SCIENTIFIC VALIDATION OF ANTI-DYSLIPIDEMIC, HYPOGLYCEMIC AND ANTI-OXIDANT ACTIVITIES OF SIDDHA HERBO-MINERAL FORMULATION *"INDHIRANI MAATHIRAI"* IN ANIMAL MODEL.

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GOVT. SIDDHA MEDICAL COLLEGE,

CHENNAI-106

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled "S**cientific Validation of Anti-Dyslipidemic, Hypoglycemic and Anti-oxidant activities of *siddha* herbo-mineral formulation *"INDHIRANI MAATHIRAI"* in animal model"** is a Bonafide and genuine research work carried out by me under the guidance of **Dr.M.D.Saravanadevi M.D(S).,** Post Graduate Department of Gunapadam, Govt. Siddha Medical College, Arumbakkam, Chennai-106 and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or other similar title.

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CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled "S**cientific Validation of Anti-Dyslipidemic, Hypoglycemic and Anti-oxidant activities of *siddha* herbo-mineral formulation *"INDHIRANI MAATHIRAI"* in animal model"** is submitted to the Tamilnadu Dr.M.G.R.Medical University in partial fulfillment of the requirements for the award of degree of M.D (Siddha) is the Bonafide and genuine research work done by **Dr.P.GNANAVEL** Under my supervision and guidance and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or other similar title.

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CHENNAI-106

ENDORSEMENT BY THE HOD AND PRINCIPAL OF THE INSTITUTION

This is to certify that the dissertation "S**cientific Validation of Anti-Dyslipidemic, Hypoglycemic and Anti-oxidant activities of *siddha* herbo-mineral formulation *"INDHIRANI MAATHIRAI"* in animal model"** is a Bonafide work carried out by **Dr.P.GNANAVEL** under the guidance of **Dr.M.D.Saravana Devi MD(s),** Post Graduate Department of Gunapadam, Govt.Siddha Medical College, Chennai - 106.

Seal and Signature of the HOD	**Seal and Signature of Principal**
Prof. Dr. R. Meenakumari M.D(S).,	**Prof. Dr. R. Meenakumari M.D(S).,**

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ABBREVIATIONS

ABCA1 gene	**ATP binding cassette transporter**
ABCG 5 & 8 gene	**ATP binding cassette subfamily G members 5 and 8**
AgNPs	**Silver Nano Particles**
ANOVA	**Analysis of Variance**
Apo C II	**Apo Protein C II**
Apo E	**Apo Protein E**
ASCVD	**Atherosclerotic Cardio vascular disease**
CAD	**Coronary Artery Disease**
CFA	**Colonisation Factor Antigen**
CK	**Creatine Kinasec**
CMC	**Carboxymethyl cellulose**
DAL	**Dalton Ascitic Lymphoma**
DMSO	**Dimethyl Sulphoxide**
DNA	**Deoxy Ribonucleic Acid**
DPPH	**2,2-Diphenyl - 1 Picrylhydrazyl**
EDS	**Energy Dispersive X-ray Spectroscopy**
EDTA	**Ethylene Diamine Tetra Acetic acid**
FFAs	**Free fatty Acids**
FTIR	**Fourier Transform Infra-red Spectroscopy**
GIT	**Gastro Intestinal Tract**
GOD-POD	**Glucose Oxidase – Peroxidase**
H2 SO4	**Sulphuric acid**
Hcl	**Hydrochloric acid**

HDL	**High Density Lipoprotein**
HIV	**Human immune deficiency virus**
HMG-CoA	**Hydroxy Methyl Glutaryl- Co A**
HNO_3	**Nitric Acid**
HPLC	**High Performance Liquid Chromatography**
HPTLC	**High Performance Thin Layer Chromatography**
IC_{50}	**Inhibitory Concentration**
ICP-MS	**Inductively Coupled Plasma Mass Spectrometry**
IDL	**Intermediate Density Lipoprotein**
IHD	**Ischemic Heart Disease**
IM	**Indhirani Maathirai**
IR	**Infrared Radiation**
KOH	**Potassium Hydroxide**
LCAT	**Lecithin Cholesterol Acyltransferases**
LD_{50}	**Lethal Dose – 50**
LDL	**Low Density Lipoprotein**
Lp (a)	**Lipoprotein (a)**
Nacl	**Sodium chloride.**
NADPH	**Nicotinamide Adenine Dinucleotide Phosphate.**
NIDDM	**Non-Insulin Dependent Diabetes Mellitus**
OECD	**Organisation for Economic Cooperation and Development**
OTC	**Over the Counter**
PCB	**Poly Chlorinated Biphenyls**
PCSK9	**Proprotein Convertase Subtilisin- like/ kexin Type 9**
PCV	**Packed Cell Volume**

pH	**Potential Hydrogen**
RBC	**Red Blood Corpuscles**
SEM	**Scanning Electron Microscope**
STZ	**Streptozotocin**
TANUVAS	**Tamilnadu Veterinary and Animal Sciences University**
TC	**Total Cholesterol**
TG	**Tri glycerides**
TLC	**Thin Layer Chromatography**
TZD	**Thiazolidinediones**
VLDL	**Very Low-Density Lipoprotein**
WBC	**White Blood C0rpuscles**
WHO	**World health organisation**
XRD	**X-ray Powder Diffraction**

CONTENTS

1. INTRODUCTION

Siddha system of medicine is one of the ancient system which are being practiced any other system in the world. The system is the mother system of Varma Maruthuvam, yoga Maruthuvam, acupuncture, acupressure, naturopathy, Reiki, etc,... Siddha system is the Unique system that definites total number of diseases that is 4448 in number which includes birth and death.[1]

The WHO defines that **"health is a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity"**

The Siddha system of medicine differs from any other system of medicine when approach to patient not only deals with physical character but also deals with psychosocial activity.

In Siddha system parts of plants, ores of minerals, poisonous elements (paadaanam), animal products are used to prepare the medicines.[2] .Addition to these the water, the air, the sand particles, the sunlight [3] and the cosmic energy also used to treat the patient.

Siddha system of medicine having the unique pharmacological aspect through the drug delivery system. The significance of drug delivery system mainly due to the particle size found in the higher order medicine like parpam and chendhooram. Most of the particle distribution found in higher order medicine nearly micro / nano in size.[4] Because of the micro / nano particle size of higher order medicine leads to the rapid solublity during the process of absorption and also enhances the ion channel mechanism.[5] After metabolism of the drug, the waste end product easily excreated by the excretory system of our body. Because of this, minimal dose of medicine that is more efficient to cure diseases and also nullifies the side effect during prolonged usage. So much of pollutant and environmental changes, our Lifestyle altered and human beings are faced the stressful and mechanical life. Because of this changes so many Lifestyle disorders may come. Obesity and Diabetes are come under this category and have the major and important role.[6]

The global prevalence of diabetes among in 2014 8.5%, in 2016, an estimated 1.6 million deaths where directly caused by diabetes. India had 69.2 million people living with diabetes as per 2015 data. In 2017 there are 451 million people with diabetes worldwide.

It was estimated that almost half of the people (49.7%) living with diabetes are undiagnosed. In 2017 approximately 5 million death worldwide were attributable in diabetes in the 20-99 years age range. The global health care expenditure on people with diabetes was estimated to be USD 850 million in 2017.[7]

Worldwide over 3 million Children's and 600 million adults are obesed. In United States 7.94 % million people are obesed and toped in the list. China got second place with 57.3 million people in this 15.3 million or children. India is the third most obese country in the world with 30 million people among this 14.4 million for children.[8]

Globally 422 million adults are living with diabetes mellitus the diabetes mellitus was classified into two major ocategories type 1 type 2. Some patient has high blood sugar level by the defects of insulin secretion. They need insulin to maintain the sugar leve. These people are in Type 1 Diabetes (or) Insulin dependent diabetes.

Some patient has high blood sugar level by the defects in action of insulin. Their body have some insulin which is not properly functioned. They were maintain the blood sugar level by the oral drugs are in type 2 diabetes (or) Non-insulin dependent diabetes. This is the most common type of diabetes nearly 85 to 90% of all cases.Type 2 diabetes was more common in males 11.4 percentage then in females 9.2 percentage in the elderly population greater than or equal to 65 year.[9]

In youngsters, less than 50 year in Urban population 12.8 % more prone to diabetic compared to rural population. 5.2 % the incidence of diabetes was higher among patient 16.9 % with hyperlipidemia.[10]

In Siddha system of medicine Diabetes mellitus was known as” Madhu Megham".

