PRECLINICAL STUDY OF SIDDHA DRUG KARPOORA SINTHAMANI MATHIRAI'S ANTI-INFLAMMATORY, ANALGESIC, ANTI-SPASMODIC AND LAXATIVE ACTIVITIES

Dissertation submitted to

THE TAMILNADU DR. MGR MEDICAL UNIVERSITY

CHENNAI-600032

In partial fulfilment of the requirements

for the award of the degree of

DOCTOR OF MEDICINE (SIDDHA)

BRANCH-II-GUNAPADAM

POST GRADUATE DEPARTMENT OF GUNAPADAM

THE GOVERNMENT SIDDHA MEDICAL COLLEGE

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OCTOBER 2019

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PALAYAMKOTTAI

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I hereby declare that this dissertation entitled **"Pre clinical study of herbo mineral drug *KARPOORA SINTHAMANI MATHIRAI* for its anti-inflammatory, analgesic, anti-spasmodic and laxative activities"** is a bonafide and genuine research work carried out by me under the guidance of **Dr. A. Kingsly M.D(s)., Reader, Head of the Department,** Post Graduate Department of *Gunapadam*, Govt. Siddha Medical College, Palayamkottai and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or other similar title.

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ACKNOWLEDGEMENT

I feel immense pleasure and gratitude in my heart to **Siddhars** for making this dissertation.

I wish to thank the esteemed authorities of the **Tamil Nadu Dr. M.G.R. Medical University, Chennai** for permitting me to undertake of Indian Medicine and Homeopathy, Chennai who flagged my dissertation with cheer.

I also wish to convey my deep gratitude to the former Principal, **Prof. Dr. R. Neelavathy, M.D.(s), Ph.D.,** and Principal **Prof. Dr. S. Victoria, M.D.(s),** of Government Siddha Medical College, Palayamkottai.

I would like to express my deep and sincere gratitude to my guide **Dr.A.Kingsly, M.D(s).,** Reader, Head of the Department, PG Gunapadam Government Siddha Medical College, Palayamkottai, for providing much needed attentions helpful suggestions for dissertation.

I would like to give special thanks to **Dr.G.Essakky pandiyan, M.D(s).,** Lecturer, Government siddha medical college, Palayamkottai, for his valuable guidance, helpful suggestions and encouragement for preclinical studies.

I would like to give special thanks to **Dr. R. Antony Duraichi, M.D(s).,** Lecturer, Grade II Government siddha medical college, Palayamkottai, for her valuable guidance, helpful suggestions and encouragement for preclinical studies.

The author is greedful to **Mr.M.Kalaivanan, M.Sc.,** Head of the Department of Pharmacology, Govt. Siddha Medical College, Palayamkottai for his suggestions and opinions regarding to the pharmacological analysis of the study.

The author is grateful to **Mrs.N.Nagaprema, M.Sc.,M.Phil.,** Head of the Department of Bio-chemistry, Govt. Siddha Medical College, Palayamkottai for her kind help and suggestion on biochemical aspect of this dissertation.

The author is very happy to thank **Dr. S. Sutha, M.Sc., Ph.D.,** Head of the Department of Herbal Botany and Herbal Pharmacognosy, Govt. Siddha Medical College,Palayamkottai for her kind help in botanical aspect of his study.

The author wants to thank **Dr. Murugesan** Scientific Officer IIT, Chennai for ICP analysis, for helping me to do the test ICP-OES,GAS&FTIR.

I express my sincere thanks to **Mr. Santhanakumar**, **M.Pharm.,** Assisant Lecturer Dept of Pharmacology, Arulmigu Kalasalingam college of pharmacy,

Krishnankoil, for their excellent help in pharmacological study, toxicity study and other guidance to do the research work.

I acknowledge my thanks to **Dr.K. Thanga mariappan, Ph.D**., Microbiology, consultant Microbiologist, vivek institute of laboratory medicine, Nagercoil. For his kind suggestion regarding with Antimicrobial studies and phyto-chemical studies of the work.

The author is greatful to the **Mrs.Poongkodi, M.L.I.S., M.phil., Librarian** Govt. Siddha Medical College,Palayamkottai and Library Assistants for help in literary collections.

I am also thankful to **Mrs.Suganthi, DMLT**, Pharmacist, and Post Graduate Department of Gunapadam for her kind co-operation to purification and preparation of the trail drug for my study and successful completion of dissertation.

I am also thankful to all my college staffs for their kind co-operation for my study. I should express my gratefulness to **All My Classmates** and **PG. Gunapadam students** for leading their helping hands whenever needed during the course of the study.

I express my sincere thanks to **Dr. M. Elakkiya, M.D(s).,** for leading her helping hands whenever needed during the course of the study.

The great walls to build my dissertation are my lovable family members they support me in all kinds to complete this work in a good way. I sincerely express my love to my respectable father **Mr. D. Kulasekaran, D.E.E.E.,** and my dearest mother **Mrs. Indrani Kulasekaran** for their sincere encouragement and inspiration throughout my research work and lifting me uphill this phase of life.

Finally, I wishes my thanks to **M.Maharaja & Mrs. Rajeswari (Maharaja DTP Services),** Palayamkottai for their marvelous work in completing this dissertation.

ABBREVIATIONS

KSM	-	KARPOORA SINTHAMANI MATHIRAI
CPCSEA	-	Committee for the purpose of control and supervision of experimental animals.
DC	-	Differential Count
ESR	-	Erythrocyte Sedimentation Rate
FTIR	-	Fourier transform infrared spectroscopy
Hb	-	Haemoglobin
IAEC	-	Institutional Animal Ethical Committee.
ICP-OES	-	Inductively coupled plasma optical emission spectrometry
Ig E	-	Immunoglobulin E
LDH	-	Lactate Dehydrogenase
MCV	-	Mean Corpuscular Volume
OECD	-	Organisation for Economic Co-operation and Development
PCV	-	Packed Cell Volume.
PGE	-	Prostaglandin E
RBC	-	Red Blood Corpuscles
SEM	-	Scanning electron microscope
CCD_S	-	Charge coupled devices.
SPME	-	Solid phase micro extraction
TCD	-	Thermal conductivity detector
FID	-	Flame Ionization detector
CCD	-	Catalytic combustion detector
LD	-	Low dose
Mg		Milligram
Kg		Kilogram
LD_{50}		Lethal Dose $_{50}$
p.o		peros
ML		Milliliter
%		percentage
R&D		Research and Development

EDTA	Ethylene Diamine Tetra Acetic Acid
M	Male
g%	Gram percentage
g	Gram
NOAEL	No-Observed-Adverse-Effect-Level
MLD	Minimum Lethal Dose
MTD	Maximum Tolerated Dose

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Proposal Number : AKCP/IAEC/10, 35, 36 / 2018-19

Date of received after modification (if any) : Nil

Date of received after second Modification : Nil

Approval date : 19.01.2018 & 03.02.2018

Animals : Rats & Guinea pig

Expiry Date : Nil

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Tamil Name	Botanical Name	Family	Part Used
Neravalam	Croton tiglium	Euphorbiaceae	Seed
Sathikkai	Myristica Fragrans	Myristicasceae	Seed
Karuvelampisin	Acacia arabica	Mimosaceae	Gum
Katrazhai	Aloe barbadensis	Lilliaceae	Leaf Juice

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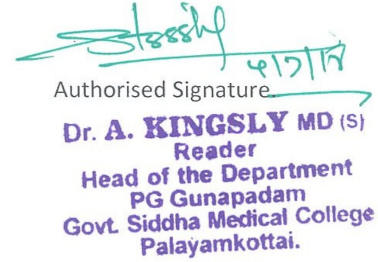
TAMIL NAME	ENGLISH NAME	CHEMICAL NAME
POORAM	CALOMEL	HYDRAGYARUM SUBCHLORIDE



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"RESEARCH METHODOLOGY & BIOSTATISTICS"

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ISSN 2589 - 3343 *A Peer Reviewed Interdisciplinary Medical Journal*

CERTIFICATE OF PUBLICATION

The board of "International Journal of Reverse Pharmacology and Health Research'' (ISSN 2589-3343, www.ijrphr.com) is herby awarding this certificate to Corresponding Author

Mahalakshmi K

in recognition of the publication of the Research/Review Paper entitled

FTIR Characterization of Siddha Medicine Sivanaramirtham

CODENJ: IJRPHR

Published in Volume **2** , Issue **2** , Jan-Mar, **2019**

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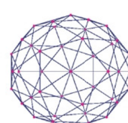
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ISSN 9582-3343

CERTIFICATE OF PUBLICATION

This certificate is hereby bestowed upon Author

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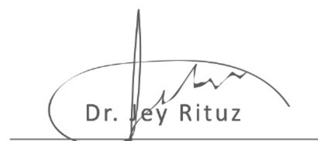
For publishing Journal article entitled

Physicochemical and Phytochemical analysis of Siddha Preparation "*Karpoora Sinthamani Mathirai*"

in Volume 1 Issue 2 , 2019 (Apr-Jun) www.biosci.in/jrbms



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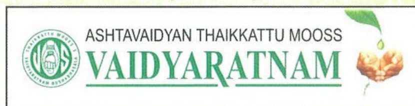

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1. INTRODUCTION

Siddha system of medicine, which has been prevalent in ancient tamil and is the foremost of all other medical system in the world. The unique nature of this system is its continuous services to humanity for more than 10,000years,in combating diseases and in maintaing the physical, mental and moral health.siddhar's are spritual scientist explored the nature . They educated this disiple and propagated siddha concepts.

The siddha system grew through the work of agathiyar,Bogar,Thirimoolar and others. Siddha's knowledge of iatrochemistry,minerals, metals and plants was stupendous. This was succesfully used system from immemorial.

The scientific evaluation is needed to validate its preciousness. The siddha system has not only the curative and preventive effects on different diseases but also paves the way for longevity and immortality. WHO has also recogonised indian system of medicine has an effective alternative medicine in the place of conventional allopathic system of medicine

According to *Siddha* the five basic elements *(panchapootham)* are present in all living and non-living things of the universe in various proportions.

Siddhars in their attempt to elevate themselves to a perfected earthly immortal (attain *siddhi*) developed techniques, which included controlled breathing *(Vaasi)*, concentration of mind, intense meditation *(Dhyanam)*, besides dietary regimen and certain postures (Yoga) for psychosomatic harmony.

They also explained five systems *(Annamaya kosham, Manomaya kosham, Pranamaya kosham, Vignagnnanamaya kosham and Anandamaya kosham).* This establishes the fact that human body is not merely a machine. But human being is composed of different dimensions namely physical, mental, supra-mental and spiritual which all should work in harmony. So that perfect health is maintained. In short, the *Siddhar's* thoroughness and extraordinary knowledge that proceeded from the metaphysics of ancient India which is eternal.

This led to the understanding of the position of man in this universe and his relationship with it.

All the things in the universe and also man are made up of *pancha-boothas* in their gross state as well as in the subtle state. In the Siddha system of medicine, all vital processes (physiological, biochemical-metabolic) have been classified under three functional heads namely *Vali, Azhal, Iyyam (vaatham, pitham, kabam)* these are called

three *Uyir-thaathukal*. It is interesting to note that one and the same basic *uyir-thaathu* differentiates itself into three functional divisions. The meaning of *Uyir-thaathu* is the route cause and basis of life.

The basic principles of the *Siddha* system consist of *panchapootham* theory, tri-humours pathology and 96 basic factors. In *Siddha* system of medicine, the understanding of the tri-humours status is very essential. The humours *vadha, pitha* and *kabha* exist in the ratio of 1:1/2:1/4 in normal physiological states in man. Any imbalance or deviation from this state leads to diseases or death will be the result.

The pulse reading *(Naadi)* forms the main diagnostic mode of *Siddha* medicine. Again this *Naadi* characteristic is also based on *Vali, Azhal, Iyyam*. All the diseases are brought under the three major divisions of *Vali, Azhal, Iyyam* as well as combination and recombination types.

The *Siddha* system is unique in that it recognizes disease condition individually as well as in strict reference to the three-*doshas* principle. All diagnostic modes are designed to take into serious consideration the symptoms, signs, characteristics of the diseased conditions and rationally evaluate them in strict accordance with the three-*dhosha* principle.

The treatment aspect involves the neutralization of affected humours.

"விரேசனத்தால் வாதம் தாழும்"

"வமனத்தால் பித்தம் தாழும்"

"நசிய அஞ்சனத்தால் கபம் தாழும்"

நோய் நாடல் நோய் முதல் நாடல் பாகம் - I

By giving viresanam (Purgative), Vatha kutrum is neutralized. By giving vamanam (emetics) pitham is neutralized. By giving Anjanam and nasiyam (application of medicine in eyes and nose), kaba kutram is neutralized.

The same has been stated as

"மிகினும் குறையினும் நோய் செய்யும் நூலோர்
வளி முதலா எண்ணிய மூன்று"

- *திருக்குறள்*

By calculating the imbalance of the three humors siddhars used to diagnose the root cause of the disease and the treatment also based on the way of diagnosis. Siddha medicines cure the root cause of the disease. So that it could be a successful medical system in the world.

The more scientific approach to the all aspects of life even before thousands of years should be appreciated and bring it to our day today life.

Herbal, medicines are being used by nearly about 80% of world populations, primarily in developing countries for primary healthcare. The herbal drugs described in *Siddha* system have been the basis of treatment of various human diseases. Selection of scientific and systematic approach for the biological evaluation of herbo mineral drug formulations based on their view in the traditional systems of medicine forms the basis for an ideal approach in the development of new drug from natural things.

According to the CDC (Council of Disease prevention and Control), more than 52.5 million peoples suffer with Arthritis today. Today number of synthetic anti-inflammatory, anti-spasmodic, analgesic drugs are available for symptomatic control of arthritis. This synthetic drug may cause side effects. Therefore the modern world is searching for suitable traditional remedies because of without any adverse effect.

In *Siddha* system, the symptoms of *Keel Vayu* can be correlated with Arthritis (Musculo Skeletal Disorder). In the literature of *Anuboga vaithiya navaneetham*, there is a preparation called, *KARPOORA SINTHAMANI MATHIRAI*, which is exclusively indicated for *Keel Vayu* in which its efficacy has to be scientifically evaluated.

2. AIM AND OBJECTIVES

AIM

The aim of this study is to do a scientific review to validate the safety and efficacy of *"Karpoora Sinthamani Mathirai "* for Anti-inflammatory, Analgeisc, Anti-spasmodic and laxative activities.

OBJECTIVES

- The main objective of the present study is to high light the safety and efficacy of *Karpoora Sinthamani Mathirai* in the treatment of *Keel Vayu*, the following methodology was adopted to evaluate the drug and its standardization studies.
- To collect the literature systematically from siddha texts as well as modern science.
- To standardize the preparation of drug according to classical siddha literature.
- To subject the drug to physico chemical and chemical analysis.
- To detect the elements present in the drug by instrumental analysis.
- To study the acute and subacute toxicity profile of *Karpoora Sinthamani Mathirai* according to OECD 423 and 407 guidelines.
- Evaluation of Anti-inflammatory activity of test drug.
- Evaluation of Analgesic activity of test drug
- Evaluation of Anti-spasmodic activity of test drug
- Evaluation of Laxative activity of test drug
- To analyse all the above study results to validate the advantage of *Karpoora Sinthamani Mathirai*

3.REVIEW OF THE LITERATURE

3.1. *POORAM* – பூரம்

(Hydrargyrum Subchloride)

3.1.1 GUNAPADAM ASPECT

Pooram does not find a place in the list of 64 padanas but is considered as one among them by the medical practitioners. It is prepared by the combination of rasam and salt.

SYNONYMS:

"துர்க்கைகளையெலியிடைச்சனிபூரந் தீட்டுவிடதாலிநக்கிபூடிமதாந்"

-நாமதீபநிகண்டு.

Durgai, Kalai, Aellidai, Sani, Pooram, Thettu, Vidathaali, Nacki, Buddimaatham.

METHOD OF SYNTHETIC PREPARATIONS (*VAIPPU)*

"தானென்றகற்பூரமொன்றுசொல்வேன் சாதகமாய்சூதம்ரெண்டுதூக்கி
வானென்றசட்டிக்குள்மூன்றுபடியுப்பை வளமாகபொடித்திட்டுநடுவேகேளு
தேனென்றசெங்கல்தூள்கால்படிதானிட்டு திரமாகக்குளித்ததிலேசூதம்விட்டு
ஏனென்றமறுசட்டிகவிழ்த்துமூடி ,யல்பாகவெழுசீலைமண்ணும்செய்யே
மண்செய்துதொண்ணூறுகடிகையப்பா வாகாகமூத்தீயுமெரித்துமைந்தா
மண்செய்தமேல்சட்டிக்குள்ளேகேளு வாகாகஉரைத்திருக்கும்வெள்ளைமெதத்
மண்செய்தநற்பூரங்குழாயில்வைத்து வாகாகபணவிடைதான்தூக்கிக்கொண்டு"

– ***Agasthiyar paripooranam 400.***

2 *palam sootham* placed in the mud pot in between 3 padi culinary salt(Nacl) and brick stone powder.Close the pot with another mud pot and seal with 7 mud *seelai* . It is burnt for 90 *kadikai* after it is cooled *pooram* is found deposited on the upper pot and the same is collected.

மயக்கமுறுமிரதகற்பூரஞ்சொல்வோம்வியாதியெல்லாந்தீர்ந்துபோம்வரிசைகேளு
மயக்கமுறஉப்புளுக்குச்செங்கல்தூளும் வரிசைபெறவழித்தெடுத்துவைத்திடாயே
வைத்ததோர்இருவகையுந்தூளாயாட்டி மைந்தனேசட்டிக்குள்பாதிநீயும்
வைத்திடுவாய்அதிற்குகையைச்சூதமப்பா வாராடாகழஞ்சுபத்துமாற்றமன்றி
வைத்தங்கேகுகைவாயில்பொடியுமிட்டு மறுசட்டிகொண்டிட்டுவாயைமூடி
வைத்துநீயேழுசீலைமண்ணும்பூசி மாயிபதம்பூசித்துஅடுப்பிலேற்றி
அடுப்பேற்றிகமலவன்னிதினந்தானொன்று அதன்பிறகுகடாக்கினியுந்தான்மூன்று
எரித்தாறியெடுத்துப்பார்சட்டிமேலே ஏறிநிற்கும்கற்பூரம்எடுத்துவைத்து.

10 *kalanju* of *sootham* placed it mud pot in between the well powdered culinary salt and brick stone powder close the mud pot with 7mud *seelai*. Then burnt it as *deepackini kamalackini* and *kaadackini* for 3 days respectively after it is cooled the *pooram* is found deposited on the upper pot and and the same is collected.

3. Sulfur 67.2gm is melted in mud pot and mercuery 336gm is added to it and kinded well and there forms a black colored powder (*kajali*). Brick stone powder is placed up to half of the level of a pot culinary salt (NaCl) 650 gm is placed over it . Mercury sulfur camphor is placed over the salt and sealed with mud paste cloth . It burnt for 12 hour with *kadackini* after it is cooled the mercurous chloride is found deposited on the upper pot and the same is collected.

ORGANOLEPTIC CHARACTORS :

Colour	-	White
Appearance	-	Hard
Potency	-	Hot
Taste	-	Salty, Pungent

ACTION:

Laxative

Tonic

Antiseptic

Diuretic

It is also an excessive bile producer.

GENERAL PROPERTIES :

“இடைவாத சூலை யெரிசூலைகுன்மந் தொடைவாழை வாதமாஞ் சோணி - யிடையாத
வொக்குரச கர்ப்பூர மொன்றே யனவொருதல் இக்குவெல்லத்தேழுநாளீ”.

“சசிவன்னகருப்பூரத்தில் சாதித்தகயஞ்சுவாசம்
பசிகலிதாபசோபம் பவுத்திரம்பிளவைகுஷ்டம்
வசிதருகிராணியோடு வளரதிசாரமேகம்
சிதருமிசிவுசூலை யிவைபலரோகம்போமே”.

“திரண்டவாதங்குடல்வாதம் தீருஞ்சந்நிபதின்மூன்று
மருண்டேகுத்துமரையாப்பு மண்டைச்சூலைகபாலவிடி
பரங்கிச்சூலைபற்கிரந்திபக்கசூலையிவைமுதல்போம்
இருண்டமேனிபொன்னிறமாம் இதுவேகற்பம்இயம்பீரே”.

calomel is taken along with jaggery for seven days,

It cures various types of throbbling pains,

Throbbling pain in the lumbar region,

Burning sensation,

Ulcer due to disorders of vatha humours,

Hepatomegaly,

Pyrexia,

Jaundice,

Basillary dysentery,

Dropsy,

Chronic ulcer,

Venereal diseases,

Indigestion,

Vomiting,

Diarrhoea,

Worm infestation,

Rheumatism,

Itching,

Constipation,

Scabies,etc.,

It is also effective in the treatment of head ache as explaind in the above tamil verses. In siddha system of medicine pooram is compared to sanjeevi because it cures hard disease like syphilis(kiranthi), uterine cancer(allgul putru), uterine ulcer(allgul ranam) and chronic non-healing ulcers.

- Anuboga Vaithiya Navaneetham 4th vol

PURIFICATION OF CALOMEL:

1.The poultice made of betel leaf(piper betel) and pepper (piper nigram) each 8.75gm,is taken and dissolved in 1.3 litre of water calomel 35gm and tied with a cloth and immersed in the liquid from the cross bar and heated. After the water is reduced to ¾ of its volume the calomel is taken out washed with water and dried to get it in purified form.

2.Calomel 35 gm is consolidified in mother's milk for 3 hours and again it is consolidified in garlic oil(thailam) for 9 hours. It is taken out as purified.

3.Before mixing calomel in *legium* preparations, it is consolidified with the juice of mukia madraspatana (*musumusukku*) and washed.

SIGNS AND SYMPTOMS OF CALOMEL POISONING:

Multiple red boils may appear on the face, ache formation, ulcers in the chest, mouth and tongue diarrhoea and dysentery, scrotal swelling and ulcer in the uvula.

ANTIDOTE FOR POISONING:

Nilapanai kilangu (curculigo orchides)	-	8.75gm
Vallarai ver (centella asiatica root)	-	8.75gm
Ponnankanni ver (alternatheria sessiles)	-	8.75gm
Kanduparangi ver (clerodendrumserratum)	-	8.75gm

All the above ingredients are mixed and boiled to make a decoction. This decoction is used twice a day for two or three weeks with suitable diet restrictions

POORAM IN OTHER PREPARATIONS

1. *CHANDA MARUTHA SENTHOORAM*

Dose : 50-100mg

Adjuvant : Honey, ginger, juice, jaggery, thirikadugu chooranam, amukkara chooranam

Indication : Arthritis, hemiplegia, parkinsonism, fever, ...etc

- Siddha vaithya thirattu

2. *LAVANGATHI MATHIRAI*

Dose : *Thuvarai alavu* (0.202gms)

Indication : Fever, pain, vatha diseases

-Kannusamy parambarai vaithiyam

3. *VELLAI ENNAI*

Dose : 15-30ml

Indication : Constipation, arthritis, ulcer, swelling

-Gunapadam (pagam-2)

4. *PRATHABA LINGA CHOORANAM*

Dose : 1-2 *kundri alavu* (130-260mg)

Adjuvant : Palm jaggery, Honey, ghee, butter, ginger juice

Indication : Fever, pain, diarrhoea, wounds, arthritis and vatha diseases

- *Anuboga vaithya navaneetham*

5.*VAAYU MATHIRAI*

Dose : ½ - 1 *Kundri alavu* (65-130mg)

Adjuvant : Palm jaggery, dried ginger paste

Indication : Arthritis, pain

6. *SOOLAI BEDHI MATHIRAI*

Dose : *Kadugu alavu* (1-2 mathirai)

Adjuvant : Jaggery , castor oil

Indication : Deep wounds, Arthritis

- *Anuboga vaithya Navaneetham (part-9)*

7. *SHANUMUGA MEZHUGU*

Dose : ½ *kundrimani alavu* (130-195mg)

Adjuvant : Palm jaggery, sugar, butter

Indication : Arthritis, joint pain, vatha diseases

- *Anuboga vaithya navaneetham (part -9)*

8. *KOOROSANAI MEZHUGU*

Dose : ½ to 1 *kundrimani alavu* (65-130mg)

Adjuvant : Palm jaggery, Butter

Indication : Hemiplegia, arthritis, gonococcal arthritis

- *Anuboga vaithya navaneetham.*

3.1.2. GEOLOGICAL ASPECT:

CALOMEL :

Calomel is a chemical compound with the formula Hg_2Cl_2. Also known as mercurous chloride (a mineral form rarely found in nature)

IUPAC name	:	Dimercury dichloride
Other name	:	Mercurous chloride
		Calomel
		Horn mercury
		Mercury (I) chloride
Chemical formula	:	Hg_2Cl_2
Molar mass	:	472.09 g/mol
Appearance	:	Heavy white rhombic crystals
Density	:	7.150 g/cm^3
Melting point	:	525°c
Boiling point	:	383 °c
Solubility in water	:	0.2 mg/100ml
Solubility	:	Insoluble in ethanol, ether
Refractive index	:	1.973
LD_{50} median dose	:	210 mg/kg (rat, oral)

CHEMICAL ASPECT:

Chemically *pooram* is a siddha drug has been identified as calomel the chief ore of mercury chemically *pooram* is known as mercury chloride or mercurous chloride.

CRYSTAL SYSTEM :

Tetragonal

HABIT :

Crystal often tabular {001}; prismatic [001]; pyramidal; equant, especially in complex twins. Complex development often exhibited. Drusy crusts of minute crystals. Massive, earthy.

TWINNING :

On {112}. as contact or interpenetrant twins often repeated and with irregular and concealed boundaries.

CLEAVAGE :

Distinct / good

{110} good but slightly uneven; {011} imperfect.

HARDNESS :

1½ - 2

TENACITY :

sectile

COLOR :

colourless, white.

OCCURRENCE:

Landsberg (moschellandsberg)

Alsenz-Obermoschel

Palatinate

Rhineland-palatinate

Germany

ASSOCIATIONS :

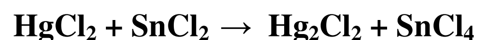
It is commonly associated with calcite, clays, limonite, montroydite, cinnabar, eglestonite, mercury, terlinguaite.

PREPARATION OF CALOMEL :

1.When mercuric chloride is heated with mercury in an iron pot, calomel is obtained.

$$HgCl_2 + Hg \xrightarrow{\Delta} Hg_2Cl_2$$

2.when mercuric chloride is treated with less amount of stannous chloride, calomel is obatained.



$$HgCl_2 + SnCl_2 \rightarrow Hg_2Cl_2 + SnCl_4$$

It can also be prepared by grinding the mixture of mercury with concentrated H2SO4 together with it, small amount of NaCl as well as mercury is added to get calomel.

3.1.3. LATERAL RESEARCH:

***In vivo* Evaluation of Analgesic, Antipyretic and Anti-inflammatory potential of *Siddha* Formulation Natural and Synthetic *Pooraparpam* in selective Rodent Model - Journal of Chemical and Pharmaceutical Research, 2016, 8(4):643-656**

ABSTRACT

Siddha system of medicine is one of the oldest medical systems of India existed separately in early times. Pooram is one among the Panchasootham (five mercurial compounds) which is widely used in Siddha preparation. The main component of the Pooram is Mercury. The mercurial compound has been in use in Siddha since many centuries and it is identified and indicated for the treatment of many diseases in ancient Siddha literature. The present study was
undertaken to assess the in vivo evaluation of analgesic, antipyretic and anti-inflammatory potential of Siddha formulation Natural and Synthetic Pooraparpamin rodents. The analgesic activity were evaluated through thermal (Eddy's hot plate test) and mechanical method (Tail clip method) of pain induction in mice, whereas antipyretic activity by yeast induced pyrexia in rats. On the other hand, anti-inflammatory activity evaluated by carrageenan and cotton pellet induced inflammation in rats. Both the test drug was administered orally at the dose of 1.15 and 2.30 mg/kg, the activity was compared with a standard reference drug Indomethacin 20 mg/Kg and Paracetamol 150mg/kg. The result obtained from the study clearly demonstrates that the Siddha formulations Natural and Synthetic Pooraparpam has promising analgesic, anti-inflammatory and antipyretic activity in tested animals. Hence it was concluded that Pooraparpamis considered to be one of the safe medicine for the clinical managementof fever, pain and inflammation associates medical conditions.

3.2. *SATHIKKAI* – சாதிக்காய்

(Myristica Fragrans)

3.2.1. GUNAPADAM ASPECT

Synonyms

Kulakkai, Jathikkai

- குணபாடம் மூலிகை வகுப்பு

சாதிக்காயின் பெயர்கள்

நல்லவாதி மலமென்றும், சீலிக்காயென்றும்
நல்லமான வாதிரோதய மென்றும் பேரு
சொல்லக்கேள், சிவசிகித மென்றும் பேருண்டாச்சு
சீரிட மென்றிதற்குப் பேர் செப்பினோம் நாம்
சொல்ல சித மென்றிதற்குப் பேரு
சுதே வனாதி தமென்றிதற்குப் பேரு
அல்லவங்கார மென்றிதற்குப் பேரு
அருளிளோம், சாதிக்காயதற்கும் பேரே

- பஞ்ச காவிய நிகண்டு

Nalavathy malam, seelikkai, vathirothayam, sivasigitham, seeridam, sitham, sudevanathitham, allalangaram.

Vernacular names

Sanskrit	-	Jati – Phalam
Malathi	-	Phalam
English	-	Nutmeg
French	-	Muscadier, Musque.
Germany	-	Achter muscatnussbaum
Kash	-	Zafal
Bom.Punj.Gaj & mah	-	Jayiphal, Javantri
Telungu	-	Jejikaya
Tamil	-	Jadikkay, Jathikkai
Malayalam	-	Jathika
Can	-	Jajikai
Kon	-	Jaiphal
Sinh	-	Jadika
Burm	-	Zadu-phu
Malay	-	Bush-pala

Pers & Arab - Sauz-ba-wawa, Zanza-ba-wawa

Partused:

Unriped fruit

Organoleptic Characters

Taste *(Suvai)* : Astringent, Pungent

Potency *(Thanmai)* : Hot*(veppam)*

Bio Transformation *(pirivu)* : Pungent

Action

- Stimulant *(Veppamundakki)*
- Carminative *(Agattuvaivagatri)*
- Narcotic *(Moorchaiundakki)*
- Aromatic *(Manamooti)*
- Aphrodisiac *(Kamam peruki)*
- Tonic *(Uramakki)*

General properties

தாதுநட்டம் பேதி சருவாசி யஞ்சிரநோய்

ஓதுசுவா சங்காசம் உட்கிரணி – வேதோ

டிலக்காய் வரும்பிணிபோம் ஏற்றமயல் பித்தங்

குலக்கா யருந்துவர்குக் கூறு

- *குணபாடம் மூலிகை வகுப்பு*

Nut meg is useful in the cases of oligospermia, diarrhoea, headache, asthma, cough, stomachache, bloated abdomen.

Traditional used

- Dicoction of nutmeg is used for dehydration in the case of vomitting and diarrhoea
- Nutmeg paste is applied around the eyes to promote eye sight.
- Nut meg oil is used for tooth ache.
- Nutmeg powder 130mg, dry ginger powder 130 mg and cumin seed powder 130mg taken internally before meals for indigestion
- For diarrhoea 2 grams of nutmeg powder is given with milk.

SATHIKKAI IN OTHER MEDICINES

1.*SATHIKKAI THAILAM*

Indication	:	Arthritis, vatha disease, Diarrhoea

- *Gunapadam mooligai vagupu*

2. *RAASA AMIRTHATHI CHOORANAM*

Dose	:	1-2 gram
Adjuvant	:	Hot water
Indication	:	Joint pain, Tuberculosis, Eczema and vatha diseases

- *Aathma Ratchamirtham ennum vaithya sarasangiragam*

3. *GARUDAN KIZHANGU ENNAI*

Dose	:	¼ - ½ owunce
Indication	:	Hemiplegia, facial palsy, joint pain, joint swelling

- *Kannusamy parambarai vaithyam*

4.*NARASIMA ILAGAM*

Adjuvant	:	Cow's ghee
Indication	:	Vatha diseases, Nervine tonic

- *Sikitcha Rathna Deepam*

5.*THALISATHI CHOORANAM*

Dose	:	1-2 gram
Adjuvant	:	Honey
Indication	:	Arthritis, 80types of vatha diseases, 40 types of pitha diseases

- *Siddha vaithya thiratu*

6. *MAHAVILVATHI LEGIYAM*

Dose	:	3 gram
Indication	:	Arthritis, Rheumatism

- *Bogar vaidhyam -700*

3.2.2. BOTANICAL ASPECT

SATHIKKAI (MYRISTICA FRAGRANS)

Classification

Taxonomical Classification

Kingdom	:	Plant kingdom
Division	:	Flowering plant
Class	:	Dicotyledonae
Subclass	:	(ஓர் அடுக்கு பூவிதழ் உடையவை) Monopetalae
Series	:	Microembryeae
Family	:	Myristicaceae
Genus	:	Myristica
Species	:	Fragrans

Habitat

Nutmeg tree is indigenous to the Malay, Peninsula, Moluccas and Penang, now cultivated in many tropical countries of both hemispheres. In India, it is grown in Madras state (Nilgiris, Coimbatore, Salem, Ramanathapuram, Tirunelveli,m Kanyakumari and Madurai Districts) a few trees are found invarious localities in kerala, Assam and other states. Preliminary trials have shown that Araku Valley (Andrapradesh), wynaad (Madrasstate) are well suited for its cultivation.

