose group) -450mg/kg b.w, Group IV (High dose group) -900mg/kg b.w. The carrageenan treated Left paw volume was measured in all three dose levels at 1^{st} hr, 2^{nd} hr, 4^{th} hr and 6^{th} hr respectively. The same procedure was repeated with standard drug (Diclofenac sodium 25mg/kg b.w) (Group 1) also.

The percentage of inhibition was calculated by the formula

$$\% \text{ Inhibition} = \frac{\text{Pre drug reading -- post drug reading} \times 100}{\text{Pre drug reading}}$$

5.7.3 Chronic anti-inflammatory activity:

Experiment animal's husbandry:[78,79,80,81]

The study was conducted in LIVEON BIOLABS PVT. LTD. Karnataka, INDIA with the experimental protocol number: LBPL-EF-047/15 for chronic anti–inflammatory study. **IAEC No: LBPL-IAEC-114-06/15**. Experiments were performed as per the guidelines prescribed by the Committee for the Purpose of conduct and Supervisions of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India. Male and female *Wistar* albino rats (7-8 weeks) Males: 170.63 – 200 g Females: 170.14 – 198.57g. Each group of rats was separately housed in polypropylene cages with Animal husbandry conditions: Temperature – 20.1-23.8^{o}C ,Humidity – 45-68 %, Air changes – 12-16 changes per hour, Light cycle – 12 hours light and 12 hours dark (as per CPCSEA guidelines). They were provided with fed *ad libitum* with AMRUT Laboratory Animal Feed manufactured by Pranav Agro Industries Limited, Sangli, Maharashtra and Reverse Osmosis purified water *ad libitum*. 7 days prior to experimentation, Animals were acclimatized to the laboratory conditions.

Cotton pellet granuloma method:

On day 1, cotton Pellets (10±1 mg each) were sterilized and impregnated with an aqueous solution of Ampicillin. Two cotton pellets were implanted subcutaneously in the groin region of rats, one on each side under anaesthesia. G3, G4 and G5 group animals were treated with test item(Nandhi mezhugu) and palm Jaggery in the ratio of at 50 mg/kg b.wt, 150 mg/kg b.wt and 500 mg/kg b.wt respectively once a day, for 7 consecutive days. G2 group animals were treated with Dexamethasone sodium @ 0.5 mg/kg and it was treated as a positive control. G1 group animals were treated as vehicle control(palm jiggery solution) and it was treated with vehicle alone. On day 8 the animals were humanely sacrificed using CO_2 exposure method, subjected to the gross necropsy and cotton pellets were collected (Specified organs were collected and preserved in 10%

NBF for histopathological evaluations). Collected cotton pellets (wet and dried) were weighed and recorded. Obtained data were subjected to statistical analysis.

Observations:

a) Clinical signs of toxicity.

b) Morbidity and mortality.

c) Individual animal, initial, weekly and final body weights were measured and recorded.

d) Gross Necropsy Cotton pellets (wet and dried) weights were recorded.

5.7.4 Anti-arthritic activity:

Experiment animal's husbandry:[82,83,84]

The study was conducted in LIVEON BIOLABS PVT. LTD. Karnataka, INDIA with the experimental protocol number: LBPL-EF-046/15for Anti-arthritic study. **IAEC No: LBPL-IAEC-110-06/15.** Experiments were performed as per the guidelines prescribed by the Committee for the Purpose of conduct and Supervisions of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India. Male Sprague Dawley (7-8 weeks) 150 -170 gram. Each group of rats was separately housed in polypropylene cages with Animal husbandry conditions: Temperature -20.1-23.8^{o}C,Humidity - 45-69 %, Air changes - 12-16 changes per hour, Light cycle – 12 hours light and 12 hours dark (as per CPCSEA guidelines). They were provided with fed *ad libitum* with AMRUT Laboratory Animal Feed manufactured by Pranav Agro Industries Limited, Sangli, Maharashtra and Reverse Osmosis purified water *ad libitum*. All the animals were acclimatized to the laboratory conditions for at least 7 days prior to experimentation.

Freund's complete adjuvant (FCA) Arthritis Model:

After acclimatization period the animals were equally distributed into 5 groups with. six animals in each group. On day 1, 0.1 ml of Freund's complete adjuvant (FCA) was injected into the sub plantar region of the left hind paw of all animals across all the groups (1.0 ml FCA consisted of 1 mg heat killed and dried mycobacterium tuberculosis, 0.85 ml paraffin oil and 0.15 ml mannide mono oleate concentration of 6mg/ml). Before administration of the vehicle, standard and test item, size of the left and right hind paw of

all the animals were measured using vernier caliper. After paw measurement, G3, G4 and G5 group animals were treated daily with test item(Nandhi mezhugu) and palm Jaggery in the ratio of 1:8 at 50 mg/kg b.wt, 150 mg/kg b.wt and 500 mg/kg b.wt respectively up to 12 days. G1 group animals were treated as Arthritis Control and it was treated with vehicle alone as similar to G3 to G5 group animals, G2 group animals were treated with the known drug Indomethacin 0.3 mg/kg, PO up to 12 days. From day 13 - day 21 the animals were not treated with vehicle, standard drug or test item. On day 22nd approximately 24 hours after the last test item administration all animals were anesthetized using CO_2, blood was collected in tubes with and without anti-coagulant (2% K_2EDTA). Blood samples without anticoagulant were centrifuged at 3000 rpm for 10 minutes. Serum samples separated and used for estimation of clinical chemistry/biochemical parameters (albumin and total protein). Blood samples with anticoagulant were used for estimation of haematology parameters (Leukocyte count, ESR and Haemoglobin). After blood collection the animals were humanely sacrificed using CO_2 asphyxiation method, subjected to the gross necropsy and knee joints or other specified organs were collected and preserved in 10% NBF for histopathological evaluations. The joints were decalcified and processed, stained with H & E stain and examined for cartilage integrity/arthritic parameters: inflammation, erosions/degeneration, fibrosis etc., and FCA administered paws were preserved in -80 ^{o}C and transferred to the monitoring scientist for the biochemical evaluation.

Body weight, paw volume measurement and evaluation of primary and secondary lesions

Body weights of the animals were measured on day 1 before the FCA administration and weekly once thereafter during the study period. Paw volume measurement for evaluation of primary and secondary lesions. For primary lesions adjuvant injected hind paw for secondary lesions non injected hind paw were measured using vernear caliper. On day 1, before and after administration FCA, both hind paws (limbs) of the all animals were assessed. On day 5, FCA administered hind paw of all the animals were assessed for primary lesions and influence of therapeutic agents on this phase. On day 12 and day 21, FCA administered hind paw and non-administered hind paw of all the animals were measured for primary lesions and secondary lesions. On day

21, the body weights were determined and the severity of the secondary lesions was evaluated visually and graded according to the following scheme:

Scoring Scheme

Organ	**Lesions**	***Score***
Nose	no swelling of connective tissue	0
	intensive swelling of connective tissue	1
Ears	absence of nodules and redness	0
	presence of nodules and redness	1
Fore paws	absence of inflammation	0
	inflammation of at least 1 joint	1
Hind paws	absence of inflammation	0
	slight inflammation	1
	moderate inflammation	2
	marked inflammation	3
Tail	absence of nodules	0
	presence of nodules	1

EVALUATION:

a) For primary lesions: The % of inhibition of paw volume of the injected left paw over vehicle control was measured at day 5.

b) For secondary lesions: The percentage inhibition of paw volume of the non-injected right paw over controls was measured at 21st Day.

c) Calculation of an arthritic index was done by summation of the scores as indicated above for each animal. The average of treated animals was compared with the control group.

The total percentage change was calculated by addition of percentage inhibition of the injected paw on day 5 + percentage inhibition of the non-injected paw on day 21 + percentage change of the arthritic index.

VASCULAR PERMEABILITY EFFECT:

Experiment animal's husbandry:[85,86,87]

The study was conducted in LIVEON BIOLABS PVT. LTD. Karnataka, INDIA with the experimental protocol number: LBPL-EF-048/15 for vascular permeability study. IAEC number **LBPL-IAEC-115-06/15** Experiments were performed as per the guidelines prescribed by the CPCSEA, Ministry of E and F, Govt. of India. Sprague Dawley (7-8 weeks) Males: 167.8 -190.6 grams Female: 172.0 – 197.0 grams. Each group of rats was separately housed in polypropylene cages with Animal husbandry conditions: Temperature -21.8-24.2^{o}C,Humidity - 46-66%, Air changes - 12-16 changes per hour, Light cycle – 12 hours light and 12 hours dark (as per CPCSEA guidelines). They were provided with fed *ad libitum* with AMRUT Laboratory Animal Feed manufactured by Pranav Agro Industries Limited, Sangli, Maharashtra and Reverse Osmosis purified water *ad libitum*. All the animals were acclimatized to the laboratory conditions at least for 7 days prior to experimentation.

Evans blue vascular permeability model:[85,86,87,88]

Test procedure: After acclimatization period the animals were equally distributed into 4 groups. Each group had 10 animals (5 Males and 5 Females)

Administration of 1% Evans blue dye solution:

60 minutes before the test item administration, 1% Evans blue dye solution@ 5ml/kg body weight was administered to all group animals through tail vein.

Administration of Vehicle and test item:

One hour after administration of 1% Evans blue dye solution, the G1 group animals were administered with vehicle alone at 10 ml per kg body weight and G2, G3 & G4 group animals were administered with test item(Nandhi mezhugu) and palm Jaggery in the ratio of 1:8 at 50 mg/kg b.wt, 150 mg/kg b.wt and 500 mg/kg b.wt respectively through oral route by gavage as a single dose.

Administration of acetic acid (0.6%):

30 minutes after the test item or vehicle administration acetic acid (0.6%) was administered to the all animals at 10 ml/kg b.wt through intra-peritoneal route.

The animals were sacrificed 30 minutes after acetic acid administration and their peritoneal cavity was washed with normal saline (Administered 5 ml/animal through intraperitoneal route) and the fluid was collected and centrifuged at 2000 rpm for 10 minutes.[89,90]

The absorbance of supernatant was measured at 620 nm using ELISA reader.

Observations:

a) Clinical signs.
b) Morbidity and mortality.
c) Individual animal, body weights were measured and recorded.
d) Gross Necropsy.
e) Measurement of dye content in peritoneal fluid using ELISA reader.

5.7.6 MTT Assay for Cell Protective and Cell Toxicity Effect:

5.7.6.1 Cyto-toxicity effect of NM:[91,92,93]

Objective

To determine the cytotoxicity effect of NM extract and LPS.

Principle

Determination of cell proliferation and cytotoxicity were measured by MTT assay which is a colorimetric assay, based on reduction of the yellow colored water soluble tetrazolium dye MTT to formazan crystals. The live cells which produce mitochondrial lactate dehydrogenase which reduces MTT to insoluble formazan crystals, which upon dissolution into an appropriate solvent exhibits purple color, the intensity of which is proportional to the number of viable cells and can be measured spectrophotometrically at 570nm.

Materials

- Cell line: RAW 264.7–Mouse macrophage cell line (From NCCS Pune)
- Cell culture medium: RPMI 1640: (#AL199A, Himedia)
- Adjustable multichannel pipettes and a pipettor (Benchtop, USA)
- Fetal Bovine Serum (#RM10432, Himedia)
- MTT Reagent (5 mg/ml) (# 4060 Himedia)
- DMSO (#PHR1309, Sigma)
- Oxaliplatin (80µM) (#O9512 SIGMA)
- D-PBS (#TL1006, Himedia)
- LPS (#L3012, Sigma)
- 96-well plate for culturing the cells (From Corning,USA)
- T25 flask (# 12556009, Biolite - Thermo)
- 50 ml centrifuge tubes (# 546043 TORSON)
- 1.5 ml centrifuge tubes (TORSON)
- 10 ml serological pipettes (TORSON)
- 10 to 1000 ul tips (TORSON)
- 96-well ELISA plate reader or spectrophotometer capable of measuring the absorbance (ELX-800 Biotek)
- Inverted microscope (Biolink)
- 37°C incubator with humidified atmosphere of 5% CO_2 (Healforce, China)

Assay controls:

(i) Medium control (without cells)

(ii) Negative control (Medium without the experimental medicine/compound, with cells)

(iii) Positive control (medium with cells and with 80 uM of Oxaliplatin**)**

Note: Extracellular reducing components such as ascorbic acid, cholesterol, alpha-tocopherol, dithiothreitol present in the culture media may reduce the MTT to formazan.

To account for this reduction, it is important to use the same medium in control as well as test wells.

Procedure for Determining Cell Cytotoxicity

Cell Seeding

1. Seed 200µl cell suspension in a 96-well plate at required cell density (20,000 cells per well), without the test agent. Allow the cells to grow for about 12 hours.
2. Add appropriate concentrations of the test agents (Mentioned in the results - Excel sheet).
3. Incubate the plate for 24 hrs at **37°C** in a 5% CO_2 atmosphere.
4. After the incubation period, takeout the plates from incubator, and remove spent media and add MTT reagent to a final concentration of 0.5mg/mL of total volume.
5. Wrap the plate with aluminium foil to avoid exposure to light.
6. Return the plates to the incubator and incubate for 3 hours.
7. (Note: Incubation time varies for different cell lines. Within one experiment, incubation time should be kept constant while making comparisons.)
8. Remove the MTT reagent and then add 100 µl of solubilisation solution (DMSO).
9. Gentle stirring in a gyratory shaker will enhance dissolution.
10. Read the absorbance on a spectrophotometer or an ELISA reader at 570nm and 630nm used as reference wavelength.

MTT assay readings and viability calculations:

11. **The Ic50 value** was determined by using linear regression equation i.e. Y =Mx+C. Here, Y = 50, M and C values were derived from the viability graph.

5.7.6.2 Cytoprotective studies by using MTT Assay

Objective

To determine the cytoprotective nature of NM extract.

Principle

MTT assay is a colorimetric assay based on reduction of the yellow colored water soluble tetrazolium dye MTT to formazan crystals, used for the determination of cytotoxicity and cell proliferation. MTT is reduced to insoluble formazan crystals, which upon dissolution into an appropriate solvent exhibit purple color, the intensity of which is proportional to the number of viable cells and can be measured spectrophotometrically at 570nm by the mitochondrial lactate dehydrogenase, which is produced by live cells.

Materials

- Cell line: RAW 264.7–Mouse macrophage cell line (From NCCS Pune)
- **Cell culture medium:**

1. RPMI 1640: (#AL199A, Himedia)

- Adjustable multichannel pipettes and a pipettor (Benchtop, USA)
- Fetal Bovine Serum (#RM10432, Himedia)
- MTT Reagent (5 mg/ml) (# 4060 Himedia)
- DMSO (#PHR1309, Sigma)
- LPS (#L3012, Sigma)
- D-PBS (#TL1006, Himedia)
- 96-well plate for culturing the cells (From Corning,USA)
- T25 flask (# 12556009, Biolite - Thermo)
- 50 ml centrifuge tubes (# 546043 TORSON)
- 1.5 ml centrifuge tubes (TORSON)
- 10 ml serological pipettes (TORSON)
- 10 to 1000 ul tips (TORSON)
- 96-well ELISA plate reader or spectrophotometer capable of measuring the absorbance (ELX-800 Biotek)
- Inverted microscope (Biolink)
- 37°C incubator with humidified atmosphere of 5% CO_2 (Healforce, China)

Assay controls:

(i) Medium control (medium without cells)

(ii) Toxicity control (medium with cells treated with 25nG/mL of LPS)

Note: The reduction of MTT to formazan was carried out by extracellular reducing components such as ascorbic acid, cholesterol, alpha-tocopherol, dithiothreitol present in the culture media. In control as well as test wells it is essential to use the same medium to account for this reduction.

Procedure for Determining Cytoprotective nature of plant extract

Cell Seeding for post treatment studies

1. Seed 200µl cell suspension in a 96-well plate at required cell density (20,000 cells per well), without the test agent. Allow the cells to grow for about 12 hours.
2. Add appropriate concentrations of LPS (Mentioned in the results - Excel sheet).
3. Incubate the plate for 24 hrs at **37°C** in a 5% CO_2 atmosphere.
4. After the incubation period, takeout the plates from incubator, and remove spent media and add appropriate concentrations of the plant extract (Mentioned in the results - Excel sheet).
5. Incubate the plate for 24 hrs at **37°C** in a 5% CO_2 atmosphere.
6. After the incubation period, takeout the plates from incubator, and remove spent media and add MTT reagent to a final concentration of 0.5mg/mL of total volume.
7. Plate should not be exposed to light.
8. Return the plates to the incubator and incubate for 3 hours.
9. (Note: Incubation time varies for different cell lines. Within one experiment, incubation time should be kept constant while making comparisons.)
10. Remove the MTT reagent and then add 100 µl of solubilisation solution (DMSO).
11. Gentle stirring needed for dissolution. Occasionally, pipetting up and down may be required to completely dissolve the MTT formazan crystals especially in dense cultures.
12. Read the absorbance on a spectrophotometer or an ELISA reader at 570nm and 630nm used as reference wavelength.

MTT assay readings and viability calculations:

13. The viability values were determined based on the OD values.

5.7.7 Gene Expression Study:

<u>Sample Collection in In-Vitro:</u>

Control (Untreated cells)

LPS (100nG/mL) alone Treated cells

NM extract alone (100uG/mL) treated cells

LPS (100nG/mL) for 24 hrs+ NM extract treated cells (100uG/mL)

Procedure:

Cell Seeding for post treatment studies

1. Seed 0.1 x 10^6 cell suspension in a 6-well plate having 2 ml of culture medium without the test agent. Allow the cells to grow for about 12 hours.
2. Add appropriate concentrations of LPS.
3. Incubate the plate for 24 hrs at **37°C** in a 5% CO_2 atmosphere.
4. After the incubation period, takeout the plates from incubator, and remove spent media and add appropriate concentrations of the plant extract.
5. Incubate the plate for 24 hrs at **37°C** in a 5% CO_2 atmosphere.
6. After incubation, the spent media is removed and the cells are washed with 1 ml of D-PBS and cells are treated with 0.5uL of trypsin-EDTA solution, Incubate the plate for 2 to 4 min at **37°C** in a 5% CO_2 atmosphere.
7. The trypsin action is neutralised by adding 1.5mL of cell culture medium and the cells are allowed for centrifugation at 2000 RPM for 5 min.
8. The supernatant is discarded and the cells are washed with D-PBS by centrifugation at 2000 RPM for 5 min.
9. Now the cells are used for RNA isolation.

Sample Collection for In-Vivo model:

Rat Paw tissues:

a) Control group

b) Standard drug treated group

Test drug (High dose) treated group

Extraction of RNA[94,95]

All the plastic wares and glass wares used for this experiment were made RNase free by treating with DEPC for overnight and autoclaving twice at 121^{o}C until the traces of DEPC removed. These were then dried in Hot Air Oven at 90^{o}C before use. The samples stored at -80^{o}C were ground to powder in liquid N_2 using sterile mortar and pestle. To the powdered sample 1ml of Trizol Reagent (Takara Bio Inc, Japan) was added mixed by grinding, transferred to a 2ml microfuge tube and incubated at room

temperature for 15 – 20 mins. It was then centrifuged at 10,000 rpm (Sigma) for 5 min and the supernatant was transferred to a fresh sterile microfuge tube. Equal volume of Chloroform :Isoamyl alcohol (24:1) was added, mixed gently and incubated in ice for 2 minutes. After centrifuging the above at 12,000 rpm for 5 minutes the supernatant was transferred to a fresh vial then incubated in ice for 2 minutes after adding 500 µl of isopropanol. The RNA was pelleted down by centrifuging the above for 10 minutes at 10,000 rpm and the pellet was vortexed gently with 300 µl of 70% of ethanol. Centrifugation at 10,000 rpm was repeated for 10 minutes and the pellet was air dried. 20µl of RNase free water was added to the pellet and heated gently (60^{o}C) to dissolve the pellet in water.

DNase treatment

The extracted RNA was treated with DNase enzyme to remove any traces of DNA contamination. One micro liter of DNase was added to above isolated RNA and incubated for 1hr at 37^{o}C and after the temperature was raised to 70^{o}C for 5 minutes to inactivate the enzyme. The RNA was stored at -20^{o}C for later use.

RNA Quanitifcation

The concentration and purity of RNA was assessed using a spectrophotometer (Sartorius). A 1µL aliquot of RNA was pipetted onto the apparatus pedestal. RNA with an absorbance ratio at 260 and 280 nm (A260/A280) between 1.8 and 2.2 is indicative of pure RNA. The concentrations of the respective samples were as mentioned below.

cDNA synthesis

After quantification, RNA was reverse transcribed using oligodT (Sigma Aldrich). Hundred nanogram of RNA was aliquot to a fresh sterile microfuge tube and 2µl of oligodT was added and incubated at 70^{o}C for 5 minutes and immediately transferred to ice. To this 2µl of dNTPs, 1µl of Reverse Transcriptase enzyme (Biolabs, New England) and 2 µl of 10x Reverse transcriptase buffer was added and made up the volume to 25µl using RNase free water. This mixture was incubated at 42^{o}C for 90 minutes and reaction was terminated by incubating at 70^{o}C for 15 minutes.

Primersynthesis and validation :

The primers in In-Vitro Study were designed as below and were synthesized and purified.

Oligo name	**No of bases**	**Sequences (5'-3')**	**GC content %**	**Tm (^{o}C)**	**Annealing Temp (^{o}C)**
TNF-alpha Accession No. L19123.1 Rattus norvegicus tumor necrosis factor-alpha gene, 5' end Product length – 713					
FP	20	GATCGGTCCCAACAAGGAGG	60	60	57
RP	20	CAAAGGCGGAGATGAGACCC	60	60.4	
Cox2 Accession No. NM_011198.4 Musmusculus prostaglandin-endoperoxide synthase 2 (Ptgs2), mRNA, complete cds Product length – 374					
FP	20	CAAGGGAGTCTGGAACATTG	50	56.03	55
RP	20	ACCCAGGTCCTCGCTTATGA	55	60.33	

The primers in In-VivoStudy were designed as below and were synthesized and purified.

Oligo name	**No of bases**	**Sequences (5'-3')**	**GC content %**	**Tm (^{o}C)**	**Annealing Temp (^{o}C)**
Beta-galacturonidase Product length 685bp					

FP	17	AAATCTGCAAAATTCCA	29.5	43.7	40
RP	21	TCATTATCCTTATGCAGAAGA	33.3	48.5	
Acid phosphatase, Accession No. M27893.1 Rat acid phosphatase mRNA, complete cds - Product length 515					
FP	20	CGCATGACACTACCCTGGTT	55	60	
RP	20	AGGCTAAACCTGTCCCTCTG	55	58.7	
Cathepsin-D Accession No. NM_134334.2 Rattus norvegicus cathepsin D (Ctsd), mRNA - Product length 668					
FP	20	CCGTCGGACTATGACGGAAG	60	59.9	
RP	20	ACAGCTCCCCGTGGTAGTAT	55	60.3	
Myeloperoxidase level Accession No. AY494708.1 Musmusculus strain DBA/2 myeloperoxidase (Mpo) gene, promoter region and partial cds Product length – 766					
FP	20	GGCATGGGACTGTTCCTGAT	55	59.74	
RP	21	CAGTCCCCAAGAGCTCAACTT	52.3	55.9	
C-reactive protein Accession No. NM_017096.3 Rattus norvegicus C-reactive protein (Crp), mRNA Product length – 425					
FP	20	GCCTTCGTATTTCCCGGAGT	55	59.8	
RP	20	GACTGATTCGCGTCAAAGCC	55	59.9	
TNF-alpha Accession No. L19123.1 Rattusnorvegicus tumor necrosis factor-alpha gene, 5' end Product length – 713					

FP	20	GATCGGTCCCAACAAGGAGG	60	60	
RP	20	CAAAGGCGGAGATGAGACCC	60	60.4	
Interferon – gamma Accession No. AH002184.2 Rattus norvegicus interferon-gamma (IFN-G) gene, complete cds Product length – 738					
FP	20	CATGAGCATCGCCAAGTTCG	55	59.7	
RP	25	AGAAAGAATGATTGGTCAAAGAGGA	36	58.4	

A gradient PCR was performed to standardize the optimum annealing temperature of the designed primer using 50 ng of synthesized cDNA keeping the temperature range of 50 – 60^{o}C and found the above mentioned annealing temperatures as optimum.

Semi quantitative Polymerase Chain Reaction

The PCR was done by optimizing the concentration of cDNA to 50ng across the sample. Each PCR reaction consisted of 1X reaction buffer (with 1.5mM $MgCl_2$), *Taq* DNA polymerase (1Unit), dNTP mix (2.5mM) 10pico moles of respective forward and reverse primers, 50ng of cDNA and the volume was made up to 20µL with PCR grade water. The β-Actin was used as a housekeeping gene for normalization.

The PCR cycle started with an initial denaturation of 94^{o}C for 5 min followed by 35 cycles of 94^{o}C for 45 sec, respective annealing temperature for 30 sec and 72^{o}C for 60 sec. Post 35 cycles a final elongation at 72^{o}C for 10 min was given to complete the reaction and was kept for holding at 4^{o}C until removed.

Agarose Gel Electrophoresis

To visualize the gene expression levels in terms of the band intensity, the PCR product was analyzed in 1.5% Agarose gel casted adding ethidium bromide as a staining agent. The PCR product was added to the wells in gel by mixing with 6x gel loading dye and 50 volts was applied until the dye front has reached more than half of the gel. The

bands were illuminated under UV light and the same was captured in a Gel documentation Unit.

The captured image was analyzed and the intensity of the bands was calculated using the GelAnalyzer software.

Gel imaging and semi quantitative analysis

The Agarose gel after electrophoresis was transferred to a Gel Documentation unit (BioBee, India) and the image was captured under UV light. The band intensity was measured using the software GelAnalyzer2010a.

Quantification:

The calculations are as follows

Step 1.

Normalizing GAPDH= GAPDH Sample - GAPDH Control

Step 2. Normalizing Samples=(Sample value - Normalized GAPDH value) - Control value

Step 3. Fold Change=(Normalized value/Control value)x100;

6. PATIENTS AND CLINICAL METHODS

6.1 Clinical study:

6.1.1 Aim

To evaluate the therapeutic efficacy of *Nandhi Mezhugu* (NM) for Uthiravatha Suronitham(Rheumatoid Arthritis) at different point of time (15^{th} ,29^{th} , 43^{th} , 60^{th} day) by observing the clinical findings.

6.1.2 Secondary objective:

To study the clinical laboratory parameters related to RA (RA Factor, Anti CCP, CRP, ESR, etc.,)

6.1.3 Study Design:

Phase II:

An open clinical study with coded Siddha Herbo Mineral Sasthric formulation NM was conducted in 40 *Uthira Vatha Suronitham* (Rheumatoid Arhritis) patients.

Dosage of NM: 500 mg with Palm Jaggery twice a day after food.

Duration of drug administration: 60 days (7 days medicine followed by 7 days drug holiday).

6.1.4 Study period:

Main study-2 years

Method of drug administration:

The medicine was given by the following fashion – 7 days medicine –followed by a break of 7 days again 7 days medicine followed by a break of 7 days likewise the medicine was repeated for 60 days.

Rationale behind *Marupathiyam* (i.e re-dieting): NM being a mercurial preparation re-dieting must be observed during administration of these drugs.

Dietary Regimen:

The patients were instructed not to take non-vegetarian diet and also reduce tamarind and salt (salt used must be roasted before consumption)as per siddha text.

6.1.5 Study place:

OPD & IPD of Ayothidoss Pandithar Hospital,

National Institute of Siddha,

Tambaram Sanitorium, Chennai-47.

6.1.6 Population and Sample:

18-60 years age group fulfilling all the inclusion criteria and passing the exclusion criteria mentioned below.

The sample consists of patients attending the OPD & IPD of Ayothidoss Pandithar Hospital, National Institute of Siddha.

Sample Size:

Phase II- 40 *Uthira Vathasuronitham* (Rheumatoid arthritis) patients

6.1.7 SELECTION CRITERIA:

Inclusion Criteria:

Age : 18-60 years

Sex : Both Male and Female

Pain and swelling in smaller and larger joints.

Symmetrical joints involvement.

More than 3 joints involvement.

Fever

Morning stiffness.

Serum positive Anti CCP

Exclusion Criteria:

Diabetes Mellitus

Hypo & Hyperthyroidism.

Hypertension.

Cardiac diseases.

Renal diseases.

Liver disorders

Neurological disorder

Pregnancy and lactation

Alcoholism

Recent treatment with steroids

Withdrawal criteria:

Not coming for regular follow up/check up

Intolerance to the drug, and development of adverse reaction during the drug trial.

Severe abdominal pain.

Any other acute illness.

Poor patient compliance and defaulters

Patients who were unwilling to continue in the course of clinical study.

Any other unforeseen circumstances rendering the patients unable to continue with the study.

6.1.8 Specification of tests:

- Anti-CCP
- Acute phase protein: C-reactive protein
- Rheumatoid factor
- Pain score (The National Initiative on Pain Control™ (NIPC™),
- Restricted movement assessment scale (BearingPoint, Atos Healthcare & DSP Copyright EBM Rheumatoid Arthritis Version 5.0 final)
- (Modified Health Assessment Questionnaire- MHAQ, Disability index) Source: Pincus T, Yazici Y, Bergman M. Development of a modified health assessment questionnaire (MHAQ) for the infrastructure of standard clinical care. Clin Exp Rheumatol. 2005;23(Suppl 39):S19-S28.

Routine Laboratory investigation:

Blood

- Hb gms%
- TC cells/cumm
- DC –P% L% E% M% B%
- ESR at 30 minutes –mm at 60 minutes-mm
- Blood sugar- Fasting mgs%
- Blood sugar- PP mgs%
- Serum cholesterol mgs%
- HDL mgs%
- LDL mgs%
- Triglycerides mgs%
- LFT, RFT

- Urine Examination
- Sugar
- Fasting
- PP
- Albumin
- Deposits
- X-Ray –Joints (if needed)

6.2 Siddha Diagnostic tool:

Patients were screened by siddha diagnostic method that is *Envagai thervu* ,was carried out in all the enrolled patients .Siddha clinical assessment was also carried out in all the enrolled cases.

6.3 Outcome:

Primary outcome:

Reduction of pain and swelling of the joints (Universal pain assessment scale-The National Initiative on Pain Control™ (NIPC™),[96]

Improvement in the movement of the affected joints [97](Restricted movement assessment scale, ref -BearingPoint, Atos Healthcare & DSP Copyright EBM Rheumatoid Arthritis Version 5.0 final)

Reduction of disability(Ref: Modified Health Assessment Questionnaire- MHAQ, Disability index) [98]

Secondary outcome:

Reduction of other clinical symptoms

Reduction of ESR and CRP

Reduction in Anti-CCP and RA Factor

7. OBSERVATION AND RESULTS

7.1. Organoleptic properties of study drug Nandhi Mezhugu

Colour: Dark brown colour; **Odour:** Resinous odour; **Taste:** Metallic taste; **Consistency**: Semisolid.

7.2. Physico-chemical analysis of Nandi mezhugu:

Table No:7.2.1. Physico-chemical analysis of Nandi mezhugu

S.No	Parameter	Mean
1.	Loss on drying at 105° C	19.156 %
2.	Total Ash	6.607 %
3.	Water soluble ash	2.95 %
4.	Acid-insoluble ash	0.93 %
5.	Water soluble extractive	39.056 %
6.	pH	3.35
7.	Rancidity	Nil
8.	Acid value	10.592
9.	Saponification value	262.62
10.	Iodine value	16.864
11.	Fat content	20.683 %
12.	Reducing Sugar	3.69 %
13.	Total Sugar	7.54 %
14	Alcohol Soluble Extractive	23.558%

7.3. PRELIMINARY PHYTOCHEMICAL ANALYSIS OF NANDHI MEZHUGU

Table No: 7.3.1.Preliminary phytochemical analysis of Nandhi mezhugu

Sl. No	Phytochemicals	Inference
1.	Steroid	**Present**
2.	Triterpene	**Present**
3.	Flavonoids	**Present**
4.	Alkaloids	**Present**
5.	Phenol	**Present**
6.	Tannin	**Present**
7.	Saponin	**Present**
8.	Coumarin	**Present**
9.	Glycoside	**Present**
10.	Quinone	**Present**

Result of Qualitative Inorganic Analysis

The qualitative inorganic analysis of the drug revealed the presence of mercury, magnesium, aluminium, calcium, sodium, potassium, copper, zinc, iron, cobalt, chloride, carbonate, nitrate, sulphate, sulphide, arsenate, acetate, silicate which are all biologically important radicles.

7.4. QUALITATIVE INORGANIC ANALYSIS OF NANDI MEZHUGU

Table No: 7.4.1. Qualitative inorganic analysis of Nandhi mezhugu

S.No	Cation/Anion	Inference
1	Mercury	**Present**
2	Magnesium	**Present**
3	Aluminium	**Present**
4	Calcium	**Present**
5	Sodium	**Present**
6	Potassium	**Present**
7	Copper	**Present**
8	Zinc	**Present**
9	Iron	**Present**
10	Cobalt	**Present**
11	Chloride	**Present**
12	Carbonate	**Present**
13	Nitrate	**Present**
14	Sulphate	**Present**
15	Sulphide	**Present**
16	Arsenate	**Present**
17	Acetate	**Present**
18	Silicate	**Present**

7.5. Heavy metal analysis

Table No: 7.5.1. Heavy metals present in Nandhi mezhugu

Heavy metal	Quantity (in ppm)
Lead (as Pb)	2.95
Arsenic (as As)	7233.42
Cadmium (as Cd)	0.01
Mercury (as Hg)	9336.61

7.6. Microbial contamination

In the microbial study, the drug was found free from *E. coli*, *Salmonella* spp., *Staphlococcus aureus* and *Enterobacteriacea*.

Table No: 7.6.1.Microbial contamination results of Nandhi mezhugu

S. No	Parameter	Detection of Microbes	WHO Limit (CFU/g)
1.	*E. coli*	Absent	10
2.	*Salmonella* spp.	Absent	None
3.	*Staphylococcus aureus*	Absent	Absent
4.	*Enterobacteriacea*	Absent	10^3
5.	Total Bacterial count	$2x10^3$CFU/gm	10^5
6.	Total Fungal count	Less than 10 CFU/gram	10^3

7.7. Pesticide residue:

Table No: 7.7.1. Pesticide residue

Pesticide residue	Level in mg/kg
alphaBHC	BLQ (LOQ : 0.01)
BetaBHC	BLQ (LOQ: 0.01)
gam BHC (Lindane)	BLQ (LOQ : 0.01)
DeltaBHC	BLQ (LOQ : 0.01)
Aldrin	BLQ (LOQ : 0.01)
Dieldrin	BLQ (LOQ : 0.01)
trans Chlordane	BLQ (LOQ : 0.01)
cis Chlordane	BLQ (LOQ : 0.01)
Endrin	BLQ (LOQ : 0.01)
Endrinaldehyde	BLQ (LOQ : 0.01)
Endrinketone	BLQ (LOQ : 0.01)
Endosulfan-I	BLQ (LOQ : 0.01)
Endosulfan-II	BLQ (LOQ : 0.01)
Endosulfansulfate	BLQ (LOQ : 0.01)
Heptachlor	BLQ (LOQ : 0.01)
Heptachlorepoxide	BLQ (LOQ : 0.01)
Dicofol	BLQ (LOQ : 0.01)
Chlorthalonil	BLQ (LOQ : 0.01)
Hexachlorobenzene	BLQ (LOQ : 0.01)
o,p"DDT	BLQ (LOQ : 0.01)

P,P"DDT	BLQ (LOQ : 0.01)
o,p"DDD	BLQ (LOQ : 0.01)
p,p"DDD	BLQ (LOQ : 0.01)
o,p"DDE	BLQ (LOQ : 0.01)
P,P"DDE	BLQ (LOQ : 0.01)
4-Bromo,2-Chlorophenol	BLQ (LOQ : 0.01)
Acephate	BLQ (LOQ : 0.01)
Chlorfenvinphos	BLQ (LOQ : 0.01)
Chlorpyrifos	BLQ (LOQ : 0.01)
Chlorpyrifos methyl	BLQ (LOQ : 0.01)
Diazinon	BLQ (LOQ : 0.01)
Dichlorvos	BLQ (LOQ : 0.01)
Dimethoate	BLQ (LOQ : 0.01)
Ethion	BLQ (LOQ : 0.01)
Etrimfos	BLQ (LOQ : 0.01)
Fenitrothion	BLQ (LOQ : 0.01)
Iprobenphos	BLQ (LOQ : 0.01)
Malathion	BLQ (LOQ : 0.01)
Methamidophos	BLQ (LOQ : 0.01)
Monocrotophos	BLQ (LOQ : 0.01)
Omethoate	BLQ (LOQ : 0.01)
Oxydemeton-methyl	BLQ (LOQ : 0.01)
Parathion ethyl	BLQ (LOQ : 0.01)
Parathion methyl	BLQ (LOQ : 0.01)

Phorate	BLQ (LOQ : 0.01)
Phosalone	BLQ (LOQ : 0.01)
Phosphamidon	BLQ (LOQ : 0.01)
Profenophos	BLQ (LOQ : 0.01)
Quinalphos	BLQ (LOQ : 0.01)
Triazophos	BLQ (LOQ : 0.01)
Phorate sulphone	BLQ (LOQ : 0.01)
Phorate sulphoxide	BLQ (LOQ : 0.01)

BLQ: Below limit of Quantification /LOQ- Limit of quantification

7.8. Quantitative Assays results of Nandhi mezhugu

Table No:7.8.1. Assays results of Nandhi mezhugu

Inorganic Compounds	Value in mg/kg
Calcium (as Ca)	3165.72
Magnesium(as Mg)	358.72
Potassium (as K)	1950.25
Aluminum (as Al)	4943.37
Copper (as Cu)	501.35
Iron (as Fe)	497.30
Zinc (as Zn)	26.290
Sulphur (as SO_2)	BLQ (LOQ : 10.0)
Chloride (as NaCl)	0.03

BLQ: Below limit of Quantification /LOQ- Limit of quantification

7.9. Test for Aflatoxins (B1,B2,G1,G2):

All the four aflatoxin were not detected in the drug.

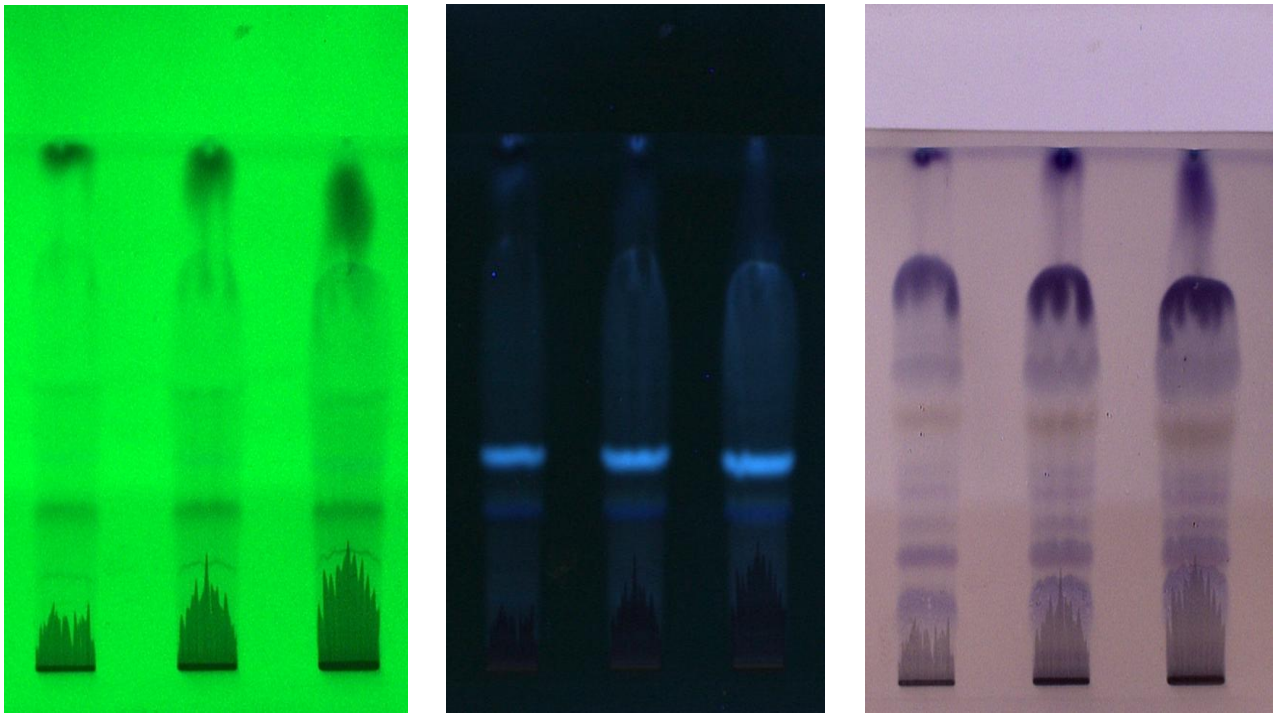
Table No:7.9.1. Test for Aflatoxins (B1,B2,G1,G2):

Test	Observed Result
Aflatoxins B1	BLQ(LOQ:0.5) µg/kg
Aflatoxins B2	BLQ(LOQ:0.5) µg/kg
Aflatoxins G1	BLQ(LOQ:0.5) µg/kg
Aflatoxins G2	BLQ(LOQ:0.5) µg/kg

BLQ: Below limit of Quantification /LOQ- Limit of quantification

7.10. Results and Discussion of TLC and HPTLC

The TLC photodocumentation of hexane extract of Nandi mezhugu under UV 254 nm showed 5 visible spots at R_f value 0.25, 0.30, 0.38, 0.50 and 0.71 (all green); under UV 366 nm showed three visible spots at R_f value 0.30 (blue), 0.38 (fluorescent blue) and 0.71 (pale blue). After derivatization with vanillin-sulphuric acid, showed 8 spots at 0.20, 0.25, 0.30, 0.35 (all purple), 0.38 (brown), 0.46, 0.57 and 0.71 (all purple).



UV 254 nm | UV 366 nm | White light after dipping in vanillin-sulphuric acid

Figure 7.10.1.TLC photodocumentation of hexane extract of Nandi mezhugu
Solvent system - Toluene : Ethyl acetate (10:0.5, v/v)

Table No: 7.10.1.TLC spots of hexane extract of Nandhi mezhugu

Under UV 254 nm		Under UV 366 nm		White light after derivatization	
R_f	Colour	R_f	Colour	R_f	Colour
-	All green	-	-	0.20	Purple
0.25		-	-	0.25	Purple
0.30		0.30	Blue	0.30	Purple
0.38		-	-	0.35	Purple
-		0.38	Fluorescent blue	0.38	Brown
0.50		-	-	0.46	Purple
-		-	-	0.57	Purple
0.71		0.71	Pale blue	0.71	Purple

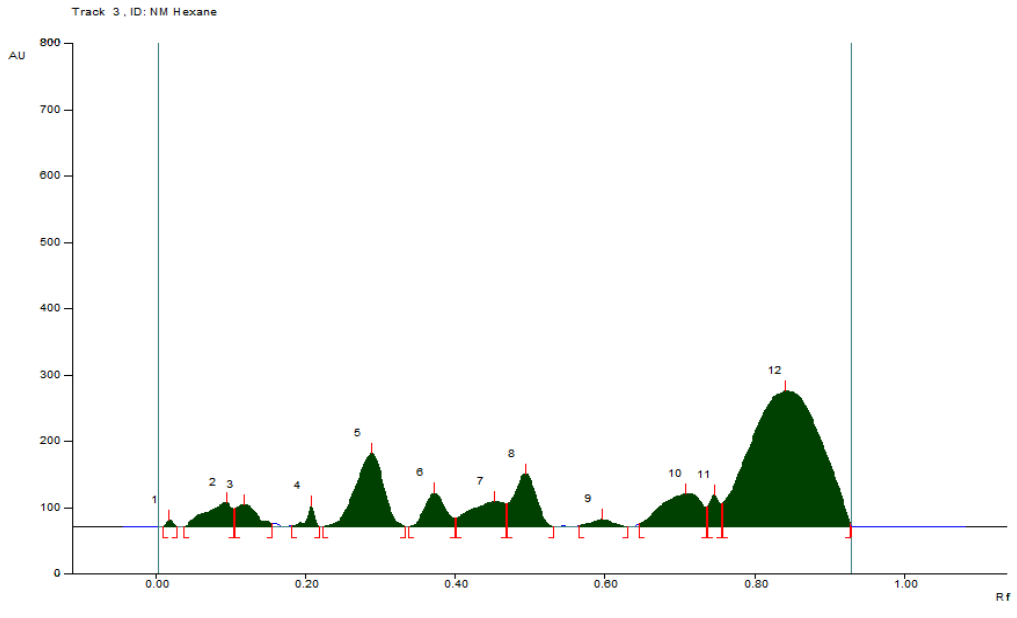


Figure: 7.10.2.HPTLC finger print profile of hexane extract of Nandi mezhugu at 254 nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	0.6 AU	0.02 Rf	10.4 AU	1.48 %	0.03 Rf	0.4 AU	76.3 AU	0.26 %
2	0.04 Rf	0.1 AU	0.10 Rf	36.7 AU	5.20 %	0.11 Rf	27.3 AU	1071.6 AU	3.65 %
3	0.11 Rf	27.7 AU	0.12 Rf	33.8 AU	4.78 %	0.16 Rf	5.4 AU	781.4 AU	2.66 %
4	0.18 Rf	1.7 AU	0.21 Rf	30.7 AU	4.34 %	0.22 Rf	0.1 AU	263.5 AU	0.90 %
5	0.22 Rf	0.3 AU	0.29 Rf	111.4 AU	15.77 %	0.33 Rf	0.2 AU	3421.0 AU	11.66 %
6	0.34 Rf	0.4 AU	0.37 Rf	50.5 AU	7.15 %	0.40 Rf	12.8 AU	1146.2 AU	3.90 %
7	0.40 Rf	13.0 AU	0.45 Rf	38.7 AU	5.48 %	0.47 Rf	35.1 AU	1443.4 AU	4.92 %
8	0.47 Rf	35.2 AU	0.50 Rf	80.1 AU	11.34 %	0.53 Rf	0.4 AU	1964.0 AU	6.69 %
9	0.57 Rf	1.3 AU	0.60 Rf	10.8 AU	1.52 %	0.63 Rf	0.1 AU	264.7 AU	0.90 %
10	0.65 Rf	4.2 AU	0.71 Rf	50.4 AU	7.13 %	0.74 Rf	29.5 AU	2243.2 AU	7.64 %
11	0.74 Rf	30.4 AU	0.75 Rf	48.5 AU	6.87 %	0.76 Rf	34.3 AU	562.2 AU	1.92 %
12	0.76 Rf	34.9 AU	0.84 Rf	204.6 AU	28.95 %	0.93 Rf	4.7 AU	16115.0 AU	54.90 %

Figure.7.10.3.R_f value of peaks with percentage peak area of HPTLC finger print profile of hexane extract of Nandi mezhugu at 254 nm

The HPTLC finger print profile of hexane extract at UV 254 nm showed 12 peaks in which the peak at R_f 0.84 was the major peak with an area of 54.90 % followed by a peak at R_f 0.29 with an area of 11.66 %. All other peaks are minor with an individual area less than 10 %.

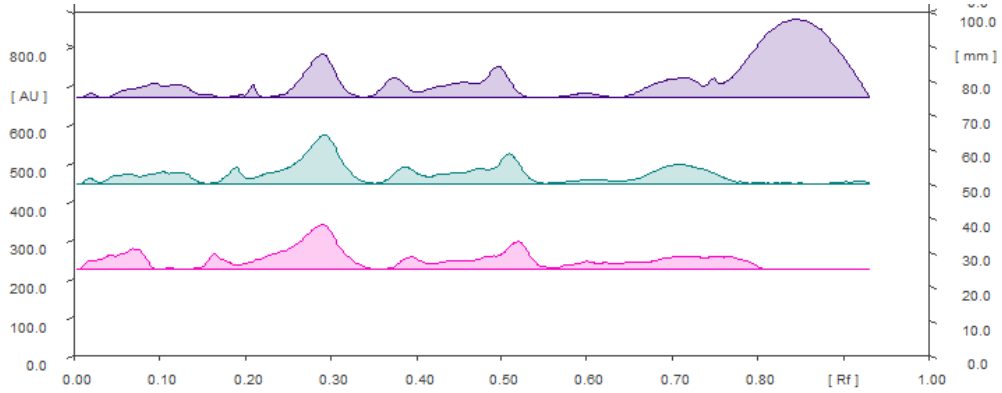


Figure.7.10.4.3D chromatogram of hexane extract of Nandi mezhugu at 254 nm

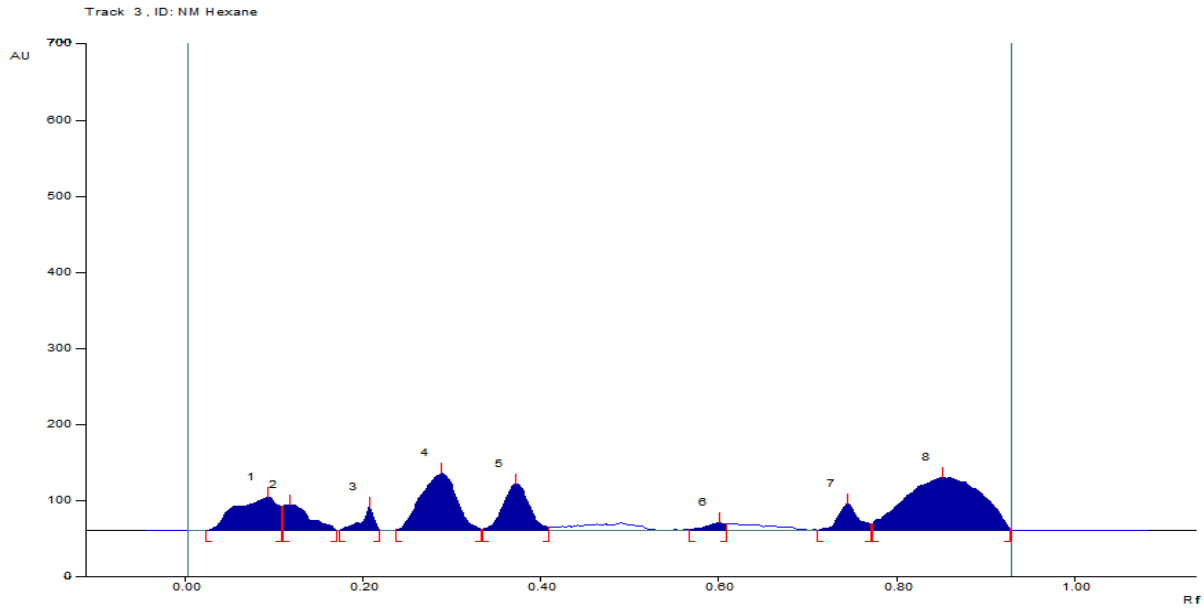


Figure.7.10.5. HPTLC finger print profile of hexane extract of Nandi mezhugu at 366 nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.03 Rf	0.3 AU	0.09 Rf	43.8 AU	12.15 %	0.11 Rf	31.7 AU	1707.3 AU	13.40 %
2	0.11 Rf	31.8 AU	0.12 Rf	34.0 AU	9.43 %	0.17 Rf	0.4 AU	859.7 AU	6.75 %
3	0.17 Rf	0.3 AU	0.21 Rf	31.1 AU	8.62 %	0.22 Rf	0.1 AU	358.4 AU	2.81 %
4	0.24 Rf	0.7 AU	0.29 Rf	75.1 AU	20.84 %	0.33 Rf	1.9 AU	2476.5 AU	19.43 %
5	0.34 Rf	2.0 AU	0.37 Rf	61.3 AU	17.01 %	0.41 Rf	4.3 AU	1477.3 AU	11.59 %
6	0.57 Rf	0.8 AU	0.60 Rf	10.3 AU	2.87 %	0.61 Rf	8.6 AU	177.1 AU	1.39 %
7	0.71 Rf	0.5 AU	0.75 Rf	35.1 AU	9.74 %	0.77 Rf	8.5 AU	644.6 AU	5.06 %
8	0.77 Rf	8.9 AU	0.85 Rf	69.7 AU	19.34 %	0.93 Rf	2.4 AU	5043.3 AU	39.57 %

Figure.7.10.6.R_f value of peaks with percentage peak area of HPTLC finger print profile of hexane extract of Nandi mezhugu at 366 nm

The HPTLC finger print profile of hexane extract at UV 366 nm showed 8 peaks in which the peak at R_f 0.85 was the major peak with an area of 39.57 % followed by a peak at R_f 0.29 (19.43 %), 0.09 (13.40 %) and 0.37 (11.59 %). All other peaks are minor with an individual area less than 10 %.

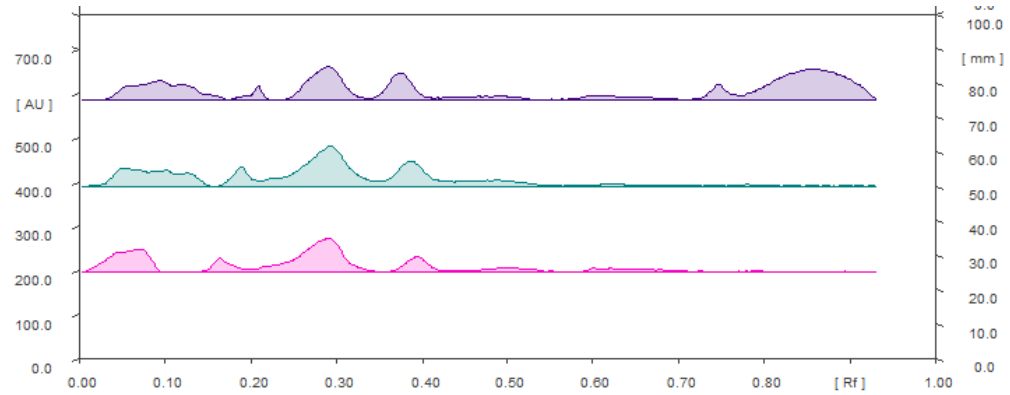


Figure.7.10.7.3D chromatogram of hexane extract of Nandi mezhugu at 366 nm

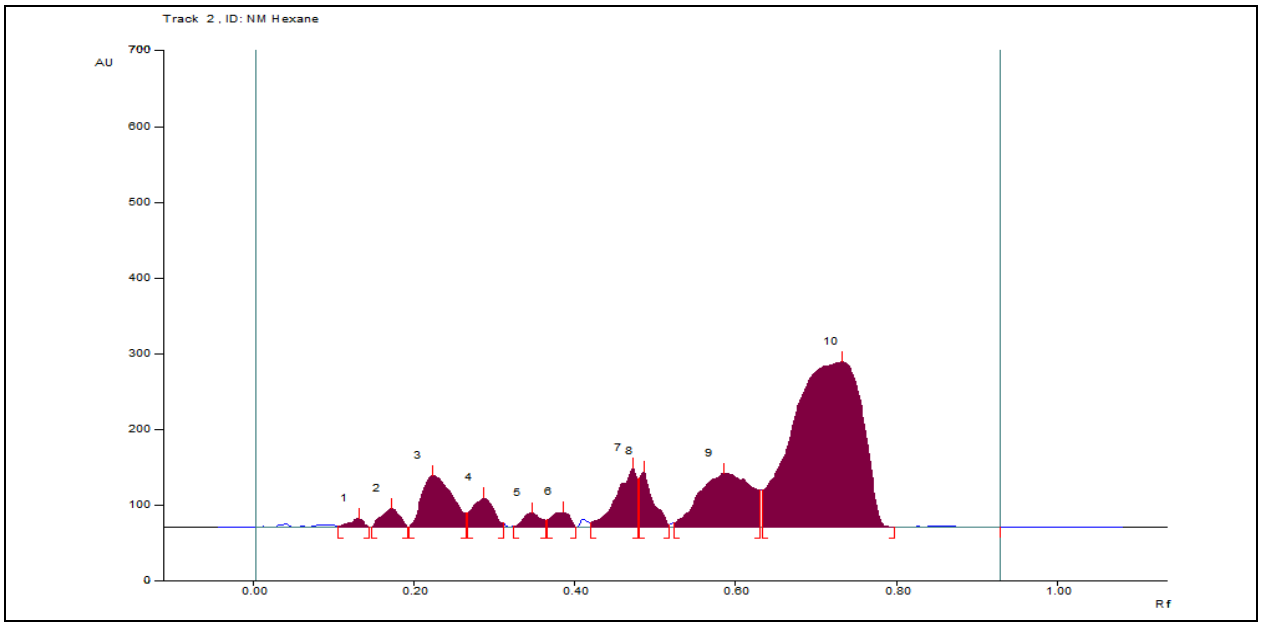


Figure.6.10.8.HPTLC finger print profile of hexane extract of Nandi mezhugu at 575 nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.11 Rf	0.9 AU	0.13 Rf	11.1 AU	1.79 %	0.15 Rf	0.5 AU	166.2 AU	0.63 %
2	0.15 Rf	0.0 AU	0.17 Rf	25.1 AU	4.03 %	0.19 Rf	0.5 AU	466.4 AU	1.76 %
3	0.20 Rf	0.5 AU	0.22 Rf	68.0 AU	10.94 %	0.27 Rf	18.9 AU	2126.5 AU	8.02 %
4	0.27 Rf	19.2 AU	0.29 Rf	38.6 AU	6.21 %	0.31 Rf	5.8 AU	844.5 AU	3.18 %
5	0.32 Rf	1.3 AU	0.35 Rf	19.0 AU	3.05 %	0.37 Rf	9.4 AU	342.0 AU	1.29 %
6	0.37 Rf	9.4 AU	0.39 Rf	19.6 AU	3.14 %	0.40 Rf	0.3 AU	388.5 AU	1.47 %
7	0.42 Rf	5.9 AU	0.47 Rf	77.9 AU	12.52 %	0.48 Rf	63.7 AU	1656.2 AU	6.25 %
8	0.48 Rf	64.4 AU	0.49 Rf	73.5 AU	11.81 %	0.52 Rf	3.3 AU	1030.4 AU	3.89 %
9	0.52 Rf	5.8 AU	0.59 Rf	71.3 AU	11.46 %	0.63 Rf	49.2 AU	3806.7 AU	14.35 %
10	0.63 Rf	49.4 AU	0.73 Rf	218.1 AU	35.05 %	0.80 Rf	0.0 AU	15692.3 AU	59.17 %

Figure.7.10.9.R_f value of peaks with percentage peak area of HPTLC finger print profile of hexane extract of Nandi mezhugu at 575 nm

The HPTLC finger print profile of hexane extract at 575 nm showed 10 peaks in which the peak at R_f 0.73 was the major peak with an area of 59.17 % followed by a peak at R_f 0.59 (14.35 %), 0.22 (8.02 %) and 0.47 (6.25 %). All other peaks are minor with an individual area less than 10 %.

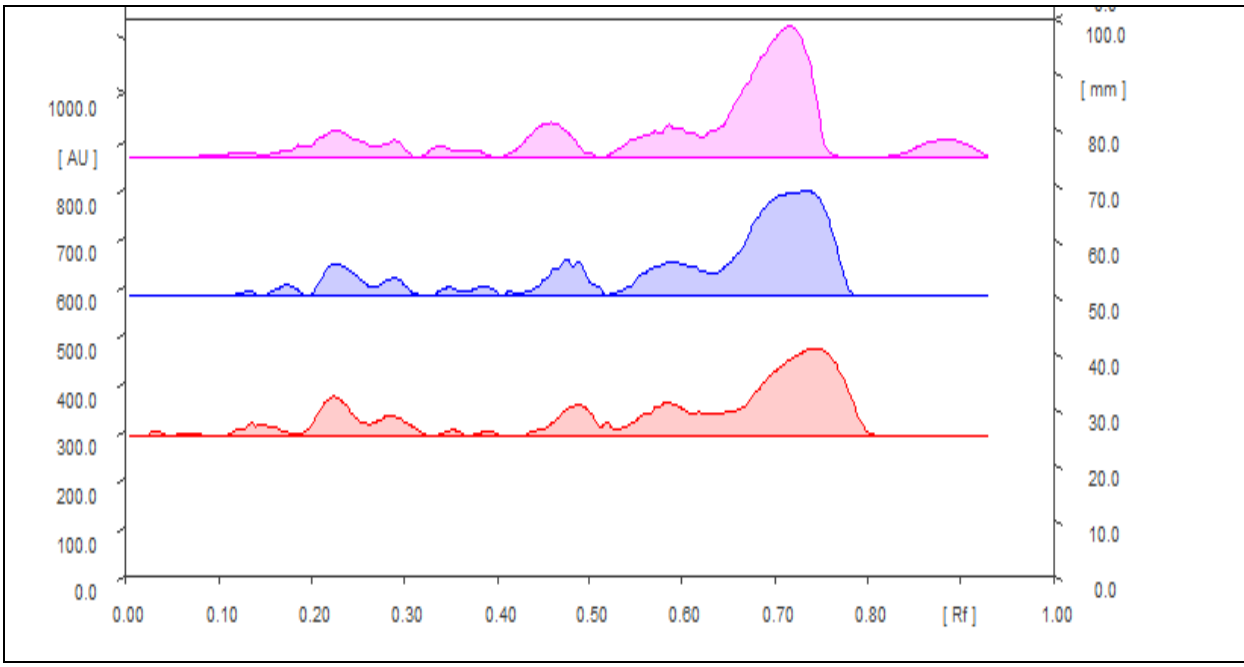
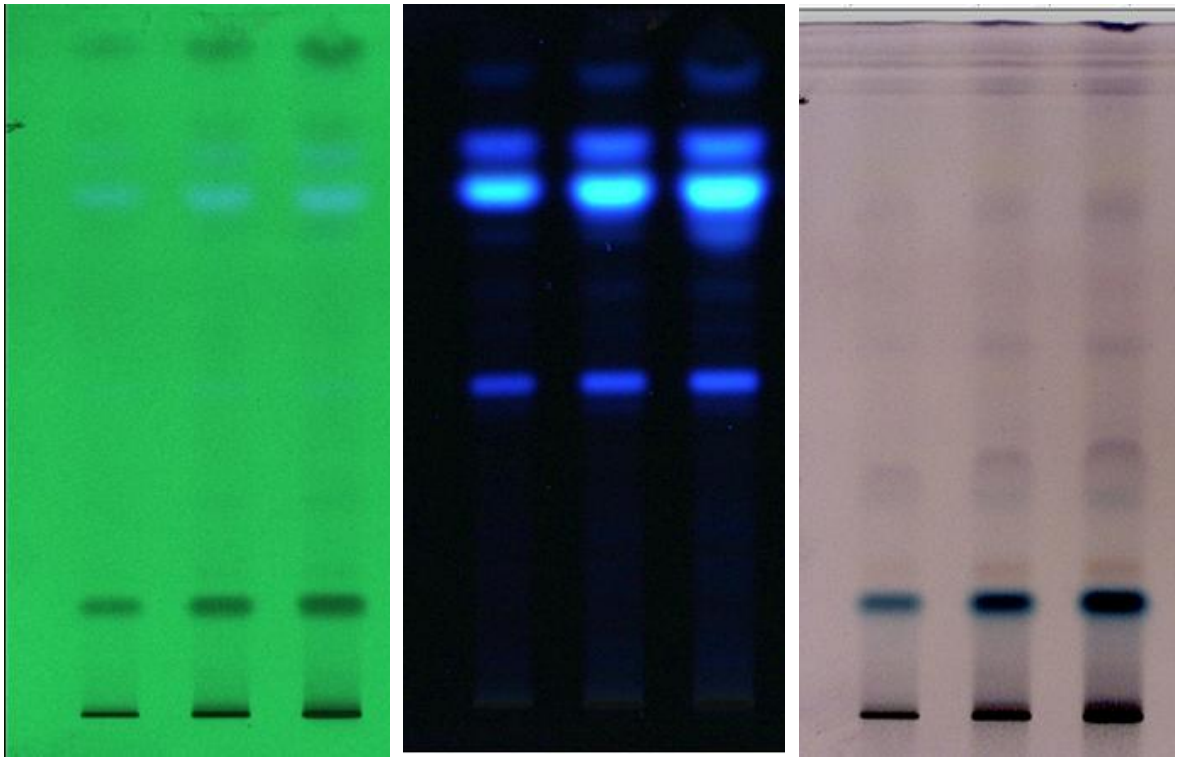


Figure.7.10.10.3D chromatogram of hexane extract of Nandi mezhugu at 575 nm



UV 254 nm | UV 366 nm | White light after dipping in vanillin-sulphuric acid

Figure.7.10.11.TLC photodocumentation of chloroform extract of Nandi mezhugu Solvent system - Toluene : Ethyl acetate (5:1.5, v/v)

Table .7.10.2.TLC spot of chloroform extract of Nandi mezhugu

Under UV 254 nm		**Under UV 366 nm**		**White light after derivatization**	
R_f	Colour	R_f	Colour	R_f	Colour
0.16	Green	-	-	0.17	Peacock blue
-	-	0.47	Fluorescent blue	0.22	Brown
-	-	-	-	0.36	Purple
-	-	-	-	0.53	Purple
0.71	Green	0.70	Fluorescent blue	-	-
0.75	Pale blue	0.76	Fluorescent blue	0.74	Purple
0.82	Pale blue	0.83	Fluorescent blue	-	-
0.85	Green	0.94	Fluorescent blue	-	-
-	-	-	-	0.91	Purple

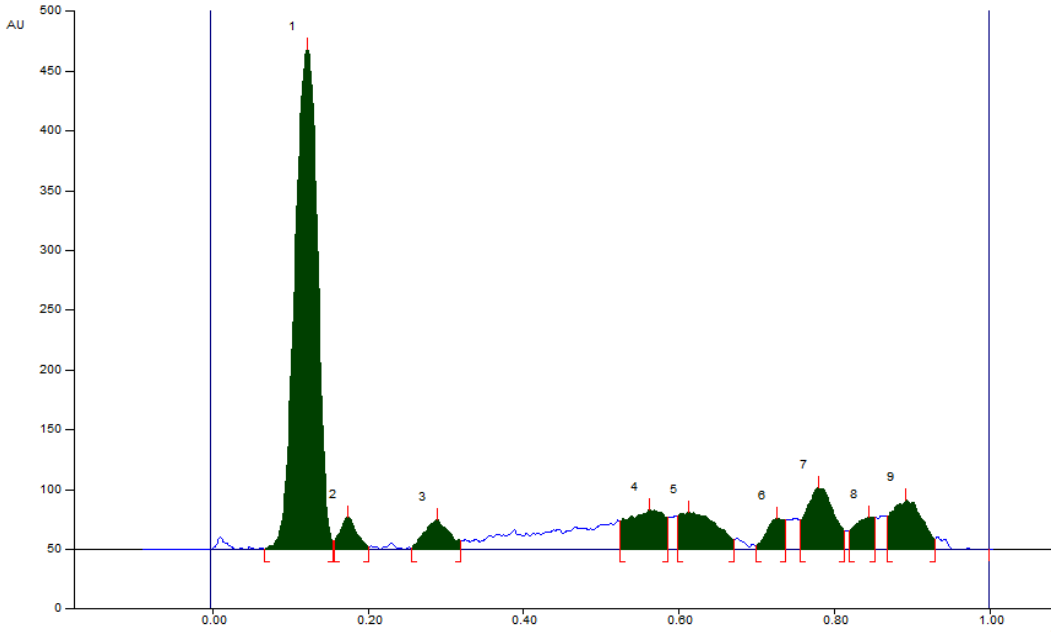


Figure.7.10.12.HPTLC finger print profile of chloroform extract of Nandi mezhugu at UV 254 nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.07 Rf	0.1 AU	0.12 Rf	418.2 AU	61.39 %	0.16 Rf	7.3 AU	11009.5 AU	56.90 %
2	0.16 Rf	7.4 AU	0.17 Rf	27.1 AU	3.98 %	0.20 Rf	2.4 AU	526.0 AU	2.72 %
3	0.26 Rf	1.4 AU	0.29 Rf	25.2 AU	3.71 %	0.32 Rf	7.5 AU	712.3 AU	3.68 %
4	0.52 Rf	23.8 AU	0.56 Rf	33.2 AU	4.88 %	0.59 Rf	26.5 AU	1408.8 AU	7.28 %
5	0.60 Rf	28.3 AU	0.61 Rf	31.0 AU	4.56 %	0.67 Rf	8.4 AU	1368.4 AU	7.07 %
6	0.70 Rf	2.8 AU	0.73 Rf	25.6 AU	3.76 %	0.74 Rf	24.3 AU	537.4 AU	2.78 %
7	0.76 Rf	24.7 AU	0.78 Rf	51.8 AU	7.60 %	0.81 Rf	15.6 AU	1664.9 AU	8.60 %
8	0.82 Rf	15.2 AU	0.85 Rf	27.2 AU	4.00 %	0.85 Rf	26.9 AU	649.3 AU	3.36 %
9	0.87 Rf	28.0 AU	0.89 Rf	41.7 AU	6.13 %	0.93 Rf	8.6 AU	1473.0 AU	7.61 %

Figure.7.10.13.R_f value of peaks with percentage peak area of HPTLC finger print profile of chloroform extract of Nandi mezhugu at UV 254 nm

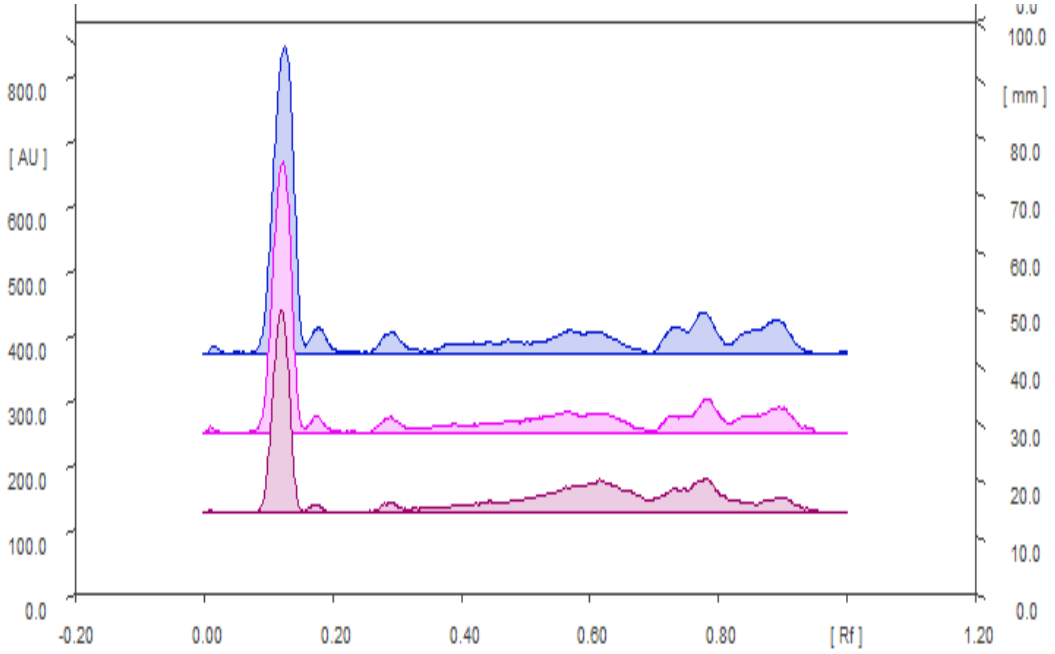


Figure.7.10.14.3D chromatogram of chloroform extract of Nandi mezhugu at UV 254 nm

The HPTLC finger print profile of chloroform extract at UV 254 nm showed 9 peaks in which the peak at R_f 0.12 was the major peak with an area of 56.90 %. All other peaks are minor appearing at R_f 0.17, 0.29, 0.56, 0.61, 0.73, 0.78, 0.85 and 0.89 with an individual area less than 10 %.