இருமியே வாதமும் பித்தமும் கூடி

மருவு சலமேக வாநதி போலாகும்

உருவம் வேராகும் உண்டவுடல் காந்திடும்

உருகவே உன்னோடு உறிஞ்சி இனிக்கும்

-திருமூலர் நாடி [11]

பார்த்திடு மூன்றும் பதிந்து மெலிந்து நிற்கில்

தேர்ந்தெடு மேகம் உள்ளே

தோன்றியே பொருந்தி மெய்யில்

-சித்த மருத்துவம் [12]

The above versus says that the vatha dhodam merge with the pitha dhodam large amount of urine may void like the stream. Hunger may come again and again. Our body tissues are excreated through urine and sweet in taste.

When discuss about the complication of Mathu megham the Saint yugimuni list out 10 major issues.

காணவே முதலவத்தைச் சரீரந் தானுங்

கனமாக பருத்து ....

-சித்த மருத்துவம் [13]

Above mentioned complication types of diabetes, first one was the bulky mass body. It was known as “Adhithoolarogam” that is obesity. Obesity is calculated by the body mass index.

$$BMI = \frac{\text{A person's body weight in kg}}{\text{A person's body height in } m^2}$$

Tabele No: 1.1 BMI Table

BMI RANGE	INDICATION
Upto 18.5	Under weight
18.5 to 25.0	Normal
25.0 to 30.0	Over weight
30.0 to 35.0	Class 1 obesity
35.0 to 40.0	Class 2 obesity
Above 40.0	Class 3 obesity

Some market available drugs are act on lipid metabolism and reduce the level of lipids in blood. They are,

1.Statins

2. Fibrates

3. Probucol.

Similarly, some drugs are acts on carbohydrate metabolism and control the sugar level and also available in the market. They are,

1. Various type of insulin

2. Glucagon

3. Sulphonyl ureas

4. Biguanides

5. Alpha glucosidase inhibitors

6. Glitazones

Regular usage of these drugs may require high dose to maintain the normal range a(or) maybe have resistant to the particular drug. So, we want to increase the dose (or) change the superior drug depends upon the patients need. In this way this drug is not active in all conditions. So, there is a need to discover a drug that will be used in long term aspects without (or) minimal adverse side effects.

In Siddha system of medicine, so many dogs are there to treat this condition. The" ***Indhirani Maathirai****"* comes under above category of medicines.

The *Indrani Maathirai* constitutes the equal ratio of

1.purasan ver,

2.kalli ver,

3.Kalarchi ver,

4.Kadugu,

5.Milagu,

6.perungayam and

7.vediyuppu.

All these purified ingredients would be e ground with karungali Kudi Neer to obtain mezhugu padham then rolled in to Maathirai. Most of the ingredients in this list have the the hypoglycemic activity and/or anti hyperlipidemic activity.

Still now proper evaluation on the drug *"**Indhirani Maathirai**"* was not done. So, the auther is interested to study the antidyslipidemic, antidiabetic and antioxidant properties of the drug in animal model.

AIM AND OBJECTIVES

AIM:

Obesity **(Adhithoola Aogam)** and diabetes mellitus **(Madhu Megham)** are the metabolic disorder oriented to our lifestyle changes Globally obesity and diabetes are the most common and fast growing health issue both on a deer prolong medication and the lifestyle modification regular usage of medicines may cause some adverse side effects. The side effects should be minimised then only we improve the health care of our society this may achieve by Siddha system of medicine ***Indhirani Maathirai*** is one of the drug side in Siddha literature to treat obesity which is not properly ever with previously the name of the study was to validate the safety and efficacy of the drug ***Indhirani Maathirai*** for Antidyslipidemic activity in triton wr 1339 induced albino rats.

OBJECTIVES:

In this study the following methodology was done to evaluate the safety and efficacy of the trial drug

- Various Siddha and modern references relevant to the study are collected.
- The trial drug was prepared honestly according to classical Siddha text.
- Validate of the physico chemical and phytochemical properties of the trial drug were done.
- To evaluate the acidic and basic radicals of the trial drug by means of biochemical analysis were done.
- Instrumental analysis of the trial drug was done to estimate the particle size of the molecule functional groups and elements which were present in it.
- According to OECD guidelines acute toxicity study and repeated oral toxicity study of the trial drug were done.
- To evaluate the pharmacological activity
    1. Antidyslipidemic activity-Triton WR 1339 induced model.
    2. Anti-Diabetic activity strip mm induced model in wistar Albino rats.
    3. Anti-oxidant activity DPPH assay were done.
- Interpret the all data which are collected through the above studies to establish the activity and potency of the drug ***Indhirani Maathirai.***

3. REVIEW OF LITRATURE

3.1. DRUG REVIEW

This chapter deals with the scientific and gunapadam aspect of the ingridiants of the Indhirani maathirai.

INGIRIDIANTS OF THE INDHIRANI MAATHIRAI

purasan ver (root of beautea monosperma)

Milagu (Piper nigrum)

Kalli ver (root of Euphorbia antiquorum)

Kalarchi ver (root of Caesalpinia bonduc)

Kadugu (Brassica juncea)

Kambiccharam (potassium nitrate)

Perungayam (ferula asafoetida)

Karungali kudineer (Acacia catechu stem decoction)

3.1.1. PURASAN VER (Butea monosperma)

SYNONYMS

Butea monosperma(Lam)

Butea frondosa Roxb ex Wild

Eeythrino monosperma Lam

Plaso monosperma

Fig.No-3.1:Beautea monosperma flower

OTHER NAMES

Palaasu, Bramathervu, Murukku, Kinguki, Kiruminasam, Seera, Palasam, Puraisu, Purosu, Punarmurukku, Vaadhabothem.

VERNACULAR NAME

Eng-Bastard Teak,Flame of forest

Tel-Moduga

Mal-Murukka maram

Pers-Dharakhte-palasina

Hin-Dhara

Kan-Muttagamara

Fig.No-3.2: Beautea monosperma plant

BOTANICAL ASPECT

CLASSIFICATION

Kingdam-Plantae

Class-Dicotyledonae

Subclass-Polypetalae

Series-Calyciflorae

Order-Rosalae

Family-Leguminosae

Subfamily-Febaceae

Genrae-Butea

Species-monosperma

Fig.No-3.3: Beautea monosperma root

HABIT

Tree

DISTRIBUTION

It is a medium sized tree native of the mountainous regions of India and Burma and now grows wild throughout India.

DESCRIPTION

Leaves are pinnate, three leaflets, each leaflet ovate-lanceolate. Flowers racemes densely brown velvety on bare branches. Calyx is dark, olive green to brown in colour and densely velvety outside. The corolla is long with silky silvery hairs outside and bright orange red. Stamens are diadelphous, anthers uniform. carpel l; style filiform, curved and stigma capitate. Pods are narrowed, thickened at the sutures, splitting round the single apical seed, lowest part indehiscent. The seeds are flat, reniform, curved.[14]

SIDDHA ASPECT

GENERAL PROPERTIES

வயிற்று கிருமி கணம் வாதமொடு சீதம்
நியத்தபடர் தாமரையும் நீங்கும்-வயிற்று
வலியிரைச்சல் வன்கரப்பான் மாறும்பித் துண்டாம்
மலிபுரசங் கொட்டைதனை வாழ்த்து.

-அகத்தியர் குணபாடம் [15]

புரசுவா தம்பித்தம் பொங்குகபங் குன்மம்
கிருமி யுடனே கிராணி-பெருவலி நோய்
ஓட்டுமதி தீபத்தை உண்டாக்கு அப்பொழுதே
தீட்டியவேற் கண்ணாய்! தெளி.