Description

Habit

Moderate sized usually dioecious evergreen aromatic tree, upto 12mm tall.

Leaves

Alternate, quite entire exstipulate, often pellucid-punctate.

Flowers

Dioecious, small, regular fascicled umbelled or panicled. Bracteoles persistent or caducoas.

Male Flowers

Perianth-3 (2-4) lobed, valvate in bud. Anthers 3 or more connate in a sessile or stropitate column head ring or disk, 2-celled.

Female flowers:

Perianth of the male, staminodes, ovary superior, free sessile, 1-celled style short or a stigma capitate, discoid or lobed, ovale, basal, erect, anatropous.

Fruit

Fleshy at length 2 rarely 4 – Valved.

Seed

Erect, enclosed in a thin or fleshy entire or lacerate often highly coloured aril, testa thin or crustaceous, albumen hard densely ruminate, embroyo basal, small, cotyledons rounded spreading often wrinkled, radicle short inferior.

Macroscopic Description of seed

Seed ellipsoid, 20-30 mm long and about 20mm broad, externally greenish brown sometimes marked with small irregular, dark brown patches or minute dark points and line slightly furrcused reticulately, a small light coloured area at one end indicating the position of the radicle. A grove running along the line of raphe to the darker chalza at the opposite end, surrounded by a thin layer of perisperm with infoldings appearing as dark ruminations in the abundant greyish brown endosperm, embryo in an irregular cavtiy, small with two widely spreading crumpled cotyledons and a small redicle, odour, strong and aromatic, taste, pungent and aromatic.

Microscopic Description of seed

Transverse section of endosperm show peripheral perisperm, of several layers of strongly flattened polyhederal cells with brown contents or containing prismatic crystals, inner layer of perisperm of thin walled parenchyma about 40 μ thick, infolding into the tissue of the endosperm to form the raninations containing numerous very large oil cells with brown cell walls, vascular strands, in the peripheral, region numerous small spiral vessels, large called, endosperm parenchymatous with occasional tannin idioblasts, with the brown walls, containing numerous simple rounded and compound starch grains, with upto about 10 componenets usually 2-8, individual grains, upto 2 μ in diameter present most of the cells with crystalline fat and often a large aleurone grain in each cell, containing a rhombic protein crystal upto 12 μ and small aleurone grains with less regular crystalloids, embryo of shrivelled and collapsed parenchyma.

Chemical constituents

The main chemical compounds are

- a-pinene, camphene, b-pinene
- Sabinene, myrcene, a-phellandrene
- a-terpinene, limonene, 1, 8 cinede, y-terpinene, linalool, terpinen-4-ol
- Safrole, methyl eugenol and myristicin.

Therapeutic uses

- Oil of nutmeg is employed for flavouring food products and liquorus
- It is used for scenting, soaps, tobacco and dental cream.
- It has been recommended for treatment of inflammation of bladder and urinary tract.
- Alcoholic extract of nutmeg shows anti-bacterial activity against micrococcus pyogenes, aqueous decoctions are toxic to cockroaches.

3.2.3. LATERAL RESEARCH:

ANTI-INFLAMMATORY ACTIVITY OF *MYRISTICA FRAGRANS* (NUTMEG) USING HRBC MEMBRANE STABILISING METHOD INT. J. PHARM. SCI. REV. RES., 44(1), MAY - JUNE 2017; ARTICLE NO. 11, PAGES: 40-42

ABSTRACT : The aim of the study is to determine the anti-inflammatory activity of *Myristica fragrans* (Nutmeg) using HRBC membrane stabilising method. Inflammation being a common symptom for various diseases has to be treated properly. The anti-inflammatory drugs come into role here in decreasing the inflammation. Nutmeg spice is a good source of minerals like copper, potassium, calcium, manganese, iron, zinc and magnesium. Since it's a natural drug and has a lot of anti-inflammatory properties having no side effects, therefore it is better than synthetic drugs. Nutmeg being a natural drug with least side effects, in comparison with the other drugs can be used in the future to produce an efficient anti-inflammatory drug.

3.3. *NERVALAM* – நேர்வாளம்

(Croton Tiglium.Linn)

3.3.1. GUNAPADAM ASPECT

Synonyms

Valam, Nepalam, Sobi, Sevalam, Sambari, Sithuruba, Sirukurinji, Thandi, Thendi, Nagathendhi, Nagakenthi, Naganam.

- *Gunapadam Mooligai Vaguppu*

Vernacular names

Tamil	:	Nervalam
English	:	Purging croton
Tel	:	Napalam
Mal	:	Nervalam
Kan	:	Japala-beeja
Sans	:	Danthit
Arab	:	Hab-ul-salantin
Pers	:	Bed-anjir-e-khati
Hind	:	Jamalgotta

Part used

Seed

Organoleptic character

Taste *(Suvai)*	:	Bitter *(Kaippu)*
Potency (*Thanmai)*	:	Hot *(Veppam)*
Bio transformation *(Pirivu)*	:	Pungent *(Kaarpu)*

Action

Purgative *(Neermalampoki)*

Rubefacient *(Dhadipundaki)*

General properties

எந்த வியாதி யினங்களையுஞ் சாடிமல
பந்த வினையைப் பரிபரித்து – வந்தவெப்பை
பாபியென மாட்டுதலால் பாடாண வெம்மையினும்
சோபிமக சோபியென்று சொல்

- தேரன் வெண்பா

Purging croton has higher toxic effects because of its purgative action. It is used to treat vadha disease, mupinni, kudaichal and other gastric disorders.

ஒதி லுதவத் துறுமலம்பன் னோய் விலகும்
பேதி மருந்திற் பெரிதாகும் - வாதமறுங்
கூர்வாளை யொத்தவிழிக் கொம்பனையே! பண்டிதர் சொல்
நேர்வாளக் கொட்டைதனை நீ

உரைத்திடு வாத நோய்க்கு முறைகெடு விடஞ்சன் னிக்கும்
நிரைத்திடு சூலை குன்ம நீண்டிடு மலக்கட் டிற்குங்
கரைத்திடு வாளந் தன்னை கதித்திடு மேகத் திற்கும்
அரைத்திடு பித்தத் தோர்க்கும் அகற்றிடு அதனைத் தானே

Purification

Croton seeds are allowed to boiled in cow dung milk then remove the outer coating and leaf like cotyledones with sheath like substance from inside. Then it is boiled in cow's milk. Then soaked in lime juice for 24 hours. The croton embryo dried and roasted in cow's ghee.

Other purification process

Croton seeds 35 gram, Amla fruit juice, eclipta prostrata leaf juice, cow's urine each 85 gram, totally 255 grams taken in a mud pot then it is closed with white cloth. Then place croton seed on it and it is boiled per 3 hours. Repeat the procedure for 3 times then it is roasted with cow's ghee.

Toxicity of Croton tiglium:

- It can cause severe diarrhoea and abdominal cramps along with burning sensation in the whole abdomen
- Unpurified croton seeds cause severe irritational inflammation in the alimentary canal. It results in Diarrhoea, Severe cramps, Severe abdominal tenderness, Ulceration in mouth, acid dyspepsia, vomitting, stupour, dizziness
- Externally it cause Itching, Blisters and Burning sensation.

Antidote

Cumin seeds roasted in honey and add sufficient amount of water to it make it dicoction.

Traditional uses:

- Croton seeds are made into paste with water mixed with honey and applied on tumours.

- Croton seeds are used with other raw drugs for anemia and constipation medicines.
- Croton seeds should not be given separately.
- Croton seeds should not be given to aged persons, younger children, diabetic patients and pregnant women.

NERVALAM IN OTHER PREPARATION

1.BALA VIRESANA KULIGAI

Dosage : *Kundrimani alavu* (130mg)

Adjuvant : Gulkanthu, sugar, jaggery, butter.

Indication : Arthritis

- *Anuboga vaithya navaneetham*

2.SANJEEVI MATHIRAI

Dose : *Kundri alavu* (130mg)

Adjuvant : urine

Indication : Snake bite, Hemmorhoids, gastric ulcer tuberculosis

- *Bharathathil marunthu sei muraigal*

3. VIRESANA BHOOPATHI

Dose : 100mg

Adjuvant : Water

Indication : Vadha and kabha diseases, abdominal distension

- *Balavagadam*

4. LINGATHI SUGA KAZHICHAL KULIGAI

Dose : ½ -1 *Kundrimani alavu* (65-130mg)

Adjuvant : Jaggery, sugar, butter

Indication : Hemiplegia, arthritis, vadha fever, kabha diseases

- *Anuboga vaithya nevaneetham*

5. SOOLAI BEDHI MATHIRAI

Dose : 1-3 *kadugu alavu* (6mg-18mg)

Adjuvant : Jaggery, castol oil.

Indication : Arthritis, urinary tract infections, non-healing wounds.

- *Anuboga vaithiya navaneetham*

3.3.2. BOTANICAL ASPECT

NERVALAM (CROTON TIGLIUM)

Taxonomical classification

Kingdom	:	Plant
Division	:	Magnoliphyta
Class	:	Magnoliopsida
Subclass	:	Rosidae
Order	:	Euphorbiates
Family	:	Euphorbiaceae
Genus	:	Croton
Species	:	Croton tiglium

Habitat

It is native to India, Srilanka, South china, Philippines and the moluccan Islands.

Description

Habit

An erect (or) more (or) loss spreading shrub (or) small tree

Leaves

Croton tiglium is evergreen. The simple leaves are alternate. They are ovate, serrulate and petiolate 5-10cm long.

Flower

It produces racemes of white five-stellate flower from January to July. Flowers small unisexual nearly 2.5cm long.

Fruit

The tree produce drupes

Macroscopic character

The seed appear as oblong, oval, approximately 12-15mm length, 5-8mm width, ventrally shows longitudinally running centrally located ridge of raphe. A small circular point located at the narrow end is the hilum. It is brown and some seeds are black in colour.

Microscopic character

The seed is spherical capsule, smooth, carunculate testa, crustaceous, albumin copious, cotyledon flat, ovary covered with stellate tomantum. Further seed show wide

and thick broadly elliptical body. It also consist of outer seed coat and inner pericarp. These two cotyledons which are flat, long, parellel to each other.

Chemical constituents

Croten oil consist chiefly of the glycerides of steric, palmitic, myristic, lauric and oleic acids. They are also present in the form of glycerin. ether the more volatile acids as formic, acetic, Isobutyric and Isovalerianic acids. The active principle is crotonic acid.

Therapeutic uses

- The seed oil and bark were widely used as a remedy for cancerous sores and tumours, carbuncles, colds, dysentery, fever, paralysis, scabies, schistosomiasis, snake bite, sore throat and toothache.
- The powdered seeds mixed with dates are eaten as a purgative
- The root is used as an abortifacient and purgative.
- The leaves are used as a poultice to treat snake bite.

3.3.3. LATERAL RESEARCH:

An Overview of the Biological and Chemical Perspectives of Croton tiglium Der Pharmacia Lettre, 2016, 8 (19):324-328

ABSTRACT

Plants and their extract have the potential to cure the infirmity of mankind. From ancient times herbal plants are used for treatment. Croton tiglium Linn belongs to the family of Euphorbiaceae is widely distributed throughout the plain of India. The various part of Croton tiglium possess different biological and chemical perspectives such as anti-tumor, anti- HIV, anti- inflammatory, antidermatophytic, antioxidant activities in case of biological perspective and Toxicity, Phytochemistry, cytotoxic, detoxification activities in chemical perspectives. This plant has great prospects for development of Ayurvedic and modern medicines.

3.4. *KARUVEL* – கருவேல்

(Acacia Arabica (Linn)

3.4.1. GUNAPADAM ASPECT

SYNONYMS

Karuvelam, Karuvel

கருவேலின் போர்தனையே கருதக் கேளு
கருமகா மேதோரி மேதச்ச மாகுங்
குருவேலாங் கிருஷ்ணப் பிறட் சோதியாகும்
குருவாகுந் தீமுறுகல் பூவுக்குள்ளே
சருவேலாஞ் சற்பாரி பசு கச்சைசகஞ்
சற்றிதப் புன்னாகக் கந்தமாகுஞ்
சிறுவேலாஞ் சிலேஷ்ம பித்த சமனியாகுஞ்
செப்பியதோர் கருவேலின் சீருமாமே.

- போகர் நிகண்டு 1200

As per the above verses the synonyms of vel are *methori, methacham, krishna piratjodhi, theemurugal, poovukulae guruvahum, charpaari, pasukachaisagam, punnagakantham, sileshmapitha samani.*

VERNACULAR NAMES

Eng name	:	Indian gum arabic tree, Babul, Black babool.
Tel	:	Nalla-tumma
Sans	:	Kala-burbura
Mal	:	Karuvolum
Hind	:	Kala-babul
Kan	:	Karijali

Part used

Tender leaf, stem bark, root, gum, seed.

Organoleptic character:

Taste *(Suvai)*	:	Astringent *(Thuvarpi)*
Potency *(Thanmai)*	:	Cool *(Thatpam)*
Bio-transformation *(Pirivu)*	:	Sweet *(Inippu)*

Action

Leaf, bark, root, seed : Astringent

Gum : Demulcent, Emollient, Nutrient, Aphrodisiac

General characters

பல்லுக் கடுத்த பலநோ யகற்றி யதைக்

கல்லுக்கு நேராகக் கட்டுமே – மல்லுக்கு

நண்மின்வனை காளெனப்போய் நாளும் வியாதிகளைச்

சண்மயில் வன்னாயு தம்.

- தேரன் வெண்பா

It cures all types of dental diseases and strengthen the gums.

Bark

தந்தம் இறுகுந் தனிச்சூதப் புண்ணாறும்

வந்தசுரம் பித்தம் மடியுங்காண் - பந்த

மருவே யகலா மலரளக மாதே

கருவேலம் பட்டைக்குக் காண்

- அகத்தியர் குணவாகடம்

It cures fever, gingivitis due to mercurial medicine, pitha diseases. It strengths the teeth.

Gum

நீர்த்தொழுகும் விந்து நிலைக்கப் புரியுமெரி

பூத்தொழுகும் வெள்ளைதனைப் போக்குமிம் - மாத்திரமோ

தேசு தருமுரஞ் செய்யும் பெரியோராற்

பேசுகரு வேலம் பிசின்

- அகத்தியர் குணவாகடம்

It cures spermatorrhoea purulent vaginal discharge with burning sensation. It improves complexion of skin and act as tonic.

Root

கருவேலின் வேர்க்குக் கடுப்பிரத்தம் மாந்தம்

பருவாதம் ஊறுகரப் பானும் - பெருகு

பெரும்பேதி யும்போகும் பேசவி னிக்கும்

கரும்பே! இதனைக் கருது

- அகத்தியர் குணவாகடம்

Decoction of the root cures vadha diseases, eczema, diarrhoea, dysentery with tenasmus, indigestion.

Traditional Formulations

1. Taking decoction of 3-4gm of tender leaves of Babla (prepared by 4gm leaves in 125ml of water boiled down to 60ml), mixed with little sugar, twice daily for three days, cures diarrhoea characterized by discharge of mucous. Some medicine may

recommend mixing 3-4 gm of bark of kuruchi (Holarrhena antidysenterica) preparing decoction.

2. Washing wound with decoction of leaves (prepared with 5-7 gm of tender Babla leaves in 3 cups of water boiled down to one cup on slow fire). cures the wounds & controls further infection by pathogens. Sprinkling fine powder to Babla leaves on the wound helps it cure quickly. sprinkling fine powder of Babla leaves on chilblain (sores in feel caused by excessive use of water) cures it.
3. Applying a hot paste of 10-12 gm of Babla leaves, 20-25gm of roasted sand and 2-3 gm of khair (cutch), twice daily for 3 days. Cures, both swelling and pain of mumps.
4. 'Solid extract' of Babla tree prepared and kept preserved, cures many diseases. Take bark of stem, bark of roots, leaves flowers and fruits, crush these and boil in water four times the quantity of the mixture. Boil down the extract to one fourth of the initial volume. Sieve and simmer the extract further, stirring all along to a consistency of molten bitumen. While still hot, add 10gm of roasted sohaga (borax) for its preservation. Keep this solid extract in airtight containers, Applying a little solid extract of Babla, diluted with water, through cotton doused in it, on gums cures their swelling.
5. Applying diluted solid extract of Babla on swelling, subsides it & removes pain.
6. Gurgling with a solution of 2-3 gm of 'solid extract' in half cup of hot water cures sores in mouth. Alternatively, boil 10-12 gm of bark of Babla in 500ml of water down to 120ml, decant the extract and gurgle, four times daily, for 3 days. This gurgle cures obstinate oral ulcerations.
7. Giving vaginal douche with a solution of 2 gm of 'solid extract' in 250ml of wate, cures leucorrhea.
8. Washing wounds in nipples of lactating mothers (caused due to vigorous sucking by infants) with decoction of bark of Babla cures it.

KARUVELAMPISIN IN OTHER PREPARATIONS

1. OOZHI MATHIRAI

i. Hydragyrum subchloride - *1 pangu*
ii. Camphor - *1 pangu*
iii. Ferula asafoetetida - *1 pangu*
iv.Phyllanthus emblica - *1 pangu*
v. Myristica fragrans - *1 pangu*
vi.Piper nigrum - *1 pangu*
vii.Syzygium aromaticum - *1 pangu*
viii.Trigonella foenum graecum - *1 pangu*
ix.Acacia arabica - *1 pangu*
x. Papaver somniferum - *1 pangu*
xi.Decoction of cannabis sativa- *1 pangu*

Preparation

Powder drugs from i to x then placed in stone morter grind it with decoction of cannabis sativa for 3 hours into fine pill rolling consistency make into 130 mg pills.

Dose : 130 mg
Adjuvant : Hot water
Indication : Cholera, diarrhoea, bacillary dysentry.

\- *Siddha vaithya thirattu*

3.4.2. BOTANICAL ASPECT

KARUVEL (ACACIA ARABICA (LINN)

Taxonomical classification

According to Benthen and Hooker's classification Acacia arabica is classified as follows

Kingdom	:	plant kingdom
Division	:	angiosperms
Class	:	dicotyledons
Sub-class	:	polypetalae
Series	:	calyciflorae
Order	:	fabales
Family	:	leguminosae/fabaceae
Sub-family	:	mimosoideae
Genus	:	acacia
Species	:	nilotica

Habitat

Indigenous to sind (Pakistan) Afghanistan and Nothern India. Wild or cultivated and naturalized throughout India but not in the most humid regions on the western coast and in the extreme Northwest where frost is too severe in winter. Also found in tropical Africa and Arabian countries.

Habit

A moderate sized, thorny, almost evergreen, wild (or) cultivated xerophytic tree with a short trunk and a spreading crown of feathery foliage.

Description

Root

Tap much branched, deep.

Stem

Erect, aerial, woody, solid, cylindrical, branched, bark dark brown, rough with deep narrow longitudinal fissures, heartwood pale red, when fresh cut nearly colourless, turning reddish brown on exposure to air

Leaves

Stipulate, cauline and ramal stipules modified into spines that are straight, 1.5-5cm long, turning white in the older branches, petiolate, alternate, 2.5-8cm long,

bipinnately compound, pinnaetly 3-6 pairs, subsessile, opposite, cup shaped glands at the base of lowest and uppermost pair, leaflets small, oblong, opposite, subsessile, entire, obtuse 1.5-4cm long, 10-20 pairs, venation unicostate reticulate.

Inflorescence

Axillary cymose heads globose, on short or medium sized peduncles, heads about 1.5cm in diameter, yellow.

Flower

Ebracteate, subsessile, small complete, hermaphrodite, regualr, actinomorphic, hypogynous, cyclic, yellow, fragrant.

Calyx

Sepals 5, gamosepalous, campanulate, 5-toothed, slightly petaloid, inferior.

Androecium

Stamens indefinite, poly androus with long filaments and anthers, minute, yellow, dithecous, versatile.

Gynoecium

Carpel, ovary superior, unilocular, sessile, green, hairy, oval with small white glands, style long, filiform, stigma minute, placentation marginal, ovules numerous.

Fruit

A lomantaceous pod, much constricted between seeds at both sutures, whitish, tomentose, 8-15cm long, stalked, fleshy, grey seeds non-endospermic, 8-12 in a single row.

Flowering

Rainy season and cold season. (June- december)

Sprouting

Never leafless but young foliage comes out in Feb-April,

Microscopical characters

Periderm

20-25 layered, cells thinwalled flattened, tanniniferous, mostly rectangular, measuring T-49-56-70 X 21-28-58µ, a few lenticels are formed by the rupturing of cork. pellogen: 8-12 layered, cells rectangular, phelloderm many layered, cells ovate to elongate, tanniniferous. Stone cells present in patches. Sclerenchyma is in the form of a ring next to secondary cortex, 2 to many layered cells polygonal, lamellated, some developing into stone cells with narrow lumen, tanniniferous, measuring T-21-28-42µ.

Macroscopical characters
Gum

Gum is obtained by making incisions into the bark. It occurs in irregular or broken tears of various sizes agglutinated masses and angular fragments or pieces. The thick pieces are opaque, the thin ones are transparent. Each tear is about 1.5cm in diameter. The gum is brittle, the taste is bland mucilaginous. Makalai gond is of the best quality, colourless, occurs in large round tears and twisted pieces of a white colour. That of an inferior quality is known as mesarigond. It is in angular pieces which are fissured, strong yellowish red or straw colour or brownish yellow with a tinge of white, soluble in water forming a sticky solution.

Chemical constituents

Various constituents are Gum : galactose, L-arabinose, L-rhamnose, and 4 aldobiocronic acids, a new arabinobinose 2-0-β-L-arabinofuranosyl L-arabinose along with 3-0-β-L-arabino pyranosyl –L-arabinose.

Bark-quercetin, gallic acid, catechin (-) epicatechin (+) dicatechin (+) leucocyanidin isolated, epigallocateatin, catechol, pyrogallol.

Seeds-glucose, galoctose, fructose, maltose, aspartic acid, glutamic acid, glycine, alanine, proline, leucine and theronine.

Therapeutic uses

- Bark is used for asthma, bronchitis, diabetes, dysentry, diarrhoea & skin diseases.
- Stem is used for tooth brush & gum for burns
- Leaves are useful for eye diseases, head ache, throat infection, urinary tract infections & gonorrhoea.
- The flowers are used for ear diseases and as tonic.
- Bark is used in haemorrhages, wounds, ulcers also used as a decoction in the treatment of diarrhoea and vaginal secretions.
- Aphrodisiac, diuretic, used to treat leprosy, leucoderma, bronchitis, seminal weakness, utero-vesical disorders.
- The bark in combination with the bark of the ficus bengalensis Linn are frequently used as a gargle in sore throat, in excessive bleeding from haemorrhoids.
- The powder of the tender pods is astringent, demulcent and has a beneficial influence over diarrhoea and dysentry
- Gum powder is used to stop bleeding from leach bites.

3.4.3. LATERAL RESEARCH

Gum Arabic Fibers Decreased Inflammatory Markers and Disease Severity Score among Rheumatoid Arthritis Patients, Phase II Trial International Journal of Rheumatology

Volume 2018, Article ID 4197537, 6 pages

Background. Rheumatoid arthritis (RA) is autoimmune inflammatory disease that attacks the synovium of the joints. Both TNF and interleukin-1 play crucial roles in the pathogenesis of RA. Gum Arabic (GA) is gummy exudates from*Acacia senegal* tree. Gum Arabic fermentation by colonic bacteria increases serum butyrate concentrations, so it is considered as prebiotic agent. Gum Arabic (GA) has anti-inflammatory activity through its derivative butyrate. To the best of our knowledge, this is the first study conducted to investigate GA intake on inflammatory markers among RA patients. *Patients andMethods.* This is clinical trial phase II in which 40

patients were enrolled aged 18 to 70 years. Patients received 30g/day GA for 12 weeks. TNF □, ESR, and complete blood count were measured and DAS-28 was calculated before and after regular GA consumption. Study was approved by the Ethical committee of NationalMedicines and Poisons Board. *Results.* This study showed significant decrease in level of serum TNF □ (p value 0.05) [95% CI, 0.65 -16.5], ESR (p value 0.011) [95% CI, 2.6 -18.89], and number of swollen and tender joints in RA patients after 12 weeks of GA intake which reflected as significant decrease in disease severity score DAS 28 P.V:0.00 [95% CI, 1.25 -1.99]. On the other hand, GA had trivial change in blood indices. *Conclusion.* Gum Arabic has favorable immunemodulator effect on rheumatoid arthritis. It can be utilized in clinical practice as adjuvant therapy. *Trial Registration.* This trial was registered with ClinicalTrials.gov Identifier:

NCT02804581 Registered at 19 June 2016, prospective registration.

3.5. *KATRAZHAI* – கற்றாழை
(Aloe Barbadensis)

3.5.1. GUNAPADAM ASPECT

Synonyms : *Kanni, Kumari*

Vernacular names

Eng : Indian aloe, curacao aloe

Tel : Kalabanda

Mal : Kattuvazha

Kan : Kathalai gidsa, lolisara

San : Kumari

Hin : Ghikauvar

Part used

Latex, juice, root

Organoleptic character

Taste *(Suvai)* : Slightly bitter *(Siru kaippu)*

Potency *(Thanmai)* : Coolant *(Thatpam)*

Bio-Transformation *(pirivu)* : Sweet *(Inippu)*

Action

Tonic *(Uramakki)*

Alternative *(Udarthetri)*

Purgative *(Neermalampooki)*

Emmenagogue *(Ruthu-undaki)*

General character

"பொல்லாமே கங்கபம்பு முச்சூலை குட்டரசம்

அல்லார்மத் கம்பகந்த ரங்குன்மம் எல்லாம்விட்

டேகு மரிக்கு மெரிச்சற் கிரிச்சரமு

மாகு மரிக்கு மருண்டு"

- தேரன் குணவாகடம்

It cures leprosy, fistula, hemmorhoides, gastric ulcer.

Traditional uses

- ❖ Aloevera leaf extract along with green cumin and sugar candy is used to treat bloody diarrhoea.
- ❖ Aloevera leaf juice is given for dehydration due to cholera and also applied exernally for swelling.
- ❖ Oil prepared from the leaf juice promotes hair growth and sleep.

KATRAZHAI IN OTHER PREPARATIONS

1. ARUMUGA CHENDOORAM

Dose	:	63-130mg
Adjuvant	:	Thirikadugu chooranam, Honey
Indication	:	Paraplegia, Hydrocele, gastric ulcer, Hemmorhoids, Chest pain, Anemia, diarrhoea, Headache, throat pain.

- *Siddha Vaithya thirattu*

2. SUYAMAKINI CHENDOORAM

Dose	:	130mg
Adjuvant	:	Ginger juice
Indication	:	Ascitis, Hemmorhoides, diarrhoea and vatha diseases

- *Siddha vaithya thirattu*

3. PATCHAI KARPOORA MATHIRAI

Dose	:	100mg
Adjuvant	:	Honey, Ginger, Juice
Indicaiton	:	Fever, 80 types of vatha diseases, 40 types of pitha diseases, 96 types of kabha diseases

- *Siddha vaithya thirattu*

4. VENPOOSANI NEI (KOOZHPANDA KIRUTHAM)

Dose	:	5-10ml
Indication	:	Leuchorrea, Tongue diseases, leprosy, tuberculosis

- *Agathiyar vaithya rathna surukkam*

5. VELVANGA PARPAM

Dose	:	65-135mg
Adjuvant	:	Butter, ghee, honey
Indication	:	Hemorrhoides and fistula

- *Hospital pharmacobia*

6. SHAYA KULANTHAGA CHENDOORAM

Dose	:	30-130mg
Adjuvant	:	Thirikadugu chooranam, Honey
Indication	:	Tuberculosis, cough, Asthma

- *Agathiyar bin-80*

7.MAGA VASANTHA KUSUMAGARAM

Dosage : 130mg

Adjuvant : Amukkara chooranam, vilam pazham, ghee

Indication : Arthritis, Indigestion, swelling, fever.

8. LINGA CHENDOORAM

Dose : ½ -1 kundri (65-130mg)

Adjuvant : Milk Halwa, Butter, wheat halwa.

Indication : Arthritis, Blood purifier and nervine tonic

- Anuboga vaithya navaneetham

9. VEERA CHENDOORAM

Dosage : ½ kundri (65mg)

Adjuvant : Palm jaggery, ginger juice, honey.

Indication : Arthritis, gastric ulcer, indigestion.

3.5.2. BOTANICAL ASPECT

KATRAZHAI (ALOE BARBADENSIS)

Toxonomical classification

Kingdom	:	Plants
Devision	:	Magnoliophyta
Class	:	Monocotyledeons
Subclass	:	Lilidae
Order	:	Liliales
Family	:	Liliaceae
Genus	:	Aloe
Species	:	Barbadensis

Habitat

An evergreen perennial, it originate from the Arabian peninsula, but grows wild in tropical climates around the world and cultivated for agriculture and medicinal uses.

Description

Habit

Aloe vera is short stemed shrubby aloe, frequently suckering and forming dense clumps.

Leaves

The leaves of the aloe vera are succulent, thick and fleshy. The slightly curved, dagger shaped foliage grows as a rosette out of the plant's base and each plant generally has 16 to 20 leaves. Each leaf grows 18 to 36 inches long and 2 to 3 inches wide. The gray-green (or) pale green upper leaf surface, sometimes variegated with small white dot, is mostly flat, while the lower leaf surface is convex. Aloevera leaf margins are slightly pinkish and have plae, firm and triangular teeth.

Flowers

Aloe vera flowers appear on spikes upto 3 feet tall. The flowers are yellowish-orange and tubular. They are arranged in an arrow shaped cluster atop the stalk. Tend to flower in spring but may bloom sporadically throughout the year.

Stems and Roots

Aloevera plants are stemless (or) have a short stem not more than about 12 inches tall. The plants do produce shallow rhizomes and stolons that allow spreading and the formation of dense calories of plants.

Root

Multiple tuberous roots store moisture while a fibrous system of small root anchors the plant and absorbs moisture and nutrients.

Fruits and seed

The fruit of the plant is a capsule that open vertically. The plant seed are ½ to 3/10 inch long, dark brown and winged.

Microscopic character

A strong cuticularized epidermis with numerous stomata on both side of surfaces which enclose a region of parenchyma containing chlorophyll, starch and occasional bundles of needle of calcium oxalate a centre region which frequently occupies about three-fifth of the diameter of the leaf, consist of large, mucilage containing parenchymatous cells which cover the doubble row of vascular bundles which lie at the junction of two previous zones and have a well marked pericycle and pericycle cells.

Macroscopic character

Perennials, stem-1.5meter high, woody, rough from leaf-remnants, leaves glaucous green often with darker spots, thick, succulent, bayonet-shaped, margin with reddish spines (or) serratures flowers racemose (or) spicate, tubular, yellowish orange-red, stamens 6, unequal, 3 longer than corolla.

Chemical constituents

❖ It contain 19 amino acids, 7 essential amino acid such as isoleucine, leucine, lysine, methionine phenylalanine, threonine, valin.

- Anthroquinones
- Enzymes (Alkaline phosphatase, cellulase, lipase etc)
- Hormones (Auxin, Gibberiellin)
- Ligiuns
- Salicylic acid
- Saponin
- Sterols and vitamins

Theraputic uses

1. 1.Extract of leaves is used for treating lymphatic obstruction, arthritis, myopathies.
2. Juice of leaves is reported to be used for treating gonorrhoea and mensural problems.
3. The pulp is also used as vermicide.
4. The phenolic and non-phenolic fraction of the leaf extract showed anti-microbial activity comparable to standard antibiotics such as neomycin, tetracycline and co-trimaezole.
5. Crushed leaves are applied on forehead for headache.

3.5.3. LATERAL RESEARCH:

EVALUATION OF ANTI-INFLAMMATORY ACTIVITY AND ANALGESIC EFFECT OF ALOE VERA LEAF EXTRACT IN RATS Thirunethiran Karpagam et al. IRJP 2 (3) 2011 103-110

ABSTRACT:

Clinical evaluation of analgesic and anti-inflammatory drugs envisages the development of side effects that makes efficacy of a drug arguable. Alternatively, indigenous drug with fewer side effects is the major thrust area of research in the management of pain and inflammation. In the present study aqueous extract of whole leaf of *Aloe vera* at various concentrations was investigated for its antiinflammatory and analgesic activities in albino wistar rats. Carrageenan and formaldehyde-induced rat paw oedema was used to evaluate the anti-inflammatory activity and tail flick, hot plate and acetic acid tests were used to assess the analgesic activity of *A. vera* leaf aqueous extracts. Whole leaf aqueous extracts at various concentrations (100, 200, 400, and 600 mg/kg of bw) significantly reduced formation of oedema induced by carrageenan and formaldehyde and granuloma formation in a dose dependent manner. Further, acetic acid-induced writhing model exhibited significant analgesic effect characterized by reduction in writhes. Whole leaf aqueous extract showed dose dependent increase in tolerance to thermal stimulus comparable to indomethacin. No mortality was observed during the acute toxicity test at
a dosage of 600mg/kg. Thus whole leaf aqueous extract of *Aloe vera* can be exploited as non toxic drug for the treatment and clinical management of inflammation and pain.