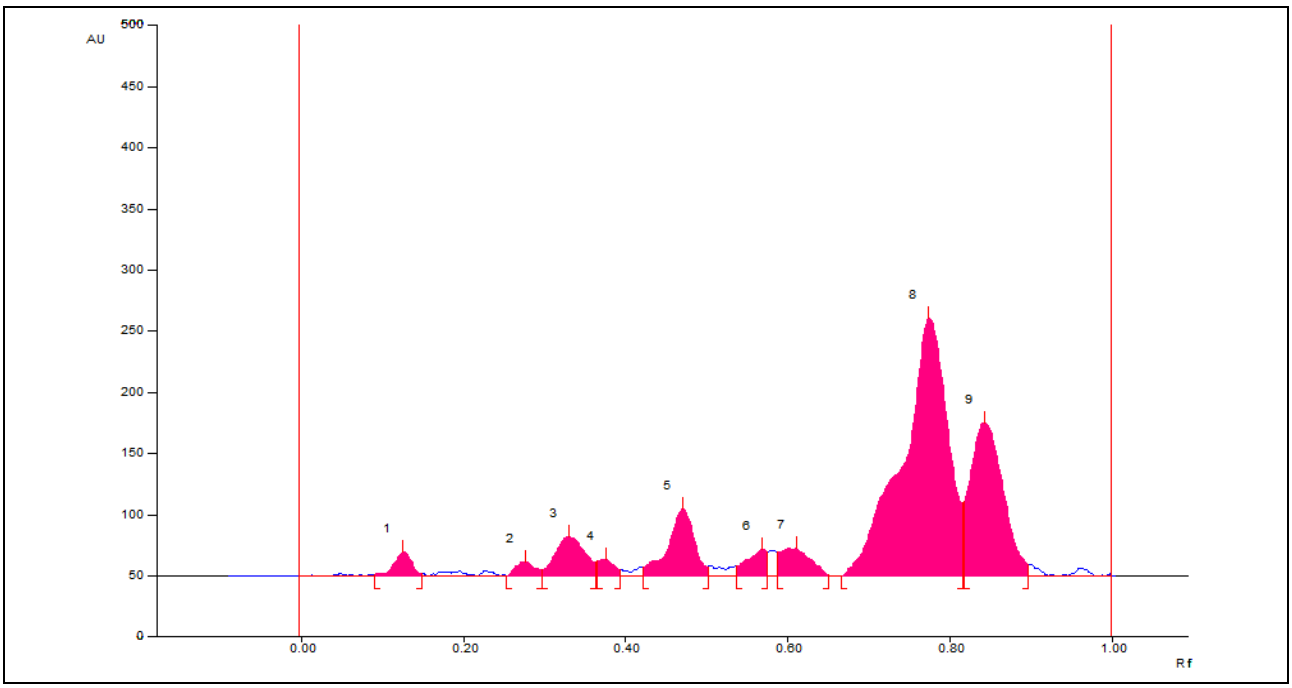


Figure.7.10.15.HPTLC finger print profile of chloroform extract of Nandi mezhugu at UV 366 nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.09 Rf	1.5 AU	0.13 Rf	19.8 AU	3.85 %	0.15 Rf	1.6 AU	394.7 AU	1.96 %
2	0.25 Rf	0.1 AU	0.28 Rf	11.7 AU	2.29 %	0.30 Rf	5.4 AU	250.5 AU	1.24 %
3	0.30 Rf	5.3 AU	0.33 Rf	32.2 AU	6.28 %	0.36 Rf	11.4 AU	1063.9 AU	5.28 %
4	0.37 Rf	12.0 AU	0.38 Rf	13.8 AU	2.69 %	0.39 Rf	4.9 AU	245.3 AU	1.22 %
5	0.42 Rf	6.7 AU	0.47 Rf	55.2 AU	10.74 %	0.50 Rf	7.5 AU	1602.7 AU	7.96 %
6	0.54 Rf	7.9 AU	0.57 Rf	22.0 AU	4.29 %	0.58 Rf	19.4 AU	483.0 AU	2.40 %
7	0.59 Rf	19.2 AU	0.61 Rf	22.6 AU	4.41 %	0.65 Rf	0.7 AU	762.0 AU	3.78 %
8	0.67 Rf	0.2 AU	0.77 Rf	210.6 AU	41.01 %	0.82 Rf	60.0 AU	10702.1 AU	53.15 %
9	0.82 Rf	60.6 AU	0.84 Rf	125.5 AU	24.43 %	0.90 Rf	9.6 AU	4630.5 AU	23.00 %

Figure.7.10.16.R_f value of peaks with percentage peak area of HPTLC finger print profile of chloroform extract of Nandi mezhugu at UV 366 nm

The HPTLC finger print profile of chloroform extract at UV 366 nm showed 9 peaks in which the peak at R_f 0.77 was the major peak with an area of 53.15 % followed by a peak at R_f 0.84 (23.00 %). Other peaks appeared at R_f 0.13, 0.28, 0.33, 0.38, 0.47, 0.57 and 0.61 with an individual area contribution of less than 10 %.

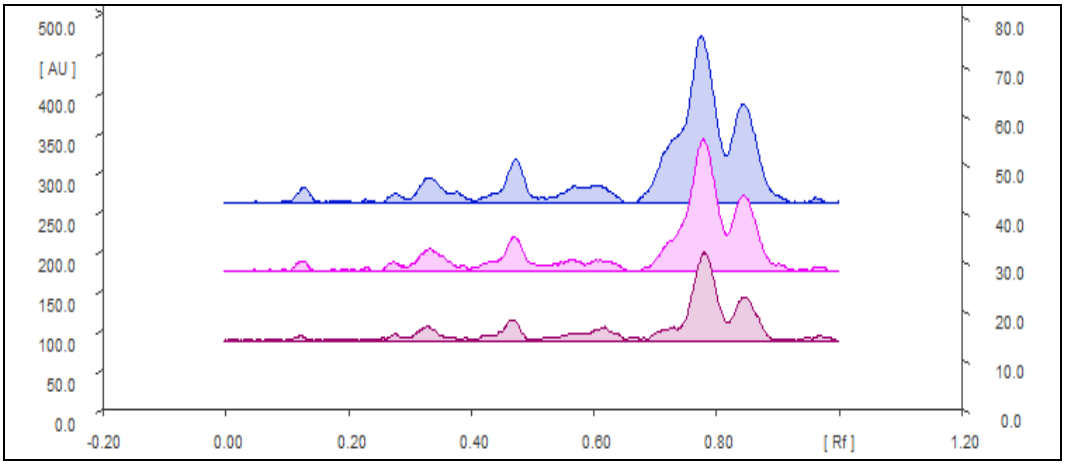


Figure.7.10.17.3D chromatogram of chloroform extract of Nandi mezhugu at UV 366 nm

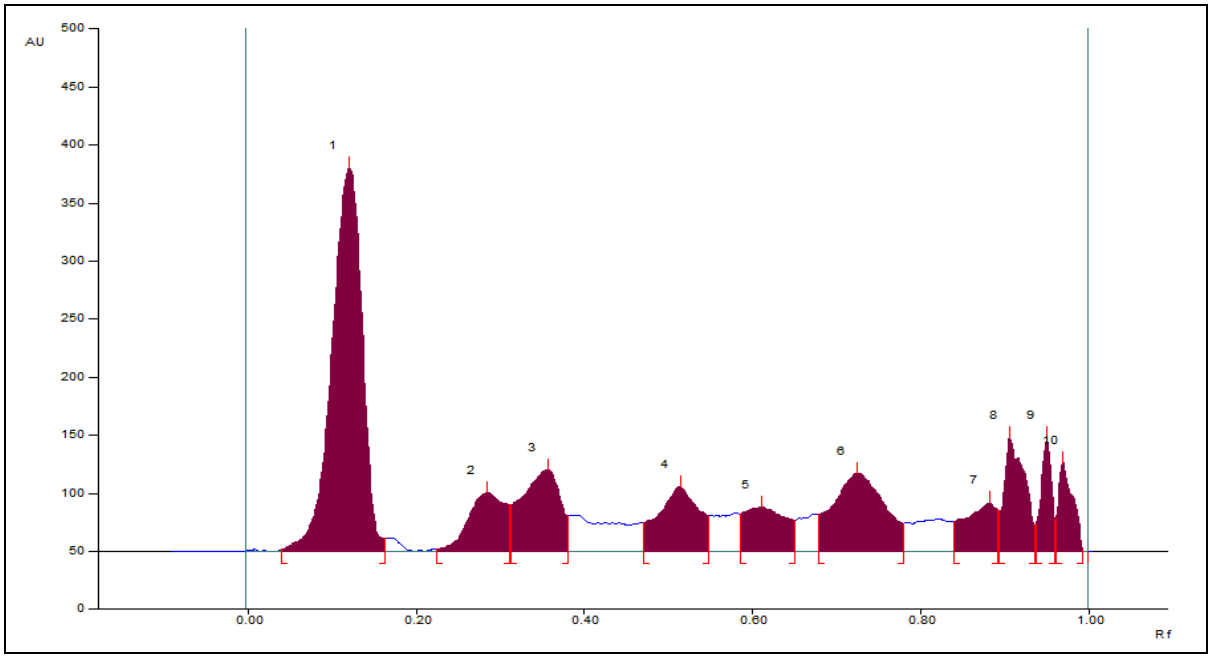


Figure.7.10.18.HPTLC finger print profile of chloroform extract of Nandi mezhugu at 575 nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.04 Rf	1.7 AU	0.12 Rf	330.5 AU	35.63 %	0.16 Rf	11.3 AU	10833.0 AU	36.74 %
2	0.23 Rf	1.9 AU	0.29 Rf	51.1 AU	5.51 %	0.31 Rf	40.0 AU	2011.3 AU	6.82 %
3	0.31 Rf	40.1 AU	0.36 Rf	70.1 AU	7.56 %	0.38 Rf	30.9 AU	2902.2 AU	9.84 %
4	0.47 Rf	25.3 AU	0.51 Rf	55.7 AU	6.01 %	0.55 Rf	30.9 AU	2405.1 AU	8.16 %
5	0.59 Rf	32.4 AU	0.61 Rf	38.5 AU	4.15 %	0.65 Rf	27.0 AU	1749.0 AU	5.93 %
6	0.68 Rf	32.1 AU	0.72 Rf	67.5 AU	7.28 %	0.78 Rf	24.2 AU	3762.6 AU	12.76 %
7	0.84 Rf	25.7 AU	0.88 Rf	42.0 AU	4.53 %	0.89 Rf	36.5 AU	1379.7 AU	4.68 %
8	0.89 Rf	35.1 AU	0.91 Rf	97.8 AU	10.54 %	0.94 Rf	22.4 AU	2161.2 AU	7.33 %
9	0.94 Rf	22.6 AU	0.95 Rf	97.7 AU	10.53 %	0.96 Rf	27.3 AU	1096.7 AU	3.72 %
10	0.96 Rf	28.2 AU	0.97 Rf	76.6 AU	8.26 %	0.99 Rf	1.5 AU	1186.2 AU	4.02 %

Figure.7.10.19.R_f value of peaks with percentage peak area of HPTLC finger print profile of chloroform extract of Nandi mezhugu at 575 nm

The HPTLC finger print profile of chloroform extract at 575 nm showed 10 peaks in which the peak at R_f 0.12 was the major peak with an area of 36.74 % followed by a peak at R_f 0.72 (12.76%), 0.36 (9.84%), 0.51 (8.16%), 0.91 (7.33%), 0.29 (6.82%) and all other peaks are minor with an individual area less than 5 %.

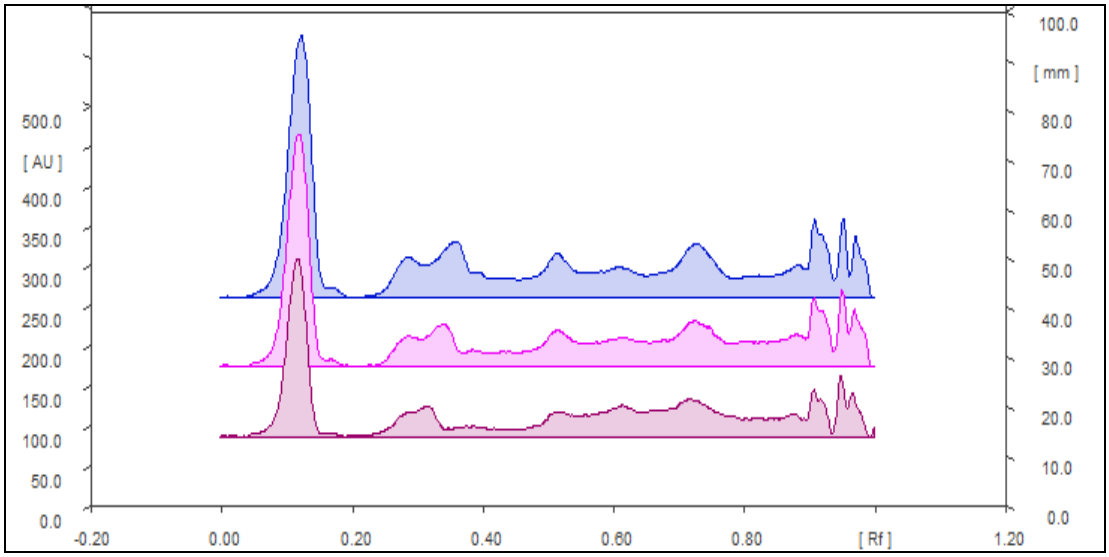
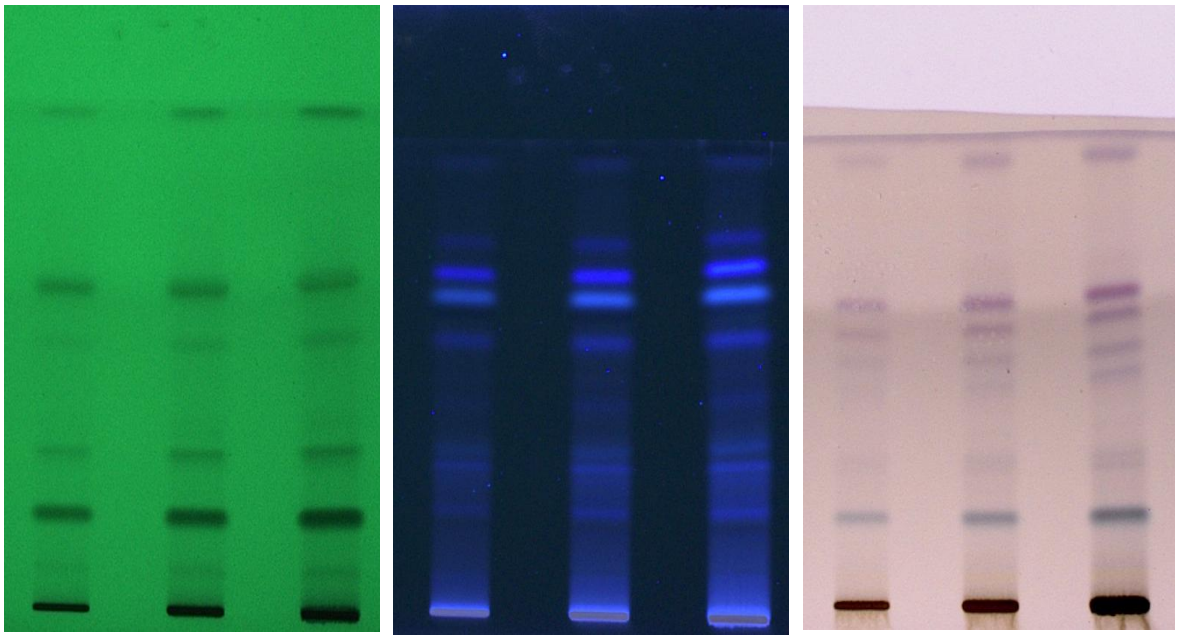


Figure.7.10.20.3D chromatogram of chloroform extract of Nandi mezhugu at 575 nm



UV 254 nm | UV 366 nm | White light after dipping in vanillin-sulphuric acid

Figure.7.10.21.TLC photodocumentation of ethanol extract of Nandi mezhugu Solvent system - Toluene : Ethyl acetate (5:1.5, v/v)

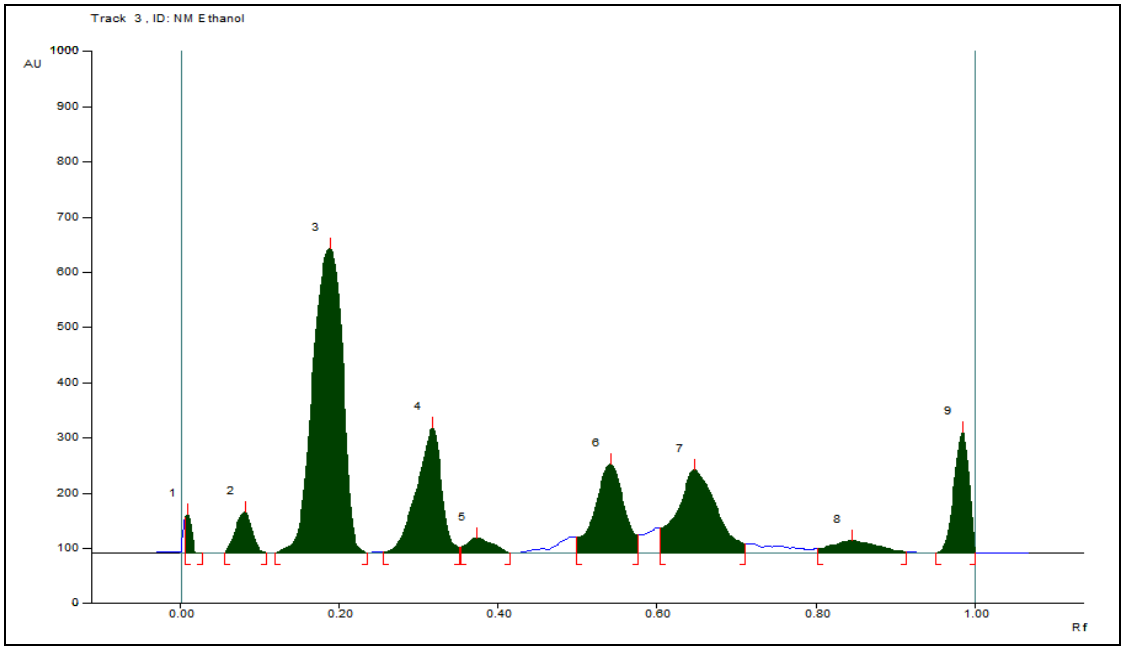


Figure.7.10.22.HPTLC finger print profile of ethanol extract of Nandi mezhugu at UV 254 nm

Track 3, ID: NM Ethanol

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	64.4 AU	0.01 Rf	70.7 AU	4.70 %	0.03 Rf	0.0 AU	431.1 AU	1.06 %
2	0.06 Rf	0.1 AU	0.08 Rf	73.8 AU	4.91 %	0.11 Rf	0.4 AU	1269.3 AU	3.11 %
3	0.12 Rf	0.2 AU	0.19 Rf	551.8 AU	36.68 %	0.24 Rf	0.2 AU	17058.4 AU	41.80 %
4	0.26 Rf	1.4 AU	0.32 Rf	227.1 AU	15.10 %	0.35 Rf	10.0 AU	5842.9 AU	14.32 %
5	0.35 Rf	10.3 AU	0.38 Rf	27.7 AU	1.84 %	0.42 Rf	0.1 AU	743.0 AU	1.82 %
6	0.50 Rf	28.6 AU	0.54 Rf	161.6 AU	10.74 %	0.58 Rf	32.6 AU	4867.2 AU	11.93 %
7	0.61 Rf	45.9 AU	0.65 Rf	151.4 AU	10.07 %	0.71 Rf	16.7 AU	6178.8 AU	15.14 %
8	0.80 Rf	7.3 AU	0.85 Rf	22.0 AU	1.46 %	0.92 Rf	2.6 AU	1076.0 AU	2.64 %
9	0.95 Rf	0.2 AU	0.99 Rf	218.3 AU	14.51 %	1.00 Rf	0.0 AU	3346.8 AU	8.20 %

Figure.7.10.23.R_f value of peaks with percentage peak area of HPTLC finger print profile of ethanol extract of Nandi mezhugu at UV 254 nm

The HPTLC finger print profile of ethanol extract at UV 254 nm showed 9 peaks in which the peak at R_f 0.19 was the major peak with an area of 41.80 % followed by a peak at R_f 0.65 (15.14%), 0.32 (14.32%), 0.54 (11.93%), 0.99 (8.20%) and all other peaks are minor with an individual area less than 5 %.

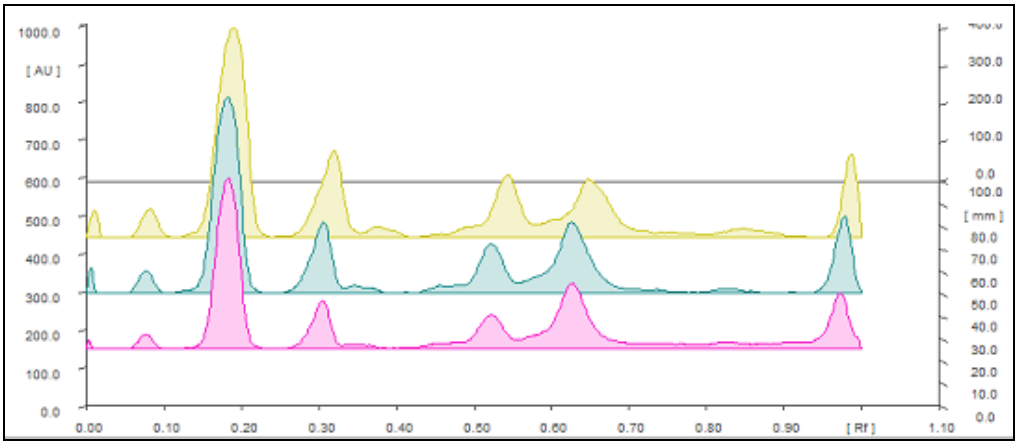


Figure.7.10.24.3D chromatogram of ethanol extract of Nandi mezhugu at UV 254 nm

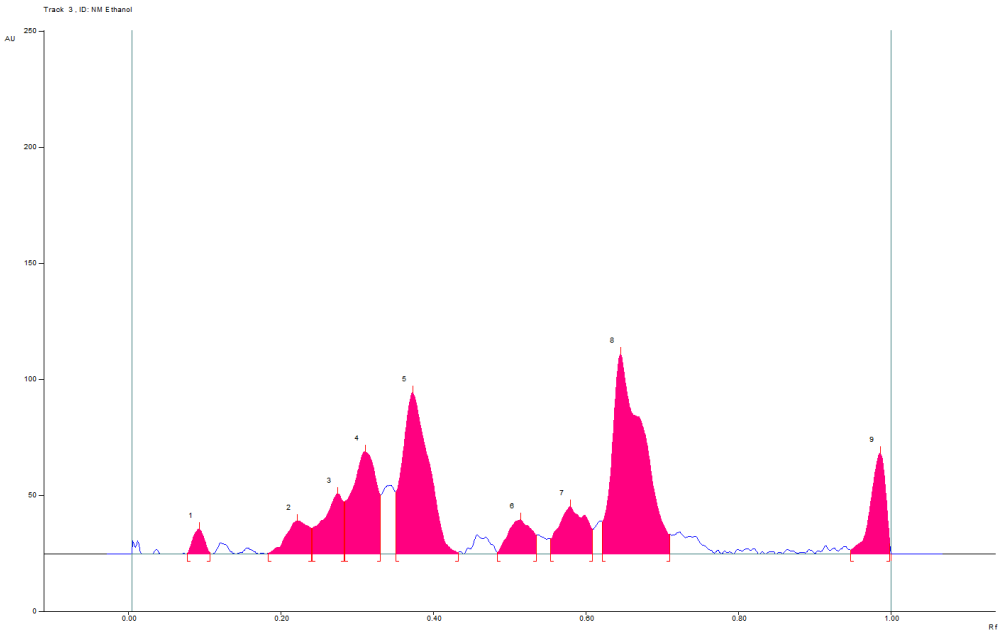


Figure 7.10.25 HPTLC finger print profile of ethanol extract of Nandi mezhugu at UV 366 nm

Track 3, ID: NM Ethanol

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.08 Rf	0.3 AU	0.09 Rf	10.8 AU	3.29 %	0.11 Rf	0.5 AU	130.0 AU	1.50 %
2	0.18 Rf	0.2 AU	0.22 Rf	14.2 AU	4.32 %	0.24 Rf	11.1 AU	348.2 AU	4.01 %
3	0.24 Rf	11.1 AU	0.28 Rf	25.9 AU	7.88 %	0.28 Rf	22.4 AU	570.6 AU	6.57 %
4	0.28 Rf	22.6 AU	0.31 Rf	43.8 AU	13.34 %	0.33 Rf	25.5 AU	1152.4 AU	13.26 %
5	0.35 Rf	26.6 AU	0.37 Rf	69.5 AU	21.14 %	0.43 Rf	0.6 AU	1967.7 AU	22.64 %
6	0.48 Rf	0.7 AU	0.52 Rf	14.6 AU	4.45 %	0.54 Rf	8.0 AU	369.9 AU	4.26 %
7	0.55 Rf	6.5 AU	0.58 Rf	20.5 AU	6.23 %	0.61 Rf	10.6 AU	598.4 AU	6.89 %
8	0.62 Rf	13.8 AU	0.65 Rf	86.1 AU	26.18 %	0.71 Rf	8.5 AU	2853.4 AU	32.83 %
9	0.95 Rf	1.6 AU	0.99 Rf	43.3 AU	13.17 %	1.00 Rf	4.4 AU	700.4 AU	8.06 %

Figure 7.10.26. R_f value of peaks with percentage peak area of HPTLC finger print profile of ethanol extract of Nandi mezhugu at UV 366 nm

The HPTLC finger print profile of ethanol extract at UV 366 nm showed 9 peaks in which the peak at R_f 0.65 was the major peak with an area of 32.83 % followed by a peak at R_f 0.37 (22.64%), 0.31 (13.26%), 0.99 (8.06%), 0.58 (6.89%), 0.28 (6.57%) and other peaks at R_f 0.52, 0.22 and 0.09 are minor with an individual area less than 5 %.

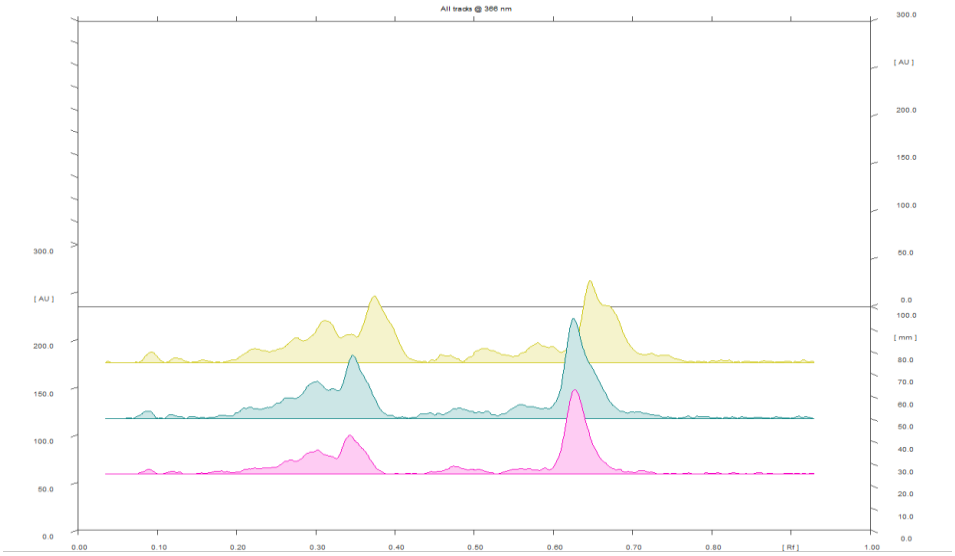


Figure.7.10.27.3D chromatogram of ethanol extract of Nandi mezhugu at UV 366 nm

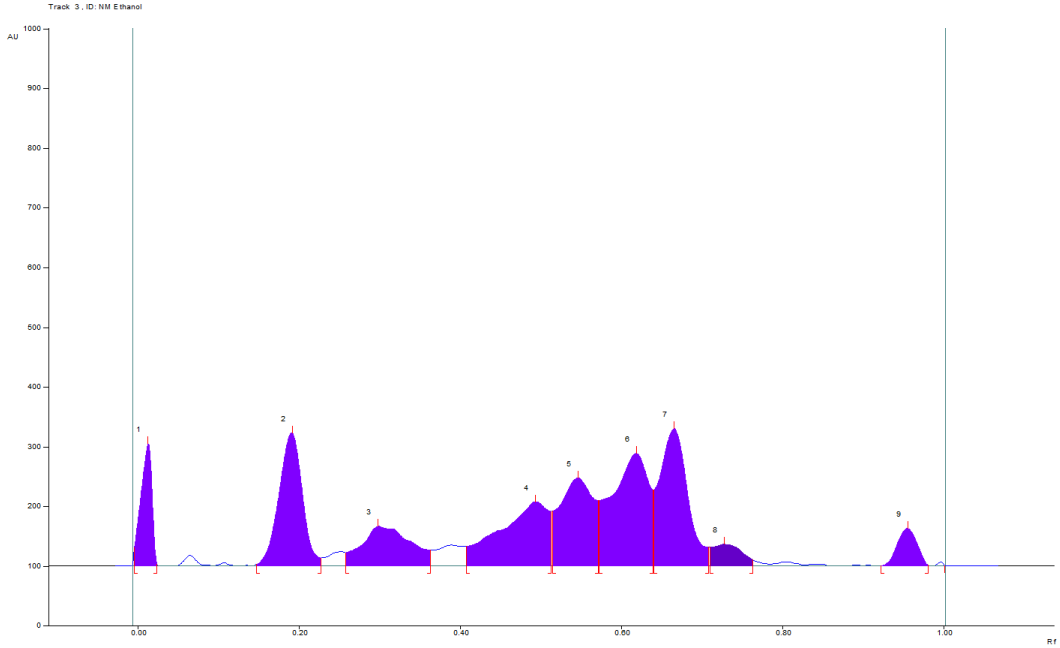


Figure 7.10.28 HPTLC finger print profile of ethanol extract of Nandi mezhugu at UV 575 nm

Track 3, ID: NM Ethanol

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.00 Rf	29.7 AU	0.01 Rf	204.6 AU	16.19 %	0.02 Rf	2.3 AU	2321.2 AU	6.16 %
2	0.15 Rf	1.9 AU	0.19 Rf	222.8 AU	17.63 %	0.23 Rf	13.1 AU	5422.2 AU	14.40 %
3	0.26 Rf	21.8 AU	0.30 Rf	66.0 AU	5.23 %	0.36 Rf	25.9 AU	3350.0 AU	8.89 %
4	0.41 Rf	32.5 AU	0.49 Rf	107.0 AU	8.47 %	0.51 Rf	91.1 AU	5392.1 AU	14.32 %
5	0.52 Rf	91.2 AU	0.55 Rf	147.2 AU	11.65 %	0.57 Rf	09.2 AU	5104.0 AU	13.55 %
6	0.57 Rf	109.4 AU	0.62 Rf	188.0 AU	14.88 %	0.64 Rf	27.2 AU	7104.3 AU	18.86 %
7	0.64 Rf	127.4 AU	0.67 Rf	229.3 AU	18.15 %	0.71 Rf	31.2 AU	6599.8 AU	17.52 %
8	0.71 Rf	31.2 AU	0.73 Rf	35.9 AU	2.84 %	0.76 Rf	10.1 AU	1098.1 AU	2.92 %
9	0.92 Rf	0.1 AU	0.96 Rf	62.8 AU	4.97 %	0.98 Rf	0.9 AU	1274.4 AU	3.38 %

Figure 7.10.29. R_f value of peaks with percentage peak area of HPTLC finger print profile of ethanol extract of Nandi mezhugu at UV 575 nm

The HPTLC finger print profile of ethanol extract at 575 nm showed 9 peaks in which the peaks at R_f 0.62 (18.86%), 0.67(17.52 %), 0.19 (14.40 %), 0.49 (14.32%), 0.55 (13.55 %) and 0.30 (8.89 %) were the major peaks. The other peaks at R_f 0.73 (2.92 %) and 0.96 (3.38 %) were minor. The peak at R_f 0.01 was not µconsidered as it is very close to the spotting position.

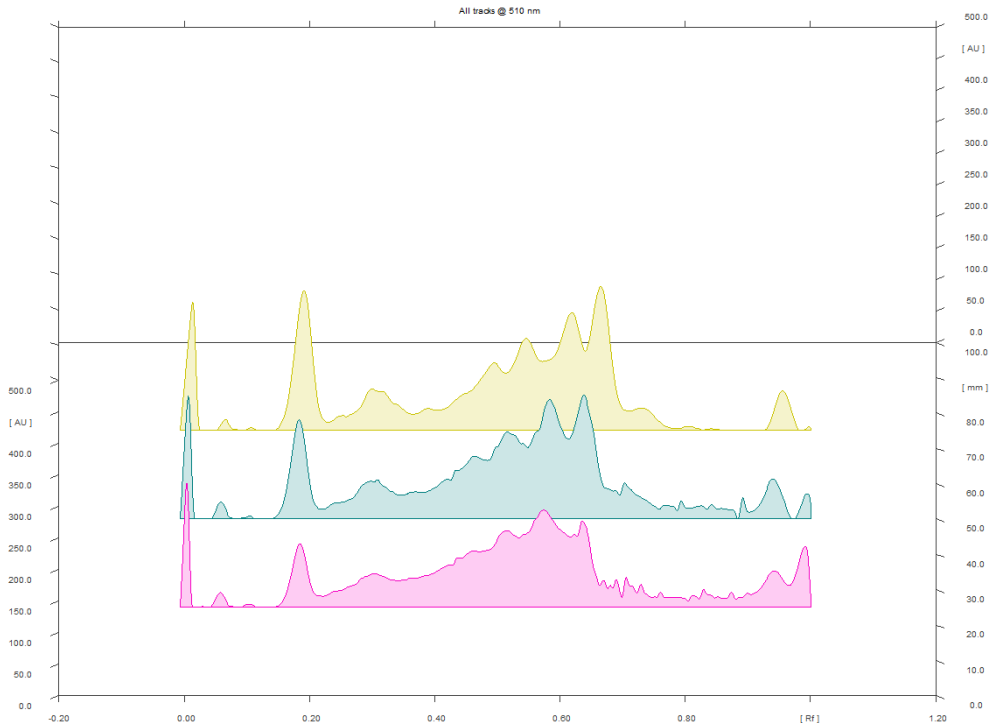


Figure.7.10.30.3D chromatogram of ethanol extract of Nandi mezhugu at UV 575nm

Any changes due to various concentrations of extract (eg- 5,10,15 micro liter) can be inferred from 3D chromatogram of the extract at UV254nm,UV 366nm,UV 575nm.

Figure.7.11.Results and Discussion:

Acute toxicity studies Table 7.11.1.Behaviour changes in acute toxicity study

Dose mg/kg	Behavioural Change																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Control (Palm jaggery solution)	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NM (50mg/kg)	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NM (300mg/kg)	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NM (2000mg/kg)	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-

(+ indicates that the particular behaviour is present and – indicates absence of the behaviour in animals)

Note:1.Alertness 2. Aggressiveness 3. Piloerection 4. Grooming 5.Gripping 6. Touch Response 7. Decreased Motor Activity 8.Tremors 9.Convulsions 10. Muscle Spasm 11. Catatonia 12. Muscle relaxant 13. Hypnosis 14.Analgesia 15.Lacrimation 16.Exophthalmos 17.Diarrhoea 18.Writhing 19. Abnormal Respiration 20. Mortality.

The limit dose of 2000 mg/kg Nandhi mezhugu (NM) did not cause mortality or any sign of acute toxicity, in the three rats on single dosing by oral route, for a short period (48 h) and long period (14 days). No behavioural changes and death were observed at the end of the treatment Table 7.11.1. Similarly, subsequent study with 50, 300 and 2000 mg/kg did not show any significant differences in bodyweight between control and treated groups during this period (Table 7.11.2).

Table 7.11.2. Feed intake chart

Days	Vehicle control (Palm jaggery solution)	Low dose NM (50mg/kgb.wt)	Mid Dose NM (300mg/ kg b.wt)	High dose NM (2000mg/kg b.wt)
1	78.4	78.6	79.1	80.0
2	74.5	74.7	75.1	76.3
3	75.4	75.3	75.5	76.1
4	73.8	73.5	74.1	77.3
5	74.8	74.9	75.3	76.3
6	73.7	73.6	74.1	76.5
7	76.2	76.1	77.2	78.4
8	77.3	78.1	76.9	77.5
9	78.1	78.3	78.9	78.1
10	72.9	73.1	74.2	76.1
11	73.4	73.5	74.2	75.2
12	75.2	75.1	76.1	77.2
13	74.1	74.3	75.3	76.7
14	75.2	75.5	76.1	76.9

There was no treatment related changes in the feed intake by all the dose groups during the observation period of 14 days.

7.12. Sub-acute toxicity study:

Table No:7.12.1:Effect of Nandhi Mezhugu on Body weight of experimental Wistar rats in Sub-acute toxicity study

Groups	Days and Body Weight in Mean and SE					
	D-0	D- 8	D- 14	D-27	D- 41	Day 56 (Wt. gain Vs Day 0)
Vehicle control (Palm jaggery solution) gm/bwt	237.6 ± 31	241.3 ± 31	244.1 ± 32	259.6 ± 39	275.8 ± 42	279.6 ± 44 (42.0 g)
Low dose (NM) 9mg/kg b.wt.	232.0 ± 17	255.4 ± 21	258.4 ± 22	272.6 ± 25	285.2 ± 27	292.8 ± 29 (60.8 g)
Mid Dose(NM) 45mg/kg b.wt.	204.1 ± 8	225.6 ± 13	228.4 ± 13	240.4 ± 17	253.3 ± 19	262.2 ± 21 (58.1g)
High dose(NM) 90mg/kg b.wt.	217.3 ± 9	244.5 ± 12	248.4 ± 13	259.5 ± 17	274.3 ± 20	282.9 ± 22 (65.6g)

All values are mean ± SE. Statistical analysis by one-way ANOVA followed by Dunnett's Multiple Comparison. *p < 0.005 is considered as significant .In the above table all are non –significant.

Recovery Groups

Groups	Day 0	Day 8	Day 14	Day 27	Day 41	Day 56 (Wt. gain Vs Day 0)	Day 69 (wt.gainVs Day56)	p-value
Recovery control (Palm jaggery solution)	246.1 ± 30	251.8 ± 31	256.0 ± 31	267.5 ± 37	281.6 ± 41	289.8 ± 42(43.7 g)	299.6 ± 44 (9.8g)	>0.05
High dose recovery(NM) 90mg/kg b.wt	205 ± 16	227.1 ± 20	231.3 ± 21	240.8 ± 23	253 ± 27	261.8 ± 30(56.8 g)	270.3 ± 31(8.5g)	>0.05

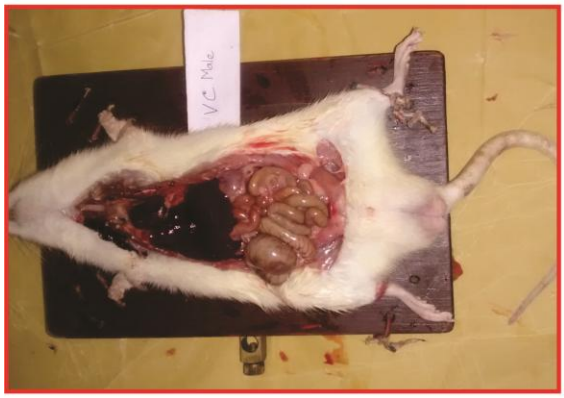
All values are mean ± SE. Statistical analysis by one-way ANOVA followed by Dunnett's Multiple Comparison. *p < 0.005 is considered as significant. While comparing the above two , it was found non-significant.

Table No:7.12.2 Feed intake of animals in Sub-acute toxicity study

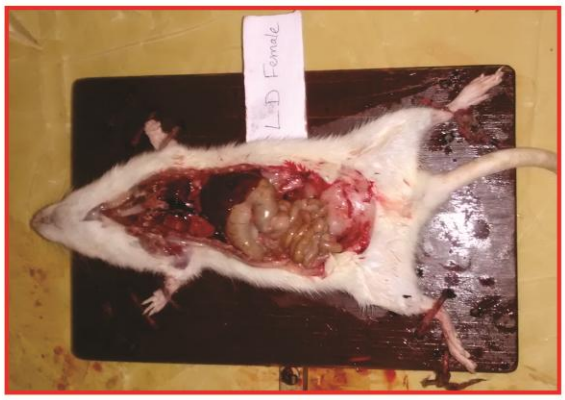
Weeks	Vehicle control	Low dose	Mid Dose	High dose	Control Recovery	High Dose Recovery
1	78.2	78	78.7	78.6	78.4	78.9
2	76.5	76.3	77.1	77.9	76.2	78.1
3	77.9	77.7	77.5	78.2	78.1	78.7
4	76.9	76.7	78.2	77.9	76.2	78.2
5	77.2	77.5	78.7	78.9	77.5	79.2
6	78.1	78.7	79.2	79.7	78.2	80.1
7	76.5	76.8	78.1	78.7	76.4	78.9
8	76.4	77.1	78.6	79.1	76.2	80.4
9	-	-	-	-	77.6	79.7
10	-	-	-	-	76.5	80.9

Figure : OBSERVATION OF GROSS PATHOLOGY IN SUB ACUTE TOXICITY STUDY OF NM IN WISTAR RATS

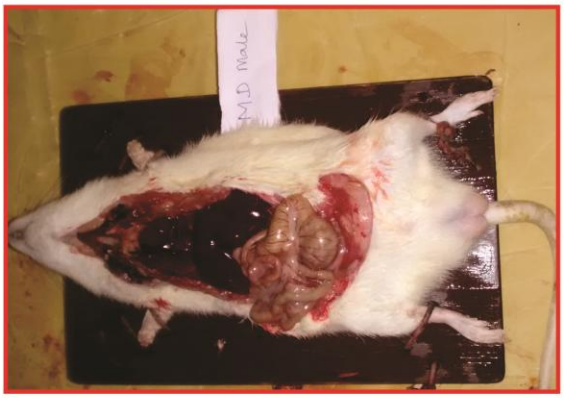
Vehicle control



Low dose

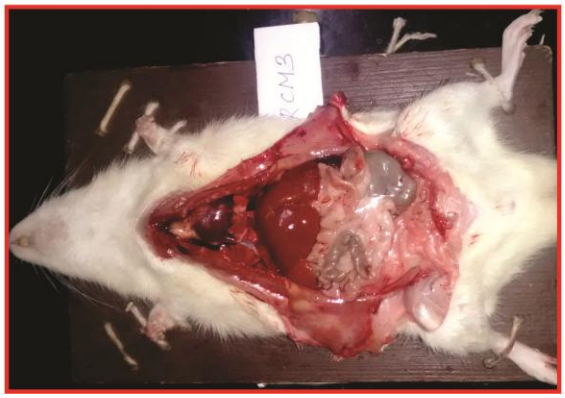


Mid dose



High dose

Recovery control

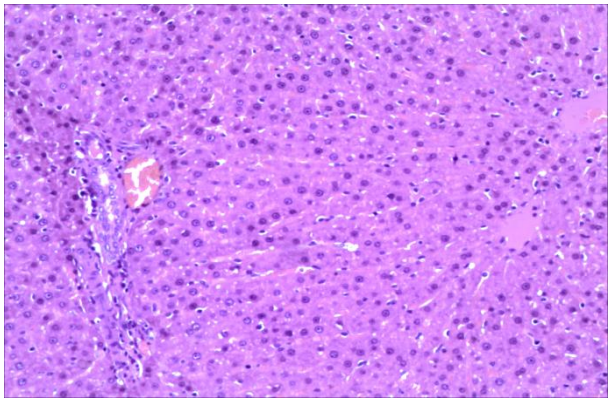


High dose recovery

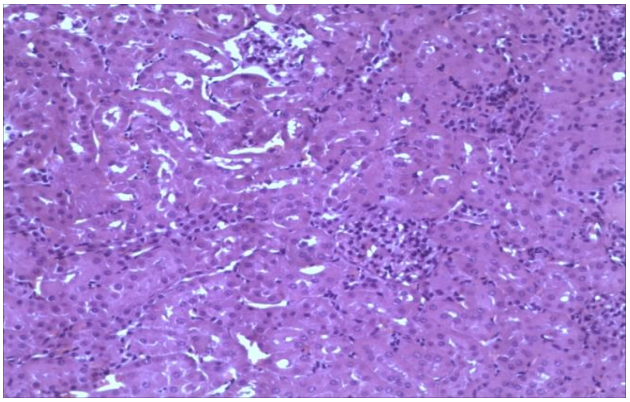
Effect of NM on Histopathalogical changes in Rat organs Sub acute Toxicity study

SPECIMEN

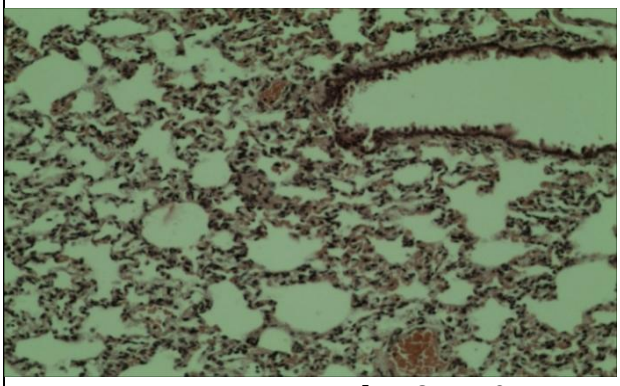
Study no: LBPL-HP-004/15



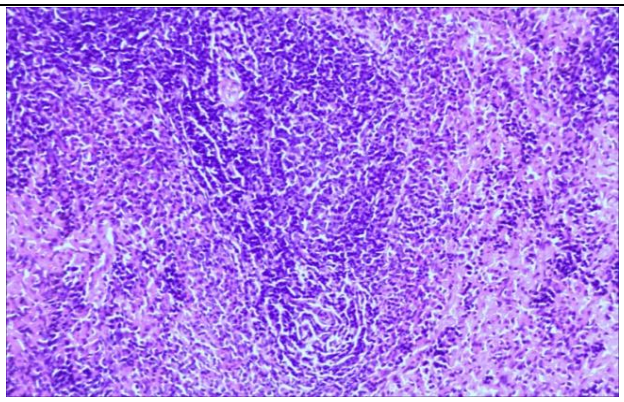
Liver: Normal, H&Ex10



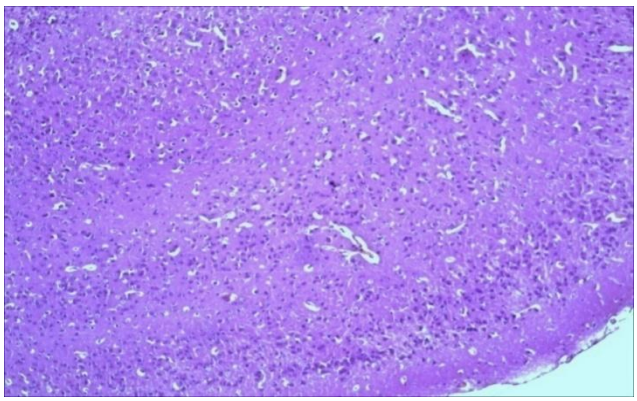
Kidney : Normal, H&Ex10



Lungs: Normal, H&Ex10

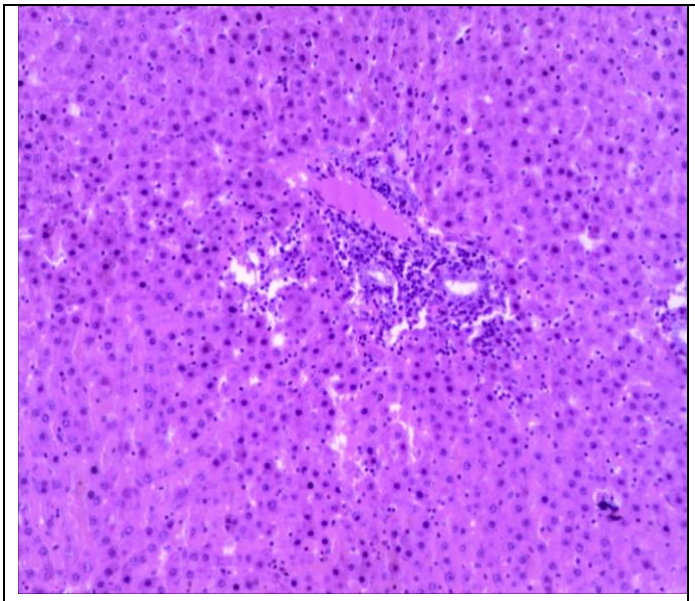
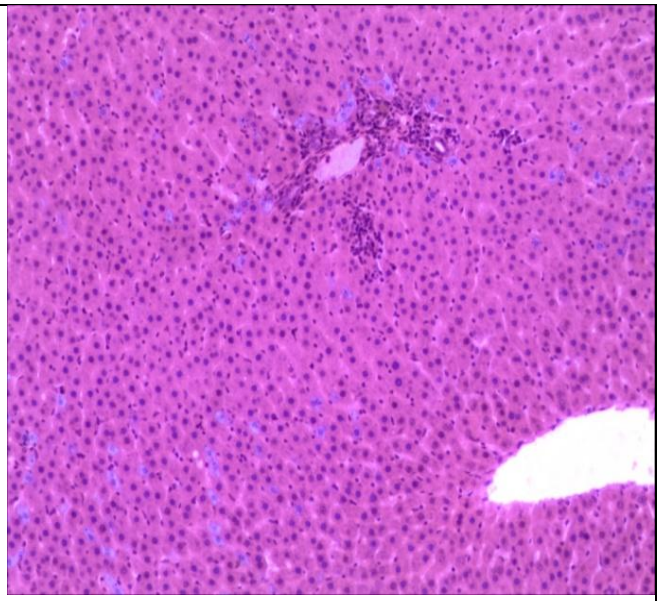
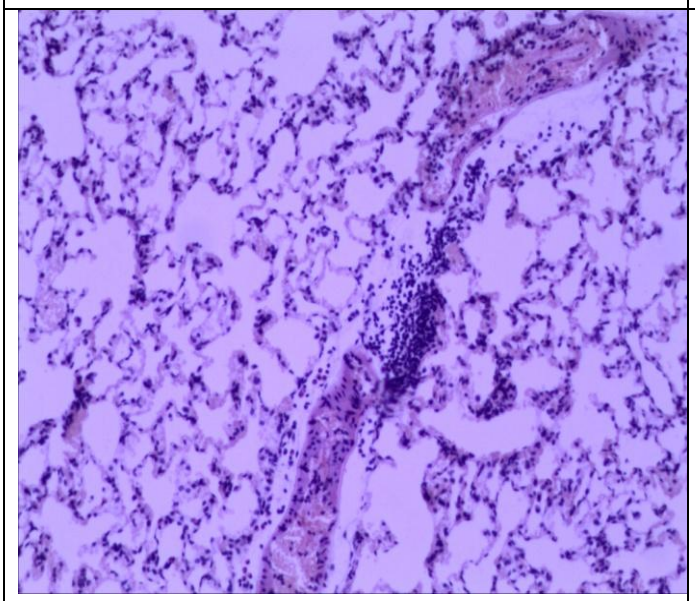
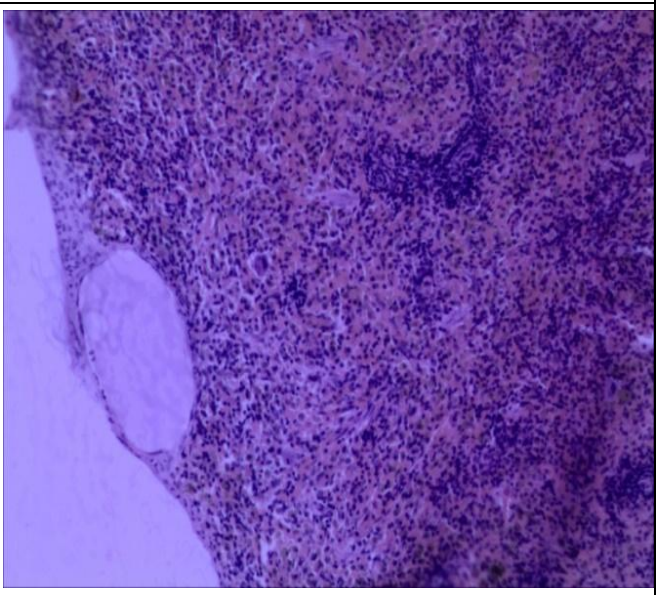


Spleen : Normal, H&Ex10

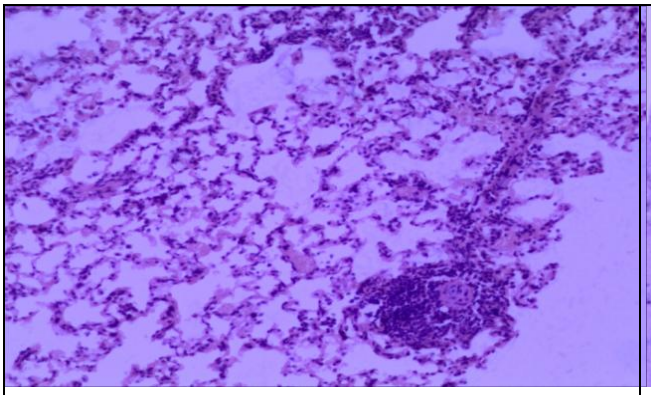
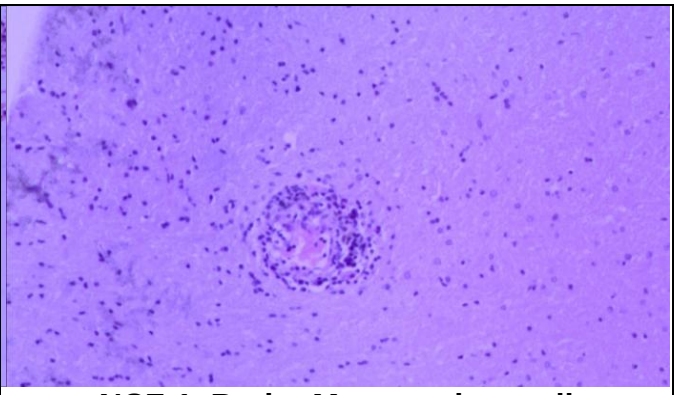
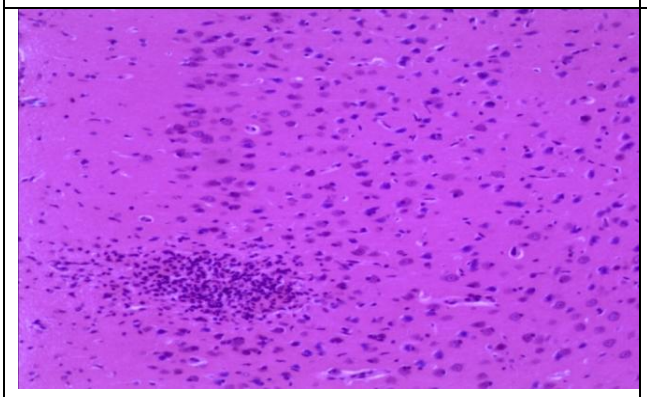
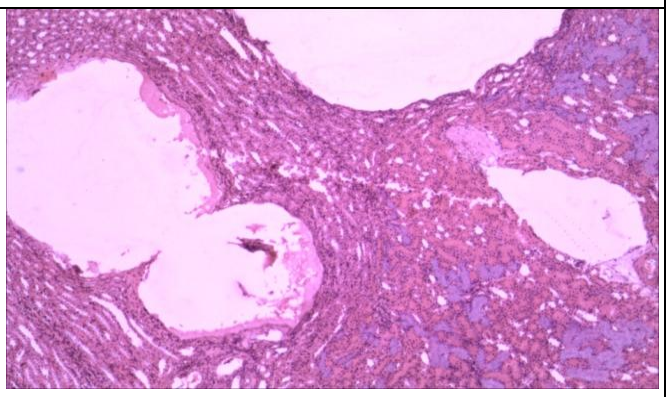
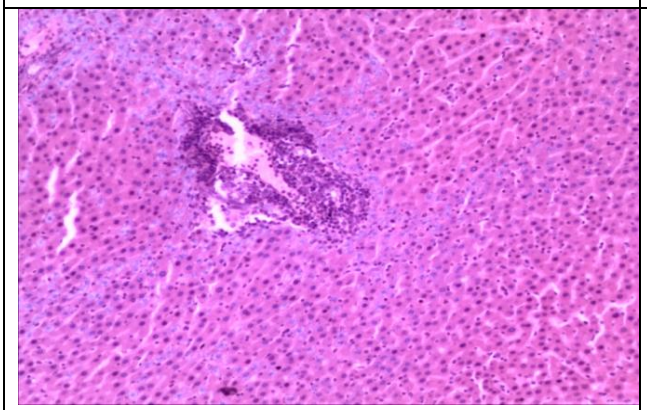
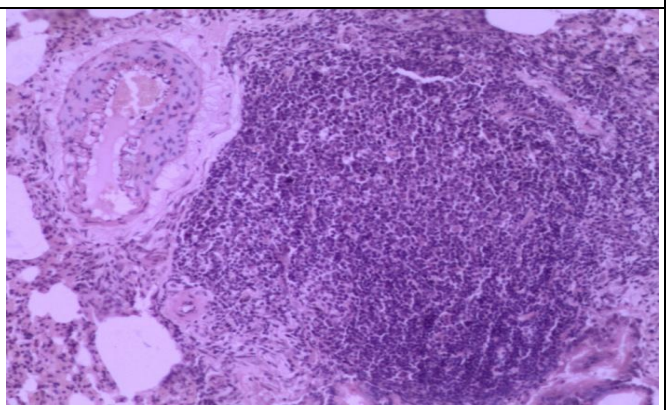


Brain : Normal, H&Ex10

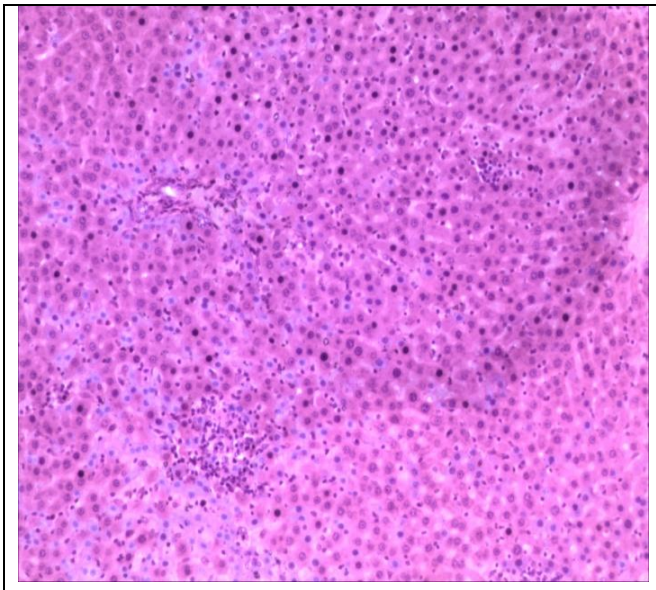
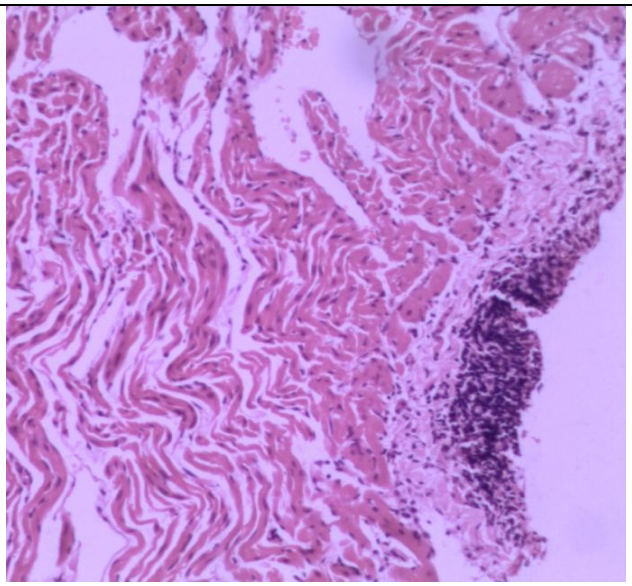
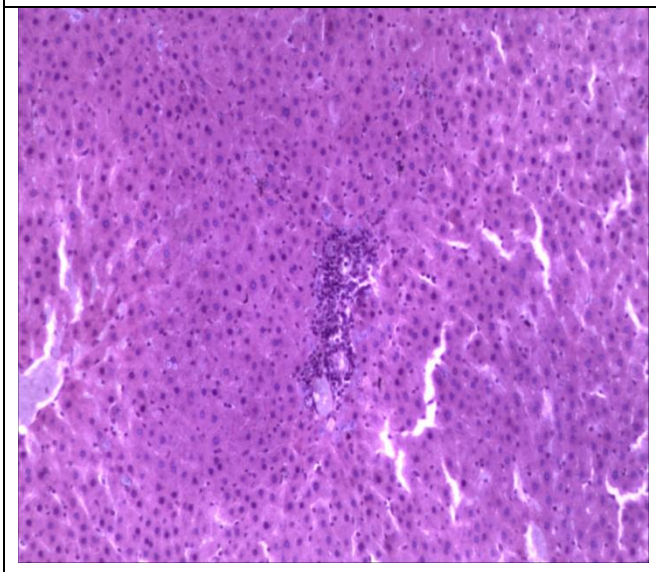
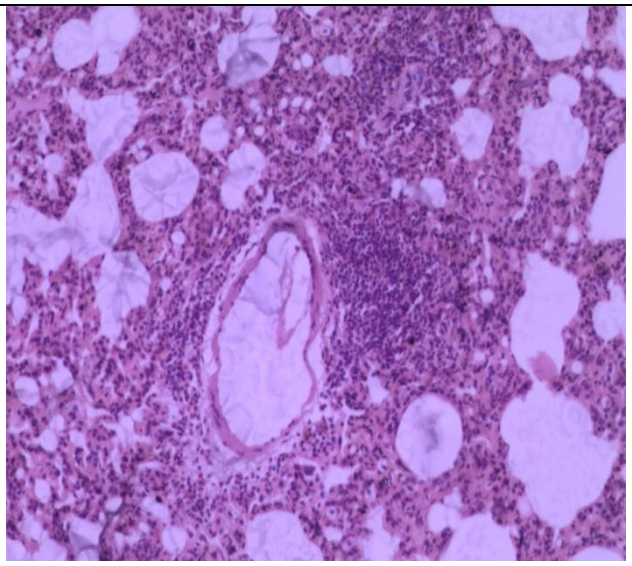
Study no: LBPL-HP-004/15 (Sub acute Toxicity study)

 **NHRF(1),Liver: Mononuclear cell infiltration, H&E X10**	 **RCF(1), Liver : Mononuclear cell infiltration, H&E X10**
 **RCF(1), Lungs: Perivascular Mononuclear cell infiltration, H&E X10**	 **RCF(1), Spleen: Cyst, H&E X10**

Study no: LBPL-HP-004/15 (Sub acute Toxicity study)

	
NHRF -2, Lungs: Perivascular Mononuclear cell infiltration, H&E X10	**NCF-1, Brain: Mononuclear cell infiltration, H&E X10**
	
NHRF (3), Brain: Mononuclear cell infiltration, H&E X10	**NHM (5), Kidney: Cysts H&E X4**
	
RCM (2), Liver: Mononuclear cell infiltration, H&E X10	**RCM (2), Lungs: Perivascular Mononuclear cell infiltration, H&E X10**

Study no: LBPL-HP-004/15 (Sub acute Toxicity study)

	
NHF(5), Liver: Mononuclear cell infiltration, H&E X10	**NHF(5), Heart: Mononuclear cell infiltration, H&E X10**
	
NHRM (2), Liver: Mononuclear cell infiltration, H&E X10	**NHF(1), Lungs: Lungs: Perivascular Mononuclear cell infiltration, H&E X10**

HISTOPATHOLOGY REPORT

In-house ref no: LBPL-HP-004/15 (Sub acute Toxicity study)

Species and strain: Rats

Tissue for histopathology: Liver, Kidneys, Lungs, Heart, Spleen, Stomach, Brain, skin, femur bone

The tissue samples from the study were preserved in 10% Neutral Buffered Formalin were processed by paraffin embedding method, sectioned and stained with Heamatoxylin & eosin stain. The stained tissue sections (Liver, Kidneys, Lungs, Heart, Spleen, Stomach, Brain, skin and femur bone) from different groups were examined microscopically for histopathological changes.

RESULTS:

The various histopathological or microscopic changes in the organs/ tissues are recorded in their respective 'Individual Animal Histopathology Report' formats.

There were no treatment related changes in the organs/tissues examined at different dose level at differnent groups. The changes noticed were considered as incidental and spontaneous in nature to this particular species and strain of animal (Refer Table 1A):

Table 1A: INDIVDUAL ANIMAL HISTOPATHOLOGY FINDINGS

Sample no.	Tissue/Organ	Histopathology findings/Observations
NHRF(1) **Nandhimezhugu High dose recovery Female**	Liver	Mononuclear cell infiltration- minimal
	Kidneys	NAD
	Lungs	NAD
	Heart	NAD
	Spleen	NAD
	Stomach	NAD
	Brain	NAD
	Skin	NAD
	Femur bone	NAD
NHRM (1) **Nandhimezhugu High dose Recovery Female**	Liver	NAD
	Kidneys	NAD
	Lungs	NAD
	Heart	NAD
	Spleen	NAD
	Stomach	NAD

	Brain	NAD
	Skin	NAD
	Femur bone	NAD
RCF (1) **Recovery control Female**	Liver	Mononuclear cell infiltration- minimal
	Kidneys	NAD
	Lungs	Preivascular Mononuclear cell infiltration- minimal
	Heart	NAD
	Spleen	Capsule-cyst, focal
	Stomach	NAD
	Brain	NAD
	Skin	NAD
	Femur bone	NAD
NHRF -2 **Nandhimezhugu High dose recovery Female**	Liver	NAD
	Kidneys	NAD
	Lungs	Preivascular Mononuclear cell infiltration- minimal
	Heart	NAD
	Spleen	NAD
	Stomach	NAD
	Brain	NAD
	Skin	NAD
	Femur bone	NAD

Note: NAD-No Abnormalities Detected, Degree of severity=1-minimal, 2-mild, 3-moderate, 4- marked/severe.

Table 1A (Cont..): INDIVDUAL ANIMAL HISTOPATHOLOGY FINDINGS

Sample no.	Tissue/Organ	Histopathology findings/Observations
NCF-1 **Nandhimezhugu Control Female**	Liver	NAD
	Kidneys	NAD
	Lungs	NAD
	Heart	NAD
	Spleen	NAD
	Stomach	NAD
	Brain	Mononuclear cell infiltration- minimal, focal
	Skin	NAD
	Femur bone	NAD
NHM (1) **Nandhi mezhugu High Dose Male**	Liver	NAD
	Kidneys	NAD

	Lungs	NAD
	Heart	NAD
	Spleen	NAD
	Stomach	NAD
	Brain	NAD
	Skin	NAD
	Femur bone	NAD
NHRF (3) **Nandhi Mezhugu High Dose Recovery Female**	Liver	NAD
	Kidneys	NAD
	Lungs	NAD
	Heart	NAD
	Spleen	NAD
	Stomach	NAD
	Brain	Mononuclear cell infiltration- minimal, focal
	Skin	NAD
	Femur bone	NAD
NCM (1) **Nandhi Mezhugu Control Male**	Liver	NAD
	Kidneys	NAD
	Lungs	NAD
	Heart	NAD
	Spleen	NAD
	Stomach	NAD
	Brain	NAD
	Skin	NAD
	Femur bone	NAD

Note: NAD-No Abnormalities Detected, Degree of severity=1-minimal, 2-mild, 3-moderate, 4- marked/severe.

Table 1A (Cont..): INDIVDUAL ANIMAL HISTOPATHOLOGY FINDINGS

Sample no.	Tissue/Organ	Histopathology findings/Observations
NHM (5) **Nandhi Mezhugu High dose Male**	Liver	NAD
	Kidneys	Cortex-Cysts, multiple
	Lungs	NAD
	Heart	NAD
	Spleen	NAD
	Stomach	NAD
	Brain	NAD
	Skin	NAD
	Femur bone	NAD
RCM (2) **Nandhi Mezhugu Recovery Control Male**	Liver	Mononuclear cell infiltration- minimal, focal
	Kidneys	NAD

	Lungs	Preivascular Mononuclear cell infiltration- minimal
	Heart	NAD
	Spleen	NAD
	Stomach	NAD
	Brain	NAD
	Skin	NAD
	Femur bone	NAD
NHRM (3) **Nandhi Mezhugu High dose recovery Control Male-3**	Liver	NAD
	Kidneys	NAD
	Lungs	NAD
	Heart	NAD
	Spleen	NAD
	Stomach	NAD
	Brain	NAD
	Skin	NAD
	Femur bone	NAD
NHF(5) **Nandhi Mezhugu High dose Female**	Liver	Mononuclear cell infiltration- minimal
	Kidneys	NAD
	Lungs	NAD
	Heart	Mononuclear cell infiltration- minimal, focal-pericardium
	Spleen	NAD
	Stomach	NAD
	Brain	NAD
	Skin	NAD
	Femur bone	NAD

Note: NAD-No Abnormalities Detected, Degree of severity=1-minimal, 2-mild, 3-moderate, 4- marked/severe.

Table 1A (Cont..): INDIVDUAL ANIMAL HISTOPATHOLOGY FINDINGS

Sample no.	Tissue/Organ	Histopathology findings/Observations
NHRM (2) **Nandhi Mezhugu High dose recovery Male**	Liver	Mononuclear cell infiltration- minimal, focal

	Kidneys	NAD
	Lungs	NAD
	Heart	NAD
	Spleen	NAD
	Stomach	NAD
	Brain	NAD
	Skin	NAD
	Femur bone	NAD
NHF (4) **Nandhi Mezhugu High dose Female**	Liver	NAD
	Kidneys	NAD
	Lungs	NAD
	Heart	NAD
	Spleen	NAD
	Stomach	NAD
	Brain	NAD
	Skin	NAD
	Femur bone	NAD
NHF(1) **Nandhi Mezhugu High dose Female**	Liver	NAD
	Kidneys	NAD
	Lungs	Preivascular Mononuclear cell infiltration- minimal
	Heart	NAD
	Spleen	NAD
	Stomach	NAD
	Brain	NAD
	Skin	NAD
	Femur bone	NAD

Note: NAD-No Abnormalities Detected, Degree of severity=1-minimal, 2-mild, 3-moderate, 4- marked/severe.

Table No: 7.12.3.Effect of Nandhi Mezhugu on Hematological Parameters of experimental Wistar rats in Sub-acute toxicity study

Groups	WBC × 10^9/L	Lymph × 10^9/L	Mon × 10^9/L	Gran × 10^9/L	Lymph %	Mono %	Gran %	RBC × 10^{12}/L	HGB g/dL
Vehicle control (Palm jaggery solution)	3.51 ± 1.1	2.56 ± 0.9	0.1 ± 0.03	0.8 ± 0.3	61.6 ± 12	2.95 ± 0.8	18.83 ± 4	5.34 ± 1.4	8.63 ± 2.9
Low dose (NM)9mg/kg b.w	8.23 ± 1.3**	5.55 ± 0.8	0.4 ± 0.07**	2.3 ± 0.4	61.9 ± 7	3.91 ± 0.4	24.19 ± 3	7.35 ± 1.3	14.56 ± 1.7**

Mid dose(NM) 45mg/kg b.w	12.02 ± 1.5**	8.91 ± 1.3**	0.5 ± 0.06**	2.7 ± 0.32**	71.9 ± 3.5	4.25 ± 0.6	23.83 ± 3	9.83 ± 0.7*	15.80 ± 1.2**
High dose(NM) 90mg/kg b.w	3 ± 0.5	2.15 ± 0.3	0.1 ± 0.02	0.8 ± 0.13	71.4 ± 2	3.63 ± 0.2	24.93 ± 2	6.66 ± 0.8	10.76 ± 1.4

All values are mean ± SE. Statistical analysis by one-way ANOVA followed by Dunnett's Multiple Comparison. *p < 0.05. **p < 0.01.