-அகத்தியர் குணபாடம் [16]

சந்திகட்கு நாடிகட்குந் தங்குசுக்கி லந்தனக்கும்
புந்தியுரங் கட்குமுரம் பூட்டுங்கா-ணுந்து
சரமதனுக் காகமநூல் சாற்றுமலர் மாதே!
பிரமதரு விற்சம்பி சின்,

-அகத்தியர் குணபாடம் [17]

EXPLANATION

Purasu regulates the three doshas.It controls the worm infection and abdominal pain.It has stomachic activity also. Butea frondosa gums have the tonic property to joints, nerves and chest region.The gums have the antrogenic property too.The seed of Butea monosperma cures the worm infestation, body achae, ring worm, eczema and peptic ulcer.The seed enhances the piththa thodam.

MODERN ASPECT

CHEMICAL CONSTITUENT:

Butin, butein, butrin, isobutrin,palasitrin, coreopsis, isocore epson, chalcones and aurones have been isolated from the flowers . The antifungal compound -medicarpin, kino-tannic acid, gallic acid, proanthocyanidins, stigmasterol,stigmasterol-D glucopyranoside

and nonadecanoic acid have been isolated from the stem bark and gum. A new lectin N-Acetyl galactosamine was isolated from the seed.[18]

MEDICINAL USES:

The flower decoction is widely used in the treatment of hepatic disorders and viral hepatitis, diarrhoea and possess anti-inflammation activity. Root combines with Pippali to be useful in the treatment of filariasis, night blindness, helminthiasis, piles, ulcers and tumours. The stem bark is used in indigenous medicine for the treatment of dyspepsia, diarrhoea, dysentery, diabetes, ulcers, sore throat and snake bites. The root bark is used as an aphrodisiac, analgesic and anthelmintic whereas the leaves possess antimicrobial property. Seed combined with honey is internally used in the case of intestinal worms especially tapeworm.[19]

3.1.2.MILAGU-PIPPER NIGRAM

OTHER NAMES:

Kalinai, Kari, Kayam, Kolagam, Thirangal, Miriyal, Sarumavandham, Vaalism, Kurumilagu, Malayali.

- குணபாடம் மூலிகை [18]

சொல்லியதோர், அருட்டென்றி ' அதற்குப் பெயர்-
சொற்பெரிய, மதங்க னென்றும் பேருண்டாச்சு
அதுலியதோர், மலைத்திருக்கலென்றும் பேரு
அட்டமா சாதி என்று மிதற்குப் பேரு
கல்வியதோர் கத்திசன் என்றும் பேரு
கத்துரும் பயனன்று நேர் வனந்தா னென்றும் பேரு
மல்லியரோர்க்கு தாயன்றி தற்குப் பேரு
வசனித்தும், மிளகினிட அதீதப் பேரே

- பஞ்சகாவிய நிகண்டு [19]

EXPLANATION

Arutendru, Mathangan, Malaiththirukkal, Attamasaathi, Katheesan, Kathurupayan, Thaai, Kalinai, Kari, Kayam, Kolagam, Thirangal, Miriyal, Sarumabandham,Valliam, Kurumilagu, Malayali.

VERNACULAR NAME

Eng-Black Pepper

Tel-Miriyalu

Mal-Kurumilagu

Kan-Menasu

Sans-Mariche

Hin-Kali-Mirch

Fig.No-3.4:Pipper nigram

BOTANICAL ASPECT

Classification

Kingdam-Plantae

Class-Dicortyledane

Subclss-Monochlamidae

Series-Microembryeae

Family-Pipraceae

Genera-Pipper

Species-nigram

Fig.No-3.5:Pipper nigram

HABIT

It is a twiner.

DISTRIBUTION

It is native to Malabar, a region in the Western Coast of South India.[20]

DESCRIPTION

Stems are stout climbing and very flexible. Leaves are elliptical to orbicular-ovate, younger leaves are cordate and all are palmately veined with 5-7 veins. Flowers are small and borne on long pendulous spikes from each node, The flowers are not showy.The berries first turn green, then red, finally turning black.[21]

PART USED

Seed, plant

SIDDHA ASPECT

PROPERTIES

Taste - bitter, pungent

Character - hot

Division - pungent

A GREEN VARIETY OF PEPPER

அளவையுறாக்காரம் அடைந்திருக்கும் வாத
விளைவையெல் லாமறுக்கும் மெய்யே - மிளகின்காய்
கண்டவர்க்கும் இன்பமாம் காரிகையே! சீழ்மூலம்
கொண்டவர்க்கு நன்மருந்தாங் கூறு.

-அகத்தியர் குணபாடம் [22]

சிதகரம் பாண்டு சிலேத்துமம் கிராணி குன்மம்
வாதம் அரூசிபித்தம் மூலம்-ஒதுசன்னி
யாசமபஸ் மாரம் அடன்மேகம் காசமிவை
நாசங் கறிமிளகினால்

-அகத்தியர் குணபாடம் [23]

கோணுகின்ற பக்கவலி குய்யவுரோ கம்வாத
சோணிதங்க ழுத்திற்குள் தோன்றுநோய் - காணரிய
காது நோய் மாதர்குன்மங் காமாலை மந்தமென்றீர்
ஏதுநோய் காயிருக்கில் ஈங்கு.

-அகத்தியர் குணபாடம் [24]

EXPLANATION

The peper cures the vadha diseases and the apses.It cures the fever with severing, anaemia, cough with expectoration, piles, anohrexia, epilepsy, urinary disorders, stroke, indigestion, jaundice.

மிளகின் பெருமை

தீயாகி எங்கும் திரியுமதை யாவத்து
மோயாம லெப்படியு முண்டாக்காற்-பாயாது
போந்திமிர்வா தங்கிரந்தி புண்ணிரும் மண்ணவர்க்கும்
காந்திமெய்வா தச்சலுப்பைக் காய்,

-தேரன் வெண்பா [25]

EXPLANATION

The peper regulates the vadha,piththa and kabha.

MODERN ASPECT

Chemical Constituent:

Pyrrolidine alkamide, Isopiperolein B,retrofractamide A, pipercide, piperchabamide , pellitorin,dehydropipernonaline, 2E, 4E, SIZE-N-isobutyl eicosatrienoic,pellitorine, trachyone, pergumidiene and isopiperolein B have been isolated from fruit.Essential oil contained.mono-terpene hydro-carbons, sabinene, B-pinene, limonene,furthermore terpinene, B-pinene, myrcene,3-carene, borneol,carvone, carvacrol, 1, 8-cineol, linalool, sesqui-terpenes, B caryophyllene, humulene-bisabolol, caryophyllene oxideketone, phenyl ether, eugenol,myristicin and safrole,bis alkaloids, pyramides D and E.[26]

Medicinal Uses:

The fruit is an important spice and flavouring agent and has also been used in the treatment of cholera a dyspepsia, as well as a variety of gastric ailments and arthritic disorders. Black pepper oil can be used to help in the treatment of pain relief, rheumatism, chills, flu, cold, increase circulation, exhaustion, muscular ache, physical and emotional coldness, nerve tonic and fevers. According to Ayurveda, fruit is useful in treatment of

asthma, chronic indigestion, colon toxins, obesity, sinus congestion, fever, intermittent fever, cold extremities, colic pain, piles, worms and sore throat. Externally, fruit paste extract can be applied to boils and other skin diseases. A pinch of pepper powder mixed with clove oil can be put in the caries to alleviate toothache. The finely ground pepper mixed with honey taken twice a day is beneficial in amnesia or dullness of intellect.[27]

3.1.3. KALLI VER-EUPHORBIA ANTIQUORUM

OTHER NAMES:

தாபித்தோம், கண்டியென்று, உச்சிரமென்றும்
தருவான, காமோதக மென்றும் பேரு
தாபிந்தோம், கற்கடக மாத ரென்றும் பேரு
நலமான, கருவு மறப்பளி யென்றும் பேரு
வாபித்தோம், கங்கைப் பாவிலை என்றும் பேரு
வாசிட்ட தி மூவி என்றும் பேரு
யாபித்த, மாக்னுடையாள மூலி என்றும் பேரு
ஆகிதமாய் சொல்லிவிட்டோம், சதுரக்கள்ளியின் பேர்

-பஞ்சகாவிய நிகண்டு [28]

EXPLANATION:

Sithiram, Komothagam, Kadarkatchi, Maadhar, Marapali, Gangaipaal, Idaivel Mooli, Mugamudaiyal Mooli.