3.6. DISEASE REVIEW

KEEL VAYU

3.6.1. SIDDHA ASPECT

Definition:

Keel vayu is a Vadha disease characterized by pain and swelling of the joints, stiffness of the muscles and joints with tenderness frequently associated with fever, insomnia etc. it may be accompanied by emaciation, anemia and restriction of joint movements and in some cases even immobility can also occur.

"வளியு மையுந் தன்னிலை கெட்டு
வலியுடன் வீக்கச் சுரமும் காய்ந்து
மூட்டுக் டோறும் முடுக்கியே நொந்து
மூட்டுக டன்னின் நீரும் சுரந்து
தாங்கொணா வலியுடன் தொந்திடு மம்மே"

(சபாபதி கையேடு)

Etiology:

"வளிதரு காய்கி ழங்கு
வரைவிலா தயிலல் கோழை
முளிதயிர் போன்மி குக்கு
முறையிலா வுண்டி கோடல்
குளிர்தரு வளியற் றேகங்
குனிப்புற வுலவல் பெண்டிர்
களித்தரு முயக்கம் பெற்றோர்
கடிசெயல் கருவியாமால்"

Classification of Keelvayu:

Classification of Keelvayu are described in different Siddha literature differently. In the text Siddha maruthuvam Keelvayu is classified and described according to Mukkutra theory.

On the basis of Mukkutra theory it is classified into 10 varieties:

1. Vali Keelvayu
2. Azhal Keelvayu
3. Iya Keelvayu
4. Vali azhal Keelvayu
5. Vali iya Keelvayu
6. Azhal vali Keelvayu
7. Azhal iya Keelvayu
8. Iya vali Keelvayu
9. Iya azhal Keelvayu
10. Mukkutra Keelvayu

வளிக்கீல்வாயு :

"வலிக்குத்தல் வீக்கங் காணும்
வாய்தொண்டை வறட்சிகாய்ச்சல்
தலைவலி மார்து டிப்புத்
தாங்கொணா வலிவீக் கந்தான்
நிலவுகாற் கணுக்கு றங்கு
நீடுதோள் முழங்கைக் காற்காம்
மலக்குடற் கட்டு வேர்வை
வாதத்தில் வாய்வி தாமே."

(சபாபதி கையேடு)

It is characterized by excruciating pain and swelling involved in toes, knee joints, hip joints, elbow joints, shoulder joints and associated with systemic disturbances like dryness of mouth, pyrexia, headache, palpitation, constipation and sweating.

அழல் கீல் வாயு :

"பித்தக்கீல் வாய்வு தன்னாற்
யிறங்குகீன் முட்டு வீங்கிச்
சித்தர்செய் மருத்து வத்துஞ்
சீர்படாத் தன்மைத் தாகித்
தத்தறு காய்ச்சல் கண்டு
சாலவே தனைதான் தந்தே
மெத்தறு சிகிச்சை தன்னால்
மென்மேல் நீங்கு மப்பா."

It is characterized by swelling of joints associated with severe pain and pyrexia. Since it is not quickly responding to medicine the prolonged medical care is said to be essential. As Pitha increases, kaba in the joint decrease and hence dryness occur. So during flexion of the joint crepitation sound " kaluk kaluk" is produced. In advanced cases it produces ankylosis of the joints and hence restriction of joint movement results.

ஐயக்கீல் வாயு:

"கருதருங் கபக்கீல் வாயு
கண்டின் உடலி ளைக்கும்
உருமெலி வாக்குங் கொள்ளும்
உண்டியைக் கருக்கும் இன்பந்
தருதுயில் நீங்கு முட்டிற்
றாங்கொணா வலுவை யாக்கும்

இருமலே விக்கல் வாந்தி

சோபைபாண் டெழுப்பும் பாரே

(சபாபதி கையேடு)

It is characterized by severe pain in the joints associated with Emaciation of the body, anorexia, insomnia, cough, hiccup-cough, vomiting, anemia and dropsy. The common sites are spinal cord, hip joint and knee joint.

வளிஅழல் கீல் வாயு:

"வாதபித் தக்கீல் வாய்வின்

வருங்குறி சாற்றக் கேளாய்

ஏதமார் மந்த மேப்பம்

இரைச்சலும் வயிற்றிற் காணும்

ஓதருங் குத்தல் வீக்கம்

ஓய்தலில் எரிச்ச லுண்டாம்

காதறு முறக்க மின்மை

காய்ச்சலும் காணுங் கண்டாய்"

Vali azhal keelvayu have the symptom of indigestion i.e., mantham, aeppam (eructation), borborygmus of the abdomen. This disease occurs due to excessive intake of foods which increases Vadha and Pitha (ex. Mutton, egg, fish, potato) and frequent intake of liquors. Laziness may also be produces this disease.

வளி ஐயக் கீல்வாயு:

"ஐயினை விலைக்கு முண்டி

யயிறலே கூதிர்க் காற்று

மெய்யினை யலைக்கு மாங்கண்

மேவலோ டோதஞ் சார்ந்த

வையத்தின் உறங்கன் மாரி

பனியினான் வாட்ட மெய்தல்

மெய்யயர் வுறவு ழைத்தல்

கவலையான் மேவு மிந்நோய்

வயங்வா தக்க பக்கீல்

வாயுவான் வலிமி குந்தே

உயங்குநீர் கோத்துக் கீல்கள்

ஓரியன் தலைபோற் காணும்

நயங்கொள்ள முடக்கல் நீட்டல்

நண்ணிடா மெய்யுங் காயும்

மயங்குறு முறக்க மின்னாம்

மன்னிய நெரிக்கட் டாமே

உடலது வெதும்பிக் கையால்

உடலது கடுத்து நொந்து

கடலுதாங் கால்க ரங்கள்

கனத்தாற்போ லுயர்ந்து காணும்

சடமது விழுந்த தாகுஞ்

சலங்கெட்டு தோட முண்டாம்

முடமதாங் கைகால் தானு

முயங்கின வாத மாமே"

(சபாபதி கையேடு)

It is characterized by pain in the joints associated with effusion of joint fluid and swelling, restricted joint movements, pyrexia, fainting, insomnia, lymphadenitis, generalized malaise, atrophy of affected limb etc. The affected joint looks like as "Fox's head".

அழல்வளிக்கீல் வாயு:

"வெயிலிடைத் திரிதல் பித்த

மிகுமுண வருந்த லுள்ளம்

பயிலுறு கவலை யாதிப்

பண்பினால் பித்த வாதம்

கயிலுறு வாய்வு தோன்றிக்

கைப்புடன் மயக்கம் வாந்தி

இயலுறு பல்லிற் செந்நீர்

இறங்கநால் நோக்கங்கொள்ளும்."

(சபாபதி கையேடு)

It is characterized by pain and swelling of the joint associated with bitter taste, vomiting, fainting, bleeding from the gums, malena, haematemesis. The common site is ankle joint pain, swelling, redness may also present.

அழல் ஐயக்கீல் வாயு:

"மிதமிலாக் கலவி யையை

மிகுவிக்கு முண்டி பித்தக்

கதமுறு செயலி வற்றிற்

காண்பித்தக் கபக்கீல் வாயு

இதமறு மயக்கம் வாந்தி

எரிசுரந் தலைநோய் வீக்கம்

மதகரி நனயின் மார்பு

துடிப்புடன் எரிவும் செய்யும்”

(சபாபதி கையேடு)

It is characterized by pain and swelling of the joints associated with fainting, vomiting, hyperpyrexia, headache and palpitation. Common sites are elbow and knee joints.

ஐய வளிக்கீல் வாயு:

“ஐவளிப் பெருக்கு முண்டி

அணங்குடன் கலவி மற்று

ஐவளி வினைமு ழக்கே

அளவிலா மதுவ ருந்தல்

மெய்யுறு மேக வாதக்

கோழையின் மிகுதி காணல்

ஐவகை கபவா தக்கீல்

வாயுவன் அறிகூ றாமல்

நரித்தலை வாத நரம்பு

கள்வாயு கோபித் தொன்றாய்

அரிந்திடுந் தலைமேல் கைகால்

அங்கமொடு வளைந்து வெம்பி

முரித்தது இறங்கி வந்து

முழங்கால் கை கால்க டுக்கும்

நரித்தலை வாத மென்று

நவின்றனர் முனிவர் தாமே”

(சபாபதி கையேடு)

It is otherwise called as *Narithalai Vadham* characterized by pain and swelling of the joints which looks like fox head and body pain.

This clinical entity mainly involves the knee joints were accumulation of blood in the joint which result in immobilization of the joint with stiffness of periarticular structures, the swelling which resembles to fox head and palpitation may occur.

ஐயத் தீக்கீல் வாயு:

“ஐயினைப் பெருக்கு முண்டி

யுயிலலாற் குளிருங் காற்றால்

மெய்யுறு கோழை மிக்கு

மிகுவலி தொண்டை கட்டல்

ஐயுறு காய்ச்சல் வாந்தி

அயர்வுடன் இருமல் வீக்கம்

செய்யுகீல் மடக்கல் நீட்டல்

செய்திடாத் துயருண்டாமே.”

It is characterized by excruciating pain in the joints associated with increased sputum, laryngitis, pyrexia, vomiting, drowsiness, cough, odema and restricted joint movements etc.

முக்குற்றக் கீல் வாயு:

“மிக்குற வியர்த்தல் மூச்சு

மேலிடல் தலைகி றுத்தல்

மக்குறு மயக்கந் தோன்றல்

வாந்தியே வாய்பி தற்றல்

பொக்குறு மலநீர் கட்டல்

பொருமிய வீக்கங் காணல்

முக்குற்றக் கீலின் வாயு

முகிழ்த்திடுங் குறிக ளாமே.”

It is the chronic ailment characterized by pain and swelling of the joints which increased day by day, suppuration of the surrounding and adjacent structures associated with excessive sweating, dyspnoea, giddiness, syncope, vomiting, delirium, constipation, anuria, odema. If this is treat improperly can lead to fatal termination.

ARTHRITIS

3.6.2. MODERN ASPECT

Diseases of Joints

A joint is where two or more bones come together, like the knee, hip, elbow, or shoulder. Joints can be damaged by many types of injuries or diseases, including

- Arthritis - inflammation of a joint. It causes pain, stiffness, and swelling. Over time, the joint can become severely damaged.
- Bursitis - inflammation of a fluid-filled sac that cushions the joint
- Dislocations - injuries that force the ends of the bones out of position

Diseases of Joints and their classifications:

Joints are subjects to various types of diseases and disorders. Many lesions which are not strictly inflammatory are still loosely termed as arthritis.

Diseases of joints can be classified as follows:

1. Infective Arthritis
2. Rheumatoid Arthritis
3. Degenerative Arthrosis (osteoarthritis)
4. Neuropathic arthropathy
5. Metabolic Arthritis
6. Arthritis in systemic disorders
7. Miscellaneous conditions
8. Hysterical joints

Degenerative Arthritis

Osteoarthritis:

Osteoarthritis is a chronic degenerative condition of the joints. It is not primarily an inflammatory disorder.

Aetiology:

The major factor leading to osteoarthritis is ;uneven distribution of load stress' across the articulating surfaces in a joints.

Osteoarthritis can be broadly grouped into

- Primary Osteoarthritis
- Secondary Osteoarthritis

Primary Osteoarthritis:

Primary osteoarthritis is due to the wear and tear changes in old age in which weight bearing joints such as the hips and knees are commonly affected. It is uncommon in non-weight bearing joints such as the shoulder and elbow. Obesity is a predisposing factor.

Osteoarthritis is a progressive process affecting the articular cartilage of ageing joints. It is characterised by focal degeneration of the articular cartilage. As the articular cartilage is cyclically loaded during movements of joints, it undergoes fatigue failure leading to fragmentation of the surface and fibrillation. In the later stage, the cartilage gets completely eroded, exposing the sclerosed (eburnated) bone and subchondral cysts are also found.

The bone undergoes reactive hypertrophy forming peripheral osteophytes. The synovial membrane undergoes hyperaemia and reactive inflammatory thickening. As there is no distructive pathology, the joints does not get ankylosed.

Secondary osteoarthritis:

Secondary osteoarthritis refers to arthritis occurring in a joint secondary to a previously occurring disease or disorder of the joint. It may occur in any age and involve any joint. Factors associated with secondary osteoarthritis are as follows:

1. Congenital malformation of joints
2. Traumatic alteration of articular surfaces causing articular incongruence
3. Loose bodies in the joint
4. Deformity of the joints, for example coxa vara, genu varum
5. Hip

   Perthes disease

   Slipped epiphysis

   Avascular necrosis of the formal head
6. Internal derangement of knee

Pathology:

The main factor associated with osteoarthritis is an abnormal increase in the mechanical stress in some part of the articulating surfaces.

- Initial changes occur in the articular cartilage. There is an increase in the water contents and decrease in proteoglycans in the cartilage, leading to progressive cartilage destruction.

- Articular cartilage destruction leads to its softening, fraying and fibrillation.
- As soon as the articualr cartilage is destroyed, the underlying subchondral bone is exposed; it looks tough, sclerotic with ivory-like smoothness (eburnation)
- Subchondral sclerosis and subchondral cysts are seen.
- Bony projections and new bone formation (osteophytes) Occur within the joints
- Thickening of the joint capsule and synovium leads to stiffness and deformity of the joint.

Clinical Features

Since the patients are usually elderly, weight- bearing joints are commonly involved.

- The Patient presents with Pain that starts insidiously and increase over time,
- Swelling- as a result of joint effusion,
- Stiffness from the thickening of capsule and synovium,
- Crepitus-while moving the joint,\
- limping-due to pain and deformity of the joint.

On examination, the joint is

- Tender and swollen,
- Synovium-thickened,
- Restricted movements,
- Deformities such as genu varum/genu valgum may be present,
- Joint instability may occur,
- Muscle wasting around the involved joint is usually seen.

Osteoarthritis Knee

Primary osteoarthritis of the knee is a common clinical problem in our country. The patient is usually a male above 50 years presents with pain, swelling and restricted movements and inability to squat in the Indian toilet. The onset is insidious starting with a mild aching pain in the joint which is relieved by rest. The other main symptom is stiffness, which is maximum at the end of the long period of rest and loosens on activity for a few minutes.

The cause of secondary osteoarthritis in the knee joint is alterations in the congruity of the articular surfaces due to various lesions, such as

- malunion of fractures involving the articular surfaces of tibia, femur or patella,
- loose bodies in the joint,
- malligament of the bone due to deformity such as genu valgum or genu varum

Clinical features

On examination, the joint is swollen and there may be an effusion into the joints. The synovium is thickened and tender. There is a tenderness in the joint line and over the attachment of the medical collateral ligament. Movements are restricted. Crepitations are felt on movement. In late cases, there is often a genu varum deformity.

Rheumatoid Arthritis:

Collagen diseases are systemic diseases affecting all connective tissues in the body.

Many of these disorders have joint manifestations. The most important of these is rheumatoid arthritis. Rheumatoid arthritis is a generalized chronic multi system disease affecting the connective tissues of the whole body with localized involvement of the musculoskeletal system.

There is inflammatory synovitis of peripheral joints, leading to cartilage damage, bone erosions and subsequent joint changes.

Aetiology:

Aetiology remains unknown. The following are put forth as possible aetiology factors:

Genetic predisposition: Rheumatoid arthritis runs in families. It is associated with Class II major histocompatability complex allele HLA-DR4 and HLA-DRBI. Genetic factors alone do not account for the disease.

Abnormal Immune response: Rheumatoid arthritis may be a manifestation of an immune-mediated response to infections caused by mycoplasma, Epstein-Barr virus, cytomegalovirus, parvovirus in a genetically predisposed individual.

Clinical Features:

Rheumatoid arthritis is more common in women and occurs between 25 and 40 years of age. It is a chronic disease with periodic acute exacerbations and remissions. Morning stiffness is very characteristic of rheumatoid arthritis. It is usually involves the small joints of the hands and feet and later on spread to the proximal joints such as the

knee, hips, elbow and shoulder. Bilateral symmetric polyarthritis is also characteristic feature.

Occasionally, it may start in the knee or hip and remain as a monoarticular lesion for some time, but soon it spread to other joints. The presenting joint is swollen and warm. There is joints line tenderness and the movement are painful and limited. There is effusion into the joint. The synovium could be felt thickened and tender. In the knee joint, the swelling cause a fusiform appearance with pericapsular swelling. In the stage, with progressive damage to the articular cartilage, there will be an increase in infection deformity, ultimately ending in ankylosis.

If the hand, the small joints are swollen and the finger assume a position of 'ulnar deviation', clinically called 'ulnar drift'. The fingers assume an' intrinsic plus' deformity which consists of flexion at the metacarpophalangeal joints and extension at the interphalangeal joints. The end stage of the disease is market by ankylosed joints.

The 'intrinsic plus' position of the finger is due to the spasm of the intrinsic muscles of the hand which later become a contracture. The other deformities in the hand are as follows:

- **Boutonniere** (buttonhole) deformity due to the rupture of the central slip of the extensor tendon resulting in flexion at the proximal interphalangeal joints with hyperextension at the distal interphalangeal joints.
- **Swan neck deformity,** where there is hyperextension at the proximal interphalangeal joints and flexion at the distal interphalangeal joints. In the late stages, he capsular contractures become fixed, resulting in a permanent crippling derformity
- Elbow- flexion deformity
- Knee-flexion deformity followed by triple subluxation: flexion, posterior subluxation and external rotation.
- Ankle-equinus deformity
- In the foot, patients develop hallux valgus of the great toe, claw toes, hammer toes and callosities.

  Other joints involved in order of frequency are as follows:

    - Foot
    - Wrist
    - Knees, elbow

- Ankle
- Hip
- Temporomandibular joints
- Interspinous facet joint (rare)

Juvenile Rheumatoid Arthritis

Juvenile rheumatoid arthritis is a clinical variant of rheumatoid arthritis in children and adolescents.

This is of the following three types:

1.Systemic type, called still's disease:

The child is usually less than 3 years, presenting with pyrexia, malaise, rashes and polyarthritis. Other feature are as follows:

Lymphadenitis

Hepatosplenomegaly

By puberty, stunting of growth occurs

2. Polyarticular type:

This involves the joints of the hands and feet. Temporomandibular joints and the cervical spine are typically involved.

3. Pauciarticular type:

This involves less than four joints, usually the large joints. This is the commonest type. Usually, medium-sized and large joints are involved.

Clinically, the orthopaedic manifestation includes stiffening of joints, premature closure and deformities of the joints. Treatment is in the same line as for the adult type.

3.7.PHARMACEUTICAL REVIEW

3.7.1. SIDDHA ASPECT OF THE FORMULATION

PILL (MATHIRAI)

DEFINITION OF PILL

The raw drugs are triturated with the juices of leaves or kudineer. They are rolled into different sizes of pills, dried and stored.

RULES OF TRITURATION

The ingredients should be first purified and powdered into a find powder and then maserated with the prescribed juices or liquid one after the other in their order. Each time it must be grinded till it becomes waxy in consistency does not adhere to the fingers or mortar and pestle, lastly it must be made into pills as prescribed and dried in shade.

ADDITION OF AROMATIC INGREDIENTS

Aromatic ingredients were added just before 24mins (1 naazhigai) of pill rolled from finely grounded paste. The aromatic ingredients added pills should allowed to dry in shade and keep them in a tightly closed container.

SIZE AND SHAPE

Usually round in shape, there fore it also called as 'Urundai'. But it may differ in some preparations. e.g. Urai mathirai. The size, shape and weight of the pills should be made as said in the literature evidence.

SHELF LIFE

1 year

3.7.2. MODERN ASPECT OF THE FORMULATION

DEFINITION OF PILLS (MATHIRAI):

A tablet is a pharmaceutical dosage form it otherwise called as caplet. Medicinal tablets are called as "pills". Originally "pills" referred specifically to a soft mass rolled into a ball shape, rather than a compressed powder.

As per Indian pharmacopeia 2007 defined the tablets are solid dosage forms each containing a unit dose of one or more medicaments. They are anticipated for oral route. A tablet consists an active medicament with excipients which are in powder form are compressed or pressed into a solid dosage form. About two third drugs prescribed are in solid dosage form and tablets include half of them.

Classification

As per IP 2007 tablets are majorly classified into following categories (Indian pharmacopoeia 2007).

1. Uncoated Tablets

This type of tablets contains single layer or more than one layer tablet consisting of active ingredient with the excipients, no additional cover is applied onto it after the compression.

2. Coated tablets

Coated types of tablets have an additional coating layer on it after the tablet was compressed, the coating layer of tablets formed with sugar, gums, resins, inactive or insoluble fillers, plasticisers, polyhydric alcohols, waxes.

3. Dispersible tablets

These are the film coated or uncoated tablets because a uniform dispersion when suspended in water.

4. Effervescent Tablets

These type of tablets which are uncoated and are planned to be dissolved and produce an dispersion before they are administered the dissolution is achieved by the reaction between an organic acid and bicarbonate which produce Co_2, thus produced Co_2 will disintegrate the tablet so which dissolves in the solution to produce an suspension which was rapidly absorbed.

5. Modified-release tablets

These types of tablets are the coated or uncoated tablets which are designed in such a way that the rate or location of the active ingredient released is modified. It includes enteric coated tablets, prolong release tablet or delay release tablet.

A. Enteric- coated tablets

These are also called as gastro resistant tablets as they resistant to the gastric juices; these are formulated by coating the tablet with anionic polymer of methylacrylic acid and their esters or by coating with cellulose acetyl pthylate. Ex. Erythromycin, NSAIDS.

B. Prolonged –release tablets

These types are otherwise called as sustain release tablets or extended release tablets was formulated in such a way that the active ingredient is released for a prolong duration of time and is available in systemic circulation after administration.

C. Delayed –release tablets.

This dosage form was planned to release the drug after some time delay or after the tablet has passed one part of the GIT into another. All enteric coated tablets are type of delayed action tablet but all delayed action of tablets was not enteric or not intended to produce enteric action.

6. Soluble tablets

These are coated or uncoated tablets which are planned to dissolve in water before they are administered.

7. Tablets for use in the mouth:

These are the tablet formulations which are planned to be show local action in the buccal cavity. These include buccal tablet, sublingual tablets and troche or lozenges. Buccal tablets are placed in between the cheek and gingival. Sublingual tablets are placed below the tongue.

Eg : glyceryl trinitrate

8. Tablets for other routes of administration

These include implantable tablets and vaginal tablet. These are inserted into the rectum or vagina for their local or systemic action.

4. MATERIALS AND METHODS

4.1. PREPARATION OF THE DRUG

Karpoora Sinthamani Mathirai has been selected from classical siddha literature Anuboga Vaithiya Navaneetham, Part – IV Page No.106

Ingredients of the test drug are *Rasa karpooram, Sathikkai, Nervalam, Karuvelam pisin, Katrazhai saru.*

COLLECTION OF THE DRUG

The raw drugs Rasa Karpooram, Sathikkai, Nervalam, Karuvelam pisin were purchased from authorized drug store in Nagercoil at Kanyakumari district.

IDENTIFICATION AND AUTHENTICATION OF DRUGS :

The raw materials were identified and authenticated by the experts of PG Gunapadam Dept, Government Siddha Medical College, Tirunelveli.

The identified raw materials were conserved in the laboratory of PG Gunapadam, Government Siddha Medical College, Tirunelveli.

INGREDIENTS:

1. *Purified Rasa Karpooram* (*Hydragyrum subchloride*) - 17 1/2 gram (5 Varakan)
2. *Sathikkai (Myristica fragrans. Houtt*) - 17 1/2 gram (5 varakan)
3. *Nervalam* (*Croton tiglium. Linn*) - 35 gram (10 varakan)
4. *Karuvelam Pisin* (*Gum of Acacia arabica. Linn*) - 3 1/2 gram (1 varakan)
5. Aloe Vera Juice (*Aloe barbadensis. Linn*) - Quantity sufficient

PURIFICATION OF DRUGS:

1) *Rasa Karpooram* (Hg_2cl_2)

The Poultice made of betel leaf (piper betle) and pepper (piper nigrum) each 8.75 gm is taken and dissolved in 1.3 litre of water, 35gm of calomel is tied with a cloth and immersed in the liquid from the cross bar and boiled. After the water is reduced to 3/4 of its volume. The calomel is taken out, washed with water and dried, to get it in purified form.

2) *Sathikkai*: (Myristica fragrans)

Scrap the outer skin and roast it in ghee

3. *Nervalam* : (Croton tiglium)

Croton seeds are allowed to boiled in cow dung milk then remove the outer coating and leaf like cotyledones with sheath like substance from inside. Then it is boiled in cow's milk. Then soaked in lime juice for 24 hours. The croton embryo dried and roasted in cow's ghee.

4) *Karuvelam Pisin*: (Gum of Acacia arabica)

The gum is dissolved in water, filtered and then dried in sunlight.

PROCESS OF PREPARATION:

All the above purified ingredients are powdered separately. Powder of Rasa Karpooram (Calomel), the powder of Myrstica fragrans and embryo of croton tiglium triturated well in a butter form. Then juice of aloe vera is added at small intervals grind it for a 6 hours. Finally add the gum of acacia Arabica powder and made in to pill upto size of Kundrimani and then stored in the air tight container.

DOSAGE:

1/2 to 1 pill

ADJUVENTS:

Sugar, Jaggery, Ghee

INDICATION:

All type of *Suram, Keel vaayu, Mudaku Vaayu, Soolai.*

SHELF LIFE :

1 Year

Ingredients of Karpoora Sinthamani Mathirai

BEFORE PURIFICATION

Pooram

Sathikkai

Nervalam

Karuvelam pisin

Katrazhai

Ingredients of Karpoora Sinthamani Mathirai

AFTER PURIFICATION

Pooram

Sathikkai

Nervalam

Karuvelam pisin

Katrazhai Saru

KARPOORA SINTHAMANI MATHIRAI

On Processing

Prepared Drug - Karpoora Sinthamani Mathirai

Drug : ***KARPOORA SINTHAMANI MATHIRAI***

4.2. STANDARDIZATION OF THE DRUG:

The standardization of the drug is essential to exhibit the purity, quality and quantity of the drug. This is basically done by chemical, physico-chemical and instrumental analysis.

4.2.1. PHYSICAL ANALYSIS

TESTING PHYSICAL CHARACTERIZATION OF SAMPLE:

Colour Examination:

Ten tablets were taken into watch glasses and positioned against white back ground in white tube light. Its colour was observed by naked eye and note in results.

Odour examination:

Ten numbers of tablets were smelled individually. The time interval among two smelling was kept two minutes to overturn the effect of previous smelling. Odour of *KARPOORA SINTHAMANI MATHIRAI.* tablet was noted in results table.

Size examination:

The diameter of ten tablets was measured by Vernier caliper. The mean value of diameter was noted. (Lohar DR-Protocol for testing ASU drugs)

Weight Variation Test:

It was carried out to make sure that, each number of tablets contains the proper amount of drug. The test was carried out by weighing the 20 tablets individually using analytical balance, then the average weight was calculated, and comparing the individual tablet weights to the average. (Sukalyan Sengupta 1988)

The percentage of weight variation is calculated by using this formula.

$$\% \text{ of wt. variation} = \frac{\text{Individual wt.} - \text{Average wt.}}{\text{Average wt.}} \times 100$$

Table. 1 ***Weight Variation limits of Tablets (IP)***

Average weight of tablets	Maximum percentage of weight difference allowed
80mg or less	$\pm$ 10.0
Between 80mg and 250mg	$\pm$ 7.5
250mg and more	$\pm$ 5.0

Accepted tablet:

Weight Variation limits of the sample not more than two tablets are outside the percentage limit and no tablet differs by more than two times the percentage limit according to the above table.

Suspected tablet:

Suspected tablet variation was not more than six tablets are outside the percentage limit and no tablet differs by more than two times the percentage limit according to the table.

Rejected tablets:

When a tablet weight variation test results showed rejected tablets mean in that test sample one tablet differs by more than two times the percentage limit according to the table or More than six tablets are outside the percentage limit. (Sukalyan Sengupta, 1988)

Solubility:

A pinch of the sample was taken in a dry test tube and shaken well with distilled water. A little amount of the sample is shaken well with con HCl and then Con.H_2SO_4. Test sample Solubility was observed.

pH Value:

Potentiometrically pH value was determined by a glass electrode and a suitable pH meter. The pH of the *KARPOORA SINTHAMANI MATHIRAI* tablet was written in results column.

4.2.2. PHYSICO CHEMICAL ANALYSIS

1. LOSS ON DRYING (INDIAN PHARMOCOEPIA, 1996)

Loss on drying is the loss in percentage w/w resulting from water and volatile matter of any kind that can be driven off under a specified condition. A glass stopper, shallow weighing bottle was weighed accurately and the quantity of the sample as specified was transferred to the bottle was weighed accurately and the quantity of the sample as specified was transferred to the bottle covered and weighed. The sample was distributed evenly and the bottle was placed in the drying chamber. The sample was then dried for a specific period of time, and the bottle was removed from the chamber and allowed to cool at room temperature in a desiccators before weighing.

2. TOTAL ASH:

Two grams of ground air dried powder of *KARPOORA SINTHAMANI MATHIRAI* was accurately weighed in a previously ignited and tared silica crucible. The drug was gradually ignited by raising the temperature to 450°C until it was white. The sample was cooled in a desiccators and weighed. The percentage of total ash was calculated with reference to air-dried drug.

a)Acid insoluble ash

The ash was boiled with 25ml of 2M hydrochloric acid for 5 minutes, the insoluble matter was collected on an ash less filter paper, washed with hot water, ignited cooled in a desiccators, and weighed. The percentage of acid insoluble ash calculated with reference to the air-dried drug.

b)Water soluble extractive

Proceed as directed for the determination of Alcohol-soluble extractive , using chloroform water instead of ethanol.

c)Alcohol soluble extractive

Macerate 5g of the air dried drug, coarsely powdered, with 100 ml of alcohol of the specified strength in a closed flask for 24 hrs, shaking frequently during six hours and allowing to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105° to constant weight. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

3. TABLET DISINTEGRATION TEST:

Each *KARPOORA SINTHAMANI MATHIRAI* tablet was placed in each of the six tubes of the basket present in the disintegration apparatus. The apparatus was operated by using water as the immersion fluid maintained at 35-39 °C. At the end of the 30 min, the basket is lifted from the fluid and the state of the tablet is observed. The disintegration time of *Karpoora Sinthamani Mathirai* was recorded.(Loher Dr).

MICROBIAL LIMIT TEST OF *KARPOORA SINTHAMANI MATHIRAI*

Evaluation of Total Aerobic Bacterial Count

1.1. Preparation of Sample for Experimental Work

Weighed 10 gm of the homogenized drug sample aseptically and dissolved in 10 ml of sterile water and made up to 100 ml with the sterile water. The insoluble drug product was suspended in 100 ml of buffered sodium chloride-peptone solution (pH 7.0).

1.2. Serial dilution of Sample

A serial dilution is the dilution of a sample, in 10-fold dilutions. From the sample, 1 ml of the sample was added to 9 ml of sterile distilled water and mixed it well. This dilution was denoted as 10^{-1} dilution. From this dilution, 1ml was taken from that mixture is added to 9 ml, and designated as 10^{-2} dilution. The same procedure was repeated up to 10^{-4}.

1.3. Isolation of Total Viable Aerobic Microbial Count

1.3.1. Isolation of Bacteria by Plate Count Method

In this test, the bacteria in sample were made to grow as colonies, by inoculating a known volume of sample into a solidifiable nutrient medium (Casein Soybean Digest agar or Nutrient agar medium) in petridish. The agar plate was prepared by mixing growth medium with agar and then sterilized by autoclaving. Once the agar was cooled to 45°C, approximately 15 to 20 ml of medium was poured into a sterile Petri dish under aseptic condition and left to solidify for 15 minutes. After solidification, each plate was smear with 0.1 ml of sample from the dilution of 10^{-1} and 10^{-2}. After inoculations, all the plates were incubated at 37°C for 24 hours. After incubation, the bacterial colonies were developed as visible to the naked eye and the number of colonies on a plate was counted using Quebec Colony Counter. Plates with an average of from 30 to 300 colonies of the target bacterium were selected for colony count. Because of the statistical problems, plates with lower than 30 colonies greater than 300 colonies were rejected

1.3.1.1. Composition of Nutrient Agar Media

Peptone	: 5.0 gm
Sodium chloride	: 5.0 gm
Beef extract	: 1.5 gm
Yeast extract	: 1.5 gm
Agar	: 15.0 gm
Distilled water	: 1000 ml
pH (at 25°C)	: 7.4±0.2

1.3.2. Isolation of Fungi

From each of the above prepared samples, 0.1 ml of sample was transferred to Sabouraud Dextrose agar (SDA) prepared with Chloramphenicol. The plates were then incubated for 5 days at room temperature (20 to 25°C). After incubation, the fungal colonies were observed and calculated.