Recovery Groups

Groups	WBC× 10^9/L	Lymph × 10^9/L	Mon × 10^9/L	Gran× 10^9/L	Lymph %	Mono %	Gran %	RBC× 10^{12}/L	HgB g/dL
Recovery Control (Palm jaggery solution)	4.4 ± 1.6	3.1 ± 1.2	0.16 ± 0.1	1.2 ± 0.4	68.3 ± 1	3.66 ± 0.3	28.1 ± 1.5	8.42 ± 1.2	12.33 ± 2.4
High dose recovery(NM) 90mg/kg b.w	3.7 ± 1.1	2.5 ± 0.8	0.16 ± 0.2	1.0 ± 0.2	66.9 ± 3	4.26 ± 0.3	28.7 ± 2.7	6.14 ± 1.4	9.95 ± 2.4

All values are mean ± SE. Statistical analysis by one-way ANOVA followed by Dunnett's Multiple Comparison. *p < 0.05. **p < 0.01

Groups	HCT %	MCV fL	MCH pg	MCHC g/dL	RDW %	PLT × 10^9/L	MPV fL	PDW	PCT %
Vehicle control (Palm jaggery solution)	27.4 ± 7.4	51.09 ± 0.39	15.13 ± 0.0	29.78 ± 1.3	12.03 ± 0.3	185.1 ± 96	6.76 ± 0.2	15.45 ± 0.2	0.12 ± 0.06
Low dose(NM) 9mg/kg b.w	38.8 ± 5.8	51.38 ± 6.7	22.04 ± 4.5*	36.71 ± 5.6	18.20 ± 4*	530.5 ± 66*	5.89 ± 0.7	13.43 ± 1.5	0.35 ± 0.04
Mid	49.59	50.25	16.07	32.05	12.51	529.4	6.57	14.9	0.35

dose(NM) 45mg/kg b.w	± 3.9*	± 0.69	± 0.2	± 0.2	± 0.4	± 87*	± 0.1	± 0.1	± 0.05
High dose(NM) 90mg/kg b.w	33.25 ± 4.2	49.83 ± 0.83	15.93 ± 0.3	32.09 ± 0.3	11.45 ± 0.3	219.7 ± 67	6.34 ± 0.1	15.02 ± 0.1	0.13 ± 0.04

All values are mean ± SE. Statistical analysis by one-way ANOVA followed by Dunnett's Multiple Comparison. *p < 0.05. **p < 0.01

Recovery Groups

Groups	**HCT %**	**MCV fL**	**MCH pg**	**MCHC g/dL**	**RDW %**	**PLT × 10^9/L**	**MPV fL**	**PDW**	**PCT %**
Recovery Control (Palm jaggery solution)	42.5 ± 6.2	50.6 ± 1.2	14.23 ± 1.5	28.31 ± 3.1	12.16 ± 0.4	319.6 ± 78.5	6.4 ± 0.1	15.03 ± 0.09	0.2 ± 0.04
High dose(NM) recovery 90mg/kg b.w	31.15 ± 7.1	50.71 ± 0.85	15.75 ± 0.46	31.18 ± 0.7	12.43 ± 0.44	349.16 ± 115	6.58 ± 0.1	15.2 ± 0.19	0.22 ± 0.07

All values are mean ± SE. Statistical analysis by one-way ANOVA followed by Dunnett's Multiple Comparison. *p < 0.05.**p < 0.01

Table No:7.12.4.Effect of Nandhi Mezhugu on biochemical parameters of experimental Wistar rats in Sub-acute toxicity study

Groups	**Glucose mg/dl**	**Urea mg/dl**	**Creatinine mg/dl**	**Cholestrol mg/dl**	**TGL mg/dl**	**HDL mg/dl**	**LDL mg/dl**
Vechicle control (Palm jaggery solution)	85.0 ± 3.6	37.0 ± 2.2	0.9 ± 0.03	78.6 ± 0.3	137.5 ± 8.5	21.0 ± 0.0	26.5 ± 1.5
Low dose (NM) 9mg/kg b.w	53.6 ± 6.1	39.5 ± 1.8	0.7 ± 0.1	82.1 ± 4.6	105.2 ± 25.5	24.6 ± 4.1	36.6 ± 6.9
Mid dose(NM) 45mg/kg b.w	92.3 ±	38.7 ±	0.8 ±	78.6 ±	111.7 ±	24.0 ±	32.8 ±

	13.6	1	0.1	1.8	10.4	0.78	1.8
High dose(NM) 90mg/kg b.w	137.3 ± 8.5**	42.2 ± 1.1*	0.7 ± 0.03	74.7 ± 3.8	84.9 ± 5.4*	25.6 ± 1.9	31.3 ± 2

All values are mean ± SE. Statistical analysis by one-way ANOVA followed by Dunnett's Multiple Comparison. *p < 0.05. **p < 0.01

Recovery Groups

Groups	Glucose mg/dl	Urea mg/dl	Creatinine mg/dl	Cholestrol mg/dl	TGL mg/dl	HDL mg/dl	LDL mg/dl
Recovery Control (Palm jaggery solution)	124.0 ± 5.3	38.6 ± 1.8	0.55 ± 0.03	82.83 ± 5.3	134.3 ± 13.4	25.25 ± 2.7	30.83 ± 7.2
High dose Recovery(NM) 90mg/kg b.w	103.8 ± 9.6	36.6 ± 2	0.49 ± 0.03	79.16 ± 6.2	168.16 ± 20.6	27.48 ± 4	32.5 ± 10.8

All values are mean ± SE. Statistical analysis by one-way ANOVA followed by Dunnett's Multiple Comparison. *p < 0.05. **p < 0.01

Groups	TRP g/dl	ALB g/dl	SBIL mg/dl	SGOT IU/L	SGPT IU/L	SAP IU/L
Vechicle control (Palm jaggery solution)	7 ± 0.1	3.4 ± 0.4	0.25 ± 0.00	159.3 ± 19.6	55.4 ± 5.2	179.3 ± 21.2
Low dose (NM) 9mg/kg b.w	7.5 ± 0.3	3.3 ± 0.2	0.20 ± 0.00	138.0 ± 7.4	65.4 ± 25.7	131.6 ± 25.3
Mid dose (NM) 45mg/kg b.w	7.8 ± 0.2	3.3 ± 0.16	0.25 ± 0.02	156.1 ± 15.6	61.1 ± 2.9	133.3 ± 3.2

High dose(NM) 90mg/kg b.w	6.8 ± 0.3	3.1 ± 0.24	0.23 ± 0.03	215.6 ± 15.6*	55.5 ± 2.6	116.0 ± 6.3

All values are mean ± SE. Statistical analysis by one-way ANOVA followed by Dunnett's Multiple Comparison. *p < 0.05. **p < 0.01

Recovery Groups

Groups	TRP g/dl	ALB g/dl	SBIL mg/dl	SGOT IU/L	SGPT IU/L	SAP IU/L
Recovery Control (Palm jaggery solution)	7.53 ± 0.52	2.9 ± 0.3	0.3 ± 0.1	193.1 ± 18	50 ± 4	111 ± 13.4
High dose Recovery(NM) 90mg/kg b.w	7.11 ± 0.19	2.8 ± 0.1	0.2 ± 1.2	211.1 ± 34	59 ± 4	109 ± 21.8

All values are mean ± SE. Statistical analysis by one-way ANOVA followed by Dunnett's Multiple Comparison. *p < 0.05. **p < 0.01

Table No 7.12.5: Effect of Nandhi Mezhugu on relative organ weight of experimental Wistar rats in Sub-acute toxicity study

Groups	Brain	Heart	Lungs	Liver	Spleen	Kidney
Vehicle control (Palm jaggery solution)	1.32 ± 0.03	0.413 ± 0.02	0.851 ± 0.03	2.86 ± 0.27	0.265 ± 0.01	0.906 ± 0.01
Low dose (NM) 9mg/kg b.w	1.32 ± 0.03	0.406 ± 0.01	0.885 ± 0.04	2.53 ± 0.18	0.257 ± 0.01	0.933 ± 0.03
Mid dose(NM)	1.29	0.385	0.831	2.66	0.269	0.848

45mg/kg b.w	± 0.03	± 0.01	± 0.03	± 0.15	± 0.01	± 0.02
High dose(NM) 90mg/kg b.w	1.28 ± 0.02	0.366 ± 0.01	0.689 ± 0.01	2.50 ± 0.13	0.253 ± 0.01	0.841 ± 0.03

Recovery Groups

Groups	Brain	Heart	Lungs	Liver	Spleen	Kidney
Recovery control (Palm jaggery solution)	1.35 ± 0.06	0.426 ± 0.04	0.811 ± 0.01	2.511 ± 0.25	0.280 ± 0.02	0.857 ± 0.04
High dose recovery(NM) 90mg/kg b.w	1.36 ± 0.05	0.460 ± 0.04	0.883 ± 0.04	2.617 ± 0.21	0.286 ± 0.01	0.999 ± 0.03

All values are mean ± SE. Statistical analysis by one-way ANOVA followed by Dunnett's Multiple Comparison. *p < 0.05. **p < 0.01

Results of sub-acute toxicity study:

There were no treatment-related toxicity signs and mortality observed in both sexes of rats treated Nandhi mezhugu(NM) at a dose of 9, 45 and 90 mg/kg b.w orally for a dosing period of 28 days(7 days drug dosing followed by 7 days drug holiday) and in the Recovery group of rats. No significant difference in body weight gain was observed between control and treated groups during the study (Table 7.12.1). Feed consumption of NM treated groups was found to be non-significant in both the sexes when compared to control (Table 7.12.2). Haematological parameters such as haemoglobin, red blood cells, white blood cells, mean corpuscular haemoglobin, though found to be statistically significant at low and mid doses as compared to the control, they were found to be well within the normal range in the rats of experimental groups (Table 7.12.3), hence the changes observed were not treatment

related effects. All the biochemical profile such as glucose, total cholesterol, triglycerides, total protein, alkaline phosphatase, creatinine, blood urea were within the normal range (Table 7.12.4) observed between control and treated groups. The levels of liver marker enzymes like SGOT and SGPT were found to be well within the normal range in rats of NM treated groups (Table 7.12.4). There were no significant differences in organ weight of brain, heart, liver, lungs, kidneys recorded between the control and NM groups (Table 7.12.5). In our study, histopathological examinations in control and high dose group revealed no abnormalities. There were no haematological, biochemical and histopathological alterations observed with NM administration even at 90 mg/kg b.w twice daily in rats for a period of 28 days compared to control. The No Observed Adverse Effect Level (NOAEL) of NM was estimated to be greater than 90 mg/kg b.w /day in rats. Hence, it is concluded that NM is safe for oral administration in rats up to 90 mg/kg b.w/day.

7.13. Sub-chronic toxicity study:

Table No:7.13.1.Effect of Nandhi Mezhugu on Body weight of experimental Wistar rats in Sub-chronic toxicity study

Groups	**On day**												
	1	7	14	28	35	42	49	56	63	70	77	84	91
Vehicle control (Palm jaggery solution)	235 ± 19	257 ± 40	259 ± 47	262 ± 49	273 ± 59	271 ± 58	276 ± 63	281 ± 65	288 ± 69	293 ± 73	299 ± 76	304 ± 79	309 ± 81
Low dose(NM) 9mg/kg b.w	187 ± 6	229 ± 18	230 ± 14	240 ± 17	244 ± 18	250 ± 20	254 ± 22	260 ± 23	266 ± 24	271 ± 25	278 ± 26	283 ± 26	290 ± 28
Middose (NM)45mg/kg b.w	230 ± 11	258 ± 15	261 ± 16	268 ± 17	273 ± 18	279 ± 19	282 ± 19	285 ± 19	290 ± 20	294 ± 21	298 ± 22	303 ± 22	308 ± 23

High dose(NM) 110mg/kg b.w	254 ± 13.3	282 ± 21	288 ± 21	297 ± 22	302 ± 24	308 ± 25	311 ± 26	316 ± 27	321 ± 28	327 ± 29	332 ± 30	336 ± 31	342 ± 32

Groups	**On day**												
	98	**105**	**112**	**19**	**126**	**133**	**140**	**147**	**154**	**161**	**168**	**172**	**179**
Vehicle control (Palm jaggery solution)	311 ± 83	310 ± 88	311 ± 91	331 ± 92	333 ± 94	334 ± 94	335 ± 87	330 ± 94	329 ± 93	331 ± 90	334 ± 88	345 ± 84	352 ± 98
Low dose 9mg/kg b.w	295 ± 28	298 ± 29	301 ± 30	302 ± 31	306 ± 32	305 ± 32	300 ± 32	297 ± 31	299 ± 30	300 ± 29	302 ± 29	308 ± 33	319 ± 33
Mid dose 45mg/kg b.w	310 ± 24	313 ± 24	316 ± 25	320 ± 26	323 ± 26	323 ± 27	318 ± 26	314 ± 26	312 ± 25	313 ± 23	316 ± 23	324 ± 25	338 ± 26
High dose 110mg/kg b.w	344 ± 33	346 ± 34	349 ± 33	351 ± 34	355 ± 34	355 ± 35	350 ± 34	345 ± 33	342 ± 32	341 ± 31	344 ± 32	354 ± 33	362 ± 34

Groups	On day												
	1	7	14	28	35	42	49	56	63	70	77	84	91
Recovery control (Palm jaggery solution)	226 ± 33	262 ± 69	251 ± 51	255 ± 52	259 ± 55	265 ± 58	269 ± 61	272 ± 63	276 ± 68	282 ± 72.3	285 ± 74	288 ± 75.2	293 ± 77
High dose Recovery(NM) 110mg/kg b.w	224 ± 19	251 ± 40	255 ± 45	263 ± 49	267 ± 50	271 ± 53	275 ± 58	278 ± 58	282 ± 63	286 ± 65	292 ± 69	297 ± 72	302 ± 76

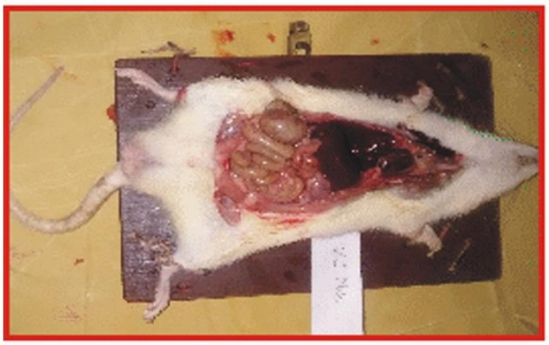
Groups	On day												
	98	105	112	119	126	133	140	147	154	161	168	175	182	193
Recovery control(Palm jaggery solution)	295 ± 14	298 ± 12	301 ± 12	302 ± 11	306 ± 11	305 ± 10	300 ± 9	297 ± 8	299 ± 10	300 ± 13	302 ± 16	310 ± 15	315 ± 14	319 ± 33
Highdose Recovery 110mg/kg b.w	311 ± 36	310 ± 39	311 ± 39	315 ± 47	317 ± 48	318 ± 48	321 ± 46	315 ± 54	314 ± 49	317 ± 51	320 ± 51	338 ± 50	345 ± 53	353 ± 98

Table No:7.13.2.Feed intake of animals in Sub chronic toxicity study

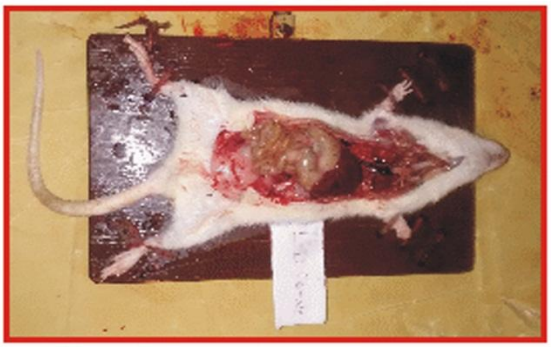
Weeks	Vehicle control	Low dose	Mid Dose	High dose	Recovery control	High Dose Recovery
1	78.9	78.8	79.2	80.2	78.7	80.1
2	77.4	77.2	77.4	79.5	77.5	79.5
3	78.5	78.9	79.4	80.1	79.1	80.2
4	76.4	77.4	78.6	79.2	78.4	79.4
5	77.9	78.2	79.8	79.9	77.2	80.4
6	76.3	77.7	78.6	078.4	76.9	79.1
7	78.2	78.9	79.7	80.4	78.2	80.2
8	77.4	77.9	78.7	79.2	77.9	79.7
9	78.7	79.2	79.4	79.9	78.2	80.4
10	78.2	78.4	78.2	78.6	77.5	79.5
11	78.9	79.1	79.7	80.3	78.7	80.1
12	77.4	78.2	78.1	79.2	77.9	79.2
13	78.2	79.2	79.8	80.5	77.5	79.9
14	77.8	78.3	78.4	79.1	78.5	78.4
15	78.7	79.4	79.6	80.7	77.7	80.2
16	77.2	78.4	78.5	79.4	76.5	79.2
17	77.6	79.3	79.9	80.4	77.9	80.3
18	77.1	78.5	78.4	79.5	76.8	79.6
19	77.8	79.7	79.6	80.3	77.7	79.9
20	77.5	78.6	78.9	79.4	76.5	79.2
21	77.9	79.4	79.4	80.2	77.5	80.1
22	77.3	78.7	78.7	79.8	77.1	79.6
23	78.2	79.7	79.6	80.5	77.8	80.5
24	77.5	78.5	78.6	79.5	77.2	79.8
25	78.0	79.5	79.5	80.4	77.9	80.4
26					77.5	79.7
27					77.8	80.5

Figure : OBSERVATION OF GROSS PATHOLOGY IN SUB CHRONIC TOXICITY STUDY OF NM IN WISTAR RATS

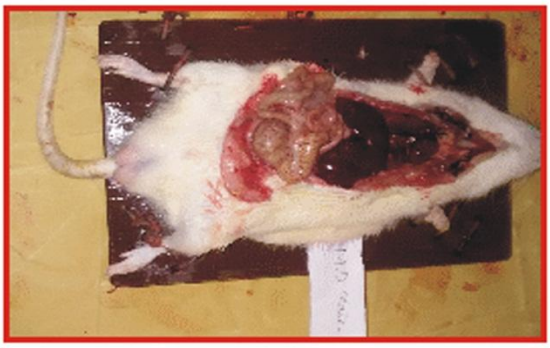
Vehicle control



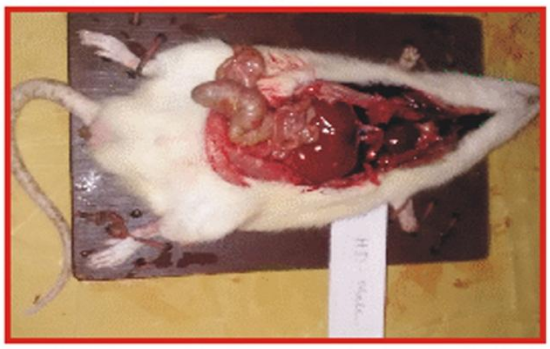
Low dose



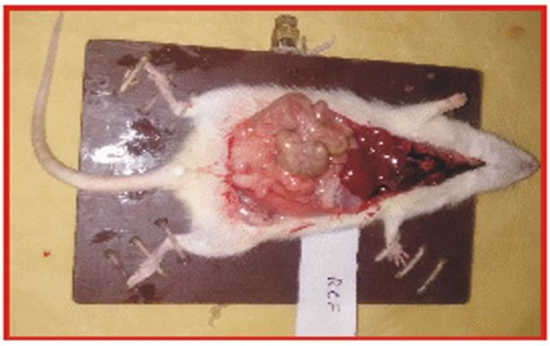
Mid dose



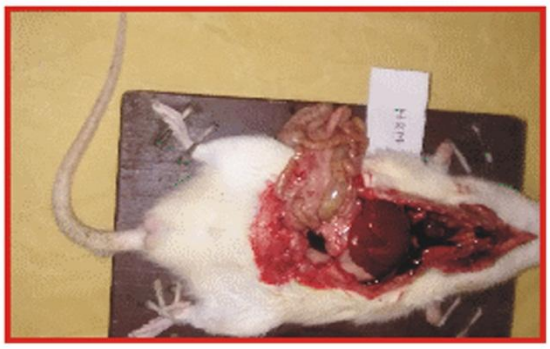
High dose



Recovery control



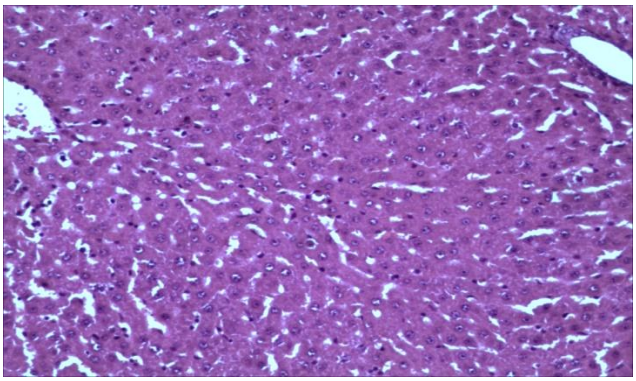
High dose recovery



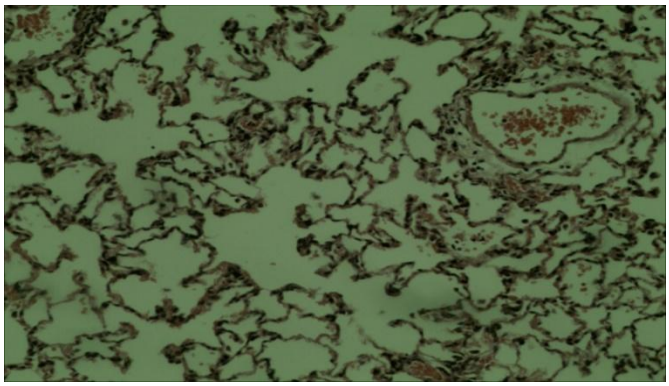
Effect of NM on Histopathalogical changes in Rat organs Sub chronic Toxicity study

SPECIMEN

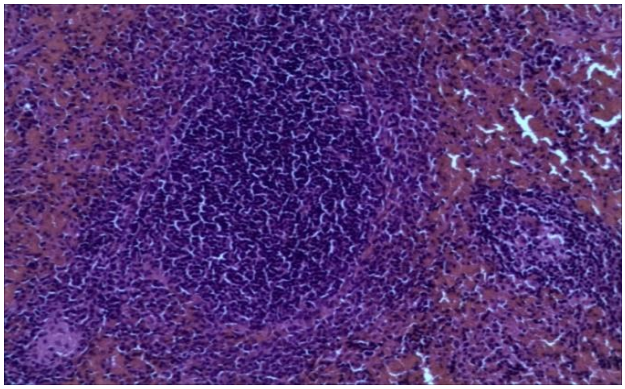
Study no: LBPL-HP-014/16 (Sub chronic Toxicity study)



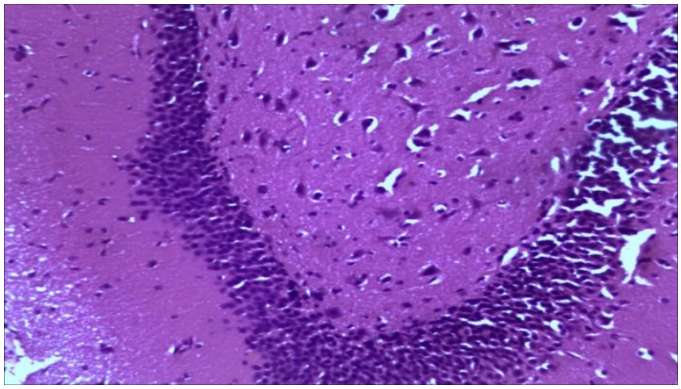
Liver : Normal, H&Ex10



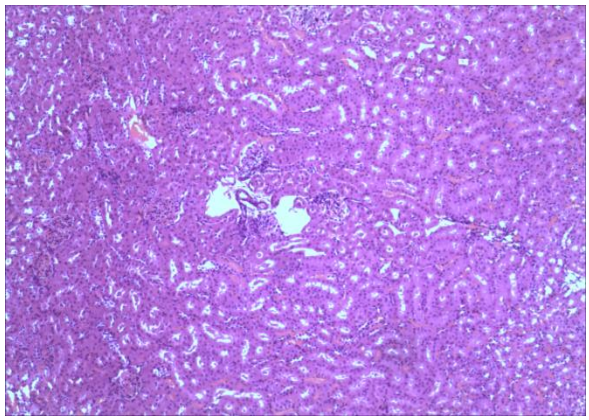
Lung : Normal, H&Ex10



Spleen : Normal, H&Ex10

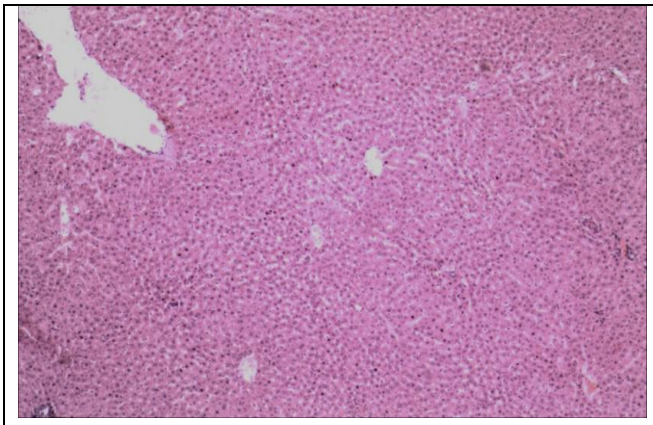
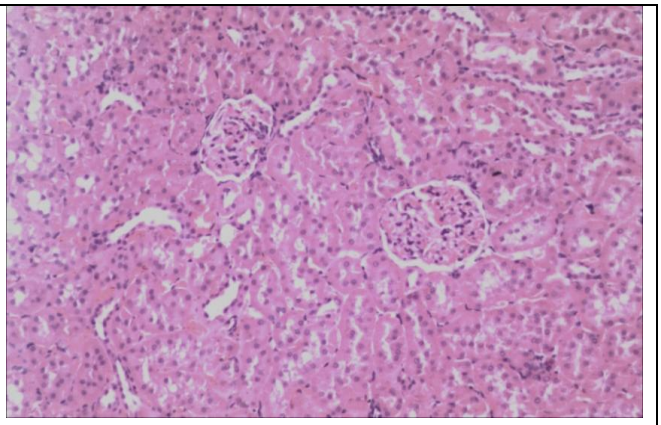
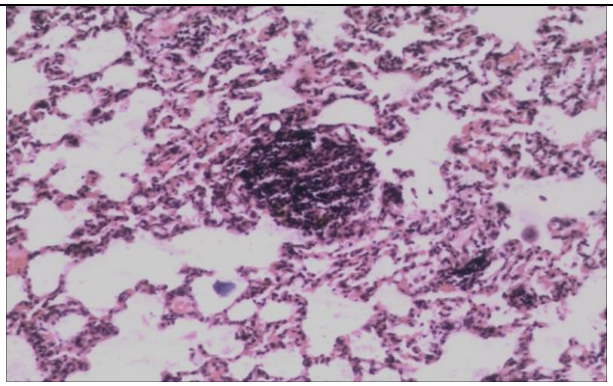
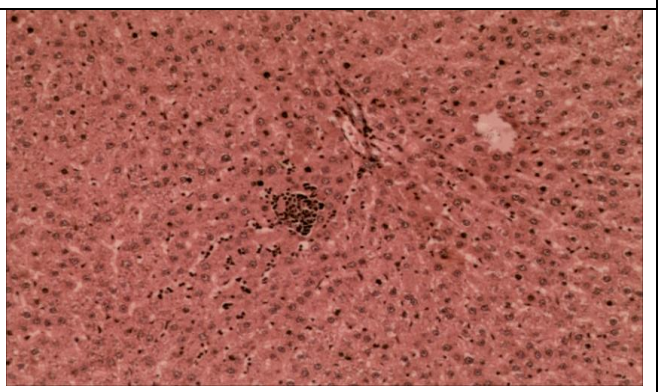
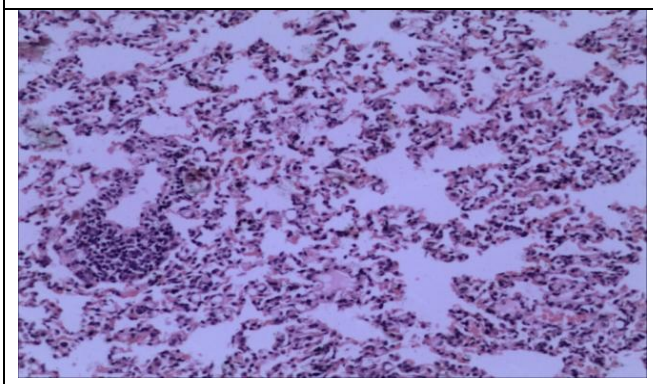
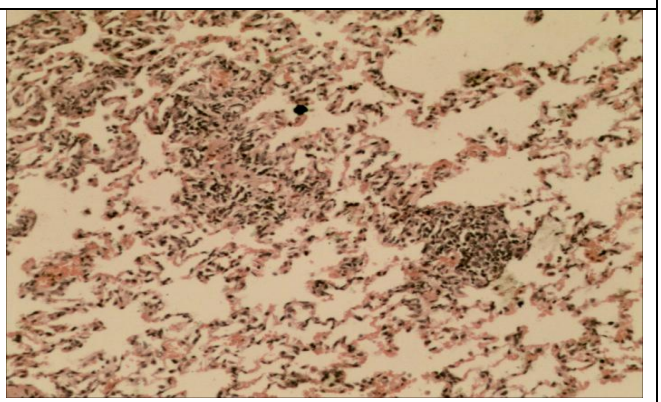


Brain : Normal, H&Ex10

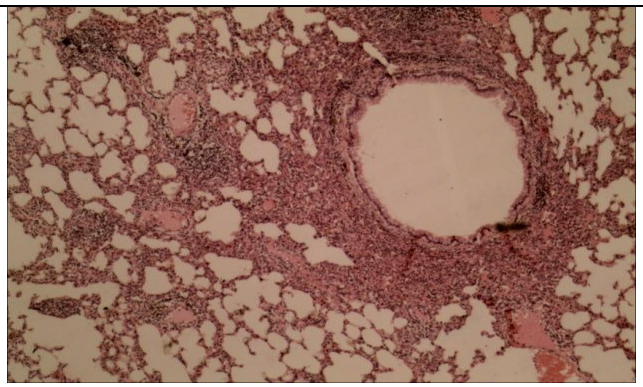
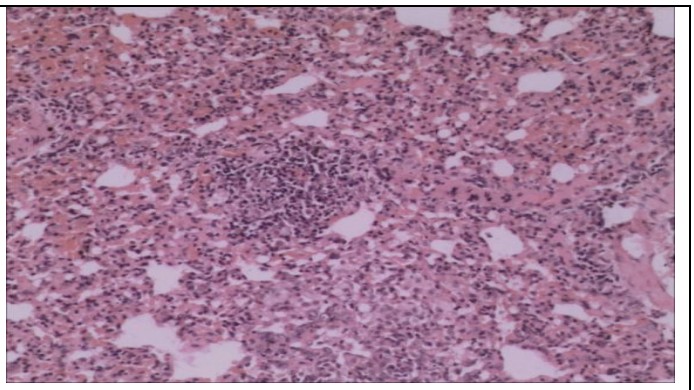
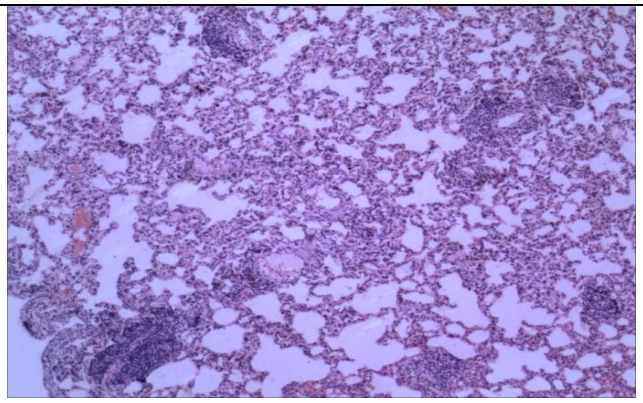
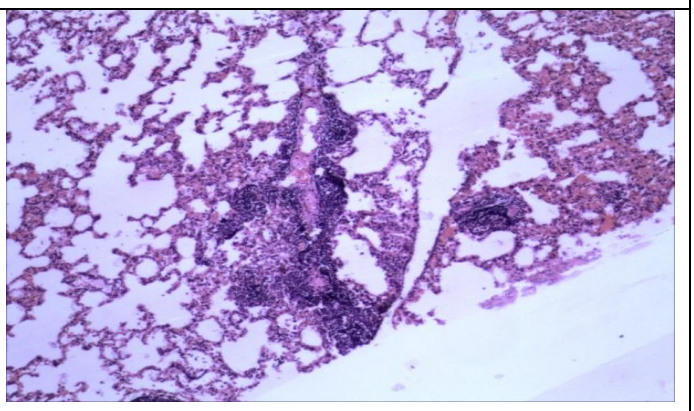
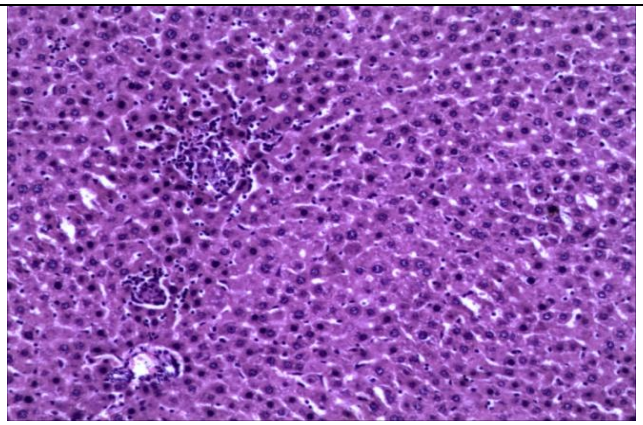
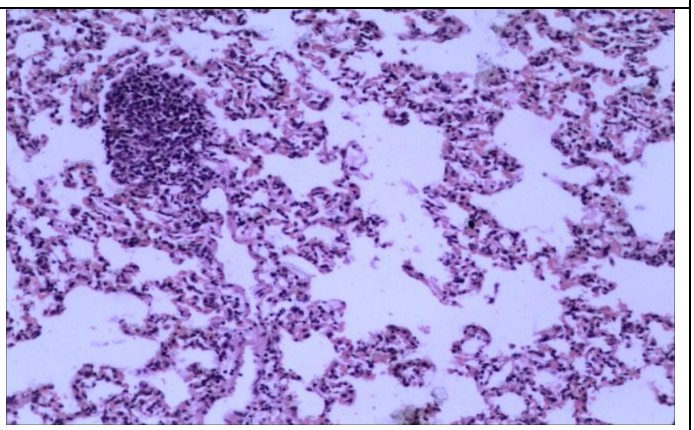


Kidney : Normal, H&Ex4

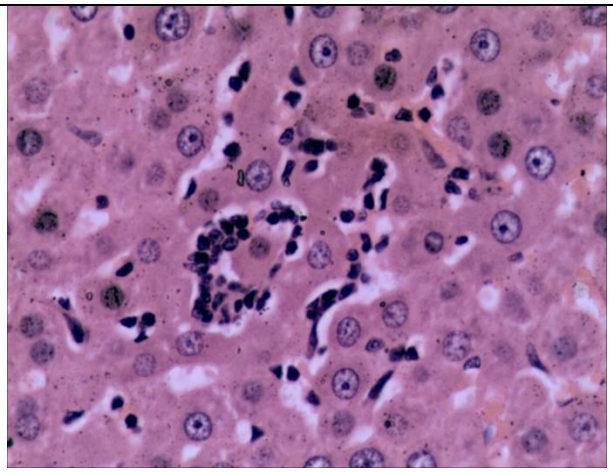
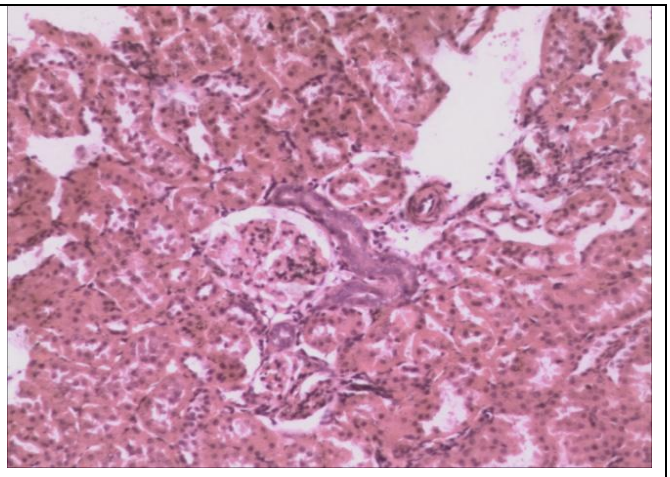
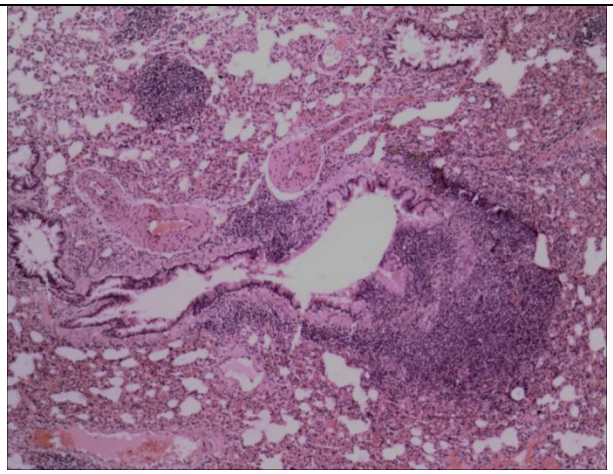
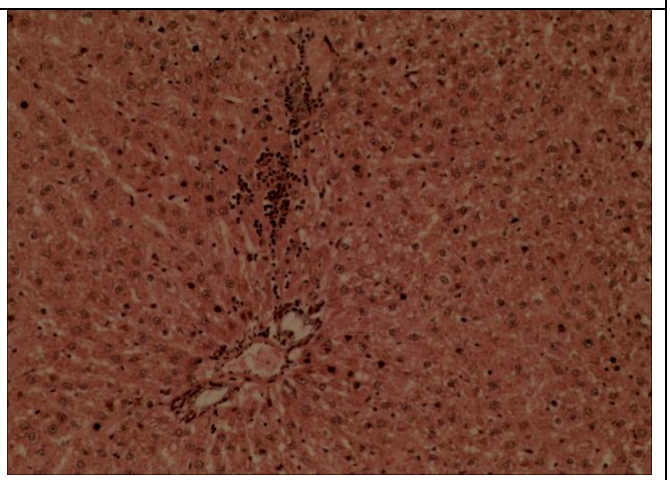
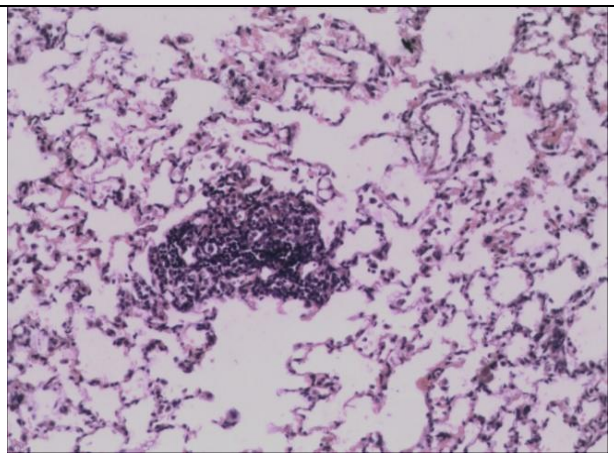
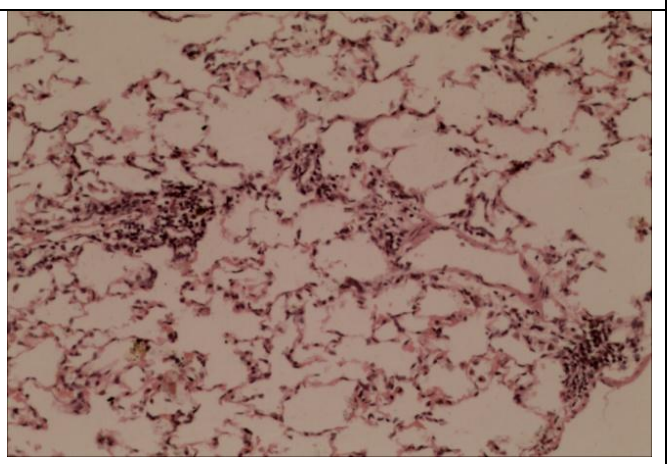
Study no: LBPL-HP-014/16 (Sub chronic Toxicity study

 HRM-B-I: NAD, H&E X4	 HRM-B-I: NAD, H&E X4
 HRM-B-III: MNC infiltration, H&E X10	 HRM-T-I: MNC infiltration, H&E X10
 HRM-T-III: MNC infiltration, H&E X10	 HRF-B-III: MNC infiltration, H&E X10

Study no: LBPL-HP-014/16 (Sub chronic Toxicity study

 HRF-B+T-III: MNC infiltration, H&E X10	 HDM-H -III: MNC infiltration, H&E X10
 HDM-H +B-III: MNC infiltration, H&E X4	 HDF-H +B-III: MNC infiltration, H&E X4
 HDF-B+T-I: MNC infiltration, H&E X10	 HDF-B+T-III: MNC infiltration, H&E X10

Study no: LBPL-HP-014/16 (Sub chronic Toxicity study

 RCM-T-I: MNC infiltration, H&E X40	 RCM-T-II: Basophilic tubule, H&E X10
 RCM-T-III:MNC infiltration, H&E X10	 VCM-T-I: MNC infiltration, H&E X10
 VCM-T-III: MNC infiltration, H&E X10	 VCF-T-III: MNC infiltration, H&E X10

HISTOPATHOLOGY REPORT

Ref Study no: LBPL-HP-014/16 (Sub chronic Toxicity study)

Organ/tissue: Liver, Kidney, Lung, Heart, Spleen, Stomach, Skin, Brain

The tissue samples were collected and preserved in neutral buffered formalin. The fixed tissues were processed, sectioned and stained with Heamatoxylin & eosin stain. The stained tissue sections from the different groups were examined microscopically for histopathological changes. The various histopathological or microscopic changes noticed in above mentioned organs/tissues from all the groups were recorded. Details of individual histopathological findings are presented in Table 1.

RESULTS:

There were no treatment related histopathological abnormalities in the organs examined microscopically. Histopathological changes noticed were within the normal histological limits for this particular species and strain.

TABLE 1B: INDIVIDUAL HISTOPATHOLOGICAL FINDINGS

Group	Slide no.	Tissue	Histopathology observations
HRM-B **High dose recovery Male**	HRM-B-I	Liver	NAD
	HRM-B-II	Kidney	NAD
	HRM-B-III	Lung	MNC infiltration <1>
	HRM-B-IV	Heart	NAD
	HRM-B-V	Spleen	NAD
	HRM-B-VI	Stomach	NAD
	HRM-B-VII	Skin	NAD
	HRM-B-VIII	Brain	NAD
HRM-T	HRM-T-I	Liver	MNC infiltration

High dose recovery Male			<1>
	HRM-T-II	Kidney	NAD
	HRM-T-III	Lung	MNC infiltration <2>
	HRM-T-IV	Heart	NAD
	HRM-T-V	Spleen	NAD
	HRM-T-VI	Stomach	NAD
	HRM-T-VII	Skin	NAD
	HRM-T-VIII	Brain	NAD
HRF-B **High dose recovery female**	HRF-B-I	Liver	NAD
	HRF-B-II	Kidney	MNC infiltration <1>
	HRF-B-III	Lung	MNC infiltration <1>
	HRF-B-IV	Heart	NAD
	HRF-B-V	Spleen	NAD
	HRF-B-VI	Stomach	NAD
	HRF-B-VII	Skin	NAD
	HRF-B-VIII	Brain	NAD
HRF-B+T **High dose recovery female**	HRF-B+T-I	Liver	NAD
	HRF-B+T-II	Kidney	NAD
	HRF-	Lung	MNC infiltration

	B+T-III		<1>
	HRF-B+T-IV	Heart	NAD
	HRF-B+T-V	Spleen	NAD
	HRF-B+T-VI	Stomach	NAD
	HRF-B+T-VII	Skin	NAD
	HRF-B+T-VIII	Brain	NAD
HDM-H **High dose male**	HDM-H-I	Liver	NAD
	HDM-H-II	Kidney	NAD
	HDM-H-III	Lung	MNC infiltration <1>
	HDM-H-IV	Heart	NAD
	HDM-H-V	Spleen	NAD
	HDM-H-VI	Stomach	NAD
	HDM-H-VII	Skin	NAD
	HDM-H-VIII	Brain	NAD
HDM-H+B **High dose male**	HDM-H+B-I	Liver	NAD
	HDM-H+B-II	Kidney	NAD
	HDM-H+B-III	Lung	MNC infiltration <2>

	HDM-H+B-IV	Heart	NAD
	HDM-H+B-V	Spleen	NAD
	HDM-H+B-VI	Stomach	NAD
	HDM-H+B-VII	Skin	NAD
	HDM-H+B-VIII	Brain	NAD
HDF-H+B **High dose female**	HDF-H+B-I	Liver	NAD
	HDF-H+B-II	Kidney	NAD
	HDF-H+B-III	Lung	MNC infiltration <2>
	HDF-H+B-IV	Heart	NAD
	HDF-H+B-V	Spleen	NAD
	HDF-H+B-VI	Stomach	NAD
	HDF-H+B-VII	Skin	NAD
	HDF-H+B-VIII	Brain	NAD
HDF-B+T **High dose female**	HDF-B+T-I	Liver	MNC infiltration <1>
	HDF-B+T-II	Kidney	NAD
	HDF-B+T-III	Lung	MNC infiltration <1>

	HDF-B+T-IV	Heart	NAD
	HDF-B+T-V	Spleen	NAD
	HDF-B+T-VI	Stomach	NAD
	HDF-B+T-VII	Skin	NAD
	HDF-B+T-VIII	Brain	NAD
RCM-T **Recovery control male**	RCM-T-I	Liver	MNC Infiltration <1>
	RCM-T-II	Kidney	Dilated tubules with eosinophilic material <1>, Basophilic tubules <1>
	RCM-T-III	Lung	MNC infiltration <1>
	RCM-T-IV	Heart	NAD
	RCM-T-V	Spleen	NAD
	RCM-T-VI	Stomach	NAD
	RCM-T-VII	Skin	NAD
	RCM-T-VIII	Brain	NAD
RCF-H **Recovery control Female**	RCF-H-I	Liver	NAD
	RCF-H-II	Kidney	NAD
	RCF-H-III	Lung	NAD
	RCF-H-IV	Heart	NAD

	RCF-H-V	Spleen	NAD
	RCF-H-VI	Stomach	NAD
	RCF-H-VII	Skin	NAD
	RCF-H-VIII	Brain	NAD
VCM-T **Vechicle control Male**	VCM-T-I	Liver	MNC infiltration (1)
	VCM-T-II	Kidney	NAD
	VCM-T-III	Lung	MNC infiltration <1>
	VCM-T-IV	Heart	NAD
	VCM-T-V	Spleen	NAD
	VCM-T-VI	Stomach	NAD
	VCM-T-VII	Skin	NAD
	VCM-T-VIII	Brain	NAD
VCF-T **Vechicle control Female**	VCF-T-I	Liver	NAD
	VCF-T-II	Kidney	NAD
	VCF-T-III	Lung	MNC infiltration <1>
	VCF-T-IV	Heart	NAD
	VCF-T-V	Spleen	NAD
	VCF-T-VI	Stomach	NAD
	VCF-T-VII	Skin	NAD
	VCF-T-VIII	Brain	NAD

Note: Severity grading: 1-minimal, 2-mild, 3-moderate, 4-marked, Distribution of lesions: [ ]: diffuse, <>-multifocal, ( )-focal, NAD-No Abnormalities Detected, MNC-Mononuclear cell infiltration

Table No:7.13.3.Effect of Nandhi Mezhugu on Hematological Parameters of experimental Wistar rats in sub-chronic oral toxicity study

Groups	WBC × 10^9/L	Lymph × 10^9/L	Mon × 10^9/L	Gran × 10^9/L	Lymph %	Mono %	Gran %	RBC × 10^{12}/L	HGB g/dL
Vechicle control(Palm jaggery solution)	6.24 ± 3.5	3.96 ± 2.8	0.24 ± 0.05	2.04 ± 0.8	60.2 ± 11.3	4.7 ± 1.4	35.14 ± 10	10.42 ± 3.6	16.98 ± 6
Low dose 9mg/kg b.w	5.71 ± 1.23	3.78 ± 0.8	0.25 ± 0.06	1.68 ± 0.39****	66.53 ± 1.3	4.05 ± 0.18	29.4 ± 1.3	7.7 ± 0.9	12.26 ± 1.4
Mid dose 45mg/kg b.w	6.62 ± 1.54	4.53 ± 1.03	0.27 ± 0.07	2.31 ± 0.57***	61.15 ± 6.8	4 ± 0.17	26.79 ± 1.63	8.54 ± 1.15	14.07 ± 1.9
High dose 110mg/kg b.w	6.36 ± 0.4	4.22 ± 1.03	0.29 ± 0.06	1.85 ± 0.2***	66.88 ± 2.7	4.28 ± 0.83	28.82 ± 1.63	9.05 ± 0.66	15.07 ± 1.4

All values are mean ± SE. Statistical analysis by one-way ANOVA followed by Dunnett's Multiple Comparison. *p < 0.05. **p < 0.01

Recovery Group

Groups	WBC × 10^9/L	Lymph × 10^9/L	Mon × 10^9/L	Gran × 10^9/L	Lymph %	Mono %	Gran %	RBC × 10^{12}/L	HGB g/dL
Recovery Control(Palm jaggery solution)	5.05 ± 1.4	3.16 ± 0.9	0.2 ± 0.07	1.66 ± 0.4	61.45 ± 1.7	4.65 ± 0.3	33.9 ± 1.6	5.9 ± 0.53	9.56 ± 0.9
High dose recovery 110mg/kg b.w	3.43 ± 1.7	2.18 ± 1.14	0.1 ± 0.06	1.15 ± 0.52	55.6 ± 5	4.5 ± 0.7	39.8 ± 4.5	4.2 ± 1.3	6.91 ± 2.2

All values are mean ± SE. Statistical analysis by one-way ANOVA followed by Dunnett's Multiple Comparison. *$p < 0.05$. **$p < 0.01$

Groups	HCT %	MCV fL	MCH pg	MCHC g/dL	RDW %	PLT × 10^9/L	MPV fL	PDW	PCT %
Vehicle control(Palm jaggery solution)	51.9 ± 18	49.6 ± 2.5	16.2 ± 0.66	32.8 ± 0.8	12.4 ± 0.9	408.2 ± 198	6.92 ± 0.4	15.3 ± 0.3	0.28 ± 0.13
Low dose 9mg/kg b.w	37.5 ± 4.1	49.2 ± 1	15.9 ± 0.3	32.5 ± 0.33	12.24 ± 0.24	301.6 ± 55.2	6 ± 0.088	15.05 ± 0.09	0.19 ± 0.03
Mid dose 45mg/kg b.w	41.9 ± 5.7	48.9 ± 0.7	16.3 ± 0.2	33.6 ± 0.17	11.6 ± 0.35	319.2 ± 62.2	6.64 ± 0.15	15.09 ± 0.15	0.2 ± 0.03
High dose 110mg/kg b.w	45.4 ± 3.7	50.0 ± 0.9	16.5 ± 0.33	33.1 ± 0.4	12 ± 0.64	242.66 ± 37.5	6.88 ± 0.12	15.37 ± 0.11	0.16 ± 0.02

All values are mean ± SE. Statistical analysis by one-way ANOVA followed by Dunnett's Multiple Comparison. *p < 0.05. **p < 0.01

Recovery Group:

Groups	HCT %	MCV fL	MCH pg	MCHC g/dL	RDW %	PLT × 10^9/L	MPV fl	PDW	PCT %
Recovery Control(Palm jaggery solution)	28.95 ± 2.6	48.7 ± 0.9	16.0 ± 0.3	33.0 ± 0.3	10.91 ± 0.5	338.3 ± 180	6.66 ± 0.1	15.36 ± 0.2	0.090 ± 0.03
High dose recovery 110mg/kg b.w	20.56 ± 6.4	48.5 ± 0.8	15.8 ± 0.4	32.7 ± 0.6	10.48 ± 0.2	170.5 ± 61	6.75 ± 0.2	15.76 ± 0.2	0.108 ± 0.03

All values are mean ± SE. Statistical analysis by one-way ANOVA followed by Dunnett's Multiple Comparison. *p< 0.05. **p< 0.01

Table No:7.13.4.Effect of Nandhi Mezhugu on serum biochemical parameters of experimental Wistar rats sub-chronic oral toxicity study

Groups	Glucose mg/dl	Urea mg/dl	Creatinine mg/dl	Cholestrol mg/dl	TGL mg/dl	HDL mg/dl	LDL mg/dl
Vechicle control(Palm jaggery solution)	172.4 ± 3.5	34.0 ± 2.4	0.7 ± 0.02	100.6 ± 5.3	131.4 ± 17	26.0 ± 2	48.4 ± 3.5
Low dose 9mg/kg b.w	149.2 ± 5.7	32.1 ± 3	0.7 ± 0.05	91.0 ± 7.5	100.5 ± 13	22.5 ± 1	48.5 ± 6.7
Mid dose 45mg/kg b.w	132.5 ± 11.3*	26.6 ± 2.7	0.7 ± 0.04	81.4 ± 3.2*	146.4 ± 13	22.2 ± 1	29.8 ± 3.0*
High dose 110mg/kg b.w	160.7 ± 6.6	27.3 ± 1.5	0.6 ± 0.02	85.7 ± 3.3	141 ± 18	20.79 ± 1*	36.7 ± 3.6

All values are mean ± SE. Statistical analysis by one-way ANOVA followed by Dunnett's Multiple Comparison. *p < 0.05. **p < 0.01

Recovery control

Groups	Glucose mg/dl	Urea mg/dl	Creatinine mg/dl	Cholestrol mg/dl	TGL mg/dl	HDL mg/dl	LDL mg/dl
Recovery Control(Palm jaggery solution)	139 ± 5.9	35 ± 0.9	0.41 ± 0.01	75 ± 4	118 ± 15	24.8 ± 1.19	31 ± 1.88
High dose Recovery 110mg/kg b.w	146 ± 9.6	37 ± 0.73	0.31 ± 0.03	76 ± 3.9	131 ± 22	26.6 ± 1.9	31 ± 4.9

All values are mean ± SE. Statistical analysis by one-way ANOVA followed by Dunnett's Multiple Comparison. *p < 0.05. **p < 0.01

Groups	TRP g/dl	ALB g/dl	SBIL mg/dl	SGOT IU/L	SGPT IU/L	SAP IU/L
Vechicle control(Palm jaggery solution)	7.04 ± 0.25	2.82 ± 0.19	0.12 ± 0.02	312.4 ± 30.7	96.0 ± 2.9	198.2 ± 18.3
Low dose 9mg/kg b.w	6.88 ± 0.28	2.83 ± 0.22	0.13 ± 0.02	236.3 ± 57.3	80.8 ± 10	170.7 ± 13.7
Mid dose 45mg/kg b.w	6.67 ± 0.11	2.84 ± 0.19	0.10 ± 0.0	238.8 ± 16.0	69.3 ± 3.2**	146.1 ± 16.2
High dose 110mg/kg b.w	6.89 ± 0.06	2.82 ± 0.09	0.11 ± 0.01	216.3 ± 13.5	67.6 ± 3.2**	114.4 ± 9.0**

All values are mean ± SE. Statistical analysis by one-way ANOVA followed by Dunnett's Multiple Comparison. *p < 0.05. **p < 0.01

Groups	TRP g/dl	ALB g/dl	SBIL mg/dl	SGOT IU/L	SGPT IU/L	SAP IU/L
Recovery Control(Palm jaggery solution)	6.8 ± 0.22	2.3 ± 0.18	0.1 ± 0.02	191.5 ± 13.3	66 ± 7.6	181.16 ± 18.4
High dose Recovery 110mg/kg b.w	6.3 ± 0.18	2.3 ± 0.14	0.1 ± 0.02	192.33 ± 23.2	67 ± 5.2	178.66 ± 12.3

All values are mean ± SE. Statistical analysis by one-way ANOVA followed by Dunnett's Multiple Comparison. *p < 0.05. **p< 0.01

Table No: 7.13.5.Effect of Nandhi Mezhugu on relative Organ weight of experimental Wistar rats in Sub-chronic oral toxicity study

Groups	Brain	Heart	Lungs	Liver	Spleen	Kidney
Vehicle control(Palm jaggery solution)	1.31 ± 0.03	0.338 ± 0.02	0.728 ± 0.04	2.359 ± 0.18	0.231 ± 0.01	0.837 ± 0.05
Low dose 9mg/kg b.w	1.30 ± 0.02	0.373 ± 0.02	0.803 ± 0.03	2.585 ± 0.22	0.244 ± 0.01	0.900 ± 0.02
Mid dose 45mg/kg b.w	1.36 ± 0.02	0.329 ± 0.01	0.712 ± 0.03	2.398 ± 0.15	0.227 ± 0.01	0.852 ± 0.05
High dose 110mg/kg b.w	1.34 ± 0.02	0.320 ± 0.01	0.754 ± 0.02	2.283 ± 0.18	0.211 ± 0.01	0.813 ± 0.03

All values are mean ± SE. Statistical analysis by one-way ANOVA followed by Dunnett's Multiple Comparison. *p < 0.05. **p < 0.01

Recovery control

Recovery control(Palm jaggery solution)	1.33 ± 0.03	0.368 ± 0.01	0.797 ± 0.03	2.546 ± 0.24	0.241 ± 0.01	0.900 ± 0.05
High dose recovery 110mg/kg b.w	1.32 ± 0.03	0.351 ± 0.01	0.786 ± 0.02	2.392 ± 0.19	0.223 ± 0.01	0.824 ± 0.04

All values are mean ± SE. Statistical analysis by one-way ANOVA followed by Dunnett's Multiple Comparison. *p < 0.05. **p < 0.01

Results of Sub chronic toxicity

There were no treatment-related toxicity signs and mortality observed in both sexes of rats treated with Nandhi mezhugu at a dose of 9, 45 and 110 mg/kg b.w orally for a period of 90 days (7 days drug dosing followed by 7 days drug holidays) and in the recovery group of rats. No significant difference in body weight gain was observed between control and treated groups during the study (Table 7.13.1). Feed consumption of NM treated groups were found to be non-significant in both the sexes when compared to control study (Table 7.13.2). Haematological parameters such as haemoglobin, red blood cells, white blood cells, mean corpuscular haemoglobin, were found to be well within the normal range in rats of experimental groups (Table 7.13.3). All the parameters in serum biochemical profiles such as glucose, total cholesterol, triglycerides, total protein, alkaline phosphatase, creatinine, blood urea were within the normal range (Table 7.13.4). The levels of liver marker enzymes like SGOT and SGPT were found to be well within the clinical range in rats of NM treated groups (Table 7.13.4). There were no significant differences in absolute/relative organ weight of brain, heart, liver, lungs, kidneys recorded between the control and NM groups (Table 7.13.5). In my study, histopathological examinations in control and high dose group revealed no abnormalities. There were no haematological, biochemical and histopathological alterations observed with NM administration even at 110 mg/kg b.w twice daily in rats for a period of 90 days compared

to control. The No Observed Adverse Effect Level (NOAEL) of NM was estimated to be greater than 110 mg/kg/day in rats. Hence, it can be concluded that NM is safe for oral administration.

7.14. Pharmacological activity:

7.14.1. Analgesic activity:

Acetic acid induced writhing method:

Groups	onset of Writhing (sec)	No of writhing
Control(0.1ml glacial acetic acid+Palm jaggery solution)	4.3±0.3	17±3.3
Standard((0.1ml glacial acetic acid +Disprin 50mg)	3.8±1.1	3±0.9****
Low dose(0.1ml glacial acetic acid+Palm jaggery solution+NM 25mg)	2.3±2.5	8.2±0.6**
Mid dose(0.1ml glacial acetic acid+Palm jaggery solution+NM 40mg)	6.8±1.6	4.7±0.2***
High dose(0.1ml glacial acetic acid+Palm jaggery solution+NM 50mg)	11.8±2.5	2.3±0.6****

All values are mean ± SE. Statistical analysis by one-way ANOVA followed by Dunnett's Multiple Comparison. **$p < 0.01$, ***$p < 0.001$,****$p < 0.0001$

Result: The high dose group (NM 50mg) showed significant analgesic activity when compared to standard drug.It was inferred by the delayed onset of writhing and the reduction in the number of writhing.

7.15. Acute anti-inflammatory activity Model

Carrageenan induced paw odema method:

Table no: 7.15.1.Effect of Nandhi mezhugu on Carrageenan induced paw odema method

Groups	0 hour Paw Volume			Left paw volume			
	Left Paw	**Right Paw**	**After Administrating Carrageenan 0.01ml Lt Paw Volume**	**1 hr**	**2 hr**	**4 hr**	**6 hr**
Standard (**Diclofenac sodium 25mg/kg b.w)**	1.022 ± 0.081	1.022 ± 0.081	1.392 ± 0.156	1.415 ± 0.081	1.267 ± 0.125	1.103 ± 0.172	0.950 ± 0.095
Low dose(NM) **90mg/kg b.w**	1.332 ± 0.137	1.223 ± 0.140	1.485 ± 0.108	1.435 ± 0.120	1.385 ± 0.223	1.413 ± 0.179	1.085 ± 0.106
Mid dose(NM) **450 mg/kg b.w**	1.263 ± 0.092	1.193 ± 0.106	1.572 ± 0.230	1.3 ± 0.161	1.382 ± 0.192	1.333 ± 0.119	0.955 ± 0.09
High dose(NM) **900mg/kg b.w**	1.15 ± 0.147	1.027 ± 0.136	1.537 ± 0.230	1.322 ± 0.173	1.268 ± 0.161	1.118 ± 0.173	0.908 ± 0.034

Values are Mean± SD

Table no: 7.15.2.Percentage of Inhibition in Carrageenan induced paw odema method

Groups	% Inhibition			
	1 Hr	2Hr	4Hr	6Hr
Standard Standard (**Diclofenac sodium 25mg/kg b.w)**	-2.3	8.08	19.07	31.48
Low dose(NM) **90mg/kg b.w**	3.24	7.09	4.73	26.69
Mid dose(NM) **450 mg/kg b.w**	15.4	10.7	13.63	37.86
High dose(NM) **900mg/kg b.w**	11.35	15.51	26.04	39.95

Paw volume was reduced significantly in high dose group with that of standard. Percentage of inhibition in high dose group was comparatively high when compared to that of standard.

7.16. Chronic anti-inflammatory activity:

Cotton pellet granuloma method:

Results:

a) Body weight (g) and Body weight gain (%):

Summary of male and female animals body weight (g) and body weight gain (%) are presented in Table-7.16.1 and Table-7.16.2

There were no biologically significant changes observed in body weight (g) and body weight gain (%) in all group animals.

b) Clinical signs observation:

All the animals were found to be normal in their health condition during entire experimental duration.

c) Weights of dry and wet weights of cotton pellets and Percentage inhibition of Granuloma tissue formation :

Summary of dry & wet (initial and final) weights of the cotton pellets of males and females are presented in table 7.16.3 & 7.16.4 respectively.

Percentage inhibition of granuloma tissue formation of males and females are presented in Table-7.16.5.

Dose dependent inhibition of granuloma tissue formation was observed in dexamethasone treated group animals and test item treated group animals when compared with the control group animals.

d) Haematology parameters:

Summary of haematology parameters are presented in Table-7.16.6 and Table-7.16.7.

Table 7.16.1

SUMMARY OF WEEKLY ANIMALS BODY WEIGHT (g) AND BODY WEIGHT GAIN (%) OF MALES

Group	Treatment and Dose(mg/kg)	DAYS (Body wt in mean and SE)		
		1	**7**	**1-7**
G1	Vehicle Control (Palm jaggery solution)	203.35 ± 12.99	214.79± 5.13	5.88 ±s 5.19
G2	Positive Control (Dexamethasone) – 0.5	203.77 ± 10.56	215.60± 11.91	5.82 ± 2.51
G3	Nandhi mezhugu – 50	195.38 ± 19.22	204.56± 14.33	5.00 ± 5.73
G4	Nandhi mezhugu-150	207.67 ± 20.04	218.77± 17.33	5.54 ± 4.13
G5	Nandhi mezhugu -500	202.23 ± 11.52	213.86± 16.80	5.65 ± 2.85

n=5; Values are Mean, ±Standard Deviation; p value

Table 7.16.2.WEEKLY ANIMALS BODY WEIGHT (g) AND BODY WEIGHT GAIN (%) OF FEMALES

Group	Treatment and Dose(mg/kg)	DAYS		
		1	**7**	**1-7**
G1	Vehicle Control(Palm jaggery solution)	182.71 ± 12.76	195.49 ± 12.26	7.08 ± 3.76
G2	Positive Control (Dexamethasone) – 0.5	184.00 ± 10.48	194.20 ± 12.27	5.60 ± 4.67
G3	Nandhi mezhugu - 50	182.07 ± 5.25	195.08 ± 13.22	7.06 ± 4.27
G4	Nandhi mezhugu- 150	186.82 ± 12.13	203.43 ± 23.84	8.77 ± 9.06
G5	Nandhi mezhugu- 500	193.97 ± 13.56	206.85 ± 9.69	6.82 ± 4.41

n=5; Values are Mean, ±Standard Deviation;

TABLE – 7.16.3 SUMMARY OF DRY AND WET COTTON PELLET WEIGHTS (mg) OF MALES

Group	Treatment and Dose(mg/kg)	INITIAL				FINAL			
		Right groin		**Left groin**		**Right groin**		**Left groin**	
		Dry	**Wet**	**Dry**	**Wet**	**Dry**	**Wet**	**Dry**	**Wet**
G1	Vehicle Control (Palm jaggery solution)	10.22 ±0.56	68.72 ±10.50	10.26 ±0.43	65.38 ±10.82	36.64 ±3.49	181.24 ±13.18	35.66 ±2.04	1 14.90 ± 16.20
G2	Positive Control (Dexamethasone) – 0.5	10.46 ±0.44	63.12 ±4.99	10.18 ±0.61	54.56 ±18.01	20.86** ±2.01	111.86* ±14.95	21.18*** ±2.17	5 0.32*** ± 21.39

G3	Nandhi mezhugu -50	10.12 ±0.50	52.90 ±13.50	10.24 ±0.32	46.88 ±4.38	29.50* ±6.64	133.26* ±11.70	28.26*** ±3.61	83.72*** ± 11.73
G4	Nandhi mezhugu -150	10.18 ±0.61	50.46 ±12.62	10.00 ±0.34	43.90 ±13.37	27.34** ±2.08	122.08* ±16.62	26.28*** ±4.33	8 3.96*** ± 27.35
G5	Nandhi mezhugu-500	10.50 ±0.25	75.18 ±11.04	10.44 ±0.61	76.28 ±14.71	23.80** ±4.21	124.84* ±12.54	24.88*** ±1.05	4 2.04*** 13.10

n=5; Values are Mean, ±Standard Deviation; p-value

TABLE – 7.16.4 SUMMARY OF DRY AND WET COTTON PELLET WEIGHTS (mg) OF FEMALES

Group	Treatment and Dose(mg/ kg)	INITIAL				FINAL			
		Right groin		**Left groin**		**Right groin**		**Left groin**	
		Dry	**Wet**	**Dry**	**Wet**	**Dry**	**Wet**	**Dry**	**Wet**
G1	Vehicle Control (Palm jaggery solution)	9.90 ±1.04	48.56 ±7.28	10.34 ±0.57	43.38 ±5.24	35.36 ±1.73	179.92 ±12.38	33.80 ±3.87	131.74 ±12.32

G2	Positive Control (Dexamethasone) – 0.5	10.34 ±0.91	44.14 ±8.29	10.82 ±0.94	48.20 ±8.50	20.84** ±1.81	120.96*** ±9.90	20.30** ±1.12	68.98*** ±18.10
G3	Nandhi mezhugu - 50	10.76 ±0.89	45.54 ±2.81	9.40 ±0.96	46.56 ±5.92	27.46** ±3.12	131.42*** ±7.67	26.74* ±4.68	86.08*** ±7.78
G4	Nandhi mezhugu- 150	10.44 ±0.85	47.68 ±7.94	10.28 ±0.18	46.18 ±6.36	24.52** ±2.09	128.40*** ±12.05	25.76** ±3.47	83.08*** ±13.42
G5	Nandhi mezhugu - 500	9.92 ±0.85	40.94 ±6.49	10.74 ±0.90	40.64 ±5.36	23.10** ±4.80	127.16*** ±13.34	24.10** ±3.53	86.90*** ±13.67

n=5; Values are Mean, ±Standard Deviation;

TABLE – 7.16.5 PERCENTAGE INHIBITION OF GRANULOMA TISSUE FORMATION OF MALES & FEMALES

Group	Treatment and Dose(mg/kg)	MALES				FEMALES			
		Right groin		**Left groin**		**Right groin**		**Left groin**	
		Dry	**Wet**	**Dry**	**Wet**	**Dry**	**Wet**	**Dry**	**Wet**
G1	Vehicle Control (Palm jaggery solution)	-	-	-	-	-	-	-	-

G2	Positive Control (Dexamethasone) – 0.5	43.07	38.28	40.61	41.82	41.06	32.77	39.94	33.09
G3	Nandhi mezhugu-50	19.49	26.47	20.75	27.56	22.34	26.96	20.89	24.26
G4	Nandhi mezhugu -150	25.38	32.64	26.30	29.08	30.66	28.63	23.79	26.19
G5	Nandhi mezhugu-500	35.04	31.12	30.23	34.37	34.67	29.32	28.70	27.17

n=5; Values are in percentage.

TABLE –7.16.6 SUMMARY OF HEAMATOLOGY PARAMETERS OF MALES

Groups	Treatment (mg/kg)	WBC (10^3 cells/µl)	RBC (10^6 cells/µl)	HgB (g/dl)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dl)	PLT (10^3 cells/µl)
G1	Vehicle Control (Palm jaggery solution)	18.90 ±6.73	7.13 ±0.42	12.68 ±0.72	41.86 ±2.29	58.74 ±1.06	17.80 ±0.66	30.30 ±0.77	779.00 ±45.59
G2	Positive Control (Dexamethasone) – 0.5	16.86 ±2.46	7.72 ±0.92	13.60 ±0.58	45.40* ±1.91	59.30 ±4.97	17.78 ±1.51	29.94 ±0.15	745.80 ±325.69
G3	Nandhi mezhugu-50	17.24 ±5.79	7.79 ±0.31	13.48 ±0.49	45.04* ±1.28	57.82 ±1.04	17.30 ±0.60	29.92 ±0.60	835.40 ±226.66
G4	Nandhi mezhugu-150	17.82 ±7.29	7.38 ±0.37	13.10 ±0.44	43.02 ±1.58	58.30 ±1.80	17.74 ±0.54	30.46 ±0.29	876.80 ±174.26
G5	Nandhi mezhugu - 500	20.06 ±4.90	7.40 ±0.35	13.46 ±0.62	43.78 ±1.96	59.22 ±2.48	18.20 ±0.95	30.78 ±0.44	900.80 ±194.52

n=5; Values are Mean, ±Standard Deviation

TABLE – 7.16.7.SUMMARY OF HEMATOLOGY PARAMETERS OF FEMALES

Group	Treatment (mg/kg)	WBC (10^3 cells/µl)	RBC (10^6 cells/µl)	HGB (g/dl)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dl)	PLT (10^3 cells/µl)
G1	Vehicle Control (Palm jaggery solution)	17.38 ±3.18	7.16 ±0.45	12.92 ±0.56	41.28 ±2.01	57.68 ±1.25	18.06 ± 0.58	31.28 ±0.55	816.60 ±130.96
G2	Positive Control (Dexamethasone) – 0.5	13.92 ±3.44	7.23 ±0.34	13.18 ±0.52	42.40 ±1.56	58.76 ±2.53	18.2 ± 0.92	31.08 ±0.26	854.60 ±123.84
G3	Nandhi mezhugu-50	15.34 ±2.60	6.79 ±0.87	12.70 ±0.87	40.32 ±2.67	59.80 ±3.75	18.4 ± 1.5	31.50 ±0.78	939.20 ±162.34
G4	Nandhi mezhugu - 150	12.28 ±2.14	6.78 ±0.71	12.58 ±1.31	39.88 ±3.34	58.94 ±2.99	18.56 ± 0.69	31.54 ±1.44	916.40 ±279.90
G5	Nandhi mezhugu - 500	19.00 ±7.10	6.83 ±0.69	12.83 ±0.64	39.67 ±3.74	58.10 ±0.66	18.7 ± 1.07	32.47 ±1.51	986.67 ±103.37

n=5; Values are Mean, ±Standard Deviation;

7.17 Vascular permeability:

Results:

Body Weight & Clinical Signs:

The summary of body weight measurements (Before treatment) and clinical signs of male and female animals is presented in Table-7.17.1.

There were no clinical signs were observed during the study period.

Gross Necropsy:

The summary of external and internal gross pathological observations of male and female animals is presented in Table-7.17.2.

There were no external and internal gross pathological changes were observed during necropsy except.

Measurement of dye content in peritoneal fluid:

The summary of dye content in peritoneal fluid of male and female animals is presented in Table-7.17.3.