VERNACULAR NAMES:

Eng- Quadrangular Spurge
Hind- Tidahara, Sehund, Tin-dhari-send
Te- Bont-jemudu, Bedda-yemudu
Duk,- Tidharl•sound
Sans- Vajratundi, Vajradathtaka, Mahasunhi
Mal- Chathura-kali

Fig.No-3.6:Euphorbia antiquorum

BOTANICAL ASPECT

Classification

Kingdam-Plantae

Class-Dicortyledane

Subclss-Monochlamidae

Series-Unisexuales

Family-Euphorbiaceae

Genera-Euphorbia

Species-antiquorum

Fig.No-3.7: Euphorbia antiquorum root

Habit

It is a shrub

Description

It is a small armed tree with whorled fleshy branches branchlets thick and broad, 3-5 winged, having sharp stipular spines leaves are small, sub-sessile, soon deciduous; involucres 3-nate, the central, flower sessile, female, the two laterals on long stout pedicles, glands 5, style bifid; fruit capsule.[29]

Part used

Stem, latex, root, root bark

SIDDHA ASPECT

Properties

Taste - pungent

Character - hot

Division - pungent

Action:

Thermogenic

Anodyne

Purgative

Emetic

Stomachic

Rubefacient

General properties

கரப்பான் சொறியுங் கடியுங் கபமும்
உரப்பன் குன்மம் கிழிக்கும்-நிரைப்பான
பேதிதருஞ் சீதமென்ற பேச்சகற்றும் பூவுலகிற்
ஓத்து சதுரக்கள்ளி தான்.

-அகத்தியர் குணபாடம் [30]

EXPLANATION

This plant cure eczema, scabies, urticaria, peptic ulcer and excess kabam.It cures diahroea also.

Latex

குட்டங் கடிசூலை குன்மமே தம்வாயு !
துட்டக் கிருமியோடு தூறுபுண்ணு - முட்ட
முதிரக் கரப்பனும்போ மொய்குழலே! நாளுஞ்
சதுரக்கள் ளிப்பாற்குத் தான்.

-அகத்தியர் குணபாடம் [31]

EXPLANATION

The latex cures leprasy, urticaria, peptic ulcer, vaadha diseases, leucorhoea, non-healing ulcers megguts and eczema.

MODERN ASPECT

Chemical constituent:

Diterpene, antiquorin, friedelan-3-B-ol, B-taraxerol, triterpenes, euphol 3-0-cinnamate, antiquol A and antiquol B, euphol, 24-methylenecyeloartanol, cycloeucalenol, (Z)-9-Nonacosene, sistosterol and p-acetoxyphenol were isolated from the latex.[32]

Medicinal uses:

The juice is acrid, anti-inflammatory, deobstruent, purgative and is useful in rheumatism, dropsy, gout, neuropathy, deafness, cough and cutaneous diseases.[33]

3.1.4KALARCHI VER

SYNONYMS

Caesalpinia bonduc (L) Roxb

Caesalpinia bonducella

Caesalpinia crista

OTHER NAME

Kaai, Vajjirapeejam, Kalar Kaai, Kacchakkaai, Guberapatchi.

VERNACULAR NAME

Eng-Bonduc Nut,Moloucca bean,Pysic nut

Tel-Gach-chakaya

Mal-Kazhanchikkara,Kalanchikkuru

Kan-Gajjaga-Kay

Sans-Kuberaleshi,Serakaraja

Arab-Akitnakit

Pers-Sagar Ghota

Hin-Kalkaranj,Katkaliji

Duk-Guchhe

Fig.No-3.8:Caesalpinia crista

BOTANICAL ASPECT

CLASSIFICATION

Kingdam-Plantae

Class-Dicotyledonae

Subclass-Polypetalae

Series-Calyciflorae

Order-Rosalae

Fig.No-3.9:Caesalpinia crista root

Family-Leguminosae

Subfamily-Caselpinasceae

Genrae-Caesalpinia

Species-crista

HABBIT

Caeselpinia crista is a thorn straggling shrub.

DISTRIBUTION

Wildely distributed in tropics.It was cultivated in India by means of seeds.[34]

DESCRIPTION

It is a large straggling, thorny shrub. The branches are armed with straight, yellow prickles. The leaves are pinnate, stipules foliaceous, pinnae 7 pairs, leaflets 3-8. The flowers are pale yellow in color, in supra-axillary racemes at the top. The fruits are inflated pods, covered with prickles, 6 cm long, 1-2 seeds per pod. The seeds are globular, hard, bluish grey with a smooth shiny surface.[35]

SIDDHA ASPECT

PROPERTIES

Taste-Bitter

Character-Hot

Division-Pungent

ACTION

Anti-periodic

Anti-spasmodic

Tonic

Febrifuge

Anthemintic

Deobstrecent

Emmenogue

GENERAL PROPERTY

விரைவாதம் சூலையரும் வெட்டையனல் ஏகும்
நிரைசேர்ந்த குன்மம் நிலையா -துரைசேர்
அழற்சி விலகும் அருந்திற் கசப்பாம்
கழற்சியிலை என்றுரைக்கும் கால்

-அகத்தியர் குணபாடம் [36]

ஏரண்ட தோடே இரங்கண்ட மெத்தவுமே
வீறண்ட மெல்லாம் விலகுங்கான்
தூரண்ட வள்ளை குழையடிபோம் வாள்விழிமாதே!
நல்ல வெள்ளை கழற்சிஇலை விள்.

-அகத்தியர் குணபாடம் [37]

கோசம் சுருங்கத் குடிலமிகு மன்னவனால்
வீசன் சுருங்காது மேலிட்ட - மோசம்
ஒழிய மருந்தாகி ஒத்தாசை செய்யும்
கழலாகிய கழற்சிக் காய்

- தேரர் வெண்பா [38]

EXPLANATION

The leaves of black type of Kalarchi reduces the orchitis,leucorrhoea and the types of peptic ulcers.The leaves of white type also reduces the orchitis.

MODERN ASPECT

CHEMICAL CONSTITUENTS

Seeds contain amino acid, Caesalpinia, bounducin, fatty oils, glycerols, palmitic acid, norcassane-type diterpenes, cassane-type diterpenes namely Norcaesalpinin A B and C were isolated from the seed kernel,Taepeenin A-I, nortaepeenin A-B and three diterpenes vinhaticoic acid, methyl vinhaticoate and ent-115-hydroxy-osa 5,15-diene were isolated from the stems and root.[39]

Medicinal Uses

Roasted seeds with castor oil is externally applied for hydrocele. Roasted seeds are internally given for hydrocele, leprosy, mitigates the condition and relieves the pain. Leaves areinternally used for liver diseases.Decoction of roots with honey is given for leucorrhoea. Leaves and stem bark considered as an anthelmintic, emmenogogue. Roasted seed powder combined with asafoetida, ghee and little amount of salt is recommended in postpartum period especially the abdominal pain. The seeds powder mixed with milk used in the case of diarrhoea.[40]

3.1.5. KADUGU (BRASSICA JUNCEA)

OTHER NAMES

Iyavi

-குணபாடம் மூலிகை வகுப்பு

விட்ட வலி விண்ணென்று இதற்கு பேரு
விளக்கினோம், அய்யாலு சீன மென்றும் பேரு
எட்டவென்றும், பள்ள னென்று இதற்குப் பேரு
எனது, மகத்தான துருஞ்சி எறிதற்குப் பேரு
நடராஜ் சிதறி எறிதற்குப் பேரு
நல்ல பத்தரவமென்றிதற்குப் பேரு
அட்டவத்தனாதி என்று, இதற்குப் பேரு
அருளினோம், புலத்தியனே கடுகின் பேரே

-பஞ்சகாவிய நிகண்டு [41]

EXPLANATION

Vitta vali, vin, iyyalu, seenam, ettam, pallan, thurunji, naattaraasa sithari, nalla paththaravam.

VERNACULAR NAMES

Eng-Indian mustard

Hin-Rayi

Kan-Sassiv

Mal-Katuku

San-Sarsapah

Tel-Avalu

Fig.No-3.10:Brassica juncea

BOTANICAL ASPECT

CLASSIFICATION

Kingdam-Planta

Class-Dicortyledane

Subclss-Polypetalae

Order-Thalamiflorae

Series-Parietales

Family-Brassicaceae

Genera-Brassica

Species-juncea

Fig.No-3.11:Brassica juncea

HABIT

Shrub

DISTRIBUTION

Cultivated throughout india.