1.3.2.1. Composition of SDA

Dextrose	; 40 gm
Peptone	: 10 gm
Agar	: 15 gm
Distilled water	: 1000 ml

1.4. Evaluation of Antimicrobial Activity of Drug

Antimicrobial activity was performed by agar well diffusion method on agar.

1.4.1 Preparation of drug extracts solutions for the experiment

The dried drugs were weighed and dissolved in sterile distilled water to prepare appropriate dilution to get required concentrations of about 10, 20 and 30µg/ml. They were kept under refrigerated condition unless they were used for the experiment.

1.4.2. Procedure for the Agar Well Diffusion Test

The antibacterial screening of the drugs were carried out by determining the zone of inhibition using agar well diffusion method. All the drug extracts were tested against four pathogenic bacterial strains of gram positive and gram negative organism by agar well diffusion method.

1.4.3. Bacterial Inoculums Preparation

Inoculums of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, and *Bacillus substilis* were prepared in nutrient broth medium and kept for incubation at 37°C for 8 hrs.

1.4.4. Agar well-diffusion method

This method was followed to determine the antimicrobial activity. Muller-Hinton Agar media plates were swabbed (sterile cotton swabs) with 8 hour old - broth culture of respective bacteria. After inoculation, wells with the size of 10 mm diameter and about 2 cm a part were made in each of these plates using sterile cork borer. Stock solution of each drug extract was prepared at a concentration of 1 mg/ml in water. About 100 µl of different concentrations of drug solvent extracts were added into the wells and allowed to diffuse at room temperature for 2 hrs. The plates were incubated at 37°C for 24 hrs. After incubation, the diameter of the inhibition zone (mm) was measured and the activity index was also calculated.

1.4.4.1. Composition of Muller Hinton Agar Media

Beef Extract	: 02.00 gm
Acid Hydrolysate of Casein	: 17.50 gm
Starch	: 01.50 gm
Agar	: 17.00 gm

1.5. Evaluation of Specified Microorganisms

1.5.1. Isolation & Identification of *Escherichia coli*

One ml of the prepared sample was added in a sterile screw-capped container containing 50 ml of nutrient broth and mixed well. Then, it was allowed to stand for 1 hour and mixed well again. After one hour, the screw caps of the bottle was loosened and incubated at 37º for 18 to 24 hours.

1.5.1.2. Primary Test

From the above prepared enrichment culture, 1.0 ml was taken and transferred aseptically into a tube containing 5 ml of Mac- Conkey broth. Inoculated tubes were incubated in a water-bath at 36º to 38º for 48 hours.

1.5.1.3. Secondary Test

From the primary test, 1.0 ml of the enrichment culture was taken and transferred aseptically in to 5 ml of peptone water. It was then incubated in a water-bath at 43.5º to 44.5º C for 24 hours and observed the tubes for acid and gas. Then, the culture was subjected to biochemical tests of imvic and the results were observed and correlated.

1.5.1.4. Alternative test

It was done by a loop full of enriched culture in the primary test was streaked on a sterile Mac-Conkey agar medium. Then, the plates were inverted and incubated at 37 º C for 24 hours. After incubation, the pink or brick red color colonies were examined and transfer them individually into the surface of Eosin Methylene Blue agar medium (EMB), on Petri dishes. Inoculated plates were inverted and incubated at 37 º C for 24 hours. After incubation, the colonies on medium were checked for their color appearance like green metallic sheen under reflected light. The colonies were subjected to confirmation by further suitable cultural and biochemical tests.

1.5.1.5. Components of Eosin Methylene Blue Agar Media

Pancreatic digest of gelatin	: 10.0 g
Dibasic potassium phosphate	: 2.0 g
Lactose	: 10.0 g
Eosin Y	: 400 mg
Methylene blue	: 65 mg
Agar	: 15.0 g
Distilled water	: 1000 ml

1.5.2. Isolation & Identification of *Salmonella* sp.

One ml of the prepared sample was added in a sterile screw-capped container containing 100 ml of nutrient broth and mixed well. Then, it was allowed to stand for 1 hour and mixed well again. After one hour, the screw caps of the bottle was loosened and incubated at 37º for 18 to 24 hours.

1.5.2.1. Primary Test

From the above prepared enrichment culture, 1.0 ml was taken and transferred aseptically into a tube containing 10 ml of Selenite F broth. Inoculated tubes were incubated in a water-bath at 36º to 38º for 48 hours. After incubation, the culture was

subcultured on two of the agar media namely Bismuth sulphate agar and Deoxy cholate citrate agar and incubated the plates at 36º to 38º for 18 to 24 hours. After incubation, colonies were observed on the medium and confirmed the genus *Salmonella* based on guidelines.

1.5.2.2. Secondary test

The suspected colonies of the primary test were subcultured on the slant of triple sugar-iron agar in test tube and in urea broth. Both media were incubated at 37ºC for 24 hours. After incubation, the results were observed according to the development of color change and acid / gas in media. The presence of Salmonella was confirmed by agglutination tests.

1.5.2.3. Composition of *Salmonella Shigella* Agar Media

Beef Extract	: 5.0 gm
Enzymatic Digest of Casein	: 2.5 g
Enzymatic Digest of Animal Tissue	: 2.5 gm
Lactose	: 10 gm
Bile salts	: 8.5 gm
Sodium Citrate	: 8.5 gm
Ferric Citrate	: 1.0 gm
Brilliant Green	: 0.00033 gm
Neutral Red	: 0.025
Agar	: 13.5 gm
Distilled water	: 1000 ml

1.5.3. Isolation and Identification of *Pseudomonas aeruginosa*

From the above prepared enrichment culture, 1.0 ml was taken and transferred aseptically into 100 ml of fluid soyabean-casein digest medium and mixed well. The inoculated tubes were incubated at 37º C for 24 hours. After incubation, the growth of bacteria was checked. From this, a loop full of culture was streaked on the surface of Cetrimide agar medium and Pseudomonas Isolation Agar medium and incubated at incubated at 37º C for 24 hours. After incubation, the colonies from the agar surface of these two media were checked for detection of fluorescein and pyocyanin.

1.5.3.1. Composition of Cetrimide Agar Media

Pancreatic digest of gelatin	: 20.0 g
Magnesium chloride	: 1.4 g
Potassium sulphate	: 10.0 g
Cetrimide	: 0.3 g
Agar	: 13.6 g
Glycerin	: 10.0 g
Distilled Water t	: 1000 ml

1.5.4. Isolation and Identification of *Staphylococcus aureus*

From the above prepared enrichment culture, a loop full of culture was taken and transferred aseptically on Mannitol salt agar and incubated at 37° C for 24 hours.. After incubation, the colonies were subjected to confirmation by hem agglutination test.

1.5.4.1. Composition of Mannitol Salt Agar Media

Pancreatic digest of gelatin	: 5.0 g
Peptic digest of animal tissue	: 5.0 g
Beef extract	: 1.0 g
D-Mannitol	: 10.0 g
Sodium chloride	: 75.0 g
Agar	: 15.0 g
Phenol red	: 25 mg
Distilled Water	: 1000 ml

4.2.3. BIO CHEMICAL ANALYSIS

PROCEDURE:

5gms of the drug was wighted accurately and placed in a 250ml clean beaker. Then 50ml of distilled water is added and dissolved well. Then it is boilded well for about 10 minutes. It is cooled and filtered in a 100ml volumetric flask and then it is make up to 100ml with distilled water. This fluid is taken for analysis.

QUALITATIVE ANALYSIS FOR BASIC RADICALS:

Test for Calcium:

2ml of the above prepared extract is taken in a clean test tube. To this add 2ml of 4% Ammonium oxalate solution. Formation of white precipitate indicates the presence of calcium.

Test for Iron (Ferric):

The extract is acidified with glacial acetic acid and potassium ferro cyanide.absence of blue colour indicates the absence of ferric iron.

Test for Iron (Ferrous):

The extract is treated with concentrated Nitric acid and ammonium thio-cyanate solution. Formation of blood red colour indicates the presence of ferrous iron.

Test for zinc:

The extract is treated with potassium ferro-cyanide. Gives absence of white precipitate indicates the absence of zinc.

QUALITATIVE ANALYSIS FOR ACIDIC RADICALS:

Test for Sulphate:

2ml of extract is added to 5% barium chloride solution. Formation of white precipitate indicates the presence of sulphate.

Test for Chloride:

The extract is treated with silver nitrate solution. Formation of white precipitate indicates the presence of chloride.

Test for Phosphate:

The extract is treated with ammonium molybdate and concentrated nitric acid. Formation of yellow precipitate indicates the presence of phosphate.

Test for carbonate:

On treating the extract with concentrated Hydrochloric acid giving absence of brisk effervescence indicates the absence of carbonate.

Test for starch:

The extract is added with weak iodine solution. Formation of blue colour indicates the presence of starch.

Test for albumin:

The extract is treated with Esbach's reagent. Gives absence of formation of yellow precipitate indicates the absence of albumin.

Test for tannic acid:

The extract is treated with ferric chloride.Gives absence of formation of bluish black precipitate indicates the absence of tannic acid.

Test for unsaturation:

The extract is treated with potassium permanganate solution. The dis-colourization of potassium permanganate indicates the presence of unsaturated compounds.

Test for the reducing sugar:

5ml of Benedict`s qualitative solution is taken in a test tube and allowed to boil for 2 minutes and added 8-10 drops of the extract and again boil it for 2 minutes. Any colour change indicates the presence of reducing sugar.

Test for amino acid:

One or two drops of the extract is placed on a filter paper and dried it well. After drying, 1% Ninhydrin is sprayed over the same and dried it well. Formation of violet colour indicates the presence of amino acid.

4.2.4. PHYTOCHEMICAL ANALYSIS OF *KARPOORA SINTHAMANI MATHIRAI*

The siddha preparation *KARPOORA SINTHAMANI MATHIRAI* was prepared and used for phytochemical analysis. Preliminary test, on the siddha preparation *KARPOORA SINTHAMANI MATHIRAI* was carried out for the presence of alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins, phenolic compounds, proteins and free amino acids, flavanoids, lignin, fixed oils and fats. The methods adopted for the estimation are as follows:

Test for Alkaloids (Evans, 1997)

A small segment of the siddha preparation *KARPOORA SINTHAMANI MATHIRAI* was mixed separately with a few drops of dilute hydrochloric acid and filtered. The filtrates were tested carefully with various alkaloidal reagents as follows:

a) Mayer's test (Evans, 1997):

To a few ml of filtrate, a drop of Mayer's reagent is added by the side of the test tube. A white or creamy precipitate indicates that the test as positive.

b) Hager's test (Wagner et al., 1996):

To a few ml of filtrate, one to 2ml of Hager's reagent is added. A prominent yellow precipitate indicates the test as positive.

c) Dragendorff's test (Waldi, 1965):

To a few ml of filtrate, one to 2ml of Dragendorff's reagent is added. A prominent yellow precipitate indicates the test as positive.

Test for Carbohydrates (Ramakrishnan et al., 1994)

A small quantity of siddha preparation *KARPOORA SINTHAMANI MATHIRAI* was dissolved separately in 5ml of distilled water and filtered. The filtrate was subjected to Molisch's test to detect the presence of carbohydrates. Filtrate was treated with 2-3 drops of 1% alcoholic alpha naphthol solution and 2ml of concentrated sulphuric acid was added along the sides of the test tube. Appearance of brown ring at the junction of 2 layers shows the presence of carbohydrates.

Test for Glycosides

The siddha preparation *KARPOORA SINTHAMANI MATHIRAI* was hydrolyzed with hydrochloric acid for few h on a water bath and the hydrolysate was subjected to Legal's and Borntrager's test to detect the presence of different glycosides.

(a) Legal's Test:

To the hydrolysate, one ml of pyridine and few drops of sodium nitro prusside solution were added and then it was made alkaline with sodium hydroxide solution. Appearance of pink to red color shows the presence of glycosides and aglycones.

(b) Borntrager's Test:

Hydrolysate was treated with chloroform and the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. Ammoniacal layer acquires pink color, shows the presence of glycosides (Evans, 1997).

Test for Phytosterols (Finar, 1986)

(a) **Liebermann Burchard Test:**

Small amount of the siddha preparation *KARPOORA SINTHAMANI MATHIRAI* was dissolved with 3ml of acetic anhydride, a few drops of glacial acetic acid and followed by the addition of few drops of concentrated sulphuric acid. Appearance of bluish green color shows the presence of phytosterols.

(b) **Salkowski Test:**

Small quantities of the siddha preparation *KARPOORA SINTHAMANI MATHIRAI* were dissolved in chloroform separately. This chloroform solution was added with few drops of concentrated sulphuric acid. The appearance of bluish green color shows the presence of phytosterols.

Test for Flavanoids

(a) **Shinoda's Test:**

Small quantity of siddha preparation *KARPOORA SINTHAMANI MATHIRAI* was treated with alcohol to that a piece of magnesium was added followed by an addition of concentrated hydrochloric acid drop wise and heated. Appearance of magenta color shows the presence of flavanoids (Harborne, 1984).

(b) **Florescence Test:** Small quantity of *KARPOORA SINTHAMANI MATHIRAI* was dissolved separately in alcohol and a drop of that extract was placed on Whatman filter paper and observed under UV light. Florescence indicates the presence of flavanoids.

Test for Tannins (Mace, 1963)

Small quantities of siddha preparation *KARPOORA SINTHAMANI MATHIRAI* was dissolved separately in water and tested for the presence of phenolic compound and tannins. In the process of testing and treating, the following observations were noted:

a) Dilute ferric chloride solution (5%) gives a dark green color. 38

b) 10% aqueous potassium dichromate solution gives yellowish brown precipitate.

c) 10% lead acetate solution gives a white precipitate.

Test for Proteins and Free Amino Acids (Fisher, 1968; Ruthmann, 1970)

Small quantities of various siddha preparation *KARPOORA SINTHAMANI MATHIRAI* was dissolved in few ml of water and the following reaction were carried out

(a) Millon's Test : To 2ml of filtrate, few drops of Millon's reagent were added. A white precipitate indicates the presence of proteins (Rasch and Swift, 1960).

(b) Ninhydrin Test: To 2ml of filtrate 2 drops of ninhydrin solution was added. A characteristic purple color indicates the presence of amino acids (Yasma and Ichikawa, 1953).

(c) Biuret Test: An aliquot of 2ml of filtrate was treated with a drop of 2% copper sulphate solution. To this, 1ml of ethanol (95%) was added, followed by excess of potassium hydroxide pellets, Pink color in the ethanol layer indicates the presence of protein (Gahan, 1984).

Tests for Fixed oils and Fats

(a) **Spot Test:**

A small quantity of siddha preparation *KARPOORA SINTHAMANI MATHIRAI* was placed between 2 filter papers. Oil stains produced with any extract shows the presence of fats and fixed oils in the *KARPOORA SINTHAMANI MATHIRAI* (Harborne, 1984).

(b) **Saponification Test:**

A small quantity of siddha preparation *KARPOORA SINTHAMANI MATHIRAI* was treated with few drops of 0.5N alcoholic potassium hydroxide along with 2 to 3 drops of phenolphthalein. Later the mixture is refluxed for about 2h. Soap formation indicates the presence of fats and fixed oils in the *KARPOORA SINTHAMANI MATHIRAI* .

Tests for Lignin

Small quantities of *KARPOORA SINTHAMANI MATHIRAI* was dissolved separately in few ml of alcoholic solution of hydrochloric acid and phloroglucinol gives red color, which shows lignin is present.

Test for Saponins (Kokate, 1999)

Frothing Test:

The siddha preparation *KARPOORA SINTHAMANI MATHIRAI* was diluted separately with 20ml of distilled water and it was agitated on a graduated cylinder for 15min. Absence of the foam formation shows the devoid of saponins.

4.2.5. INSTRUMENTAL ANALYSIS

Fig. No. 1 SCANNING ELECTRON MICROSCOPE (SEM)

The microstructure of the powders was examined using a Hitachi S 3000H scanning electron microscope

Introduction:

The scanning Electron Microscope is one of the most versatile instruments available for the examination and analysis of the micro structural characteristics of solid objects. The primary reason for the SEM's usefulness is the high resolution which can be obtained when bulk objects are examined; values of the order of 5nm (50degreeA) are usually quoted for commercial instruments. Advanced research instruments have been described which have achieved resolutions of about 2.5nm (25 degree A). Any solid material can be studied. Sample size is limited to specimens less than about 10μm in diameter

Principle:

The beam is then rastered over the specimen in synchronism with the beam of a cathode ray tube display screen. The eleastically scattered secondary electrons are emitted from the sample surface and collected by a scintillator, the signal from which is used to modulate the brightness of the cathode ray tube. In this way the secondary electron

emission from the sample is used to form an image on the CRT display screen. (Goldstein, et. al., 1992)

SEM MECHANISM

Procedure:

An electron beam passing through an evacuated column is focused by electromagnetic lenses onto the specimen surface. Since an electron is a charged particle, it has a strong interaction with the specimen (due to coulomb interaction).So when an electron beam images on a specimen, it is scattered by atomic layers near the surface of the specimen. As a result, the direction of electron motion changes and its energy is partially lost. Once an incident electron (primary electron) enters a substance, its direction of motion is influenced by various obstructions (multiple scattering), and follows a complicated trajectory which is far from a straight line. Also, when electrons with the same energy are incident on the specimen surface, a portion of electrons is reflected in the opposite direction (back scattered) and the remainder is absorbed by the specimen (exciting X- rays or other quanta in the process). If the specimen issufficiently thin, the electron can pass all the way through the specimen (transmitted electrons, scattered or non-scattered).

The depth at which various signals are generated due to electron beam – specimen interaction indicates the diffusion area of the signals in the specimen in addition to the local chemistry of the specimen. Secondary electrons mainly indicate information about the surface of a specimen. Since secondary electrons do not diffuse much inside the specimen, they are most suitable for observing the fine-structures of the specimen surface. That is to say, sharp scanning images with high resolution can be expected from secondary electrons, because of the smaller influence on resolution by their diffusion.

As the incident electron energy increases, the probability of incident electrons Colliding with elemental components of the specimen and releasing secondary electrons also increases. In other words, as the incident energy increases, the emission of electrons from the specimen also increases. However, as the energy increases beyond a certain level, the incident electrons penetrate deeper into the specimen with the result that the specimen derived electrons use up most of their energy to reach the specimen surface.

Consequently, the electron emission yield decreases. Therefore, the peak secondary electron emission yield occurs at a specific entry level of the incident electrons.

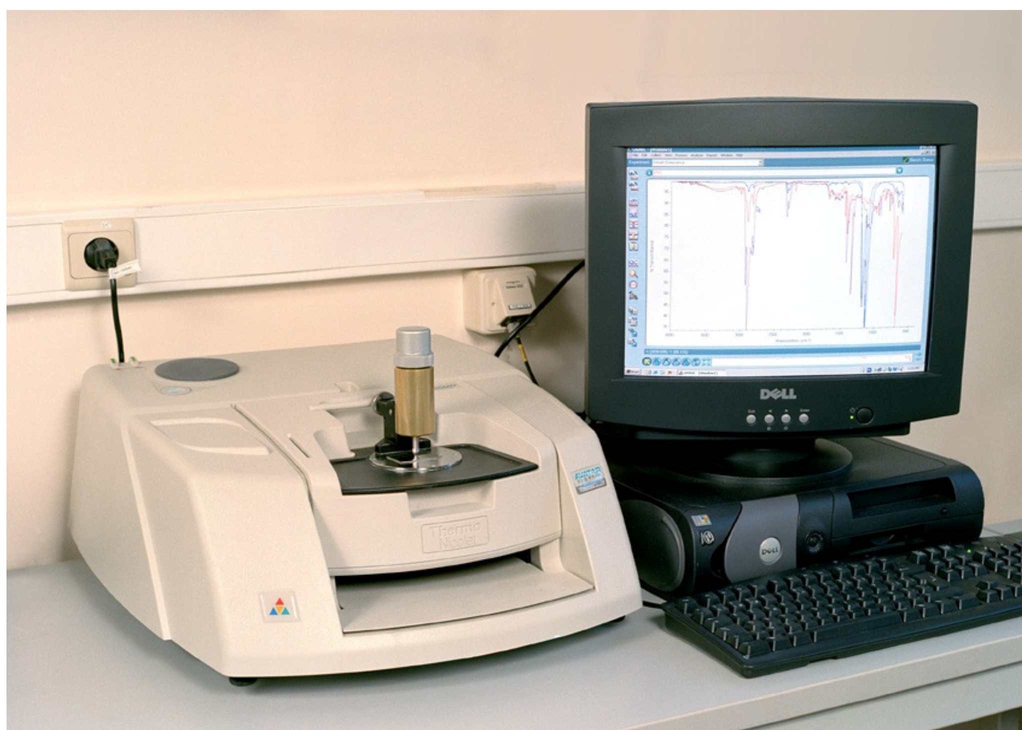
In order to verify the existence of a substance and recognize its shape, the image contrast must be well defined. In other words, even if a system boasts extremely high resolution, if image contrast is poor, it would be extremely difficult to determine the existence of a substance, let alone recognize its shape. Another important feature of the SEM is the three-dimensional appearance of the specimen image, which is a direct result of the large depth of field.

Applications:

The SEM is capable of examining objects at very low magnification. This feature is useful in viewing particle size and shape of any composition at various stages of preparation in *Siddha* system as well as other fields.The large depth of field available in the SEM makes it possible to observe 3-dimensional objects in stereo. Today, a majority of SEM facilities are equipped with X-ray analytical capabilities. Thus topographic crystallographic and compositional information can be obtained rapidly, efficiently and simultaneously from the same area.

The author was chosen this analysis for detecting Particle size of the classical *Siddha* herbo-mineral drug *KARPOORA SINTHAMANI MATHIRAI*. SEM results of *KARPOORA SINTHAMANI MATHIRAI* were represented in results section.

Fig. No. 2 FOURIER TRANSFORM-INFRA RED SPECTROSCOPY (FT-IR)



Introduction:

Vibrational spectroscopy is an extremely useful tool in the elucidations of molecular structure .The spectral bands can be assigned to different vibrational modes of the molecule. The various functional groups present in the molecule can be assigned by a comparison of the spectra with characteristic functional group frequencies. As the positions of the bands are directly related to the strength of the chemical bond, a large number of investigations including intermolecular interactions, phase transitions and chemical kinetics can be carried out using this branch of spectroscopy. In IR spectroscopy, the resonance absorption is made possible by the change in dipole moment accompanying the vibrational transition. The Infrared spectrum originates from the vibrational motion of the molecule. The vibrational frequencies are a kind of fingerprint of the compounds. This property is used for characterization of organic, inorganic and biological compounds. The band intensities are proportional to the concentration of the compound and hence qualitative estimations are possible. The IR spectroscopy is carried out by using Fourier transform technique.

Principle:

Infra red spectroscopy involves study of the interaction of electromagnetic radiation with matter. Due to this interaction, electromagnetic radiation characteristic of the interacting system may be absorbed (or emitted). The experimental data consist of the nature (frequency of wave length) and the amount (intensity) of the characteristic radiation absorbed or emitted. These data are correlated with the molecular and electronic structure of the substance and with intra- and inter molecular interactions.

Source : Nernst Glower

Beam splitter : It is made up of a transparent material. Thin films of Silicondepositedon Potassium bromide (KBr) Bromide (KBr) Detectors: DeutratedTriGlycine Sulphate (DTGS).

Scan Range : MIR 450to 4000 cm-1

Resolution : 4.0 cm-1

Sample required : 50mg, solid or liquid

Sampling Techniques: There are a variety of techniques for sample preparation physical form of the sample to be analyzed.

Solid : KBr or Nujol mull method.

Liquid : Csl / TIBr Cells

Gas : Gas cells

Measurements Techniques:

The procedure for recording the %T or %A is as follows:

1. Air is first scanned for the reference and stored. The sample is then recorded and finally the ratio of the sample and reference data is computed to give required % T or % A at various frequencies.
2. Study of substances with strong absorbance bands and weak absorbance bands aswell as possible.
3. Small amount of samples are sufficient
4. High resolution is obtained.

Procedure:

Typically,1.5 mg of protein, dissolved in the buffer used for its purification, were centrifuged in a 30 K Centric on micro concentrator (Amicon) at 3000_g at 4oC until a volume of approximately 40 Al.

1. Then, 300 Al of 20 mM buffer, prepared in H_20 or $2H_20$, pH or p2H 7.2, were added and the sample concentrated again. The p2H value corresponds to the pH meter reading + 0.4. The concentration and dilution procedure was repeated several times in order to completely replace the original buffer with the This buffer.
2. The washings took 24 h, which is the time of contact of the protein with the 2H20medium prior FT-IR analysis. In the last washing, the protein was concentrated to fine a volume of approximately 40 Al and used for the infrared measurements.The concentrated protein sample was placed in CaF2 windows and a 6 Am tin spacer or a 25 Am Teflon spacer for the experiments in H20 or 2H20, respectively. FT-IR spectra were recorded by means of a Perkin-Elmer -Spectrum-1 FT-IR spectrometer using a deuteratedtriglycinesulfate detector.
3. At least 24 h before, and during data acquisition, the spectrometer were continuously purged with dry air at a dew point of 40°C. Spectra of buffers and samples were acquired at 2 cm_1 resolution under the same scanning and temperature conditions. In the thermal denaturation experiments, the temperature was raised in 5°C steps from 20 to 95°C.
4. Before spectrum acquisition, samples were maintained at the desired temperature for the time necessary for the stabilization of temperature inside the cell (6 min).Spectra were collected and processed using the SPECTRUM software from Perkin-Elmer. Correct subtraction of H_20 was judged to yield an approximately flat baseline at 1900-1400 cm_ 1, and subtraction of $2H_20$ was adjusted to the removal of the$2H_20$ bending absorption close to 1220 cm_ 1

KBr Method

1. The sample is grounded using an agate mortar and pestle to give a very fine powder.
2. The finely powder sample is then mixed with about 100mg dried KBr salt.
3. The mixture is then pressed under hydraulic press using a die to yield a transparent disc and measure about 13mm diameter and 0.3mm in thickness.

Nujol Mull Method:

1. The sample is ground using an agate mortar and pestle to give a very fine powder.
2. A small amount is then mixed with nujol oil to give a paste and this paste is thenapplied between two sodium chloride plates.
3. The plates are then placed in the instrument sample holder ready for scanning.

Liquids:

1. Viscous liquids can be smeared in the cell and directly measured.
2. For dilute solutions, liquid cells and variable path length cells are employed.

Applications:

Infrared spectrum is useful in identifying the functional groups like -OH, -CN, -CO, -CH, -NH2, etc. Also quantitative estimation is possible in certain cases for chemicals, pharmaceuticals, petroleum products, etc. Resins from industries, water and rubber samples can be analyzed.

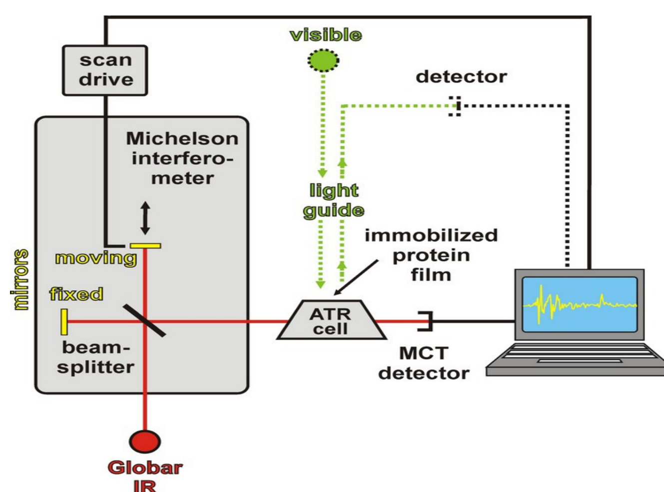


Fig. No. 3 Mechanism of FTIR analyzer

Analytical Capabilities:

1. Identifies chemical bond functional groups by the absorption of infrared radiation which excites vibrational modes in the bond.
2. Especially capable of identifying the chemical bonds of organic materials
3. Detects and identifies organic contaminants.
4. Identifies water, phosphates, sulphates, nitrates, nitrites, and ammonium ions
5. Detection limits vary greatly, but are sometimes $<10^{13}$ bonds/cm^3 or sometimes sub monolayer .Useful with solids, liquids, or gases.

Fig. No. 4 INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROMETRY(ICP-OES):

Introduction:

Inductively coupled plasma optical emission spectrometry (ICP-OES) is an analytical technique used for the detection of trace metals. It is a type of emission spectroscopy that uses the inductively coupled plasma to produce excited atoms and ions that emit electromagnetic radiation at wavelengths characteristic of a particular element. The intensity of this emission is indicative of the concentration of the element within the sample.

Mechanism:

The ICP-OES is composed of two parts: ICP and the optical spectrometer. The ICP torch consists of 3 concentric quartz glass tubes. The output or "work" coil of the radiofrequency (RF) generator surrounds part of this quartz torch. Argon gas is typically used to create the plasma.

When the torch is turned on, an intense electromagnetic field is created within the coil by the high power radio frequency signal flowing in the coil. This RF signal is created by the RF generator which is, effectively, a high power radio transmitter driving the "workcoil" the same way a typical radio transmitter drives a transmitting antenna. The argon gas flowing through the torch is ignited with a Tesla unit that creates a brief

discharge are through the argon flow to initiate the ionization process. Once the plasma is "ignited", the Tesla unit is turned off.

The argon gas is ionized in the intense electromagnetic field and flows in a particular rotationally symmetrical pattern towards the magnetic field of the RF coil. Stable, high temperature plasma of about 7000 K is then generated as the result of the inelastic collisions created between the neutral argon atoms and the charged particles. A peristaltic pump delivers an aqueous or organic sample into a nebulizer where it is changed into mist and introduced directly inside the plasma flame. The sample immediately collides with the electrons and charged ions in the plasma and is itself broken down into charged ions. The various molecules break up into their respective atoms which then lose electrons and recombine repeatedly in the plasma, giving off radiation at the characteristic wavelengths of the elements involved.

Within the optical chamber(s), after the light is separated into its different wavelengths (colours), the light intensity is measured with a photomultiplier tube or tubes physically positioned to "view" the specific wavelength(s) for each element line involved, or, in more modern units, the separated colours fall upon an array of semiconductor photo detectors such as charge coupled devices (CCDs). In units using these detector arrays, the intensities of all wavelengths (within the system's range) can be measured simultaneously, allowing the instrument to analyse for every element to which the unit is sensitive all at once. Thus, samples can be analysed very quickly.

The intensity of each line is then compared to previously measured intensities ofknown concentrations of the elements and their concentrations are then computed by interpolation along the calibration lines. In addition, special software generally corrects for interferences caused by the presence of different elements within a given sample matrix.

Applications :

ICP-OES is used in the determination of metals, arsenic present in Traditional medicines, and trace elements bound to proteins. ICP-OES is widely used in minerals processing to provide the data on grades of various streams, for the construction of mass balances.

The author used it for elemental identification and quantitative compositional information of the *KARPOORA SINTHAMANI MATHIRAI.*

X-RAY POWDER DIFFRACTION

(XRD) INSTRUMENTATION

X-ray diffractometers consist of three basic elements: an X-ray tube, a sample holder and an X-ray detector.

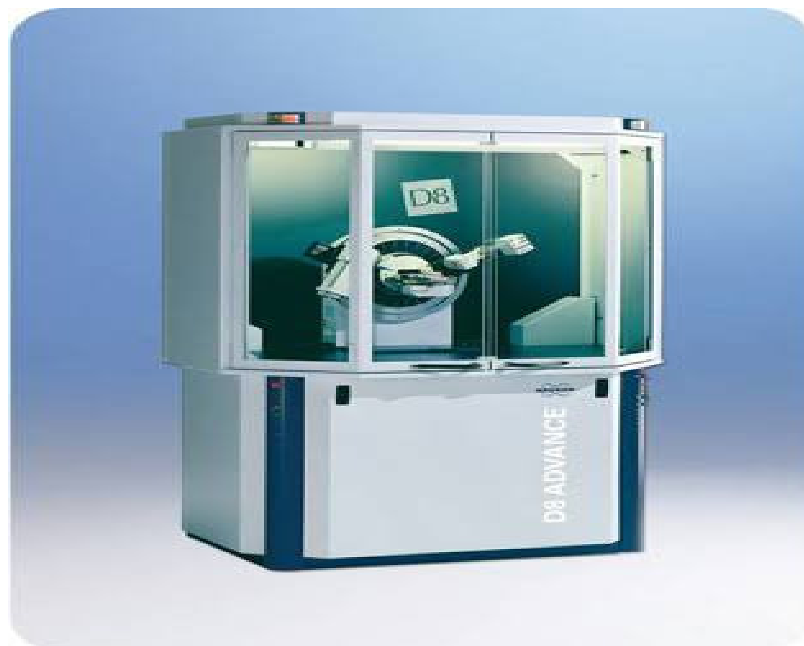


Fig.No. 5 Bruker's X-ray Diffraction D8-Discover instrument.