Graphical representation is presented in (Figure-7.17.1)

Percentage Inhibition:

Percentage inhibition of dye migration was calculated using Following formula:

$$\% \text{ Inhibition} = \frac{(\text{Mean OD of Control group} - \text{Mean OD of Treatment group})}{\text{Mean OD of Control group}} \times 100$$

Group	Males	Females
G2	49.33	24.53
G3	64.97	53.00
G4	55.92	72.72

TABLE No.: 7.17.1 SUMMARY OF BODY WEIGHT (g) AND CLINICAL SIGNS RECORD

Group	Treatment	Dose (mg/kg)	No. of Animals	Body weight (g) and Clinical signs			
				Male		Female	
G1	Control (Palm jaggery solution)	0	5+5	178.1 ±4.6	N	190.3 ±5.9	**N**
G2	Nandhi Mezhugu	50	5+5	183.7 ±6.8	N	191.5 ±7.1	**N**
G3		150	5+5	183.7 ±6.8	N	189.9 ±8.4	**N**
G4		**500**	**5+5**	**176.6 ±4.8**	**N**	**177.6 ±7.6**	**N**

N: Normal

TABLE No.: 7.17.2. SUMMARY OF GROSS NECROPSY

Group	Treatment	Dose (mg/kg)	No. of Animals	GROSS PATHOLOGICAL CHANGES			
				Male		Female	
				External	**Internal**	**External**	Internal
G1	Control (Palm jaggery solution)	0	5+5	NAD	NAD	NAD	**NAD**
G2	Nandhi Mezhugu	50	5+5	NAD	NAD	NAD	**NAD**
G3		150	5+5	NAD	NAD	NAD	**NAD**
G4		**500**	**5+5**	**NAD**	**NAD**	**NAD**	**NAD**

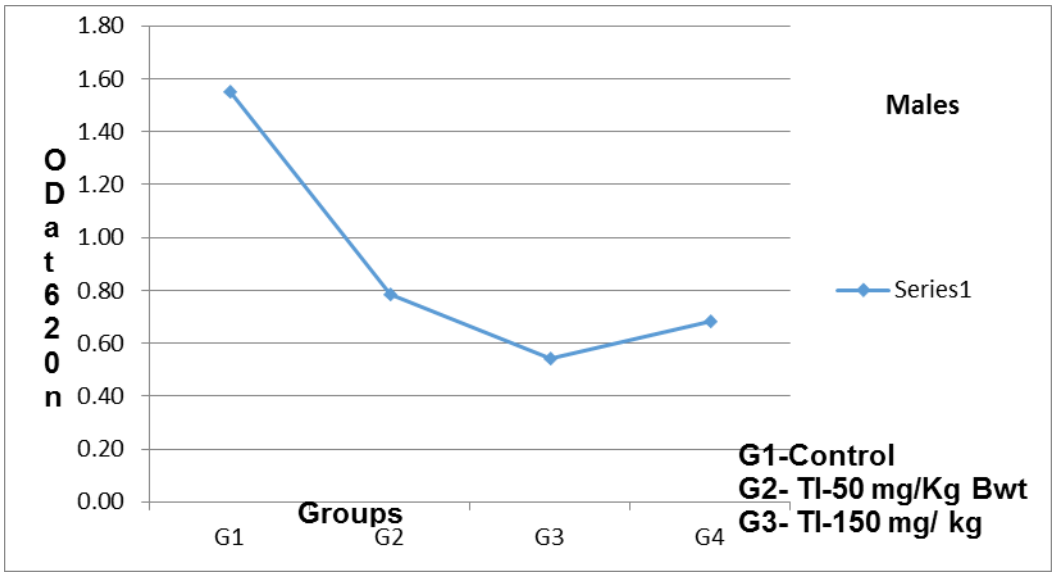
NAD: No abnormalities detected

TABLE No.: 7.17.3 SUMMARY OF ABSORBANCE OF DYE CONTENT IN PERITONEAL FLUID

Group	Treatment	Dose (mg/kg)	No. of Animals	COLOUR ABSORBANCE	
				Male	Female
G1	Control (Palm jaggery solution)	0	5+5	1.55	**1.67**
				±0.53	**±0.77**
G2	Nandhi Mezhugu	50	5+5	0.79*	**1.26**
				±0.33	**±0.98**
G3		150	5+5	0.54**	**0.79****
				±0.46	**±1.05**
G4		500	5+5	0.68*	**0.46****
				±0.32	**±0.28**

Level of Significance: ** = p<0.01, * = p<0.05

Figure 7.17.1 GRAPHICAL REPRESENTATION OF ABSORBANCE OF DYE CONTENT IN PERITONEAL FLUID



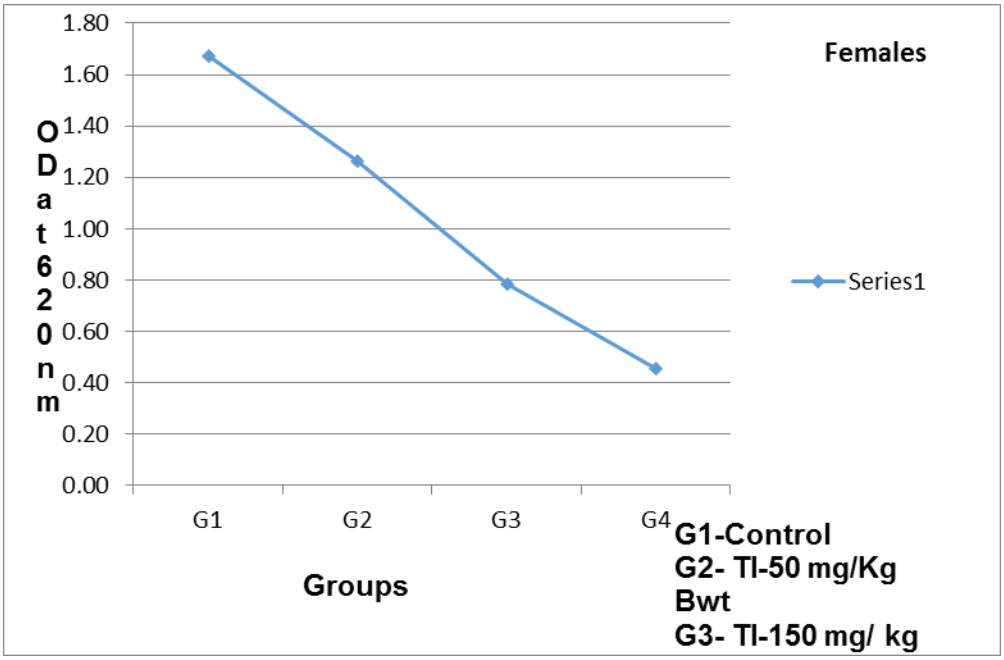


Figure 7.17.4.FORMULATION DETAILS

Group & Treatment	1.0% Evans blue dye solution	Vehicle / Test item (Dose mg/kg body weight)	Acetic acid (0.6%)	No. of Animals	Test Item (mg)	Vehicle (ml/group/ day)	Total Volume (ml/group/ day)
G1 – Control (Palm jaggery solution)	5.0 ml/kg Bwt	-	10 ml/kg Bwt	10	-	25	25
G2 – Nandhi Mezhugu		50		10	125	25	25
G3 – Nandhi Mezhugu		150		10	375	25	25
G4 – Nandhi Mezhugu		500		10	1250	25	25

7.18.Freund's Complete adjuvant (FCA) Arthritis Model Results:

7.18.1.Body Weight & Body weight gain

The summary of weekly body weight and body weight gain measurements of animals are presented in Table-7.18.1.

There were no treatment related statistical significance observed in all treatment groups when compared with the control group animals.

7.18.2.Clinical signs and Mortality observations

The summary of clinical signs and mortality observations of animals are presented in Table-7.18.2.

There were no treatment related abnormal clinical signs were observed across all the treatment group animals during the experiment period except swelling of paw oedema due to administration of FCA.

7.18.3.Paw measurements and scoring system

Paw measurements (mm) on day-1, 5 & day 21 and percentage of inhibition on day-5 & day 21

The summary of paw measurements and percentage inhibition of all group animals are presented in Table-7.18.3.

7.18.4 Arthritis index

The summary of Arthritis index of all group animals is presented in Table-7.18.4.

7.18.5 Total percentage change

The summary of Total percentage change of treated group animals is presented in Table-7.18.5. Dose dependent related changes were observed in treatment group animals.

7.18.6 Clinical pathology

Hematology

The summary of haematology parameters of all group animals is presented in Table-7.18.6. There were no significant changes across all the groups when compared with the G1 group animals.

7.18.7 Clinical chemistry

The summary of clinical chemistry parameters of all group animals is presented in Table-7.18.7. There were no statistical significant changes observed in clinical chemistry parameters in treatment group animals when compared with the control animals.

7.18.8 Pathology

Group	Treatment and Dose(mg/kg)	DAYS				
		Treatment period				
		Body Weight (g)			**Body gain (%)**	
		Day 1	**Day 8**	**Day 15**	**Day 1-8**	**Day 8-15**
G1	Arthritis Control (Palm jaggery solution)	159.27 ±9.07	180.8 ±17.0	212.0 ±14.9	13.4 ±5.5	17.6 ±6.4
G2	Reference drug (Indomethacin) – 0.3	157.27 ±5.66	175.0 ±6.6	197.4 ±7.6	11.3 ±3.1	12.9 ±4.7
G3	Nandhi Mezhugu – 50	160.98 ±9.03	183.0 ±11.4	202.0 ±10.0	13.7 ±4.2	10.7 ±7.2
G4	Nandhi Mezhugu - 150	158.83 ±7.84	169.2 ±11.0	199.8 ±15.0	6.7 ±8.1	18.5 ±12.4
G5	Nandhi Mezhugu – 500	159.83 ±8.21	181 ±14.0	205 ±19.0	13.7 ±10.8	13.0 ±1.8

Gross pathology

The summary of gross pathological observations of all group animals is presented in Table-7.18.8.There were no external and internal gross pathological changes noticed during the necropsy except for the swelling of paws.

Histopathology

The summary of histo-pathological observations of all group animals is presented in Table-7.18.9.There was a moderate degree of arthritis induction at G1 group. There was mild degree of improvement in the arthritis condition at G2 when compared to the G1 group. There was a marginal degree of reduction in the arthritis score at G3, G4 and G5 groups.

TABLE –7.18.1 SUMMARY OF ANIMALS WEEKLY BODY WEIGHT (g) AND BODY WEIGHT GAIN (%)

n=6; Values are Mean±Standard Deviation;

TABLE –7.18. 2 SUMMARY OF CLINICAL SIGNS AND MORTALITY OBSERVATIONS

Group	Treatment and Dose(mg/kg)	DAYS		
		Day 1-10	**Day 10-20**	**Mortality**
G1	Arthritis Control (Palm jaggery solution)	N	N	0/6
G2	Reference drug (Indomethacin) – 0.3	N	N	0/6
G3	Nandhi Mezhugu – 50	N	N	0/6

G4	Nandhi Mezhugu - 150	N	N	0/6
G5	Nandhi Mezhugu – 500	N	N	0/6

N-Normal

TABLE – 7.18.3 SUMMARY OF PAW MEASUREMENTS (mm) ON DAY-1, DAY-5 & DAY-21 AND PERCENTAGE OF INHIBITION ON DAY-5 & DAY-21

Group	Treatment (mg/kg)	PAW MEAUSREMTS (mm)						PERCENTAGE OF INHIBITION (%)	
		Day-1		**Day-5**		**Day-21**			
		Left hind paw	**Right hind paw**	**Left hind paw**	**Right hind paw**	**Left hind paw**	**Right hind paw**	**Percentage inhibition of injected paw on day 5**	**Percentage inhibition of non - injected paw on day 21**
G1	Arthritis Control (Palm jaggery solution)	8.63 ±0.39	4.55 ±0.24	8.11 ±0.31	4.52 ±0.31	6.24 ±0.26	4.44 ±0.28	-	-
G2	Referencedrug (Indomethacin) – 0.3	8.61 ±0.81	4.74 ±0.13	6.80 ±0.62	4.44 ±0.15	5.73 ±0.25	4.42 ±0.16	16.25	7.01
G3	Nandhi Mezhugu -50	8.71 ±0.91	4.28 ±0.11	7.34 ±0.62	4.12 ±0.07	6.15 ±0.25	4.64 ±0.16	9.59	3.89

G4	Nandhi Mezhugu - 150	9.06 ±0.77	4.44 ±0.12	7.29 ±0.66	4.27 ±0.12	5.63 ±0.32	4.52 ±0.19	10.17	2.66
G5	Nandhi Mezhugu - 500	9.07 ±0.80	4.48 ±0.21	7.11 ±0.57	4.25 ±0.11	5.51 ±0.25	4.51 ±0.25	12.33	5.70

n=6;Values are Mean±Standard Deviation;

TABLE – 7.18.4 ARTHRITIS INDEX

Group	Treatment (mg/kg)	Arthritis index					
		Ear	**Nose**	**Tail**	**Fore paws**	**Hind Paws (Only Left Hind paw)**	**Sum**
G1	Arthritis Control(Palm jaggery solution)	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	3.0 ±0.0	3.0 ±0.0
G2	Reference drug (Indomethacin) – 0.3	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	2.0 ±0.0	2.0 ±0.0
G3	Nandhi Mezhugu -50	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	2.6 ±0.5	2.6 ±0.5
G4	Nandhi Mezhugu - 150	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	2.0 ±0.0	2.0 ±0.0
G5	Nandhi Mezhugu - 500	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	2.0 ±0.0	2.0 ±0.0

n=6;Values are Mean±Standard Deviation;

TABLE – 7.18.5 TOTAL PERCENTAGE CHANGE

Group	Treatment (mg/kg)	TOTAL PERCENTAGE CHANGE (Percentage inhibition of injected paw on day 5+ Percentage inhibition of non - injected paw on day 21+ Percentage change of the arthritis index)			
		Percentage inhibition of injected paw on day 5 (%)	**Percentage inhibition of non - injected paw on day 21 (%)**	**Percentage of Inhibition Arthritis index (%)**	**Total Percentage Change (%)**
G1	Arthritis Control (Palm jaggery solution)	-	-	-	-
G2	Reference drug (Indomethacin) – 0.3	16.25	7.01	33.33	56.60
G3	Nandhi Mezhugu -50	9.59	3.89	14.29	27.77
G4	Nandhi Mezhugu - 150	10.17	2.66	33.33	46.16
G5	Nandhi Mezhugu - 500	12.33	5.70	33.33	51.36

n=6;Values are Mean±Standard Deviation;

TABLE –7.18 6 SUMMARY OF HAEMATOLOGY PARAMETERS

Gr	Treatment (mg/kg)	Total (WBC) (10^3 cells/µl)	Total (RBC) (10^6 cells/µl)	HGB (g/dl)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dl)	PLT (10^3 cells/µl)	ESR (mm/hour)
G1	Arthritis Control (Palm jaggery solution)	12.23 ± 1.66	7.87 ± 0.31	13.80 ± 0.25	44.43 ± 1.41	56.43 ± 1.22	17.55 ± 0.63	31.08 ± 0.90	752.67 ± 153.17	0.27 ± 0.16
G2	Reference drug (Indomethacin) – 0.3	13.80 ± 2.59	7.93 ± 0.23	13.88 ± 0.38	43.93 ± 1.00	55.47 ± 1.56	17.53 ± 0.63	31.60 ± 0.41	627.17 ± 236.86	0.22 ± 0.15
G3	Nandhi Mezhugu - 50	14.05 ± 3.67	7.38 ± 0.61	13.12 ± 1.02	43.17 ± 3.41	58.52 ± 2.00	17.80 ± 0.87	30.42 ± 1.35	668.67 ± 113.99	0.17 ± 0.08
G4	Nandhi Mezhugu – 150	11.37 ± 1.46	7.57 ± 0.54	13.50 ± 0.77	43.12 ± 1.91	57.03 ± 1.75	17.87 ± 0.61	31.30 ± 0.60	682.33 ± 160.39	0.18 ± 0.13
G5	Nandhi Mezhugu – 500	12.95 ± 2.89	7.09 ± 0.70	13.27* ± 0.69	41.83 ± 2.35	59.25 ± 3.41	18.78* ± 0.98	31.72 ± 0.48	634.83 ± 209.50	0.17 ± 0.08

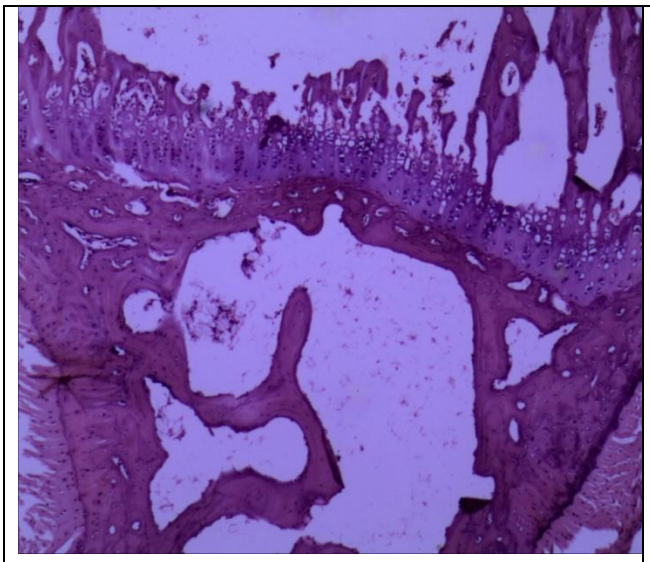
n=6;Values are Mean±Standard Deviation; P<0.05*;

TABLE – 7.18.7 SUMMARY OF CLINICAL CHEMISTRY PARAMETER

Group	Treatment (mg/kg)	TOTAL PROTEIN (g/dl)	ALBUMIN (g/dl)
G1	Arthritis Control (Palm jaggery solution)	6.58 ±0.71	3.60 ±0.27
G2	Reference drug (Indomethacin) – 0.3	7.69 ±1.62	3.43 ±0.40
G3	Nandhi Mezhugu – 50	8.19 ±1.08	3.49 ±0.15
G4	Nandhi Mezhugu – 150	7.56 ±0.84	3.76 ±0.20
G5	Nandhi Mezhugu – 500	7.30 ±0.98	3.75 ±0.62

n=6;Values are Mean±Standard Deviation

Study No.: LBPL-EF-046/15 FCA **HISTOPATHALOGY SPECIMEN**



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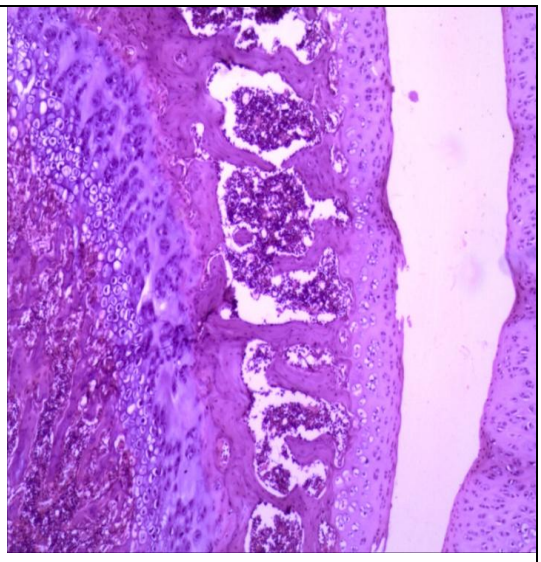

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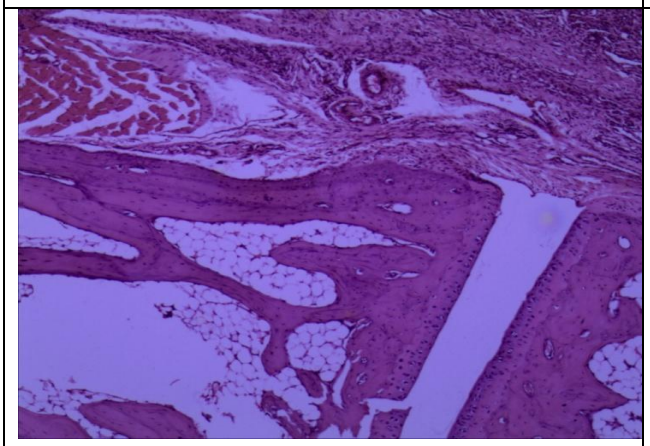

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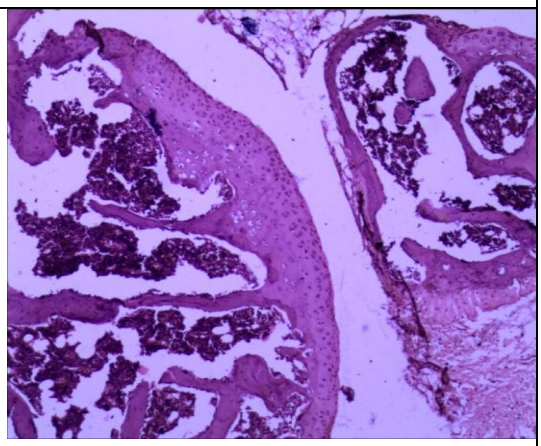

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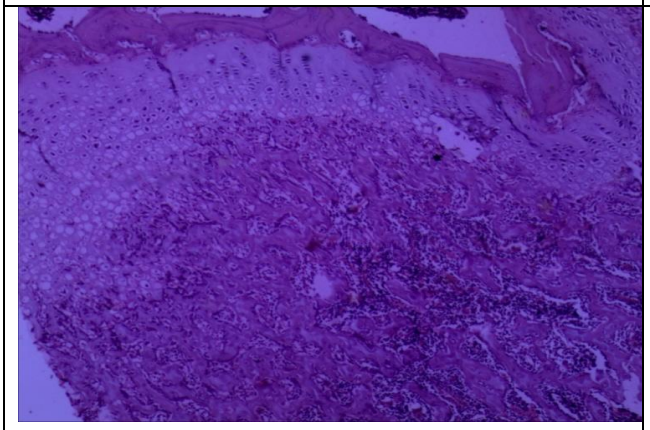

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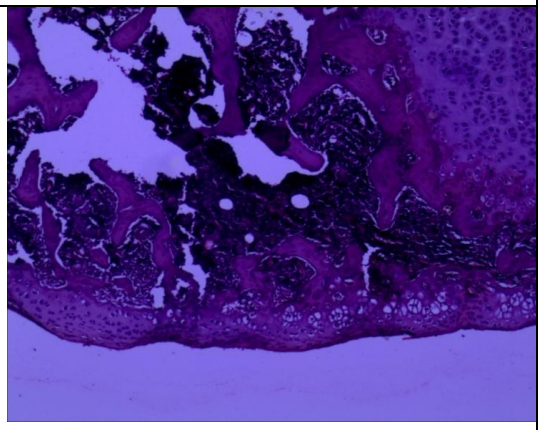

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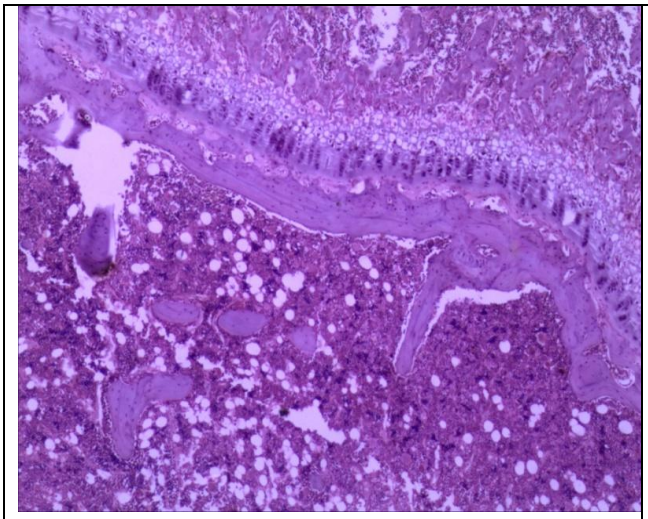
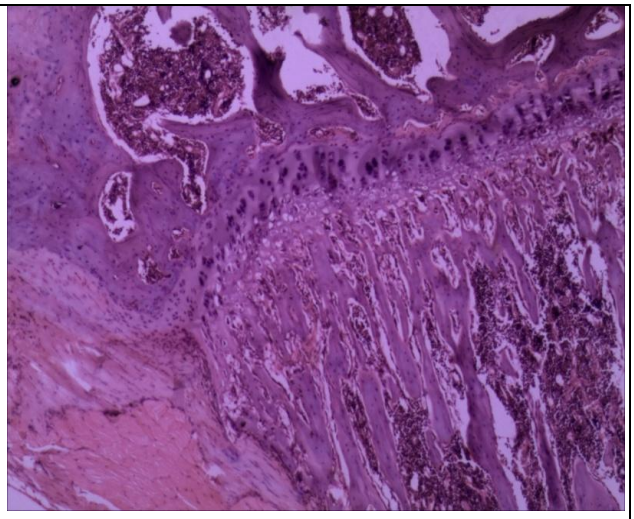
Study No.: LBPL-EF-046/15 FCA **HISTOPATHALOGY SPECIMEN**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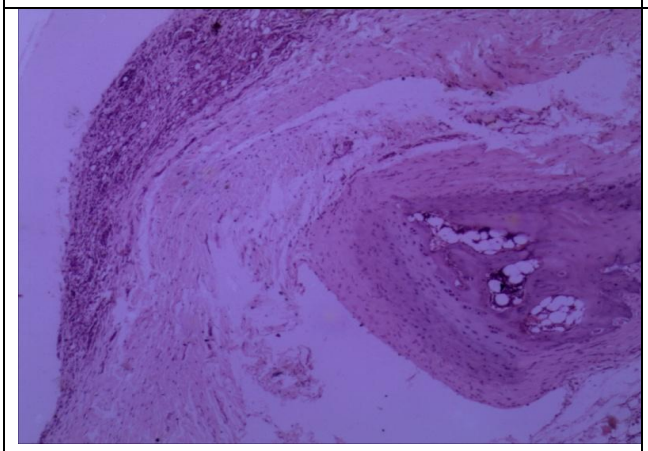
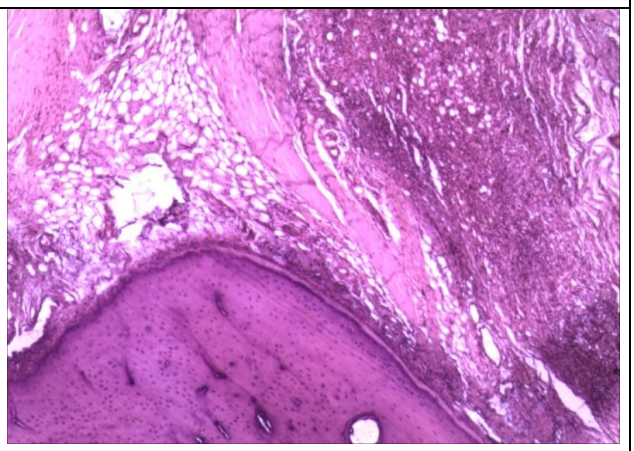
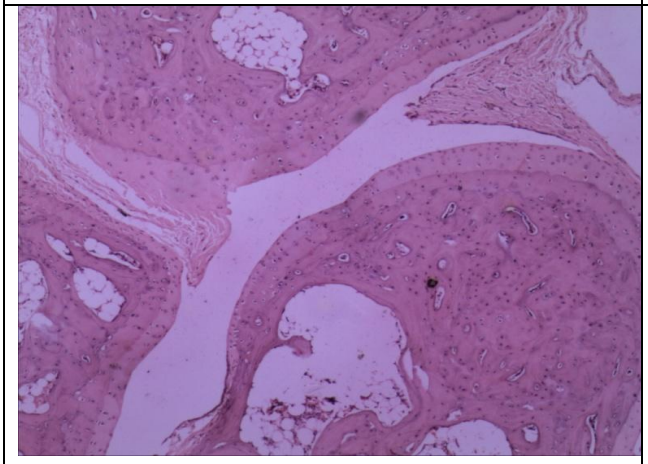
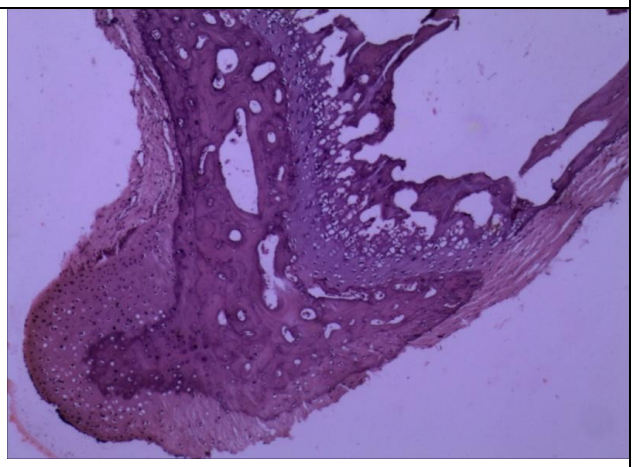
	
GIII,A.No.13-III, H&E x4	GIV,A.No.21-III, H&E x10
	
GIV,A.No.24-II, H&E x4	**GV,A.No.27-I, H&E x4**

Study No.: LBPL-EF-046/15 FCA **HISTOPATHALOGY SPECIMEN**

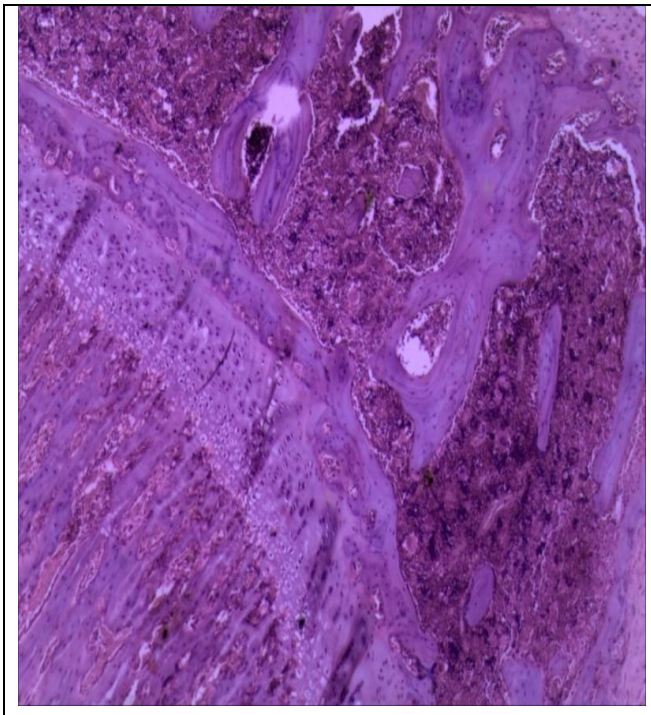
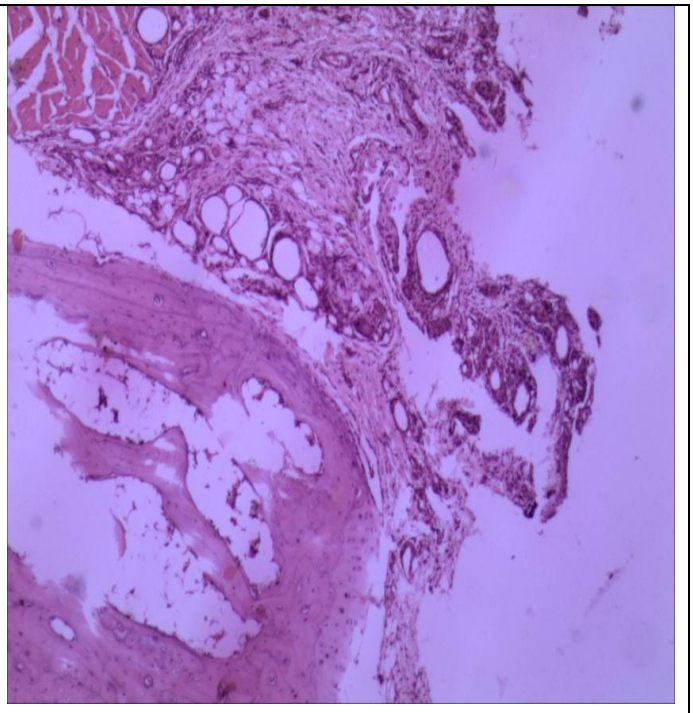
	
GV,A.No.29-III, H&E x4	**GV,A.No.30-II, H&E x4**

TABLE – 7.18.8 SUMMARY OF GROSS PATHOLOGICAL OBSERVATIONS

Group	Treatment (mg/kg)	External	Internal
G1	Arthritis Control (Palm jaggery solution)	NAD	NAD
G2	Reference drug (Indomethacin) – 0.3	NAD	NAD
G3	Nandhi Mezhugu – 50	NAD	NAD
G4	Nandhi Mezhugu – 150	NAD	NAD
G5	Nandhi Mezhugu – 500	NAD	NAD

n=6;NAD – No abnormalities detected

TABLE –7.18.9 SUMMARY OF HISTOPATHOLOGICAL OBSERVATIONS

Group	Treatment (mg/kg)	Mean Arthritic score
G1	Arthritis Control (Palm jaggery solution)	3.0
G2	Reference drug (Indomethacin) – 0.3	1.8
G3	Nandhi Mezhugu - 50	2.0
G4	Nandhi Mezhugu - 150	1.5
G5	Nandhi Mezhugu - 500	1.5

7.19.MTT Assay:

7.19.1 Cytoprotective effect of Nandhi mezhugu:

Table No:7.19.1.1Cytoprotective effect of Nandhi mezhugu extract vs 25nG/mL of LPS

	Blank	Untreated	LPS 25 ng/ml	Concentration Unit: µg/ml		
				50	100	150
Reading 1	0.024	1.795	1.75	1.748	1.777	1.553
Reading 2	0.008	1.793	1.761	1.736	1.716	1.505
Mean	0.016	1.794	1.7555	1.742	1.7465	1.529
Mean OD-Mean B	NA	1.778	1.7395	1.726	1.7305	1.513
SD		0.001414214	0.007778175	0.008485	0.043134	0.033941
SEM		0.001000151	0.005500831	0.006001	0.030505	0.024004
Viability%	NA	100	97.83464567	97.07537	97.32846	85.09561

Table No:7.19.1.2 Cytoprotective effect of Nandhi mezhugu extract vs 50 nG/mL of LPS

	Blank	Untreated	LPS 50 ng/ml	Concentration Unit: µg/ml		
				50	**100**	**150**
Reading 1	0.024	1.795	1.743	1.748	1.767	1.565
Reading 2	0.008	1.793	1.749	1.736	1.767	1.445
Mean	0.016	1.794	1.746	1.742	1.767	1.505
Mean OD-Mean B	NA	1.778	1.73	1.726	1.751	1.489
SD		0.001414214	0.004232641	0.004242641	0	0.084852814
SEM		0.001000151	0.005500831	0.003000453	0	0.060009062
Viability%	NA	100	97.83464567	97.35658043	98.48143982	83.74578178

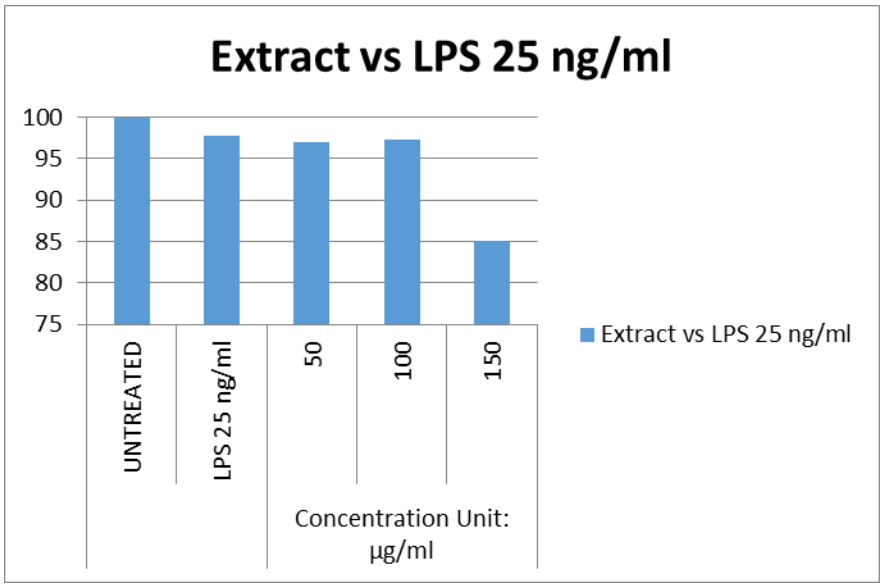


Figure No:7.19.1.1Cytoprotective effect of Nandhi mezhugu extract vs 25nG/mL of LPS

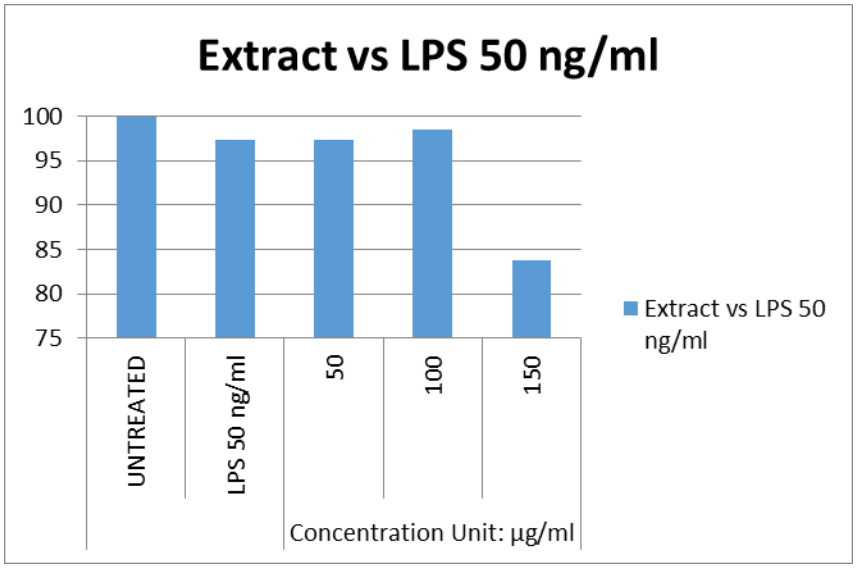


Figure No:7.19.1.2 Cytoprotective effect of Nandhi mezhugu extract vs 50 nG/mL of LPS

Table No:7.19.1.3 Cytoprotective effect of Nandhi mezhugu extract vs 100 nG/mL of LPS

	Blank	Untreated	LPS 100 ng/ml	Concentration Unit: µg/ml		
				50	**100**	**150**
Reading 1	0.024	1.795	1.72	1.802	1.832	1.687
Reading 2	0.008	1.793	1.722	1.758	1.798	1.632
Mean	0.016	1.794	1.721	1.78	1.815	1.6595
Mean OD-Mean B	NA	1.778	1.705	1.764	1.799	1.6435
SD		0.001414214	0.001414214	0.031112698	0.024041631	0.038890873
SEM		0.001000151	0.001000151	0.022003323	0.017002568	0.027504153
Viability%	NA	100	95.89426322	99.21259843	98.48143982	92.43532058

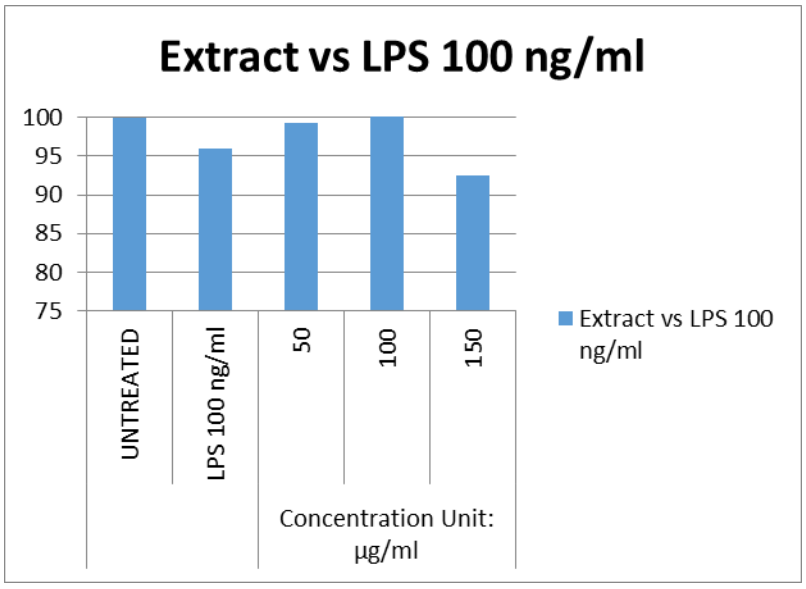


Figure No:7.19.1.3 Cytoprotective effect of Nandhi mezhugu extract vs 100 nG/mL of LPS

7.19.2. Cytotoxicity Effect of Nandhi mezhugu compared with LPS:

7.19.2.1 Cytotoxicity Effect of LPS:

Table No:7.19.2.1 Cytotoxicity Effect of LPS:

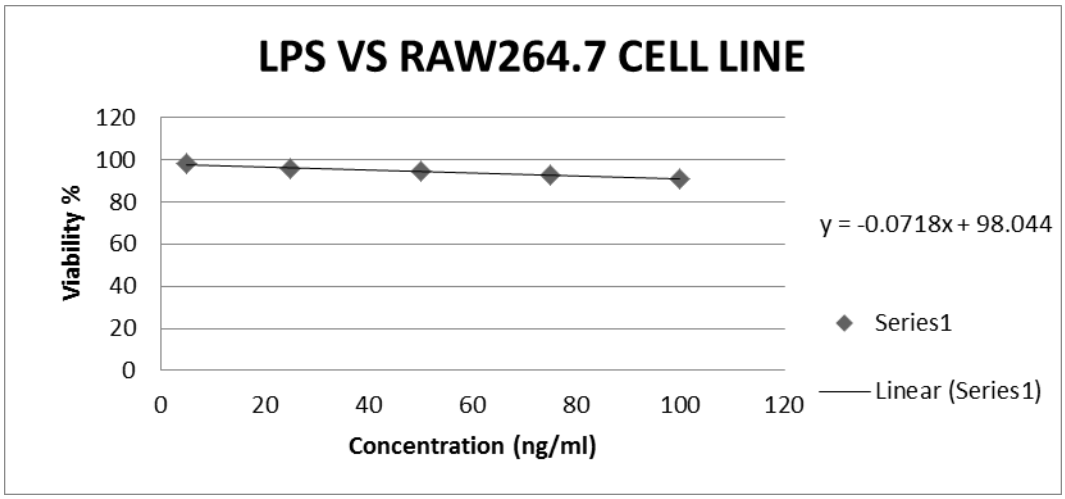


Figure No: 7.19.2.1 Cytotoxicity Effect of LPS:

Table No: 7.19.2.2 Cytotoxicity Effect of Nandhi mezhugu

	Blank	Untreated	Oxaliplatin (80µM)	Concentration unit: ng/ml				
				62.5	125	250	500	1000
Reading 1	0.011	0.655	0.344	0.657	0.633	0.627	0.613	0.355
Reading 2	0.004	0.669	0.357	0.644	0.638	0.623	0.616	0.339
Mean	0.0075	0.66175	0.3505	0.6505	0.6355	0.625	0.61	0.347
Mean OD-Mean B	NA	0.65425	0.343	0.643	0.628	0.6175	0.607	0.3395
SD		0.010253048	0.00919223 88	0.00919 2	0.006 364	0.009 192	0.0077 78	0.0113 14
SEM		0.007251095	0.0065009 82	0.00650 1	0.004 501	0.006 501	0.0055 01	0.0080 01
Viability%	NA	100	52.426442 49	98.2804 7	97.82 193	96.29 347	82.690 1	51.891 48

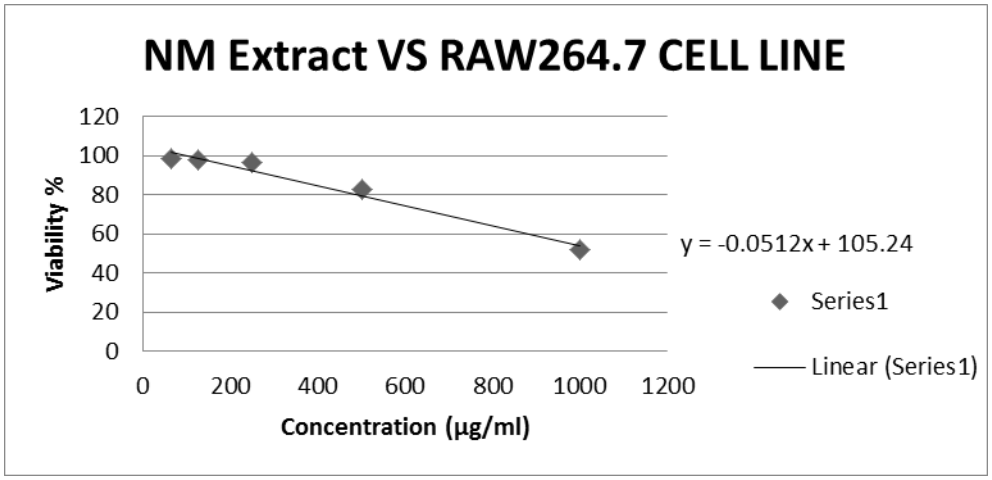


Figure No: 7.19.2.2 Cytotoxicity Effect of Nandhi mezhugu

7.20. Gene expression study:

7.20.1.Gene expression study of Nandhi melugu in-vivo model :

Results

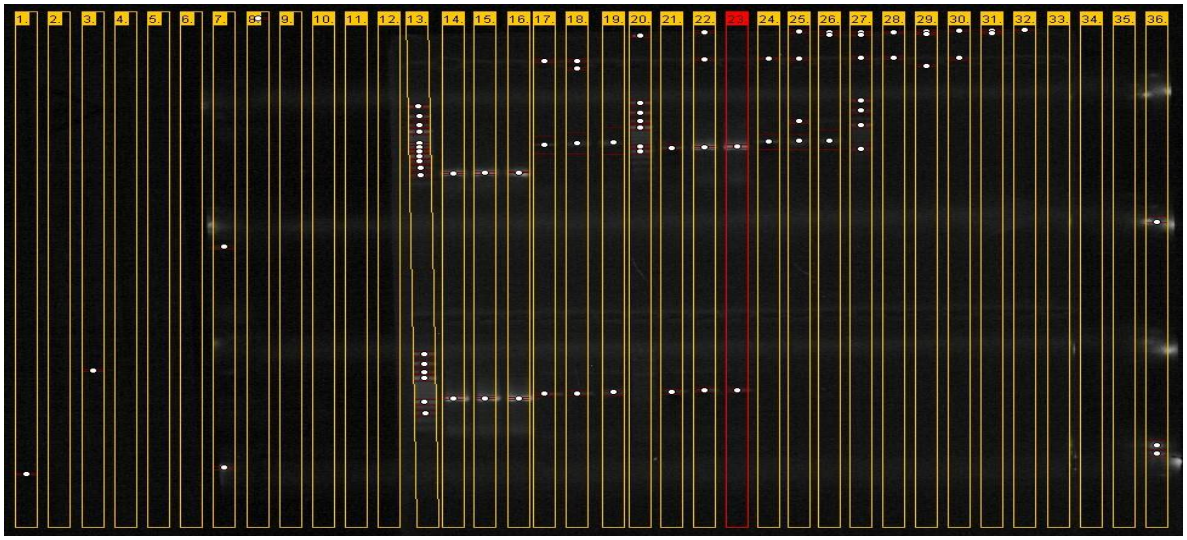


Figure 7.20.1.1 Gel analyzer view in in vivo gene expression study

Here, the sample refers to Treated/Positive control treated

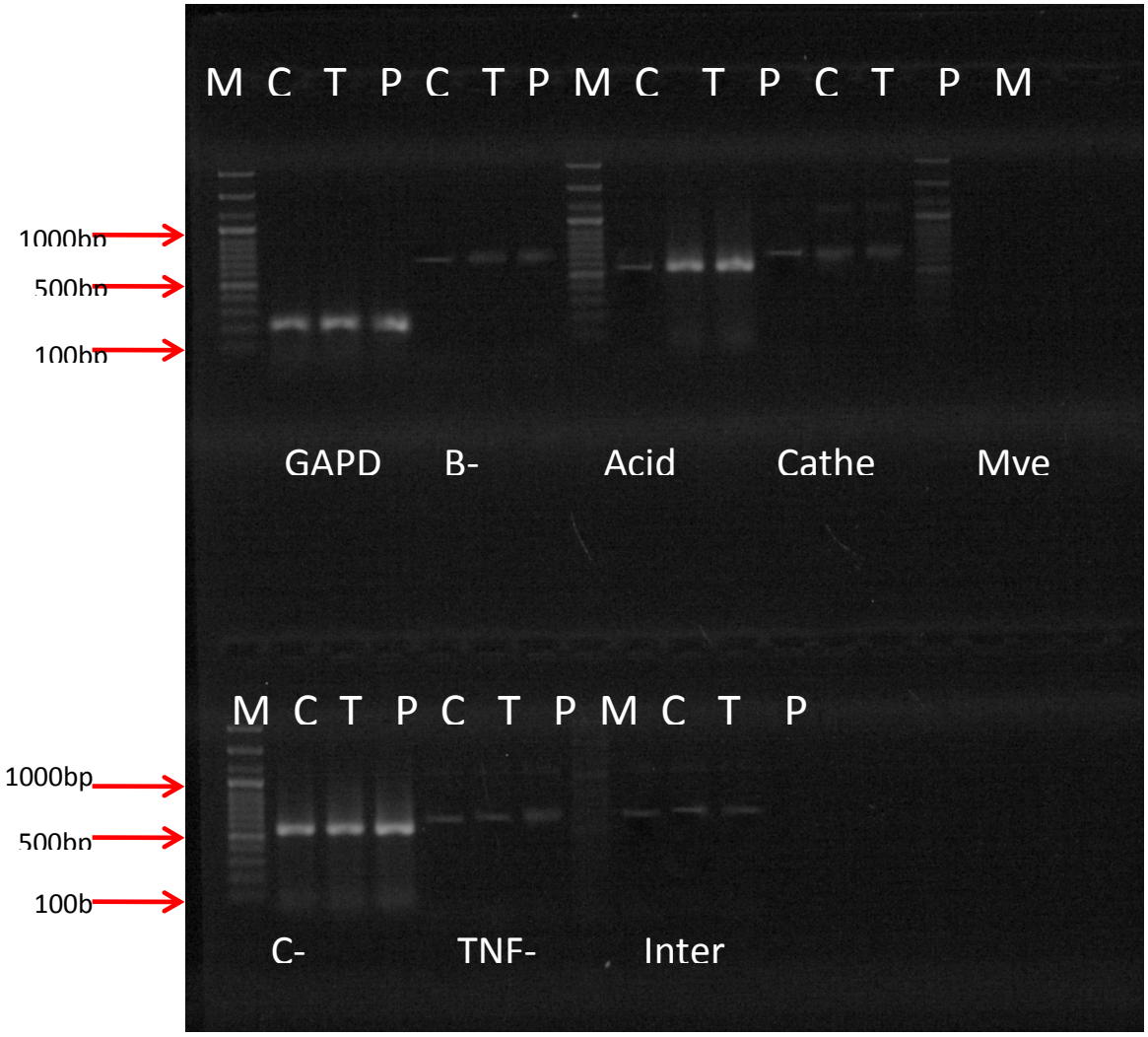


Table No: 7.20.1.1 Gene Expression effect of Nandhi mezhugu

Gene	Control	Treated	Fold change	Positive	Fold Change
GAPDH	638	841		542	
Beta-galacturonidase	2191	2635	11.00	3241	52.30
Acid phosphatase	731	1075	19.29	1324	94.25
Cathepsin-D	1951	2272	6.05	2572	36.75
Myeloperoxidase level	0	0	0.00	0	
C-reactive protein	1812	982	-57.01	1120	-32.89
TNF-alpha	522	896	32.76	987	107.47
Interferon – gamma	704	716	-27.13	525	-11.79

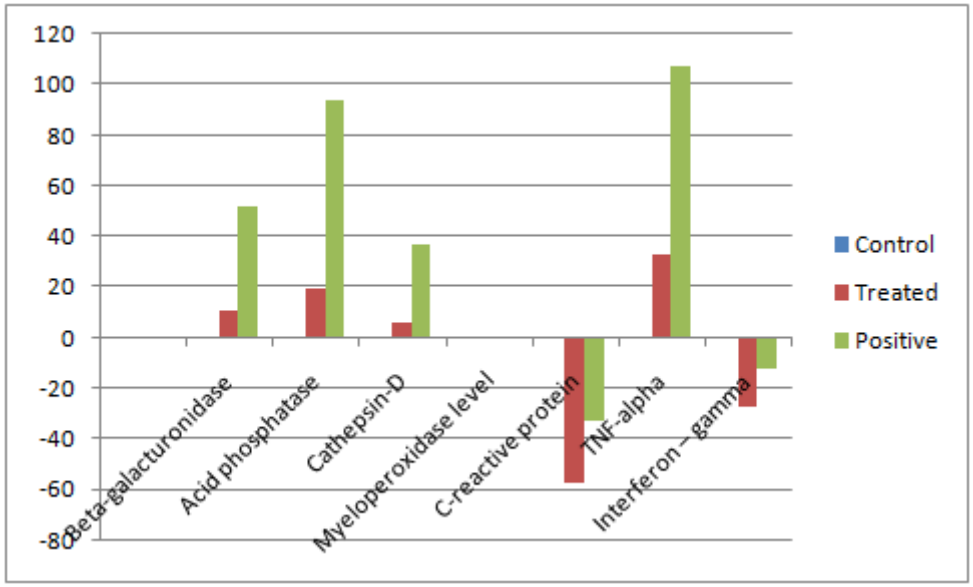
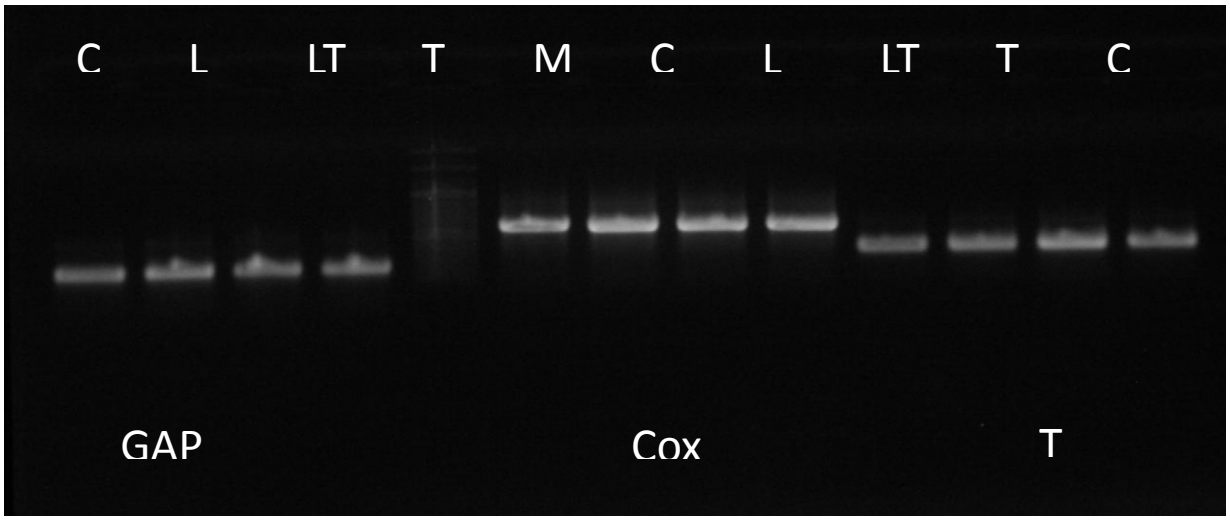
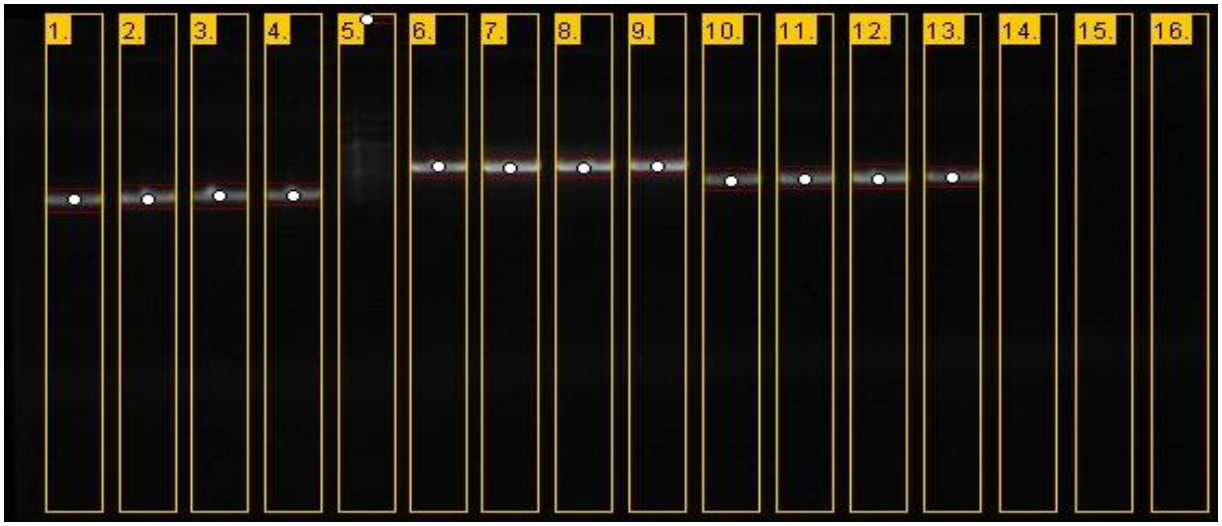


Figure No: 7.20.1.2 Gene Expression effect of Nandhi mezhugu



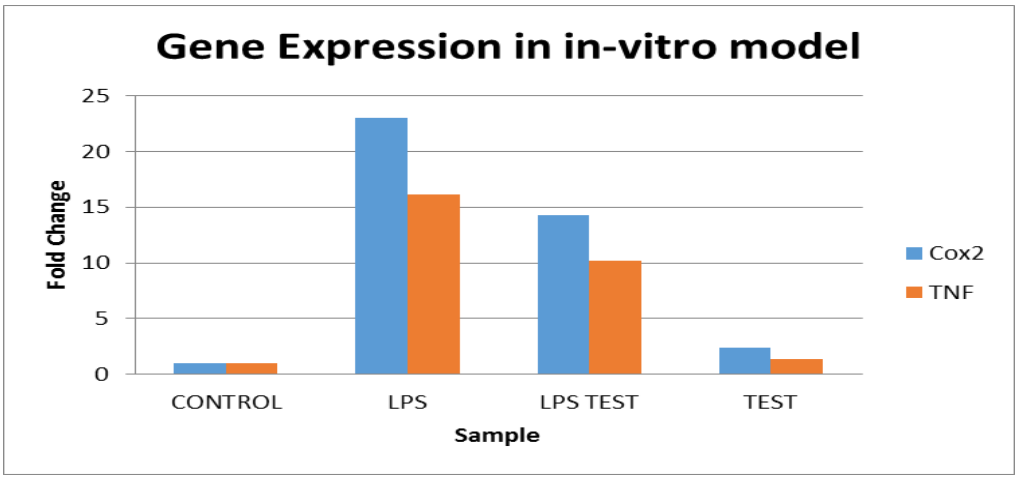


Here, the sample refers to LPS/LPS test/Test treated

Table No: 7.20.2.1.Effect of Nandhi mezhugu in in -vitro gene expression study:

Sample	**CONTROL**	Flod change	**LPS**	Fold Change	**LPS TEST**	Fold Change	**TEST**	Fold Change
GAPDH	2432		2416		2540		2504	
Cox2	3552	1	4354	23.03	4168	14.30	3710	2.42
TNF	2396	1	2767	16.15	3375	10.18	2501	1.38

Figure No: 7.20.2.2.Effect of Nandhi mezhugu in in -vitro gene expression study:



In vitro cell line assay:

In cytotoxicity assay in a LPS induced inflammation in the RAW 264.7–Mouse macrophage cell line study .Nandhi Mezhugu showed a cytotoxicity at 500 µg/ml and above, which is too high concentration to correlate to the in vivo studies. The concentrations less than 250 µg/ml were considered for the cytoprotective cell line assay where the expression of the inflammatory biochemical markers such as COX-2 and TNF-alpha were studied. Nandhi Mezhugu at 100 µg/ml showed a decrease in inflammatory markers to the extent of 14% and 10% of COX-2 and TNF-alpha respectively. This result suggests that the Nandhi Mezhugu preparation can be used as an alternative treatment for the inflammatory conditions.

In Vivo –Rat Paw oedema assay:

In rat paw oedema bio-assay, there was a dose dependent decrease in the inflammation was observed. The highest dose studied in this bio-assay was 500 mg/kg which is almost equivalent to the standard drug indomethacin. The pro-inflammatory and anti-inflammatory gene expression studies were performed in the rat paw treated with Nandhi Mezhugu. A slight increase in the gene expression of Beta-galacturonidase, Acid phosphatase, and Cathepsin-D at 11,19, and 6% respectively which is biologically non-significant, whereas there was a decrease in C-reactive protein and Interferon-gamma gene expression at 57 and 27% respectively considered due to anti-inflammatory effect of Nandhi Mezhugu. An increase in TNF-alpha which is a pro-inflammatory marker which is expected to reduce with the treatment, however the increase in the level could not be explained. Further studies are required to understand the pathway of anti-inflammatory effect of Nandhi mezhugu.

7a.CLINICAL STUDY

Clinical Study of *Nandhi mezhugu*:

Subject enrolment

215 patients were screened for Rheumatoid arthritis, among them 80 patients were included in the study .Out of them 60 patients were enrolled. Among 60 patients 5 patients were withdrawn their consent and 15 patients failed to come for follow up. The remaining 40 patients were completed the full duration of study in OPD and IPD of Ayothidoss pandithar hospital, National Institute of Siddha, Tambaram sanatorium, Chennai-47. 40 patients were observed in the present study. The observations were made and tabulated with regards to the following features:

- Gender distribution(Sex distribution)
- Age distribution
- Kaalam distribution
- Food habits
- Socio economic status
- Occupational status
- Distribution of thinai(Land)
- Paruvakaalam(Seasons)

- Imporigal
- Impulangal
- Kanmendrium
- Kanmavidayam
- Kosangal
- Seven udal thathukkal
- Distribution of mukkutram
- Deranged vatham
- Deranged pitham
- Deranged kabam
- Envagaithervugal
- Neerkuri, Neikuri
- Clinical features
- Clinical signs
- Clinical symptoms
- Pain score
- Grade for Restricted movements
- MHAQ
- EULAR Score
- Haematological parameters
- Biochemical parameters
- LFT
- RFT
- Hb, ESR
- Anti CCP,RA,CRP

Outline of the Study

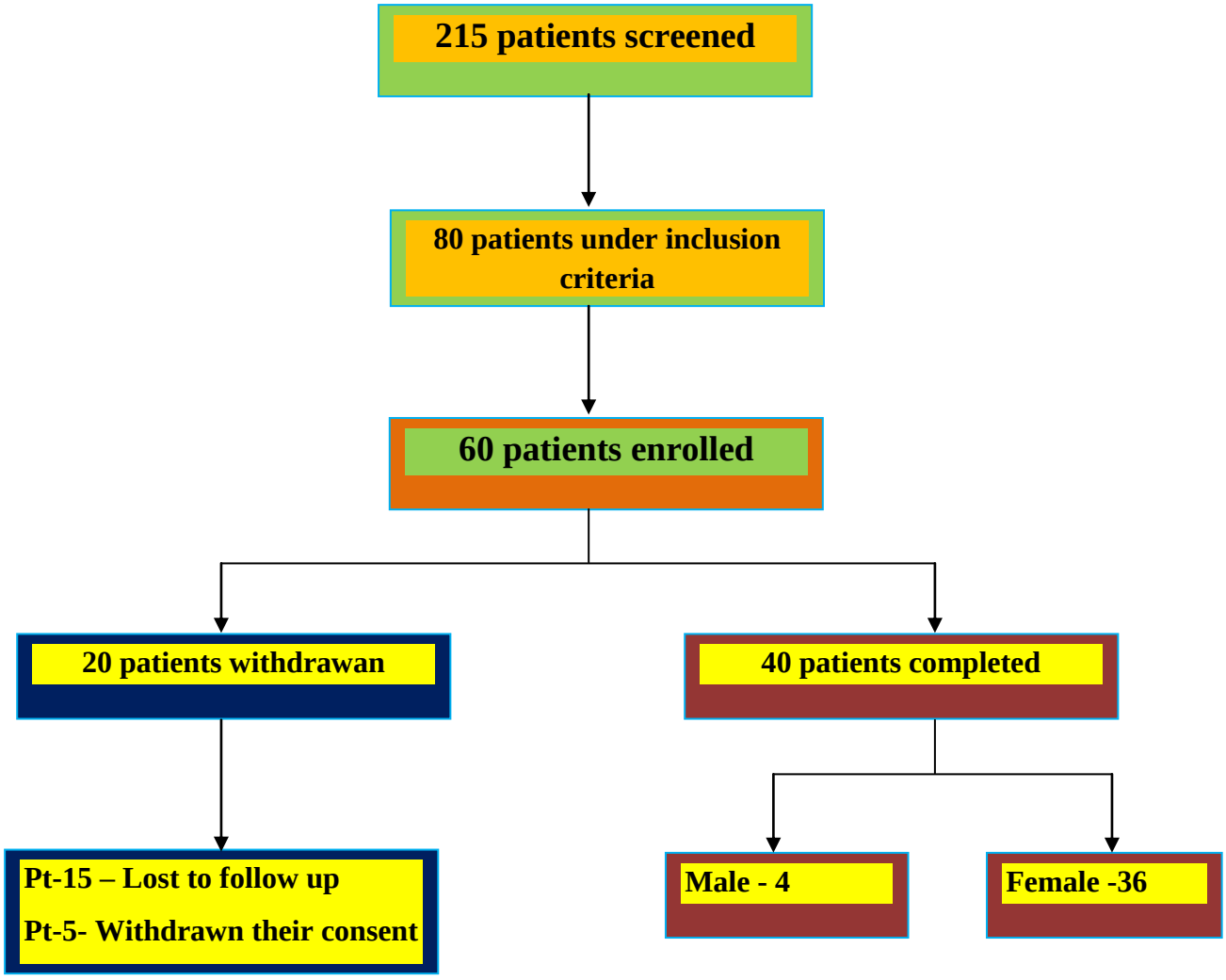


Chart: Flow chart of patient recruitment for clinical study

Demograpic characteristics

7a.1 Gender distribution:

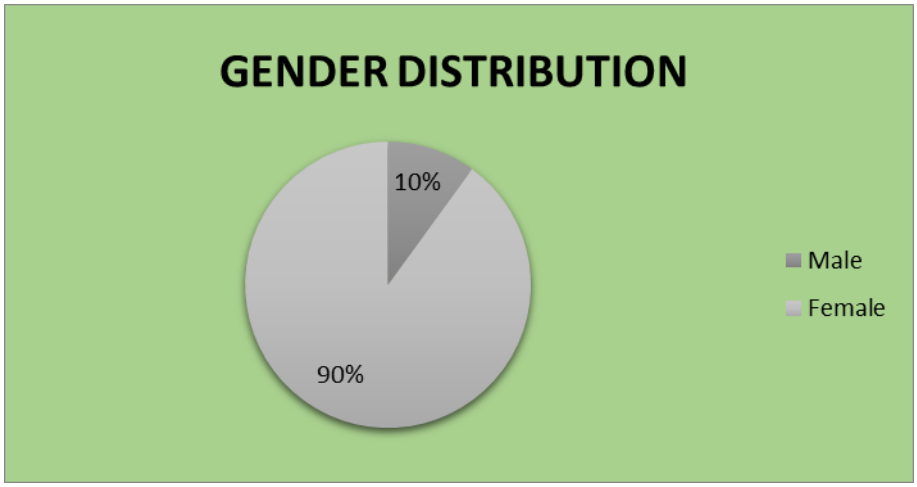


Figure:7a.1 shows that among 40 cases, 4(10%) cases were male and 36(90%) cases were females.

7a.2 Age distribution:

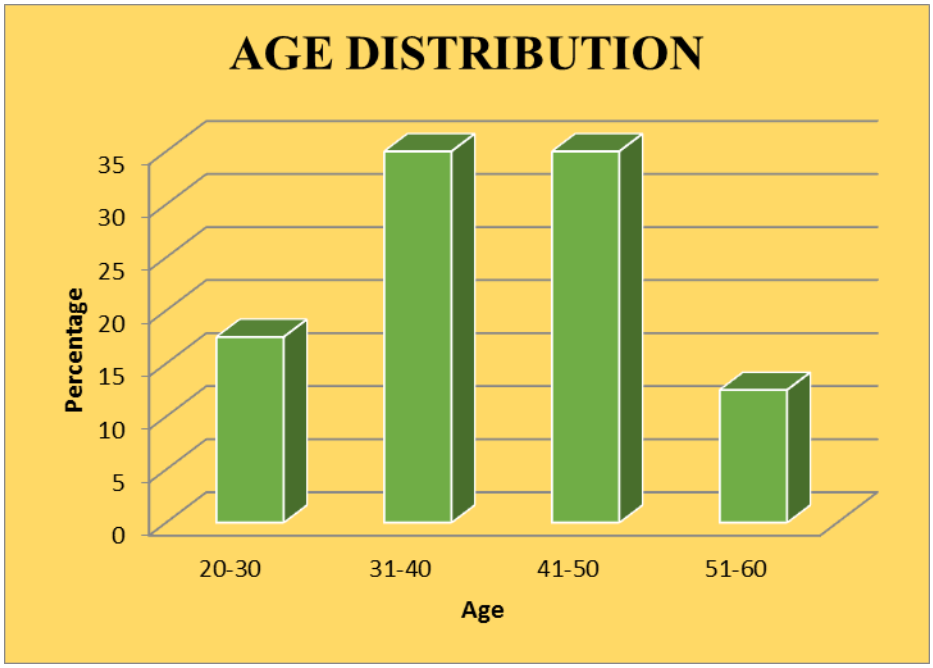


Figure 7a.2 shows that among 40 patients 35% of cases in 31-40 years of age, 35% of cases in 41-50 years of age, 17.5% of cases between 20-30 years of age and 12.5% of cases in 51-60 years of age.

7a.3 Distribution of Thinai(Land):

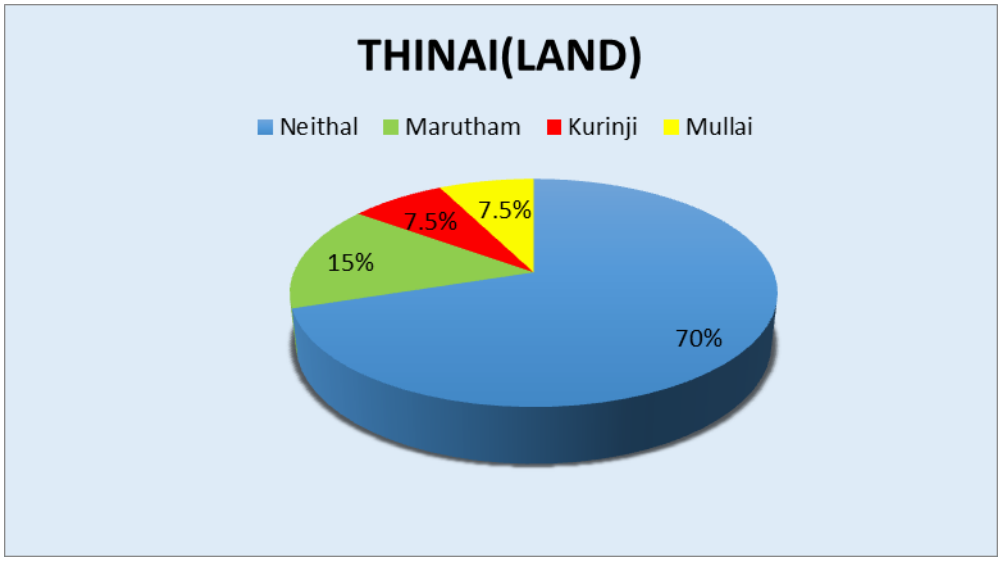


Figure 7a.3 shows among 40 cases 70% of cases from Neithal, 15% of cases from Marutham, 7.5% of cases from Mullai and 7.5% of cases from Kurinji.

7a.4 Kaalam(Age) distribution:

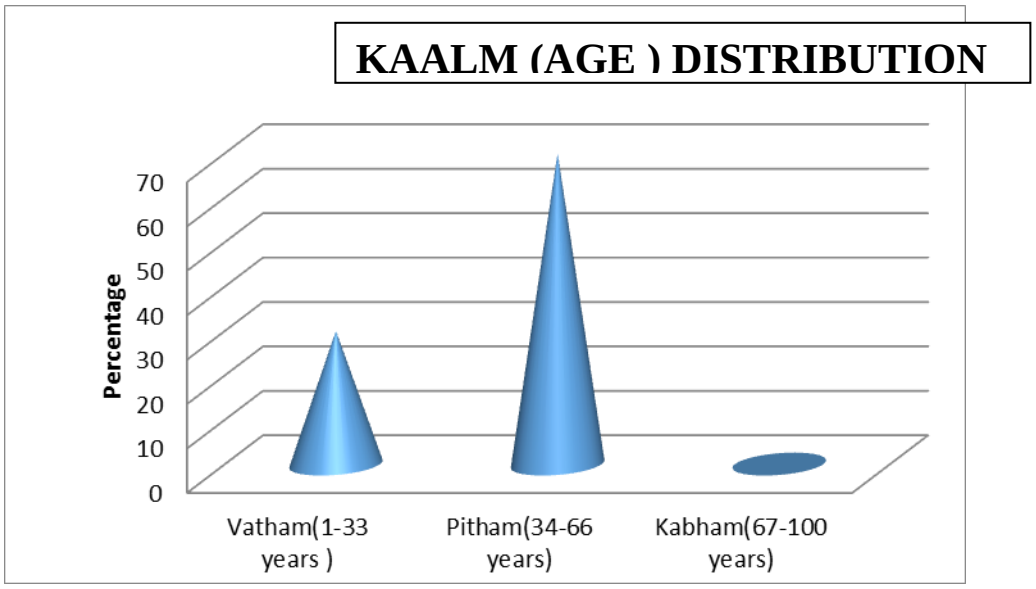


Figure 7a.4 shows among 40 patients 30% of patients comes under Vatha kaalam (1-33 years of age) and 70% of patients comes under Pitha kaalam (34-66 years of age).