DESCRIPTION

A glabrous annual with a few bristles at the base upto 1.5m in height, basal leaves long, broadly ovate, coarsely denate, persistant middle leaves oblong, 8 denate, upper leaves broadly linear, entire.Flowers yellow in racemes, fruits siliqua, breaking away from below upwards.Seeds attached to the replum.[42]

PARTS USED

Seed, seed oil.

SIDDHA ASPECT

PROPERTIES

Taste - Sour

Character - Heat

Division – Sour

ACTION

Emetic

Digestive

Stimulant

Rubefacient

GENERAL PROPERTIES

கடிப்பகை யெனவரு கடுகினை நாளு
மிளகில வணத்தொடு மூன்றுமொன் றாக்கி
யடுபுன லருந்துமு னயிலவை கறைதொறும்
வளிமுதன் மூலிகை வலியெலா மகனும்
பச்சடி முதற்கறி பண்பினா லயிலினு
நிச்சய மாயுணி கழப்பிணி யறுக்குமே

-தேரன் காப்பியம் [43]

இடிகாச நாசிச்சூ ரீளைதபம் பித்தங்
கடிவாத சீதங் கடுப்போட்டுடலிற்
படுகோட்டு நோயென்னும் பங்கிவைக ளைப்புண்
கடுகோட்டு மேன் மருந்து காண்,

-அகத்தியர் குணபாடம் [44]

மந்தமயக் கம்வாதம் வாய்நீர்ச் சுழற்றலறு
முந்து சுகப்பிரச வங்களுண்டா -மிந்துநுதன்
மானே! கிராணிகுன்ம மாறுமுத் தோட்டமும்போம்
தானே கடுகிற்குத் தான்.

-அகத்தியர் குணபாடம் [45]

வெண் கடுகு

பாலக்கிர கம்பூத பைசாச மோட்டிவிடும்
ஆல விஷங்கடிக ளாற்றுங்காண்-கோலமிகு
வெள்ளைச் செவிமாதே மாபங்க நோய்விலக்கும்
வெள்ளைக் கடுகின் விதி

-அகத்தியர் குணபாடம் [46]

EXPLANATION

- It cures with expectoration rainy days urticaria myalgia laziness concentrated saliva diarrhoea abdominal pain and delirium.Excuse dysentery joint disease indigestion and hiccup. Every day we take mustard with salt and pepper along with hot water that that will cure 3 dhodam.
- It is the diuretic and emitec property also.
- We take 2 gram mustard and titrated with water and have vomit may come.The paste applied in cloth. When the cloth applied in ARM legs that will be cured inflammation present in the site. The cloth was applied on the chest that will be cured hiccups.

3.1.6. KAMBICHARAM

Six times processed (distilled) vediyuppu is known as kambicharam.[47]

VEDIYUPPU (POTTASIUM NITRATE)

OTHER NAMES:

Pottiluppu, Inangan, Padairasan, Boomikoorma Navachara mithru

PREPARATION:

The sand containing the crude salt is placed in a mud pot. Water is added into it and mixed well. and a straw is placed inside the pot and filtered. The filterated mixture is heated to get the salt.

The potassium nitrate salt is used for the preparation of explosives It is also used for cooling alcohol and to polish the gold ornaments.

Fig.No-3.12:Potassium nitrate

Purification:

1. Salt - 100g
2. Water - 400g
3. Fermented butter milk - 100g
4. Lime juice - 100g

Water is added to the salt and boiled on a hearth with mild flames The white of eggs (4 Nos.) is added to every 1,400 gm of salt and the bubbles appearing with impure substances are removed with a wooden spoon

The ingredients are then transferred to another pot sealed with mud pasted cloth, filtered and kept in places without aeration. Next day the water is filtered and the salt is dried in sun shade. This process is repeated for seven times to get it purified.

OTHER METHOD

Potassium nitrate (1part), sea water or water (2 parts) are taken Potassium nitrate is finely powdered and dissolved in water. The clear fluid is poured in white coloured iron pot and heated till a semi-solid consistency is obtained. This is then poured in a copper pot and placed in a cool place; now the salt will form; the salt is taken out and dissolved in 2 parts of water and heated as mentioned above, this process is repeated for five to seven times to get it purified

The purified salt will appear as white thin rods. When placed in the tongue, it gives a cooling effect.

PROPERTIES AND USES

"மல்லாரு மட்டகுன்ம மாருத ரக்கட்டி
கல்லா மதைப்புநீர்க் கட்டருக - லெல்லாமே
கம்பிகம்பி யென்றுங் கருவுண்டா மங்கிநின்ற
கம்பிகம்பி யென்றுரைக்குங் கால்.

-குணபாடம் தாது சீவம் [48]

"சூதக வாயுவொடு சோணிதத்தின் வாதமும்போம்
வாத வலி குன்மம் மாறுங்கண்-மீதாங்
கொடிய வயிறிழியுங் கோழைகப மேகும்.
வெடியுப்பு தன்னை விளம்பு."

-குணபாடம் தாது சீவம் [49]

EXPLANATION

- Potassium nitrate salt has got demulcent, diuretic and diaphoretic properties. This should be given by dissolving in large quantities of water
- The salt is also useful in the treatment of eight types of gunman, uterus fibroids, anorexia, anaemia, urinary tract infections, dysuria, strangury, ascites, menopausal disorders, abdominal distention and asthma
- It improves fertility in women.
- The salt is also effective in fever, swellings, rheumatic disorders haemorrhage, gonorrhoea, eye diseases and sore throat.

DOSAGE:

650 mg - 1300 mg.

MEDICINAL USES

- Equal ratio of salt and tamarind are dissolved in water and boiled and the mixture is taken in semi-solid consistency and applied over the swelling and sprain by which haematoma will subside.
- The salt is also used as one of the ingredients in tooth powders
- Insects entered in the ear, generative organs, anus will come out on spraying the salt water
- Dissolved salt water is given through the rectum to remove the worms in the large intestine
- The salt is also used as a preservative for fish, mutton and vegetables
- The salt is dissolved in water and given for the silver nitrate poisoning

3.1.7. **PERUNGAYAM (Ferula asafoetida)**

OTHER NAMES

Valeegam, Abhiyagraham, Ramadem, Gandhi, Kayam, Sandenasam, Boodhanasam.

- குணபாடம் மூலிகை வகுப்பு

அருளினோம், சோம நாதி என்றும் பேரு
ஆட கண்டகாரி யாதி என்றும் பேரு
எருளினோம் இந்தத் தூனி என்றும் பேரு
இறங்கு ராமென்ற தற்குப் பேருண்டாச்சு
உருளினோம் உக்கித மென்றிதற்குப் பேரு
உசிதமுள்ள குந்ததமென்றதற்குப் பேரு
குருளினோம் குட்டாமென்றதற்குப் பேரு
கூறினோம் பெருங்காய அதீதப் பேரே

- பஞ்ச காவிய நிகண்டு[50]

EXPLANATION

Somanathi, Aadakanda kaari, Aadhi,Indhuli, Kooraam, Ukkitham, Kunthatham, Kuttaam.

VERNACULAR NAMES

Tam-Perungayam
Eng-Asafoetida
Tel-Inguva
Mal-Perungayam
Kan-Ingu
Hin-Hing

Fig.No-3.13:Ferula asafoetida

BOTANICAL ASPECT

CLASSIFICATION

Kingdam-Plantae

Class-Dicortyledane

Subclss-Polypetalae

Series-Calyciflorae

Order-Umbellales

Family-Apiaceae

Genera-Ferula

Species-asafoetida

Fig.No-3.14:Ferula asafoetida

HABIT

It is a shrub.

DISTRIBUTION

Cultivated in the north west part of india.

DESCRIPTION

A tree attaining height of 2-4 m.Stem stout and much branched.Leaves puberulas and minutely olandrelar or somewhat termantoce, the eaotical ones large and teenatisect,with segements oblongand ofuse.Umbels are fleshy peduncles, 20-30, flowers yellow.[51]

PARTS USED

Leaves,stem,root and gum resin.