X-rays are generated in a cathode ray tube by heating a filament to produce electrons, accelerating the electrons towards a target by applying a voltage and bombarding the target material with electrons. When electrons have sufficient energy to dislodge inner shell electrons of the target material, characteristic X-ray spectra are produced. These spectra consist of several components, the most common being K_{α} and K_{β}. K_{α} consists in part of $K_{\alpha 1}$ and $K_{\alpha 2}$. $K_{\alpha 1}$ has a slightly shorter wavelength and twice the intensity of $K_{\alpha 2}$. The specific wavelengths are characteristic of the target material (Cu, Fe, Mo, Cr). Filtering, by foils or crystal monochrometers, is required to produce monochromatic X-rays needed for diffraction. $K_{\alpha 1}$ and $K_{\alpha 2}$ are sufficiently close in wavelength such that a weighted average of the two is used. Copper is the most common target material for single-crystal diffraction, with CuK_{α} radiation = $1.5148A^{0.}$ These X-rays are collimated and directed onto the sample. As the sample and detector are rotated, the intensity of the reflected X-rays is recorded. When the geometry of the incident X-rays impinging the sample satisfies the Bragg Equation, constructive interference occurs and a peak in intensity occurs. A detector records and processes this X-ray signal and

converts the signal to a count rate which is then output to a device such as a printer or computer monitor.

The geometry of an X-ray diffractometer is such that the sample rotates in the path of the collimated X-ray beam at an angle θ while the X-ray detector is mounted on an arm to collect the diffracted X-rays and rotates at an angle of 2θ. The instrument used to maintain the angle and rotate the sample is termed a goniometer. For typical powder patterns, data is collected at 2θ from -5^0 to 70^0, angles that are present in the X-ray scan.

Applications:

X-ray powder diffraction is most widely used for the identification of unknown crystalline materials (e.g. minerals, inorganic compounds). Determination of unknown solids is critical to studies in geology, environmental science, material science, engineering and biology.

Other applications include:

1. Characterization of crystalline materials
2. Identification of the fine-grained minerals such as clays and mixed layer clays that are difficult to determine optically.
3. Determination of unit cell dimensions.
4. Measurement of sample purity.

With specialized techniques, XRD can be used to:

1. Determine crystal structures using Rietveld refinement
2. Determine of modal amounts of minerals (quantitative analysis)
3. Make textural measurements such as the orientation of grains in a polycrystalline sample.

Strengths and Limitations of X-ray Powder Diffraction:

Strengths:

1. Powerful and rapid (<20 min) technique for identification of an unknown minerals.
2. In most cases, it provides an unambiguous mineral determination.
3. Minimal sample preparation is required.
4. XRD units are widely available.
5. Data interpretation is relatively straight forward.

Limitations:

1. Homogenous and single phase material is best for identification of an unknown
2. Must have access to a standard reference file of inorganic compounds (d-spacings, *hkls)*
3. Requires tenths of a gram of material which must be ground into a powder.
4. Foe mixed materials, detection limit is -2% of sample.
5. For unit cell determinations, indexing of patterns for non-isometric crystal systems is complicated.
6. Peak overlay may occur and worsens for high angle 'reflections'.

User's Guide-Sample Collection and Preparation:

Determination of an unknown requires: the material, an instrument for grinding and a sample holder.

1. Obtain a few tenths of a gram (or more) of the material, as pure as possible.
2. Grind the sample to a fine powder, typically in a fluid to minimize inducing extra strain (surface energy) that can offset peak positions, and to randomize orientation.
    a. Powder less than -10 µm (or 200-mesh) in size is preferred.
3. Place into a sample holder or onto the sample surface.
    a. Packing of the fine powder into a sample holder. Smear uniformly onto a glass slide, assuring a flat upper surface.
4. Pack into a sample container
5. Sprinkle on double sticky tape
    a. Typically the substance is amorphous to avoid interference
6. Care must be taken to create a flat upper surface and to achieve a random distribution of lattice orientations unless creating an oriented smear.
7. For unit cell determinations, a small amount of a standard with known peak positions (that do not interfere with the sample) can be added and used to correct peak positions.

Data Collection, Results and Presentation:

Data collection:

The intensity of diffracted X-rays is continuously recorded as the sample and detector rotate through their respective angles. A peak in intensity occurs when the mineral contains lattice planes with d-spacings appropriate to diffract X-rays at that value of θ. Although each peak consists of two separate reflections ($K_{\alpha1}$ and $K_{\alpha2}$), at small values of 2θ the peak locations overlap with $K_{\alpha2}$ appearing as a hump on the side of $K_{\alpha1}$. Greater separation occurs at higher values of θ. Typically these combined peaks are treated as one. The 2λ position of the diffraction peak is typically measured as the center of the peak at 80% peak height.

Data reduction:

Results are commonly presented as peak positions at 2θ and X-ray counts (intensity) in the form of a table or an *x-y* plot (shown above). Intensity (*I*) is either reported as peak height intensity, that intensity above background, or as integrated intensity, the area under the peak. The relative intensity is recorded as the ratio of the peak intensity to that of the most intense peak (*relative intensity* $=I/I_1 \times 100$).

The d-spacing of each peak is then obtained by solution of the Bragg equation for the appropriate value of λ. Once all d-spacings have been determined, automated search/match routines compare the *ds* of the unknown to those of known materials. Because each mineral has a unique set of d-spacings, matching these d-spacings provides an identification of the unknown sample. A systematic procedure is used by ordering the d-spacings in terms of their intensity beginning with the most intense peak. Files of d-spacings for hundreds of thousands of inorganic compounds are available from the International Centre for Diffraction Data as the Powder Diffraction File (PDF). Many other sites contain d-spacings of minerals such as the American Mineralogist Crystal Structure Database. Commonly this information is an integral portion of the software that comes with the instrumentation.

4.3. TOXICOLOGICAL STUDIES

4.3.1. ACUTE TOXICITY STUDY IN FEMALE WISTER RATS TO EVALUATE TOXICITY PROFILE OF *KARPOORA SINTHAMANI MATHIRAI*

OBJECTIVES

The aim of this Study is to evaluate the toxicity of the test substance ***Karpoora Sinthamani Mathirai*** , when administered orally to Female Wister Rats with different doses, so as to provide a rational base for the evaluation of the toxicological risk to man and indicate potential target organs.

Guidelines followed:

(a) OECD Guidelines No. 423,

Study Design and Controls:

1) Female Wister Rats in controlled age and body weight were selected.
2) ***Karpoora Sinthamani Mathirai*** was administered at **5 mg/kg, 50 mg/kg, 300 mg/kg,1000 mg/kg, 2000 mg/kg,** body weight as (Water) as suspension along with blank.
3) The results were recorded on day 0, with single oral dosing period of 14 days.

EXPERIMENTAL PROCEDURE

1. ANIMALS

Supply

A total of 15 Female Wister Rats with an approximate age of 6 weeks and purchased from Central Animal House, Arulmigu Kalasalingam college of pharmacy, krishnankoil. On their arrival a sample of animals was chosen at random and weighed to ensure compliance with the age requested. The mean weights of Female Wister Rats were 100-150 g respectively. The animals were housed in metabolic cages (55 x 32.7 x 19 cm), with sawdust litter, in such a way that each cage contained a maximum of 3 animals of the same sex.

All animals underwent a period of 20 days of observation and acclimatization between the date of arrival and the start of treatment. During the course of this period, the animals were inspected by a veterinary surgeon to ensure that they fulfilled the health requirements necessary for initiation of the Study.

Housing

The Female Wister Rats were housed in metabolic cages (55 x 32.7 x 19 cm), placed on racks. From the week before initiation of the treatment, each cage contained a maximum of 3 rats of the same sex and treatment group.

Each cage was identified by a card, color coded according to the dose level. This card stated the cage number, number and sex of the animals it contained, Study number, test substance code, administration route, dose level and Study Director's name, date of the arrival of the animals and initiation of treatment.

The temperature and relative humidity were continuously monitored. Lighting was controlled to supply 12 hours of light (7:00 to 19:00 hours) and 12 hours of dark for each 24-hour period.

The cages corresponding to each experimental group were distributed on racks in such a manner that external factors, such as environmental conditions, were balanced as far as possible.

2. DIET

All the rats had free access to a pelleted rat diet. The diet was analyzed by the manufacturer to check its composition and to detect possible contaminants.

Water

The water was offered ad libitum in bottles.

3. ADMINISTRATION ROUTE AND PROCEDURE

The test substance was administered orally. The Female Wister Rats belonging to the control group were treated with the vehicle (Water) at the same administration volume as the rest of the treatment groups.

Table – 2 Numbering and Identification

The animals were marked on body with picric acid solution prepared in water. The marking within the cage was as below.

Table – 2 Numbering and Identification

Group No	Animal Marking
1	Head
2	Body
3	Tail

The group no., cage no., sex of the animal and animal no. were identified as indicated below using cage label and body marking on the animals

Cage No	Group No	Animal Marking	Sex
1	I	H,B,T	Female
2	II	H,B,T	Female
3	III	H,B,T	Female
4	IV	H,B,T	Female
5	V	H,B,T	Female

Doses

The doses for the study were selected based on literature search and range finding study. Following the period of fasting, the animals were weighed and then drug was administered orally as single dose using a needle fitted onto a disposable syringe of approximate size at the following different doses.

GROUP	DOSE
Group-I	5 mg/kg
Group-II	50 mg/kg
Group-III	300 mg/kg
Group-IV	1000 mg/kg
Group-V	2000 mg/kg

The test item was administered as single dose. After single dose administration period, all animals were observed for day 14.

Dose Preparation

Karpoora Sinthamani Mathirai was added in distilled water and completely dissolved to form oral for administration. The dose was prepared of a required concentration before dosing by dissolving, in distilled water. It was mixed well. The preparation for different doses was vary in concentrations to allow a constant dosage volume.

Administration

The test item was administered orally to each Female Wister rats as single dose using a needle fitted onto a disposable syringe of appropriate size at the following different doses. The concentration was adjusted according to its body weight. The volume was not exceeding 10 ml/kg bodyweight. Variability in test volume was minimized by adjusting the concentration to ensure a constant volume at all dose levels.

Observation period

All animals were observed for any abnormal clinical signs and behavioral changes. The appearance, change and disappearance of these clinical signs, if any, were recorded for approximately 1.0, 3.0 and 4.0 hours post-dose on day of dosing and once daily thereafter for14 days. Animals in pain or showing severe signs of distress were humanely killed. The cageside observation was included changes in skin, fur, eyes and mucous membranes, occurrence of secretions and excretions. Autonomic activity like lacrimation, piloerection, pupil size and unusual respiratory pattern, changes in gait, posture, response to handling, presence of clonic or tonic movements, stereotypes like excessive grooming and repetitive circling or bizarre behavior like self-mutilation, walking backwards etc were observed. At the 14th day, sensory reactivity to stimuli of different types (e.g. auditory, visual and proprioceptive stimuli) was conducted. Auditory stimuli responses were measured by clicker sound from approximately 30 cm to the rats; visual stimuli response were measured with the help of shining pen light in the eye of rats and placing a blunt object near to the eye of rats. Response to proprioceptive stimuli was measured by placing anterior/dorsal surface of animals paw to the table edge. The responses of reactions for these three exercises were normal in animals belonging to both the controls as well as drug treatment dose groups.

Mortality and Morbidity

All animals were observed daily once for mortality and morbidity at approximately 1.0, 3.0 and 4.0 hours post dose on day of dosing and twice daily (morning and afternoon) thereafter for 14 days.

4.3.2. SUB-ACUTE TOXICITY STUDY IN WISTER RATS TO EVALUATE TOXICITY PROFILE OF KARPOORA SINTHAMANI MATHIRAI

Objective

The objective of this '**Sub-Acute Toxicity Study of *Karpoora Sinthamani Mathirai* on Wister Rats**' was to assess the toxicological profile of the test item when treated as a single dose. Animals should be observed for 28 days after the drug administration. This study provides information on the possible health hazards likely to arise from exposure over a relatively limited period of time.

Test Guideline Followed

OECD 407 Method - Sub-Acute Toxic Class Method (Repeated Dose 28-Day Oral Toxicity Study in Rodents)

Test Item Detail

Karpoora Sinthamani Mathirai

Test System Detail

The study was conducted on 5 male 5 female Wister rats. These animals were selected because of the recommended rodent species for oral studies as per followed guideline and availability of Animals 8-12 weeks old male and female rats were selected after physical and behavioral examination. The body weight range was fallen within ± 20% of the mean body weight at the time of Randomization and grouping. The rats were housed in standard laboratory condition in Polypropylene cages, provided with food and water *adlibitum* in the Animal at Central Animal House, Arulmigu Kalasalingam College of Pharmacy, Krishnankoil. The experimental protocol was approved by Institutional Animal Ethical Committee as per the guidance of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, government of India.

Acclimatization

The animals were selected after veterinary examination by the veterinarian. All the selected animals were kept under acclimatization for a week.

Randomization & grouping

One day before the initiation of treatment (days 0- last day of acclimatization), the selected animals were randomly grouped into three different groups containing minimum 5 male and 5 female animals per group.

The animals were marked on body with picric acid solution prepared in water. The marking within the cage was as below.

Table – 3 Numbering and Identification

Case No	Group No	Animal Marking
1	CONTROL	H,B,T,HB,NM (Male) H,B,T,HB, NM (Female
2	Low dose of *KARPOORA SINTHAMANI MATHIRAI* 300mg/kg	H,B,T,HB,NM (Male) H,B,T,HB, NM (Female)
3	Middle dose of *KARPOORA SINTHAMANI MATHIRAI* 600mg/kg	H,B,T,HB,NM (Male) H,B,T,HB, NM (Female)
4	High dose of *KARPOORA SINTHAMANI MATHIRAI* 900 mg/kg	H,B,T,HB,NM (Male) H,B,T,HB ,NM (Female)

The group no., cage no., sex of the animal and animal no. were identified as indicated below using cage label and body marking on the animals:

Case No	Group No	Animal Marking	Sex
1	CONTROL	H,B,T,HB,NM H,B,T,HB, NM	Male Female
2	Low dose of *KARPOORA SINTHAMANI MATHIRAI* 300mg/kg	H,B,T,HB,NM H,B,T,HB, NM	Male Female
3	Middle dose of *KARPOORA SINTHAMANI MATHIRAI* 600mg/kg	H,B,T,HB,NM H,B,T,HB, NM	Male Female
4	High dose of *KARPOORA SINTHAMANI MATHIRAI* 900 mg/kg	H,B,T,HB,NM H,B,T,HB ,NM	Male Female

Husbandry

Housing

The Wister rats were housed in standard polypropylene cages with stainless steel top grill. Paddy husk was used as bedding. The paddy husk was changed at least twice in a week. From the week before initiation of the treatment, each cage contained a maximum of 5 rats of the same sex and treatment group.

Environmental conditions

The animals were kept in a clean environment with 12 hour light and 12 hour dark cycles. The air was conditioned at 22 ± 3^{0}C and the relative humidity was maintained between 30-70% with 100% exhaust facility. The cages corresponding to each experimental group were distributed on racks in such a manner that external factors, such as environmental conditions, were balanced as far as possible.

Feed & feeding schedule

Feed was provided *adlibitum throughout* the study period, except over night fasting (18-20 hours) prior to dose administration. After the substance has been administered, food was with held for a further 3-4 hours.

Water

The water was offered *adlibitum* in bottles. There was periodically analyzed to detect the presence of possible contaminants

Doses

The doses for the study were selected based on literature search and range finding study. Following the period of fasting, the animals were weighed and then extract was administered orally as single dose using a needle fitted on to a disposable syringe of approximate size at the following different doses.

Test Group	**Dose to Animals (mg/kg body –weight / day**	**Number of Animals**
Group – I	CONTROL	10 (5 Male and 5 Female)
Group – II	Low dose of *KSM* 300mg/kg	10 (5 Male and 5 Female)
Group – III	Middle dose of *KSM* 600mg/kg	10 (5 Male and 5 Female)
Group - IV	High dose of *KSM* 900 mg/kg	10 (5 Male and 5 Female)

The test item was administered as single dose. After single dose administration period, all animals were observed for 28 days.

Dose Preparation

Karpoora Sinthamani Mathirai was added in distilled water and completely dissolved to for oral for administration. The dose was prepared of a required concentration before dosing by dissolving ***Karpoora Sinthamani Mathirai*** in distilled water. It was mixed well. The preparation for different doses was vary in concentrations to allow a constant dosage volume.

Administration

The test item was administered orally to each rat as single dose using a needle fitted on to a disposable syringe of appropriate size at the following different doses. The concentration was adjusted according to its body weight. The volume was not exceeding 10 ml/kg body weight. Variability in test volume was minimized by adjusting the concentration to ensure a constant volume at all dose levels.

OBSERVATIONS

These observations were also performed on week-ends. The observations included but were not limited to changes in skin and fur, in the eyes and mucous membranes, in the respiratory, circulatory, central nervous and autonomous systems, somatomotor activity and behavior.

Clinical signs of toxicity

All the rats were observed at least twice daily with the purpose of recording any symptoms of ill- health or behavioral changes. Clinical signs of toxicity daily for 28 days.

Food intake

Prior to the beginning of treatment, and daily, the food intake of each cage was recorded for period of 28 days and the mean weekly intake per rats was calculated.

Water intake

Water intake was checked by visual observation during the Study. In addition, the water consumption in each cage was measured daily for a period of 28 days.

Bodyweight:

The body weight of each rat was recorded one week before the start of treatment, and during the course of the treatment on the day of initial, 3rd, 7th, 10th, 14th, 17th, 20th, 24th and 28th days (day of sacrifice). The mean weights for the different groups and sexes were calculated from the individual weights.

Blood Collection

Blood was collected through retro-orbital sinus from all the animals of different groups on 28th day. The blood was collected in tubes containing Heparin/EDTA as an anticoagulant. Animals were fasted over night prior to the blood collection.

LABORATORY STUDIES

During the 4th week of treatment, samples of blood were withdrawn from the orbital sinus of 10 rats from each group, under light ether anesthesia after fasting for 16 hours. The blood samples are used to evaluate Hematological parameters like RBC, WBC, and PLATELETS etc….. The collected blood samples also centrifuged 10000 rpm in 10 minutes to separate the serum. The separated serum used to evaluate biochemical parameters like SGOT, SGPT, ALP and BILIRUBIN etc……..

Hematology

The following hematological parameters were analysed using Autoanalyser

Hb	:	Haemoglobin (g %)
PCV	:	Packed Cell Volume
WBC	:	White Blood Corpuscles (x103/cmm)
RBC	:	Red Blood Corpuscles (x106/cmm)
		Blood Platelet count (x103/cmm)

Differential WBC count:

N	:	Neutrophils (%)
L	:	Lymphocytes (%)
M	:	Monocytes (%)
E	:	Eosinophils (%)
RDW	:	Red Cell Distribution Width.
MPV	:	Mean Platelet Volume

Clinical Biochemistry:

The following clinical Bio parameters were analysed using Auto analyser

Total serum protein (g/dl)

ALT/SGPT	:	Alanine amino transferase (U/L)
AST/SGOT	:	Aspartate amino transferase (U/L)
ALP	:	Alkaline serum phosphatase (U/L)
CHL	:	Cholesterol (mg/dL)
HDL	:	High density lipoprotein
TG	:	Triglyceride

TERMINAL STUDIES

Sacrifice and macroscopic examination

On completion of the 4 weeks of treatment, 18 Wister rats were sacrificed by ether inhalation. A full autopsy was performed on all animals which included examination of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents both *in situ* and after evisceration. As the number of animals exceeded the number that could be sacrificed in one day, the autopsies were carried out over three consecutive days at the end of the treatment period.

Organ weights:

After the macroscopic examination the following organs were weighed after separating the superficial fat: Brain, Heart, Spleen Kidneys, Testes, Liver, Lungs, pancreas and stomach.

4.4. PHARMACOLOGICAL STUDY

4.4.1. ANTI-INFLAMMATORY ACTIVITY OF SIDDHA PREPARATION KARPOORA SINTHAMANI MATHIRAI ON CARRAGEENAN INDUCED HIND PAW OEDEMA IN RATS

The anti-inflammatory activities of siddha preparation Karpoora Sinthamani Mathirai at a dose of 100 mg/kg and 200 mg/kg were evaluated using carrageenan-induced paw edema method. The inflammation was readily produced in the form of edema with the help of irritant such as carrageenan. Carrageenan is a sulphated polysaccharide obtained from sea weed (Rhodophyceae) and when injected cause the release of prostaglandins by the way it produces inflammation and edema.

REQUIREMENTS:

Animal	:	Albino rat (180-200 g)
Drugs and chemicals	:	Carrageenan (1%w/v), Diclofenac sodium (standard), Carboxy methyl cellulose (1%w/v), Digital plethysmo meter. U G O Basile (Italy)
Test compounds	:	Siddha preparation *Karpoora Sinthamani Mathirai*

PROCEDURE:

Anti-inflammatory study of *Karpoora Sinthamani Mathirai* was studied in healthy albino rats.

Six rats were selected for each group and divided into IV groups. To the first group distilled water was given and kept as control. The second group was given the standard drug diclofenac a dose of 5 mg/kg body weight. The third and fourth group was treat with the test drug extermelly. Before the application of the drug the Hind paw volume of all rats was measure .This was done by dipping the Hind paw upto the tibio dorsal junction in a mercury plethysmography. Subcutaneous injection of 0.1 ml of 1%w/v carrageenin in water was made in to planter surface of the left hind paw of each rat. Three hours after injection the Hind paw volume was measured once again. The difference between the initial and final volume would show the amount of inflammation.

Taking the volume in the control group as 100% of inflammation the inflammatory or anti-inflammatory effect of the test group is calculated, injection of 0.1 ml of 1%w/v of carrageenin in water was made into planter surface of both the hind paw if each rat.

Three hours after carrageenin injection, the hind paw volume was measured once again. Difference between the initial and final value were noted and compared.

The method is more suitable for studying anti-inflammatory activity on acute inflammation.

The result of the drug is compared with the standard as well as control group.

4.4.2. ANALGESIC ACTIVITY OF KARPOORA SINTHAMANI MATHIRAI BY HOT PLATE METHOD

Analgesic activity of siddha preparation Karpoora Sinthamani Mathirai was evaluated by Hot plate method.

REQUIREMENTS

Animal	:	Wistar Albino Rat (4 – 5 weeks)
Drugs and chemicals	:	1% Tween-80 solution in water, 10ml/kg, p.o. Diclofenac sodium 10 mg/kg, p.o. and the test siddha preparation Karpoora Sinthamani Mathirai drug of 100 mg/kg, p.o. & 200 mg/kg, p.o. respectively

METHOD:

Hot plate method:

Animals

Young wister albino rats of either sex aged 4-5 weeks, average weight 100-150 gm were used for the experiment. The rat were purchased from the animal TANVASU . They were kept in standard environmental condition (at 24.0±0°C temperature & 55-65% relative humidity and 12 hour light/12 hour dark cycle) for one week for acclimation after their purchase and fed ICDDRB formulated rodent food and water ad libitum. The set of rules followed for animal experiment were approved by the institutional animal ethical committee (Zimmermann, 1983).

Experimental animals of either sex were randomly selected and divided into four groups designated as group-I, group-II, group-III and group-IV consisting of five Rats in each group for control, positive control and test sample group respectively. Each group received a particular treatment i.e. control (1% Tween-80 solution in water, 10ml/kg, p.o.), positive control (Diclofenac sodium 10 mg/kg, p.o.) and the test sample (drug of 100 mg/kg, p.o. & 200 mg/kg, p.o. respectively). The animals were positioned on Eddy's hot plate kept at a temperature of 55±0.5 0C. A cut off period of 15 s (Franzotti *et al.*, 2000) was observed to avoid damage to the paw. Reaction time was recorded when animals licked their fore or hind paws, or jumped prior to and 0, 30, 60 and 90 min after oral administration of the samples (Eddy *et al.*, 1953; Kulkarni, 1999; Toma *et al.*, 2003).

4.4.3. IN-VITRO ANTI-SPASMODIC ACTIVITY OF KARPOORA SINTHAMANI MATHIRAI ON EXCISED GUINEA PIG ILEUM

ISOLATION OF GUINEA PIG ILEUM:

Guinea pig were anesthetized and sacrificed by cervical displacement followed by exsanguinations. The ileum was dissected out, immersed in tyrode's solution and cleaned off the mesentery. Respective segments of 2-3 cm long were mounted in a 25ml tissue organ bath, filled with a mixture of 95% O_2 and 5% Co_2 and maintained at 37°C. The composition of Tyrode's solution (in mm for 1 lit) was 9 mg KCl, 0.1mg NaCl, 0.1mg $NaHCo_3$, 0.42mg NaH_2PO_4, 0.6 mg Glucose and pH value was 7.4.

ANTI-SPASMODIC ACTIVITY ASSAY PROCEDURE:

1. Firstly concentration dependent responses of acetylcholine awere recorded (with dose of 0.1ml, 0.2ml, 0.4ml, 0.8ml, 1.6ml, 3.2ml) using Sherrington's recording drum with a frontal writing level. Contact time of 60 sec, and base line of 30 sec time cycle were opted for proper recording of the responses in presence of plane Tyrode's solution as stock – I solution.
2. Then same concentration dependent responses of acetylcholine (Ach) using same procedure for a mixture of Tyrode's solution+ KSM extract (with a concentration of 1mg/ml) as a stock-II solution were recorded.
3. Lastly the same concentration dependent responses of Ach for a mixture of Tyrode's solution+ Atropine (as a standard antispasmodic agent) as a stock-III solution were recorded.

4.4.4. LAXATIVE ACTIVITY OF KARPOORA SINTHAMANI MATHIRAI

Requirements:

Animal	:	Rat (180 – 220gm)
Drugs and Chemicals	:	Sodium Picosulphate, Siddha preparation
		Karpoora Sinthamani Mathirai

Method:

Rats fasted for 12 h before the experiment were placed individually in cages lined with clean filter paper. Rats were divided in five groups with the I group acting as the control and administered saline (5 ml/kg, p.o.) that acted as the negative control. The II group received sodium picosulfate (5 mg/kg, p.o.), this served as the positive control. The III, IV and V groups received 100, 200 and 300 mg/kg per os of the *KSM*. The administration was done using metal oropharyngeal cannula. The faeces production (total number of normal as well as wet faeces) in all five groups was monitored for 16 h[7].

Statistical Analysis

Results were expressed as mean ± SEM. The data were analyzed by using one way analysis of variance (ANOVA) followed by Newmann keul's multiple range tests. P values < 0.05 were considered as significant.

5. MICROBIOLOGICAL ANALYSIS

ANTI - MICROBIAL STUDIES

Aim

To study the Anti-microbial action of "*Karpoora Sinthamani Mathirai*" done by **"Agar well diffusion method"** – Kirby – bauyermethod.

Components of Muller Hinton agar medium

Beef extract	-	300gms/lit
Agar	-	17 gms/lit
Starch	-	1.5 gms/lit
Casein Hydrolysate	-	17.5 gms/lit
Distilled water	-	1000 ml
PH	-	7.6

Procedure:

The method of antibacterial activity study is UPS Diffusion Method. Antibiotic discs are prepared with known concentration of antibiotic are placed on agar plates that has been inoculated with the known pathogenic micro organism. The antibiotic diffuses through the agar producing an antibiotic concentration, gradient antimicrobial susceptibility is proportional to the diameter of the inhibitory zone around the disc. If the microorganism which grows up to the edge of the disc are resistant to the antimicrobial agent. The recommended medium in this method is Muller Hinton Agar, its PH should be between 7.2-7.6 and should be poured to uniform thickness of 4mm in the petri plate (25ml).

Methodology:

Muller Hinton Agar plates are prepared and Pseudomonas, Staphylococcus aureus, Escherichia coli, is inoculated separately.

The prepared disc of *Karpoora Sinthamani Mathirai* are placed over the incubated plate using sterile forceps and incubated for 24 hours at 37 degree celcius. The plates after 24 hours incubation are observed for the zone of inhibition.

6. RESULTS AND DISCUSSION

6.1. STANDARDISATION OF *KARPOORA SINTHAMANI MATHIRAI*

The test drug ***Karpoora Sinthamani Mathirai*** had been subjected to various studies to establish the works of Siddhar`s to be true. Literary collections, physico-chemical and elemental analysis, pharmacological study, toxicological study and antimicrobial study are done to prove the activity of ***Karpoora Sinthamani Mathirai*** as an anti-inflammatory, analgesic, anti-spasmodic and laxative activities.

Table – 4 Physico Chemical Standardisation.

SL. NO.	PARA METER	RESULTS
1.	Organo leptic characters	
	a. Color	Brown
	b. Odour	Pleasant odour
	c. taste	Salt, bitter
	d. Sense of touch	Hard
	e. Appearance	Round
1.	Physico chemical standard	
	a. Loss on drying at 70°C	7.40 %
	b. Ash	
	i. Total ash	7.96%
	ii. Acid insoluble ash	0.90 %
	iii. Water soluble	7.85 %
	c. Extractive value	
	i. Ethanol soluble extractive	8.10 %
	ii. Water soluble extractive	9.30 %
	d. pH value (1% solution)	7.140

Interpretation:

The physical parameters like colour, odour touch, appearance revealed that *Karpoora Sinthamani Mathirai* is a Brown, Pleasant odour, hard to touch and having the PH 7.140 slightly alkaline Ph, Round in appearance.

Determination of loss on drying normal:

The loss on drying test is designed to measure the amount of volatile matters in a sample when the sample is dried under specified conditions moisture is one of the major factors. Responsible for the deterioration of the drugs and formulations low moisture content is always desirable for higher stability of days.

The percentage of loss on drying was (7.40%) within acceptable range to thus implying that the formulation can be stored for a long period and would not easily be attacked by microbes.

Total Ash:

Ash values are helpful in determining the quality and purity of crude drugs. in this trial drug *KARPOORA SINTHAMANI MATHIRAI* (The minerals that present in the trial drug are calcium, chloride, sulphate). The salts, Ca+, Cl-, Sulphate are not harmful one. In this trial drug *KARPOORA SINTHAMANI MATHIRAI* is used as a condensation from water extraction . So only water soluble trace elements present here in a very few trace levels. The total ash value was 7.96%.

Acid insoluble Ash:

Acid insoluble ash values represents detecting the presence of silica and oxalate in a drugs. In my drug the silica and oxalate that is the acid insoluble ash is very low on 0.90 ± 0.011. So the drug has high quality.

Water soluble ash:

Water soluble ash also indicate the purity of the drug water soluble ash higher than acid insoluble ash represents good quality of the drug which is *KARPOORA SINTHAMANI MATHIRAI* is 7.85 %. So water soluble ash is higher than acid insoluble ash.

b)Water soluble extractive

Proceed as directed for the determination of Alcohol-soluble extractive , using chloroform water instead of ethanol water soluble extractive Karpoora Sinthamani Mathirai is 9.30 %.

c)Alcohol soluble extractive

Macerate 5g of the air dried drug, coarsely powdered, with 100ml of alcohol of the specified strength in a closed flask for 24 hrs, shaking frequently during six hours and allowing to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105°,to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug. Alcohol soluble extractive Karpoora Sinthamani Mathirai is 8.10 %.

d)Determination of pH

5 gms of *Karpoora Sithamani Mathirai* was weighted accurately and placed in clear 100ml beaker. Then 50ml of distilled water was added to it and dissolved well. after 30 minutes it was then applied into pH meter at standard buffer solution of 4.0, 7.0 and 9.0. Repeat the test 4 times and average was recorded. The pH of Karpoora Sinthamani Mathirai is 7.140.

Microbial Limit Tests

Table 1: Results of Microbial Contamination Test

S.No.	Test Particulars	Colony Counts (CFU/ g)	Limits Value (CFU/g)
1.	Total Viable Aerobic Bacterial Count	4×10^{2}	1×10^{5}
2.	Total Viable Fungal Count	3.5×10^{2}	1×10^{3}

Results of Specific Pathogens Test

S.No.	Test for Specified Pathogens	Colony Counts (CFU/ g)	Limits Value (CFU/g)
1.	*Salmonella* sp.	No growth	-
2.	*Staphylococcus aureus*	No growth	-
3.	*Escherichia coli*	No growth	-
4.	*Pseudomonas aeruginosa*	No growth	-

Interpretation :

The total bacterial count and the total fungal count of the drug were found to be within the WHO prescribed limits which indicate that the drug is free from microbial contamination. The other pathogens like Escherichia coli, Salmonella sps, Pseudomonas aeruginosa and Staphylococcus aureus were found to be completely absent in the drugs

Disintegration:

The disintegration of test sample under the specifications not more than 15 minutes. In the present analysis the *KARPOORA SINTHAMANI MATHIRAI* disintegration on only 10m 50 sec.