7a.5 Noiutrakaalam:

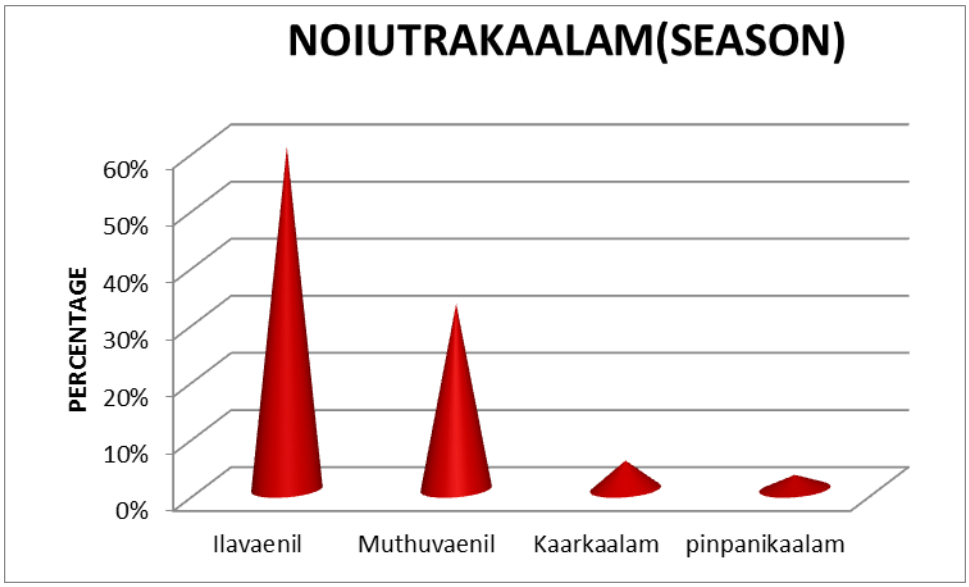


Figure 7a.5 shows among 40 patients 60% of patients reported in Ilavaenil (April 15-June 14), 32.5% patients affected in Muthuvaenil kaalam (June15-August 14), 5% of patients affected in Kaarkaalam (August 15-October 14) and 2.5% of patients affected in Pinpanikaalam (February 15- April 14).

7a.6 Distribution of Food Habits:

Figure 7a.6 shows 97.5% of patients were non-vegetarian and 2.5% of patients were vegetarian.

7a.7.Duration of illness:

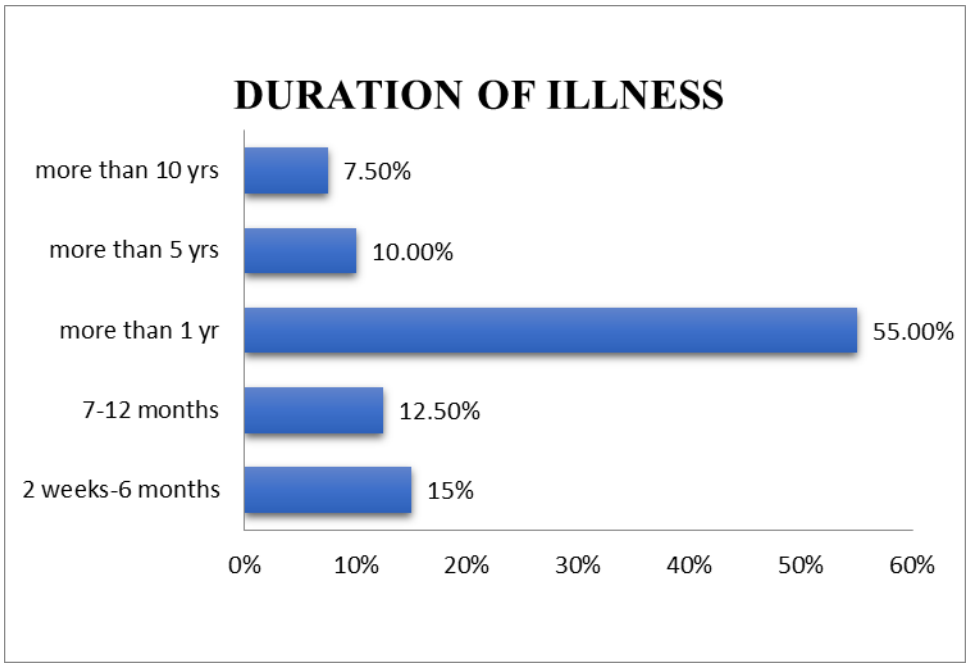


Figure 7a.7 shows that 7.5% of patients having symptoms more than 10 years, 10% of cases having symptoms more than 5 years, 55% of patients having symptoms more than 1 year, 12.5% of patients having symptoms with 7-12 months and 15 % of patients having symptoms with 2 weeks-6 months.

7a.8 Body constitution:

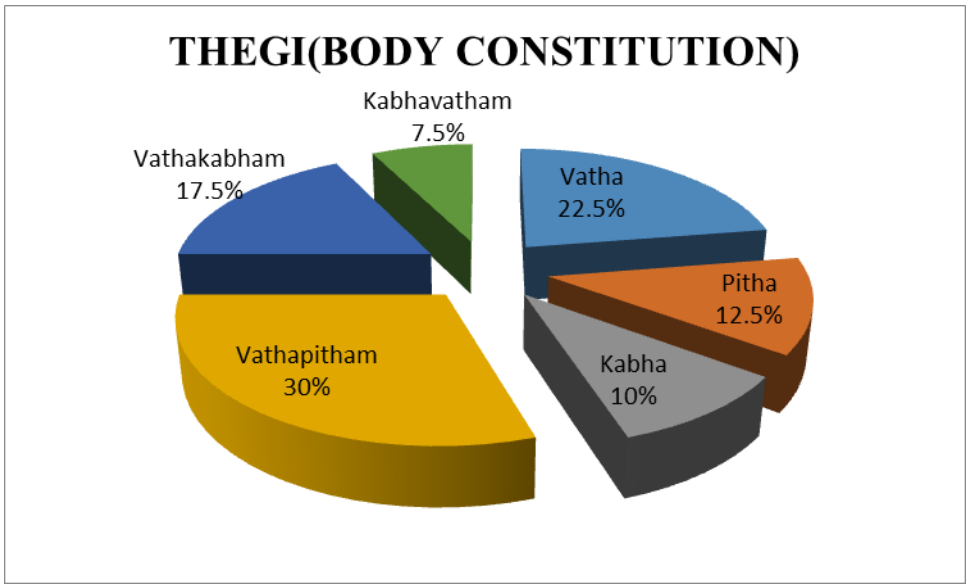


Figure 7a.8 shows that 30% of cases had Vathapitha thegam, 22.5% of cases had Vatha thegam, 17.5% of cases had Vathakabha thegam, 12.5% of cases had Pitha thegam, 10% of patients had Kabha thegam and 7.5% of cases had Kabhavaatha thegam.

7a.9Distribution of Socio Economic Status:

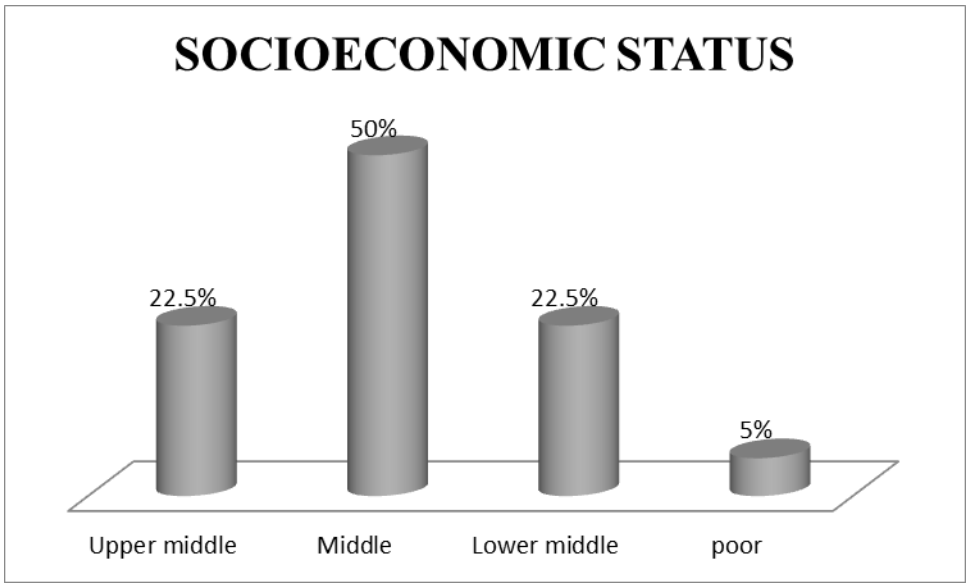


Figure 7a.9 shows that 50% of cases coming under Middle class , 22.5% of cases coming under Upper middle class, 22.5% of cases coming under Lower middle class and 5% of cases coming under Poor class.

7a.10 Distribution of Occupational status:

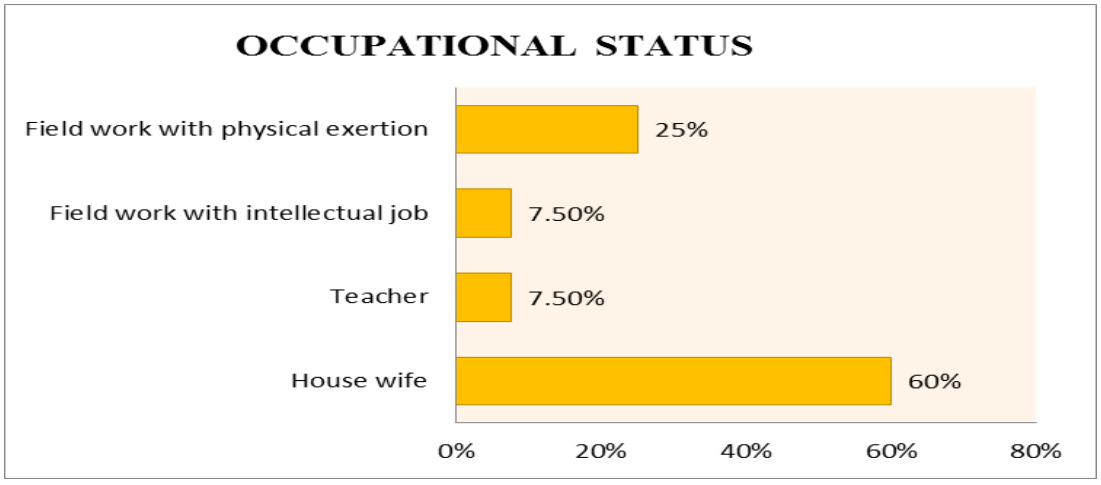


Figure 7a.10 shows that 60% of cases were Housewives.7.5% of cases were in Field work with intellectual job category, 7.5% of cases working as a Teacher, 25% of patients were in Field work with physical exertion category.

7a.11Gunam Distribution:

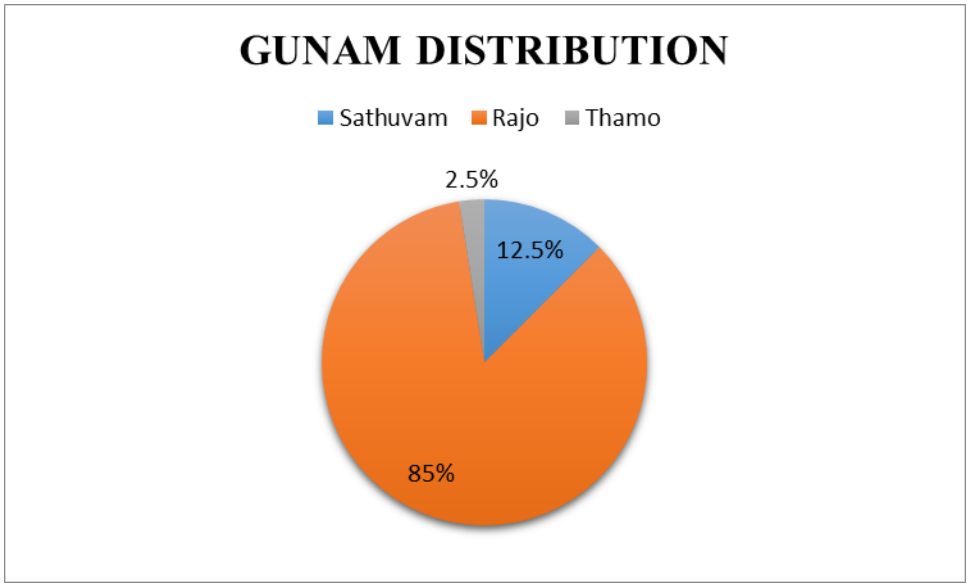


Figure 7a.11 shows that 85% had Rajo gunam(courage,honesty,perseverance), 12.5% of patients had Sathuva gunam(patience,selfcontrol,humility), and 2.5% of patients had Thamo gunam(excessive sleep,laziness,anger).

7a.12 Imporigal (5 Sense Organs):

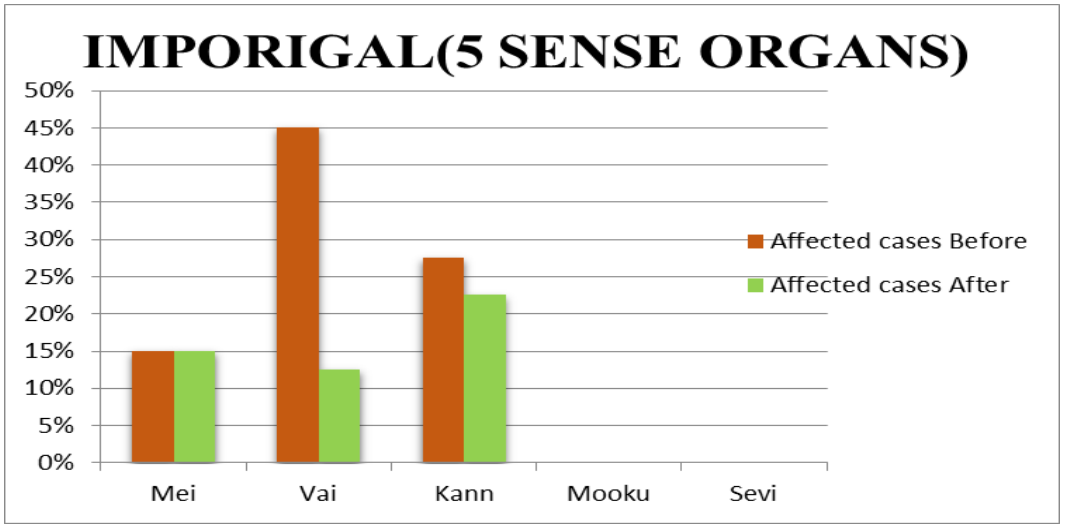


Figure 7a.12 shows, percentage of affected Mei(Skin), Vai(mouth) , Kann(Eye) before treatment(BT) and after treatment (AT)

Vai(mouth) tongue was affected (coated, sore, pallor, dryness) in BT- 45% and AT- 12.5% of cases,

Mei(Skin) was affected(dryness, pallor) in BT- 15% and AT -15% of cases.

Kann(Eye) was affected (pale conjunctiva, redness, squint) in BT- 27.5% and AT - 22.45% of cases.

7a.13 Impulangal (5 Senses):

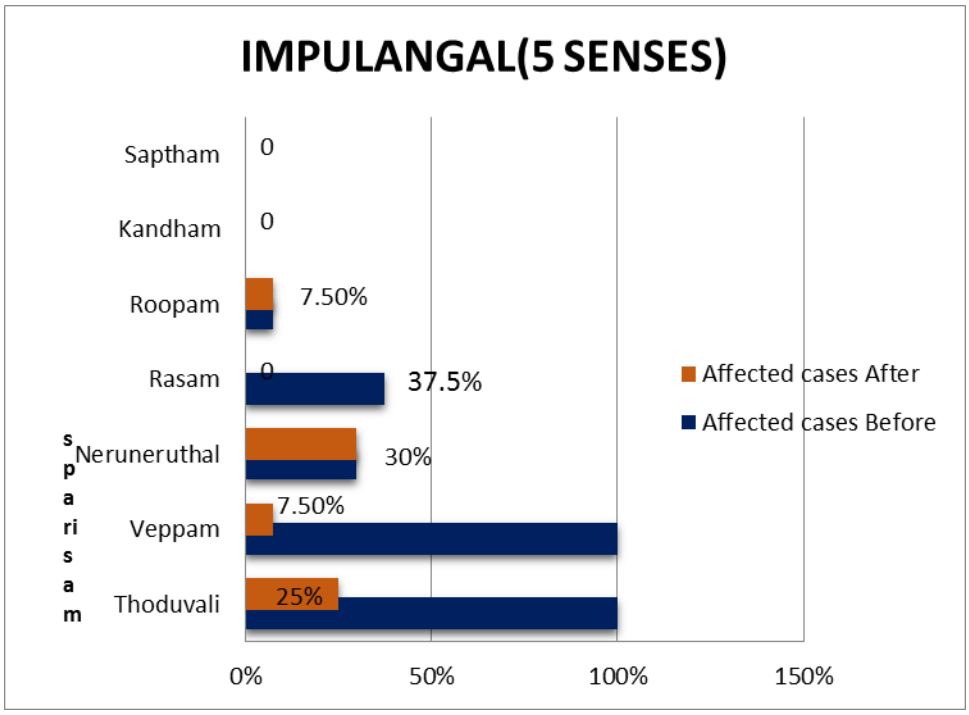


Figure 7a.13 shows % of affected Sparisam(Touch), Rasam(Taste), Roopam(Vision) before treatment(BT) and after treatment (AT)

Sparism(Touch sense) was affected (Thoduvali-tenderness, Veppam-local heat, Neru neruthal-Crepitation) in BT- 100% and AT-25% of cases, BT- 100% and AT-7.5% of cases, BT- 30% and AT-30% of cases respectively.

Rasam(Taste sense) was affected (Suvai inmai-Tastelessness, Vai kasathal-Bitter taste, Vai pulithal-sour taste, Naa erichal-burning sensation) in BT-37.5 % and AT -0% of cases.

Kann(Visual sense) was affected (Maaru kan(squint) in BT- 7.5% and AT -7.5% of cases.

7a.14.Kanmenthiriyangal (Organs of Action):

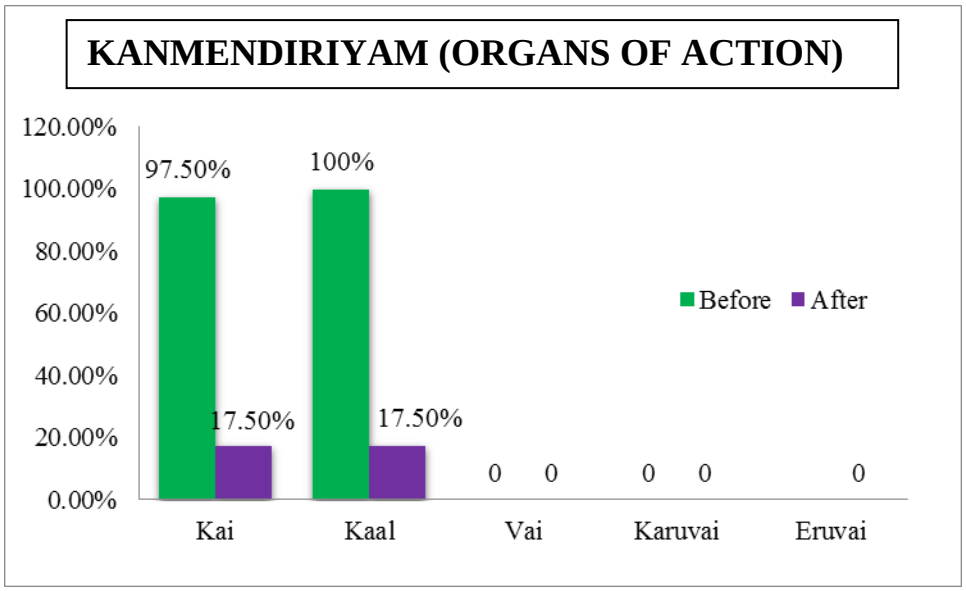


Figure 7a.14 shows percentage of affected Kai(upper limb), Kaal(Lower limb) before treatment(BT) and after treatment (AT)

Kai(Upper limb) was affected(swollen joints and muscle spasm of Upper limb) in BT-97.5 % and AT -17.5% of cases.

Kaal(Lower limb) was affected(swollen joints and muscle spasm of lower limb) in BT 100 % and AT -17.5% of cases.

7a.15 Kanmavidayam(Motor Action):

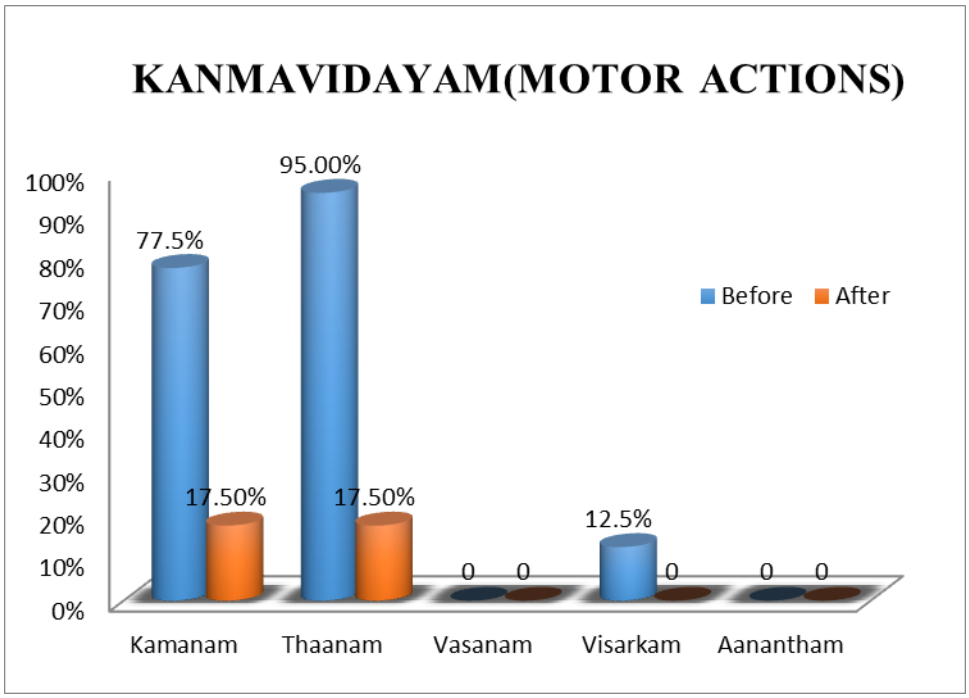


Figure 7a.15 shows percentage of affected Kamanam(actions of lower limb), Thaanam(actions of Upper limb), Visarkam(Defecation) before treatment(BT) and after treatment (AT)

Kamanam(actions of lower limb) was affected(Abduction, adduction, flexion, extension, standing, walking, running etc.,) in BT-77.5 % and AT -17.5% of cases.

Thanam(actions of Upper limb) was affected(Abduction, adduction, flexion, extension, rotation, gripping, fisting etc.,) in BT 95 % and AT -17.5% of cases.

Visarkam(Defecation) was affected(constipation) in BT 12.5% and AT -0% of cases.

7a.16 Kosangal(Sheaths or Coverings of Aathma(soul):

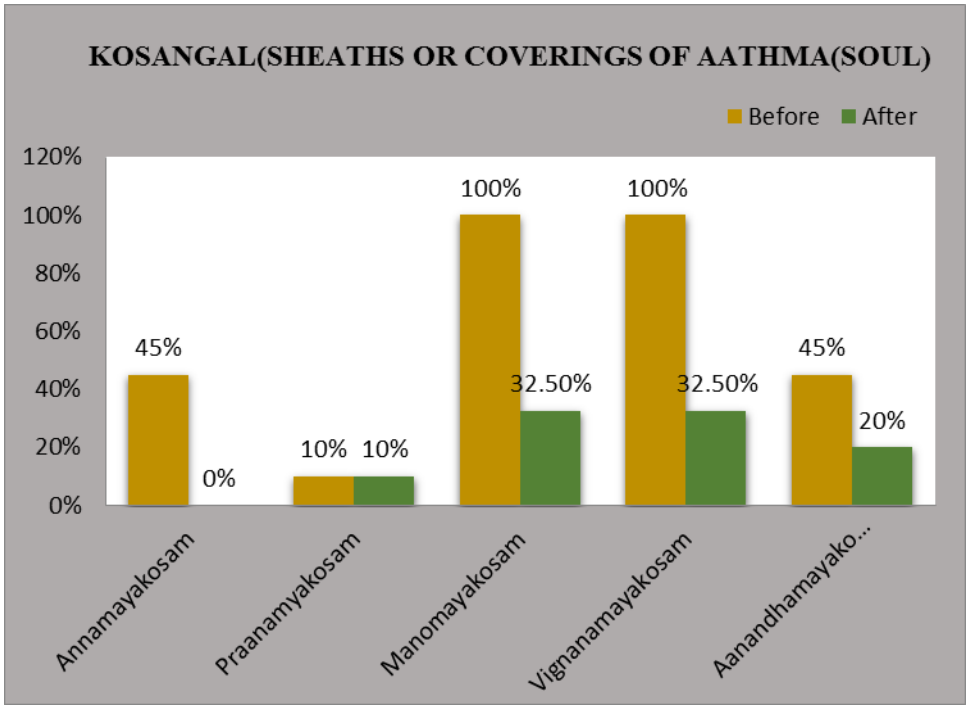


Figure 7a.16 shows percentage of affected Annamayakosam (Sheath of food-It is the body constituted by the 7 physical constituents.This food of sheath helps in the digestion of food which is separated into essence and excrement and nourishes the 7 physical constituents), Pranamayakosam (Sheath of breath/praanan-It is the body constituted by the combination of Praanan and Kanmenthiriyam. This helps to take in the vital air from outside and spreads it all over the body,collects the vayu(i.e.CO_2)produced as a result of metabolic function from all over the body and expels it from the body)., Manomayakosam(Sheath of mind-It is the body constituted by the Manam(mind) and Gnanenthiriyam.The heart is said to be the seat of the mind. The heart, with the help of the vascular system, circulates the blood throughout the body. So the mind is said to have control or influence over the cardio-vascular system (N.B: According to some yogis, the mind has no specific seat and it pervades all the tissues.) Vignanamaya kosam(Sheath of

Knowledge- It is the body constituted by the Buddhi and Gnanenthiriyam. Seated in the brain knowledge, with the support of Kanmenthiriyam and Gnanenthiriyam, rules over the other kosams), Aanandhamayakosam (Sheath of bliss-It is constituted by the Praana vayu and Suzhuthi(a sleep like state).It indicates the maintaining of a blissful state with the application of knowledge.

Before treatment (BT) / After treatment (AT)

Annamayakosam was affected (Anorexia) in BT-45% and AT-0%

Praanamayakosam was affected (Dyspnoea) in BT-10% and AT-10%

Manomayakosam was affected (Stress) in BT-100% and AT-32.5%

Vignanamayakosam was affected (Depression) in BT-100% and AT-32.5%

Aanandhamayakosam was affected (Sleep disturbances) – in BT- 45% and AT - 20%

Note: Kosam- It implies coverings of the aathma which experiences the different levels of consciousness ranging from the gross physical body to subtle levels of mind which includes emotional and spiritual aspects. It is the experience leading from individual consciousness to cosmic consciousness.

7a.17.1.Uyirthatukkal–Vatham

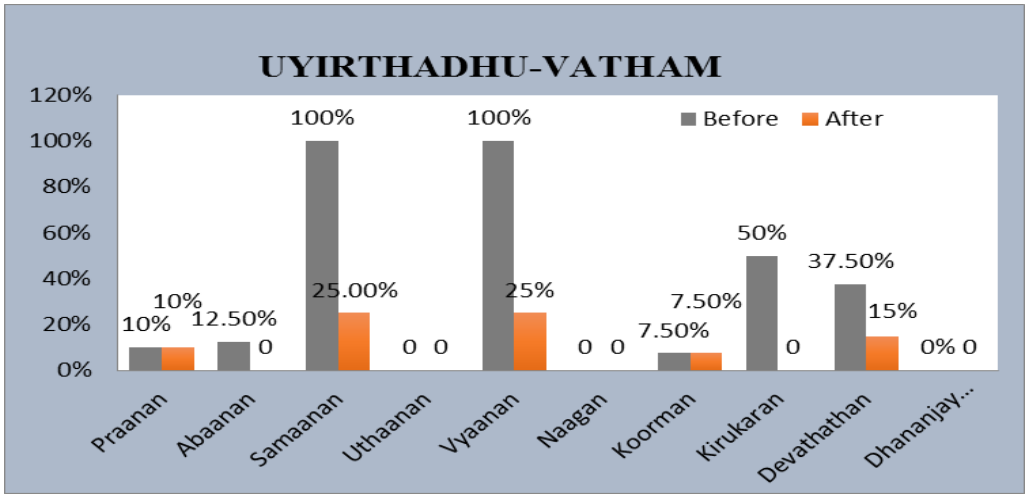


Figure 7a.17 shows percentage of affected Praanan(It helps in inspiration, expiration and digestion), Abaanan(It helps in the act of urination, defecation, discharge of semen during sexual act), Udhaanan(It responsible for the reflexes like cough, sneeze, hiccup and vomiting, Viyaanan(It performs movements of both movable and immovable parts of the body, Samaanan(controls all the other vayus, balance the six tastes, water and foodstuffs during the process of digestion and reaches them to their sites of action, Naagan(It acts as an instrument for Anthakaranam(Manam, Puthi, Sitham, Agankaaram),responsible for one's intelligence. It activates the brain to learn all kinds of arts and to sing good songs),

Koorman(It emanates from manam(mind) acts on the eye and produces blinking and good vision. It responsible for the act of yawning and closing of the mouth), Kirukaran(It acts on the tongue to produce salivary secretion and on the nose to produce nasal(mucous) secretion. It produces much hunger. Devathathan (It emanates the form of circle it produces laziness and tiredness on waking up from bed),

Before treatment -BT/After treatment-AT.

Praanan was affected (Dyspnoea on exertion) in BT-10% and AT-10% due to moderate iron deficiency anaemia (Hb level 8-9.5 gm/dL).

Abaanan was affected (Constipation) in BT-12.5% and AT-0%.

Samaanan was affected (Anorexia, controlling other vaayus was affected- Praanan, Abaanan, Vyanan, Naagan, Koorman, Kirukaran, Devathaththan) in BT-100% and AT-25%.

Vyaanan was affected (Tenderness and Restricted movements of the minor and major joints of upper and lower limbs) in BT-100% and AT- 25%.

Koorman was affected (defective vision) in BT- 7.5% and AT-7.5%.

Kirukaran was affected (Anorexia and dryness of the tongue) in BT-50% and AT-0%.

Devathathan was affected (Tiredness) in BT-37.5% and AT-15%.

7a.17.2 Pitham:

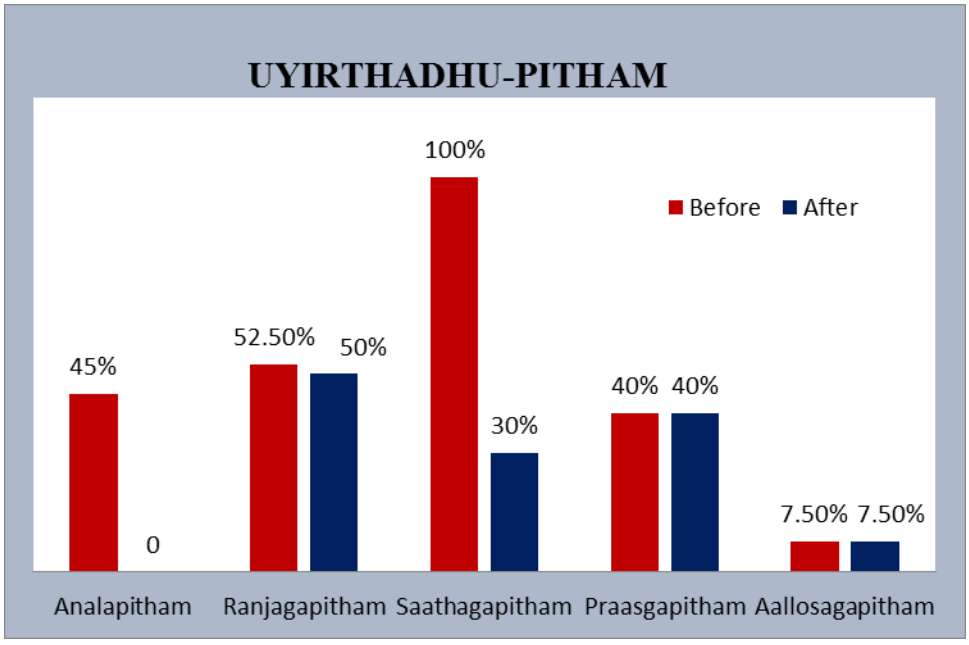


Figure 7a.17.2 shows affected Anal pitham (Located in the digestive organ , helps in the process of digestion.), Ranjaka pitham(It has the characteristic of increasing the blood volume. Located in the stomach and intestine, it gives the juice, which is separated from

the digested food, the typical red colour.), Saathaka pitham(It has the characteristic of performing or executing an act.), Praasakam(Located on the skin, it gives lustre to the skin.), Alosakam(It has the characteristic of enabling the eyes to perceive the objects.)

Anal pitham was affected (Anorexia) in BT-45% and AT-0%

Ranjaka pitham was affected (Anemia) in BT-52.5% and AT-50%

Saathaka pitham was affected (difficult to perform routine works) in BT-100% and AT-30%

Praasakapitham was affected (loss of luster and dryness of skin) in BT-40% and AT-40%

Alosakapitham was affected (Myopia, squint) in BT-7.5% and AT-7.5%.

7a.17.3 Iyyam:

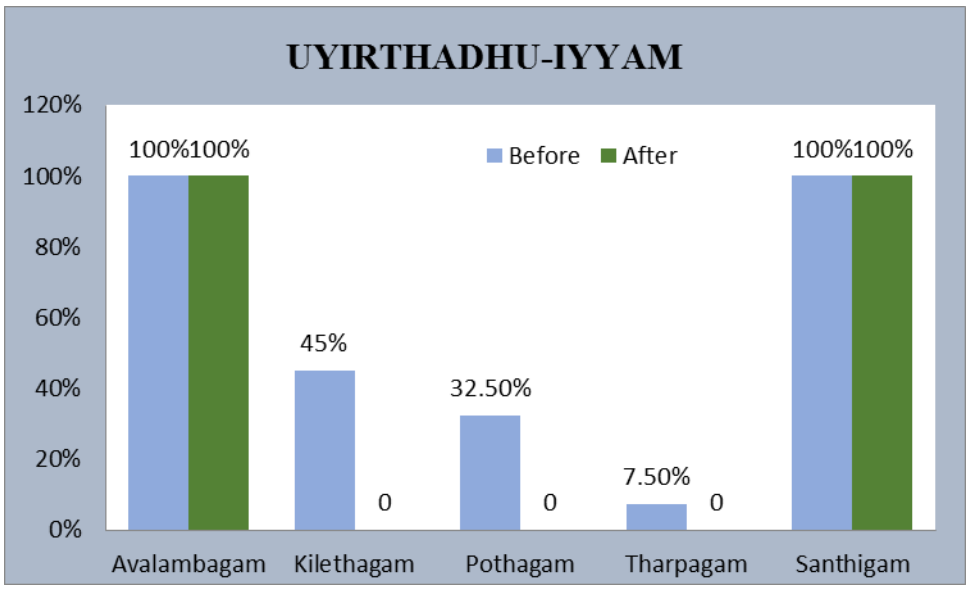


Figure 7a.17.3 shows affected Avalambagam(It supports the other 4 Kabams), Kilethagam(located in the stomach, it wets the food and water ingested and renders them softness), Pothagam(located in the tongue, it perceives the sense of taste of foods and liquids taken in by mouth), Tharpakam(Located in the head, it cools the eyes), Santhikam(Located in the joints, it gives lubrication and helps in extension, flexion and rotation).

Avalambagam was affected (It supports other kabams-Tharpagam, Santhikam, etc) in BT-100% and AT-100%

Kilethagam was affected (Anorexia) in BT-45% and AT-0%

Pothagam was affected (Tastelessness , Bittertaste sense) in BT-32.5% and AT-0%

Tharpagam was affected (Burning sensation of eyes) in BT-7.50% and AT-0%

Santhikam was affected (Tenderness and restricted movement) in BT-100% and AT-100%.

7a.18.Seven Udalthadhukkal:

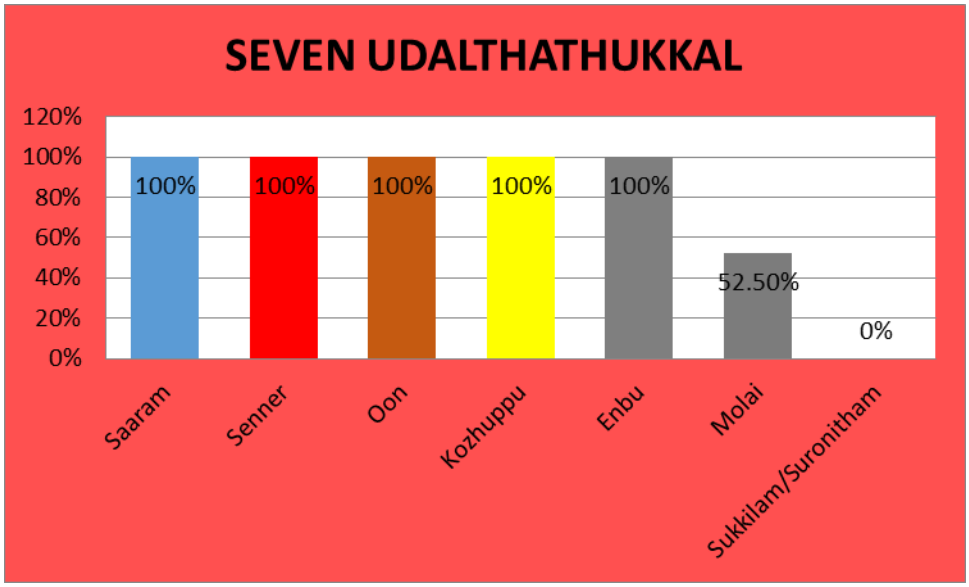


Figure 7a.18 shows In Seven Udal thathus, Saaram was affected leading to lethargic and depressed conditions. Senneer was affected leading to nervousness, dryness of skin, diminution of bodily luster. Oon was affected(muscle spasm) ,resulting in difficulty to perform routine duties. Kozhuppu was the main thathu affected , lead to cause crepitations(due to diminution of the pulpy semifluid material) and difficulty in movements of the joints.. Enbu thathu was affected, leading to restricted movements of the joints and joint stiffness. Moolai thath(bone marrow) was affected, leading to Anaemia.

Saaram, Senneer, Oon, Kozhuppu, Enbu were affected in 40 cases and Moolai was affected in 21 cases.

7a.19 EnvagaiThervu (Eight Fold Examination):

7a.19.1 NaadiNadai (Pulse Play):

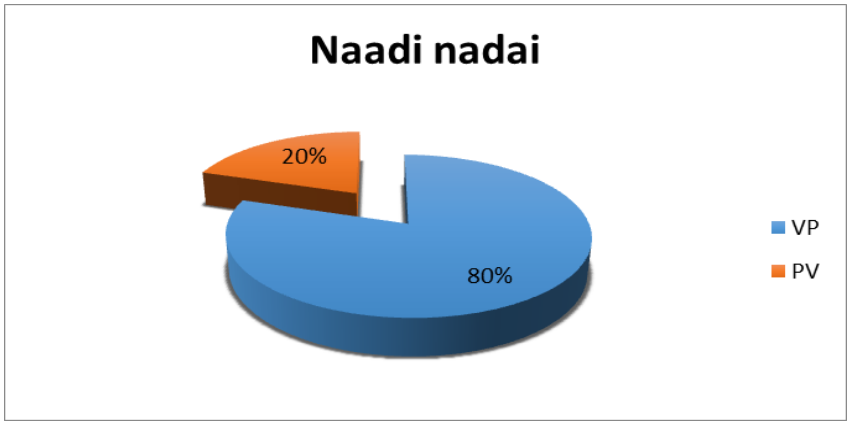


Figure 7a.19.1 Among the 40 cases, 80% of cases had Vatha pitha naadi, 20% of cases had Pithavatha naadi.

7a.19.2 Sparism(Mei kuri -Physical signs):

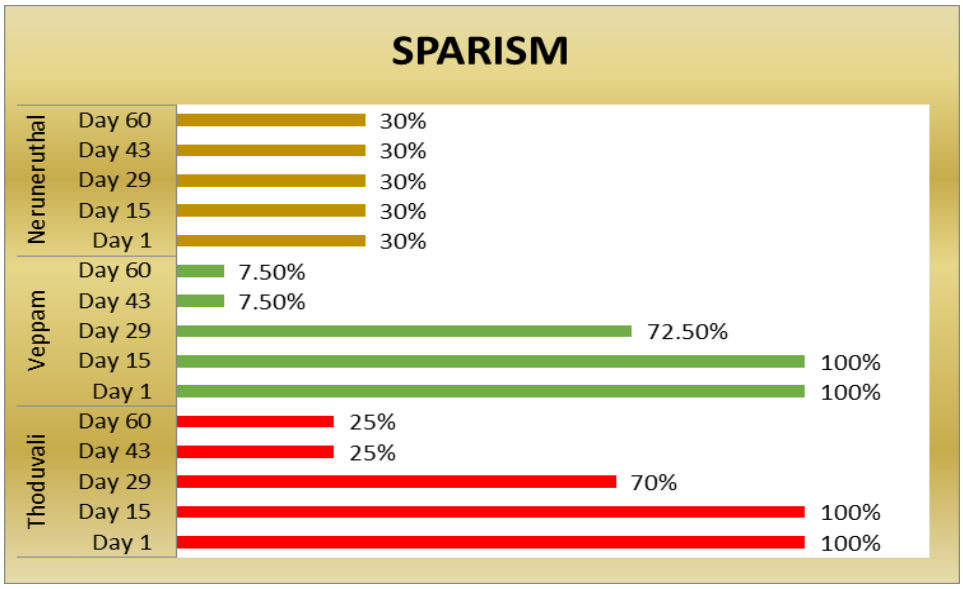


Figure 7a.19.2 shows **Thoduvali (Tenderness)** Among 40 cases, 70% cases had tenderness till 29th day of treatment and further 14 days treatment, only 25% of cases had tenderness.

Veppam (Warmth)

Among 40 cases, initially 100% of cases had veppam, 72.5% of cases had veppam (warmth) at 29th day of treatment and further 14 days of treatment only 7.5% of cases had veppam.

Neruneruthal (Crepitation):

From Day 1 –Day 60 only 30% of cases had Neruneruthal(Crepitation).

7a.19.3 Naa (Tongue):

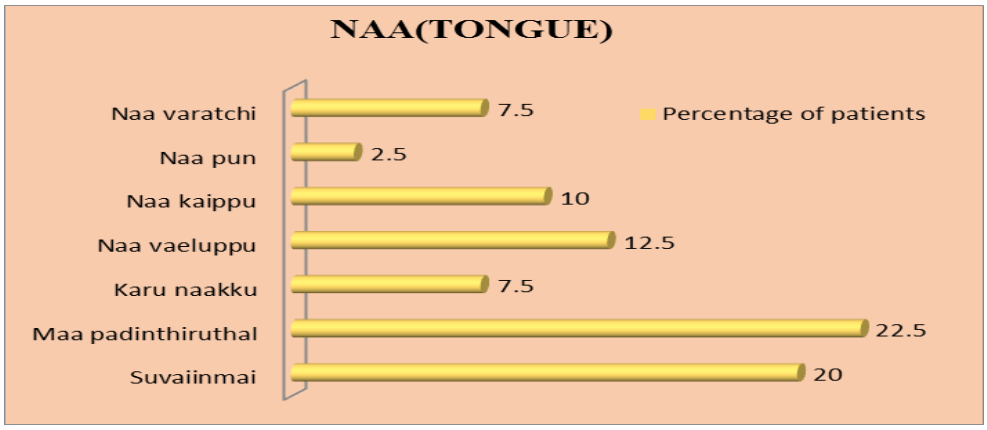


Figure 7a.19.3 shows out of 40 patients 22.5% of patients had Maa padinthiruthal (coated tongue), 20% of patients had Suvai inmai(tastelessness), 12.5% of patients had Naa Vaeluppu(Pallor tongue), 10% of patients had Naa Kaippu(Bitter taste), 7.5% of patients had Naa Varatchi(Dryness of tongue), 7.5% of patients had Karu naakku(Pigmented tougue), 2.5% of patients had Naa pun.

7a.19.4 Niram(Complexion)

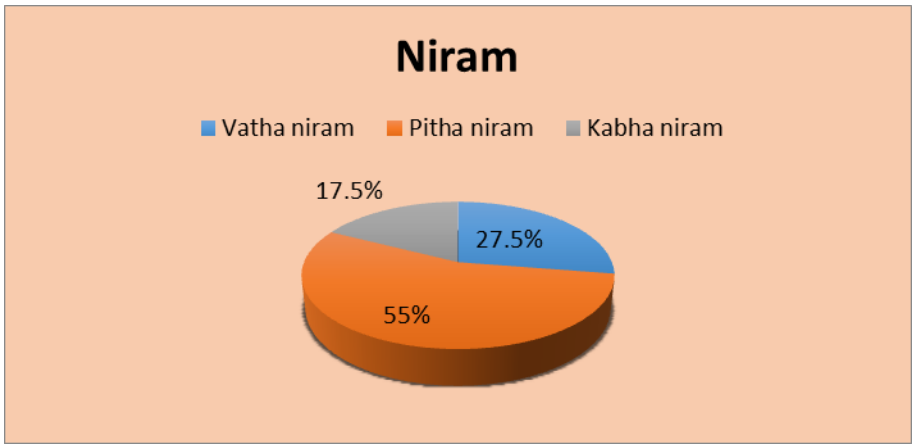
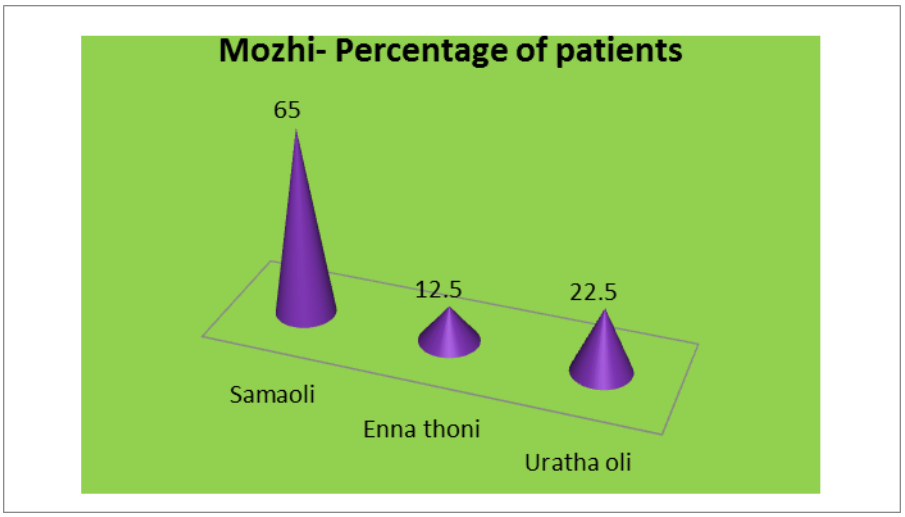


Figure 7a.19.4 Among 40 cases 27.5% of cases had Vathaniram, 55 % of cases had Pithaniram and 17.5% of cases had Kabhaniram.

7a.19.5 Mozhi:



Voice (mozhi)

Figure shows 7a.19.5 Among 40 cases, 65% cases had Samaoli (medium pitched voice), 12.5% cases had Ennathoni (low pitched voice) and 22.5% cases had Uratthaoli (high pitched voice).

7a.19.6 Vizhi (Eyes):

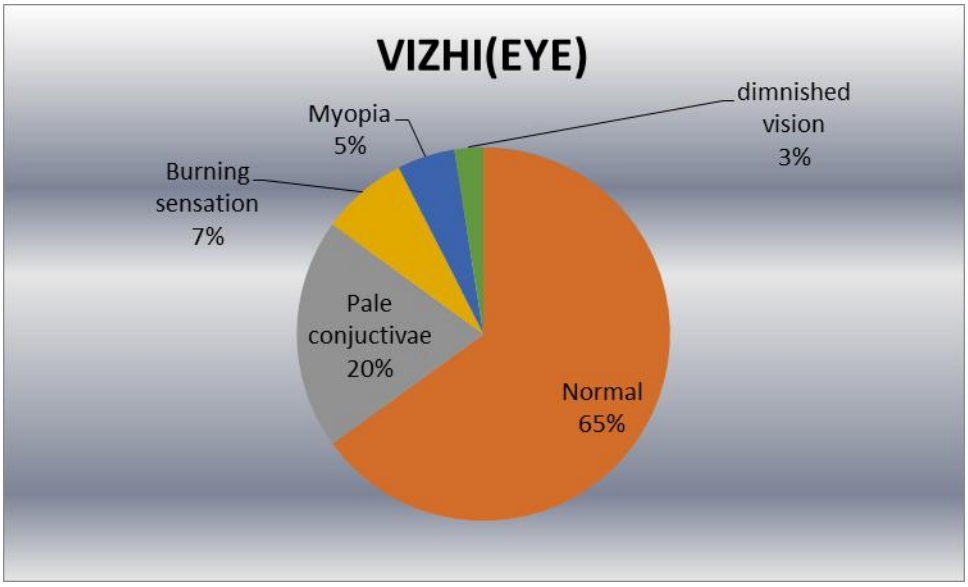


Figure shows 7a.19.6 Among 40 cases, 65% of cases had normal eyes, 20% of cases had Imai inaipaadala vaeluppu(pale conjunctivae), 7% cases had Kann eritchal (burning of eyes), 5% of cases had Kittap parvai (myopia) and 3% of cases had Paarvai kuraipaadu (diminished vision).

7a.19.7 Malam (Stools)

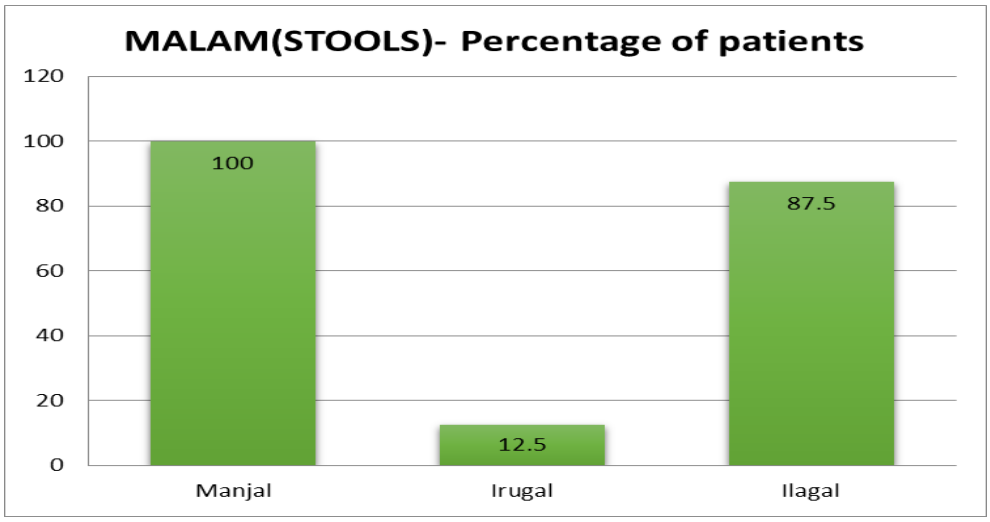


Figure shows 7a.19.7 Among 40 cases, 100% cases had Yellow (Manjal) coloured stools throughout the treatment period

- Among 40 cases, 12.5 % cases had constipation in first 3 visits and became normal in further course of treatment.
- Among 40 cases, 87.5 % cases had normal bowel habits and became 100% normal at the end of the treatment.

7a.19.8.1 Neerkuri (Urine-Physical characteristics):

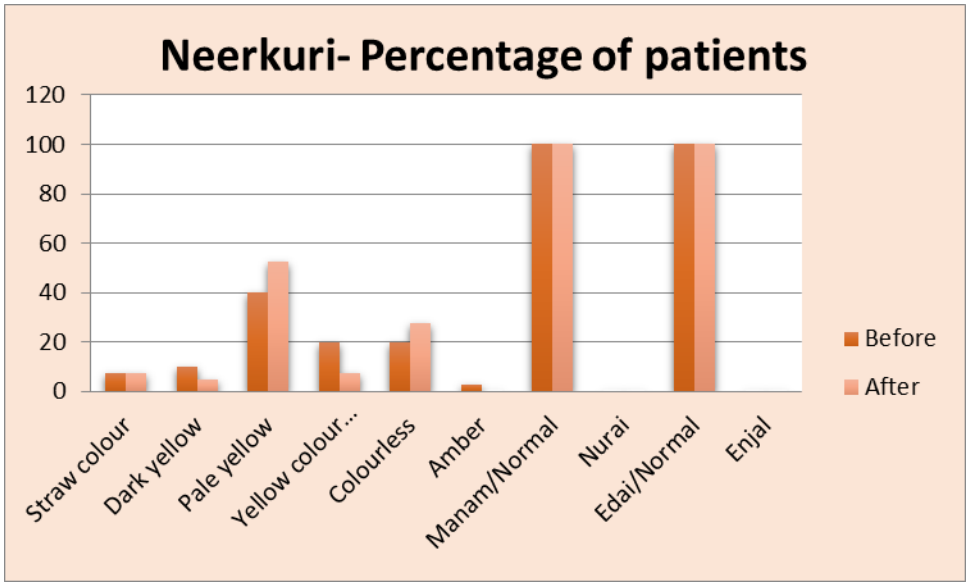


Figure shows 7a.19.8.1

Colour of the urine

Among 40 cases, 40% cases had pale yellow coloured urine, 20% cases had colourless urine, 7.5 % cases had straw coloured urine, 20% cases had yellow coloured

urine, 10% cases had dark yellow coloured urine and 2.5% cases had amber coloured urine in before treatment. 52.5% cases had pale yellow coloured urine, 27.5% cases had colourless urine, 7.5 % cases had straw coloured urine, 7.5% cases had yellow coloured urine and 5% cases had dark yellow coloured urine in after treatment.

Smell of the urine

Among 40 cases, 100% of cases had normal odour(ammonical odour) in before and after treatment.

Volume (Alavu) of urine

Among 40cases, 100% of cases had normal volume of urine in before and after treatment.

Turbidity (Nurai) of urine

Among 40cases, 100% of cases had clear urine in before and after treatment.

Deposits (Enjal) in urine:

Among 40cases, 100% of cases reported normal in before and after treatment.

7a.19.8.2 Neikkuri (Oil on Urine sign):

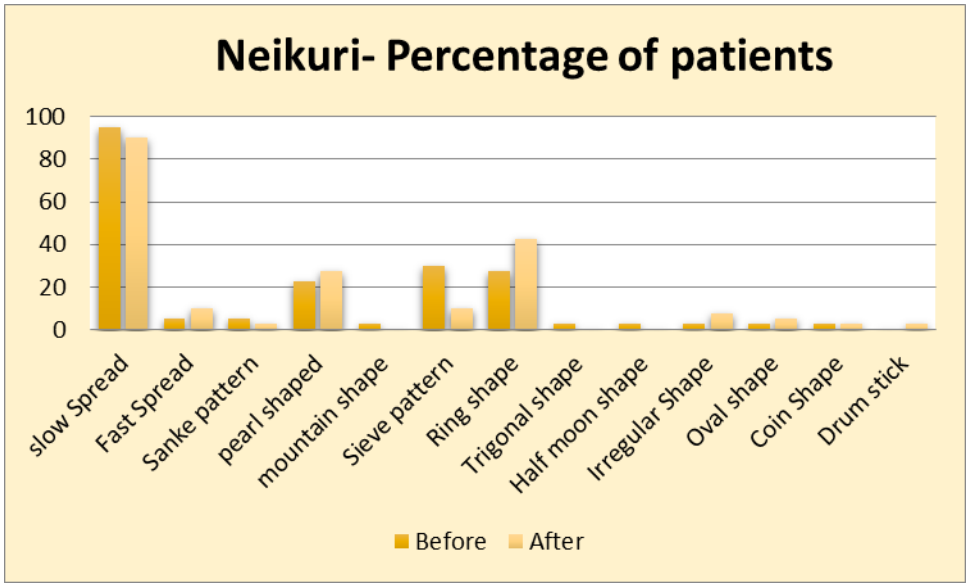


Figure 7a.19.8.2

In before treatment

Spreading pattern

Among 40cases, 95% of cases had slow spreading pattern in before treatment and 5% of cases had fast spreading pattern.

Shape

5% had snake pattern, 22.5% of cases had pearl shaped, 2.5% of cases had mountain shaped, 30% of cases had sieve pattern, 27.5% cases had ring shaped, 2.5% of cases had trigon shaped, 2.5% cases had half-moon shaped, 2.5% cases had irregular shaped, 2.5% cases had oval shaped and 2.5% cases had coin shaped and 0% of cases had drumstick pattern.

In after treatment

Spreading pattern

90% cases had slow spreading, 10% cases had fast spreading.

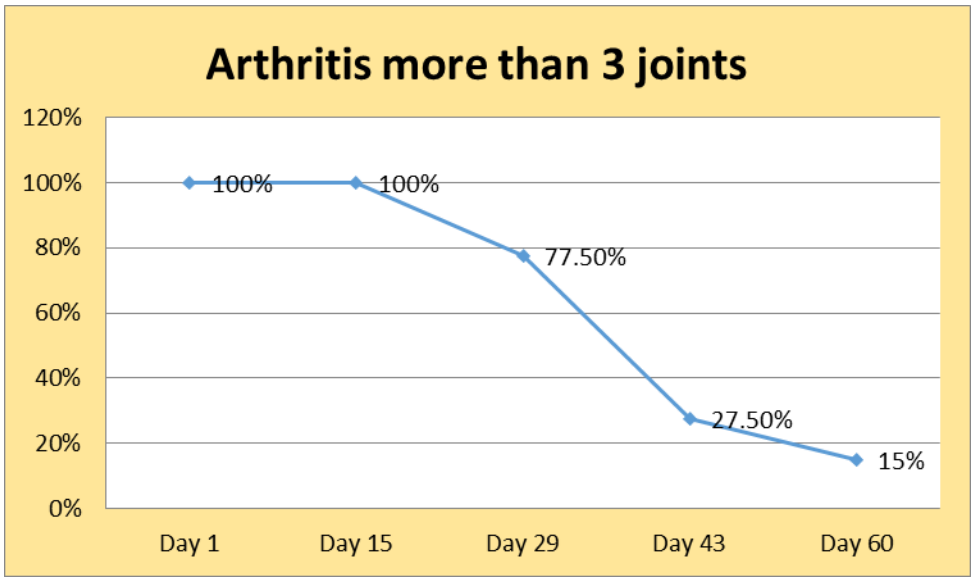
Shape

2.5% of case had Snake pattern, 27.5% of cases had Pearl shaped, 0% of cases had Mountain shaped, 10% of cases had Sieve pattern, 42.5% cases had Ring shaped, 0% of cases had Trigone shape, 0% cases had half-moon, 7.5% cases had irregular, 2.5% cases had Oval shape and 2.5% cases had coin shaped and 2.5% of cases had Drumstick pattern.

7a.20. Physical functions assessment

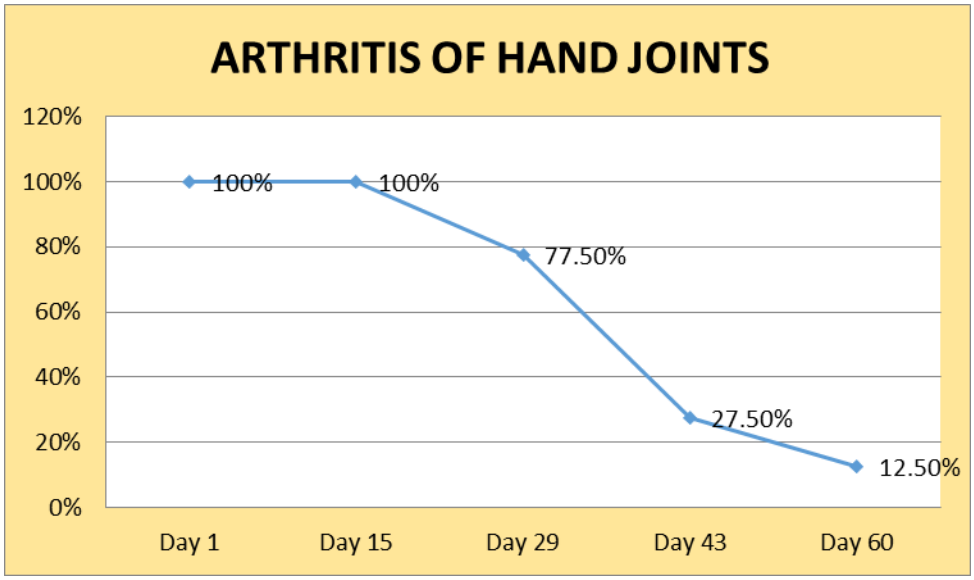
7a.20.1Clinical Features:

7a.20.1.1Arthritis of more than 3 joints:



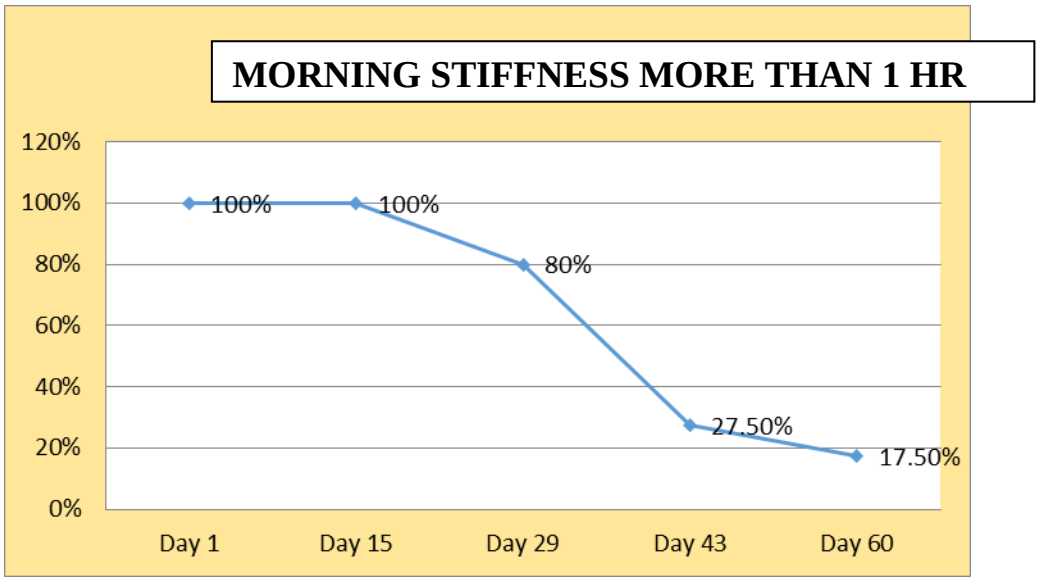
At the baseline period, 100 % of patients had pain and inflammation in more than three joints. At the end of treatment with NM i.e., 60th day (7 days dosing followed by 7 days drug holiday) only 15% of patients had the pain and inflammation in more than three joints.

7a.20.1.2 Arthritis of hand joints



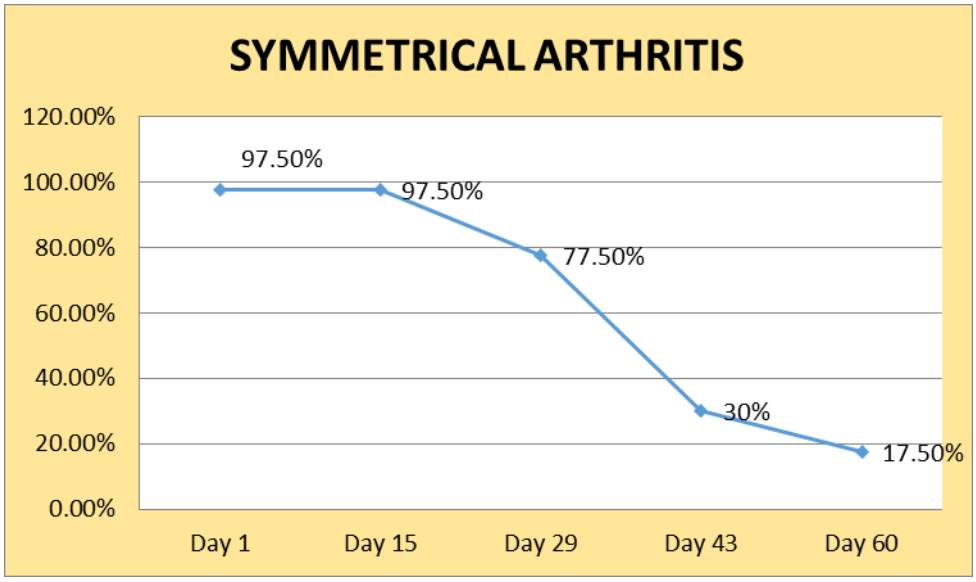
At the baseline period, 100 % of patients had pain and inflammation of hand joints. During the treatment period, in all the patients, pain and inflammation of hand joints were reduced gradually. Only 12.5% of patients had pain and inflammation of hand joints at the end of NM treatment.

7a.20.1.3 Morning stiffness> 1 hr:



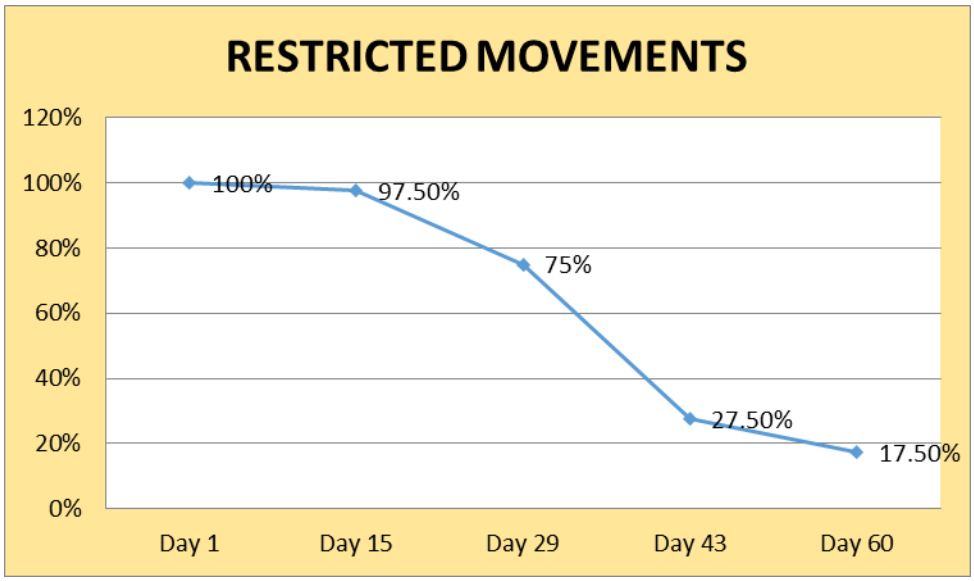
At the baseline period, 100 % of patients had morning stiffness in the joints. During the treatment with NM for 60 days, only 17.5%.of patients had the morning stiffness at the end of the treatment..

7a.20.1.4 Symmetrical arthritis:



At the baseline period, 97.5 % of patients had involvement of arthritis in the symmetrical joints. During the treatment with NM for 60 days, the involvement of arthritis in symmetrical joints was reduced into 30% of patients at 43rd day. Only 17.5% of patients had symmetrical joints involvement after the end of 60 days treatment.

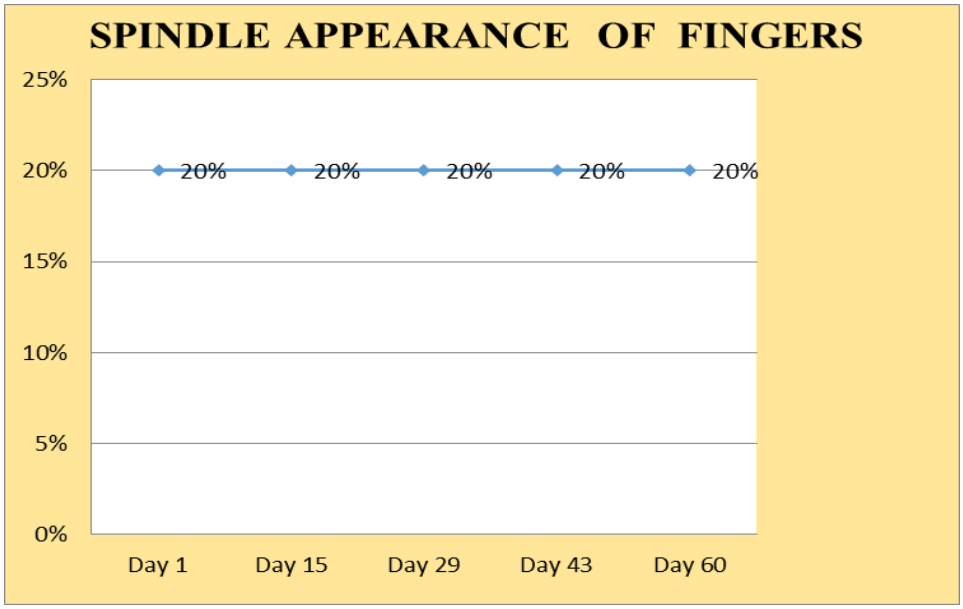
7a.20.1.5 Restricted movements:



At the baseline period, 100 % of patients had restricted movements of the joints. During the treatment with NM for 6o days, only 27.5% of patients had restricted movements in

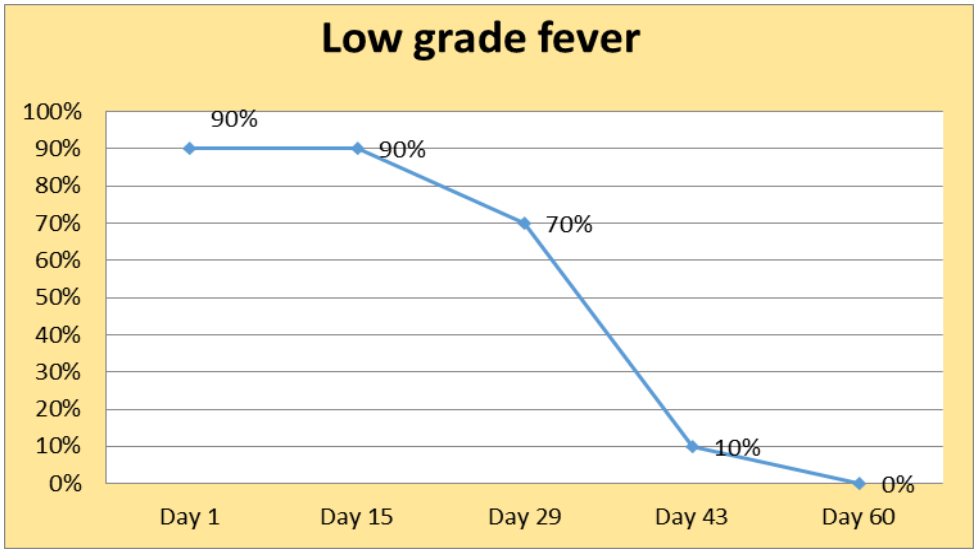
the joints at 43rd day of treatment and at the end of the treatment period, only 17.5% of patients had restricted movements.

7a.20.1.6Spindled appearance of fingers:



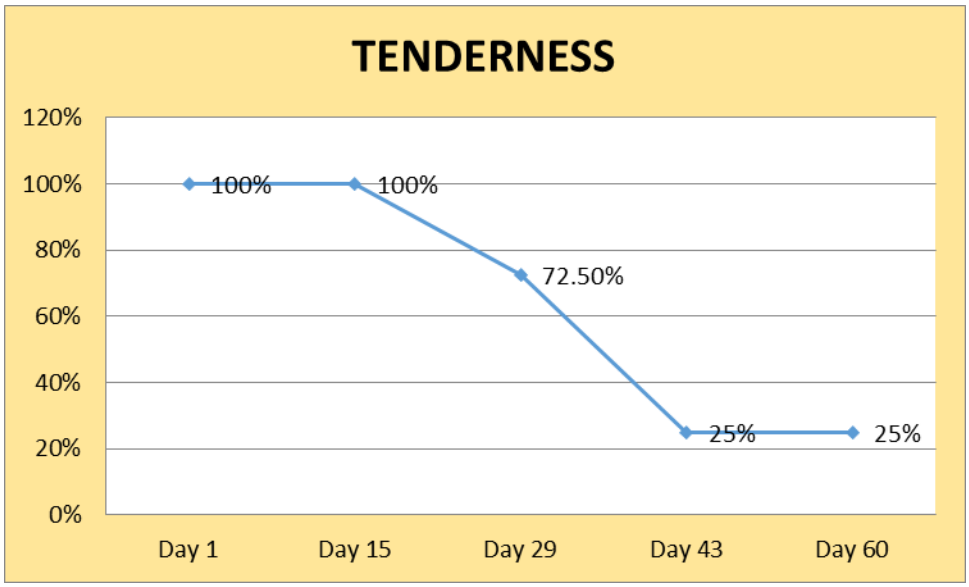
At the baseline period, 20 patients had spindled appearance of fingers. No change was observed in all the 20 patients even after the treatment period of 60 days.