SIDDHA ASPECT

PROPERTIES

Taste-Bitter,Pungent

Character-Hot

Divison-Pungent

ACTION

Expectorant

Carminative

Anthelmintic

Diuretic

GENERAL PROPERTIES

தந்தவே தந்த மூலத்தெழும்பிணி
பருவகாலம் விருச்சிகங்கீடம்மா
மந்தம் வாதம் உதாவர்த்தம் அல்குல் நோய்
மார்பணங்கட்ட குன்மம் மகோதரம்
உந்து கர்ப்பத்தின் வித்திரஞ்சூலைச்சூர்
உதிரப்பூச்சி சிலேத்துமத்துறும் வலி
வந்தமெய்க்கடுப் போடிவைமுற்றுமே
மாயுநாறுநற் காயங்கிடைக்கினே.

- தேரையர் குணபாடம் [52]

INDICATION

Cures gastritis, uterine disorders, ascites,worms,vatha diseases.

MODERN ASPECT

CHEMICAL CONSTITUENTS

Luteolin and its 7-O-B-D-glycopyranoside(fruits) seven S containing compound in essential oil. Ferrasiferol A,gummosin, kamolonol, mogoltaclone, polyanthia, umbelliferone (gum), foetidin.[53]

MEDICINAL USES

- Asafoetida given along with egg to cure dry cough.
- Equal quandity of aloe juice,pepper,asafoetida cures amenorrhoea.
- Asafoetida heated with gingely oil and used as a ear drops cures ache.

- It is used as anti-spasmodic,aphrodisiac,carminative, diuretic, expectorant, emmanogogue and nerve tonic.
- It is remedy for historia and nerve disorders of women and children and in the advanced stages of whoophing cough.
- It is used in colic pain,spasmodic movements of the bowels, infantile convulsions, aasthma and bronchitis

3.1.8. KARUNGAALI (Accasia catechu)

SYNONYMS

Acacia catechu (L) Willd, Oliv.

Senegalia catechu

catechuoides (Roxb.)Prain

Acacia catechuoides (Roxb.) Benth.

Acacia sundra (Roxb.) Bedd.

Acacia wallichiana DC

Mimosa catechu Lif.

Mimosa catechu uses Roxb.

Fig.No-3.15:Acacia catechu

VERNACULAR NAMES

Eng - black catechu, cutch tree

Tel - chandra

Mal - karungali

Kan - ladies

Sans - chandiran

Hind - katha

Duk - kher

Fig.No-3.16: Acacia catechu

BOTONICAL ASPECT

CLASSIFICATION

Kingdom - plantae

Class - dicotyledonae

Sub class - polymerase

Series - calyciflorae

Order - rosales

Family - leguminaceae

Sub family - mimosaceae

Genera - Acacia

Species - chetachu

HABIT

It is a deciduous shrub or tree, grows to 2-6 m tall with a flat to rounded crown.

DISTRIBUTION

Widly distributed in tropics and sub tropics espectually in india and burma.

DISCRIPTION

The tree has many branches and erect twigs spreading within the upright part. The branchlets have thorns just below the nodes: either three thorns with the central one hooked downwards and laterais curved upwards, or a single thorn with laterals absent. Leaves are small, grey-green, alternate and bipinnate. Pinnae occur in (2-) 3-8 (-12) pairs, and leaflets in 7-25 pairs. The rachis sometimes has prickles. The white or cream-colored flowers occur on spikes. Pods are indehiscent, yellowish to brown, flat papery and oblong; seeds are nearly round.[54]

PART USED:

Gum and stem bark, root

ACTION:

Demulcent and emollient.

SIDDHA ASPECT

PROPERTIES

Taste - astringent

Character - cold

Division - pungent

GENERAL PROPERTIES

Aquous extract of root

குட்டங் கயரோகங் குன்மம் பெருவயிறு
நெட்டை புழு திமிரு நீரிழிவும்-விட்டே
யருங்கான கத்தேகு மஞ்சுகமே! நல்ல
கருங்காலி நீரதனைக் கண்டு.

-அகத்தியர் குணபாடம் [55]

Bark

மேகநீ ரைக்கெடுக்கும் வீறுதந்த மூலத்துள்
ஆகிய வேதனையையாற்றுங்காண்-சோகம்
தருங்காச மீளையிவை சாரா தகற்றுங்
கருங்காலிப்பட்டையது காண்

-அகத்தியர் குணபாடம் [56]

Gums

கருங்காலி யின்பிசின்றான் காரார் கரப்பான்
வருமேக நீரிழிவை மாற்றும் விரைவாய்ப்
பெரும்பாடடைப நீக்கும் பெருந்தாது விருத்தி
அரும்பு மெனக்கண் டறி.

-அகத்தியர் குணபாடம்[57]

Root

கைப்புற் றிருக்குங் கருங்காலி வேருக்கு
மைப்புழுவும் பாகம் மதுநீரும்-மெய்க்குளெழு
குஷ்டுமுதி ரப்போக்குங் கூவியழு தகலும்
மட்டுறுபூங்கோதாய்! வழுத்து.

-அகத்தியர் குணபாடம்[58]

EXPLANATION

- Across accept of root of karungali cures leprosy Ascites worm infestation diabetes and peptic ulcer numbness due to anaemia

- The seed of hydnocarpus laurifolia boil in decoction of bark of karungali, the oil may come. The oil is used to leprosy internal and externally
- The decoction is used 2 diabetes and blood purification and also strengthens the body
- The gum of karungali used in Eczema diabetes menorrhagia the gum has spermatogenic activity
- The root of karungali used in diabetes CA tongue worm infestation can amoebic dysentery

MODERN ASPECT

CHEMICAL CONSTITUENT:

The naturally occurring association of the smaller molecular weight of arabinogalactan and glycoprotein were from isolated gum.[59]

MEDICINAL USES:

Gum is used externally for burns, sore nipples, cough, diarrhoea, dysentery, haemorrhage and externally to cover inflamed areas. Gum arabic is used in the food industry as a flavour fixative and emulsifier, to prevent crystallization of sugar in confections, as a stabilizer in frozen dairy products, for viscosity.

3.2. DISEASE REVIEW

3.2.1. SIDDHA ASPECT OF THE DISEASE

ADHI THOOLA ROGAM

In Siddha system of medicines, dyslipidaemia condition is correlated with ***Adhi thoola rogam*.**

Adhi – Excess

Thoola - Body (muscle mass)

Rogam - Ailment

SYNONYMS

Mikkakozhuta udambu - Excessive accumulation of fat in the body. Athitoolam, Mikkaparithauambu - Excessive development of muscular tissues.[60]

SYMPTOMS

Adiposity in abdomen, thigh, breast and gluteal regions. decrease in happiness, increased fatigue, tiredness, decrease in intelligence, shaking of the body parts while walking, increased kapha-pitha disorders.it also brings dosham of sperm and ovum result in in distribution of conception.

DYSLIPIDEMIC DRUGS USED IN SIDDHA MEDICINE

- Ekku chendooram
- *Amukkura podi*
- *Thuthuvalai Karpam*
- *Saccharum spontaneum*
- *Bauhinia purpurea*
- *Shuddha guggulu*
- *Vrikshamla*

3.2.2. MODERN ASPECT OF THE DIESASE

LIPIDS

Lipids {Greek: lipos-fat} are of great importance to the body as the chief concentrated storage form of energy, besides their role in cellular structure and various other biochemical functions. As such, lipids are a heterogenous group of compounds and, therefore it is rather difficult to define them.

Lipids may be regarded as organic substance relatively insoluble in water, soluble in organic solvents (alcohol, ether etc.), actually or potentially related to fatty acids and utilised by the living cells.

CLASSIFICATION OF LIPIDS

Lipids are broadly classified in to simple, complex, derived and miscellaneous lipids, which are further divided in to different groups.

1.**SIMPLE LIPIDS:** Esters of fatty acids with alcohols. These are mainly of two types.