BIO CHEMICAL ANALYSIS

Table – 5 Results of Preliminary test for basic and acidic radicals

S.NO	EXPERIMENT	INFERENCE
1.	Test for Calcium	**Present**
2.	Test for Sulphate	**Present**
3.	Test for Chloride	**Present**
4.	Test for Carbonate	Absent
5.	Test for Starch	**Present**
6.	Test for Ferric Iron	Absent
7.	Test for Ferrous Iron	**Present**
8.	Test for Phosphate	Absent
9.	Test for Albumin	Absent
10.	Test for Tannic Acid	Absent
11.	Test for Unsaturated Compounds	**Present**
12.	Test for Reducing Sugar	Absent
13.	Test for Amino Acid	**Present**
14.	Test for Zinc	Absent

INTERPRETATION:

The biochemical analysis of *Karpoora Sinthamani Mathirai* contains the following chemical constituents, Sulphate, Chloride, Starch, Ferrous iron, Unsaturated compound and Amino acid.

CALCIUM:

- ❖ Maintenance of plasma calcium level within normal range is of vital importance because neuro-muscular excitability is dependent on plasma calcium level.

- ❖ 99% of calcium is used to keep our bones and teeth strong, thereby supporting skeletal structure and function. The rest of the calcium in our body plays a major role in muscle contraction, nerve function and blood clotting.
- ❖ Proper levels of calcium in body over a lifetime can prevent osteoporosis.

SULPHATE:

- ❖ It significantly improves the joint function. It helps in reduce the pain and inflammation.
- ❖ Sulphate's primary biological role in halting or reversing joint degeneration.
- ❖ Nutritionally essential element
- ❖ Functional in the form of sulphur containing amino acids.

STARCH:

- ❖ Starches functions much like dietary fibre. They provide nutrition for the beneficial bacteria in the colon, keeping them thriving and health. Dietary fibre in starch reduces effects of haemorrhoids, diverticulosis & controls blood pressure.

FERROUS IRON :

- ❖ Iron help to preserve many vital functions in the body, including general energy and focus, gastrointestinal processes, the immune system and regulation of body temperature.

CHLORIDE:

- ❖ Calcium- activated chloride channels (Ca-Cl) are thought to regulate neuronal excitability and recently chloride regulation in DRG (Dorsal root ganglion) neurons has attracted much attention in pain research.
- ❖ Chloride forms the chief anion of the extracellular fluid and exists along with sodium mostly.
- ❖ Regulates acid base balance.
- ❖ Formation of HCl in gastric juice
- ❖ Help to preserve normal neuromuscular irritability by maintaining a state of equilibrium, on account of their relative proportion in ECF and ICF.

UNSATURATED COMPOUND:

- A free phenolic hydroxyl group is essential for scavenging oxygen free-radicals and is also essential for inhibiting leukocyte chemotaxis,
- That inhibition of leukocyte chemotaxis may be involved in the anti-inflammatory action of phenolic compounds, and that one of the anti-inflammatory actions of phenolic compounds is the prevention of the production of oxygen free-radicals by leukocytes.

AMINO ACID:

- Amino acids are involved in protein synthesis.
- Amino acid nourishes smooth muscles of GIT
- The body can also use aminoacid for energy when lack of carbohydrates and fats

PHYTOCHEMICAL STUDY OF *KARPOORA SINTHAMANI MATHIRAI*

The *Karpoora Sinthamani Mathirai* was subjected to qualitative chemical investigation Details of the various tests performed for the presence of phytoconstituents is shown in Table 7.

Table – 6 Phytochemical tests for *Karpoora Sinthamani Mathirai*

Tests	***Karpoora Sinthamani Mathirai***
Alkaloids	
Mayer's test	+ve
Dragendroff's test	+ve
Hager's test	+ve
Carbohydrates and glycosides	
Molisch test	+ve
Legal's test	-ve
Borntrager's test for anthraquinones	-ve
Phytosterols	
Liebermann-Burchard test	-ve
Salkowski test	-ve
Flavanoids	
Shinoda test Magnesium turnings and hydrochloric acid (Presence of red color)	-ve
Fluorescence test	-ve
Tannins	
Ferric chloride test	-ve
Potassium dichromate test	-ve
Lead acetate test	+ve
Proteins	
Millon's test	-ve
Biuret test	-ve

Ninhydrin test	-ve
Fixed oils and fats	
Spot test	-ve
Saponification test	+ve
Lignin	
Phloroglucinol test	-ve
Saponins	
Frothing test	-ve

(+ve) indicates the presence of phytochemical, (-ve) indicates the absence of phytochemical.

INTERPRETATION:

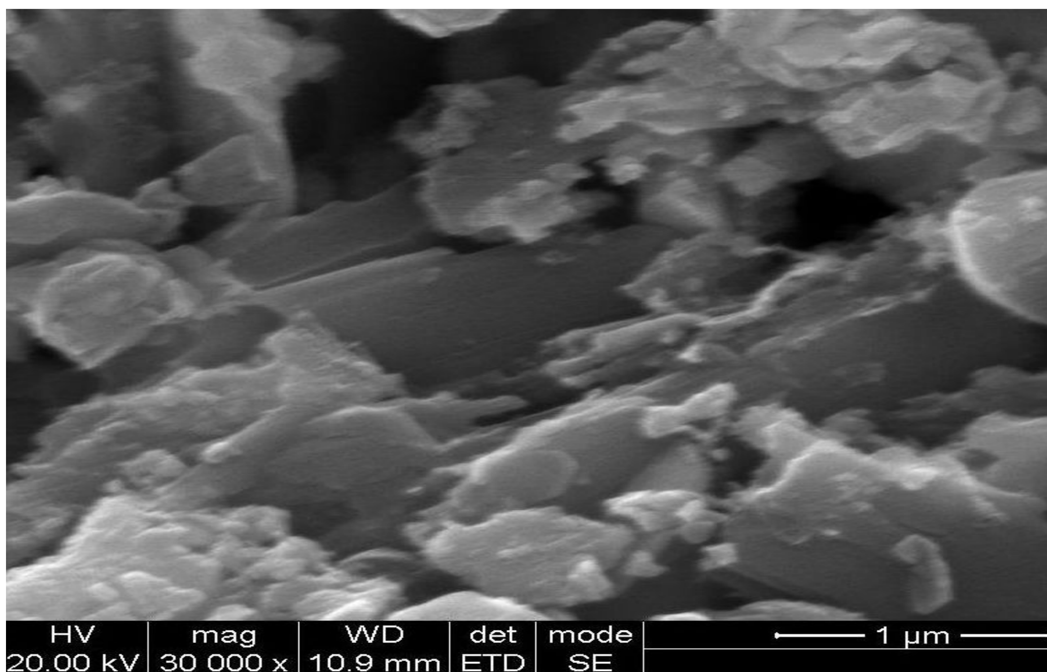
Alkaloids-decreased gastric acid secretion and inhibit the gastric motility

Tannins-Tannins react with tissue promote tissue proteins.

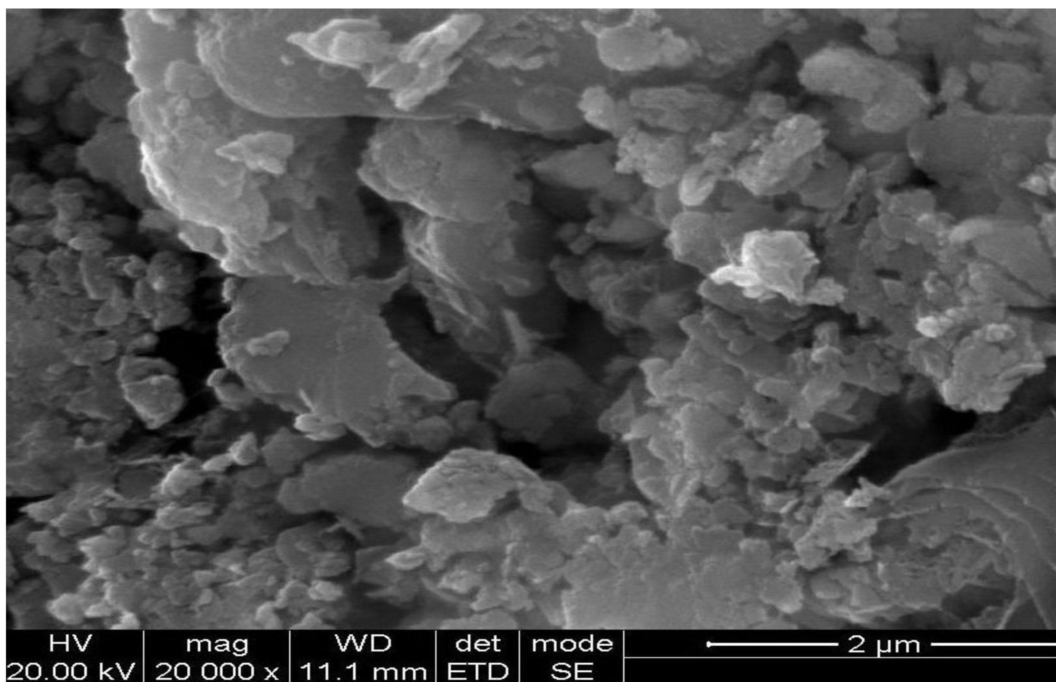
This study revealed the presence of active phytochemicals in Karpoora Sinthamani Mathirai such as alkaloids, carbohydrates, glycosides, tannins, lignins, fixed oils and fats.

INSTRUMENTAL ANALYSIS

SCANNING ELECTRON MICROSCOPE (SEM)



SEM -30000 Magnification



SEM -20000 Magnification

Figure - 6 Showing SEM Results of Trial Drug

(KARPOORA SINTHAMANI MATHIRAI)

INTERPRETATION :

The morphology of the *Karpoora Sinthamani Mathirai* samples can be determined by Environmental SEM (FEI Quanta). A representative portion of each sample must be sprinkled onto a double side carbon tape and mounted on aluminium stubs, in order to get a higher quality secondary electron image for SEM examination. The SEM photographs revealed that particles were spherical in shapes and sizes were in the range from 1µm to 5µm.Although the particle sizes of different batches showed similarity, it seems that these particles were aggregates of much smaller particles.

When dispersed in an aqueous medium, these preparations form a negatively charged hydrophobic particle suspension. This hydrophobicity gave these particles a tendency to aggregate together to form larger particles. ***Karpoora Sinthamani Mathirai*** exhibited larger sizes and agglomeration of the particles. Therefore, the comparatively larger size may be due to the agglomeration of the particles by repeated cycles of calcinations involved in preparation. SEM analysis of the ***Karpoora Sinthamani Mathirai*** shows most of the particles present in the sample are nano size, average particle size is **2 - 1µm**.

FOURIER TRANSFORM-INFRARED SPECTROSCOPY(FTIR)

Fourier Transform Infra-Red Spectroscopy (FTIR) analysis results in absorption spectra that provide information about the functional group and molecular structure of a material IR relates with the sample and the bonds among atoms in the molecule stretch and bend, absorbing infrared energy and creating the infrared spectrum. It is of two kinds of bending and stretching.

FT-IR is a very useful tool in the recognition of the functional groups of bio molecules, thus aiding in their structural elucidation, so confirming the presence of active molecules responsible for the therapeutic activity of Siddha drugs. The results of Table no: 7 and Fig no:7 shows the presence of functional group and inorganic compounds of ***Karpoora Sinthamani Mathirai***

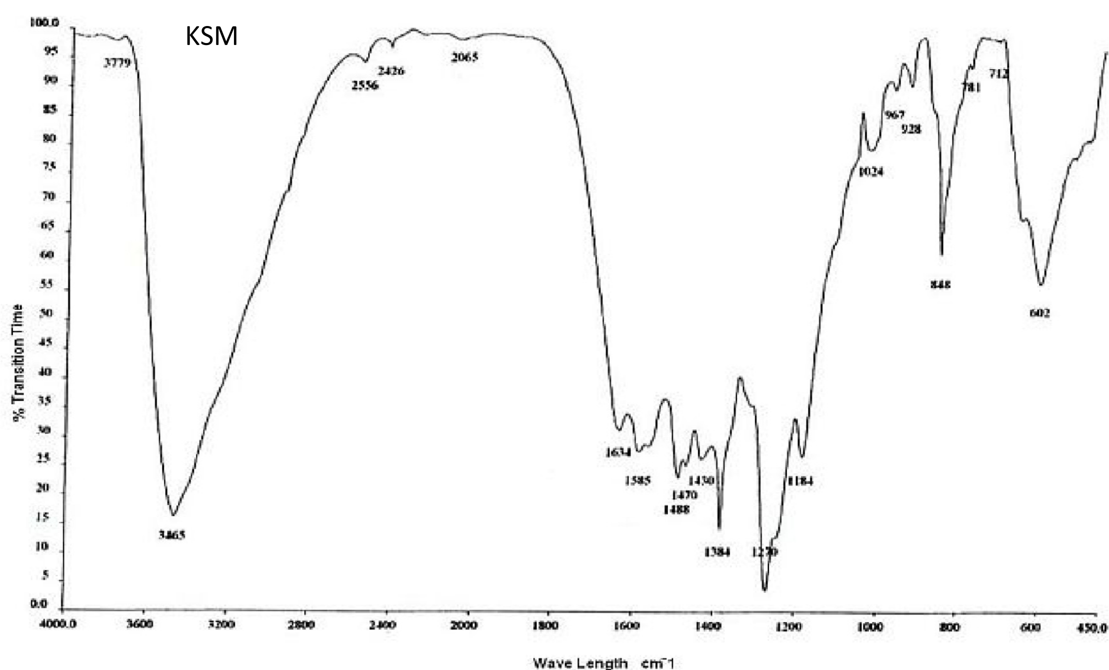


Figure -7 Showing FTIR Image of *KARPOORA SINTHAMANI MATHIRAI*

Table – 7 Interpretation of FTIR Spectrum

S.No	Frequency	Bond	Functional Group
1.	3779	-	Unknown compound
2.	3465	O-H Stretch	Hydrogen Bonded - Alcohol Phenol
3.	2556	O-H Stretch	Carboxylic acid
4.	2426	-	Unknown compound
5.	2065	-	Unknown compound
6.	1634	N-H Bend	Primary amines
7.	1585	$-NO_2$	Aliphatic nitro group
8.	1488	C-C-Stretch	Aromatics
9.	1470	C-H Bend	Alkanes
10.	1430	C-H Bend	Alkanes
11.	1384	NO_2 Symmetrical stretch	Aliphatic nitro compounds
12.	1220	C-O Stretch	Alcohols, ether, carboxylic acid, ester
13.	1184	C-O Stretch	Alcohols, ether, carboxylic acid, ester
14.	1044	C-N Stretch	Aliphatic amines
15.	967	=C-H Bend	Alkenes
16.	928	O-H Bend	Carboxylic acid
17.	848	C-H Bend	Phenyl ring substitution bands
18.	781	C-Cl Stretch	Alkyl halides
19.	712	C-H Bend	Aromatics
20.	602	Actylenic C-H Bend	Alkynes

INTERPRETATION:

1. FTIR instrumental analysis was done. The test drug was identified to have 20 peaks. They are the functional groups present in the trial drug *Karpoora Sinthamani Mathirai* .

2. It confirms that *Karpoora Sinthamani Mathirai* constitutes Alcohol, Phenol, Carboxylic acid, Primary amine, Aliphatic Nitro groups, Aromatics, Alkanes, Aliphatic Nitro compounds, ether, Aliphatic amine, Alkenes, Phenyl ring, Alkyl halides, Alkynes as functional groups.

Ether:

- Ether is a class of organic compound characterized by an oxygen atom bonded to two alkyl or aryl group.
- Diethyl ether inhibits alcohol dehydrogenase and thus slows the metabolism of ethanol. It depresses the myocardium and increases tracheobronchial secretion.
- Codine a potent pain relieving drug is the methyl ether of morphine.

Carboxylic acid :

It is a classical organic compounds that are characterised by the presence of carboxyl groups (-COOH) in them. They make up series fatty acids which are extremely good for human health.The omega3 and the omega 6 are the essential fatty acids which are not produced by the body. They help in maintaining the cell membrane and control nutrient use along with metabolism.

Phenols:

Phenol is active against a wide range of micro organisms. .It is a powerful Anti-septic, Antiferment and disinfectant.Prevents the decomposition of albuminous fluids by bacteria.It is a neurolytic agent, applied in order to relieve spasms and chronic pains.

Amines:

Acts as a neuro transmitter.Involved in protein synthesis.Amines play an important role in reducing abdominal pain,bloating.

Alkanes:

They protect against bacteria and fungal infections.

Alcohols

It has anti microbial action.Acts as a antiseptic agent.

Aldehydes:

Aldehydes normally have Anti-microbial activities.They are readily absorbed by the Gastro intestinal tract.

Aromatics:

Aromatics are good pain relievers.It has Anti-pyretic, Anti-inflammatory, Auto-immune activities.

ICP-OES of *KARPOORA SINTHAMANI MATHIRAI*

Karpoora Sinthamani Mathirai (wt:0.41210g)

Elements	Wavelength (nm)	Concentration
Al	396.152	BDL
As	188.979	BDL
Ca	315.807	121.100 mg/l
Cd	228.802	BDL
Cu	327.393	BDL
Fe	238.204	21.354 mg/l
Hg	253.652	BDL
K	766.491	03.071 mg/l
Mg	285.213	01.104 mg/l
Na	589.592	04.180 mg/l
Ni	231.604	BDL
Pb	220.353	BDL
P	213.617	86.307 mg/l
S	180.731	41.204 mg/l
Zn	206.200	3.256 mg/l

BDL: Below Detectable Limit(Normal-1ppm)

1% = 10000ppm,

1ppm = 1/1000000 or 0.0001%

Toxic metals and the permissible limits

Heavy metals	WHO & FDA limits
Arsenic(As)	10ppm
Mercury(Hg)	1ppm
Lead (pb)	10ppm
Cadmium (Cd)	0.3ppm

INTERPRETATION

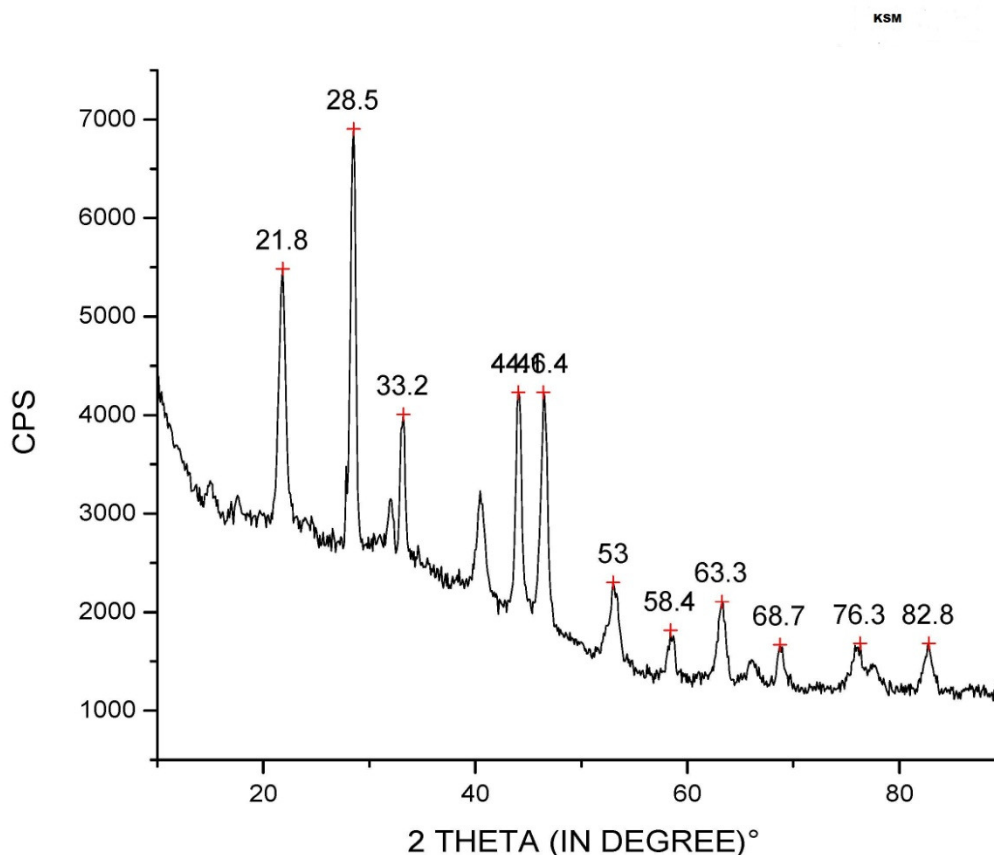
This result indicates the presence of Calcium, Iron, Potassium, Sodium, Magnesium, Phosporus, Sulphur, Zinc

- **Calcium** is essential for maintaining the necessary level of bone mass. The body is constantly using calcium for the muscles and nerves. Maintenance of plasma calcium level within normal range is of vital importance because neuro-muscular excitability is dependent on plasma calcium level.
- **Phosphorous**, it decreases urine calcium, reduces demineralization of bone and increases calcium balance. It is an important constituent of phosphate buffers in the blood and urine. It is required for the formation of certain physiologically important phosphorus containing compounds like phospholipids, coenzymes and enzymes of intermediary metabolism. Contributes formation of ATP, ADP and creatine phosphate. [24]
- **Sodium and Potassium**: In the presence of Sodium and Potassium regulate the acid-base balance of the body fluids . They help to preserve the neuromuscular irritability by maintaining a state of equilibrium on account of their relative proportion in the ECF and ICF.
- **Magnesium** is a cofactor that regulates diverse biochemical reactions in the body, including protein synthesis, muscle, nerve function, blood glucose control and blood pressure regulation
- **Zinc** may be regarded as an antioxidant, protects the body against free radical damage and cell damage. Zinc is important for a healthy immune system. It enhances absorbtion of iron.It can produce healthy veins and arteries that enhance the blood circulation
- **Sulphate** may prevent the occurrence of any infection, sulphate is potent anti oxidant activity in human body.

XRD (X-Ray Diffraction):

X-Ray powder diffraction is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions.

Fig. No. 8 XRD –Results of *KSM*



This XRD finger print shows both the similarities and differences of the sample successfully and is a valuable primary tool for checking the quality control of Herbo-mineral formulations. The different peaks show the presence of minerals in the samples.

TOXICITY STUDIES

EVALUATION OF ACUTE TOXICITY STUDY OF *KARPOORA SINTHAMANI MATHIRAI*

8. Effect of Acute Toxicity Study (14 Days) of *KARPOORA SINTHAMANI MATHIRAI*

Table no –8.1 Physical and behavioral examinations.

Group no.	**Dose(mg/kg)**	**Observation sign**	**No. of animal affected.**
Group-I	5mg/kg	Normal	0 of 3
Group- II	50mg/kg	Normal	0 of 3
Group-III	300mg/kg	Normal	0 of 3
Group-IV	1000mg/kg	Normal	0 of 3
Group-V	2000mg/kg	Normal	0 of 3

Table no-8.2 Home cage activity

Functional and Behavioural observation	**Observation**	**5mg/kg Group (G-I)**	**50mg/kg (G-II)**	**300mg/kg (G-III)**	**1000mg/kg (G-IV)**	**2000mg/kg (G-V)**
		Female n=3	**Female n=3**	**Female n=3**	**Female n=3**	**Female n=3**
Body position	Normal	3	3	3	3	3
Respiration	Normal	3	3	3	3	3
Clonic involuntary Movement	Normal	3	3	3	3	3
Tonic involuntary Movement	Normal	3	3	3	3	3
Palpebral closure	Normal	3	3	3	3	3
Approach response	Normal	3	3	3	3	3
Touch response	Normal	3	3	3	3	3
Pinna reflex	Normal	3	3	3	3	3
Tail pinch response	Normal	3	3	3	3	3

Table no-8.3 Hand held observation

Functional and Behavioral observation	Observation	Control	5 mg/kg (G-I)	50 mg/kg (G-II)	300mg/kg (G-III)	1000mg/kg (G-IV)	2000mg/kg (G-V)
		Female n=3	Female n=3	Female n=3	Female n=3	Female n=3	Female n=3
Reactivity	Normal	3	3	3	3	3	3
Handling	Normal	3	3	3	3	3	3
Palpebral closure	Normal	3	3	3	3	3	3
Lacrimation	Normal	3	3	3	3	3	3
Salivation	Normal	3	3	3	3	3	3
Piloerection	Normal	3	3	3	3	3	3
Pupillary reflex	Normal	3	3	3	3	3	3
Abdominal tone	Normal	3	3	3	3	3	3
Limb tone	Normal	3	3	3	3	3	3

Table no-8.4 Mortality

Group no	Dose no(mg/kg)	Mortality
Group-I	5(mg/kg)	0 of 3
Group-II	50(mg/kg)	0 of 3
Group-III	300(mg/kg)	0 of 3
Group-IV	1000(mg/kg)	0 of 3
Group-V	2000(mg/kg)	0 of 3

RESULT:

From acute toxicity study it was observed that the administration of *KARPOORA SINTHAMANI MATHIRAI* at a dose of 2000 mg/kg to the rats do not produce drug-related toxicity and mortality. So No-Observed-Adverse-Effect- Level (NOAEL) of *KARPOORA SINTHAMANI MATHIRAI* is 2000 mg/kg.

DISCUSSION

KARPOORA SINTHAMANI MATHIRAI was administered single time at the dose of 5mg/kg, 50mg/kg , 300mg/kg, 1000mg/kg and 2000mg/kg to rats and observed for consecutive 14 days after administration. Doses were selected based on the pilot study and literature review. All animals were observed daily once for any abnormal clinical signs. Weekly body weight and food consumption were recorded. No mortality was observed during the entire period of the study. Data obtained in this study indicated no significance physical and behavioural signs of any toxicity due to administration of***KARPOORA SINTHAMANI MATHIRAI*** at the doses of 5mg/kg, 50mg/kg , 300mg/kg, 1000mg/kg and 2000mg/kg to rats.

At the 14th day, all animals were observed for functional and behavioral examination. In functional and behavioral examination, home cage activity, hand held activity were observed. Home cage activities like Body position, Respiration, Clonic involuntary movement, Tonic involuntary movement, Palpebral closure, Approach response, Touch response, Pinna reflex, Sound responses, Tail pinch response were observed. Handheld activities like Reactivity, Handling, Palpebral closure, Lacrimation, Salivation, Piloercetion, Papillary reflex, abdominal tone, Limb tone were observed. Functional and behavioral examination was normal in all treated groups. Food consumption of all treated animals was found normal as compared to normal group.

Body weight at weekly interval was measured to find out the effect of ***KARPOORA SINTHAMANI MATHIRAI*** on the growth rate. Body weight change in drug treated animals was found normal.

INTERPRETATION:

KARPOORA SINTHAMANI MATHIRAI was administered single time at the dose of 5mg/kg, 50mg/kg , 300mg/kg, 1000mg/kg and 2000mg/kg to rats and observed for consecutive 14 days after administration. Doses were selected based on the pilot study and literature review. All animals were observed daily once for any abnormal clinical signs. Weekly body weight and food consumption were recorded. No mortality was observed during the entire period of the study. Data obtained in this study indicated no significance physical and behavioural signs of any toxicity due to

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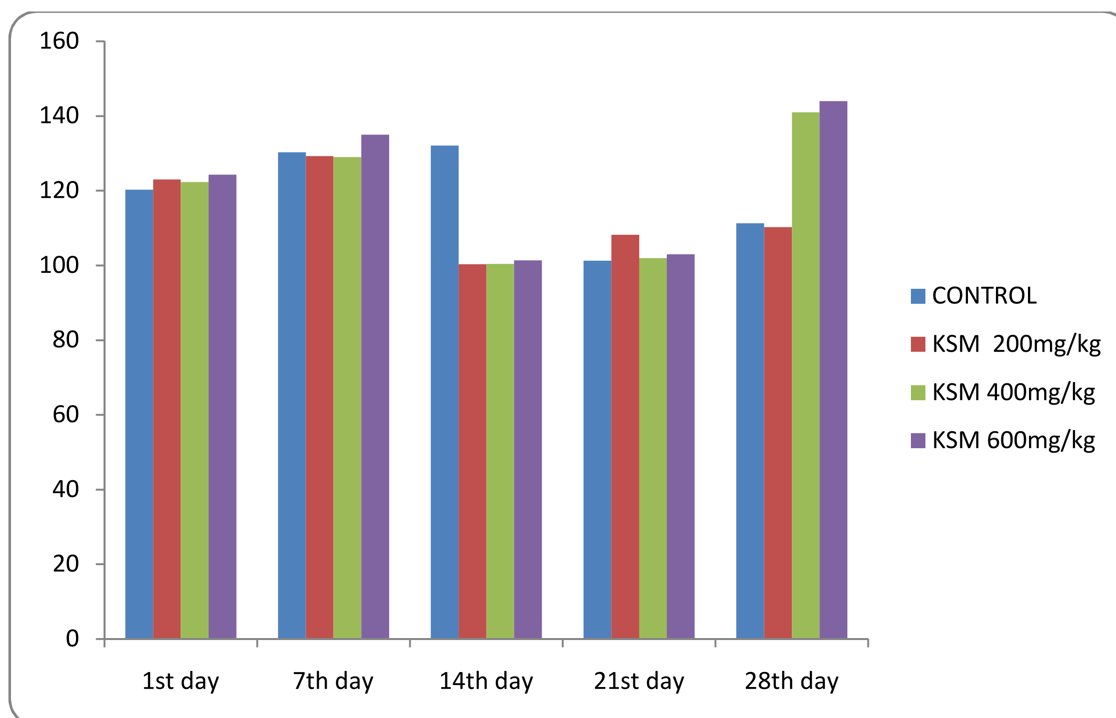
Body weight at weekly interval was measured to find out the effect of ***KARPOORA SINTHAMANI MATHIRAI*** on the growth rate. Body weight change in drug treated animals was found normal.

SUB-ACUTE TOXICITY STUDY IN WISTAR RATS TO EVALUATE TOXICITY PROFILE OF *KARPOORA SINTHAMANI MATHIRAI*

Table 9.1.EFFECT OF SUB- ACUTE DOSE (28 DAYS)OF *KARPOORA SINTHAMANI MATHIRAI ON* BODY WEIGHT IN GRAM

GROUP	CONTROL	LOW	MID	HIGH
1st day	120.3±1.03	123±1.543	122.3±2.231	124.3±2.23
7th day	130.3±1.03	129.3±1.343	129±2.113	135±2.11
14th day	132.1±1.004	100.3±1.12	100.4±2.012	101.4±2.012
21st day	101.3±2.120	108.2±1.501	102±1.131	103±1.13
28th day	111.3±1.041	110.3±1.202	141±2.0405	144±2.040

Values are expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by Dunnett's(n=6); $^{ns}p>0.05$, $*p<0.05$, $**p<0.01$, $***p<0.001$, calculated by comparing treated groups with control group.

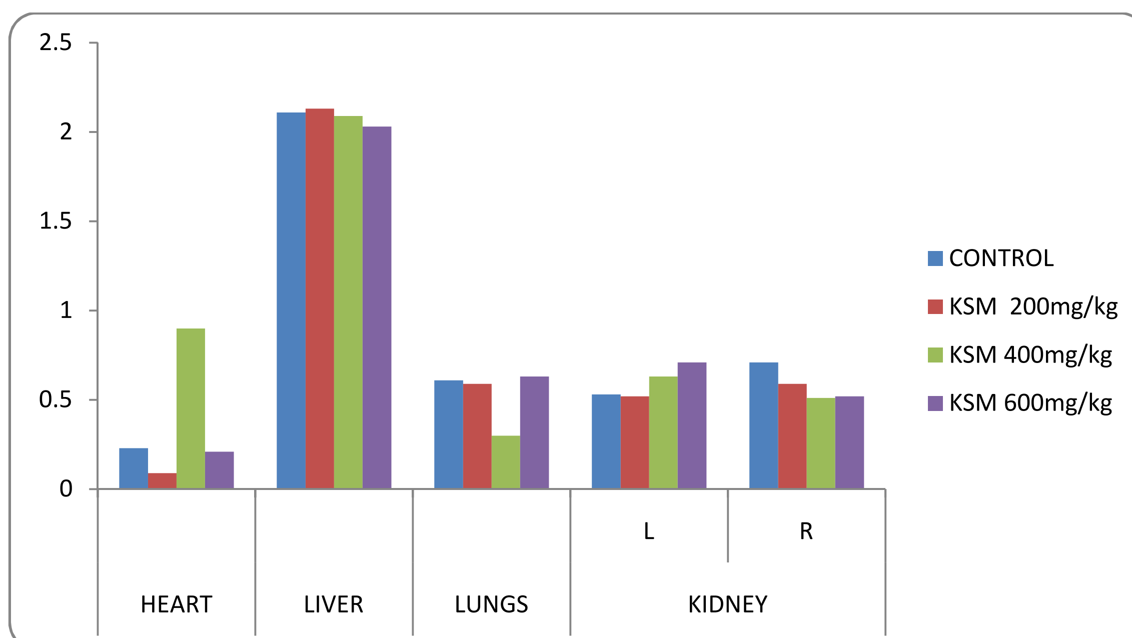


EFFECT OF SUBACUTE DOSE (28 DAYS) OF *KARPOORA SINTHAMANI MATHIRAI*

Table : 9.2. *KARPOORA SINTHAMANI MATHIRAI* ON ORGAN WEIGHT (PHYSICAL PARAMETER) IN GRAM

GROUP		CONTROL	LOW	MID	HIGH
HEART		0.23±0.02	0.09±0.04	0.90±0.11	0.21±0.02
LIVER		2.11± 0.23	2.13±0.23	2.09±0.01	2.03± 0.23
LUNGS		.61±0.10	0.59±0.14	0.30±0.24	0.63±0.10
KIDNEY	L	0.53±0.02	0.52±0.03	0.63±0.02	0.71±0.02
	R	0.71±0.024	0.59±0.02	0.51±0.024	0.52±0.024

Values are expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by Dunnett's(n=6); $^{ns}p>0.05$, $*p<0.05$, $**p<0.01$, $***p<0.001$, calculated by comparing treated groups with control group.