7a.20.1.7Low grade fever



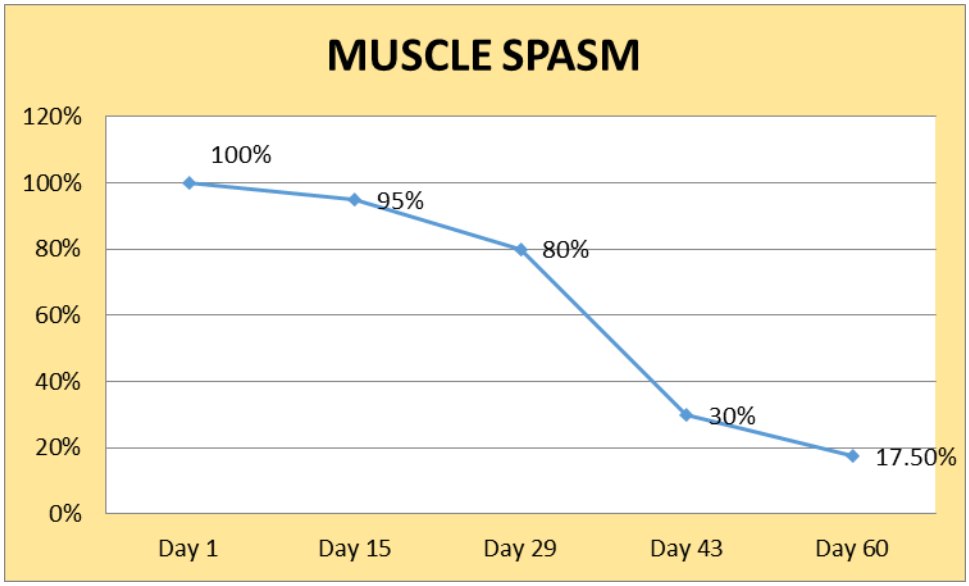
At the baseline period, 90 % of patients had intermittent low grade fever. After 60 days of treatment with NM, none of the patients had reported of having low grade fever.

7a.20.1.8 Tenderness



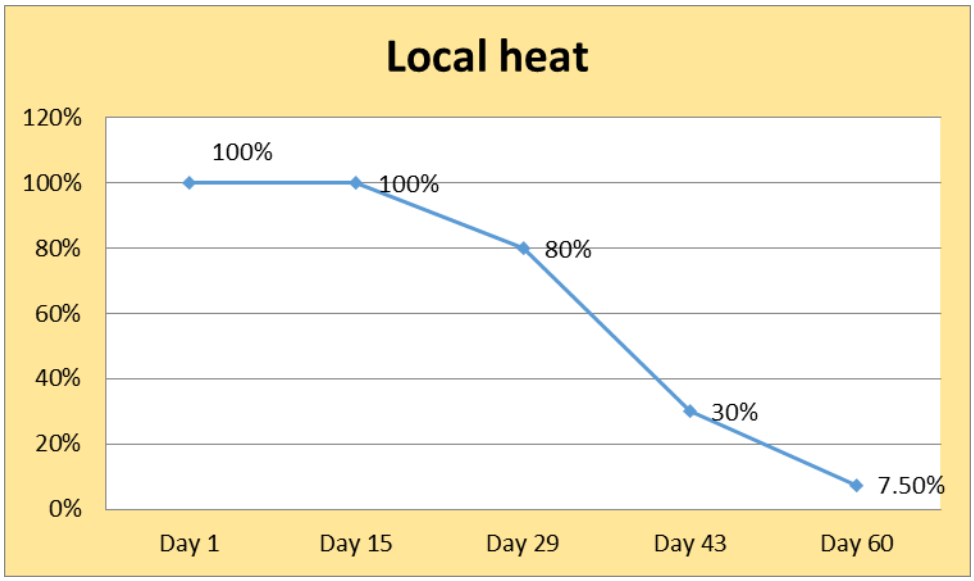
At the baseline period, 100 % of patients had tenderness in the joints. But at the end of 60 days treatment with NM, only 25% of the patients had tenderness in the joints.

7a.20.1.9 Muscle spasm



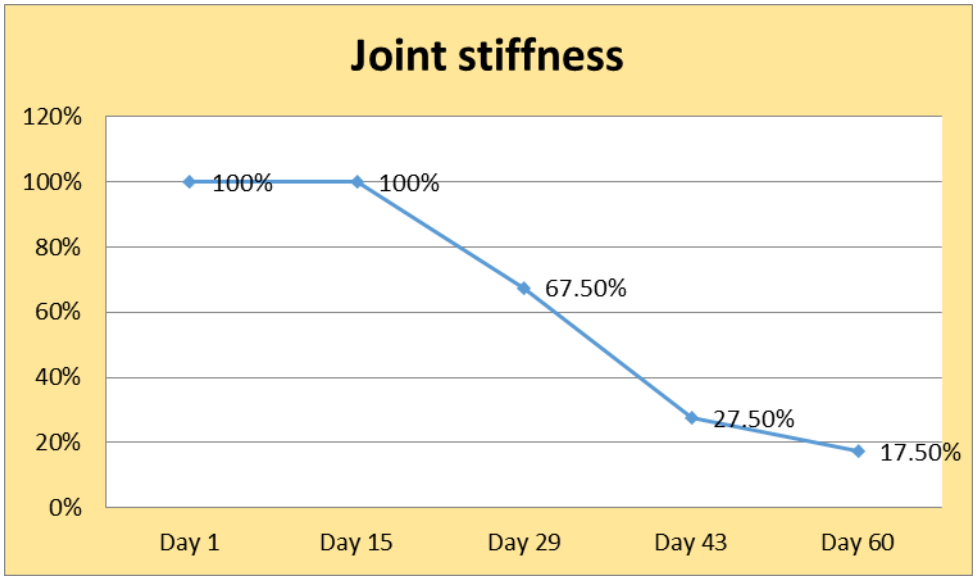
At the baseline period, 100 % of patients had muscle spasm in the affected joints. During the treatment with NM for 60 days, only17.5% of patients had muscle spasm in the affected joints.

7a.20.1.10 Local heat



At the baseline period, 100 % of patients had local heat. During the treatment with NM for 60 days, only 7.5%.of patients had local heat over the affected joints.

7a.20.1.11Joint stiffness



At the baseline period, 100 % of patients had joint stiffness. During 60 days of treatment with NM, only 25% of patient had stiffness of the joints at 43rd dayand· at the end of 60th day ,only17.5% of patients had jsoint stiffness.

7a.21 MHAQ:(Modified health assessment questionnaire (MHAQ)) score

Before treatment:

Are you able to	**without ANY difficulty <0>**	**with SOME difficulty <1>**	**with MUCH difficulty <2>**	**unable TO DO <3>**
Q1 Dress yourself, including tying shoelaces and doing buttons?				**2.425**
Q2 Get in and out of bed?			**1.975**	
Q3 Lift a full cup or glass to your mouth?				**2.025**
Q4 Walk outdoors on flat ground?			**1.275**	
Q5 Wash and dry your entire body?			**1.7**	
Q6 Bend down to pick up clothing from the floor?			**1.65**	
Q7 Turn regular faucets on and off?			**1.325**	
Q8 Get in and out of a bus, car, train, or airplane?			**1.65**	
Sub-total			**9.575**	**4.450**
Total	**14.025**			

Q1, Q2, Q3, Q4, Q5, Q6, Q7, Q8- the mean of 40 patients

After treatment

Are you able to	without ANY difficulty <0>	with SOME difficulty <1>	with MUCH difficulty <2>	unable TO DO <3>
Q1 Dress yourself, including tying shoelaces and doing buttons?		0.325		
Q2 Get in and out of bed?		0.375		
Q3 Lift a full cup or glass to your mouth?		0.333		
Q4 Walk outdoors on flat ground?		0.4		
Q5 Wash and dry your entire body?		0.475		
Q6 Bend down to pick up clothing from the floor?		0.525		
Q7 Turn regular faucets on and off?		0.4		
Q8 Get in and out of a bus, car, train, or airplane?		0.425		
Sub-total		3.25		
Total	3.25			

Q1, Q2, Q3, Q4, Q5, Q6, Q7, Q8- the mean of 40 patients

Table No:7a.21.1 Modified health assessment questionnaire(MHAQ)

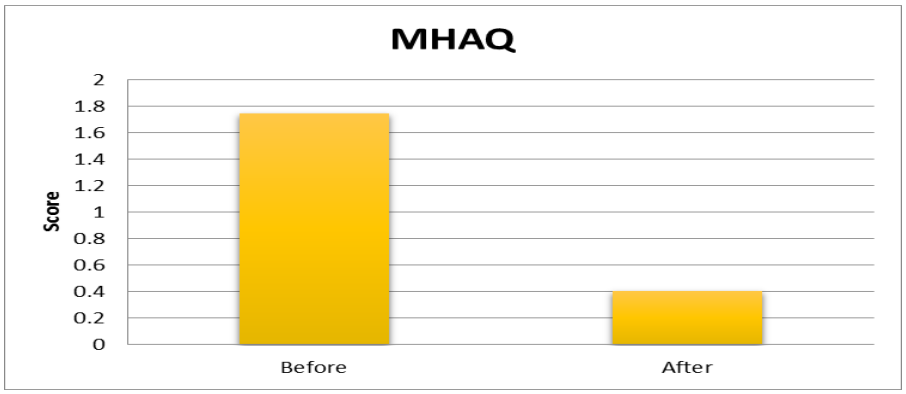
MHAQ	Before treatment	After treatment	p-value
Q1	**2.425**	**0.325**	p<0.0001
Q2	**1.975**	**0.375**	p<0.0001
Q3	**2.025**	**0.333**	p<0.0001
Q4	**1.275**	**0.4**	p<0.0001
Q5	**1.7**	**0.475**	p<0.0001
Q6	**1.65**	**0.525**	p<0.0001
Q7	**1.325**	**0.4**	p<0.0001
Q8	**1.65**	**0.425**	p<0.0001
Total	**14.025**	**3.25**	p<0.0001

Q1, Q2, Q3, Q4, Q5, Q6, Q7, Q8- each value denotes the mean of 40 patients

MHAQ	Total	Total/8 (mean of 8 questions)
Before treatment	14.025	1.75
After treatment	3.25	0.406

- MHAQ score less than 0.3 is considered normal.

Figure 7a.21.1.Modified health assessment questionnaire(MHAQ)

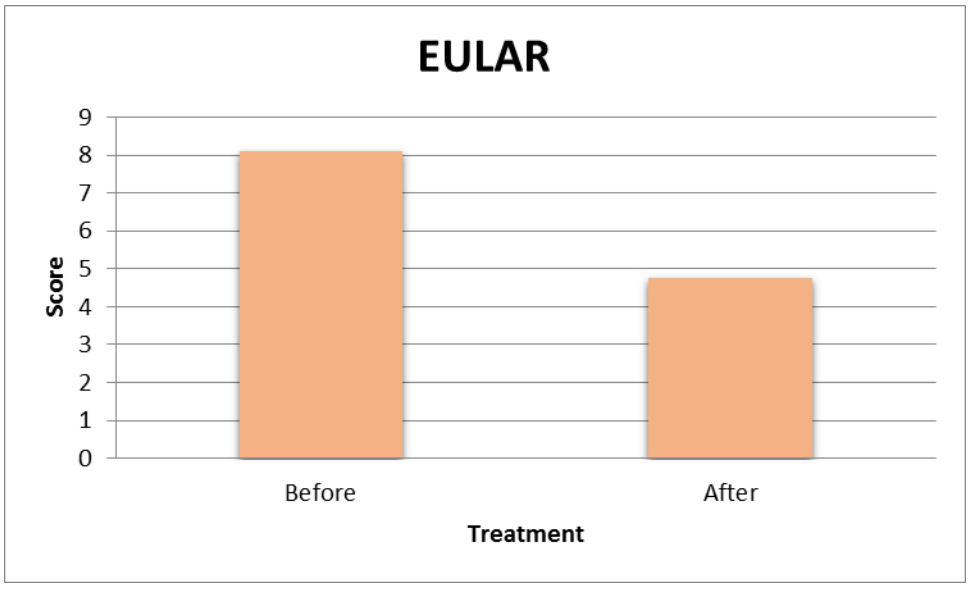


The mean MHAQ score was recorded after treatment(0.406) and compared with the score of before treatment(1.75). The mean score was significantly reduced from the base line after treatment with NM 500 mg/kg, orally with Palm Jaggery, twice daily for 60 days(7 days drug dosing followed by 7 days drug holiday). In this study the mean of MHAQ after treatment for 40 patients was 0.406. So it was inferred that after treatment, the patients were improved in their disability index.

7a.22 Disease Activity - EULAR Score

Before Treatment	8.125
After Treatment	4.775

Figure 7a.22.1 EULAR score



****P value < 0.0001

The mean EULAR score was recorded after treatment and compared with the score of before treatment.for 40 patients.The mean score was reduced from 8.125 to 4.775 after treatment with NM 500 mg/kg, orally with Palm Jaggery, twice daily for 60 days(7 days drug dosing followed by 7 days drug holiday).The reduction in EULAR score could be attributed to treatement effect.

7a.23.Pain Score:(UNIVERSAL PAIN ASSESSMENT SCALE-The National Initiative on Pain Control™ (NIPC™)),

Table no: 7a.23.1 Pain score

Pain score	Day 1	Day 15	Day 29	Day 43	Day 60
0: No Pain	0	0	0	6	30
1-3: Mild Pain	0	0	14	30	7
4-6: Moderate Pain	2	25	26	4	3
7-9: Severe Pain	34	15	0	0	0
10: Worst Pain	4	0	0	0	0
Total no.of. patients	40	40	40	40	40

Figure no:7a.23.1 P ain score

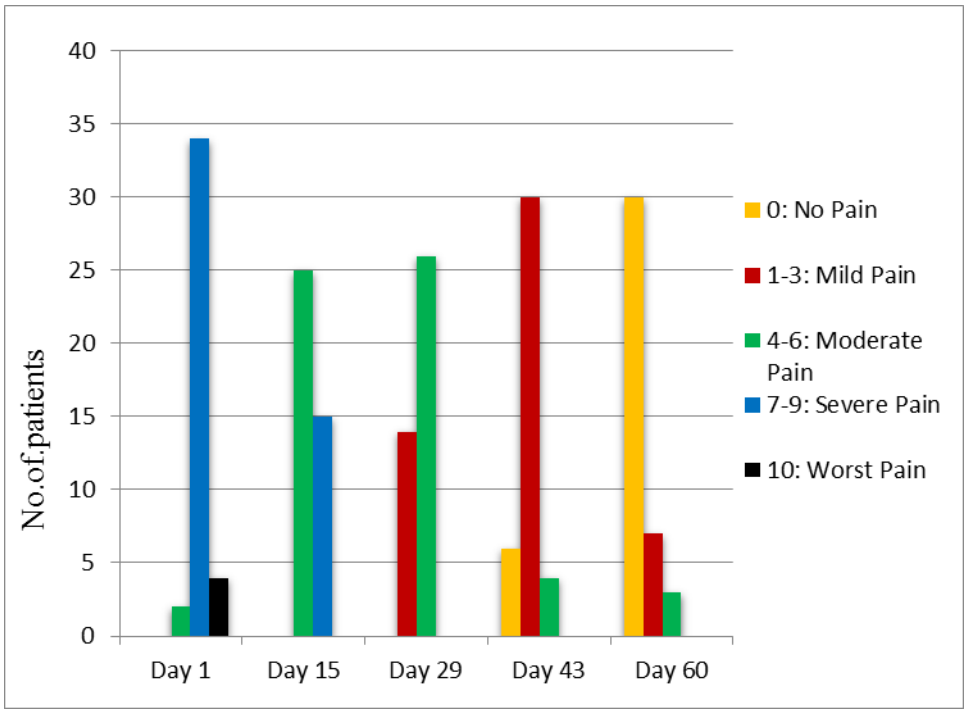


Table 7a.23.2 Pain score before and after treatment

Pain score	Before treatment D1	After treatment D60	Inference
0: No Pain	--	30	2 from moderate pain and 28 from severe pain
1-3: Mild Pain	--	7	1 from worst pain and 6 from severe pain
4-6: Moderate Pain	2	3	3 from worst pain
7-9: Severe Pain	34	--	Nil
10: Worst Pain	4	--	Nil
T. no .of. patients	40	40	

After treatment pain score status of forty patients:

In after treatment, out of four patients who had worst pain, reported moderate pain (3 patients) and mild pain (1 patient)

Out of thirty four patients, who had severe pain, twenty eight patients reported no pain and six patients reported mild pain.

Out of two patients, who had moderate pain, reported no pain.

7a.24 Restricted Movement Assessment Scale: (Ref:BearingPoint, Atos Healthcare & DSP Copyright EBM Rheumatoid Arthritis Version 5.0 final)

Grade I - Able to perform normal duties.

Grade II - Moderate Restriction- Self-care is possible.

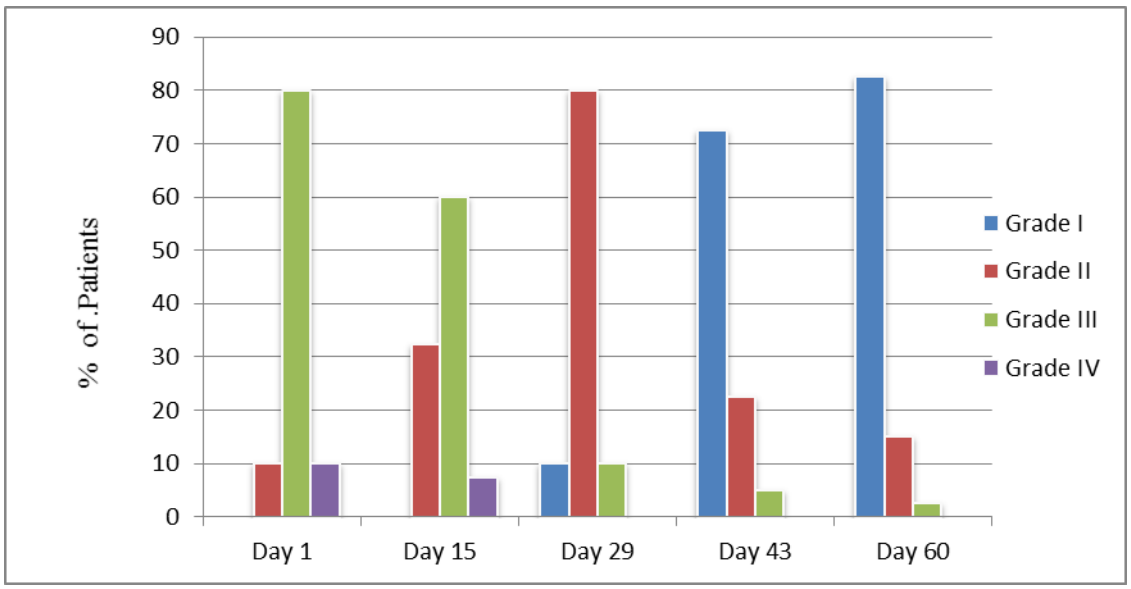
Grade III – Marked restriction – Limited self-care/ some assistance required.

Grade IV – Confined to bed or wheel chair.

Table 7a.24.1.Restricted Movement Assessment Scale

Grade	Day 1	Day 15	Day 29	Day 43	Day 60	% patients
Grade I	--	--	4	29	33	82.5
Grade II	4	13	32	9	6	15
Grade III	32	24	4	2	1	2.5
Grade IV	4	3	--	--	--	--
Total no. of. Patients	40	40	40	40	40	100

Figure 7a.24.2.Restricted Movement Assessment Scale



As per the restricted movement assessment scale,in before treatment,four patients (10%) were in GradeII(moderate restriction-self care is possible), thirty two patients(80%) were in Grade III(marked restriction-limitted self care-some assistance required) and four patients(10%) were in GradeIV(confined to bed/wheel chair). After treatment thirty three patients (82.5%,) were found to perform normal duties(GradeI) ,six patients(15%) were able to care themselves(GradeII) and one patient(2.5%) needed some assistance(Grade III).

7a.25.1Haematological parameters:

Table 7a.25.1Haematological parameters:

S.No	Variables/ Units	Measurements	Before n=40	After n=40	p Value
1	Hb g/dl	Mean Median Range SD SEM	11.56 12 8.5-14.5 1.56 0.25	11.58 12 8.48-14.3 1.51 0.24	0.82
2	TC Cells/cumm	Mean Median Range SD SEM	8050 7750 4600-4900 1736 274.5	7502 7300 4900 11300 1600 252.9	*0.0346
3	DC- Polymorphs %	Mean Median Range SD SEM	66.2 66 40-86 7.81 1.23	65.4 65 44-78 6.23 0.99	0.3315
4	Lymphocytes %	Mean Median Range SD SEM	28.2 28 12-55 7.8 1.23	29.02 30 15-54 6.9 1.08	0.4518

5	Eosinophils %	Mean Median Range SD SEM	3.55 4 0-5 1.18 0.19	3.85 4 1.5-7 1.20 0.19	0.4171
6	Monocyte %	Mean Median Range SD SEM	1.95 2 0-7 1.50 0.24	1.55 1 0-7.3 1.54 0.24	0.1218
7	Total RBC Cells/cumm	Mean Median Range SD SEM	3.8 4.5 3.8-5.3 0.45 0.07	3.3 4.03 3.3-5.3 0.50 0.08	*0.0490
8	PCV %	Mean Median Range SD SEM	37.05 36.95 29.5-27.2 3.98 0.63	36.41 37.3 27.2-44.4 3.99 0.63	0.0593
9	MCV fL/red cell	Mean Median Range SD SEM	81.3 80.9 64.8-90.27 6.19 0.98	82.28 82.6 66.7-99.2 5.99 0.95	*0.0363

10	MCH pg/cell	Mean Median Range SD SEM	25.32 25.05 18.1-18.8 2.83 0.45	25.89 26 18.8-30.4 2.68 0.42	**0.002
11	MCHC g/dL	Mean Median Range SD SEM	31.6 31.3 27.1-33.4 1.52 0.24	31.5 31.6 27.8-36.3 1.64 0.26	*0.04
12	PLT cells/cumm	Mean Median Range SD SEM	3.604 3.3 2.1-6.7 1.12 0.18	3.785 3.5 2.23-6.5 1.16 0.18	*0.02
13	AEC cells	Mean Median Range SD SEM	295.7 299 98-440 92.99 14.7	300.1 94.6 110-520 14.87 14.9	0.77
14	ESR mm/hr	Mean Median Range SD SEM	48.1 44.5 10-100 26.01 4.113	33.78 32 10-85 19.67 3.11	****0.0001

*p value <0.05, **p value <0.01, ****p Value<0.0001 are considered as significant

Figure 7a.25.1 ESR

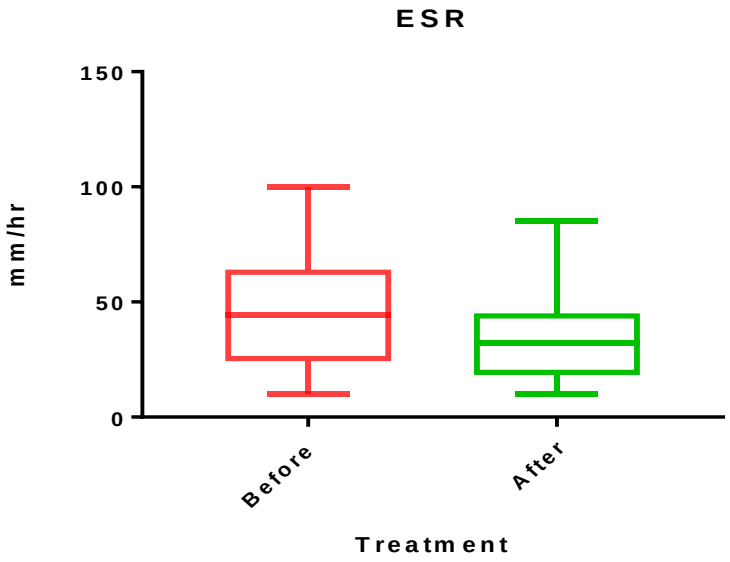
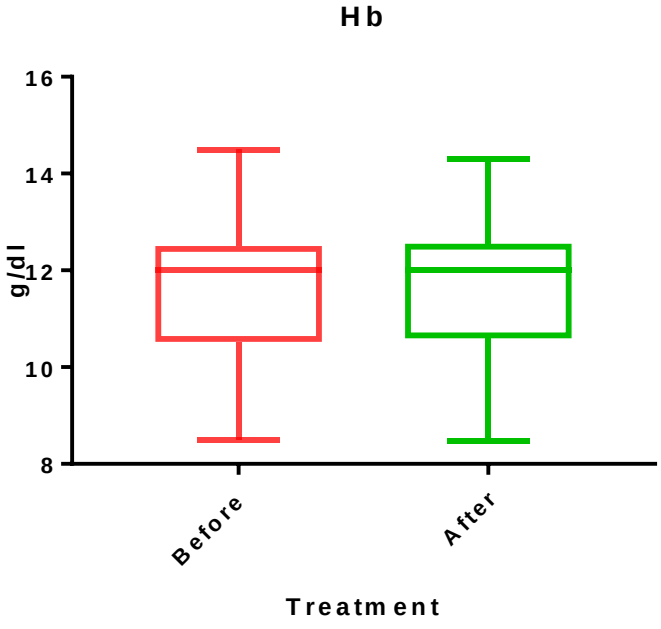


Figure 7a.25.2 Hb



In Haematology significant reduction was observed in TC, TRBC, MCH, MCHC, PLT, ESR and their respective p values are 0.03, 0.046, 0.002, 0.04, 0.02, 0.0001.

7a.26.1Biochemical parameters:

Table 7a.26.1Biochemical parameters:

S.No	Variables	Measurements	Before n=40	After n=40	P Value
1	FBS mg/dl	Mean	92.18	93.95	0.25
		Median	92	93.5	
		Range	80-108	79-109	
		SD	8.45	8.317	
		SEM	1.34	1.32	
2	PPBS mg/dl	Mean	116.7	113.5	0.18
		Median	118.5	112.5	
		Range	91-135	89-135	
		SD	12.51	11.89	
		SEM	1.98	1.88	
3	T. Cholesterol mg/dl	Mean	175.2	159.4	**0.006
		Median	171	151.5	
		Range	99-272	109-250	
		SD	42.35	36.82	
		SEM	6.697	5.82	
4	HDL mg/dl	Mean	40.88	37.67	0.06
		Median	39	9.03	
		Range	25-60	1.43	
		SD	8.23	9.03	
		SEM	1.30	1.43	
5	LDL mg/dl	Mean	99.42	89.32	*0.02
		Median	94	89.5	

		Range	48-150	44-129	
		SD	24.46	22.7	
		SEM	3.87	3.589	
6	VLDL mg/dl	Mean	23.56	23.76	0.87
		Median	22	22.3	
		Range	10-45	12-45	
		SD	8.19	8.05	
		SEM	1.30	1.28	
7	TGL mg/dl	Mean	120.6	116.8	0.50
		Median	110.5	112.1	
		Range	55-224	59-200	
		SD	41.31	34.91	
		SEM	6.53	5.52	

*p value <0.05, **p value <0.01, ****p Value<0.0001 are considered as significant.

Discussion: While comparing the Total cholesterol level before and after treatment, there was significant reduction was observed (p Value 0.006), and LDL also significantly reduced (p value 0.02).

7a.27.Liver function test (LFT):

S.No	Variables	Measurements	Before n=40	After n=40	p Value
1	T.Bilirubin mg/dl	Mean	0.56	0.59	0.22
		Median	0.5	0.6	
		Range	0.24-1	0.3-1	
		SD	0.17	0.18	
		SEM	0.03	0.03	

2	D.Bilirubin mg/dl	Mean	0.21	0.22	0.58
		Median	0.2	0.2	
		Range	0.09-0.4	0.1-0.5	
		SD	0.08	0.09	
		SEM	0.01	0.01	
3	I.Bilirubin mg/dl	Mean	0.35	0.37	0.38
		Median	0.3	0.3	
		Range	0.1-0.7	0.2-0.71	
		SD	0.13	0.12	
		SEM	0.02	0.02	
4	SGOT U/L	Mean	19.38	17.03	0.06
		Median	18.5	15	
		Range	10-37	6-37	
		SD	6.71	6.14	
		SEM	1.06	0.97	
5	SGPT U/L	Mean	18.53	15.44	*0.01
		Median	18	14.5	
		Range	7-32	7-30	
		SD	6.14	6.89	
		SEM	0.97	1.09	
6	ALP U/L	Mean	82.6	84.38	0.63
		Median	80.5	80	
		Range	40-148	43-140	
		SD	22.88	19.34	
		SEM	3.61	3.06	

Table 7a.28 Renal function test (RFT)

S.No	Variables	Measurements	Before n=40	After n=40	p Value
1	Total Protein g/dl	Mean	7.093	7.095	0.99
		Median	7	7.05	
		Range	6-8.7	6-9.1	
		SD	0.78	0.72	
		SEM	0.12	0.11	
2	Albumin g/dl	Mean	3.99	4.02	0.40
		Median	3.73	3.93	
		Range	3.1-4.8	2.9-4.6	
		SD	0.39	0.30	
		SEM	0.06	0.05	
3	Globulin g/dl	Mean	3.11	3.05	0.62
		Median	3	3	
		Range	2-4.9	1.8-5	
		SD	0.81	0.79	
		SEM	0.13	0.12	
4	Urea mg/dl	Mean	17.58	15.24	***0.0003
		Median	17.5	15.5	
		Range	9-25	7-22	
		SD	3.69	3.79	
		SEM	0.58	0.56	
5	Creatinine mg/dl	Mean	0.76	0.73	0.29
		Median	0.8	0.7	
		Range	0.6-1	0.48-1	
		SD	0.10	0.14	
		SEM	0.01	0.02	

6	Uric acid mg/dl	Mean Median Range SD SEM	.24 4.05 2.7-7 1.13 0.18	4.24 4.2 1.9- 6.9 1.05	0.98

*p value <0.05 is considered as significant.

Others:

1	Calcium mg/dl	Mean Median Range SD SEM	9.81 10 7.2-11.5 0.97 0.15	9.59 11.3 7.7-11.3 0.97 0.15	0.069
2	Phosphate mg/dl	Mean Median Range SD SEM	3.67 3.4 2.5-5.9 0.75 0.12	3.61 3.5 2-5.8 0.88 0.14	0.99

While comparing the SGPT before and after, there was significant reduction was observed.(p Value -0.01)

7a.28.Renal function test(RFT):

***p Value <0.001 is considered as significant.

While comparing Urea level before and after, there was significant reduction was observed. (p Value -0.0003)

7a.29.Anti CCP,RAF,CRP

Table 7a.29.Anti CCP,RAF,CRP

S.No	Variables	Measurements	Before n=40	After n=40	p Value
1	Anti CCPSS mg/dL	Mean	360.6	344.1	0.33
		Median	187.8	376.8	
		Range	29.8-500	9.21-500	
		SD	172.1	167.3	
		SEM	31.95	31.07	
2	CRP mg/dL	Mean	16.42	13.83	0.080
		Median	11.85	11	
		Range	1-65.3	1.1-55.9	
		SD	15.24	13.18	
		SEM	2.69	2.33	
3	RAF mg/dL	Mean	171.7	153.1	*0.032
		Median	107.3	91.4	
		Range	3.6-562.1	4.1-636.6	
		SD	158.2	155.1	
		SEM	27.96	27.42	

*p value <0.05 is considered as significant.

While comparing RAF before and after treatment, there was significant reduction was observed. (p value 0.032)

Figure 7a.29.1 Anti CCP

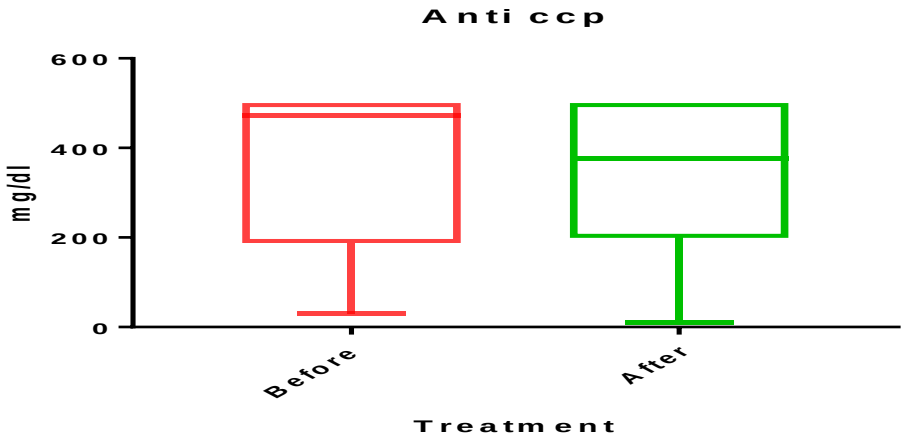


Figure 7a.29.2 Rheumatoid factor :

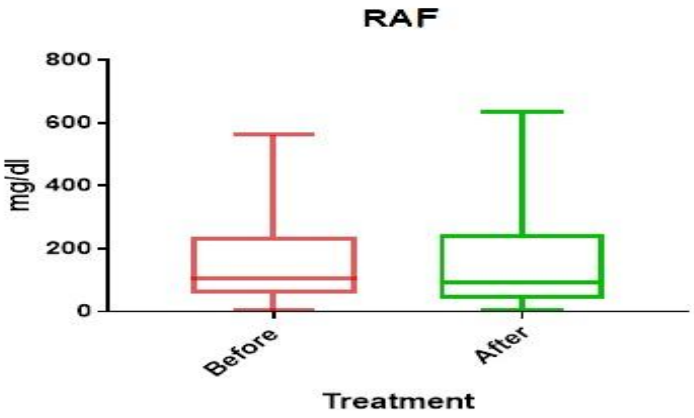
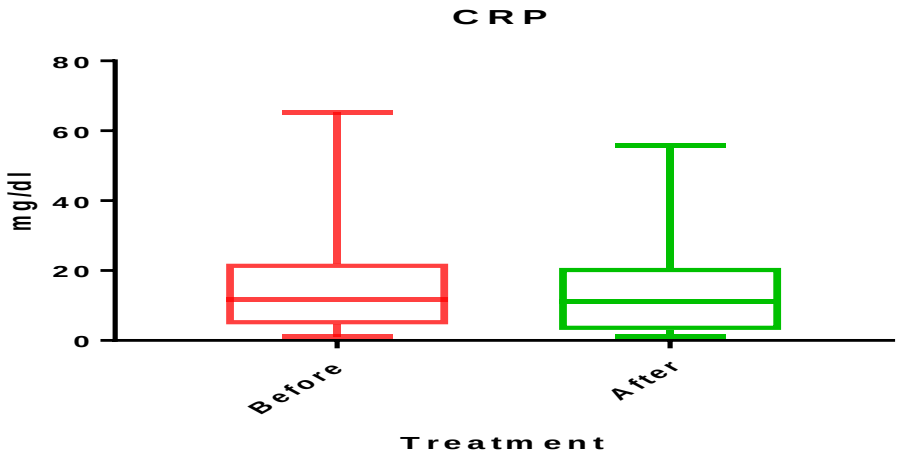


Figure 7a.29.3 C - Reactive Protein



8. DISCUSSION

Siddhar Yugi Munivar, in his Yugi Vaithiya Chinthamani, classified the Vatha diseases into 80 types;[1] one such disease is Uthira Vatha Suronitham. Uthira Vatha Suronitham is a systemic illness caused by the derangement of vatham which manifests as pain, swelling, tenderness and limitation of movements in major and minor joints, low grade fever, tiredness, and mental depression. The above mentioned signs and symptoms of Uthiravatha Suronitham may be correlated to rheumatoid arthritis in modern science.

As mentioned by Yugi Munivar[4] in his verses on the aetiology of vatha disease, the socio-economic (starvation and doing hard work like lifting heavy weights), psychological (disrespecting elders and neglecting parents and teachers and defying Vedic scriptures), environmental (seasonal changes), and lifestyle factors (consumption of bitter astringent, rancid and salty food, alcohol, day time sleep and night-time overwork) causes immunological modification thereby leading to infection, which is highly correlated to the aetiology of RA as said in modern science.

Nandhi mezhugu is a cost- effective and time-tested Siddha herbo-mineral formulation and is well-known for its unique action against Uthira Vatha Suronitham (Rheumatoid Arthritis) as evidenced by several decades of its use in clinical practice. For global acceptance, scientific validation of Nandhi Mezhugu,[53,54,55] by safety and efficacy studies is the need of the hour.

The required raw drugs for the preparation of Nandhi Mezhugu were procured from the “The Indian Medical Practitioner’s Co-operative Society, Pharmacy and Stores” (IMPCOPS), Chennai-41.The metals and mineral raw drugs were identified and authenticated by Smt.R.Shakila, RO chemistry in Siddha Central research Institute, Arumbakkam, Chennai-106. The Herbal raw drugs were identified and authenticated by Dr.Sasikala Ethirajalu, RO-Scientist II (Pharmacognosy) in Siddha Central research Institute, Arumbakkam, Chennai-106.The ingredients were purified as per the techniques stated in the Siddha literature[99]. The raw drugs were purified at The Indian Medical Practitioner’s Cooperative Pharmacy. The medicine Nandhi Mezhugu was prepared at The Indian Medical Practitioner’s Cooperative Pharmacy (IMPCOPS) as per the SOP mentioned in Siddha Vaidhiya Thirattu.The drug was subjected to physico-chemical analysis as per AYUSH Guidelines,[60,61]

The organoleptic characters such as colour, odour, taste, and consistency were recorded. All the results of physio-chemical parameters were as follows: the loss on drying was observed to be 19.156% and fat content 20.683%. It was understood that the high value of loss on drying may be due to the fat content. The total ash was calculated as 6.607% which indicated the content of total inorganics. The water soluble ash value of 2.95 % showed the content of water soluble inorganic salts like sodium chloride, etc. The acid insoluble ash value was calculated as 0.93%. The water soluble and alcohol soluble extractives which were estimated to be 39.056 % and 23.558 % respectively indicated the presence of high polar secondary metabolites like glycosides, sugars, tannins, saponins, alkaloids, etc. The calculated acid value, saponification value and iodine value were indicative of purity of the ghee used for the preparation and showing the number of milligrams of free acids and saponifiable unsaturated acid in the drug. Though the reducing sugar (3.69 %) and total sugar (7.54%) values were expected to promote the growth of organisms, the drug did not become rancid due to its acidic nature (low pH value of 3.35). As a result, the shelf life of the drug would be increased. The phytochemical analysis[62] of extract of Nandhi Mezhugu revealed the presence of secondary metabolites, viz., Steroid, Triterpene, Flavonoid, Alkaloids, Phenol, Tannin, Saponin, Coumarin, Glycoside and Quinone which improve the therapeutic efficacy of the drug.

The qualitative inorganic[63] analysis of the drug, which was carried out as per the methods mentioned in standard practical guide, revealed the presence of mercury, magnesium, aluminium, calcium, sodium, potassium, copper, zinc, iron, cobalt, chloride, carbonate, nitrate, sulphate, sulphide, acetate, silicate and arsenate which are all biologically important radicles. Calcium controls nerve impulse transmission, muscle action and prevents convulsive muscular contraction.[100] Magnesium plays a role in the stability of all polyphosphate compounds in the cells, [101] including those associated with the synthesis of DNA and RNA. It prevents osteoporosis and muscle spasms. Potassium prevents stroke, osteoporosis, hypertension, and renal stone. Copper maintains [102]the strength of connective tissues throughout the body and plays a role in production of haemoglobin. Copper also scavenges free radicals and may reduce or help prevent some of the damage. Iron is used for oxygen transport in the blood through haemoglobin. Aluminium is involved in the action of enzymes such as succinic dehydrogenase which is involved in porphyrin synthesis. Acetate is the most common building block for

biosynthesis of fatty acids. Sulphate and sulphides prevent inflammation**.** [103]Quantitative assays for Calcium, Magnesium**,** Potassium**,** Aluminium**,** Copper**,** Iron and Zinc**,** were observed in ICP-OES using standards.[104] Sulphur (as SO_2) was estimated by following AOAC 990.28 method and Chloride (as NaCl) was calculated by following AOAC 950.52 method. Quantitative assay results of Nandhi Mezhugu revealed the presence of calcium, may due to the added ingredient Nandukkal (Crab's fossil),in the drug; presence of potassium and aluminium may be due to the added ingredients padikaram (Common Alum- Aluminum Potassium sulphate), Kuthiraipal padanam (Potassium Aluminium Silicate) and Vediuppu.(Potassium nitrate); occurrence of copper was due to the presence of Mayil thutham(copper sulphate), and ponnimilai(copper pyrite); occurrence of iron and zinc was due to the presence of palm jiggery and pal thutham (zinc sulphate) respectively. Occurrence of Sulphur and chloride was due to presence of lingam(Mercuric sulphide), Thalagam (Arsenic trisulphide), Gowri padanam (Arsenic pentasulphide), Gandhagam(Sulphur), Padigaram(Aluminum Potassium sulphate), Rasa Chendooram (Red sulphide of mercury), Mayil thutham (copper sulphate), palthutham (zinc sulphate) and pooram((Mercurous chloride), Kariuppu (Sodium chloride) respectively.

Quantitative results of heavy metal analysis revealed that the content of lead and cadmium were within the admissible limit. While the content of arsenic and mercury were high due to the presence of the ingredients *Rasam (mercury), Lingam* (Mercuric sulphide), *Pooram* (Mercurous chloride), *Thalagam*(Arsenic trisulphide), *Manosilai (Arsenic disulphide), Vellai padanams (Arsenic trioxide), gowri padanam(Arsenic penta sulphide), Kalmatham (Hydrous cobalt arsenate)* and *Rasa chenduram/sinduram (Red sulphide of mercury)*. WHO has mentioned the limits of heavy metals only for herbal raw material and food substances not for the traditional mineral formulations. The products used in the traditional Indian Medical Systems are that they are mostly used in compound forms and are multi-component mixtures including minerals in some of the formulations and that substantial information is available regarding their prior human use vouchsafing safety and efficacy of these formulations. Therefore, an approach different from that for evaluation of synthetic drugs is required. It needs to be emphasized that since the substance to be tested is already in use in Indian systems of medicine or has been described in their texts, the need for testing its toxicity in animals has been considerably reduced--- says the Ethical guidelines for biomedical research on human subjects,ICMR,2006. In the microbial study, the drug was found free from *E. coli*,

Salmonella spp., *Staphlococcus aureus* and *Enterobacteriacea*. The total bacterial count and the total fungal count were within the permissible limits.

Various pesticide residues of organo-chlorine and organo-phosphorous viz., alpha BHC, beta BHC, gamma BHC(Lindane), delta BHC, Aldrin, Dieldrin, trans Chlordane, CIS-Chlordane, Endrin, Endrinaldehyde, Endrinketone, Endosulfan-I, Endosulfan-II, Endosulfansulfate, Heptachlor, Heptachlorepoxide, Dicofol, Chlorthalonil, Hexachlorobenzene, o,p"DDT, P,P"DDT, o,p"DDD, p,p"DDD, o,p"DDE, P,P"DDE, 4-Bromo,2-Chlorophenol, Acephate, Chlorfenvinphos, Chlorpyrifos, Chlorpyrifos methyl, Diazinon, Dichlorvos, Dimethoate, Ethion, Etrimfos, Fenitrothion, Iprobenphos , Malathion, Methamidophos, Monocrotophos, Omethoate, Oxydemeton-methyl, Parathion ethyl, Parathion methyl, Phorate, Phosalone, Phosphamidon, Profenophos, Quinalphos, Triazophos, Phorate sulphone Phorate sulphoxide were checked by following AOAC 2007.01 methods were found to be below limit of quantification (BLQ). The limit of quantification(LOQ) for the above mentioned pesticidal residue is 0.01 ppm. All the tested organochlorine pesticides, organophosphorus pesticides were found to be lower than the limit of quantification, ie., 0.01 ppm and hence the drug Nandhi mezhugu is found to be safe as an internal medicine .

Aflatoxins such as B1, B2, G1and G2 were checked using AOAC 2008.02 methods. All the four aflatoxins were not present in the drug.

The TLC photo-documentation of hexane extract of Nandi Mezhugu under UV 254 nm showed 5 visible spots at R_f value 0.25, 0.30, 0.38, 0.50 and 0.71 (all green); under UV 366 nm showed three visible spots at R_f value 0.30 (blue), 0.38 (fluorescent blue) and 0.71 (pale blue). After derivatization with vanillin-sulphuric acid, showed 8 spots at 0.20, 0.25, 0.30, 0.35 (all purple), 0.38 (brown), 0.46, 0.57 and 0.71 (all purple).

The HPTLC finger print profile of hexane extract at UV 254 nm showed 12 peaks in which the peak at R_f 0.84 was the major peak with an area of 54.90 % followed by a peak at R_f 0.29 with an area of 11.66 %. All other peaks are minor with an individual area less than 10 %.

The HPTLC finger print profile of hexane extract at UV 366 nm showed 8 peaks in which the peak at R_f 0.85 was the major peak with an area of 39.57 % followed by a peak at R_f 0.29 (19.43 %), 0.09 (13.40 %) and 0.37 (11.59 %). All other peaks are minor with an individual area less than 10 %.

The HPTLC finger print profile of hexane extract at 575 nm showed 10 peaks in which the peak at R_f 0.73 was the major peak with an area of 59.17 % followed by a peak at R_f 0.59 (14.35 %), 0.22 (8.02 %) and 0.47 (6.25 %). All other peaks are minor with an individual area less than 10 %.

The HPTLC finger print profile of chloroform extract at UV 254 nm showed 9 peaks in which the peak at R_f 0.12 was the major peak with an area of 56.90 %. All other peaks are minor appearing at R_f 0.17, 0.29, 0.56, 0.61, 0.73, 0.78, 0.85 and 0.89 with an individual area less than 10 %.

The HPTLC finger print profile of chloroform extract at UV 366 nm showed 9 peaks in which the peak at R_f 0.77 was the major peak with an area of 53.15 % followed by a peak at R_f 0.84 (23.00 %). Other peaks appeared at R_f 0.13, 0.28, 0.33, 0.38, 0.47, 0.57 and 0.61 with an individual area contribution of less than 10 %.

The HPTLC finger print profile of chloroform extract at 575 nm showed 10 peaks in which the peak at R_f 0.12 was the major peak with an area of 36.74 % followed by a peak at R_f 0.72 (12.76%), 0.36 (9.84%), 0.51 (8.16%), 0.91 (7.33%), 0.29 (6.82%) and all other peaks are minor with an individual area less than 5 %.

The HPTLC finger print profile of ethanol extract at UV 254 nm showed 9 peaks in which the peak at R_f 0.19 was the major peak with an area of 41.80 % followed by a peak at R_f 0.65 (15.14%), 0.32 (14.32%), 0.54 (11.93%), 0.99 (8.20%) and all other peaks are minor with an individual area less than 5 %.

The HPTLC finger print profile of ethanol extract at UV 366 nm showed 9 peaks in which the peak at R_f 0.65 was the major peak with an area of 32.83 % followed by a peak at R_f 0.37 (22.64%), 0.31 (13.26%), 0.99 (8.06%), 0.58 (6.89%), 0.28 (6.57%) and other peaks at R_f 0.52, 0.22 and 0.09 are minor with an individual area less than 5 %.

The HPTLC finger print profile of ethanol extract at 525 nm showed 9 peaks in which the peaks at R_f 0.62 (18.86%), 0.67(17.52 %), 0.19 (14.40 %), 0.49 (14.32%), 0.55 (13.55 %) and 0.30 (8.89 %) were the major peaks. The other peaks at R_f 0.73 (2.92 %) and 0.96 (3.38 %) were minor. The peak at R_f 0.01 was not µconsidered as it is very close to the spotting position.

The HPTLC finger print profile of hexane extract, chloroform extract, ethanol extract showed several peaks since it is a poly-herbo-mineral formulation it is difficult to read the individual peaks and it is done to assure the quality of the study drug.

Any changes due to various concentrations of extract (eg 5,10,15 µl) can be inferred from 3D chromatogram of the extract at UV254,UV366 and UV 575.

In continuation of the standardisation of study drug, the safety of the study drug had been studied as per OECD guidelines 423,407,408 (acute, sub-acute sub-chronic toxicity studies). As per OECD test guideline 423, acute oral toxicity study was performed on Nandhi Mezhugu in 3 dose levels. It was observed that there were no mortality and toxicity signs up to 2000 mg/kg. NM can be classified under category-5 since the LD50 [69] value was greater than 2000 mg/kg in accordance with Globally Harmonized System of Classification and Labelling of chemicals [70] and this provided us a direct relevance for protecting human and animal health. Therefore, it can be concluded that Nandhi Mezhugu when administered at single dose was non-toxic and can be used safely as oral formulation.

The 28-day repeated dose oral toxicity study was performed as per OECD test guideline 407 in both male and female wistar albino rats. The NM administered twice daily with Palm Jaggery solution as vehicle for 28 days of treatment duration as per Siddha literature, where 7 days test drug administration was followed by 7 days drug holiday. Few parameters such as feed consumption, haematology and serum biochemical parameters showed a statistical significance in low and mid doses, which can be considered as incidental effect as there was no dose dependent relationship and the values were within the normal range for the rats used in the experimental studies. There were no treatment related abnormalities in liver, renal function and other biochemical parameters, suggesting that NM was non-toxic up to the high dose tested. Histopathological studies provided supportive evidence for biochemical and haematological observations. The organ weights were found to be non-significant between the control and NM treated rats in both 28 days and 90 days toxicity studies. No abnormality was recorded with respect to gross or histopathological examinations of all organs examined. Since there were no signs

of toxicity with respect to haematology, clinical chemistry, organ weight, gross and histopathological examinations noted in NM Recovery group, it can be inferred that NM did not produce delayed onset of toxicity in both 28 days and 90 days toxicity studies. Based on these results, the No Observed Adverse Effect Level (NOAEL) of "NM" was greater than 110 mg/kg/day. ICP-OES – detection of heavy metal traces in animal tissue samples(Brain, Liver, Kidney)in Sub-chronic toxicity studies (High dose group)of Nandhi Mezhugu reported the absence of traces of heavy metal such as lead, cadmium, mercury, arsenic.

In Siddhar's science of longevity, kayakarpa drugs (Kayam=body; Karpam=Rejuvenation) play a key role, in the prevention of degeneration and promotion of longevity [105]. Semicarpus *anacardium* L.f.(Serankottai) [106,107,108,109], the chief ingredient and other herbal ingredients namely Zingiber *officinalis* Rosc.*(*chukka)[110], Elettaria *cardamiomum* (L.)Maton(Ellam) [111], Cuminum *cyminum* L.(jeerakam)[112], Withania *somifera* (L.)Dunal (ammukkura)[113], *Trachyspermum ammi* (L.)Spargue(Kurosani omam) [114], Plumbago *zeylanica* L.(sithiramoolam) [115], are the rejuvenating herbs (Kayakarpa mooligaigal) contain polyphenols, flavonoids and steroidal compounds [116] which protect the vital organs such as liver, kidney etc. from oxidative stress. Pirssonite (Pooneeru –Fuller's earth) [51] one of the mineral ingredients of Nandhi Mezhugu has been categorized under rejuvenator of mineral origin (muppu=super salt). Even though the purified mercurial and arsenical compounds were added in a very small quantity in the preparation of NM, it is interesting to note that the above mentioned kayakarpa ingredients are well known potent detoxifying agent of mercury and arsenic as per the Siddha literature e.g. curcumin powder used for purification of mercury contribute to maintain the healthy status of liver and kidney [117]. The purified arsenical and mercurial ingredients were further finely processed by the ingredients such as Indigofera tinctoria L. (avuri), *Anacyclus pyrethrum(L.) Lag.(*akkiragaram), *Zingiber officinale Rosc.*,*Piper nigrum L.*,*Piper longum L.* ,(thirikadugu- chukka,milagu, thippili) ,*Elettaria cardamomum(L.)Maton* (elam,,*Premna), herbacea Roxb.(*siruthekku),*Myristica fragrans Houtt.* (jathikkai),*Plumbago zeylanicaL.* (chithiramoolam), pooneeru(Fuller's earth), kariuppu (Sodium chloride) ,(padikaram –Alum)Aluminium Potassium Sulphate and honey which possessed detoxifying properties [105]. They also enriched the potential of the NM and yielded a qualitatively safe Siddha herbo-metallic mineral formulation NM ,established through the screening of biochemical and haematological parameters in

rodents as per the above safety studies (acute, sub-acute, sub-chronic toxicity studies). The toxic effects of mercury were neutralized in the presence of sulphur which was one of the ingredients in NM [118]. From other safety studies on herbo-metalo-mineral formulations[118,119], It was evident that if the preparation of such formulations were carried out as per Standard Operating Procedures (They undergo thorough extensive purification and preparation methods which involve crushing, boiling, and repeated incinerations in earthen pots at specified temperatures to make the minerals ready for human consumption) mentioned in classical literature, they will not produce any toxic effects. In the prepared NM, the essential elements such as Na, K, Ca, Mg, Cu, Fe and Zn had also been found in μg/g amounts and trace amounts of arsenic and mercury seemed to remain chelated with organic ligands derived from medicinal herbs by alchemic processes making these elements biologically assimilable. If the Siddha herbo-metallic-mineral formulations containing biologically produced nanoparticles are taken along with palm jiggery, honey, ghee and milk they become easily assimilable and their harmful effects are removed enhancing their biocompatibility. It is well established that several metals play a vital role in the biochemical processes as well as in the cure of many diseases. **It is believed that widely used heavy metals such as Hg and As in traditional systems of medicine act as a catalyser, which stimulates catalytic activity by their presence in the intestines without ever reaching the bloodstream. It is probably this property that renders many of the highly toxic metals into the nontoxic form. It is believed that the toxic effects of these medicines are neutralized by the medium of honey/ghee/milk** and provide a natural and effective alternative to synthetic allopathic drugs [118]**.**

Acetic acid induced writhing method was preferred to evaluate the analgesic efficacy of the Nandhi Mezhugu(NM) in mice. It was inferred that, in high dose group (NM 50mg + PJ solution) there was delayed onset of writhing and the number of writhings were also reduced. The effect was highly dose dependent. The high dose group showed significant analgesic activity when compared to standard drug.

Acute anti-inflammatory activity was studied by Carrageenan induced paw odema method, the paw volume was reduced significantly in high dose group(NM) 900mg/kg b.w with that of standard **(**Diclofenac sodium 25mg/kg b.w). Percentage of inhibition in high dose group was comparatively high when compared to that of the standard. Chronic anti-inflammatory activity was studied by Cotton pellet

granuloma method: There were no biologically significant changes observed in body weight (g) and body weight gain (%) in all group animals. All the animals were found to be normal in their health condition during the entire experiment. Haematology parameters were well within the normal range . Dose dependent inhibition of granuloma tissue formation was observed in dexamethasone treated group animals and Nandhi Mezhugu treated group animals when compared with the control group animals. Based on the results of the cotton pellet granuloma study, there was a significant decrease in the final weight of cotton pellet (right groin and left groin of animals) at G3, G4 and G5 (NM-50,150,500mg/Kg b.w respectively) when compared to G1(Vehicle control group--palm jaggery solution) group in both male and female animals. However, in the reference standard of treated group (G2-Dexamethasone sodium @ 0.5 mg/kg) an increase in the weight of cotton pellet was observed in comparison to control group (G1).

In Freund's Complete adjuvant (FCA) Arthritis study, the arthritis was established in the G1(vehicle control group-palm jaggery solution) group animals and also across other groups(G2-reference drug Indomethacin-0.3mg/kg b.w, G3,G4,G5-NM-50,150,500mg/kg b.w respectively) before the initiation of the treatment. The sum of arthritis indices noticed were 3.0, 2.0, 2.6, 2.0 and 2.0 in control(G1)standard(G2), low dose(G3),mid dose(G4) and high dose (G5) respectively. The total percentage change in inhibition of arthritis was 51.36% in high dose group with respect to control (0% inhibition). When compared with standard (56.60%), the test drug NM showed significant inhibition. Histologically, there was a moderate degree of arthritis induction at G1 group. There was mild degree of improvement in the arthritis condition at G2 when compared to the G1 group. There was a marginal degree of reduction in the arthritis score in G3, G4 and G5 groups

In vascular permeability study, the intra-peritoneal injection of 0.6% acetic acid induced increased permeability of vascular system resulting in dilation of the blood vessels of all the animals across all groups in both sexes. This was evidenced by the presence of Evans blue dye in peritoneal fluid. Administration of the test item (NM-50,150,500mg/kg b.w to G2,G3,G4 respectively) caused a significant decrease in vascular permeability in G2, G3 and G4 in males and in G3 and G4 in females when compared to G1 group (palm jaggery solution).

In in-vitro cytotoxicity assay in a LPS induced inflammation in the RAW 264.7–Mouse

macrophage cell line study .Nandhi Mezhugu showed cytotoxicity at 500 µg/ml and above, which is too high concentration to correlate to the in vivo studies. The concentrations less than 250 µg/ml were considered for the cyto-protective cell line assay where the expression of the inflammatory biochemical markers such as COX-2 and TNF-alpha were studied. Nandhi Mezhugu at 100 µg/ml showed a decrease in inflammatory markers to the extent of 14% and 10% of COX-2 and TNF-alpha respectively. This result suggests that the Nandhi Mezhugu preparation can be used as an alternative treatment for the inflammatory conditions.

In in-vivo –Rat Paw oedema bio-assay, there was a dose dependent decrease in the inflammation was observed. The highest dose studied in this bio-assay was 500 mg/kg which is almost equivalent to the standard drug indomethacin. The pro-inflammatory and anti-inflammatory gene expression studies were performed in the rat paw treated with Nandhi Mezhugu. A slight increase in the gene expression of Beta-galacturonidase, Acid phosphatase, and Cathepsin-D at 11,19, and 6% respectively which is biologically non-significant, whereas there was a decrease in C-reactive protein and Interferon-gamma gene expression at 57 and 27% respectively considered due to anti-inflammatory effect of Nandhi Mezhugu. An increase in TNF-alpha which is a pro-inflammatory marker which is expected to reduce with the treatment; however the increase in the level could not be explained. Further studies are required to understand the pathway of anti-inflammatory effect of Nandhi mezhugu.

An open labelled, non-randomized, clinical study (without control) on 40 cases of Uthiravatha suronitham or Rheumatoid arthritis was conducted, to determine the safety and therapeutic efficacy of Nandhi melugu (NM), in out-patient and in-patient departments of Maruthuvam, Ayothidoss Pandithar hospital, National Institute of Siddha, Tambaram S anatorium,Chennai-47. The study drug NM was given for 60 days (7 days drug dosing followed by 7 days drug holiday). Out- patients were asked to visit OPD once in 2 weeks and in-patients were taken care of every day by daily visits. Clinical assessment and prognosis were recorded during their visits. Clinical laboratory investigations were carried out before and after treatment. A total of 66.6% (40/60) patients were followed as per the study protocol and completed 60 days of treatment with NM. 5 patients refused to sign consent form and 15 patients did not come for regular follow-up and blood investigations. There were (4/40) 10% male and (36/40) 90% female patients. At the baseline 100% of both male and female patients were tested high

positive for Anti CCP, RAF and high ESR ,raised CRP level. After 60 days of treatment, the haematology report showed a significant reduction in the high value of ESR(****p value -0.0001).While comparing the RAF before and after treatment, there was significant reduction observed (*p value-0.032).

The observations regarding siddha parameters and demographic details were seen as follows.

Gender distribution –The incidence of the disease was far higher in female (90%) than in male(10%) .

Age incidence-among the 40 patients under study is as follows: 35% of patients belonged to the age between 31 and 40 , 35% of patients between 41 and 50 , 17.5% of patients between 20 and 30 and 12.5% of cases between 51and 60 .

Thinai (Land distribution)- among the 40 patients under study---- 70% of patients hailed from Neithal (Coastal belt), 15% from Marutham (plains), 7.5% from Mullai (Forest range) and 7.5% from Kurinji (Hilly terrain).

Kaalam-wise age distribution (according to Siddha science)) – most of the patients i.e. (70%) under study belonged to Pitha kaalam of 34-66 years ,only 30% of patients to Vatha kaalam of 1-33 years .

Paruvakalangal (Seasonal incidence) – Out of 40 patients, 2.5% of patients were treated during Pinpani kaalam (February 15- April 14), 60% of patients during Ilavaenil kalam (April 15- June 14), 32.5% patients during Muthuvaenil kaalam (June15-August 14) and 5% of patients during Kaar kaalam (August 15-October 14).

Food habits---97.5% of patients were non-vegetarian and 2.5% of patients were vegetarian.

Thegi (Body constitution) --30% of cases had Vatha pitha thegam, 22.5% of cases had Vatha thegam, 17.5% of cases had Vatha kabha thegam, 12.5% of cases had Pitha thegam, 10% of patients had Kabha thegam and 7.5% of cases had Kabha vaatha thegam.

Socio economic status - 50% of cases were middle class , 22.5% upper middle class, 22.5% lower middle class and 5% of cases were Poor .

Occupational status - 60% of cases were housewives,7.5% of cases were field workers of intellectual job category, 7.5% of cases were Teachers and 25% of patients were field workers of physical exertion category.

Gunam (Character) -- 85% of cases had Rajo gunam (courage, honesty, perseverance), 12.5% of patients had Sathuva gunam (patience, self-control, humility), and 2.5% of patients had Thamo- gunam(excessive sleep, laziness, anger).

Duration of illnesss - 7.5% of patients had been suffering from symptoms for more than 10 years, 10% of patients for more than 5 years, 55% of patients for more than 1 year, 12.5% of patients for 7 to 12 months and 15 % for 2 weeks to 6 months.

Aimporigal (Five sense organs) --- The condition of Aimporigal with reference to five sense organs were studied in all the patients.

Vai (mouth) -- 45% of patients had any one of the following signs and symptoms like coated or pallor or sore or dryness of the tongue before treatment and only 12.5% of patients had any one of the above signs and symptoms after the treatment.

Mei (Skin) -- In 15% of patients, dryness or pallor of the skin was observed before and after the treatment.

Kann (Eye) -- 27.5% of patients had any one of the following conditions like pale conjunctivae or redness of the eye or squint before treatment and in 22.45% of patients had pale conjunctivae , squint after treatment.The patients showed improvement from redness of eye condition.

Aimpulangal (Five senses) -- The condition of Aimpulangal with reference to five senses were studied in all the patients.

Sparism(Mei kuri (Touch sense) -Physical signs): It was affected and it was perceived through palpation i.e thoduvali-tenderness, veppam-local heat, neru neruthal - crepitation.

All the 100% of patients had tenderness in the affected joints before treatment and only 25% of them had it even after treatment.

Similarly before treatment, presence of local heat over the affected joints was perceived in all the 100% of patients and only 7.5% of patients had it even after treatment.

30% of patients had crepitation in the affected joints both before and after treatment..

Rasam(Taste sense) -- 37.5 % of patients had any one of the following symptoms i.e.Suvai inmai-Tastelessness, or Vai kasatthal- bitter taste or Vai pulithal-sour taste or Naa erichal-burning sensation of the tongue before treatment and became normal after the treatment.

Kann-(Visual sense) 7.5% of patients had defective vision due to myopia(kitta paarvai-near vision) before and after the treatment.

.Kanmenthiriyangal (the organs of action): The condition of kanmenthiriyangal with reference to five organs of action were studied in all the patients. 97.5 % of patients had

swollen joints and muscle spasm of the upper limb (kai) before treatment and only 17.5% of patients had the symptoms after treatment.

100% of patients had swollen joints and muscle spasm of lower limb (kal) before treatment and in only 17.5% of patients the symptoms continued even after treatment.

Kanmavidayam (Motor actions) -- The condition of kanmavidayam with reference to five motor actions were studied in all the patients.

77.5% of patients had difficulty in kamanam (actions of lower limbs) i.e., abduction, adduction, flexion, extension, standing, walking, running, etc. before treatment and in only 17.5% of patients, the symptoms persisted even after the treatment.

95 % of patients, had difficulty in Thaanam (actions of upper limb) abduction, adduction, flexion, extension, rotation, gripping, fisting, etc., before treatment and in only 17.5 % of patients, the symptoms persisted even after the treatment. .

Visargam-- (Defecation) was affected in 12.5% of patients who had constipation before treatment and were completely relieved of constipation after the treatment (0%)

Kosangal (Sheaths or Coverings of Aathma (soul)**:** The conditions of kosangal with reference to five kosams were studied in all the patients.

Annamayakosam -- (Sheath of food- it is the body constituted by the 7 physical constituents. This food of sheath helps in the digestion of food which is separated into essence and excrement and nourishes the 7 physical constituents.

Praanamayakosam (Sheath of breath/praanan- it is the body constituted by the combination of Praanan and Kanmenthiriyam. This helps to take in the vital air from outside and spreads it all over the body, collects the vayu (i.e.CO_2) produced as a result of metabolic function from all over the body and expels it from the body).

Manomaya kosam (Sheath of mind-it is the body constituted by the Manam (mind) and Gnanenthiriyam. The heart is said to be the seat of the mind. The heart, with the help of the vascular system, circulates the blood throughout the body. So the mind is said to have control or influence over the cardio-vascular system (N.B: According to some yogis, the mind has no specific seat and it pervades all the tissues.)

Vignanamaya kosam (Sheath of knowledge- it is the body constituted by the Buddhi and Gnanenthiriyam. Seated in the brain, knowledge, with the support of Kanmenthiriyam and Gnanenthiriyam, rules over the other kosams).

Aanandhamayakosam (Sheath of bliss-it is constituted by the Praana vayu and Suzhuthi (a sleep like state). It indicates the maintaining of a blissful state with the application of knowledge.

Annamayakosam was affected (Anorexia) in 45% of patients before treatment and no patients (0%) had the symptom after the treatment.[120]

Praanamayakosam was affected (Dyspnoea due to moderate iron deficiency anaemia) in 10% of patients before and after treatment.

Manomayakosam was affected (Stress) in 100% of patients before treatment and 32.5% of patients had not been relieved from stress even after the treatment.

Vignanamayakosam was affected (Depression due to disease process) in 100% of patients before treatment and the depression continued in 32.5% of patients even after the treatment.

Aanandhamayakosam was affected (Sleep disturbances) in 45% of patients before treatment and 20% of patients had sleep disturbances even after the treatment .

Note: Kosam- It implies coverings of the aathma which experiences the different levels of consciousness ranging from the gross physical body to subtle levels of mind which includes emotional and spiritual aspects. It is the experience leading from individual consciousness to cosmic consciousness.

Uyirthatukkal–Vatham--The condition of Vaatham with reference to ten vayus were studied in all the patients.

The **Praanan** helps in inspiration, expiration and digestion, **Abaanan** helps in the act of urination, defecation, discharge of semen during sexual act, **Udhaanan** is responsible for the reflexes like cough, sneeze, hiccup and vomiting, **Viyaanan** performs movements of both movable and immovable parts of the body, **Samaanan** controls all the other vayus, balances the six tastes, water and foodstuffs during the process of digestion and reaches them to their sites of action, **Naagan** acts as an instrument for Anthakaranam (Manam, Puthi, Sitham, Agankaaram) and is responsible for one's intelligence. It activates the brain to learn all kinds of arts and to sing good songs), **Koorman** emanates from manam (mind), acts on the eye and produces blinking and good vision. It responsible for the act of yawning and closing of the mouth, **Kirukaran** acts on the tongue to produce salivary secretion and on the nose to produce nasal (mucous) secretion. It produces much hunger. **Devathathan** emanates in the form of circle and it produces laziness and tiredness on waking up from bed.

(Before treatment -BT/ After treatment-AT).

Praanan was affected (Dyspnoea on exertion) BT-10% and AT-10% due to moderate iron deficiency anaemia (Hb level 8-9.5 gm/dl).

Abaanan was affected (Constipation) BT-12.5% and AT-0%.

Samaanan was affected (anorexia, controlling other vaayus like praanan, abaanan, vyanan, koorman, kirukaran, devathaththan) in 100% of patients in before treatment and only 25%.of patients had derangement of samaanan. in after treatment.

Vyaanan was affected (Tenderness and Restricted movements of the minor and major joints of upper and lower limbs) BT-100% and AT- 25%.

Koorman was affected (defective vision) BT- 7.5% and AT-7.5%.

Kirukaran was affected (Anorexia and dryness of the tongue) BT-50% and AT-0%.

Devathathan was affected (Tiredness) BT-37.5% and AT-15%.

Uyirthatukkal–Pitham or Azhal :The condition of pitham with reference to five pithams were studied in all the patients.

Anal pitham -- located in the digestive organ, helps in the process of digestion; Ranjaka pitham -- has the characteristic of increasing the blood volume. Located in the stomach and intestine, it gives the juice, which is separated from the digested food, the typical red colour; Saathaka pitham -- has the characteristic of performing or executing an act; Praasakam -- located on the skin, it gives lustre to the skin; Alosakam -- has the characteristic of enabling the eyes to perceive the objects.

Anal pitham was affected (Anorexia) BT-45% and AT-0%

Ranjaka pitham was affected (Anemia) BT-52.5% and AT-50%

Saathaka pitham was affected (difficult to perform routine works) BT-100% and AT-30%

Praasakapitham was affected (loss of luster and dryness of skin) BT-40% and AT-40%

Alosakapitham was affected (Myopia, squint) BT-7.5% and AT-7.5%.

Uyirthatukkal–Kabam or Iyyam:The condition of kabam with reference to five kabams were studied in all the patients.

Avalambagam -- It supports the other 4 Kabams); Kilethagam -- located in the stomach, it wets the food and water ingested and renders them soft; Pothagam -- located in the tongue, it perceives the sense of taste of foods and liquids taken in by mouth; Tharpakam -- located in the head, it cools the eyes; Santhikam -- located in the joints, it gives lubrication and helps in extension, flexion and rotation.

Avalambagam was affected (It supports other kabams-Tharpagam, Santhikam, etc) BT-100% and AT-100%

Kilethagam was affected (Anorexia) BT-45% and AT-0%

Pothagam was affected (Tastelessness , Bittertaste sense) BT-32.5% and AT-0%

Tharpagam was affected (Burning sensation of eyes) BT-7.50% and AT-0%

Santhikam was affected (Tenderness and restricted movement) BT-40% and AT-40%.

The condition of seven udalthathukkal were studied in all the patients.

In Seven Udal thathus, Saaram wsa affected leading to lethargic and depressed conditions. Senneer was affected leading to nervousness, dryness of skin, diminution of bodily luster. Oon was affected (muscle spasm), resulting in difficulty to perform routine duties. Kozhuppu was the main thathu affected leading to crepitations (due to diminution of the pulpy semifluid material) and difficulty in movements of the joints.. Enbu thathu was affected, leading to restricted movements of the joints and joint stiffness. Moolai thathu (bone marrow) was affected, leading to Anaemia.

Saaram, Senneer, Oon, Kozhuppu, Enbu was affected in 40 cases and Moolai was affected in 21 cases.

EnvagaiThervu (eight fold esxamination):

NaadiNadai (Pulse Play): among 40 cases, 80% of cases had Vatha pitha naadi, 20% of cases had Pithavatha naadi.

Sparism(Mei kuri -Physical signs):Thoduvali (Tenderness)- among 40 cases, 70% cases had tenderness till 29th day of treatment and after further 14 days treatment, only 25% of cases had tenderness.

Veppam (Warmth)-among 40 cases, initially 100% of cases had veppam, 72.5% of cases had veppam (warmth) at 29th day of treatment and after further 14 days of treatment only 7.5% of cases had veppam.

Neruneruthal (Crepitation)- from Day1–Day60 only 30% of cases had Neruneruthal(Crepitation).

Naa (Tongue):among 40 patients 22.5% of patients had Maa padinthiruthal (coated tongue), 20% of patients had Suvai inmai(tastelessness), 12.5% of patients had Naa Vaeluppu(Pallor tongue), 10% of patients had Naa Kaippu(Bitter taste), 7.5% of patients had Naa Varatchi(Dryness of tongue), 7.5% of patients had Karu naakku(Pigmented tougue), 2.5% of patients had Naa pun.

Niram(Complexion)- among 40 cases 27.5% of cases had Vathaniram, 55 % of cases had Pithaniram and 17.5% of cases had Kabhaniram.

Mozhi- among 40 cases, 65% cases had samaoli (medium pitched voice), 12.5% cases had ennathoni (low pitched voice) and 22.5% cases had uratthaoli (high pitched voice).

Vizhi (Eyes)- among 40 cases, 65% of cases had normal eyes, 20% of cases had Imai inaipaadala vaeluppu(pale conjunctivae), 7% cases had Kann eritchal (burning of eyes), 5% of cases had Kittap parvai (myopia) and 3% of cases had Paarvai kuraipaadu (diminished vision).

Malam (Stools)- among 40 cases, 100% cases had Yellow (Manjal) coloured stools throughout the treatment period, 12.5 % cases had constipation in first 3 visits and became normal in further course of treatment.

among 40 cases, 87.5 % cases had normal bowel habits and became 100% normal at the end of the treatment.