(a) FATS AND OILS (*Triacylglycerlols*)

(b) WAXES

2. COMPLEX OR COMPOUND LIPIDS:

These are esters of fatty acids with alcohols containing additional groups such as phosphate, nitrogenous base, carbohydrate, protein etc. They are further divided as follows

(a) Phospholipids: It is further classified into:

1. Glycerophospholipids
2. sphingophospolipids

(b) Glycolipids

(c) Lipoproteins

(d) Other complex lipids

3.DERIVED LIPIDS

4.MISCELLANEOUS LIPIDS

5.NEUTRAL LIPIDS

FUNCTIONS OF LIPIDS:

- They are the concentrated fuel reserve of the body (triacylglycerols).
- Regulate the permeability of membrane (phospholipids and cholesterol).
- They serve as a source of fat-soluble vitamins (A, D, E and K).
- Lipids are important as cellular metabolic regulators (steroid hormones and prostaglandins).

- Lipids protect the internal organs, serve as insulating materials, and give shape and smooth appearance to the body.[61]

LIPOPROTEINS

Lipoproteins consists of lipids and proteins. The function as transport vehicles lipids in blood plasma.

STRUCTURE OF LIPOPRTEINS

Neutral lipid core surrounded by a coat shell of phospholipids, apoproteins and cholesterol.

CLASSIFICATION OF LIPOPROTEIN

Five major classes of lipoproteins are identified in human plasma, based on their separation by electrophoresis.

1) Chylomicrons
2) Very low-density lipoprotein
3) High density lipoproteins
4) Low density lipoproteins
5) Free fatty acids—albumin

Table No:3.1. Lipoprotein Patterns (Fredrickson Phenotypes)

	Elevated Lipoprotein(s)	**Elevated Lipids**
I	Chylomicrons	TGs
IIa	LDL	Cholesterol
IIb	LDL and VLDL	TGs and cholesterol
III	VLDLand chylomicron remnants	TGs and cholesterol
IV	VLDL	TGs
V	Chylomicrons and VLDL	TGs and cholesterol
LDL = low-density lipoprotein; TGs = triglycerides; VLDL = very-low-density lipoprotein.[62]		

METABOLISM OF LIPOPROTEIN

A general of lipoprotein metabolism is depicted. Chylomicrons are synthesized in the small intestine during the course of fat absorption. They contain apoprotein B48 name is given since this apoprotein contains 48% of protein coded by apo B gene. Chylomicrons are produced when nascent particles combine with apo C II and apo E, derived from HDL.

ROLE OF LIPOPROTEIN LIPASE:

It hydrolyses a portion of triacylglycerols present in chylomicrons and VLDL to liberate **free** fattyacids and glycerol. Lipoprotein lipase is activated by apo C II.

UPTAKE OF CHYLOMICRON REMNANTS BY LIVER:

The chylomicron remnants are taken uo by receptors present on the hepatocytes of liver.

CONVERSION OF VLDL TO LDL

During the course of VLDL metabolism intermediate density lipoprotein is formed which lose apo-E and get connected to LDL.

Cholestrol Ester Transfer Protein

CETP is synthesized in the liver and it facilitates the exchange of components between different the exchange lipoproteins.

LDL Receptors and Supply of Cholesterol to Tissues

The most important function of LDL is to supply cholesterol to the extrahepatic tissues.

DEFICIENCY OF LDL RECEPTORS:

A defect in LDL receptor results in the elevation of plasma LDL, hence plasma cholesterol. However, plasma triacylglycerol concentration remains normal. Deficiency LDL receptors is observed in a type IIa hyper beta lipoproteinaemia. This disorder is associated with a very high risk of atherosclerosis.

The evidence implicating hypercholesteremia in atherogenesis includes the following observations:

1. The dominant lipids in atheromatous plaques are cholesterol esters.
2. Genetic defects in lipoprotein uptake and metabolism that because hyperproteinaemia is associated with accelerated atherosclerosis.

3.Lowering serum cholesterol by diet or drugs slows the rate of progression of atherosclerosis, causes regression of some plaques, and reduces the risk of cardiovascular events.

The mechanisms by which hyperlipidaemia contributes to atherogenesis include the following:

Oxidised LDL is indigested by macrophages through a scavenger receptor, distinct from the LDL receptor, resulting in foam -cell formation. In addition, oxidised LDL stimulates the release of growth factors, cytokines, and chemokines by ECs and macrophages that increase monocyte recruitment into lesions. Finally, oxidised LDL is cytotoxic to ECs and smooth muscle cells (SMCs) and can induce EC dysfunction.

The importance of oxidised LDL in atherogenesis is suggested by its accumulation within macrophages at all stages of plaque formation. Moreover, antioxidant therapy (β-carotene and vitamin E) protects against atherosclerosis in animal models, but it does not appear to be effective for preventing IHD.

DYSLIPIDEMIA

Dyslipidemia is *elevation* of plasma cholesterol, triglycerides (TGs), or both, or a low HDL cholesterol level that contributes to the development of atherosclerosis. Causes may be primary (genetic) or secondary. Diagnosis is by measuring plasmalevels of total cholesterol, TGs, and individual lipoproteins. Treatment involves dietary changes, exercise, and lipid-lowering drugs.

CLASSIFICATION

Dyslipidemias were traditionally classified by patterns of elevation in lipids and

lipoproteins. A more practical system categorizes dyslipidemias as primary or secondary and characterizes them by,

- Increases in cholesterol only (pure or isolated hypercholesterolemia)
- Increases in TGs only (pure or isolated hypertriglyceridemia),
- Increases in both cholesterol and TGs (mixed or combined hyperlipidemias)

Both primary and secondary causes contribute to dyslipidemias in varying degrees. For example, in familial combined hyperlipidemia, expression may occur only in the presence of significant secondary causes.

PRIMARY CAUSES

Primary causes are single or multiple gene mutations that result in either overproduction or defective clearance of triglycerides and LDL or in underproduction or excessive clearance of HDL.

The names of many primary disorders reflect an old nomenclature in which lipoproteins were detected and distinguished by how they separated into alpha (HDL) and beta (LDL) bands on electrophoretic gels.

SECONDARY CAUSES

Secondary causes contribute to many cases of dyslipidemia in adults.

The secondary cause of dyslipidemia in developed countries is sedentary lifestyle with excessive dietary intake of saturated fat, cholesterol, and trans fats.

Trans fats are polyunsaturated or monounsaturated fatty acids to which hydrogen atoms have been added; they are used in some processed foods and are as atherogenic as saturated fat.

Other common secondary causes of dyslipidemia include

- Alcohol overuse
- Hypothyroidism
- Primary biliary cirrhosis and other cholestatic liver diseases
- Drugs, such as thiazides, beta-blockers, retinoids, highly active antiretroviral agents, cyclosporine, tacrolimus, estrogen and progestins, and glucocorticoids

- Secondary causes of low levels of HDL cholesterol include cigarette smoking, anabolic steroids, HIV infection, and nephrotic syndrome.
- chronic kidney disorders

SIGNS AND SYMPTOMS:

- Dyslipidemia itself usually causes no symptoms but can lead to symptomatic vascular disease, including coronary artery disease (CAD), stroke, and peripheral arterial disease.
- High levels of triglycerides (> 1000 mg/dL [> 11.3 mmol/L]) can cause acute pancreatitis.
- High levels of LDL can cause arcus corneae and tendinous xanthomas at the Achilles, elbow, and knee tendons and over metacarpophalangeal joints. Other clinical findings seen in patients with high LDL (eg, in familial hypercholesterolemia) include xanthelasma (lipid rich yellow plaques on the medial eyelids).
- Xanthelasma can also occur in patients with primary biliary cirrhosis and normal lipid levels.
- Patients with the homozygous form of familial hypercholesterolemia may have arcus corneae, tendinous xanthomas and xanthelasma plus planar or tuberous xanthomas.
- Planar xanthomas are flat or slightly raised yellowish patches. Tuberous xanthomas are painless, firm nodules typically located over extensor surfaces of joints.
- Patients with severe elevations of TGs can have eruptive xanthomas over the trunk, back, elbows, buttocks, knees, hands, and feet. Patients with the rare dysbetalipoproteinemia can have palmar and tuberous xanthomas.
- Severe hypertriglyceridemia (> 2000 mg/dL [> 22.6 mmol/L]) can give retinal arteries and veins a creamy white appearance (lipemia retinalis).

Extremely high lipid levels also give a lactescent (milky) appearance to blood plasma. Symptoms can include paresthesias, dypsnea, and confusion.