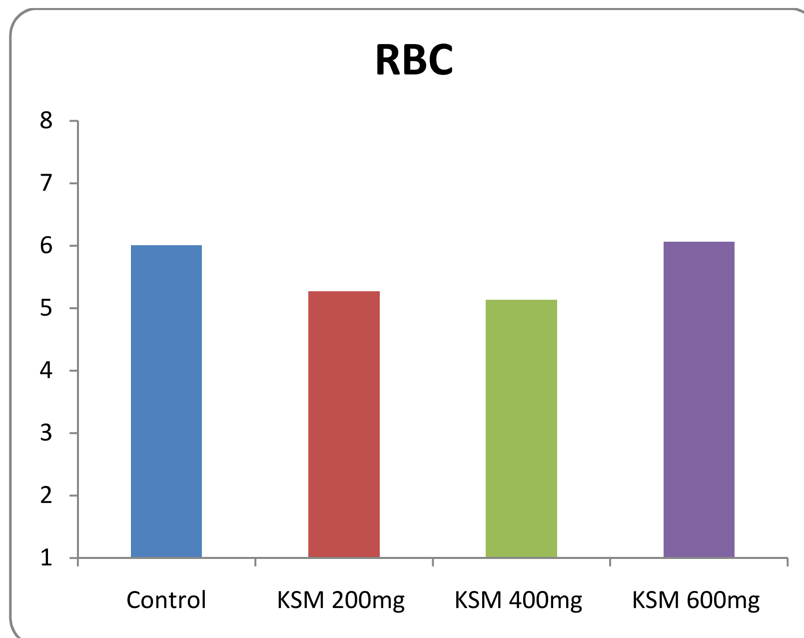


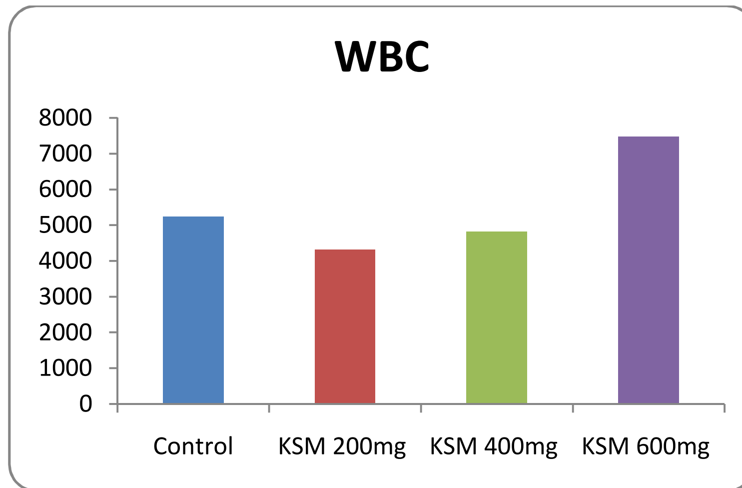
EFFECT OF SUB- ACUTE DOSE (28 DAYS) OF *KARPOORA SINTHAMANI MATHIRAI ON* HAEMATOLOGICAL PARAMETERS

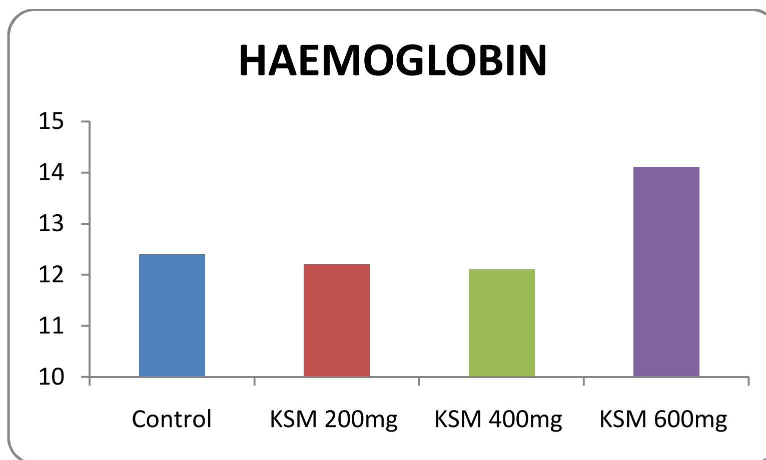
Table no 9.3

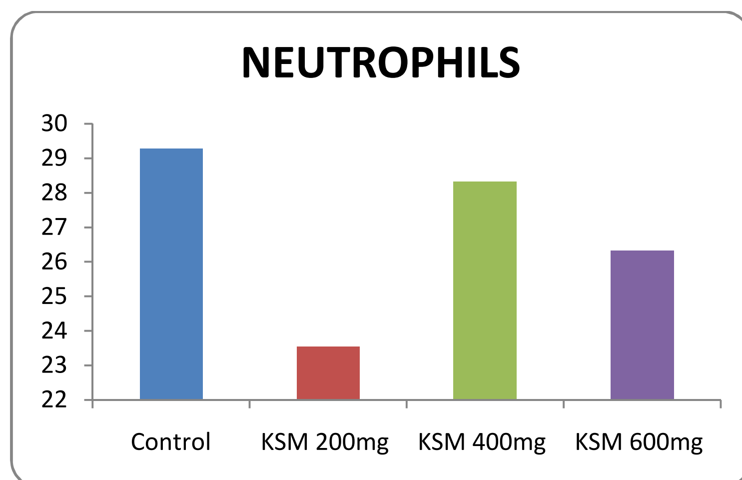
GROUP	CONTROL	KSM 200mg/kg	KSM 400mg/kg	KSM 600mg/kg
RBC ($X10^6/\mu L$)	6.01±0.40	5.27±0.20	5.13±0.21	6.06±0.21
WBC ($X10^3/\mu L$)	5232.41±23.32	4314.04±23.22	4828.25±32.35	7468.25±32.35
HB (g/dl)	12.40±0.45	12.20±0.43	12.11±1.03	14.11±1.03
Neutrophils(%)	29.27±1.20	23.54±1.41	28.32±2.22	26.32±2.22
Eosinophils(%)	1.33±0.11	1.90±0.14	1.24±0.12	1.30±0.12
Monocyte(%)	2.89±0.15	3.09±0.30	3.44±0.40	3.57±0.40
Lymphocyte(%)	67.46±3.32	66.27±3.51	66.93±3.32	64.69±3.32

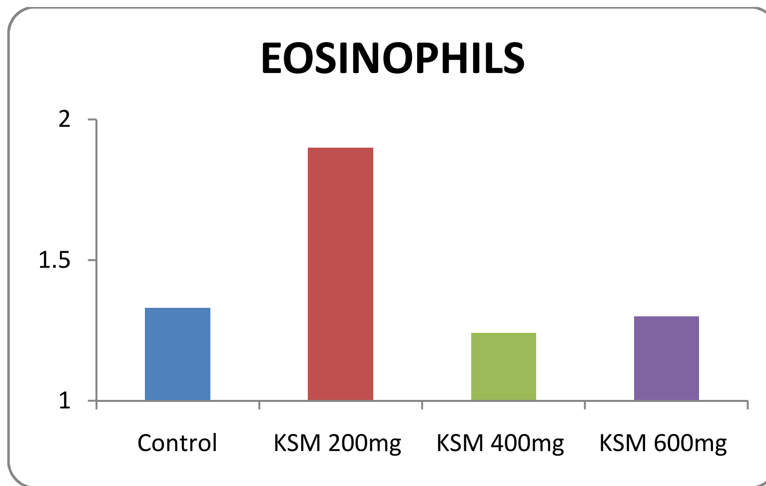
Values are expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by Dunnett's(n=6); $^{ns}p>0.05$, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, calculated by comparing treated groups with control group.

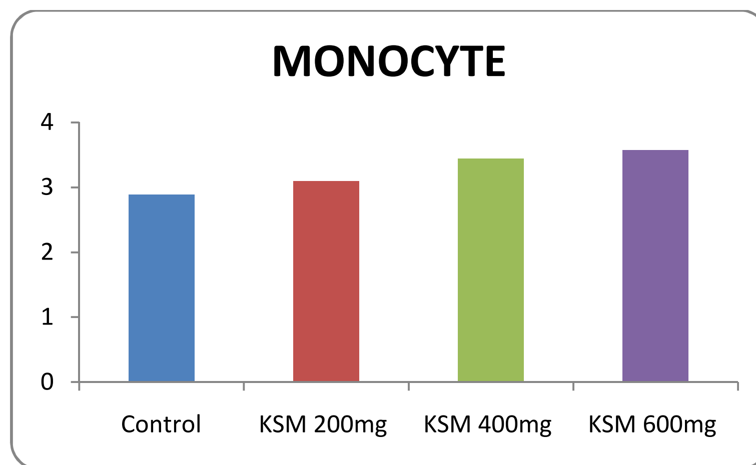



WBC
8000
7000
6000
5000
4000
3000
2000
1000
0
Control
KSM 200mg
KSM 400mg
KSM 600mg


HAEMOGLOBIN
15
14
13
12
11
10
Control
KSM 200mg
KSM 400mg
KSM 600mg


NEUTROPHILS
30
29
28
27
26
25
24
23
22
Control
KSM 200mg
KSM 400mg
KSM 600mg


EOSINOPHILS
2
1.5
1
Control
KSM 200mg
KSM 400mg
KSM 600mg


MONOCYTE
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3
2
1
0
Control
KSM 200mg
KSM 400mg
KSM 600mg

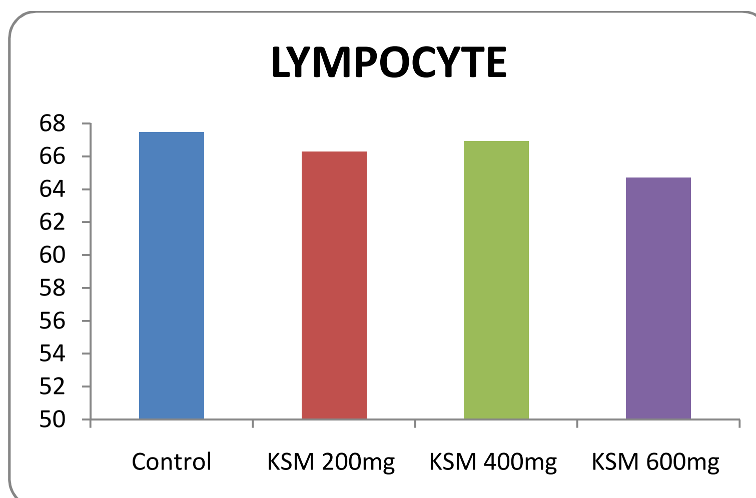
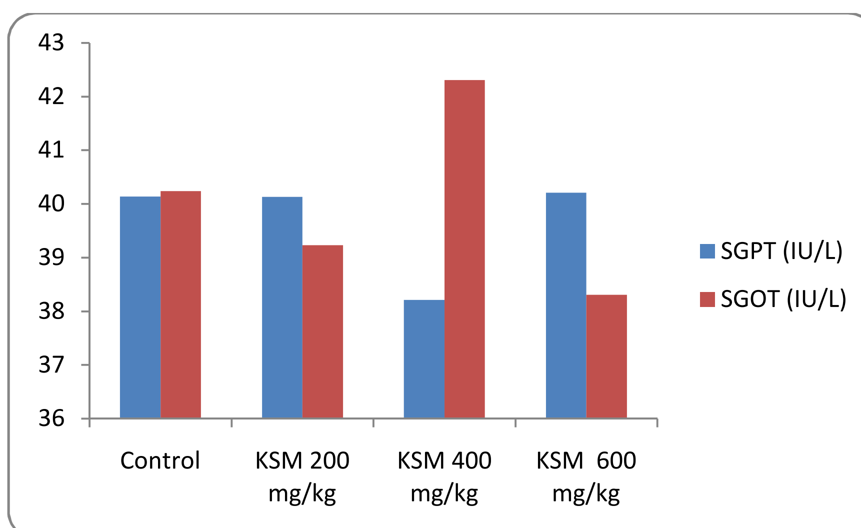
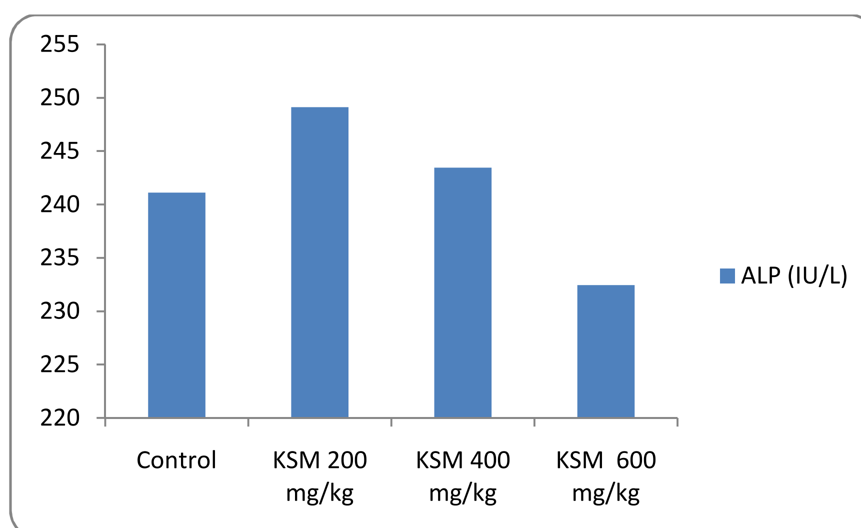
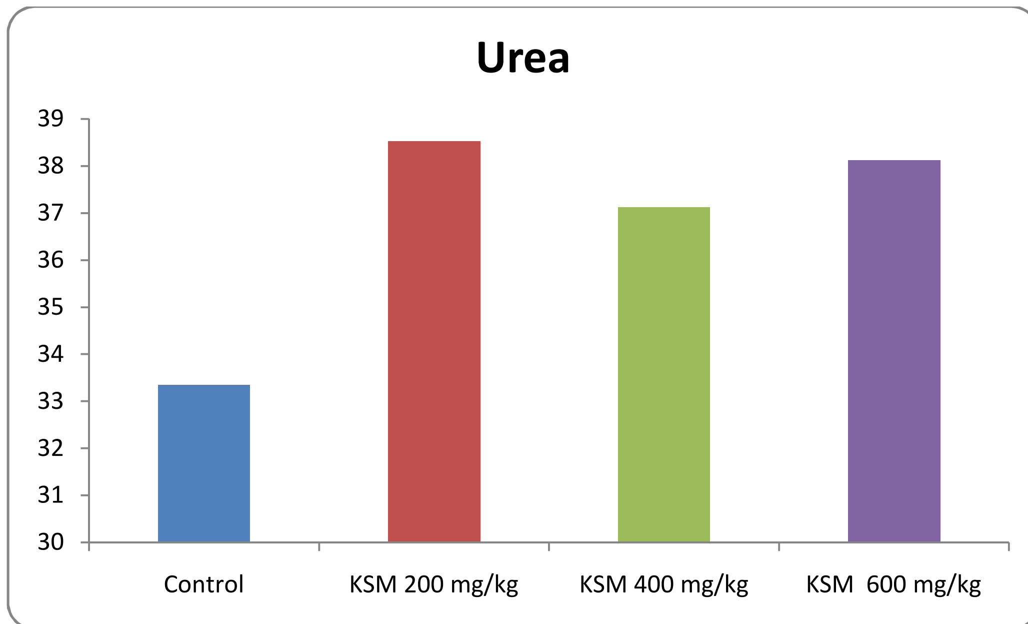

LYMPOCYTE
68
66
64
62
60
58
56
54
52
50
Control
KSM 200mg
KSM 400mg
KSM 600mg

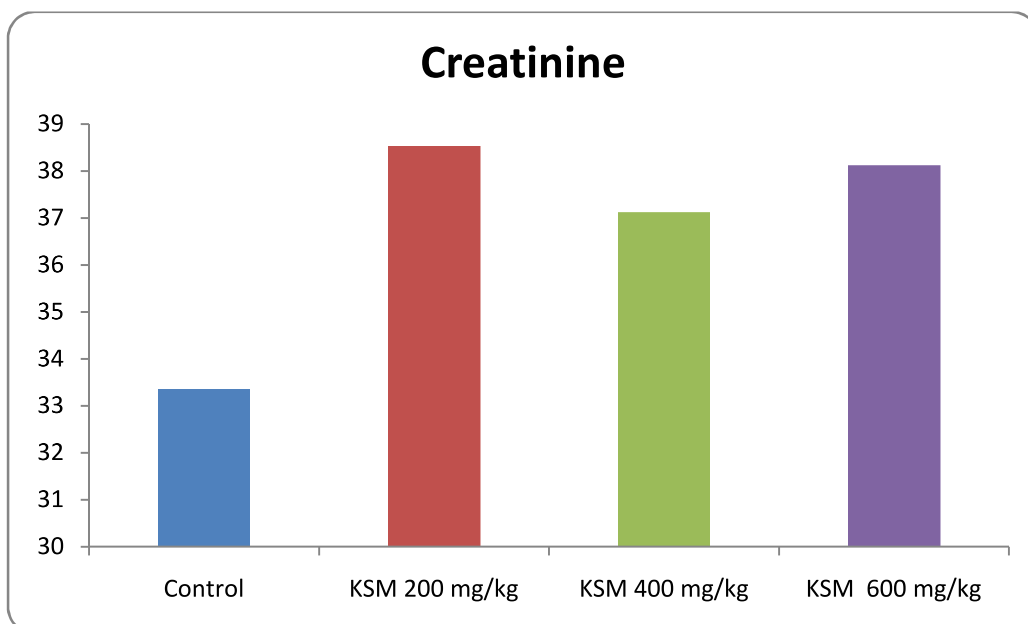
Table :9.4 EFFECT OF SUB- ACUTE DOSE (28 DAYS) OF *KARPOORA SINTHAMANI MATHIRAI* ON BIOCHEMICAL PARAMETERS

Drug Treatment	SGPT (IU/L)	SGOT(IU/L)	ALP(IU/L)	Urea (mg/dl)	Creatinine(mg/dl)
Control	40.14±3.02	40.24±4.31	241.12±11.32	33.35±3.00	0.54±0.03
LOW	40.13±3.22	39.23±4.01	249.11±12.42	38.53±2.42	0.60±0.04
MID	38.21±4.44	42.31±2.21	243.45±4.14	37.12±2.22	0.45±0.04
HIGH	40.21±4.44	38.31±2.21	232.45±4.14	38.12±2.22	0.56±0.04






Urea
39
38
37
36
35
34
33
32
31
30
Control
KSM 200 mg/kg
KSM 400 mg/kg
KSM 600 mg/kg


Creatinine
39
38
37
36
35
34
33
32
31
30
Control
KSM 200 mg/kg
KSM 400 mg/kg
KSM 600 mg/kg

EFFECT OF SUB- ACUTE DOSE (28 DAYS) OF *KARPOORA SINTHAMANI MATHIRAI* BIOCHEMICAL PARAMETERS

GROUP	CONTROL	*KSM* (200mg/kg)	*KSM* (400mg/kg)	*KSM* (600mg/kg)
TOTAL BILIRUBIN (mg/dl)	0.508±0.2457	0.858±0.2827	0.819±0.3376	0.904±0.199

Values are expressed as mean ± SEM Statistical significance (p) calculated by one-way ANOVA followed by Dunnett's(n=6); $^{ns}p>0.05$, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, calculated by comparing treated groups with control group.

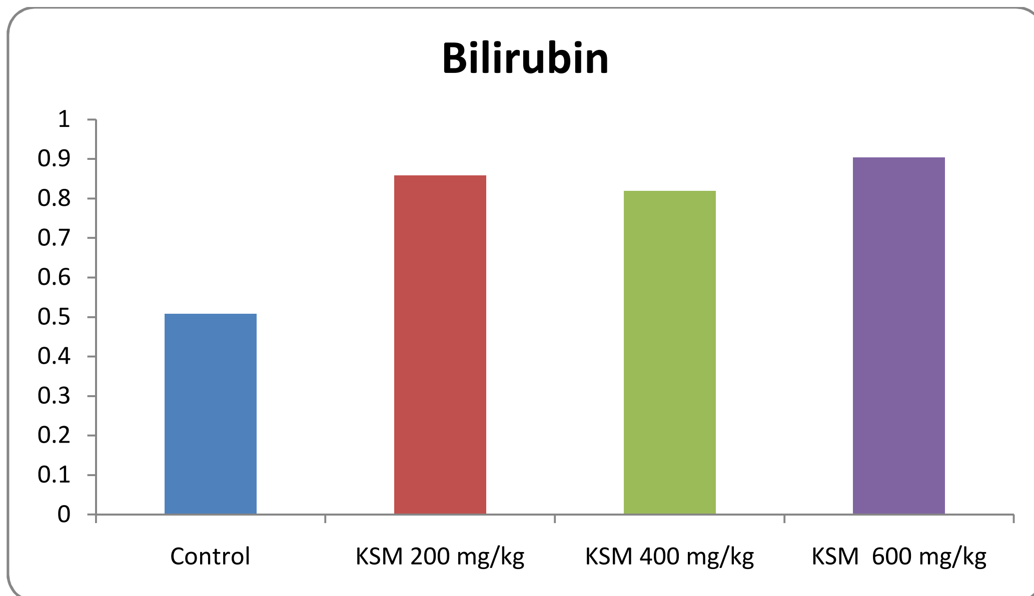


Table: 9.5. EFFECT OF SUB- ACUTE DOSE (28 DAYS) OF ON FOOD INTAKE IN GRAM

GROUP	CONTROL	L	M	H
1st DAY	17.33±13.6110	18.1672±15.3	11.10±22.71	16.5±8.62
7th DAY	14.5±12.	9.863±13.67	15.73±10.853	10.17±15.41
14th DAY	17.83±8.72	9.83±15.28	9±14.96	18.72±9.981
21st DAY	10.87±13.4	14±8.466	14.88±10.43	18.17±9.02
28th DAY	11.10±11.38	17.38±11.50	9±8.90	9±7.57

Values are expressed as mean ± SEM Statistical significance (p) calculated by one-way ANOVA followed by Dunnett's(n=6); $^{ns}p>0.05$, $*p<0.05$, $**p<0.01$, $***p<0.001$, calculated by comparing treated groups with control group

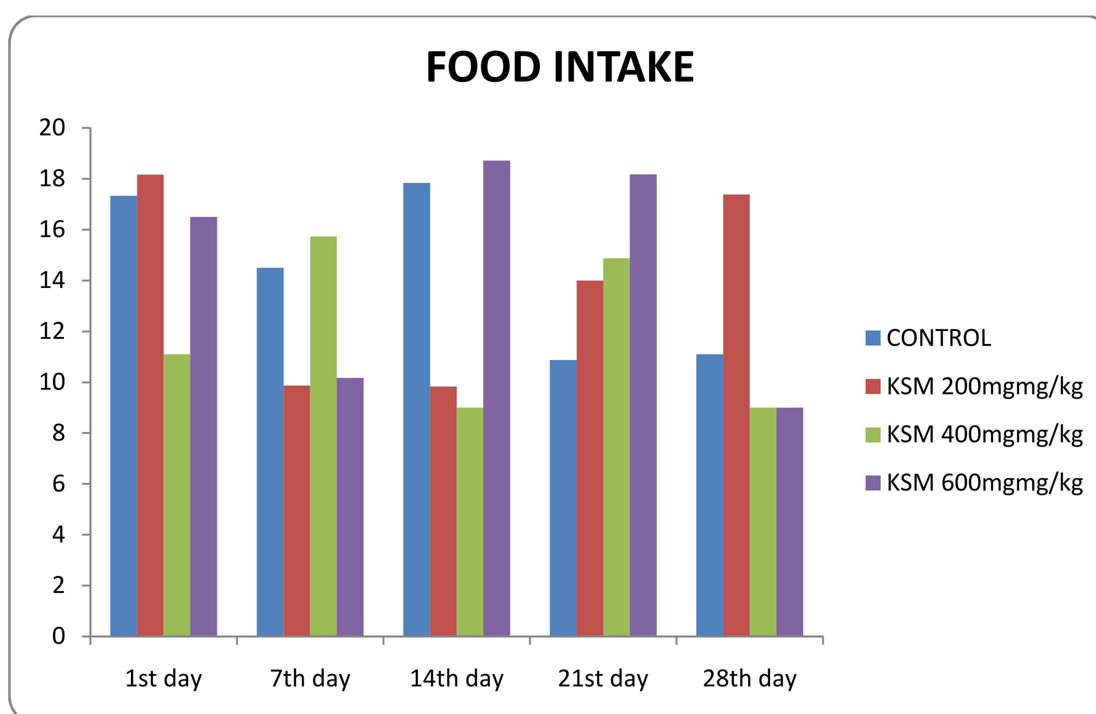


Table: 9.6. Effect of Sub- Acute Dose (28 Days) Of *KARPOORA SINTHAMANI MATHIRAI* On Water Intake in ml

GROUP	CONTROL	*KSM* (200mg/kg)	*KSM* (400mg/kg)	*KSM* (600mg/kg)
1st DAY	98.±13.10	89.72±14.3	102.10±21.79	67.5±7.623
7th DAY	85.5±11.78	100.863±12.60	76.66±9.85	81.617±14.41
14th DAY	58.3383±8.717	90.8363±14.28	80±13.92	89.162±881
21st DAY	91.6687±12.49	85±8.2	65.88±9.50	89.17±8.72
28th DAY	82.10±11.30	88.38±114	80±0.861	70±7.5

Values are expressed as mean ± SEM Statistical significance (p) calculated by one-way ANOVA followed by Dunnett's(n=6); $^{ns}p>0.05$, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, calculated by comparing treated groups with control group.

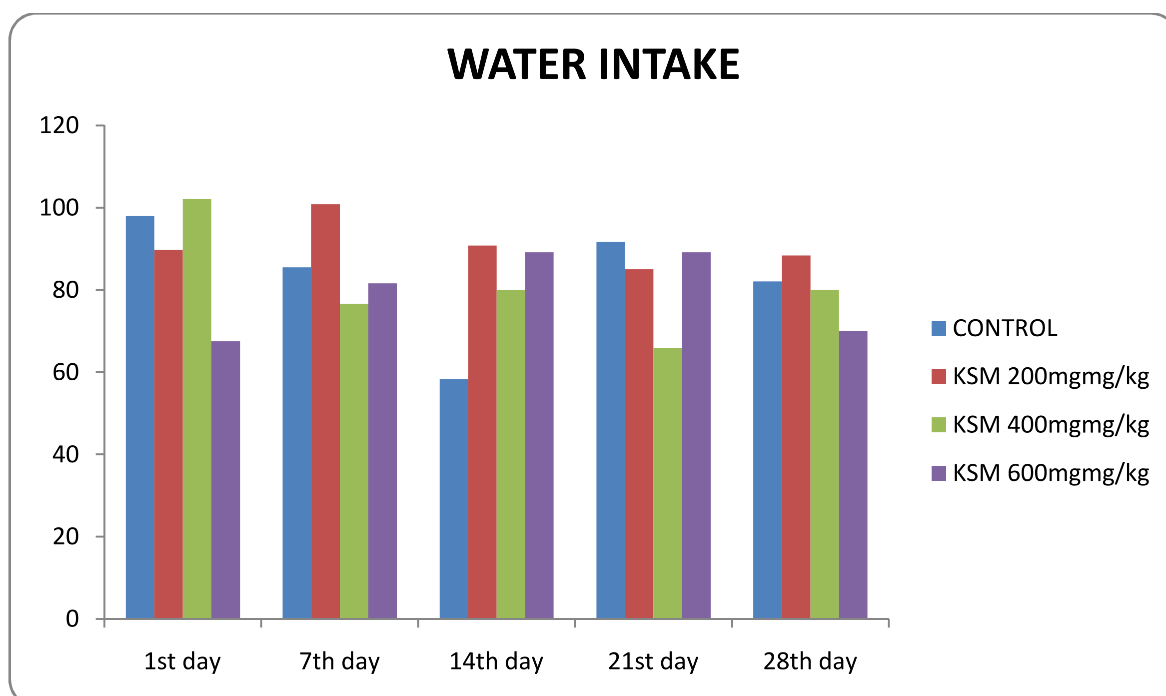
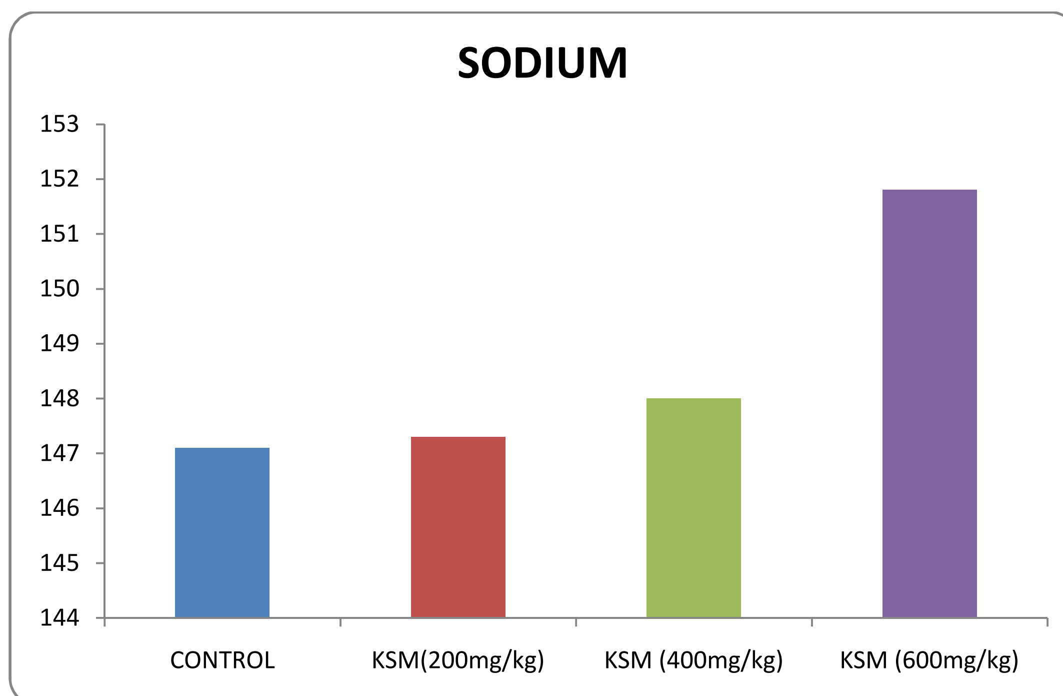
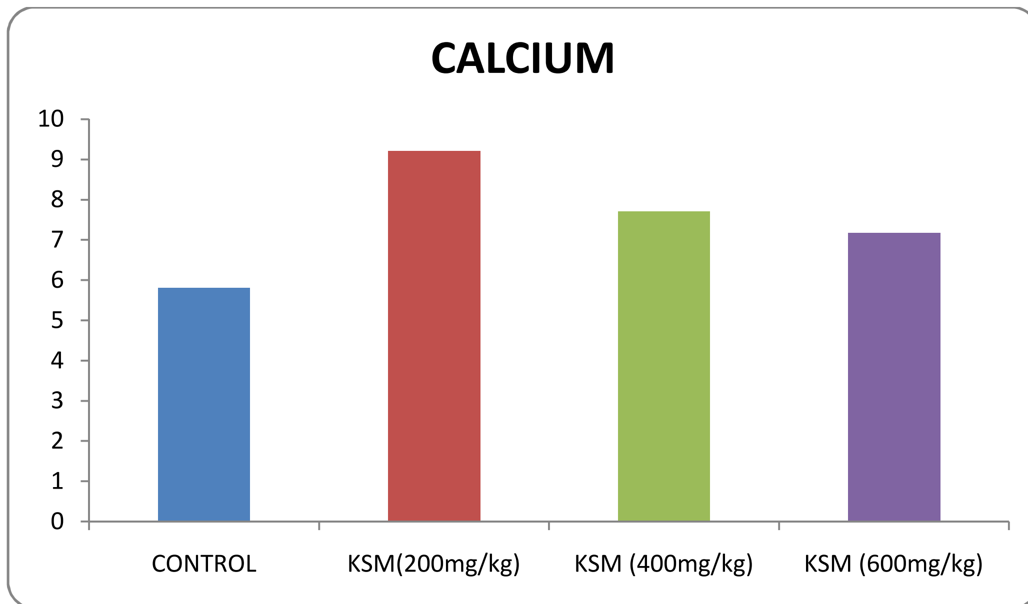


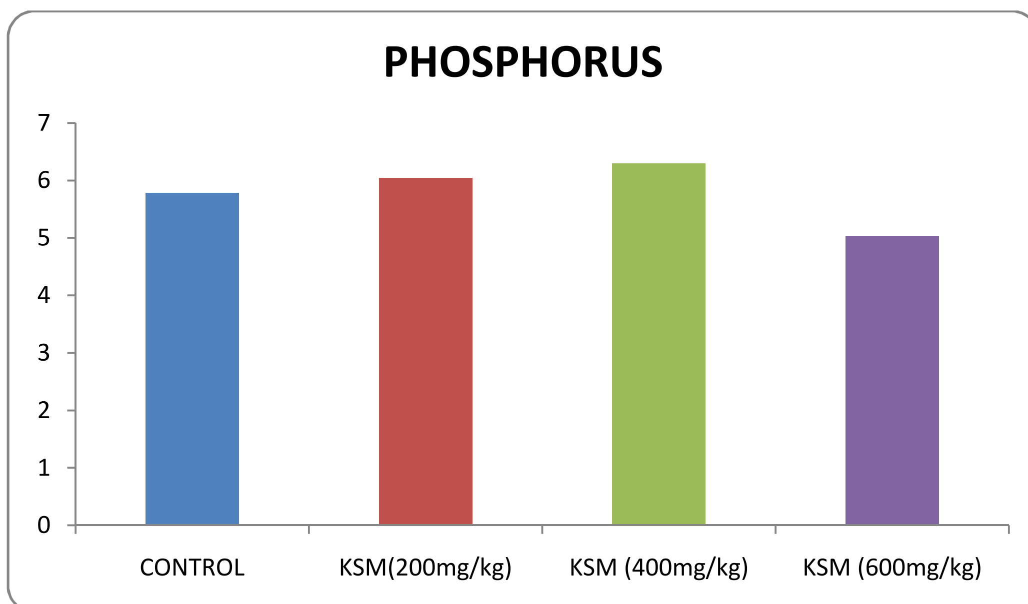
Table: 9.7 EFFECT OF SUB ACUTE DOSES (28 DAY) OF *KARPOORA SINTHAMANI MATHIRAI* ON ELECTROLYTES: -

GROUP	CONTROL	*KSM* (200mg/kg)	*KSM* (400mg/kg)	*KSM* (600mg/kg)
Sodium (mg/dl)	147.10±055	147.30±0.672	148±0.71	151.80±0.70
Calcium(mg/dl)	5.80±0.137889	9.20±0.13***	7.7±0.129**	7.180±0.19611***
Phosphorus (U/L)	5.78±0.023017	6.0410±0.21	6.30 ± 0.035491^{ns}	5.037±0.32502*

Values are expressed as mean ± SEM Statistical significance (p) calculated by one-way ANOVA followed by Dunnett's(n=6); NS- non-significant, *p<0.05, **p<0.01, ***p<0.001 calculated by comparing treated groups with control group.