Neerkuri (Urine-Physical characteristics): Colour of the urine- among 40 cases, 40% cases had pale yellow coloured urine, 20% cases had colourless urine, 7.5 % cases had straw coloured urine, 20% cases had yellow coloured urine , 10% cases had dark yellow coloured urine and 2.5% cases had amber coloured urine before treatment. 52.5% cases had pale yellow coloured urine, 27.5% cases had colourless urine, 7.5 % cases had straw coloured urine, 7.5% cases had yellow coloured urine and 5% cases had dark yellow coloured urine after treatment

Smell of the urine- among 40 cases, 100% of cases had normal odour(ammonical odour) before and after treatment.

Volume (Alavu) of urine-among 40cases, 100% of cases had normal volume of urine before and after treatment.

Turbidity (Nurai) of urine- among 40cases, 100% of cases had clear urine , before and after treatment.

Deposits (Enjal) in urine: among 40cases, 100% of cases reported normal before and after treatment.

Neikkuri (Oil on Urine sign): before treatment-Spreading pattern

Among 40cases, 95% of cases had slow spreading pattern before treatment and 5% of cases had fast spreading pattern.

Shape

5% of cases had Snake pattern, 22.5% of cases had Pearl shaped, 2.5% of case had Mountain shaped, 30% of cases had Sieve pattern, 27.5% cases had Ring shaped, 2.5% of cases had Trigon shape, 2.5% of case had half-moon shaped, 2.5% case had irregular shaped, 2.5% case had oval shaped and 2.5% case had coin shaped and 0% of cases had Drumstick pattern.

After treatmentSpreading pattern

90% cases had slow spreading, 10% cases had fast spreading.

Shape-2.5% of case had Snake pattern, 27.5% of cases had Pearl shaped, 0% of cases had Mountain shaped, 10% of cases had Sieve pattern, 42.5% cases had Ring shaped, 0% of cases had Trigone shaped, 0% cases had half-moon shaped, 7.5% cases had irregular shaped, 2.5% cases had oval shaped and 2.5% cases had coin shaped and 2.5% of cases had Drumstick pattern.

Physical functions assessment was carried out before and after treatment in all the patients with respect to the clinical features : (i) arthritis of more than 3 joints--at the baseline period, 100 % of patients had pain and inflammation in more than three joints. At the end of treatment with NM i.e., 60th day (7 days dosing followed by 7 days drug holiday) only 15% of patients had the pain and inflammation in more than three joints.

(ii) Arthritis of hand joints --at the baseline period, 100 % of patients had pain and inflammation of hand joints. During the treatment period, in all the patients, pain and inflammation of hand joints were reduced gradually. Only 12.5% of patients had pain and inflammation of hand joints at the end of NM treatment.

(iii) Morning stiffness> 1 hr -- at the baseline period, 100 % of patients had morning stiffness in the joints. During the treatment with NM for 60 days, only 17.5%.of patients had the morning stiffness at the end of the treatment..

(iv) Symmetrical arthritis--at the baseline period, 97.5 % of patients had involvement of arthritis in the symmetrical joints. During the treatment with NM for 60 days, the involvement of arthritis in symmetrical joints was reduced into 30% of patients at 43rd day. Only 17.5% of patients had symmetrical joints involvement after the end of 60 days treatment.

(v) Restricted movements--at the baseline period, 100 % of patients had restricted movements of the joints. During the treatment with NM for 6o days, only 27.5% of patients had restricted movements in the joints at 43rd day of treatment and at the end of the treatment period, only 17.5% of patients had restricted movements.

(vi) Spindled appearance of fingers -- at the baseline period, 20 patients had spindled appearance of fingers. No change was observed in all the 20 patients even after the treatment period of 60 days.

(vii)Low grade fever-- at the baseline period, 90 % of patients had intermittent low grade fever. After 60 days of treatment with NM, none of the patients had reported of having low grade fever.

(viii) Tenderness--at the baseline period, 100 % of patients had tenderness in the joints. At the end of 60 days treatment with NM, only 25% of the patients had tenderness in the joints.

(ix) Muscle spasm--at the baseline period, 100 % of patients had muscle spasm in the affected joints. During the treatment with NM for 60 days,only17.5% of patients had muscle spasm in the affected joints.

(x) Local heat-- at the baseline period, 100 % of patients had local heat. During the treatment with NM for 60 days, only 7.5%.of patients had local heat over the affected joints.

(xi) Joint stiffness--at the baseline period, 100 % of patients had joint stiffness. During 60 days of treatment with NM ,only 25% of patient had stiffness of the joints at 43rd day and at the end of 60th day ,only17.5% of patients had joint stiffness

(xii) MHAQ:(Modified health assessment questionnaire (MHAQ) score

MHAQ score less than 0.3 is considered normal.

The mean MHAQ score was recorded after treatment(0.406) and compared with the score of before treatment(1.753) for 40 patients. The mean score was significantly reduced from the base line after treatment with NM 500 mg/kg, orally with Palm Jaggery, twice daily for 60 days(7 days drug dosing followed by 7 days drug holiday). In this study the mean of MHAQ after treatment for 40 patients was 0.406. So it was inferred that after treatment, the patients were improved in their disability index.

(xiii) Disease Activity - EULAR Score

The mean EULAR score was recorded after treatment and compared with the score of before treatment for 40 patients.The mean score was reduced from 8.125 to 4.775 after treatment with NM 500 mg/kg, orally with Palm Jaggery, twice daily for 60 days(7 days drug dosing followed by 7 days drug holiday)..

(xiv) pain score status of forty patients was assessed by universal pain assessment scale.sIn after treatment, out of four patients who had worst pain, reported moderate pain (3 patients) and mild pain (1 patient)

Out of thirty four patients, who had severe pain, twenty eight patients reported no pain and six patients reported mild pain.

Out of two patients, who had moderate pain, reported no pain.

(xv) Restricted Movement Assessment Scale**:** (Ref:BearingPoint, Atos Healthcare & DSP Copyright EBM Rheumatoid Arthritis Version 5.0 final)

Grade I - Able to perform normal duties.

Grade II - Moderate Restriction- Self-care is possible.

Grade III **–** Marked restriction **–** Limited self-care/ some assistance required.

Grade IV **–** Confined to bed or wheel chair.

As per the restricted movement assessment scale,in before treatment,four patients (10%) were in GradeII(moderate restriction-self care is possible), thirty two patients(80%) were in Grade III(marked restriction-limitted self care-some assistance required) and four patients(10%) were in GradeIV(confined to bed/wheel chair).

After treatment thirty three patients (82.5%,) were found to perform normal duties(GradeI) ,six patients(15%) were able to care themselves(GradeII) and one patient(2.5%) needed some assistance(Grade III)

Haematological parameters: After 60 days of treatment ,the haematology report showed a significant reduction in the high value of ESR(****p value - 0.0001). While comparing the serum RAF before and after treatment ,there was significant reduction was observed (*p value-0.032).

9. SUMMARY

The study drug Nandhi mezhugu(NM) was tested for preclinical and clinical study, The findings of the drug were given as follows.

- The raw drugs for NM were purified and the medicine NM was prepared at IMPCOPS as per the SOP mentioned in Siddha Vaidhiya Thirattu.
- The physico-chemical analysis of the drug revealed that the drug possessed high value for loss on drying , total ash indicated the presence of inorganic substances and the water soluble ash value showed the presence of water soluble inorganic salts like sodium chloride. The water soluble and alcohol soluble extractives indicated the presence of high polar secondary metabolites like glycosides, sugars, tannins, saponins and alkaloids. The calculated acid value, saponification value and iodine value were indicative of purity of the ghee used for the preparation and showed the number of milligrams of free acids and saponifiable unsaturated acid in the drug. The shelf life of the drug is increased.
- The phyto-chemical analysis of the extract of Nandhi mezhugu revealed the presence of secondary metabolites, viz., Steroid, Triterpene, Flavonoid, Alkaloids, Phenol, Tannin, Saponin, Coumarin, Glycoside, Quinone, which improve the therapeutic efficacy of the drug.
- The qualitative inorganic analysis of the drug showed the presence of mercury, magnesium, aluminium, calcium, sodium, potassium, copper, zinc, iron, cobalt, chloride, carbonate, nitrate, sulphate, sulphide, acetate, silicate and arsenate which are biologically important radicles.
- Quantitative results of heavy metal analysis revealed that the contents of lead and cadmium were within the admissible limits, whereas the contents of arsenic and mercury were high due to the presence of the ingredients *Rasam (mercury), Lingam* (Mercuric sulphide), *Pooram* (Mercurous chloride), *Thalagam* (Arsenic trisulphide), *Manosilai (Arsenic disulphide), Vellai padanams (Arsenic trioxide), gowri padanam(Arsenic penta sulphide), Kalmatham (Hydrous cobalt arsenate)* and *Rasa chenduram/sinduram (Red sulphide of mercury).*
- Quantitative assays showed the presence of Calcium, Magnesium**,** Potassium**,** Aluminium**,** Copper**,** Iron and Zinc . Sulphur (as SO_2) and Chloride (as NaCl).
- In the microbial study, the drug was found free from *E. coli, Salmonella* spp., *Staphlococcus aureus* and *Enterobacteriacea*.
- All the four aflatoxins were not detected in the drug.

- Various pesticide residues were lower than the limit of quantification.
- The TLC photo-documentation and the HPTLC finger print profile of hexane extract, chloroform extract, ethanol extract of Nandhi Mezhugu showed several peaks. Though it is difficult to read the individual peaks, it is done to assure the quality of the study drug.
- The 28-days and 90 days repeated doses oral toxicity studies in Wistar albino rat inferred that NM did not produce delayed onset of toxicity in both 28 days and 90 days toxicity studies. Based on these results, the No Observed Adverse Effect Level (NOAEL) of "NM" was greater than 110 mg/kg/day.
- ICP-OES – detection of heavy metal traces in animal tissue samples (Brain, Liver, Kidney) in Sub-chronic toxicity studies (High dose group) of Nandhi Mezhugu reported the absence of traces of heavy metals such as lead, cadmium, mercury and arsenic.
- Acetic acid induced writhing method was preferred to evaluate the analgesic effect of NM in mice. It was inferred that, in high dose group (NM 50mg + PJ solution) there was delayed onset of writhing and the number of writhings were also reduced. The effect was highly dose dependent. The high dose group showed significant analgesic activity when compared to standard drug.

- Acute anti-inflammatory activity was studied by Carrageenan induced paw odema method, the paw volume was reduced significantly in high dose group (NM) 900mg/kg b.w with that of standard **(**Diclofenac sodium 25mg/kg b.w). Percentage of inhibition in high dose group was high when compared to that of the standard.

- Chronic anti-inflammatory activity was studied and inferred that dose dependent inhibition of granuloma tissue formation.
- In Freund's Complete adjuvant (FCA) Arthritis study, the arthritis was established in the G1(vehicle control group-palm jaggery solution) group animals and also across other groups (G2-reference drug Indomethacin-0.3mg/kg b.w, G3,G4,G5-NM-50,150,500mg/kg b.w respectively) before the initiation of the treatment. The total percentage change in inhibition of arthritis was 51.36% in high dose group with respect to control (0% inhibition). When compared with standard (56.60%), the test drug NM showed significant inhibition. Histologically, there was a moderate degree of arthritis induction at G1 group. There was mild degree of improvement in the arthritis condition at G2 when compared to the G1 group. There was a marginal degree of reduction in the arthritis score in G3, G4 and G5 groups.
- In vascular permeability study, the intra-peritoneal injection of 0.6% acetic acid induced increase in permeability of vascular system resulting in dilation of the blood vessels of all

the animals across all groups in both sexes. This was evidenced by the presence of Evans blue dye in peritoneal fluid. Administration of the test item (NM-50,150,500mg/kg b.w to G2, G3, G4 respectively) caused a significant decrease in vascular permeability in G2, G3 and G4 in males and in G3 and G4 in females when compared to G1 group (palm jaggery solution).

- In in-vitro cytotoxicity assay a LPS induced inflammation in the RAW 264.7–Mouse macrophage cell line study. Nandhi Mezhugu showed cytotoxicity at 500 µg/ml and above, which is too high a concentration to correlate to the in-vivo studies. The concentrations less than 250 µg/ml were considered for the cyto-protective cell line assay where the expression of the inflammatory biochemical markers such as COX-2 and TNF-alpha were studied. Nandhi Mezhugu at 100 µg/ml showed a decrease in inflammatory markers to the extent of 14% and 10% of COX-2 and TNF-alpha respectively. This result suggests that the Nandhi Mezhugu preparation can be used as an alternative treatment for the inflammatory conditions.
- In in-vivo –Rat Paw oedema bio-assay, a dose dependent decrease in the inflammation was observed. The highest dose studied in this bio-assay was 500 mg/kg which is almost equivalent to the standard drug indomethacin. The pro-inflammatory and anti-inflammatory gene expression studies were performed in the rat paw treated with Nandhi Mezhugu. A slight increase in the gene expression of Beta-galacturonidase, Acid phosphatase, and Cathepsin-D at 11%, 19%, and 6% respectively is biologically non-significant, whereas a decrease in C-reactive protein and Interferon-gamma gene expression at 57% and 27% respectively is considered due to anti-inflammatory effect of Nandhi Mezhugu. An increase in TNF-alpha, which is a pro-inflammatory marker, is expected to reduce with the treatment; however the increase in the level could not be explained. Further studies are required to understand the pathway of anti-inflammatory effect of Nandhi mezhugu.
- An open labelled, non-randomized, clinical study (without control) on 40 cases of Uthiravatha suronitham or Rheumatoid arthritis was conducted, to determine the safety and therapeutic efficacy of Nandhi melugu (NM), in out-patient and in-patient departments of Maruthuvam, Ayothidoss Pandithar hospital, National Institute of Siddha, Tambaram Sanatorium, Chennai-47. The study drug NM was given for 60 days (7 days drug dosing followed by 7 days drug holiday). Out- patients were asked to visit OPD once in 2 weeks and in-patients were taken care of every day by daily visits. Clinical

assessment and prognosis were recorded during their visits. Clinical laboratory investigations were carried out before and after treatment. A total of 66.6% (40/60) patients were followed as per the study protocol and completed 60 days of treatment with NM. 5 patients refused to sign consent form and 15 patients did not come for regular follow-up and blood investigations. There were (4/40) 10% male and (36/40) 90% female patients. At the baseline 100% of both male and female patients were tested high positive for Anti CCP, RAF and high ESR and raised CRP level. After 60 days of treatment, the haematology report showed a significant reduction in the high value of ESR (****p value -0.0001). While comparing the RAF before and after treatment, there was significant reduction observed (*p value-0.032).

The observations were made and tabulated with regard to the following features:

- Gender distribution(Sex distribution)
- Age distribution
- Kaalam distribution (Vaatha, Pitha, Kaba kaalam)
- Food habits
- Socio-economic status
- Occupational status
- Distribution of thinai(Land)
- Paruvakaalam (Seasons)
- Aimporigal
- Aimpulangal
- Kanmenthirium
- Kanmavidayam
- Kosangal
- Seven udal thathukkal
- Distribution of mukkutram
  - Deranged vatham
  - Deranged pitham
  - Deranged kabam
- Envagai thervugal
- Neerkkuri, Neikkuri
- Clinical features
- Clinical signs

- Clinical symptoms
- Pain score
- Grade for restricted movements
- MHAQ
- EULAR Score
- Haematological parameters
- Biochemical parameters
- LFT
- RFT
- Hb, ESR
- Anti CCP, RA, CRP

- MHAQ:(Modified health assessment questionnaire (MHAQ) score

MHAQ score less than 0.3 is considered normal.The mean MHAQ score was recorded after treatment (0.406) and compared with the score before treatment (1.753) for 40 patients. The mean score was significantly reduced from the base line after treatment with NM 500 mg/kg, orally with Palm Jaggery, twice daily for 60 days (7 days drug dosing followed by 7 days drug holiday). In this study the mean of MHAQ after treatment for 40 patients was 0.406. So it was inferred that after treatment, the patients were improved in their disability index.

- Disease Activity - EULAR Score

The mean EULAR score was recorded after treatment and compared with the score before treatment for 40 patients.The mean score was reduced from 8.125 to 4.775 after treatment with NM 500 mg/kg, orally with Palm Jaggery, twice daily for 60 days (7 days drug dosing followed by 7 days drug holiday)..

- He Pain score status of forty patients was assessed by universal pain assessment scale. Out of 4 patients who had worst pain, 3 patients reported moderate pain and 1 patient mild pain after treatment.

  Out of 34 patients, who had severe pain, 28 patients reported no pain and 6 patients reported mild pain.

  2 patients, who had moderate pain, reported no pain.

- Restricted Movement Assessment Scale**:** (Ref: BearingPoint, Atos Healthcare & DSP Copyright EBM Rheumatoid Arthritis Version 5.0 final)

Grade I - Able to perform normal duties.

Grade II - Moderate Restriction- Self-care is possible.

Grade III – Marked restriction – Limited self-care/ some assistance required.

Grade IV – Confined to bed or wheel chair.

As per the restricted movement assessment scale, before treatment, 4 patients (10%) were in GradeII (moderate restriction-self care is possible), 32 patients (80%) were in Grade III (marked restriction-limitted self care-some assistance required) and 4 patients (10%) were in GradeIV (confined to bed/wheel chair).

After treatment 33 patients (82.5%,) were found to perform normal duties (Grade I), 6 patients (15%) were able to care for themselves (GradeII) and only one patient (2.5%) needed some assistance (Grade III).

10.CONCLUSION

The raw ingredients of the study drug, Nandhi Mezhugu (NM), were purchased and prepared at the Indian Medical Practitioner's Cooperative Pharmacy (IMPCOPS) society, as per standard operating procedure mentioned in the text Siddha Vaithiya Thirattu and standardised as per Ayush guidelines. The safety studies - acute, sub-acute, sub-chronic of NM, done as per OECD guidelines.Acute toxicity findings showed no mortality and toxicity signs up to 2000 mg/kg in acute oral toxicity study. NM can be classified under category-5, since the LD50 value was greater than 2000 mg/kg in accordance with Globally Harmonized System of Classification and Labelling of chemicals (GHS 2015] and this provided us a direct relevance for protecting human and animal health. NM did not produce delayed onset of toxicity in both 28 days and 90 days repeated oral toxicity studies. Based on these results, No Observed Adverse Effect Level (NOAEL) of "NM" was greater than 110 mg/kg/day. ICP-OES –study for detection of heavy metal traces in animal tissue samples (Brain, Liver, Kidney) in Sub-chronic toxicity studies (High dose group) of NM reported the absence of traces of heavy metals such as lead, cadmium, mercury and arsenic.

NM showed significant analgesic and anti-inflammatory (acute and chronic) and anti- arthritic activities in animal models. In vascular permeability study, NM caused a significant decrease in vascular permeability. In in-vitro cytotoxicity assay, in a LPS induced inflammation in the RAW 264.7–Mouse macrophage cell line study, NM showed cytotoxicity at 500 µg/ml and above. NM at 100 µg/ml showed a decrease in inflammatory markers to the extent of 14% and 10% of COX-2 and TNF-alpha respectively. This result suggests that the Nandhi Mezhugu preparation can be used as an alternative treatment for the inflammatory conditions. The pro-inflammatory and anti-inflammatory gene expression studies performed in the rat paw treated with Nandhi Mezhugu, showed a decrease in C-reactive protein and Interferon-gamma gene expression which is considered due to anti-inflammatory effect of Nandhi Mezhugu.

An open labelled, non-randomized, clinical study (without control) was conducted in 40 cases of Uthiravatha suronitham (or Rheumatoid arthritis), to determine the safety and therapeutic efficacy of Nandhi mezhugu (NM). The study drug NM was given for 60

days (7 days drug dosing followed by 7 days drug holiday). At the baseline, 100% of both male and female patients were tested high positive for Anti CCP, RAF and high ESR, and raised CRP level. After 60 days of treatment, the haematology report showed a significant reduction in the high value of ESR (****p value -0.0001). While comparing the RAF before and after treatment, a significant reduction was observed (*p value-0.032). The quantitative outcome of MHAQ score, Universal pain assessment scale score, Restricted movement assessment scale score and EULAR score showed significant reduction after treatment and proved the therapeutic efficacy of NM for the treatment of RA.

10. RECOMMENDATIONS

In further, unexplained areas of gene expression and cell line studies of NM in anti –inflammatory, anti-rheumatoid pathways will also be studied.

Due to time constraint this study drug was given for a period of 60days (as per literature 7days drug dosing followed by 7days drug holiday/patient). In future by recording the impact of NM in serum Anti CCP titre value, by extending the duration of treatment up to 6 months with large human sample size.

Based on the above study findings, this research will be continued by doing a double blind randomized controlled clinical trial to improve the quality of life in RA patients, then Nandhi Mezhugu as an anti-rheumatoid drug, will be a evidence based medicine for global recognization .

11. BIBLIOGRAPHY

1. Kuppusamymudhaliyar KN. Pothu maruthuvam. Department of Indian medicine and Homoeopathy.Chennai. 2007; 7th ed; 608-609.
2. Venugopal. Noi naadal noi mudhalnaadal.Part 1,Department of Indian medicine and Homoeopathy.Chennai-106
3. Sambasivam pillai TV. Siddha medical dictionary(Tamil-English). Department of Indian medicine and Homoeopathy.Chennai.2016;4th print.
4. Yugi,YugiVaithiyaSinthamani;1st ed; Dec 1998;Thamarai noolagam, Chennai; version 243- 244.Page no:92-130
5. Handout on Health: Rheumatoid Arthritis February 2016, National Institute of Arthritis and Musculoskeletal and skin diseases. Available at http://www.niams.nih.gov/ health_info/rheumatic_disease/
6. Dr Ananya Mandal, MD. Rheumatoid Arthritis Epidemiology,November 2016, Available at http://www.news-medical.net/health/Rheumatoid-Arthritis-Epidemiology.aspx
7. Dr. Cristiano Zerbini MD, International Osteoporosis Foundation (IOF)**.** Biologic drugs may help prevent progressive bone loss in patients with RA, November, 2016. Available at http://www.news-medical.net/news/20161107/Biologic-drugs-may-help prevent-progressive-bone-loss-in-patients-with-RA.aspx
8. DebrodhSymmons 1 Colin Mathers 2 Bruce Pfleger 3 The Global Burden of Rheumatoid arthritis in the year 2000. WHO statistics (2007) : 8-12
9. Kasper BraunwaldFauci Hauser Longo Jameson Harrison's Principles of Internal Medicine.Vol II 16 th edit.P.1970
10. Brooks PM.MJA Practice Essentials-Rheumatology, Sydney: Australasian Medical Publishing Company Limited, 1997.
11. Agasthiyar 2000. 1stpart. Thanjai Saraswathy mahal,1958;page no:9-14.
12. Agasthiyar kanma kandam-300, 2nd ed, Thandayuthapaani thirukkovil,Palani, India maruthuva Iyakkunar aluvalagam, Chennai, 1976
13. Theraiyar Segarappa 1st edition; Central council for Research in Ayurvedha and Siddha, New Delhi-24.
14. Shanmugavelu M, Noi Nadal Noi Mudhal Naadal- Part II.3rd ed, Department of Indian medicine and Homoeopathy.Chennai-106, 2003.p no:585,550-613.

15. Theraiyar, Theraiyar Vagadam, Thandayuthapaani thirukkovil,Palani, Ist edtion, Nov 1975.
16. Sapapathy mudhaliyar, Jeeva Rakshamirtham Page No :13
17. Shanmugavelu M,Thanvanthri vaithiyam, Noi Nadal Noi Mudhal Naadal- Part II. Department of Indian medicine and Homoeopathy. Chennai.
18. S.P.Ramachandran,YugiVaithiyacinthamani-800,Thamarai noolagam,2nd edition, July 2013, Page No:122.
19. T.V. Sambasivampillai Tamil and English Dictionary of Medicine, Chemistry, botany and allied sciences, Department of Indian Medicine and Homeopathy (volume I- First edition 1931.
20. Agasthiyar , Agasthiyar Vaithiya Vallathi-600, ,Central council for Research in Ayurvedha and Siddha, New Delhi-16. Ist edtion,1980
21. Pathaartha Guna Chinthamani, Department of Indian medicine and Homoeopathy.Chennai-106, 2007, p.no:362.
22. S.P.Ramachandran, *Thanjai Navarathina Vaidhiyaraja chinthamani*-800, A.Rangaswamy Mudhaliyar and Son's, part 1,page no:21-22.
23. Agathiyar,*Agathiyar Vaithiya Rathina surukkam*, *Thamarainoolagam* Chennai-26, 2nd edition,Feb 1998,Song No:36-38, 55-57, 114-118, 149-150. Page No: 12-14, 18-19, 40-42, 53.
24. Dr.K.N.Kuppusamymudhaliyar, Dr.K.S.Uththamaraayan, *Siddha Vaidhiya Thirattu*, Department of indian medicine and Homoeopathy, 1st edition, Reprinted 2009, Page no:305, 204-213, 128, 257, 165-166, 228-229, 187-193, 30-31, 203-204, 41, 193-194.
25. Agathiyar, *Agathiyar Paripooranam*-400 ,B.RathinaNayakar and Son's Chennai-79,2008, Song No:195-201, 261-264, 281-282, 351-352, 324-325, 126-129, Page No:37, 48, 63, 59, 51.
26. Dr.R.Thiyagarajan, *Gunapaadam Thadhu jeeva vaguppu*,Deptarment of Indian Medicine and Homeopathy ,Part 2 and 3 P.No:58
27. Therayar,*TherayarYamagaVenbha*,Department of Indian Medicine and Homeopathy,
28. R.Thiyagarajan,A.Anandhan and G.Perumal,*TherayarMahaKarisal*, Department of Indian Medicine and Homeopathy, Song No:50,Page.No:132-134.
29. Therayar,*Therayar Karisal* 300 ,B.RathinaNayakar and Son's Chennai-79,Song No:2 Page.No:8.

30. Anonymous, Hospital Pharmacoepia, Department of Indian Medicine and Homeopathy,Chennai-106, P.No:133.
31. Therayar,*Therayar Thyla Varga surukkam*, Baskara and Son's, C.T.Arumugam pillai,Chennai,2nd edition,1927, Song No:79, Page.No:101,128,87,48,
32. Boogar,*BoogarVaithiyam* 700,B.RathinaNayakar and Son's Chennai-79,2012, Song No:175-187,Page.No:33-36
33. Pulipani,*Pulipaani vaithiyam*-500, B.RathinaNayakar and Son's Chennai-79,2008,Song No:324-330,Page No:60-61.
34. Yugi,*Yugi Karisal*-151,Department of Indian Medicine and Homeopathy Song No:16-24 Page.No:4-7.
35. S.S.Anandhan,R.Thiyagarajan, *TherayarPaadalThirattu*, Department of Indian Medicine and Homeopathy,2nd edition,1975, P.No:28.
36. Dr.K.S.Murugeshamudaliyar Dr.Pon Guru Siroonmani, *BalaVaagadam*, Department of Indian Medicine and Homeopathy,4th edition,2007, P.No:79-80.
37. Dr.Murugeshamudaliyar,*GunapadamMooligaivaguppu*, Department of Indian Medicine and Homeopathy Part I ,Edition, page No:69,94,186,449,285,498,678,760,766,785, 397, 801,824.
38. Gerd-Rudiger burmester, Arthur G Pratt, Hans Ulrich Scherer, Jacod M van Laar, Rheumatoid arthritis , Chapter -9, Pathogenesis and Clinical Features, Page No:206-231.
39. Johannes W J Bijlsma, Textbook on Rheumatic Diseases, Eular score BMJ Publishing Group Ltd. London, WCIH9JR.UK. 1st edtion, 2013.
40. Muthukaruppapillai,*Vaidiyasaarasangiragam*,Madurai tamil sanga miththira salai,2nd edition,1929,Page.No:496-497
41. Mona Semalty, Ajay Semalty, Ashutosh Badola, Geeta Pant Joshi, and M. S. M. RawatPharmacognosy Review Semecarpus anacardium Linn.: A review. 2010 Jan-Jun; 4(7): 88–94 Available at http://www.ncbi.nlm.nih.gov/pmc / articles /PMC32 499 08/ ta ble/T1/
42. Saraf MN, Ghooi RB, Patwardhan BK.Studies on the mechanism of action of Semecarpus anacardium in rheumatoid arthritis.J Ethnopharmacol. 1989 Apr;25(2):159-64 Available at http://www.ncbi.nlm.nih.gov/pubmed/16595901/
43. Rostogi & Mehrotra, Compendium of Indian Medicinal Plants, Vol 3,p.580, PID, New Delhi, 1980-1984
44. Nayar & Chopra, Glossary Indian Medicinal Plants, Vol I, p 225, PID, New Delhi,1956.

45. The Indian material medica, Dr.K.M.Nadkarni, volume 1,3rd edition reprinted 2005,Popular Prakashan pvt.ltd . page no: 1175-1181,1308,969,965-969,475-475,80,840-842,97-98,1292,1196,1009,670-672,478,25-27,830-834,845-857,408-410,969-971,990-993,965-969,901-922,557-559,396-399,461,478,497,473,

46. The Indian material medica, Dr.K.M.Nadkarni,3rd edition reprinted 2005,Popular Prakashan pvt.ltd vol:2 page no: 308

47. Jeba Singh G,Avuri (Indigofera tinctoria) Zahid National Health Portal Dec 11 2016 Available at www.medicinalplants.in

48. Sunil kumar, Vinod Gauttam, Mukunda Sudhakar Rao Bande, Kunal Nepali, Ankit Tyagi and Kanaya Lal Dhar.Estimation of crotepoxide in the fruit of Piper attenuatum Buch.-Ham. Using HPTLC and APLC. Current Pharmaceutical Analysis, 12(4):343-348.

49. Jayaram Siva,Benefits of palm jaggery(panai vellam,karupeti)Posted 28th April 2013, Available at http://saffronjuice.blogspot.in/

50. R. Srivastava, H. Ahmed, R. K. Dixit, Dharamveer, and S. A. Saraf Pharmacogn Review Crocus sativus L.: A comprehensive review 2010 Jul-Dec; 4(8): 200–208**.**

51. Swarnamariyammal I.Boogar ellayirathil Siddha maruthuva kanimangal,Ulaga Siddha Arakkattalai,Chennai;2016;for Review page No

52. Thiyagarajan R, Anaivari Anandan R, Thulasimani M. Siddha Materia Medica(mineral and animal kingdom). Department of Indian medicine and Homoeopathy.2008.

53. Anonymous, Vaidhiya yoga ratnavali, Impcops, Chennai, P.no: 402-403.

54. Kuppusamy mudhaliyar KN, Uththamaraayan KS. Siddha Vaidhiya Thirattu. Department of indian medicine and Homoeopathy.2009; 1; 183-189.

55. Anonymous .The Siddha Formulary of India. Department of ISM & H, Ministry of Health & Family Welfare, Government of India, New Delhi.1992;1:70-71

56. Thiyagarajan R, Anaivari Anandan R, Thulasimani M. Siddha Materia Medica(mineral and animal kingdom). Department of Indian medicine and Homoeopathy.2008;2; 235, 407, 252, 266, 295, 326, 272, 223, 221, 424, 351, 286, 419, 342, 422, 32, 461.

57. Kannusamyam pillai S. Sikitcha Rathina Deepam.B.Rathinanayakar and Son's. 2007;2;38,32,28,29,31,34

58. Aanaivaari anandhan. Sarakku Suththi Muraigal.Department of Indian medicine and Homoeopathy. 2008;1;48,6,7.

59. Deva Asirvatham Samuel, M.D(S) – MarundhuSeiIyalumKalaium published by Directorate of Indian Medicine & Homeopathy.P.328

60. Lohar DR.Pharmacopoeial laboratory for Indian medicine.Department of Ayurvedha, yogaand Naturopathy, Siddha, Unani andHomoeopathy (AYUSH), Ministry of health and family welfare. 2011; 20.

61. Anonymous .Quality Control Methods for Medicinal Plant Materials. World Health Organisation, Geneva, Switzerland.1998.

62. Harborne JB .Phytochemical Methods. Chapman and Hall, 1973. Wagner H, Bladt S (editors). Plant Drug Analysis. Springer, Berlin, London. 1996 ;2.

63. Feigl F. Anger V. Spot tests in inorganic analysis. Elsevier: Amsterdom, 1972;2.

64. Stahl I .Thin layer chromatography, A Laboratory Hand Book (student edition). Springer-Verlag, Berlin, Germany. 1969 ;52-86.

65. Sethi PD .High Performance Thin Layer Chromatography .Vol. X. CBS Publishers and Distributors, New Delhi, India.1996;1;1-56.

66. OECD, OECD Guideline for Testing of Chemicals, Acute Oral Toxicity-Acute Toxic Class Method: TG 423-Adopted,OECD, Paris, France, 2001.

67. OECD, OECD Guideline for Testing of Chemicals, Repeated Dose 28-day Oral Toxicity Study in Rodents: TG 407-Adopted, OECD, Paris, France, 1995.

68. OECD, OECD Guideline for Testing of Chemicals, Repeated Dose 90-day Oral Toxicity Study in Rodents: TG 408-Adopted, OECD, Paris, France, 1995.

69. S. K. Gulkarni, Hand Book of Experimental Pharmacology, Vallabh Prakashan, 3rd edition, 1999.

70. Globally Harmonized system of Classification and Labelling of chemicals(GHS). Health and Environmental Hazards Classification criteria for substances and mixtures ppt;2015;6;10

71. Stanley O.Aniagu, Florence C. Nwinyl, David D, Akumka, Gloria A, Ajoku, Sunday Dzarma, KazeemS.Izebe, Matthew Ditse, Patrick E.C, Nwaneri, Charles Wambebe and KaryniusGamaniel., 2005. Toxicity Studies in rats fed nature cure bitters. African Journal of Biotechnology 4, 72-78

72. Koster R, Anderson M, Beer EJ (1959). Acetic acid for analgesic screening. Fed. Proc., 18: 412-418.
73. Hajare SW, Chandra S, Tandon SK, Sarma J, Lal J, Telang AG. Analgesic and antipyretic activities of *Dalbergia Sissoo* leaves. Indian J Pharmacol 2000;32:357-60.
74. Vinegar R, Schreiber W, Hugo R. Biphasic development of carrageenin oedema in rats. J Pharmacol Exp Ther 1969;166:96-103.
75. Jana U, Chattopadhyay RN, Shaw BP. Preliminary studies on antiinflammatory activity of *Zingiber officinale Rosc.*, *Vitex negundo Linn*, and *Tinospora cordifolia* (Willid) miers in albino rats. Indian J Pharmacol 1999;31:232-3.
76. Winter CA, Porter CC. Effect of alterations in the side chain upon antiinflammatory and liver glycogen activities of hydrocortisone esters. Journal of the American Pharmaceutical Association. 1957;46:515–519.
77. Patil KR, Patil CR. Anti-inflammatory activity of bartogenic acid containing fraction of fruits of Barringtonia racemosa Roxb. in acute and chronic animal models of inflammation. J Tradit Complement Med. 2016 Apr 4;7(1):86-93.
78. Cotton pellet granuloma Anti-inflammatory activity of piperinearvind manohar mujumdar, jayant nilkanth dhuley, vinaykumar keshav deshmukh, palghat hariharan raman, suresh ramnath naik japanese journal of medical science and biologypanese journal of medical science vol.43 (1990) no. 3 p 95-100. Available online at http://doi.org/10.7883/yoken1952.4.
79. Hicks R (1969) The evaluation of inflammation induced by material implanted subcutaneously in the rat. J Pharm Pharmacol 21:581–588
80. Meier R, Schuler W, Desaulles P (1950) Zur Frage des Mechanismus der Hemmung des Bindegewebswachstums durch Cortisone. Experientia 6:469–471.
81. Ramesh B. Nidavani, Mahalakshmi AM, Mallappa Shalawadi Vascular permeability and Evans blue dye: a physiological andpharmacological approach Journal of Applied Pharmaceutical Science Vol. 4 (11), pp. 106-113, November, 2014 Available online at http://www.japsonline.com
82. Bendele A.M Animal models of rheumatoid arthritis 2001; 1(4):377-385
83. Spector WG. The Granulomatous Inflammatory Exudate. Int RevExpPathol1969;8:51-5.
84. Carl M. Pearson Fae D. Arthritis and Rheumatology, Wood Studies of polyarthritis and other lesions induced in rats by injection of mycobacterial adjuvant. I. General clinical

and pathologic characteristics and some modifying factors, American college of rheumatology,Oct 1959.

85. Dvorak HF. How tumors make bad blood vessels and stroma.Am J Pathol, 2003; 162: 1747-1757. Evans blue material safety data sheet[http://datasheets.scbt.com/sc-203736.pdf].
86. Ramesh BN, Mahalakshmi AM, Mallappa HS, Biradar BS,Imad MU. Role of Inflammatory Mediators as Therapeutic Targets for Rheumatoid Arthritis: An Updated Review. World J Pharm Pharma Sci 2013;2(5):2526-2538.
87. Nagy JA, Dvorak AM, Dvorak HF. VEGF-A(164/165) andPlGF: roles in angiogenesis and arteriogenesis. Trends Cardiovasc Med,2003; 13: 169-175.
88. Matsuda R, Nishikawa A, Tanaka H. Visualization ofdystrophic muscle fibres in mdx mouse by vital staining with Evans blue:Evidence of apoptosis in dystrophin-deficient muscle. J. Biochem. 1995;118: 959-964.
89. Brown MA, Mitar DA, Whitworth JA. Measurement of plasmaVolume in pregnancy. Clin Sci, 1992; 83: 29-34.
90. Oztas B, Kaya M, Camurcu S. Influence of profoundhypothermia on the blood-brain barrier permeability during acute arterial hypertension. Pharmacol Res, 1992; 26: 75-84.
91. Berridge MV, Tan AS. Characterization of the cellular reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT): subcellular localization, substrate dependence, and involvement of mitochondrial electron transport in MTT reduction. Arch Biochem Biophys. 1993;303(2):474–82.
92. Tada H, Shiho O, Kuroshima K, et al. An improved colorimetric assay for interleukin 2. J. Immunol. Methods. 1986;93:157–65.
93. Hansen MB, Nielsen SE, Berg K. Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. J. Immunol. Methods. 1989;119:203–210.
94. Nisarat Siriwatanametanon, Bernd L. Fiebich, Thomas Efferth, Jose M. Prieto, Michael HeinrichTraditionally used Thai medicinal plants: *In vitro* anti-inflammatory, anticancer and antioxidant activities.
95. Michael J. ThunS. Jane Henley Carlo Patrono Nonsteroidal Anti-inflammatory Drugs as Anticancer Agents: Mechanistic, Pharmacologic, and Clinical Issues Volume 94Issue 4 20 February 2002.

96. Universal pain assessment scale .The National Initiative on Pain Control™ (NIPC™)
97. Restricted movement assessment scale, BearingPoint, Atos Healthcare & DSP Copyright EBM Rheumatoid Arthritis Version 5.0 final
98. Pincus T, Yazici Y, Bergman M. Development of a multi-dimensional healthassessment questionnaire (MD-HAQ) for the infrastructure of standard clinical care. Clin Exp Rheumatol. 2005;23(Suppl 39):S19-S28.
99. Kannusamy Pillai, Kannpusamy Parambarai Vaithiyam (P.226-230)
100. Calcium biological importance, Available at http://nautilus.fis.uc.pt/st2.5/scenes-e/elem/e02040.html
101. Magnesium in biology, From Wikipedia, the free encyclopediaAvailable athttps://en.wikipedia.org/wiki/Magnesium_in_biology
102. **JoskoOsredkarand NatasaSustar** ' Journal of Clinical Toxicology Copper and Zinc, Biological Role and Significance of Copper/Zinc Imbalance
103. Acetate From Wikipedia, the free encyclopedia Available at https://en.wikipedia.org/wiki/Acetate
104. Lakshmi kantham T et al. "Standardization of Nandhi Mezhugu, a poly herbomineralSiddha formulation". J Pharmacy Research 2016; 10(10):666-673. Availablefrom:http://jprsolutions.info.
105. Thiyagarajan R.Siddha maruthuvam sirappu. Palani Thandayuthapani swamy thirukoil Siddha maruthuva Nool Veliyeettu gulu.1986;1-13
106. Siger.C.P, Younces.M,Effect of bioflavonoids on Lipid Peroxidation Induces by GSH Depletion, Proc.It. Bioflavonoids Symposium, Knwch, FRG, 1981,403-409.
107. Umarani.M,Shanthi.P,Sachdanantham.P,Protective Effect of Kalpaamruthaa In Combating The Oxidative Stress Posed By Aflatoxin B1-induced Hepatocellular, Carcinoma with special reference to flavonoid structure-activity relationship, Live Int-28,200-13.
108. Mukherji.B, The Wealth Of India Alchemy And Its Medicinal Users, 1, Sri Satguru Publication, New Delhi,1998; 388.
109. Goudgaon.N.M, Lamture.J.B and Nayak.U.R, Semecarpus anacardium as an Anticancer Agent: Epoxy Derivatives of the Monoene and Ddiene Bhilawanols, Indian Drugs, 22(11), 1984,556.
110. Byoung Seok hong,jeong Ho Kim, Na Yeon Kim, Bong-Gyu Kim, Youyhoon Chong and Joong-Hoon Ahn, Characterized of Uridine –Diphosphate Dependent Flavonoid

Glucosyltranferase from Oryza sativa, Journal of Biochemistry and Molecular Biology;2007;40(6);870-874.

111. Politeo.O, Jukic.M, Milos.M, Chemical composition and Anti-oxidant Activity of Essential Oils of Twelve Spice Plants, Croa. Chem.Acta, 79;2006;545-552.
112. Irfan Ali khan and Atiya Khanum, Anti-Diabetic Plants of India , Ukaaz Publication, Hyderabad, 2005;84.
113. Etheresia Pretorius, Hester Magdalena Oberholzer, Petrus Johannes Bedker, Comparing the cytotoxic Potential of Withania somnifera Water and Methanol Extracts, Ar.J.Trad.CAM;2009;6(3);275-280.
114. Kumaravel R, Siddha maruthuvam Part-3 Sirappiyial, Tamil Valarchi Kazhagam, Chennai.1st Edition;2004;7
115. Shanmugavelan A. Siddhar's Science of Longevity and Kalpa Medicine, Department of Indian medicine and Homoeopathy. Chennai;1992;2;92-94.111-119.
116. Sankaranarayanan S.Mooligai Thavarangalin Mukkiyathuvamum athan Vethi Porutkalum.Harishi Publication.Chennai;2009;2;1,13,28,60,75,83,84,107.
117. Rakthi agarwal.Sudhir k.Goel.Jai Raj Behari.Detoxification and antioxidant effects of curcumin in rats experimentally exposed to mercury. Wiley interscience. Mar 2010 Available at www.interscience.wiley.com
118. Kumar A. Nair AGC .reddy AVR. Garg AN. Bhasmas Unique Ayurvedic Metallic–Herbal Preparations,Chemical Characterization. May 10, 2005
119. Gajendra kumar Yogendra kumar gupta.Evidence for safety of Ayurvedic herbal, herbo-metallic and Bhasma preparations on neurobehavioral activity and oxidative stress in rats .October 2012.Available athttps://www.researchgate.net/publication/236978234_Evidence_for_safety_of_Ayurvedic_herbal_herbometallic_and_Bhasma_preparations_on_neurobehavioral_activity_and_oxidative_stress_in_rats.
120. Ramaswamy RS, Glimpses of Thathuvam-96, The South India Saivasiddhantha Works Pub Society Limited.

PRECLINICAL AND CLINICAL EVALUATION OF SAFETY AND EFFICACY OF SIDDHA HERBOMINERAL FORMULATION NANDHI MEZHUGU IN THE TREATMENT OF UTHIRA VATHA SURONITHAM (*RHEUMATOID ARTHRITIS)*

CASE RECORD FORM

FORM I

SCREENING AND SELECTION PROFORMA

1. O.P.No / I.P No ----------

2. S. No --------

3. Name ----------

4. Age/sex ----------

5. Occupation / Income ----------

6. Address:

---

7. Contact No / E-mail ----------

8. Selection Criteria

Eligibility

Age Eligible for Study	:	18 Years to 60 Years
Genders Eligible for Study	:	Both
Accepts Healthy Volunteers	:	No

Inclusion criteria:

Major

	Yes	No
• Onset of arthritis after the age of 16 years	☐	☐
• Positive for rheumatoid factor (RF)	☐	☐
• Anti CCP	☐	☐
• Either an erythrocyte sedimentation rate (ESR) Of greater than 30 mm/hr OR C-reactive protein Level greater than 1.0 mg/dl (normal less than 0.4)	☐	☐
• Diagnosis of Uthira Vadha Suronitham, As defined by fulfilling at least more than Two articular involvements	☐	☐

Minor

• Patients giving informed consent (Signed or oral witnessed, according To local regulations) before any Protocol specific procedures.	☐	☐
• .Morning stiffness	☐	☐
• Willing to intake study medication	☐	☐
• Willing to follow diet restriction	☐	☐

Exclusion Criteria

- Diabetes Mellitus
- Hypertension
- Hypo & Hyperthyroidism
- Cardiac disease
- Renal disease
- Liver disorders
- Neurological disorders
- Pregnancy and lactation
- History of alcohol or substance abuse
- Active infection, or chronic or persistent infection that might worsen with immunosuppressive treatment
- Patients who have already participated in a new drug study in the past 3 months.

Subject withdrawal criteria

- Subject seeking any other treatment without informing the Investigator.
- Clinical failure after 1 month of treatment as worsening or no amelioration of signs and symptoms.
- Request of the subject.
- Repeated protocol criteria violation and non-compliance with its specification.
- Subject is failed to come for follow up.
- Serious adverse events/ reactions/ Inter current illness where continuation of study possess serious risk to the subject.
- Subject who develop signs and symptoms of Hyper sensitivity.
- Subject who become pregnant during the study period.

Date: Signature

FORM II

CLINICAL ASSESSMENT FORM

By

Dr. T.Lakshmi kantham, MD(s)

Investigator's signature and stamp

Date:

A. DEMOGRAPHIC DATA:

Age/Sex	:	
Weight	:	kg
Height	:	cm
BMI	:	
Address & Contact No	:	

B. CASE HISTORY:

Presenting Complaints:

Relevant past History

Personal History:

Concurrent illness and medications:

C. GENERAL EXAMINATION:

1. Temperature :
2. Heart rate :
3. Pulse rate :
4. Respiratory rate :
5. Blood pressure :
6. Pallor :
7. Icterus :
8. Cyanosis :
9. Clubbing :
10. Pedal oedema :
11. Lymphadenopathy :

EXAMINATION OF VITAL ORGANS

- CVS
- RS
- GIT
- Genito urinary
- CNS

THE ACR/EULAR 2010 CLASSIFICATION CRITERIA FOR RA:

To be applied to patients: (1) who have ≥ 1 joint with definite synovitis, excluding the DIP joints, first MTP joints, and first CMC joints, and (2) in whom the synovitis cannot be explained by another disease.

Criteria	**Score**
A. Joint involvement:	
1 large joint	**0**
2- 10 large joints[a]	**1**
1-3 small joints(With or without involvement of large joints)	**2**
4-10 small joints[b](With or without involvement of large joints)	**3**
>10 joints(at least 1 small joints)	**5**
B. Serology(at least 1 test result is needed for classification): Negative RF and negative anti-CCP antibodies	**0**
Low-positive RF or low-positive anti-CCP antibodies[c]	**2**
High-positive RF or high-positive anti-CCP antibodies[d]	**3**
C. Acute phase reactants: Normal CRP level and normal ESR	**0**
Abnormal CRP level or abnormal ESR	**1**
D. Duration of symptoms: <6 weeks	**0**
≥6 weeks	**1**

ACR, American College for Rheumatology; EULAR, European League Against Rheumatism; RA, rheumatoid arthritis; DIP, distal interphalangeal; MTP, metatarsophalangeal; CMC, Carpometacarpal; MCP, metacrpophalangeal; PIP, proximal interphalangeal; RF, Rheumatoid factor; anti-CCP, anti-cyclic citrullinated protein; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.

[a]Large joints= shoulder, elbow, hips, knees, ankles.

[b]Small joints= MCPs, PIPs, Second-fifth MTPs, thumb IPs, wrists.

[c]Low-positive is ≤ 3 times the upper limit of normal

[d]High-positive is > 3 times the upper limit of normal

SIDDHA SYSTEM OF EXAMINATION

1. THEGI (BODY CONSTITUTION):

2. NILAM (LAND WHERE THE PATIENT LIVED MOST):

3. KAALAM:

4. GUNAM:

5. IMPORIGAL (SENSORY ORGANS):

	Day1	Day15	Day29	Day43	Day60
Mei (Skin)					
Vai (Tongue)					
Kann (Eye)					
Mooku (Nose)					
Sevi (Ear)					

6.IMPULANGAL (SENSES)

	Day1	Day15	Day29	Day43	Day60
Sparisam					
Rasam					
Roobam					
kandham					
saptham					

7.KANMENDRIYANGAL (MOTOR ORGANS) :

	Day1	Day15	Day29	Day43	Day60
Kai					
Kaal					
Vai					
Eruvai					
Karuvai					

8.KANMAVIDAYANGAL

	Day1	Day15	Day29	Day43	Day60
Kamanam					
Thaanam					
Vasanam					
Visarkam					
Anandham					

9. KOSANGAL (SHEATH):

	Day1	Day15	Day29	Day43	Day60
Annamaya kosam					
Pranamaya kosam					
Manomaya kosam					
Vignanamaya kosam					
Ananthamaya kosam					

10. SEVEN UDAL THAATHUKKAL (SEVEN SOMATIC COMPONENTS)

	Day1	Day15	Day29	Day43	Day60
Saaram					
Senneer					
Oon					
Kozhuppu					
Enbu					
Moolai					
Sukkilam / Suronitham					

11. UYIR THAATHUKKAL: **[THREE HUMORS] (VALI/ AZHAL/ IYYAM)**

A) VALI

	Day1	Day15	Day29	Day43	Day60
Praanan					
Abaanan					

Samaanan					
Udhaanan					
Viyaanan					
Naagan					
Koorman					
Kirukaran					
Devathathan					
Dhananjeyan					

B) AZHAL

	Day1	Day15	Day29	Day43	Day60
Analakam					
Ranjakam					
Saathakam					
Prasakam					
Aalosakam					

C) IYYAM

	Day1	Day15	Day29	Day43	Day60
Avalambagam					
Kilethagam					
Pothagam					

Tharpagam					
Santhigam					

12. ENVAGAI THERVU: [EIGHT TYPES OF EXAMINATION]

I. NAADI: [PULSE PERCEPTION]

	Day1	Day15	Day29	Day43	Day60
NAADI					

II. SPARISAM: [PALPATION]

	Day1	Day15	Day29	Day43	Day60
Thoduvali					
Veppam					
Creptation					

III. NAA: [TONGUE]

	Day1	Day15	Day29	Day43	Day60
NAA					

IV.NIRAM: [COMPLEXION]

V.MOZHI: [VOICE]

VI.VIZHI: [EYES]

VIZHI	Day1	Day15	Day29	Day43	Day60

VII. MALAM: [BOWEL HABITS / STOOLS]

	Day1	Day15	Day29	Day43	Day60
Niram					
Irugal					
Ilagal					
Others					

VIII. MOOTHIRAM [URINE EXAMINATION]

NEERKKURI:

Neerkkuri	Day1	Day15	Day29	Day43	Day60
Niram					
Manam					
Edai					
Nurai					
Enjal					

NEIKKURI:

Neikkuri	Day1	Day15	Day29	Day43	Day60
Spreading pattern					
Shape					

PRECLINICAL AND CLINICAL EVALUATION OF SAFETY AND EFFICACY OF SIDDHA HERBOMINERAL FORMULATION NANDHI MEZHUGU IN THE TREATMENT OF UTHIRA VATHA SURONITHAM (*RHEUMATOID ARTHRITIS)*

CLINICAL ASSESSMENT FORM

S.NO	CLINICAL FEATURES	Day1	Day15	Day29	Day43	Day60
1.	Arthritis of more than 3 joints					
2.	Arthritis of hand joints					
3	Morning stiffness > 1 hr					
4.	Symmetrical arthritis					
5.	Restricted movements					
6.	Spindled appearance of fingers					
7.	Anorexia					
8.	Low grade fever					
9.	Subcutaneous nodules in specific places					

CLINICAL EXAMINATION

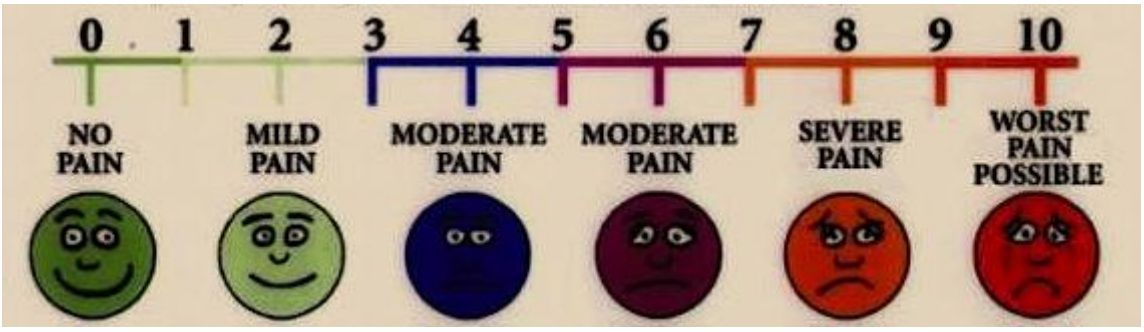
INSPECTION

	Day1	Day15	Day29	Day43	Day60
Tenderness					
Muscle spasm					
Local heat					
Local Lymphadenopathy					
Pitting oedema					
Joint stiffness					
Nodules					

CLINICAL SYMPTOMS:

	Day 1	Day 15	Day 29	Day 43	Day 60
Pain Onset:					
Early morning Stiffness					
Nature of pain					
Stiffness					
Tenderness					
Restriction:					

UNIVERSAL PAIN ASSESMENT SCALE:



	Day1	Day15	Day29	Day43	Day60
0 : No Pain					
1-3 : Mild pain					
4-6: Moderate pain					
7-10 : Severe pain					

The National Initiative on Pain Control™ (NIPC™)

RESTRICTED MOVEMENT ASSESSMENT SCALE:

GRADATION OF MOVEMENTS

Grade 1 – Able to perform normal duties

Grade II – Moderate Restriction – Self care is possible

Grade III – Marked restriction – Limited self care/some assistance required.

Grade IV – Confined to bed or wheel chair

	Day1	Day15	Day29	Day43	Day60
Grade 1					
Grade 2					
Grade 3					
Grade 4					

MODIFIED HEALTH ASSESSMENT QUESTIONARRE

Before treatment

sAre you able to	**without ANY difficulty <0>**	**with SOME difficulty <1>**	**with MUCH difficulty <2>**	**unable TO DO <3>**
Dress yourself, including tying shoelaces and doing buttons?				
Get in and out of bed?				
Lift a full cup or glass to your mouth?				
Walk outdoors on flat ground?				
Wash and dry your entire body?				
Bend down to pick up clothing from the floor?				
Turn regular faucets on and off?				
Get in and out of a bus, car, train, or airplane?				
Sub-total				
Total				

Source: Pincus T, Yazici Y, Bergman M. Development of a multi-dimensional health assessment questionnaire (MD-HAQ) for the infrastructure of standard clinical care. Clin Exp Rheumatol. 2005;23(Suppl 39):S19-S28.

After treatment

Are you able to	without ANY difficulty <0>	with SOME difficulty <1>	with MUCH difficulty <2>	unable TO DO <3>
Dress yourself, including tying shoelaces and doing buttons?				
Get in and out of bed?				
Lift a full cup or glass to your mouth?				
Walk outdoors on flat ground?				
Wash and dry your entire body?				
Bend down to pick up clothing from the floor?				
Turn regular faucets on and off?				
Get in and out of a bus, car, train, or airplane?				
Sub-total				
Total				

How to score the MHAQ

1.Ask the patient to complete the entire questionnaire by circling the appropriate responses while in the waiting room prior to his/her visit.

2.Add the totals from each of the four columns to get the patient's MHAQ score.

3.Divide this score by 8 and result should fall between 0.0 and 3.0. the higher the score, the greater the overall disability. MHAQ scores <0.3 are considered normal, although the average MHAQ in the general population increases with the age.

PRECLINICAL AND CLINICAL EVALUATION OF SAFETY AND EFFICACY OF SIDDHA HERBOMINERAL FORMULATION NANDHI MEZHUGU IN THE TREATMENT OF UTHIRA VATHA SURONITHAM (*RHEUMATOID ARTHRITIS)*

FORM-III

LABORATORY INVESTIGATIONS

1. O.P No: _____ Reg.No_____ Serial No_____

2. Name: _____

3. Age : _____ years

4. Sex:

5. Date of assessment: _____

BLOOD INVESTIGATIONS		**NORMAL VALUES**	**BEFORE TMT (WITH DATE)**	**AFTER TMT (WITH DATE)**
HB(gm/dl)		**M:12-15** **W:11.5-14**		
T.WBC (cells/cu.mm)		**4000-11000**		
DIFFERENTIAL COUNT (%)	**Polymorphs**	**40-75**		
	Lymphocytes	**20-40**		
	Monocytes	**2-10**		
	Eosinophils	**1-6**		
	Basophils	**0-1**		

T.RBC(million cells/cu.mm)		**M:4.0-5.5** **W:3.5-4.5**		
ESR(mm/hour)	**½ hr.**	**M:6-12** **W:7-18**		
	1 hr.			

Blood Investigations		**Normal Values**	**Before TMT(WITH DATE)**	**After TMT (WITH DATE)**
Blood glucose (mg/dl)	**Fasting**	**70-110**		
	PP	**80-140**		
	Random	**80-120**		
RFT (mg/dl)	**Blood urea**	**16-50**		
	Serum creatinine	**0.6-1.2**		
	Serum uric acid	**4-5.8**		
LFT (mg/dl)	**Total bilirubin**	**0.2-1.2**		
	Direct bilirubin	**0.1-1.2**		
	Indirect bilirubin	**0.2-0.7**		
	SGOT	**0-40**		
	SGPT	**0-35**		
	Alkaline phosphatase	**80-290**		

Urine	**Albumin**	**Nil**		
	Sugar	**Nil**		
	Deposits	**Epi cells** **1-2 cells/hpf** **Pus cells** **1-2 cells/hpf**		
Motion	**Ova**	**Nil**		
	Cyst	**Nil**		
	Occult blood	**Nil**		

SPECIFIC EXAMINATION

	BEFORE TMT	**AFTER TMT**
1.CRP		
2.RAFactor		
3.Anti CCP		

RADIOLOGICAL EXAMINATION:

X-Ray –Of the affected joints

ECG

Date: Signature of the Doctor

PRECLINICAL AND CLINICAL EVALUATION OF SAFETY AND EFFICACY OF SIDDHA HERBOMINERAL FORMULATION NANDHI MEZHUGU IN THE TREATMENT OF UTHIRA VATHA SURONITHAM (*RHEUMATOID ARTHRITIS)*

FORM-IV

CONSENT FORM FOR PARTICIPATION IN A

CLINICAL TRIAL

I …….exercising my free power of choice, hereby give my consent to be included as a subject in the clinical trial of a drug namely **Nandhi mezhugu** for the treatment of Uthira vatha suronitham(Rheumatoid arthritis)**.** I understand that I may be treated with this drug for the disease. I am suffering from Uthiravatha suronitham (Rheumatoid arthritis)**.** which has been informed to my satisfaction by the attending physician and the purpose of the clinical trial, the nature of drug treatment and follow up including the laboratory investigations to monitor and safeguard my body functions. I have received, read, and understood the contents of the Information sheet, Dietary and General Advice Sheet.

I am also aware of my right to opt out of the trial at any time during the course of the trial without having to give the reasons for doing so. At the same time, I am also aware of my termination from the trial on the condition of my failure to co-operate with the investigators for the already explained conditions to me.

Date……. Thumb impression /Signature of the patient

Date…… Signature:

Date: Name of the patient:

Station: Signature of the Principal Investigator

II-ம் கட்ட மருத்துவ ஆய்விற்கு உட்படுவதற்கான ஒப்புதல் படிவம்

(CONSENT FROM – PHASE – II-Tamil Version)

..... .ஆகிய நான் என்னுடைய சுயதீனமான தேர்வு செய்யும் உரிமையின் படி உதிர வாத சுரோண¢தம் நோய்க்கு நந்தி மெழுகு மருந்தை எனக்கு கொடுத்து பரிசோதிக்க என் முழு மனதுடன் சம்மதம் தெரிவிக்கிறேன்.

நான் உதிர வாத சுரோணிதம் என்ற நோய் நிலையில் துன்பப்படுவதை மருத்துவர் விளக்க புரிந்து கொண்டேன். ஆய்வின் நோக்கம், சிகிச்சைக்குப் பின் மருத்துவர்கள் மேற்கொள்ளும் பின்தொடர்வு, ஆய்வுக்கூட பரிசோதனை விவரங்கள் எல்லாவற்றையும் விளக்கப் பெற்றேன். ஆய்வின் நடுவில் என் சொந்த விருப்பத்தின் பேரில் வெளியேற எனக்கு முழு உரிமை உண்டு என்பதையும் அறிந்து கொண்டேன். முதலில் விளக்கப்பட்ட ஆய்வு விதிமுறைகளுக்கு ஒத்துழைக்க மறுப்பின் ஆய்விலிருந்து வெளியேற்றப்படுவேன் என்பதையும் அறிந்து கொண்டேன். தகவல் படிவம், மருந்து, உணவு மற்றும் பொது அறிவுரை படிவங்களை பெற்று வாசித்து நன்கு விளங்கிக் கொண்டேன்.

Date:

Thumb Impression /Signature of Patient

Date:

Name of Patient:

Signature of Principal Investigator

PRECLINICAL AND CLINICAL EVALUATION OF SAFETY AND EFFICACY OF SIDDHA HERBOMINERAL FORMULATION NANDHI MEZHUGU IN THE TREATMENT OF UTHIRA VATHA SURONITHAM (*RHEUMATOID ARTHRITIS)*

FORM-V

WITHDRAWAL FORM

Name : OPD/IPD No:

Age: Reg. No:

Date of trial commencement:

Date of withdrawal from trial:

Reason(s) for withdrawal

1. Long absence at reporting: Yes/No

2. Irregular treatment: Yes/No

3. Shift of locality: Yes/No

4. Complication/Adverse reactions if any: Yes/No

5. Poor patient compliance: Yes/No

Date: Signature of the Principal Investigator

PRECLINICAL AND CLINICAL EVALUATION OF SAFETY AND EFFICACY OF SIDDHA HERBOMINERAL FORMULATION NANDHI MEZHUGU IN THE TREATMENT OF UTHIRA VATHA SURONITHAM (*RHEUMATOID ARTHRITIS)*

FORM-VI

ADVERSE REACTION FORM/PHARMACO VIGILANCE FORM

Serial No:

Name : OPD/IPD No:

Age/Gender : Reg.No:

Date of trial commencement:

Date of Adverse Reaction occur:

Date of withdrawal from trial:

Description of Adverse reaction:

Date: Signature of the Principal Investigator

PRECLINICAL AND CLINICAL EVALUATION OF SAFETY AND EFFICACY OF SIDDHA HERBOMINERAL FORMULATION NANDHI MEZHUGU IN THE TREATMENT OF UTHIRA VATHA SURONITHAM (*RHEUMATOID ARTHRITIS)*

Form-VII
DRUG COMPLIANCE FORM

S.no	Date	Drug issued	Not consumed
Day1			
Day8			
Day15			
Day22			
Day29			
Day36			
Day43			
Day50			
Day57and58			

Darkened space indicates intake of drug

Coloured space intake of drug

Signature of the Principal Investigator

PRECLINICAL AND CLINICAL EVALUATION OF SAFETY AND EFFICACY OF SIDDHA HERBOMINERAL FORMULATION NANDHI MEZHUGU IN THE TREATMENT OF UTHIRA VATHA SURONITHAM (*RHEUMATOID ARTHRITIS)*

FORM-VIII

(PATIENT INFORMATION SHEET)

தகவல் படிவம்

- உதிரவாத சுரோணிதம் என சித்த மருத்துவ நூல்களில் குறிப்பிடப்படும். இந்நோய் பொதுவாக மூட்டு வாதம் என்ற பெயரில் வழக்கத்தில் அழைக்கப்படுகிறது.இந்நோயின் குறிகுணங்கள் நவீன மருத்துவர்களால் Rheumatoid Arthritis என்றழைக்கப்படும் நோயின் குறிகுணங்களை ஒத்துள்ளது.
- இந்நோய் ஆண், பெண் என இரு பாலரையும் பாதித்தாலும், ஆண்களை விட பெண்களில் இந்நோய் அதிகமாக வருகின்றது. வயது வரம்பை பொறுத்தவரையில் 18 வயதிலிருந்து 60 வயது வரை உள்ளவர்களுக்கு இந்நோயின் பாதிப்பு அதிக அளவில் காணப்படுகிறது.
- இந்நோயானது நம் உடலின் நோய் எதிர்ப்புசக்தியானது நம் உடலுக்கு எதிராக எதிர்வினை புரிவதால் உண்டாகிறது என கண்டறியப்பட்டுள்ளது. இந்நோய் மற்றவர்களுக்குத் தொற்றாது. இந்நோயினால் பாதிக்கப்பட்ட நபருக்கு ஆரம்பநிலையில் சிறு சுரம், கை விரல் மூட்டுகள், கால் விரல் மூட்டுகள், கணுக்கால் மூட்டுகள், கழுத்தின் முள்ளந்தண்டு மூட்டுகள், இடுப்பின் முள்ளந்தண்டு மூட்டுகள் என உடலின் சிறிய மற்றும் பெரிய என்பு மூட்டுகளில் வலி, வீக்கம், கை கால்களை அசைக்கமுடியாமை முதலியன ஏற்படும்.
- நோய் முன்னேறுகின்ற நிலையில் வலி, வீக்கத்தால் கை கால்களை அசைக்காமல் வைத்திருக்கும் காரணத்தாலும், நோயி தாக்கத்தாலும், என்பு மூட்டுகள் கரடு கட்டி நிரந்தரமாக பயன்படுத்தமுடியாமல் நோயலியை முடங்கச் செய்வதால் இந்நோயினை மூட்டுவாதம் என்ற பெயரில் அழைக்கின்றனர்.

- இந்நோய்க்கு சித்த மருத்துவதில் “நந்தி மெழுகு” என்று அழைக்கப்படும் மருந்து சிறப்பாக காலங்காலமாக கொடுப்படுகிறது. இம்மருந்து அரசு தரச்சான்றிதல் பெற்ற மருந்து செய்கூடத்தில் தயாரிக்கப்பட்டு, பல்வேறு தரச் சோதனைகளுக்கு உட்படுத்தப்பட்டு, பயன்பாட்டிற்கு வந்துள்ளது.

மருந்துண்ணும் முறை:

1. நந்தி மெழுகு 500 மிகி அளவு 2 வேளை மட்டும் காலை, மாலை என(12 மணி நேர இடைவெளியில்) பனைவெல்லத்தில், உணவுக்குப் பின் உட்கொள்ள வேண்டும்.
2. 7 நாட்கள் இடைவிடாமல் இம்மருந்து கொடுக்கப்பட்டு 7 நாட்கள் மருந்தில்லா இடைவெளி விடப்படும். பின் மறுபடியும் 7 நாட்கள் இடைவிடாமல் இம்மருந்து கொடுக்கப்பட்டு 7 நாட்கள் மருந்தில்லா இடைவெளி விடப்படும். இம்மாதிரியாக இம்மருந்து மொத்தம் 60 நாட்கள் மருந்துண்ணும் காலத்திற்கு ஏற்ப கொடுக்கப்படும்.

மருந்துண்ணும் காலத்தில் கடைபிடிக்க வேண்டியவைகள்:

1. மருந்து உட்கொள்ளும் காலத்தில் 4 நாட்களுக்கு ஒரு முறை நெல்லிக்காய்த் தைலம் தேய்த்து தலைமுழுக வேண்டும்.
2. மருந்தில்லா இடைவெளி துவங்கும் நாளில் ஒமப்பால்(ஒமத்தை பாலி ஊறவைத்து, அரைத்து தலையில் தேய்த்து குளிக்க வேண்டும்) தேய்த்து தலை முழுக வேண்டும்.
3. எண்ணெய் தேய்த்து முழுகும் பொழுதும் மருந்துண்ணும் காலத்திலும் விலக்க வேண்டியவை

   காரமான உணவு வகைகள், இறைச்சி, மீன், கருவாடு, கோழி, தேங்காய், கொத்தவரை, எள், கொள்ளு, கடலை, மொச்சை, கீரைத்தண்டு, வெண்பூசணி, கடுகு, மாங்காய், பாகற்க்காய், அகத்திக்கீரை, எண்ணெய் பதார்த்தங்கள், கிழங்கு வகைகள், மஞ்சள் பூசணிக்காய், புளி, உப்பு ஆகியவற்றை தவிர்க்க வேண்டும்.
4. மருந்துண்ணும் காலத்திலும், மறு பத்திய காலத்திலும் புளியை அறவே நீக்க வேண்டும். மறுபத்திய காலத்தில்

வறுத்த கல்லுப்பு, கோடம்புளியை குறைந்த அளவு உணவில் சேர்த்து கொள்ள வேண்டும்.

5. மறுபத்திய காலத்தில் அதிக புளிப்பில்லாத மோர் அதிகம் அருந்தலாம். இளநீர், மாதுளம்பழம், பேரீச்சம் பழம் முதலிய பழ வகைகள், பச்சை காய்கறிகள் முதலியவற்றை அதிகம் சேர்த்து கொள்ள வேண்டும்.
6. மன உளைச்சல், கோபம் முதலியவற்றை தவிர்க்க வேண்டும்.
7. மருந்துவம் தொடங்குவதற்கு முன்னும், 60 வது நாளிலும் (மருந்துவம் முடியும் நாளிலும்) நோயாளி மருத்துவ ஆய்வுக்கூட பரிசோதனைகளுக்கும், ஒளி ஊடுகதிர் ஆய்வுக்கும் உட்படுத்தப்படுவர்.
8. ஒவ்வொரு முறையும் மருந்து உண்டபின் மருந்துண்ணும் ஒழுங்கு படிவத்தில் மருந்துண்ட தேதி, நேரம் ஆகியவற்றை குறித்து கொண்டு 15 நாட்களுக்கொரு முறை மருத்துவரிடம் கையொப்பம் பெற்றிட வேண்டும்.
9. 15 நாட்களுக்கொரு முறை மருத்துவரை அணுகி ஆய்வு மருந்தை பெற்றுக் கொள்ள வேண்டும்.
10. நோயாளிக்கு மருந்துகள் ஒவ்வாத சூழ்நிலையில் நோயாளி இடையில் சிகிச்சைக்கு விருப்பமின்றி போனாலும் மருத்துவ அறிவுரைகளுக்கு ஒத்துழைக்க மறுத்தாலும் அந்நோயாளி ஆய்விலிருந்து விடுவிக்கப்பட்டு அவரது விருப்பத்தின் பேரில் பொது வெளி நோயாளர் பிரிவில், தேசிய சித்த மருத்துவ நிறுவனத்தில் சிகிச்சைக்காக அனுப்பி வைக்கப்படுவார்.

PRECLINICAL AND CLINICAL EVALUATION OF SAFETY AND EFFICACY OF SIDDHA HERBOMINERAL FORMULATION NANDHI MEZHUGU IN THE TREATMENT OF UTHIRA VATHA SURONITHAM (*RHEUMATOID ARTHRITIS)*

FORM-IX

DIETARY & GENERAL ADVICE

உணவு மற்றும் பொது அறிவுரைகள்

கொள்ளக் கூடாதவை:

1. காய்கறிகள்:

சுரைக்காய் பாகற்காய் வாழைக்காய்

முள்ளங்கி கொத்தவரக்காய்

வெண்டைக்காய் வெள்ளரிக்காய்

சவ்சவ் மாங்காய்

மஞ்சள்பூசணி நாரத்தை

2. கிழங்கு வகைகள்: அனைத்து கிழங்கு வகைகளும்

3. கீரை வகைகள்: அகத்திக் கீரை, தண்டுக் கீரை

4. பழங்கள்:

மாம்பழம் அன்னாசி முலாம்பழம்

பலாப்பழம் ஆரஞ்சு

திராட்சை எலுமிச்சை

5. பயிறு வகைகள்:

மொச்சை வேர்கடலை

பட்டாணி கொண்டைக்கடலை

காராமணி கடலைபருப்பு

தட்டைப்பயிறு துவரம்பருப்பு

6. தானிய வகைகள்: பச்சை அரிசி, ராகி, கம்பு

7. மசாலா உணவு வகைகள்

8. புளி, உப்பு பதார்தங்கள், ஊறுகாய், வெள்ளைச்சீனி, இனிப்பு

பண்டங்கள், எண்ணெயில் பொரித்த பண்டங்கள்.

9. காபி, தேயிலை பானங்கள், குளிர்ந்த செயற்கை பானங்கள்

10. மாவு பதார்தங்கள்: அரிசி உணவு, கடலை மாவு, மைதா

11. ரவை, சேமியா

12. தயிர், குளிரூட்டப்பட்ட உணவுகள், பதப்படுத்தப்பட்ட உணவுகள்

13. குளிர்சாதன வசதிகள்(AIR CONDITION), குளிர்ந்த காற்று, குளிர்ந்த நீர்

14. அசைவ உணவுகள்: கடல்மீன், கருவாடு, கோழிக்கறி, ஆட்டுக்கறி.