CALCIUM
10
9
8
7
6
5
4
3
2
1
0
CONTROL
KSM(200mg/kg)
KSM (400mg/kg)
KSM (600mg/kg)


PHOSPHORUS
7
6
5
4
3
2
1
0
CONTROL
KSM(200mg/kg)
KSM (400mg/kg)
KSM (600mg/kg)

RESULTS:

CLINICAL SIGNS:

All animals in this study were free of toxic clinical signs throughout the dosing period of 28 days.

Mortality:

All animals in control and in all the treated dose groups survived throughout the dosing period of 28 days.

Body weight:

Results of body weight determination of animals from control and different dose groups exhibited comparable body weight gain throughout the dosing period of 28 days.

Food consumption:

During dosing and the post-dosing recovery period, the quantity of food consumed by animals from different dose groups was found to be comparable with that by control animals.

Organ Weight:

Group Mean Relative Organ Weights (% of body weight) are recorded in Table No.9.2 Comparison of organ weights of treated animals with respective control animals on day 29 was found to be comparable similarly.

Hematological investigations:

The results of hematological investigations conducted on day 29 revealed following significant changes in the values of different parameters investigated when compared with those of respective controls; however, the increase or decrease in the values obtained was within normal biological and laboratory limits or the effect was not dose dependent.

Biochemical Investigations:

Results of Biochemical investigations conducted on the day 29th and recorded in Table no 9.4 revealed the following significant changes in the values of hepatic serum enzymes studied. When compared with those of respective control. However, the increase or decrease in the values obtained was within normal biological and laboratory limits.

INTERPRETATION:

1) All the animals from control and all the treated dose groups up to 15ml/kg survived throughout the dosing period of 28 days.

2) No signs of toxicity were observed in animals from different dose groups during the dosing period of 28 days.

3) Animals from all the treated dose groups exhibited comparable body weight gain with that of controls throughout the dosing period of 28 days.

4) Food consumption of control and treated animals was found to be comparable throughout the dosing period of 28 days

5) Haematological analysis conducted at the end of the dosing period on day 29th, revealed no abnormalities attributable to the treatment.

6) Biochemical analysis conducted at the end of the dosing period on day 29th, no abnormalities attributable to the treatment.

7) Organ weight data of animals sacrificed at the end of the dosing period was found to be comparable with that of respective controls.

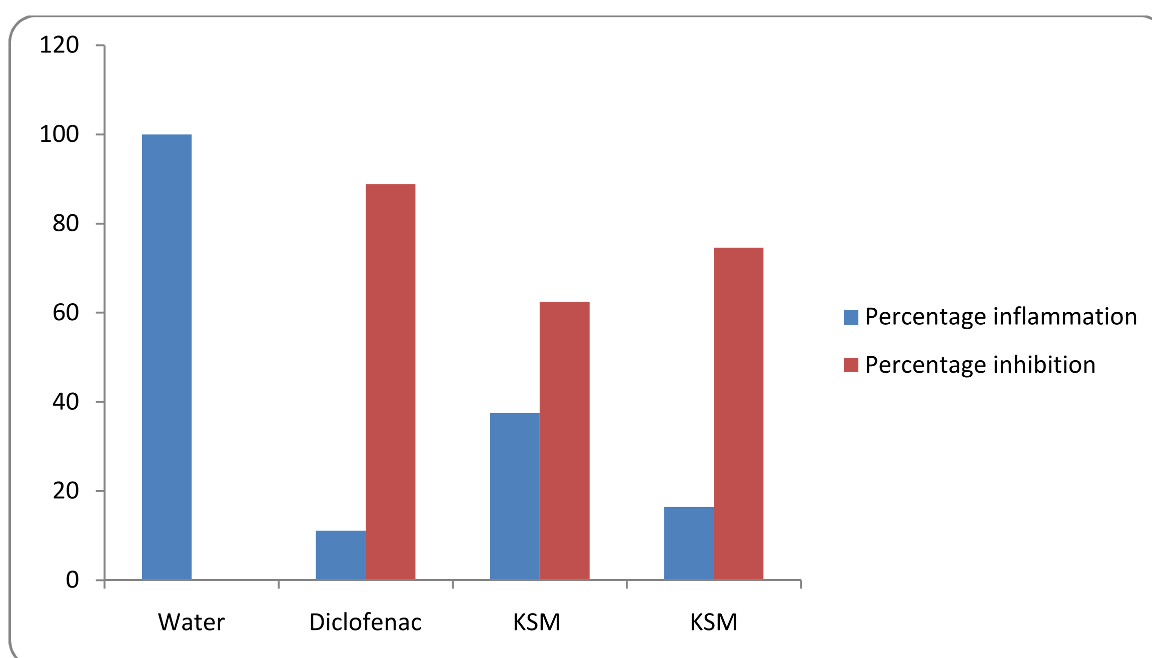
PHARMACOLOGICAL RESULTS

ANTI-INFLAMMATORY ACTIVITY OF SIDDHA PREPARATION KARPOORA SINTHAMANI MATHIRAI ON CARRAGEENAN INDUCED HIND PAW OEDEMA IN RATS

Table : 10 Study of Acute –Inflammatory By Hind Paw Method

Serial no	Name of drugs/groups	Dose/100 gram body weight	Initial reading average	final reading average	Mean difference	Percentage inflammation	Percentage inhibition
1	Water	2 ml	0.9	1.2		100	-
2	Diclofenac	5 mg/kg	0.9	0.8	0.2	11.1	88.9
3	KSM	100mg/kg	0.8	1.1	0.30	37.5	62.5
4	KSM	200mg/kg	0.8	0.7	0.1	16.4	74.6

Figure : 10 Study of Acute –Inflammatory By Hind Paw Method



INTERPRETATION:

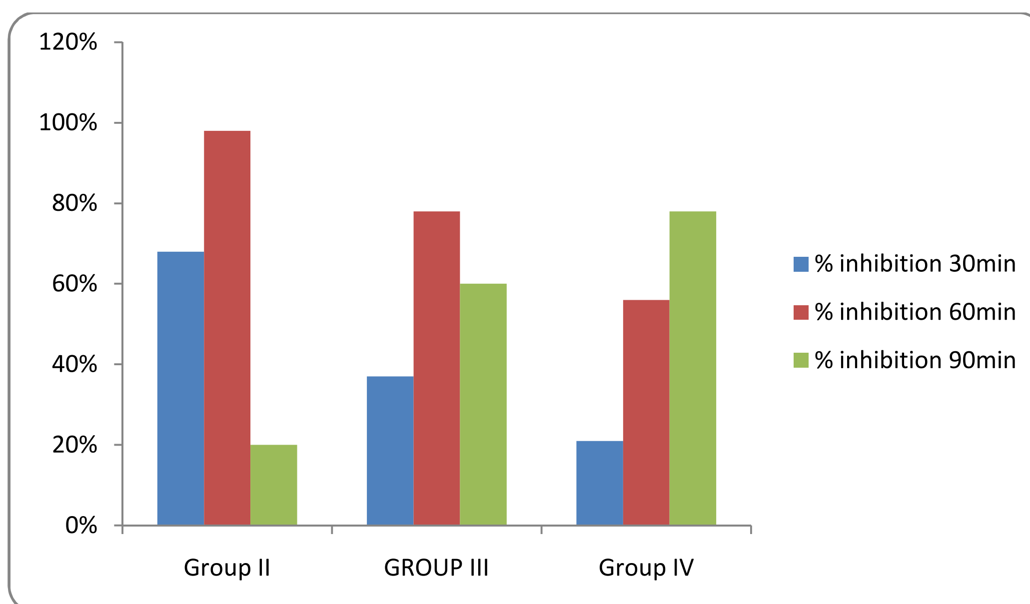
The Anti-inflammatory activity of siddha formulation Karpoora Sinthamani Mathirai at 100 mg/kg & 200 mg/kg were tested for their Anti-inflammatory activity by using carrageenan induced Hind paw method and the result are tabulated in Table. No.10 The results revals that both 100 mg/kg & 200 mg/kg dose of siddha preparation of Karpoora Sinthamani Mathirai possesses signification Anti-inflammatory activity when compared to control group.

ANALGESIC ACTIVITY OF KARPOORA SINTHAMANI MATHIRAI BY HOTPLATE METHOD

Table :11 Analgesic activity of karpoora sinthamani mathirai

GROUP	DOSE	Mean latency before and after drug administration				% inhibition		
		0 min	30 min	60 min	90 min	30min	60min	90min
Group I	Vehicle	3.96±0.20	4.35±0.26	3.86±0.198	4.28±0.267	-	-	-
Group II	10	4.04±0.88	7.32±0.65	7.67±0.645	5.37±1.008	68%	98%	20%
GROUP III	100	3.91±0.04	5.97±0.25	6.89±0.776	6.87±0.617	37%	78%	60%
Group IV	200	3.77±0.08	5.27±0.82	6.04±0.514	7.63±0.465	21%	56%	78%

Figure : 10 Study of Analgesic Activity



Statistical analysis

The results of statistical analysis for animal experiment were expressed as mean ± SEM and were evaluated by ANOVA followed by Dunnet's multiple comparisons. The results obtained were compared with the vehicle control group. The $p<0.05$, 0.001 were considered to be statistically significant

Result

Results of hotplate test are presented in Table for drug *Karpoora Sinthamani Mathirai* respectively. The drug were found to exhibit a dose dependent increase in latency time when compared with control. At 90 minutes, the percent inhibition of two different doses of *Karpoora Sinthamani Mathirai* (100 and 200 mg/kg body weight) was 60% & 78% respectively. The results were found to be statistically significant ($p<0.001$)

INTERPRETATION:

Karpoora Sinthamani Mathirai possess significant analgesic and anti-inflammatory potential as evidenced from the present preclinical study. These findings support the use of *Karpoora Sinthamani Mathirai* in traditional system of medicine for the management of pain and inflammatory conditions. Further studies are needed to be carried out in other animal models of pain and inflammatory to validate its efficacy and to identify the active phytoconstituents in the formulation and their targets in pain and inflammatory pathways.

ANTI-SPASMODIC ACTIVITY (SMOOTH MUSCLE RELAXANT) OF KARPOORA SINTHAMANI MATHIRAI

Table No: 12

Dose Response Relationship Observations of Acetylcholine

Sl.No	Concentration/dose	Acetylcholine
		Response (cm)
1	0.1 ml	3.8 cm
2	0.2 ml	4.0 cm
3	0.4 ml	4.2 cm
4	0.8 ml	5.0 cm
5	1.6 ml	5.0 cm

Table No: 13

Dose Response Relationship Observations of Atropine

Sl.No	Concentration/dose	atropine
		Response (cm)
1	0.1 ml	-
2	0.2 ml	-
3	0.4 ml	-
4	0.8 ml	-
5	1.6 ml	-

Table No: 14

Dose Response Relationship Observations of Acetylcholine and *KARPOORA SINTHAMANI MATHIRAI*

Sl.No	Concentration/dose	Acetylcholine + *KSM*
		Response (cm)
1	0.1 ml +0.1 ml	2.6 cm
2	0.2 ml +0.2 ml	3.0 cm
3	0.4 ml +0.4 ml	5.6 cm
4	0.8 ml +0.8 ml	5.8 cm
5	1.6 ml + 1.6 ml	623 cm

Comparative Dose Response of Acetylcholine followed by *KARPOORA SINTHAMANI MATHIRAI*

Table No:15

Sl. No	Treatment	Dose(ml)	response
1	Acetylcholine	0.1 ml	3.8 cm
2		0.2 ml	4.0 cm
3		0.4 ml	4.2 cm
4		0.8 ml	5.0 cm
5		1.6 ml	5.0 cm
6	Acetylcholine +*KARPOORA SINTHAMANI MATHIRAI*	0.1 ml+0.1 ml	2.6 cm
7		0.2 ml+0.2 ml	3.0 cm
8		0.4 ml+0.4 ml	5.6 cm
9		0.8 ml+0.8 ml	5.8 cm
10		1.6 ml+1.6 ml	6.23cm

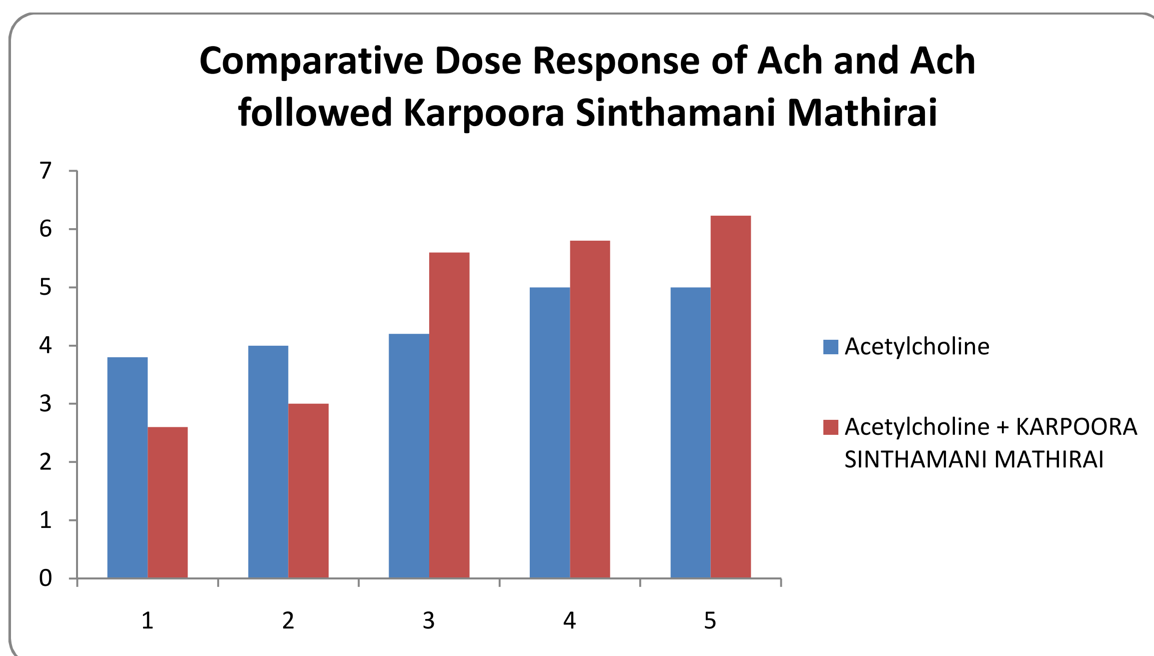


Figure 11: Comparative dose response relationship of Acetylcholine and ***KARPOORA SINTHAMANI MATHIRAI*** on excised rat ileum.

RESULTS:-

Effect of Acetylcholine on excised rat ileum reflected an increase in spasmodic activity (response) with an increase in dose.

INTERPRETATION:

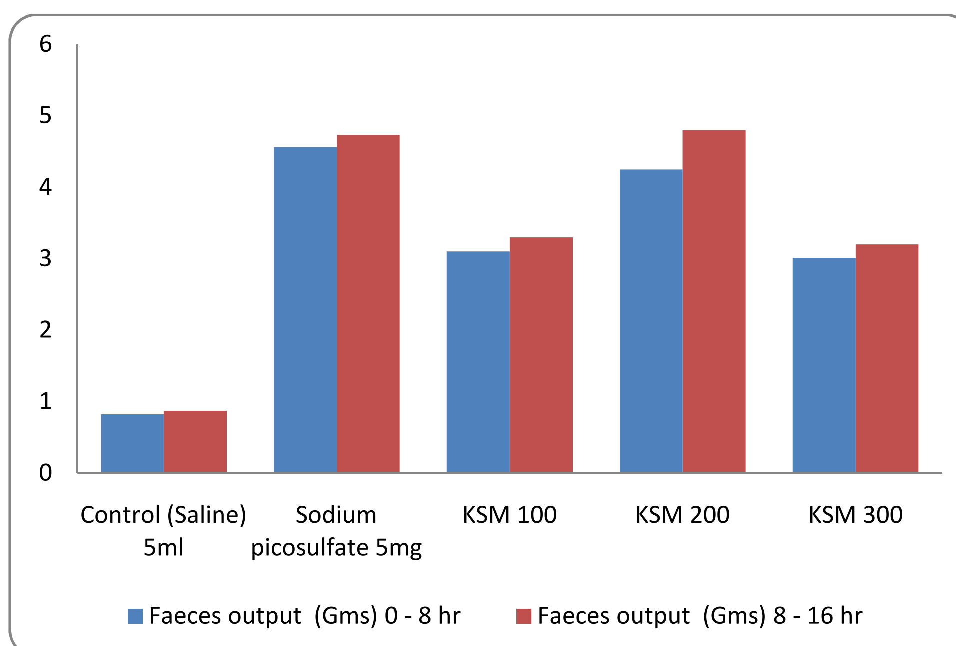
From the present study results it was observed that acetylcholine (Ach) alone causes contraction of excised rat ileum but when acetylcholine was given in presence of ***KARPOORA SINTHAMANI MATHIRAI*** there was a marked decrease in contraction of ileum was observed. This revealed that ***KARPOORA SINTHAMANI MATHIRAI*** possess a high degree of anti-spasmodic (smooth muscle relaxant) activity by blocking cholinergic receptors.

LAXATIVE ACTIVITY OF KARPOORA SINTHAMANI MATHIRAI

Table 16: Laxative Activity of KSM in Rats

Group (Treatment)	Dose (mg/kg)	Faeces output (g)	
		0 - 8 hr	8 - 16 hr
Control (Saline)	5 ml/kg	0.82 ± 0.04	0.87 ± 0.2
Sodium picosulfate	5 mg/kg	4.56 ± 0.01	4.73 ± 35
KSM	100 mg/kg	3.1 ± 0.12	3.3 ± 0.1
KSM	200 mg/kg	4.25 ± 0.30	4.8 ± 0.79
KSM	300 mg/kg	3.01 ± 0.01	3.2 ± 0.01

Figure 12. : Laxative Activity of drug in Rats



DISCUSSION

The siddha formulation Karpoora Sinthamani Mathirai was studied for its laxative activity in Wistar Albino rats. The laxative activity was assessed by measuring the wet faeces in all test drugs

Administered groups: The siddha formulation Karpoora Sinthamani Mathirai showed significant ($P<0.01$) dose dependent laxative activity as compare to normal control animals. The laxative activity produced by the siddha formulation Karpoora Sinthamani Mathirai was similar to that of the reference control sodium picosulfate. The laxative activity of siddha formulation Karpoora Sinthamani Mathirai was studied in rats.

The results showed that an oral administration of the siddha formulation Karpoora Sinthamani Mathirai produced significant and dose dependant increase in faeces output of rats. Sodium picosulfate is a member of the poly phenolic group of stimulant laxatives. Following oral administration, it is converted in the colon to an active form through the action of bacterial enzymes. As a result, its effects are directed the colon, where it stimulates peristalsis and, in common with other laxatives, reduces water reabsorption leading to the softening of stools. The results suggest that the siddha formulation Karpoora Sinthamani Mathirai might also be produced its laxative activity by reducing the water reabsorption in the colon which might soften the stool.

INTERPRETATION

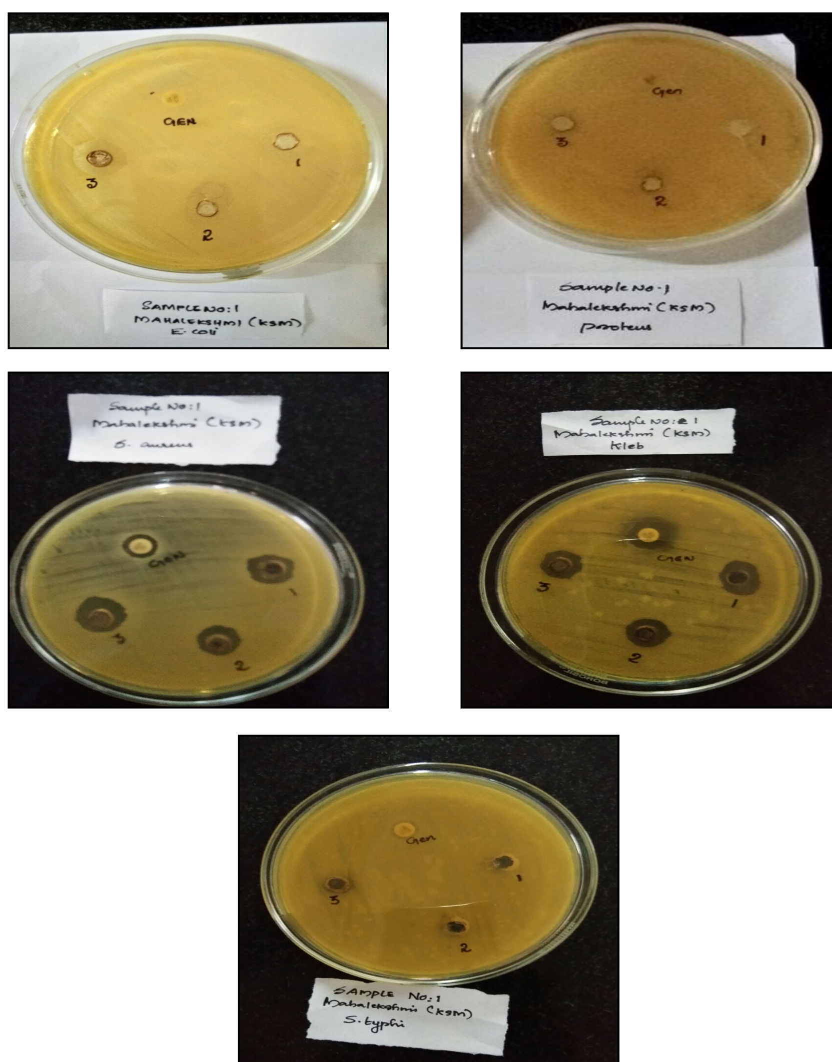
From the result it could be concluded that oral administration of siddha formulation Karpoora Sinthamani Mathirai shows significant laxative activity in rats. Further phyto-chemical studies are required to isolate the active compounds responsible for laxative activity which could be a major contribution to prove the claims in Indian systems of medicine.

ANTIMICROBIAL ACTIVITIES

Table 17: Antimicrobial Activities of Drug by Agar Well Diffusion Method

S.No.	Test Pathogens	Result	Zone of Inhibition (mm) at 30µl	
			Positive Control (Gentamycin)	Size of Inhibition
1.	*Escherichia coli*	Sensitive	20 mm	12 mm
2	*Klebsiella pneumoniae*	Sensitive	22 mm	14 mm
3	*Staphylococcus aureus*	Sensitive	21 mm	10 mm
4	*Pseudomonas aeruginosa*	Sensitive	22 mm	9 mm
5.	*Salmonella typhi*	Sensitive	19 mm	14 mm

FIGURE : 13 ANTI-MICROBIAL ACTIVITY RESULT



INTERPRETATION

Both Gram positive and Gram negative bacteria *E.coli, Klebsiella pneumoniae* and *Staphylococcus aureus, Pseudomonas aeruginosa and Salmonella typhi* were found to be sensitive when compared to the standard drug Gentamycin (Broad spectrum) (Table 17). The herbal drug ***Karpoora Sinthamani Mathirai*** exhibited broad spectrum activity against bacterial pathogens at 100mg / ml concentration of the drug.

7. SUMMARY

In this dissertation work, I have selected **"KARPOORA SINTHAMANI MATHIRAI "** from the Siddha literature **Anuboga Vaithiya Navaneetham, Part – IV**, authored by Abdhula Sayubu PM, Thamarai Noolagam, Chennai (2006) 106 for the evaluation of safety, efficacy and therapeutic potency in "KEEL VAYU" for its anti – inflammatory, analgesic, anti-spasmodic and laxative activities.

"Karpoora Sinthamani Mathirai " is a herbo-mineral preparation. it is very effective and traditionally used in the treatment of "Keel Vayu" (Arthritis).

Collection of literature reviews regarding the trial medicine carried out in siddha aspect, geochemical aspect and pharmaceutical review.

All the ingredients of the test drug "Karpoora Sinthamani Mathirai " were collected from Nagercoil, Traditional drug store. Each ingredient are verified and authenticated by the Gunapadam exeperts from Department of Gunapadam of Government Siddha Medical College, Palayamkottai. The test drug was prepared as per the procedure given in the literature.

As per siddha literature "Keel Vayu" is a disease caused due to domination vatham to neutralize deranged vatha kutram. The drug "Karpoora Sinthamani Mathirai " has hot (வெப்பம்) in potency it is essential to neutralize vatha kutram.

Physiochemical analysis of "Karpoora Sinthamani Mathirai " shows brown in colour, sense of touch is hard, pleasan odour, round in appearance with salt, pungent, bitter, astringent taste. the loss on drying (LOD) is 7.40% which shows low moisture content present in the prepared medicine. Increased moisture content is the issue for instability of drug and lesser shelf-life of a drug. Since, Karpoora Sinthamani Mathirai has been well prepared it could get maximum stability and better shelf-life. Longer shelf-life for mathirai mentioned in siddha literature is thus justified from the above observation.

The acid-insoluble ash limit test is designed to measure the amount of ash insoluble to diluted hydrochloric acid. Acid-insoluble ash value of the prepared formulation (0.90%) shows that a very small amount of the inorganic component is insoluble in acid. It indicates that adulteration of raw ingredients by substances, such as silica and husk, is very less, and a low acid-insoluble ash value may also affect the amount of the component absorbed in the gastro-intestinal canal when taken orally.

The test drug Karpoora Sinthamani Mathirai having, lower the acid insoluble value better will be the drug quality.

- Total viable aerobic counts within the normal level. (Normal- $4x10^2$CFU/g.)
- Total Enterobacteriaceae counts is Nil
- Total fungal count within the normal level. (Normal-$3.5x10^2$ CFU/g.)
- Specific pathogens Salmonella sp., Staphylococcus aureus, E. coli, Pseudomonas aeruginosa are Nil.
- So it can be stored for a long period and would not easily be attacked by microbes. It is hygienically good for taking as internal medicine.
- Water soluble extract shows, water is a better solvent of extraction for the formulation.
- The alkaline (7.140) nature of the drug is very useful in gastric juice.

- **Physio chemical** and **bio-chemical analysis** of Karpoora Sinthamani Mathirai shows the presence of calcium, sulphate, ferrous iron, chloride, starch, Unsaturated compounds, amino acids and all the above minerals might be responsible for the effectiveness of the drug.

- The Pharmacological review were done to establish the different methodologies adopted in the preclinical evaluation of the test drug

- FTIR reveals the presence of alcohols,phenols,carboxylic acids,amines, aliphatic nitro groups, alkanes, aromatics, alkyl halides groups which has anti-inflammatory activity.

- ICP-OES analysis of these drug shows heavy metals Like Arsenic, cadmium, Nickel, copper and lead are found in below detecting level. The toxic metals are found in BDL. It reveals the drug in safer for long term use. The elements like magnesium are also found in below detecting level. The phosphorus is involved in tissue repair, Sodium chloride regulates acid-base balance. calcium has got potent anti- inflammatory activity.
- SEM analysis result shows most of the particles present in the sample is micro size, average particle size is 2 to 1 microns. So very minimal quantity of the medicine is enough to treat the disease.
- Anti-microbial study of the test drug carried out by Agar well-diffusion method. It is observed that "Karpoora Sinthamani Mathirai" is sensitive to Escherichia coli, klebsiella pneumoniae, staphylococcus aureus, pseudomonas aeruginosa, salmonella typhi. The Karpoora Sinthamani Mathirai has significant anti-bacterial activity.
- Pharmacological analysi*s* o*f* Karpoora Sinthamani Mathirai shows that the drug has got significant anti-inflammatory activity and excellent analgesic activity and good anti-spasmodic activity and laxative activity.
- According to siddha aspect, Keel Vayu is a disease caused by derangement of vadha humors chiefly and kabha humour the drug has salt, bitter taste. Salt taste neutralize vadha humour and bitter taste normalise kabha humour. In this case vadha and kabha humours are gradually normalised by administration of Karpoora Sinthamani Mathirai . Neutralization of affected humours by giving Viresanam (purgative) vatha kuttram is neutralize the drug Karpoora Sinthamani Mathirai having ingredients with laxative and purgating action such as (Pooram, Nervalam and Kattrazhai) because of this actions vatha kutram is neutralized by administration of Karpoora Sinthamani Mathirai .
- Acute oral toxicity of Karpoora Sinthamani Mathirai observed no toxicity. Acute and sub acute toxicity study of Karpoora Sinthamani Mathirai represents non toxic and safe drug in Wistar albino rats.

8. CONCLUSION

From the literature review, Physico-chemical, Bio-chemical, Instrumental analysis, Microbiological, Toxicological, Pharmacological studies, it has been concluded that The trial drug is a **Karpoora Sinthamani Mathirai** in herbo mineral form, selected from the text book of "*Anuboga vaithiya navaneetham* Part IV" authored by Hakeem.P.M.Abdulla saheeb and the results supported the study has got a good Anti-inflammatory, Analgesic, Anti-spasmodic activity, laxative activity and hence effective for Keel Vayu.

9. FUTURE SCOPE

Preclinical evaluation of the test drug **Karpoora Sinthamani Mathirai** has been done by bio-chemical, physio-chemical, instrumenta, pharmacologica, toxicological and microbial standard presecribed procedures. In future the drug has to validated by extensive clinical trials as per WHO guidelines. This **Karpoora Sinthamani Mathirai** is to be used very much to treating all types of arthritis.

Having made up of nano particles, **Karpoora Sinthamani Mathirai** holds extra ordinary promise for the presentation and treatment Keel Vayu. Thus the ancient wisdom siddhars will remains as one important source of future medicine and therapeutics.

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