15. மன உளைச்சல், கோபம் முதலியவற்றை தவிர்க்க வேண்டும்.

16. ஒய்வு எடுக்கவும்

கொள்ளக் கூடியவைகள்:

1. இந்துப்பு

2. கோடம்புளி

3. நாட்டுதக்காளி

4. காய்கறிபிஞ்சுகள்: கத்திரி, அவரை, முருங்கை etc

5. பழங்கள்: ஆப்பிள், மாதுளை, பப்பாளி, அத்தி, பேரீச்சு, வாழைப்பழம்-கற்பூரவள்ளி

6. முடக்கறுத்தான், சிறுகீரை, பொன்னாங்காணி, முருங்கை,

கீழ்க்காய்நெல்லி, மஞ்சள்கரிசாலை

7. பால்

8. மோர்

9. மசாலாப்பொருட்கள்: பெருங்காயம், சீரகம், மிளகு

10. தானியவகைகள்: புழுங்கல் அரிசி, கார் அரிசி, சிவப்பரிசி, கோதுமை,

11. பயிறு வகைகள்: சிறுபயிறு, தோல் உளுந்து

12. முளைகட்டிய சிறுபயிறு

13. தேன், கருப்பட்டி

14. எண்ணெய் குளியல் வாரத்திற்கு 2 முறை.

NATIONAL INSTITUTE OF SIDDHA

(An Autonomous Body under Department of AYUSH)
Ministry Of Health & Family Welfare, Government of India

Tambaram Sanatorium, Chennai - 600 047
Tel : 044-22411611 Fax : 044-22381314
E-mail : nischennaisiddha@yahoo.co.in
Website : www.nischennai.org

Dr.T.Lakshmikantham, M.D(S), Lecturer

DECISION

Opinion of the Institutional Ethics Committee – Please Check one
Approved Approval _____ Modifications required prior to approval (Please specify on space below) _____ Disapproval Date of review: 23-06-2011 Signed: K. Thamizhchelvi 23/6/11 (Please print name) Dr. K. MANICKAVASAKAN, Secretary / Dr. V. SUBRAMANIAN, Chairperson 25-Jun-2011 submission (Please delete as appropriate, Chairperson, Secretary)
Modifications needed Modification given to the Guide and Candidate
The research proponent is hereby informed that the Institutional Ethics Committee will require the following: 1) All adverse drug reactions (ADRs) that are both serious and unexpected to be reported promptly to the IEC within 7 working days. 2) The progress report to be submitted to the IEC at least annually 3) Upon completion of the study, a final study status report needs to submitted to the IEC.

(10)

CERTIFICATE

This is certify that the project title. Preclinical and clinical Evaluation of Safety and Efficacy of Siddha Herbo Mineral Formulation Nandhi Merzhugu in the treatment of Uthira Vatha Suronitham [Rheumatoid Arthritis] **has been approved by the IAEC.**

Prof. Dr. K. Manickavasakam, M.D(S), — **Name of Chairman/Member Secretary IAEC:**

Dr. B. Jayachandran Dare. — **Name of CPCSEA nominee:**

Signature with date

K. Manickavasakam 14/6/11 — **Chairman/Member Secretary of IAEC:**

CPCSEA nominee:

NATIONAL INSTITUTE OF SIDDHA CHENNAI - 600 047

(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by Office)

NATIONAL INSTITUTE OF SIDDHA

INSTITUTIONAL ANIMAL ETHICS COMMITTEE

CERTIFICATE

F. No:NIS/6-24/Res/IAEC Date:05.12.2013

Project Title: **"Scientific Evaluation of Safety and efficacy of a Sastric Siddha Herbomineral formulation – Nandhi Mezhugu in the treatment of Uthiravatha suronitham (Rheumatoid Arthritis)"**

Scholar name: Dr.T.Lakshmikantham,

Part time Ph.D Scholar,

National Institute of Siddha,

Chennai – 600 047

IAEC Reg Number: 1248/ac/09/CPCSEA dated . 21.05.2009

IAEC approved Number: 1248/AC/09/CPCSEA -9/Dec 2013/5

No of animals approved

Rats: 120

Mice:

Rabbits:

Guinea pigs:

Dr.K.Manickavasakam 05/12/13

Dr.P.Kumarasamy 05/12/13

Name of Chairman/Member Secretary IAEC:

Name of CPCSEA nominee:

Signature with date

Chairman/Member Secretary IAEC

CPCSEA nominee

NATIONAL INSTITUTE OF SIDDHA CHENNAI 600 047

சித்த மருத்துவ மைய ஆராய்ச்சி நிலையம், அரும்பாக்கம், சென்னை - 600 106
सिध्द केन्द्रीय अनुसंधान संस्थान, अरुम्बाक्कम, चेन्नई- 600 106
Siddha Central Research Institute
Arignar Anna Govt. Hospital Campus, Arumbakkam, Chennai-600 106
(Central Council for Research in Siddha, Department of AYUSH,
Ministry of Health & Family Welfare, Govt. of India)
Phone: 044-26214925, Tele Fax: 044-26214809, E.mail: crisiddha@gmail.com, Web: www.crisiddha.tn.nic.in

18.12.2013

Certificate

Certified that the market samples procured from "Indian Medical Practitioner's Co-operative Pharmacy & Stores, Chennai - 41 and submitted by **Dr. T. Lakshmikantham**, M.D(S), Lecturer / Research Scholar, Department of Maruthuvam, National Institute of Siddha, Tambaram Sanatorium, Chennai – 47 are identified / authenticated as below:

1.	Pooram	Mercurous chloride
2.	Kalnar Seemai	Magnesium calcium silicate
3.	Ganthagam	Sulphur
4.	Gowripashanam	Arsenic pentasulphide
5.	Kuthiraipal pashanam	Potassium Aluminium Silicate
6.	Vellaipashanam	Arsenic trioxide
7.	Padikaram	Aluminium Potassium Sulphate
8.	Thalagam	Arsenic trisulphide
9.	Lingam	Mercuric sulphide
10.	Rasa sindooram	Rasa sindooram (Prepared Medicine)
11.	Rasa Chendooram (Raw material)	Red Sulphide of mercury
12.	Ponnimilai	Copper pyrite
13.	Pooneeru	Fuller's earth
14.	Manosilai	Arsenic disulphide
15.	Palthutham	Zinc sulphate
16.	Mayilthutham	Copper sulphate
17.	Kalmatham	Hydrous Cobalt Arsenate
18.	Pachai karpooram	Borenol
19.	Nandukkal	Crab's fossil
20.	Kariuppu	Sodium Chloride
21.	Vediuppu	Potassium nitrate

22.	Karchunnam	Lime stone
23.	Korosanam	Gall stone of bull
24.	Kaadi neer	Acetic acid
25.	Rasam	Mercury
26.	Ten	*Apies mellifica* - Honey
27.	Ney	*Bos indicus* - Ghee
28.	Pasumpaal	*Bos indicus* - Cow's milk
29.	Pasu nir	*Bos indicus* - Cow's Urine
30.	Pasu saanam	*Bos indicus* - Cow's dung
31.	Mor	*Bos indicus* - Butter Milk

R. Shakila
Research Officer (Chemistry)

(Dr. S. Jega Jothi Pandian)
Research Officer (Scientist 2) - I/c

சித்த மருத்துவ மைய ஆராய்ச்சி நிலையம், அரும்பாக்கம், சென்னை - 600 106
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Phone: 044-26214925, Tele Fax: 044-26214809, E.mail: crisiddha@gmail.com, Web: www.crisiddha.tn.nic.in

18.12.2013

CERTIFICATE

Certified that the market samples supplied by the Indian Medical Practitioner's Cooperative Pharmacy & Stores, Chennai - 41 and submitted by Dr. J. Lakshmikantham, M.D(S), Lecturer/Research Scholar, Department of Maruthuvam, National Institute of Siddha, Tambaram Sanatorium, Chennai–47 are identified / authenticated as below:

1.	Akkirakaram	*Anacyclus pyrethrum* (L.) Lag. (Root)
2.	Nattu Amukkara	*Withania somnifera* (L.) Dunal (Root)
3.	Ativitayam	*Aconitum heterophyllum* Wall. ex Royle (Root)
4.	Avuri elai	*Indigofera tinctoria* L. (Leaf)
5.	Catamancil	*Nardostachys jatamansi* (D. Don) DC. Syn. *N. grandiflora* DC. (Rhizome)
6.	Catikkai	*Myristica fragrans* Houtt. (Kernel)
7.	Catipattiri	*Myristica fragrans* Houtt. (Aril)
8.	Cerankottai	*Semecarpus anacardium* L.f. (Fruit)
9.	Chevviyam	*Piper nigrum* L. (Root)
10.	Chukku	*Zingiber officinale* Rosc. (Rhizome)
11.	Cirakam	*Cuminum cyminum* L. (Fruit)
12.	Cirutekku	*Premna herbacea* Roxb. (Root)
13.	Cittarathai	*Alpinia officinarum* Hance (Rhizome)
14.	Elarisi	*Elettaria cardamomum* (L.) Maton (Seed)
15.	Elumicham pazham	*Citrus aurantifolia* (Christm.) Swingle (Fruit)
16.	Etti vittu	*Strychnos nux-vomica* L. (Seed)

17.	Ilavankam	*Syzygium aromaticum* (L.) Merr. & L.M. Perry Syn. *Eugenia caryophyllata* Thunb. (Flower bud)
18.	Kacakaca vitai	*Papaver somniferum* L. (Seed)
19.	Kattu milaku	*Piper attenuatum* Buch. –Ham. ex. Miq. – (Fruit)
20.	Katukkai	*Terminalia chebula* Retz. (Fruit)
21.	Kodiveli ver	*Plumbago zeylanica* L. – (Root)
22.	Kopparai tenkai	*Cocos nucifera* L. (Dried kernel)
23.	Kovai elai	*Coccinia grandis* (L.) Voigt (Leaf) Syn. *C. indica* W. & A.
24.	Kurocani omam	*Trachyspermum ammi* (L.) Sprague (Seed)
25.	Milaku	*Piper nigrum* L. (Fruit)
26.	Nellikkai	*Phyllanthus emblica* L. Syn. *Emblica officinalis* Gaertn. (Fruit)
27.	Nervalam	*Croton tiglium* L. (Seed)
28.	Pagal elai	*Momordica charantia* L. (Leaf)
29.	Perumcirakam	*Foeniculum vulgare* Mill. (Fruit)
30.	Sirukeerai	*Amaranthus tricolor* L. Syn. *A. tricolor var*. tristis (L.) Thell. (Whole plant)
31.	Talica pattiri	*Taxus wallichiana* Zucc. Syn. *T. baccata* L. (Leaf)
32.	Tanrikkai	*Terminalia bellirica* (Gaertn.) Roxb. (Fruit)
33.	Tippili	*Piper longum* L. (Fruit)
34.	Tippilik kattai	*Piper longum* L. (Stem)
35.	Vaividankam	*Embelia ribes* Burm.f. (Fruit)
36.	Vellaip puntu	*Allium sativum* L. (Bulb)
37.	Vetrilai	*Piper betle* L. (Leaf)

(R.Shakila)
Research Officer (Chemistry)

(Dr. S. Jega Jothi Pandian)
Research Officer (Scientist 2)-I/c

CERTIFICATE

THIS IS TO CERTIFY THAT THE PROJECT TITLED "ANTI-ARTHRITIC EFFICACY EVALUATION OF TEST ITEM AGAINST ADJUVANT INDUCED ARTHRITIS IN RATS "HAS BEEN APPROVED BY THE IAEC.

Name of Chairman/Member Secretary IAEC:

Dr. K. Y. MATHUR, M.V.Sc
Managing Director and Head
Laboratory Services
Liveon Biolabs Private Limited
Antharasanahalli, Tumkur,
Karnataka, INDIA

Name of CPCSEA nominee:

Dr. P. SARAVANAN
Senior Scientist
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Signature with date:

Chairman / Member Secretary of IAEC:

CPCSEA Nominee:

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CERTIFICATE

This is to certify that the project titled "EFFICACY EVALUATION OF A TEST ITEM AGAINST TO THE COTTON PELLET INDUCED GRANULOMA WISTAR RATS" has been approved by the IAEC.

Name of Chairman/Member Secretary IAEC:

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This is to certify that the project titled "VASCULAR PERMIABILITY TEST IN RATS" has been approved by the IAEC.

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Hebbal, BANGALORE 560 024.

144/239

Clinical Trial Details (PDF Generation Date :- Thu, 28 Jul 2016 04:13:53 GMT)

CTRI Number	Pending -
Last Modified On	
Post Graduate Thesis	No
Type of Trial	Interventional
Type of Study	Siddha
Study Design	Other
Public Title of Study	NANDHI MEZHUGU FOR RHEUMATOID ARTHRITIS
Scientific Title of Study	PRECLINICAL AND CLINICAL EVALUATION OF SAFETY AND EFFICACY OF SIDDHA HERBO-MINERAL FORMULATION- NANDHI MEZHUGU IN THE TREATMENT OF UTHIRA VATHA SURONITHAM (RHEUMATOID ARTHRITIS)

Secondary IDs if Any

Secondary ID	Identifier
NIL	NIL

Details of Principal Investigator or overall Trial Coordinator (multi-center study)

Details of Principal Investigator	
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Source of Monetary or Material Support

Source of Monetary or Material Support
> AYOTHIDOSS PANDITHAR HOSPITAL

Primary Sponsor

Primary Sponsor Details	
Name	AYOTHIDOSS PANDITHAR HOSPITAL
Address	NATIONAL INSTITUTE OF SIDDHA TAMBARAM SANATORIUM, CHENNAI-600047
Type of Sponsor	Research institution and hospital

Details of Secondary Sponsor

Name	Address
NIL	NIL

Countries of Recruitment

List of Countries
India

Sites of Study

Name of Principal Investigator	Name of Site	Site Address	Phone/Fax/Email
Dr T LAKSHMIKANTHAM	AYOTHIDOSS PANDITHAR HOSPITAL	NATIONAL INSTITUTE OF SIDDHA, TAMBARAM SANATORIUM, CHENNAI-600047. Kancheepuram TAMIL NADU	9444466880 drlakshmiramaswamy@gmail.com

Details of Ethics Committee

Name of Committee	Approval Status	Date of Approval	Is Independent Ethics Committee?
INSTITUTIONAL ETHIC COMMITTEE	Approved	23/03/2011	No

Regulatory Clearance Status from DCGI

Status	Date
Not Applicable	No Date Specified

Health Condition / Problems Studied

Health Type	Condition
Patients	UTHIRAVATHASURONITHAM(RHEUMATOID ARTHRITIS)

Intervention / Comparator Agent

Type	Name	Details
Intervention	NANDHI MEZHUGU	500mg with palm jaggery BD oral route 60 days.
Comparator Agent	nil	nil

Inclusion Criteria

Inclusion Criteria	
Age From	18.00 Year(s)
Age To	60.00 Year(s)
Gender	Both
Details	1.Pain and swelling in smaller and major joints. 2.Symmetrical joint involvement. 3.More than 3 joints involved. 4.Fever. 5.Morning stiffness. 6.Serum positive Anti CCP

Exclusion Criteria

Exclusion Criteria

Details	1.Diabetes mellitus. 2.Hypo & Hyperthyroidism. 3.Hypertension. 4.Cardiac disease. 5.Renal disease. 6.Liver disorder. 7.Neurological disorders. 8.Pregnancy and Lactation. 9.Alcoholism. 10.Recent treatment with steroids.

Method of Generating Random Sequence	Other
Method of Concealment	Case Record Numbers
Blinding/Masking	Open Label

Primary Outcome

Outcome	Timepoints
Reduction of pain and swelling of the joints,and improvement in the movement of the affected joints.	PRE STUDY SCREENING AND AFTER TREATMENT

Secondary Outcome

Outcome	Timepoints
1.Reduction of other clinical symptoms. 2.Reduction in Anti-CCP and RA factor. 3.Reduction of ESR and CRP	PRE STUDY SCREENING AND AFTER TREATMENT

Target Sample Size	**Total Sample Size=**40 **Sample Size from India=**40
Phase of Trial	Phase 2
Date of First Enrollment (India)	22/03/2015
Date of First Enrollment (Global)	No Date Specified
Estimated Duration of Trial	**Years=**1 **Months=**6 **Days=**0
Recruitment Status of Trial (Global)	Not Applicable
Recruitment Status of Trial (India)	Open to Recruitment
Publication Details	NONE
Brief Summary	The drug was prepared as per standard operating procedure at GMP certified pharmacy and the preclinical studies (safety and efficacy)of the trail drug were carried out as per OECD guidelines.It is a single Non -Randomised, Open clinical trail to determine the efficacy of the siddha drug NANDHI MEZHUGU [Prepared from herbo -mineral constituents] in patients with Uthiravathasuronitham[Rheumatoid arthritis].In this trail 40 patients are recruited and administrated with 500 mgs of internal medicine for 60 days[7 days medicine and 7 days drug holiday].During the trail period if any AE/SAE/SUSAR will be noticed then I will refer the patient to the Pharmacovigilance Department in National Institute of Siddha and further management will also be given in NIS OPD/IPD. The entire trail is monitored by the research monitoring committee of NIS. During this trail all safety and efficacy parameters will be recorded in the CRF. After completion of the trail all the study related data will be analysed statistically.The outcome of this trail will be published in Indian Journal of Medical Research.

URKUND

Urkund Analysis Result

Analysed Document: FINAL THESIS TLK 26-04-2017).doc (D27621393)
Submitted: 2017-04-27 12:22:00
Submitted By: drlakshmiramaswamy@gmail.com
Significance: 8 %

Sources included in the report:

Thesis full draft.docx (D27175036)
A Preclinical and Clinical Evaluation Of Vidathari Chooranam A Siddha Herbo Mineral Preparation On Kaanakadi Urticaria.docx (D26302983)
Medicinal Plants Al & AR activity 1.docx (D26263134)
Master Thesis Project 2014.doc (D13309515)
http://gnowfglins.com/2014/01/28/how-to-make-all-natural-mouthwash/
http://www.google.com/patents/US20060137702
http://www.herbsatwork.com/product-details/jiwadayad-tonechurna.html
http://ijcrims.com/pdfcopy/may2016/ijcrims6.pdf
http://ijarbs.com/pdfcopy/june2016/ijarbs14.pdf
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3249908/
http://www.ncbi.nlm.nih.gov/pubmed/16595901/

Instances where selected sources appear:

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Prof. Dr. G.GANAPATHY MD(S)
Ph.D Guide / Former Professor & H.O.D.
No.151/1, Golden Jubilee Flats,
Anna Nagar West, Chennai-600040.

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Review Article

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A REVIEW OF RHEUMATOID ARTHRITIS AND MEDICINES IN SIDDHA SYSTEM

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ABSTRACT

The Siddha system is a treasure trove of several medicines for innumerable diseases that need to be scientifically validated. The saint *Yugi* classified different types of neuro musculo skeletal diseases under 80 types of *Vatha* diseases in his text *YugiVaithiyacinthamani*. *UthiraVathasuronitham* is one among them. The signs and symptoms of *UthiraVathasuronitham* may be correlated with that of Rheumatoid Arthritis in modern science. This paper deals with single herb therapy and poly Siddha herbo-mineral formulations that have been mentioned in siddha literature and have been clinically well established. This paper establishes the effectiveness of Siddha system of medicine as the best choice for the treatment of RA. This will create awareness among the Rheumatology associations and researchers worldwide.

KEYWORDS: Siddha Medicine, Rheumatoid Arthritis, *Vatha* diseases, Herbs, Herbo-mineral formulations.

1. INTRODUCTION

Rheumatoid Arthritis is a chronic disease which will affect the normal person to live a normal healthy life, due to its worst complications.[1] RA affects nearly 1% of population worldwide due to its debilitating nature, in advance stages; the disease burden is considerable in economics and health expenditure terms. In developed countries 0.5-1% of adults are affected. Incidence of new cases ranges from 5-50 per 100,000 adults in developed countries.[2] Bone loss is one of the most harmful effects induced by chronic inflammation as well as the medication taken to rheumatoid arthritis, such as glucocorticoids. It is therefore important that, we gain the better understanding of, which medications used to treat patients with chronic inflammation are less likely to impact negatively on bone health. One study has shown that continuous treatment with prednisone at 10 mg /day during 90 days or more increase the risk of vertebral fractures 17- folds and hip fractures 7- folds. At this juncture the intervention with the siddha system of medicine gives relief from the chronic inflammation and to retain the bone density.[3] Since the treatment for RA is a long term one, the siddha medications are completely secure and safe without any adverse effects, unlike NSAIDs which is the medication used for temporary pain relief worldwide but after long term use they can be very dangerous, can damage liver, kidney and may led to fatalities. Siddha system of medicine does not cause any harmful effects and is very effective in limiting the pain but also improves mobility of the joints to achieve better and healthy life.

Siddha is more than a system of physical medicine, because it removes distress and diseases. In Siddha system, Siddhars classified the diseases on the basis of affected vital humors(Vali, azhal, Iyam), organs (eye and ear disease etc), *kosam*(systems like Gastro intestinal system, Respiratory system etc) in to 4448[4]. The saint *Yugi* classified different types of neuro musculo skeletal diseases under 80 types of *Vatha* diseases in his text *YugiVaithiyacinthamani.*[5] *UthiraVathasuronitham* is one among them. The signs and symptoms of *UthiraVathasuronitham* may be correlated with that of Rheumatoid Arthritis in modern science. This is the first system to emphasis health as the perfect state of Physical, Psychological, Social and Spiritual component of human being which was mainly needed for RA which is mainly caused by stress .Worldwide so many Rheumatology medical associations have been formed to find a new treatment and management strategies for RA.[1] This paper deals with single herb therapy and poly Siddha herbo-mineral formulations that have been mentioned in siddha literature and have been clinically well established.

2. RHEUMATOID ARTHRITIS DISEASE IN SIDDHA SYSTEM OF MEDICINE

Vatham was generated below the Abdomen and spread all over the body and responsible for the movements of the body. Saint *Yugi* in *Yugi VaidhiyaChinthamani* classified *Vatha diseases*(neuro muscular skeletal diseases) as 80 types. *VathaSuronitham* is the condition dealt under *vatha* disease .*Yugi* classified *VathaSuronitham* in to seven types[5]

- *Vathasuronitham*
- *UthiraVathasuronitham*
- *SithuVathasuronitham*
- *VaigithaVathasuronitham*
- *PaithiyaVathasuronitham*
- *SlethumaVathasuronitham*
- *UtharaVathasuronithamira*

Uthira Vatha suronitham is one type of *Vatha suronitham* . This disease is caused by imbalance of humors that is elevated *vatham* and *pitham.*

Clinical Features of *UthiraVathasuronitham*[5]

"*Vaigithamaaikkanaikkaalumuzhangalthaanu*
Markadanthsandthupuravadiyumveengich
Saeigithamaanjsiruviralgalmigavumnondhu
Sinthaithadumaariyaesalippundaagum
Paigithamaampaithiyaththilvathamminjip
Baaramaaiurpaviththuazhalundaagum
Uyikithamaaiasanamathuthaanumvaenda
Uthiravathasuronithathinunaarchchiyaamae"-Song No-319

- YugiVaithiyaChinthamani

Table 1: Correlation of *Uthiravathasuronitham* and Rheumatoid arthritis.[1][6]

S.No	Symptoms for UthiraVathasuronitham	Symptoms for Rheumatoid Arthritis
1.	"*Vaigithamaaik kanaikkaalu muzhangal thaanu Markadanth sandthu puravadiyum veengich*	Swelling of Ankle, Knee, and smaller joints of the Hand. Flexion of distal inter- phalangeal joints and Extension of Proximal inter phalangeal joints looks like Apes Hand-Swan neck Deformity.
2.	*Saeigithamaanj siruviralgal migavum nondhu*	Pain and tenderness of minor joints especially phalanges.
3.	*Sinthai thadumaariyae salip pundaagum*	Depression and Apathetic mood.
4.	*Paigithamaam paithiyaththil vatham minjip Baaramaaiurpaviththu*	Signs of Inflammation (Elevation of Pitham) pain and restricted movements of the joints (Elevation of vatham).
5.	*Azhalundaagum*	Fever
6.	*Uyikithamaai asanamathu thaanum vaenda*	Loss of appetite

3. SIDDHA MEDICINES FOR RHEUMATOID ARTHRITIS.

Herbo-Mineral Formulations for RA: In Siddha system there are several herbo mineral formulations mentioned for *Vatha* Disease. Some major formulations are given in the following Table.2

Table 2: Herbo mineral formulations for RA.

S.No	Name of the Formulations	Reference text	Page no /Song no
1.	*AadathodaiManappagu*	*Siddha Vaidhiya Thirattu* [7]	P.no:257
2.	*AttathiChooranam*	*Thanjai Vaidhiyarajachinthamani* [8] Part1	P.no:21-22
3.	*AyaChendooram*	*AgathiyarParipooranam-400* [9]	S.no:261-264
4.	*AyaveeraChendooram*	*Gunapaadam* Part 2 and 3 [10]	P.no:58
5.	*ElathiChooranam*	*Agathiyar Vaithiya Rathinasurukkam* [11]	S.no:149-150
6.	*GandhagaParpam*	*Anubhogavaithiyanavaneetha* [12] Part 6	P.no:28
7.	*GandhagaRasayanam*	*Pulipaani-500* [13]	S.no:324-330
8.	*GundhirigaThylam*	*Hospital Pharmacoepia* [14]	P.no:133
9.	*KaalamegaNaarayanaChendooram*	*Vaidiyasaarasangiragam* [15]	P.no:496-497
10.	*KorosanaiMathirai*	*Agathiyar Vaithiya Rathinasurukkam*[11]	S.no:149-150
11.	*KoushigarKulambu*	*Siddha vaidhiya Thirattu* [7]	P.no:204-213
12.	*LaguvisamushitiThylam*	*Therayar Thyla Vargasurukkam* [16]	S.no:79 P.no:101
13.	*LingaChendooram*	*Gunapaadam* Part 2 and 3 [10]	P.no:159
14.	*LingaPathangam*	*Therayar Karisal 300* [17]	S.no:2 P.no:8

15.	*MahavallathiLeghiyam*	*Boogar Vaithiyam 700* [18]	S no:175-187
16.	*MahaveeraMezhugu*	*Siddha VaithiyaThirattu* [7]	P.no:203-204
17.	*MayanaThylam*	*Therayar Thyla Vargasurukkam* [16]	S.no:79 P.No:130
18.	*MehanaathaKuligai*	*Siddha Vaithiya Thirattu* [7]	P.no:41
19.	*MerugulliThylam*	*Therayar Thyla Vargasurukkam* [16]	P.no:75
20.	*MoosambraPattru*	*Siddha Vaithiya Thirattu* [7]	P.no:305
21.	*MuthuChippiParpam*	*Siddha Vaithiya Thirattu* [7]	P.no:128
22.	*MuthuParpam*	*Therayar Maha Karisal* [19]	P.no:132-134 S.no:50
23.	*NandhiMezhugu*	*Siddha Vaithiya Thirattu* [7]	P.no:183-187
24.	*NavauppuMezhugu*	*Siddha Vaithiya Thirattu* [7]	P.no:193-194
25.	*PachaikarpooraMathirai*	*Siddha VaithiyaThirattu* [7]	P.no:30-31
26.	*PanchasoothaMezhugu*	*Yugi Karisal-151* [19]	S.no:16-24
27.	*ParangipattaiRasayanam*	*AgathiyarVaithiyaRathinasurukkam* [11]	S.no:114-118
28.	*PoorakKattu*	*Gunapaadam* Part 2 and 3 [10]	P.no:163
29.	*Rasa Mezhugu*	*Agathiyar Paripooranam-400* [9]	S.no:126-129
30.	*SeenthilChooranam*	*Agathiyar Paripooranam-400* [9]	S.no:324-325
31.	*SivanarAmirtham*	*Siddha VaithiyaThirattu* [7]	P.no:165-166
32.	*ThalagaParpam*	*Agathiyar Paripooranam-400* [9]	S.no:195-201
33.	*ThalisathiChooranam*	*Siddha VaithiyaThirattu* [7]	P.no:228-229
34.	*ThalisathiVadagam*	*TherayarPaadalThirattu* [21]	P.no:28
35.	*ThangaParpam*	*Gunapaadam* Part 2 and 3 [10]	P.no:109-110
36.	*ThettrankottaiLeghiyam*	*Agathiyar Paripooranam-400* [9]	S.no:281-282
37.	*UlogamandooraChendooram*	*TherayarYamagaVenbha* [22]	P.no:150
38.	*VaathakesariThylam*	*TherayarThylaVargasurukkam*[16]	S.no:9, P.No:48
39.	*VathaRakshanan*	*AgathiyarVaithiyaRathinasurukkam*[11]	S no:36-38
40.	*VelliChendooram*	*Gunapaadam* Part 2 and 3[10]	P.no:129-130
41.	*VelvangaChunnam*	*Gunapaadam* Part 2 and 3[10]	P.no:122
42.	*ViresanaPoobathi*	*BalaVaagadam*[23]	P.no:79-80

4. ANTI-*VATHA* HERBS

Drugs which are prevent the *Vatha* Diseases in the body mentioned in *Gunapadam* Part I *Mooligaivaguppu.*[24] Some important herbs which prevent *Vatha* diseases are mentioned in Table 3.

Table 3: Herbs Prevent Vatha Diseases.

S.No	Herbs	Botanical Name	Part Used
1.	*Aamanakku*	*Ricinus communis*, Linn.	Leaves,roots
2.	*Aanaipuli*	*Adansonia digitata*, Linn.	Leaves
3.	*Kadambu*	*Anthocephalus cadamba* Roxb	Seed
4.	*Chinni*	*Acalypha fruticosa*, Forsk	Root
5.	*Kattaamanakku*	*Jatropha curcus*, Linn.	Leaves,root
6.	*Chevvamanakku*	*Ricinus tanarius*, Linn.	Leaves,root
7.	*Thakkolam*	*Illicium veram*, Hook.f.	Seed
8.	*PirappanKizhangu*	*Calamus rotang* Linn.	Rhizome
9.	*Mizhagu*	*Piper nigram* Linn.	Seed
10.	*Musuttai*	*Rivea ornate* (Roxb)W.& A	WholePlant
11.	*Merugu*	*Alocasia indica*, Schott.	Rhizome
12.	*Maikonrai*	*Poinciana pulcherrima*, Linn.	Flower,Bark
13.	*VathaNaarayanan*	*Delonix elata*, (L) Gamble	Leaves
14.	*Vizhuthi*	*Cadaba trifoliate* (Roxb)W.& A.	Leaves and Fruit

5. CONCLUSION

For the painless better future of RA patients, Siddha system of medicine has opened a venue in the treatment with promising Siddha formulations and Single herb therapy consisting of *kayakalpa* drugs (Rejuvenators) there by promoting the health status of RA without any untoward adverse effects and ensure long life without any complication and disability. It is interesting to note that the siddha medicines are made from easily available sources such as plant, animal, marine, metal and mineral kingdoms. Siddha medicines are easily available, cost effective and efficacious. It is apt to adhere the siddha system of medicine for the long term treatment of RA. These medicines necessitate so many studies to evaluate the safety and efficacy. This paper establishes the effectiveness of Siddha system of medicine as the best choice for the treatment of RA. This will create awareness among the Rheumatology associations and researchers worldwide.

REFERENCE

1. S.P.Ramachandran,*YugiVaithiyacinthamani*-800, *Thamarai noolagam*,2nd edition, July 2013; Page No:122
2. Dr. M. Shanmugavelu, *NoiNadal Noimudhal nadal*, Department of Indian Medicine and Homeopathy, Part 2,3rd edition., 2003; 585.
3. Handout on Health: Rheumatoid Arthritis February 2016; National Institute of Arthritis and Musculoskeletal and skin diseases. Available at http://www.niams.nih.gov/health_info/rheumatic_disease/
4. Dr Ananya Mandal, MD. Rheumatoid Arthritis Epidemiology, November 2016; Available at http://www.news-medical.net/health/Rheumatoid-Arthritis-Epidemiology.aspx
5. Dr. Cristiano Zerbini MD, International Osteoporosis Foundation (IOF)**.** Biologic drugs may help prevent progressive bone loss in patients with RA, November, 2016; Available at http://www.news-medical.net/news/20161107/Biologic-drugs-may-help-prevent-progressive-bone-loss-in-patients-with-RA.aspx
6. T.V. Sambasivampillai Tamil and English Dictionary of Medicine, Chemistry, botany and allied sciences, Department of Indian Medicine and Homeopathy (volume I- First edition 1931.
7. Dr.K.N.Kuppusamymudhaliyar,Dr.K.S.Uththamaraayan, *Siddha Vaidhiya Thirattu*, Department of indian medicine and Homoeopathy, 1st edition, Reprinted 2009; Page no 305: 204-213, 128, 257, 165-166, 228-229, 187-193, 30-31, 203-204, 41, 193-194.
8. S.P.Ramachandran, *Thanjai Navarathina Vaidhiyaraja chinthamani*-800, A.Rangaswamy Mudhaliyar and Son's, part 1,page no:21-22
9. Agathiyar, *Agathiyar Paripooranam*-400, B. Rathina Nayakar and Son's Chennai-79, 2008; Song No:195-201, 261-264, 281-282, 351-352, 324-325, 126-129, Page No:37, 48, 63, 59, 51, 26.
10. Dr. R. Thiyagarajan, *Gunapaadam Thadhu jeeva vaguppu*, Deptarment of Indian Medicine and Homeopathy, Part 2 and 3 P.No:58
11. Agathiyar,*Agathiyar Vaithiya Rathina surukkam*, *Thamarai noolagam* Chennai-26, 2ndedition, Feb 1998; Song No:36-38, 55-57, 114-118, 149-150. Page No: 12-14, 18-19, 40-42, 53.
12. Hakeem Mohamed Abdhula Sayubu PM, *Anubhoga vaithiya navaneetham*, *Thamarai noolagam*, Chennai, Part 6, P.No:28
13. Pulipani, *Pulipaani vaithiyam*-500, B. Rathina Nayakar and Son's Chennai-79, 2008; Song No: 324-330, Page No:60-61
14. Anonymous, Hospital Pharmacoepia, Department of Indian Medicine and Homeopathy, Chennai-106, P.No:133.
15. Muthukaruppapillai, *Vaidiya saara sangiragam*, Madurai tamil sanga miththira salai,2nd edition, 1929; Page.No:496-497
16. Therayar, *Therayar Thyla Varga surukkam*, Baskara and Son's, C.T.Arumugam pillai, Chennai, 2nd edition, 1927; Song No:79, Page.No:101,128,87,48,
17. Therayar, *Therayar Karisal* 300, B.RathinaNayakar and Son's Chennai-79, Song No:2 Page.No:8.
18. Boogar, *Boogar Vaithiyam* 700,B.RathinaNayakar and Son's Chennai-79, 2012; Song No:175-187, Page.No:33-36
19. R. Thiyagarajan, A. Anandhan and G. Perumal, *Therayar Maha Karisal*, Department of Indian Medicine and Homeopathy, Song No:50, Page.No: 132-134.
20. Yugi, *Yugi Karisal*-151,Department of Indian Medicine and HomeopathySong No: 16-24, Page.No:4-7
21. S.S.Anandhan,R.Thiyagarajan, *Therayar Paadal Thirattu*, Department of Indian Medicine and Homeopathy,2nd edition, 1975; P.No:28
22. Therayar, *Therayar Yamaga Venbha*, Department of Indian Medicine and Homeopathy, Vol 3, P.No:150
23. Dr. K.S.Murugeshamudaliyar Dr. Pon Guru Siroonmani, *Bala Vaagadam*, Department of Indian Medicine and Homeopathy, 4th edition, 2007; P.No:79-80
24. Dr. Murugeshamudaliyar, *Gunapadam Mooligai vaguppu*, Department of Indian Medicine and Homeopathy Part I, Edition, page No:69,94,186,449, 285,498,678,760,766,785,397,801,824.

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Standardization of Nandhi Mezhugu, a poly herbomineral Siddha formulation

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ABSTRACT

Background: Standardisation is essential for scientific validation of any poly herbo mineral formulation. Nandhi mezhugu is a classical Siddha herbo mineral formulation has indication for many diseases such as all types of arthritis, male and female reproductive tract disorders, different types of cancers eg ovarian cancer, testicular cancer, cancer penis, cancer cervix, oral and cheek cancers, fistula, hydrocele, chronic ulcers, skin diseases eg eczema, leucoderma, diabetic carbuncle, chronic ulcers, Hanson's disease etc. **Aim**: The aim of this study was to standardise Nandi mezhugu. **Materials and Methods:** The drug was prepared as per the procedure mentioned in Siddha Literature and then subjected to the following analysis such as physico chemical, heavy metals, pesticide residue, aflatoxin, qualitative phytochemical, qualitative inorganic analysis, TLC photo documentation and HPTLC finger print profile. **Results and conclusion:** The drug was free of microbial contamination and aflatoxins and pesticide residues. Hence the drug was safe for consumption.

KEYWORDS: Serankottai, Etti kottai, Rasa chendooram, Nervalam, Nandukkal, Pooneeru.

1. INTRODUCTION:

Recent years have witnessed that, there is an exponential growth and demand in traditional medicine due to the new global trend of "Return to Nature". It has been estimated that eighty percent of the world population are using herbal and complementary medicines for their primary healthcare needs, which provides a new sphere of growth for traditional medicine[1].

The Siddha system of medicine is being time tested and still cater to the health needs of the society. For Global acceptance, this system of medicine has to undergo scientific validation through quality control measures of the medicinal raw drugs as well as standard operating procedures for preparing Siddha medicines. Nandi mezhugu is one such drug which is to be standardized. It is enlisted in Siddha Formulary of India, Part I. it is prescribed for various ailments[2][3]. Nandhi Melugu was subjected to the following analysis such as physico chemical, heavy metals, pesticide residue, aflatoxin, qualitative phytochemical, qualitative inorganic analysis, TLC photo documentation and HPTLC finger print profile as per WHO[4] and AYUSH Guidelines[5] .

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2. MATERIALS AND METHODS

2.1. Collection and Authentication of Raw Drugs

All the ingredients of Nandi Mezhugu were purchased from Indian Medical Practitioners Co-operative Pharmacy and Stores Limited Sales (IMPCOPS), Chennai-600041. The metals and mineral raw drugs were identified and authenticated in the chemistry lab; the herbal raw drugs were identified and authenticated by Dr.Sasikala Ethirajalu Research Officer-Scientist II (Pharmacognosy) in Siddha Central research Institute, Arumbakkam, Chennai-106.

2.2 . Preparation of the drug Nandhi Mezhugu

Ingredients:

Table 1: Ingredients of Nandhi mezhugu

Sl.No.	Ingredients	Source	Quantity
1.	Purified Marking Nut (Serankottai) *	*Semecarpus anacardium* L.f. (Fruit)	1Kg
2.	Purified Nux Vomica (Ettikkottai) seeds **	*Strychnos nux-vomica* L. (Seed)	315 gms
3.	Ghee (Nei)	*Bos indicus* – Ghee	1400 gms
4.	Common Alum (Padikaram) *	Aluminium Potassium Sulphate	1120 gms
5.	Palm Jaggery (Panai Vellam)	*Borassus flabellifer* L.(Palm Jaggery)	2240 gms
6.	Honey (Then)	*Apies mellifica* – Honey	1400 gms
7.	Ponnimilai parpam *	Calx of Copper pyrites	53 gms
8.	Kalnar Parpam *	Calx of Magnesium calcium silicate	53 gms
9.	Kalmatha Parpam***	Calx of Hydrous Cobalt Arsenate	53 gms
10.	Nandukkal Parpam *	Calx of Crab's fossil	53 gms
11.	Pachai karpooram*	Borneol	53 gms
12.	Kungumap poo**	*Crocus sativus* L. (Style & Stigma)	53 gms
13.	Gorochan (Korochanam)*	Gall stone of bull	53 gms
14.	Prepared Rasa sindooram	Rasa sindooram	140 gms
15.	Chukku***	*Zingiber officinale* Rosc. (Rhizome)	35 gms
16.	Milaku**	*Piper nigrum* L. (Fruit)	35 gms
17.	Tippili***	*Piper longum* L. (Fruit)	35 gms
18.	Elarisi**	*Elettaria cardamomum* (L.) Maton (Seed)	35 gms
19.	Beetle killer roots (Siruthekku)**	*Premna herbacea* Roxb. (Root)	35 gms
20.	Yew leaves (Thalisa Pathiri) **	*Taxus wallichiana* Zucc. Syn. *T. baccata* L. (Leaf)	35 gms
21.	Henbane niger (Kurosani Omam) **	*Trachyspermum ammi* (L.) Sprague (Seed)	35 gms
22.	Vaividankam**	*Embelia ribes* Burm.f. (Fruit)	35 gms
23.	Athividayam (Atis) **	*Aconitum heterophyllum* Wall. ex Royle (Root)	35 gms
24.	Jathikkai (Nutmeg)**	*Myristica fragrans* Houtt. (Kernel)	35 gms
25.	Jathipattri(Mace)**	*Myristica fragrans* Houtt. (Aril)	35 gms
26.	Karunjeeragam***	*Nigella sativa* L.(Seed)	35 gms
27.	Cirakam***	*Cuminum cyminum* L. (Fruit)	35 gms
28.	Ilavankam**	*Syzygium aromaticum* (L.) Merr. & L.M. Perry Syn. *Eugenia caryophyllata* Thunb. (Flower bud)	35 gms
29.	Chevviyam**	*Piper nigrum* L. (Root)	35 gms
30.	Kattu milaku**	*Piper attenuatum* Buch. –Ham. ex Miq. – (Fruit)	35 gms
31.	Kodiveli ver**	*Plumbago zeylanica* L. – (Root)	35 gms
32.	Tippilik kattai**	*Piper longum* L. (Stem)	35 gms
33.	Kacakaca	*Papaver somniferum* L. (Seed)	35 gms
34.	Perumcirakam**	*Foeniculum vulgare* Mill. (Fruit)	35 gms
35.	Nervalam*	*Croton tiglium* L. (Seed)	35 gms
36.	Cittarathai**	*Alpinia officinarum* Hance (Rhizome)	35 gms
37.	Catamancil**	*Nardostachys jatamansi* (D. Don) DC. Syn. *N. grandiflora* DC. (Rhizome)	35 gms
38.	Akkirakaram**	*Anacyclus pyrethrum* (L.) Lag. (Root)	35 gms
39.	Nattu Amukkara**	*Withania somnifera* (L.) Dunal (Root)	35 gms
40.	Rasa Chendooram (Raw material) *	Red Sulphide of mercury	25gms

Sl.No.	Ingredients	Source	Quantity
41.	Pooneeru *	Fuller's earth	25gms
42.	Mayilthutham*	Copper sulphate	25gms
43.	Palthutham **	Zinc sulphate	25gms
44.	Rasam***	Mercury	25gms
45.	Pooram*	Mercurous chloride	25gms
46.	Lingam*	Mercuric sulphide	25gms
47.	Manosilai*	Arsenic monosulphide	25gms
48.	Ganthagam*	Sulphur	25gms
49.	Thalagam*	Arsenic trisulphide	25gms
50.	Kuthiraipal pashanam***	Potassium Aluminium Silicate	25gms
51.	Vellaipashanam *	Arsenic trioxide	25gms
52.	Gowripashanam *	Arsenic pentasulphide	25gms
53.	Avuri elai	*Indigofera tinctoria* L. (Leaf)	Q.S

Drugs were purified as per the Siddha literatures * Siddha Materia Medica, **Sikitcha Rathina Deepam, ***Sarakku Suththi Muraigal[6],[7],[8].

Preparation:

(a) Fry items 1 & 2 in 3 ingredients .Ground 1 & 2 into a fine paste.
(b) Ground 4, 14-39 in to fine powder separately.

(c) Ground 40-52 in to a paste with juice of drug no 53 for 6 hours and were made cakes and dried. Covered the cakes with the paste of drug no 53 and subjected to calcination process, then the product was taken and powdered.

(d) Syrup of palm jaggery was prepared and the ingredients (a),(b) and (c) and the calxes were added, after that ghee and honey were added. To this mixture powders of 11, 12, 13 were added, thoroughly mixed and stored in Air tight Container.[2],[3]

2.3. Organoleptic properties

The organoleptic characters such as colour, odour, taste, consistency were observed.

2.4. Physico Chemical Parameters:

Loss on drying at 105°C, total ash, water soluble ash, acid-insoluble ash, water soluble extractive, alcohol soluble extractive, rancidity, acid value, saponification value, iodine value, pH, total solid, fat content , reducing sugar, total sugar were carried out as per the procedures mentioned in standard references (WHO, Protocol for testing)[4][5][9]

2.5 Assays:

Quantitative assays for Calcium, Magnesium, Potassium, Aluminium, Copper, Iron and Zinc, were observed in ICP-OES using standards. Sulphur (as SO_2) was estimated by following AOAC 990.28 method and Chloride (as NaCl) was calculated by following AOAC 950.52 method.

2.6 . Qualitative Phytochemical Analysis:

Various tests for different types of secondary metabolites, viz., Steroids, terpinoids, alkaloids, flavanoids, etc were carried out as per the procedures quoted in standard organic book. (Ref Harborne)[10]

2.7. Qualitative Inorganic Analysis:

Qualitative test for various cations and anions were carried out as per the methods mentioned in standard practical guide. (Ref : Feigl)[11]

2.8 . Heavy metal Analysis:

Tests for heavy metals, viz., lead, cadmium, arsenic and mercury were carried out in ICP-OES instrument (Perkin Elmer Optima 3000 DV).

2.9. Microbial contamination:

Tests for total bacterial /fungal counts *E. coli, Salmonella* spp., *Staphylococcus aureus* and *Entero bacteriacea* were done. [9]

2.10. Pesticide residues:

Various pesticide residues of organo chlorine and organo phosphorous viz., alphaBHC, betaBHC, gam BHC(Lindane), deltaBHC, Aldrin, Dieldrin, trans Chlordane, cis Chlordane, Endrin, Endrinaldehyde, Endrinketone, Endosulfan-I, Endosulfan-II, Endosulfansulfate, Heptachlor, Heptachlorepoxide, Dicofol, Chlorthalonil, Hexachlorobenzene, o,p"DDT, P,P"DDT, o,p"DDD, p,p"DDD, o,p"DDE, P,P"DDE, 4-Bromo,2-Chlorophenol, Acephate, Chlorfenvinphos, Chlorpyrifos, Chlorpyrifos methyl, Diazinon, Dichlorvos, Dimethoate, Ethion, Etrimfos, Fenitrothion, Iprobenphos, Malathion, Methamidophos, Monocrotophos, Omethoate, Oxydemeton-methyl, Parathion ethyl, Parathion methyl, Phorate,

Phosalone, Phosphamidon, Profenophos, Quinalphos, Triazophos, Phorate sulphone , Phorate sulphoxide were checked by following AOAC 2007.01 methods.

2.11 . Tests for Aflatoxins:

Aflatoxins such as B1,B2,G1and G2 were checked using AOAC 2008.02 methods.

2.12 TLC Photodocumentation[12]/HPTLC Finger print profiling[13]

Sample preparation

Four gm of the drug was extracted successively by hexane, chloroform and ethanol using Soxhlet apparatus. The extracts were filtered freed from solvents and made up to 10 ml in standard flasks using the respective solvents.

TLC plate

Aluminium plate precoated with silica gel $60F_{254}$ of 0.2 mm thickness (Merck) was used for the TLC/HPTLC analysis.

Developing chamber

Camag's twin trough chamber was used for the development.

Solvent system

Many solvent systems were tried for a better separation and the same was achieved in Toluene : Ethyl acetate (10 : 0.5, v/v) for hexane extract; Toluene : Ethyl acetate (5:1.5, v/v) for chloroform extract and ethanol extract.

Derivatization reagent

For derivatization vanillin-sulphuric acid reagent was used (1 gram vanillin dissolved in a mixture of ethanol and sulphuric acid with the composition 95 ml : 5 ml).

Instrument

Linomat 5 automatic applicator, CAMAG's visualizer, CAMAG's scanner 030618 attached with WINCATS software were the instruments used for photo documentation and HPTLC finger printing. CAMAG's plate heater was used for derivatization.

Procedure

5 µl, 10 µl and 15 µl of the hexane, chloroform and ethanol extracts were applied on three different plates as 10 mm bands with 8 mm distance in between and developed up to 8 cm in the above mentioned solvent systems. The air dried developed plates were visualized under UV 254 and 366 nm for documenting TLC chromatograms. The plates were scanned in UV 254 nm (all extracts) & 366 nm (hexane and chloroform) and the finger print profiles were recorded. Then the plates were dipped in vanillin-sulphuric acid reagent and heated in an oven at 105ÚC until the development of colored spots. TLC photo documentation in white light after derivatization were recorded and finger print profiles at 575 nm (hexane and chloroform) were also recorded.

3. RESULTS AND DISCUSSION:

3.1. Organoleptic properties

Colour: Dark brown colour; **Odour:** Resinous odour; **Taste:** Metallic taste; **Consistency**: Semisolid.

3.2. Physicochemical Properties

All the results of physico chemical parameters are presented in table 2. The loss on drying was observed as 19.156% and fat content was observed as 20.683%. It is understood that the high value of loss on drying due to the fat content. The total ash was calculated as 6.607% which indicates the content of total inorganics. The water soluble ash value of 2.95 % shows the content of water soluble inorganic salts like sodium chloride, etc. The acid insoluble ash value was calculated as 0.93%. The water soluble and alcohol soluble extractives were estimated as 39.056 % and 23.558 % respectively indicating the presence of high polar secondary metabolites like glycosides, sugars, tannins, saponins, alkaloids, etc. The calculated acid value, saponification value and iodine value were indicative of purity of the ghee used for the preparation and showing the number of milligrams of free acids and saponifiable acid and number of unsaturation in the drug. Though the reducing sugar (3.69 %) and total sugar (7.54%) values are indicative of promoting the growth of organisms, the drug was free from rancidity and the pH value (10 % solution) of 3.35 indicates the acidic nature of the drug. Hence the susceptibility of microbial growth due to presence of sugar may be decreased by the acidity and the shelf life of the drug would be increased.

Table 2. Physicochemical results of Nandhi mezhugu

Sl.No	Parameter	Mean (n=2)
2	Loss on Drying at 105° C	19.16%
3	Total Ash	6.61%
4	Water soluble ash	2.95%
5	Acid-insoluble ash	0.93%
6	Water soluble extractive	39.06%
7	Alcohol soluble extractive	23.56%
8	Rancidity	Nil
9	Acid value	10.592
10	Saponification value	262.62
11	Iodine value	16.864
12	Ph	3.35
13	Total solid	80.84%
14	Fat content	20.68%
15	Reducing Sugar	3.69%
16	Total Sugar	7.54%

3.3. Qualitative Phytochemical Analysis

The extract of Nandi mezhugu was subjected to various phytochemical tests as per the standard procedure (Ref. Harborne book). All the tested secondary metabolites were present in the drug which would improve the therapeutic efficacy of the drug.

3.4. Qualitative Inorganic Analysis

The qualitative inorganic analysis of the drug revealed the presence of mercury, magnesium, aluminium, calcium, sodium, potassium, copper, zinc, iron, cobalt, chloride, carbonate, nitrate, sulphate, sulphide, arsenate, acetate, silicate and arsenate which are all biologically important radicles.

3.5. Heavy metal analysis

The content of lead and cadmium are within the admissible limit of WHO standards. While the content of arsenic and mercury are high due to the reasons they are added in the drug in the form of rasam, lingam, pooram, thalagam, manosilai, vellai pashanam, gowri pashanam, kalmatham and rasa chenduram/sinduram. But they are not present in the elemental form and hence non toxic. In continuation of the standardisation of trail drug we had studied the safety of the trail drug as per OECD guidelines(acute, sub-acute sub-chronic toxicity studies) showed non-toxic effect in rodents(unpublished). The result (table 3) from the safety study provided was encouraging and opened a venue in the management of auto immune disease like Rheumatoid arthritis that needs long term treatment with Nandhi mezhugu. Physico-chemical forms of heavy metals in the indigenous medicine is totally different from the known Physico-chemical forms of that metal.[14]

Table 3. Heavy metals present in Nandhi mezhugu

Heavy metal (in ppm)	Quantity (in ppm)	WHO limit
Lead (as Pb)	2.95	10
Arsenic (as As)	7233.42	3
Cadmium (as Cd)	0.01	0.3
Mercury (as Hg)	9336.61	1

3.6 . Microbial contamination

In the microbial study, the drug was found free from *E. coli, Salmonella* spp., *Staphlococcus aureus* and *Enterobacteriacea*. The results are shown in the table 4. The total bacterial count and the total fungal count were within the permissible limits of WHO standards. Hence the drug is safe for consumption.

Table 4. Microbial contamination results of Nandhi mezhugu

S. No	Parameter	Value	WHO Limit (CFU/g)
1.	*E. coli*	Absent	10
2.	*Salmonella* spp.	Absent	None
3.	*Staphylococcus aureus*	Absent	Absent
4.	*Enterobacteriacea*	Absent	103
5.	Total Bacterial count	2 x 10^3	105
6.	Total Fungal count	Less than 10	103

3.7 Pesticide residue

All the tested organochlorine pesticides organophosphorus pesticides were found to be lower than the limit of quantification, ie., 0.01 ppm and hence safe as internal medicine.

3.8 Assays

Presence of calcium was detected which may due to added nandukkal in the drug; presence of potassium and aluminium may be due to padikaram, kuthiraipal padanam, vediuppu. Occurrence of copper is due to mayilthutham, ponnimilai; presence of iron and zinc is due to added of palm jaggery, palthutham respectively. Sulphur and chloride occurrence is due to added of lingam, thalagam, gowripasanam, gandhagam, padigaram, rasa chendooram, mayilthutham, palthutham and pooram, kariuppu respectively (table 5).

Table 5. Assays results of Nandhi mezhugu

Calcium (as Ca)	3165.72	mg/kg
Magnesium(as Mg)	358.72	mg/kg
Potassium (as K)	1950.25	mg/kg
Aluminum (as Al)	4943.37	mg/kg
Copper (as Cu)	501.35	mg/kg
Iron (as Fe)	497.3	mg/kg
Zinc (as Zn)	26.29	mg/kg
Sulphur (as SO_2)	BLQ (LOQ : 10.0)	mg/kg
Chloride (as NaCl)	0.03	g/100g

3.9 . Test for Aflatoxins (B1,B2,G1,G2)

All the four aflatoxin were not detected in the drug. As the total fungal count was within the permissible limit, txins were not promoted in the drug and is free from these aflatoxins.

3.10. TLC/HPTLC

The TLC photodocumentation of hexane extract of Nandi mezhugu under UV 254 nm showed 5 visible spots at R_f value 0.25, 0.30, 0.38, 0.50 and 0.71 (all green); under UV 366 nm showed three visible spots at R_f value 0.30 (blue), 0.38 (fluorescent blue) and 0.71 (pale

blue). After derivatization with vanillin-sulphuric acid, showed 8 spots at 0.20, 0.25, 0.30, 0.35 (all purple), 0.38 (brown), 0.46, 0.57 and 0.71 (all purple) (table 6, fig 1 and 2-10).

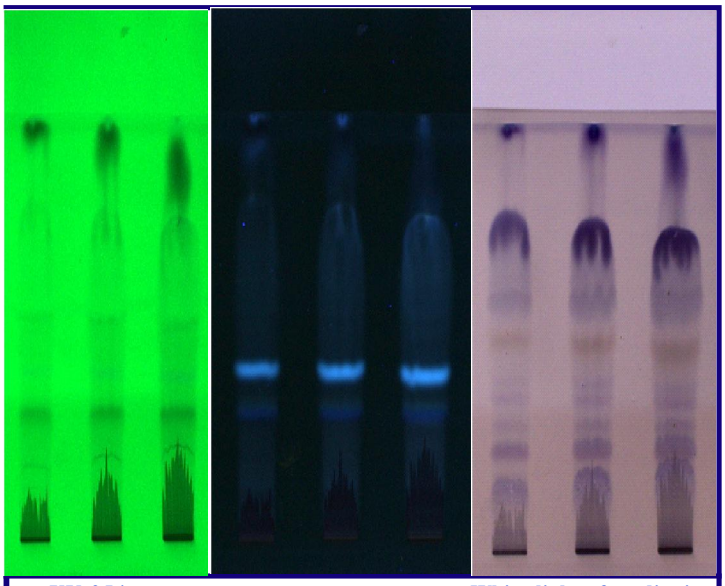

Fig.1.TLC photo documentation of hexane extract of Nandi mezhugu

Table 6. TLC results of Nandhi mezhugu

Under UV 254 nm		Under UV 366 nm		White light after derivatization	
R_f	Colour	R_f	Colour	R_f	Colour
-		-	-	0.2	Purple
0.25	All green	-	-	0.25	Purple
0.3		0.3	Blue	0.3	Purple
0.38		-	-	0.35	Purple
-		0.38	Fluorescent blue	0.38	Brown
0.5		-	-	0.46	Purple
-		-	-	0.57	Purple
0.71		0.71	Pale blue	0.71	Purple

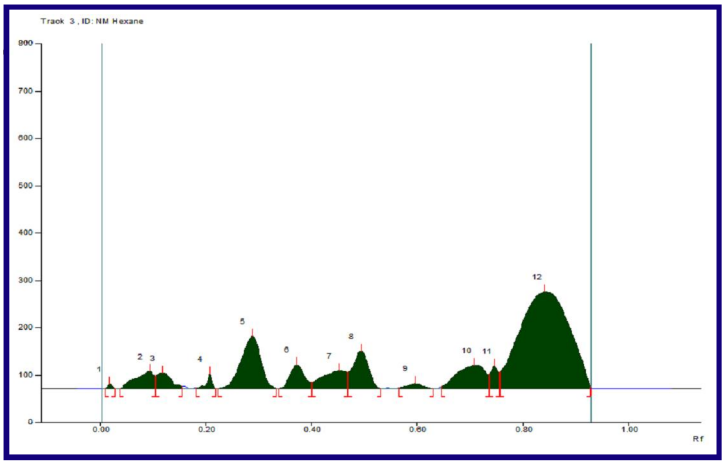

Fig. 2. HPTLC finger print profile of hexane extract of Nandi mezhugu at 254 nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	0.6 AU	0.02 Rf	10.4 AU	1.48 %	0.03 Rf	0.4 AU	76.3 AU	0.26 %
2	0.04 Rf	0.1 AU	0.10 Rf	36.7 AU	5.20 %	0.11 Rf	27.3 AU	1071.6 AU	3.65 %
3	0.11 Rf	27.7 AU	0.12 Rf	33.8 AU	4.78 %	0.16 Rf	5.4 AU	781.4 AU	2.66 %
4	0.18 Rf	1.7 AU	0.21 Rf	30.7 AU	4.34 %	0.22 Rf	0.1 AU	263.5 AU	0.90 %
5	0.22 Rf	0.3 AU	0.29 Rf	111.4 AU	15.77 %	0.33 Rf	0.2 AU	3421.0 AU	11.66 %
6	0.34 Rf	0.4 AU	0.37 Rf	50.5 AU	7.15 %	0.40 Rf	12.8 AU	1146.2 AU	3.90 %
7	0.40 Rf	13.0 AU	0.45 Rf	38.7 AU	5.48 %	0.47 Rf	35.1 AU	1443.4 AU	4.92 %
8	0.47 Rf	35.2 AU	0.50 Rf	80.1 AU	11.34 %	0.53 Rf	0.4 AU	1964.0 AU	6.69 %
9	0.57 Rf	1.3 AU	0.60 Rf	10.8 AU	1.52 %	0.63 Rf	0.1 AU	264.7 AU	0.90 %
10	0.65 Rf	4.2 AU	0.71 Rf	50.4 AU	7.13 %	0.74 Rf	29.5 AU	2243.2 AU	7.64 %
11	0.74 Rf	30.4 AU	0.75 Rf	48.5 AU	6.87 %	0.76 Rf	34.3 AU	562.2 AU	1.92 %
12	0.76 Rf	34.9 AU	0.84 Rf	204.6 AU	28.95 %	0.93 Rf	4.7 AU	16115.0 AU	54.90 %

Fig. 3. R_f value of peaks with percentage peak area of HPTLC finger print profile of hexane extract of Nandi mezhugu at 254 nm

The HPTLC finger print profile of hexane extract at UV 254 nm showed 12 peaks in which the peak at R_f 0.84 was the major peak with an area of 54.90 % followed by a peak at R_f 0.29 with an area of 11.66 %. All other peaks are minor with an individual area less than 10 %.

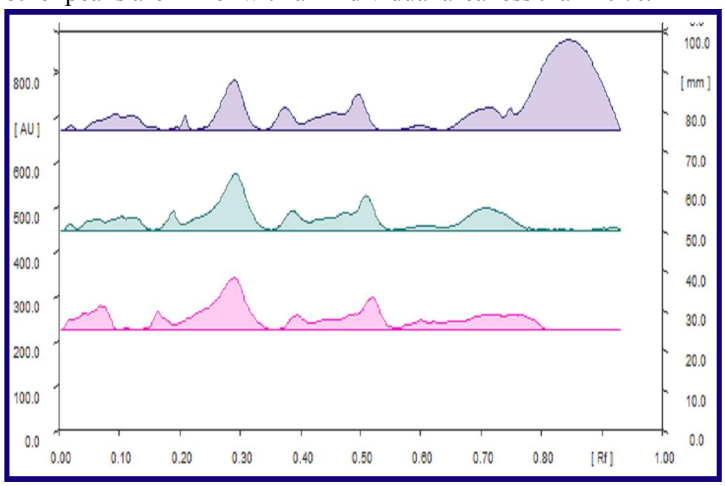

Fig. 4. 3D chromatogram of hexane extract of Nandi mezhugu at 254 nm

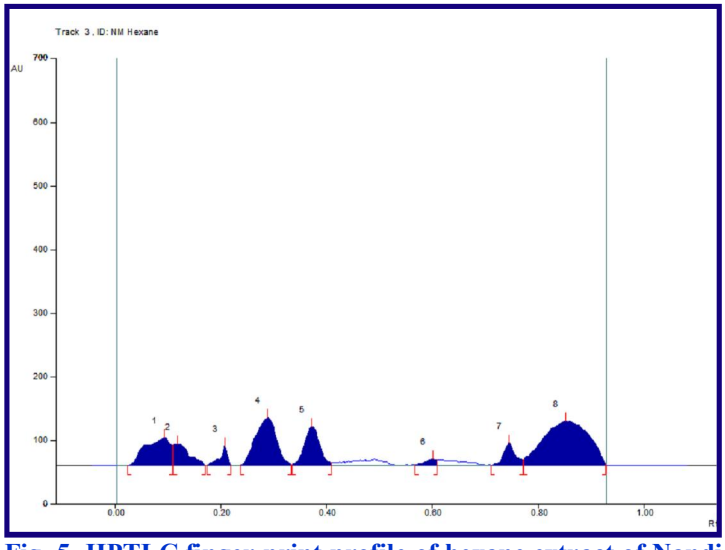

Fig. 5. HPTLC finger print profile of hexane extract of Nandi mezhugu at 366 nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.03 Rf	0.3 AU	0.09 Rf	43.8 AU	12.15 %	0.11 Rf	31.7 AU	1707.3 AU	13.40 %
2	0.11 Rf	31.8 AU	0.12 Rf	34.0 AU	9.43 %	0.17 Rf	0.4 AU	859.7 AU	6.75 %
3	0.17 Rf	0.3 AU	0.21 Rf	31.1 AU	8.62 %	0.22 Rf	0.1 AU	358.4 AU	2.81 %
4	0.24 Rf	0.7 AU	0.29 Rf	75.1 AU	20.84 %	0.33 Rf	1.9 AU	2476.5 AU	19.43 %
5	0.34 Rf	2.0 AU	0.37 Rf	61.3 AU	17.01 %	0.41 Rf	4.3 AU	1477.3 AU	11.59 %
6	0.57 Rf	0.8 AU	0.60 Rf	10.3 AU	2.87 %	0.61 Rf	8.6 AU	177.1 AU	1.39 %
7	0.71 Rf	0.5 AU	0.75 Rf	35.1 AU	9.74 %	0.77 Rf	8.5 AU	644.6 AU	5.06 %
8	0.77 Rf	8.9 AU	0.85 Rf	69.7 AU	19.34 %	0.93 Rf	2.4 AU	5043.3 AU	39.57 %

Fig. 6. R_f value of peaks with percentage peak area of HPTLC finger print profile of hexane extract of Nandi mezhugu at 366 nm.

The HPTLC finger print profile of hexane extract at UV 366 nm showed 8 peaks in which the peak at R_f 0.85 was the major peak with an area of 39.57 % followed by a peak at R_f 0.29 (19.43 %), 0.09 (13.40 %) and 0.37 (11.59 %). All other peaks are minor with an individual area less than 10 %.

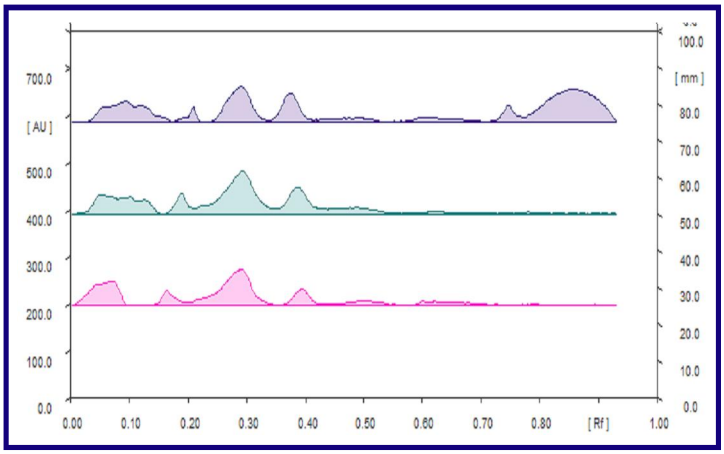

Fig. 7. 3D chromatogram of hexane extract of Nandi mezhugu at 366 nm

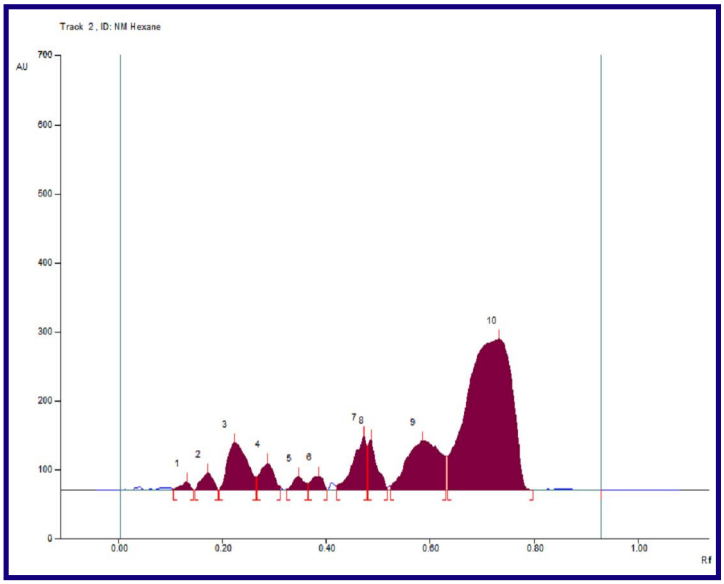

Fig. 8. HPTLC finger print profile of hexane extract of Nandi mezhugu at 575 nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.11 Rf	0.9 AU	0.13 Rf	11.1 AU	1.79 %	0.15 Rf	0.5 AU	166.2 AU	0.63 %
2	0.15 Rf	0.0 AU	0.17 Rf	25.1 AU	4.03 %	0.19 Rf	0.5 AU	466.4 AU	1.76 %
3	0.20 Rf	0.5 AU	0.22 Rf	68.0 AU	10.94 %	0.27 Rf	18.9 AU	2126.5 AU	8.02 %
4	0.27 Rf	19.2 AU	0.29 Rf	38.6 AU	6.21 %	0.31 Rf	5.8 AU	844.5 AU	3.18 %
5	0.32 Rf	1.3 AU	0.35 Rf	19.0 AU	3.05 %	0.37 Rf	9.4 AU	342.0 AU	1.29 %
6	0.37 Rf	9.4 AU	0.39 Rf	19.6 AU	3.14 %	0.40 Rf	0.3 AU	388.5 AU	1.47 %
7	0.42 Rf	5.9 AU	0.47 Rf	77.9 AU	12.52 %	0.48 Rf	63.7 AU	1656.2 AU	6.25 %
8	0.48 Rf	64.4 AU	0.49 Rf	73.5 AU	11.81 %	0.52 Rf	3.3 AU	1030.4 AU	3.89 %
9	0.52 Rf	5.8 AU	0.59 Rf	71.3 AU	11.46 %	0.63 Rf	49.2 AU	3806.7 AU	14.35 %
10	0.63 Rf	49.4 AU	0.73 Rf	218.1 AU	35.05 %	0.80 Rf	0.0 AU	15692.3 AU	59.17 %

Fig. 9. R_f value of peaks with percentage peak area of HPTLC finger print profile of hexane extract of Nandi mezhugu at 575 nm

The HPTLC finger print profile of hexane extract at 575 nm showed 10 peaks in which the peak at R_f 0.73 was the major peak with an area of 59.17 % followed by a peak at R_f 0.59 (14.35 %), 0.22 (8.02 %) and 0.47 (6.25 %). All other peaks are minor with an individual area less than 10 %.

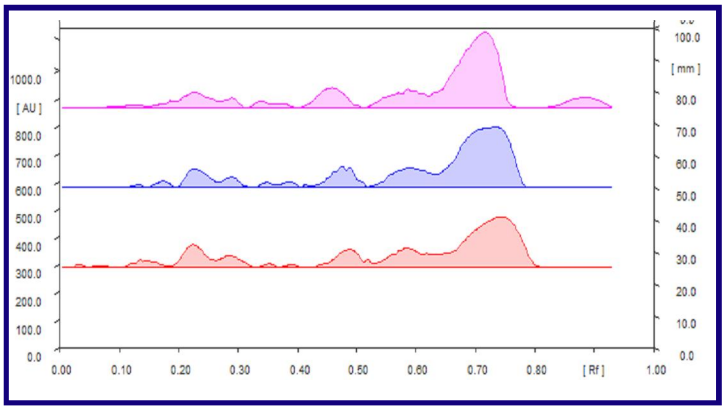

Fig. 10. 3D chromatogram of hexane extract of Nandi mezhugu at 575 nm

4. CONCLUSION:

Based on the above results, it is known that the drug Nandhi mezhugu has validated the traditional claim. The result from the physico chemical and safety study is encouraging and pave the way in the management of auto immune disease like Rheumatoid arthritis .

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REFERENCES:

1. Anonymous. Development of Standard Siddha Terminologies. National Institute of Siddha.Chennai.2014; 1; 1.

2. Kuppusamy mudhaliyar KN, Uththamaraayan KS. Siddha Vaidhiya Thirattu. Department of indian medicine and Homoeopathy.Chennai. 2009; 1; 183-189.
3. Anonymous .The Siddha Formulary of India. Department of ISM & H, Ministry of Health & Family Welfare, Government of India, New Delhi. 1992;1:70-71.
4. Organization Mondiale De La Sante, Quality control methods for medicinal plants materials,559, rev. 1, Original English, World Health Organization. 1992; 159.
5. Lohar DR. Pharmacopoeial laboratory for Indian medicine.Department of Ayurvedha, yoga and Naturopathy, Siddha, Unani and Homoeopathy (AYUSH), Ministry of health and family welfare.New delhi. 2011; 20.
6. Thiyagarajan R, Siddha Materia Medica(mineral and animal kingdom). Department of Indian medicine and Homoeopathy.Chennai. 2008;2; 235, 407, 252, 266, 295, 326, 272, 223, 221, 424, 351, 286, 419, 342, 422, 32, 461.
7. Kannusamyam pillai S. Sikitcha Rathina Deepam. B.Rathinanayakar and Son's.Chennai. 2007;2; 38,32,28,29, 31,34
8. Sarakku Suththi Muraigal. Department of Indian medicine and Homoeopathy. Chennai. 2008;1;48,6,7.
9. Anonymous .Quality Control Methods for Medicinal Plant Materials. World Health Organisation, Geneva, Switzerland.1998.
10. Harborne JB .Phytochemical Methods. Chapman and Hall, 1973. Wagner H, Bladt S (editors). Plant Drug Analysis. Springer, Berlin, London. 1996 ;2.
11. Feigl F. Anger V. Spot tests in inorganic analysis. Elsevier: Amsterdom, 1972;2.
12. Stahl I .Thin layer chromatography, A Laboratory Hand Book (student edition). Springer-Verlag, Berlin, Germany. 1969 ;52-86.
13. Sethi PD. High Performance Thin Layer Chromatography .Vol. X. CBS Publishers and Distributors, New Delhi, India.1996;1;1-56.
14. Gajendra kumar Yogendra kumar gupta.Evidence for safety of Ayurvedic herbal, herbo-metallic and Bhasma preparations on neurobehavioral activity and oxidative stress in rats .October 2012.Available at https://www.researchgate.net/publication/236978234_Evidence_for_safety_of_Ayurvedic_herbal_herbo-metallic_and_Bhasma_preparations_on_neurobehavioral_activity_and_oxidative_stress_in_rats.

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