BILE ACID SEQUESTRANTS

Bile acid sequestrants block intestinal bile acid reabsorption, forcing up-regulation of hepatic LDL receptors to recruit circulating cholesterol for bile synthesis. They are proved to reduce cardiovascular mortality.

Cholestyramine, colestipol, and colesevelam (but to a lesser degree), interfere with absorption of other drugs—notably thiazides, beta-blockers, warfarin, digoxin, and thyroxine—an effect that can be decreased by administration at least 4 h before or 1 h after other drugs. Bile acid sequestrants should be given with meals to increase their efficacy.

CHOLESTEROL ABSORBATION INHIBITORS

Cholesterol absorption inhibitors such as ezetimibe, inhibit intestinal absorption of cholesterol and phytosterol. Ezetimibe usually lowers LDL cholesterol by 15 to 20% and causes small increases in HDL and a mild decrease in triglycerides.

PCSK9 MONOCLOAL ANTIBODIES

PCSK9 monoclonal antibodies are available as subcutaneous injections given once or twice per month. These drugs keep PCSK9 from attaching to LDL receptors, leading to improved function of these receptors. LDL cholesterol is lowered by 40 to 70%. A cardiovascular outcomes trial with evolocumab showed a decrease in cardiovascular events in patients with prior atherosclerotic cardiovascular disease.

DIETARY SUPPLIMENTS

Dietary supplements that lower LDL cholesterol levels include fiber supplements and commercially available margarines and other products containing plant sterols (sitosterol and camphesterol) or stanols. The latter reduce LDL cholesterol by up to 10% without affecting HDL or TGs by competitively displacing cholesterol from intestinal micelles.

FUTURE THERAPIES

Future therapies to reduce LDL include peroxisome proliferator–activated receptor agonists that have thiazolidinedione-like and fibrate-like properties, LDL-receptor activators, LPL activators, and an adenosine triphosphate-citrate lyase/adenosine monophosphate activated kinase modulator.

Cholesterol vaccination (to induce anti-LDL antibodies and hasten LDL clearance from serum) and gene transfer are conceptually appealing therapies that are under study but years away from being available for use.

ELEVATED LDL CHOLESTEROL IN CHILDREN

- Dietary treatment for children with LDL cholesterol > 110 mg/dL (> 2.8 mmol/L). Drug therapy is recommended for children > 8 yr and with either of the following:
- Poor response to dietary therapy, LDL cholesterol ≥ 190 mg/dL (≥ 4.9 mmol/L), and no family history of premature cardiovascular disease
- LDL cholesterol ≥ 160 mg/dL (> 4.13 mmol/L) and a family history of premature cardiovascular disease or ≥ 2 risk factors for premature cardiovascular disease.

HIGHER HDL

Treatment includes lifestyle changes such as an increase in exercise and weight loss. Alcohol raises HDL cholesterol but is not routinely recommended as a therapy because of its many other adverse effects. Drugs may be successful in raising levels when lifestyle changes alone are insufficient, but it is uncertain whether raising HDL levels reduces mortality.

TREATMENT FOR SECONDARY CAUSES OF DYSLIPIDEMIA

- Treatment of diabetic dyslipidemia should always involve lifestyle changes and statins to reduce LDL cholesterol.
- To decrease the risk of pancreatitis, fibrates can be used to decrease TGs when levels are > 500 mg/dL (> 5.65 mmol/L).
- Metformin lowers TGs, which may be a reason to choose it over other oral antihyperglycemic drugs when treating diabetes.

- Some thiazolidinediones (TZDs) increase both HDL cholesterol and LDL cholesterol. Some TZDs also decrease TGs.
- These antihyperglycemic drugs should not be chosen over lipid-lowering drugs to treat lipid abnormalities in patients with diabetes but may be useful adjuncts.
- Patients with very high TG levels and less than optimally controlled diabetes may have a better response to insulin than to oral antihyperglycemic drugs.

MONITORING TREATMENT:

Lipid levels should be monitored periodically after starting treatment. No data support specific monitoring intervals but measuring lipid levels 2 to 3 mo after starting or changing therapies and once or twice yearly after lipid levels are stabilized is common practice.

If statin-induced muscle damage is suspected, statin use is stopped and CK may be measured. When muscle symptoms subside, a lower dose or a different statin can be tried. If symptoms do not subside within 1 to 2 wk of stopping the statin, another cause should be sought for the muscle symptoms (eg: polymyalgia rheumatica) [65]

Treatment of dyslipidemia in patients with hypothyroidism, chronic kidney disease, liver disease, or a combination of these disorders involves treating the underlying disorders primarily and lipid abnormalities secondarily. Abnormal lipid levels in patients with low-normal thyroid function (high-normal TSH levels) improve with hormone replacement. Reducing the dosage of or stopping drugs that cause lipid abnormalities should be considered.

3.3. PHARMACEUTICAL REVIEW

Purification processes in Siddha system (*suddhiseithal)*

The process of detoxification or purification of the drug is called '*suddhiseithal*' in Siddha medical terminology.

Nature has created innumerable plant, herbs, metals, poisonous substances, minerals, salts and other organic substances. The Siddha had selected such of those things which can render relief to innumerable ailments of mankind suffered. Any matter in nature has to be utilized for medicine purposes the properties which may cause bad effects should

be neutralized or eliminated. That's why every raw drug used in Siddha medicine is purified before preparing it as a medicine.

Purification

The exact part of the herb which has been prescribed should alone be taken for medicine. There should not be other impurities like mud, sand or any such thing. If it is green leaf, dried or decomposed leaves or insects should be eliminated. Care should be taken in identifying the herbs properly.

As a general rule, when anything is subjected to be processed by using heat, soaking either alone or with some other substance, some chemical reaction may take place. In these process impurities, toxins would be eliminated and the substances become purified. Hence some of the poisonous herbs which are purified by using heat.

Importance of purification

The drugs when subjected to heat like roasting or soaked in liquids undergo some chemical reactions. Such as oxidation of toxic substances to non-toxic substances, elimination of certain poisonous chemicals to non- poisonous substances. In these ways not only the toxicity, impurities or removed but also enhance the potency of the drugs.

Mathirai :[57]

The ingredients should be first purified and powdered into a fine powder and then macerated 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.

Other names : *Kuligai*

Rule of tablet preparation

- Before preparing mathirai, the raw drugs required are powdered separately and then mixed to attain homogeneity, then grinded together until it reaches waxy in consistency.
- The fine paste which does not stick to the mortar should be considered as the right consistency for rolling pills.

- If any of the preparation of pills, if mercury and sulfur are the key ingredients, mercury should be grinded first and sulfur is added to it and grinded and further continued.
- In case of inclusion of hard raw drugs, they should be grinded first; the reason behind this if hard substances are added to smooth substances, they will not be grinded finely.
- Croton seeds if included can be grinded at the last: because croton on grinding releases oil; if added early the effect of medicine will be suppressed, so it should be added finally.
- Aromatic substances like camphor, lac, musk, are added just before 24 minutes the paste reaches waxy consistency.
- The paste should not adhere to the mortar nor does it adhere to the pestle.

Storage

They should be stored in well stopper glass vials, with relevant labels and instructions.

Shelf life of medicines

Medicines can be classified into internal and external medicines. They are each in 32 types. Mathirai comes under the category of internal medicines. The shelf life of medicines indicates the potency of medicines. The medicines even though seems to be fresh is not efficacious after sometime. So, the medicines should not use after certain period.

THE SHELF LIFE OF *MATHIRAI*,

As per Siddha literature *Agamarunthu padal* in *Gunapadam Thathu-seevam* text

"*உயர்சூர ணம்பிட்டு வடகம் வெண் ணெய்நான்கி*
னுயிர்மூன்று திங்களெண்ணெய்
விரலிடுமு யர்ந்தமாத் திரைகடுகு பக்குவம்
.....
கொள்ளாறு மோராண்டு.....

- குணபாடம் தாது சீவம் வகுப்பு

From above the quote the shelf life of Mathirai is 1 yr. But according to Ayush guidelines the shelf life is 2 yr.

Traditional tests for *Mathirai*

Characters

- Non sticky on rolling
- No cracks over the surface after drying
- Shall be rolled uniformly over the plane surface.

Based on these characters the drug is assessed as the appropriate one for medication.

Table No 3.2: Testing parameters for *Mathirai*-AYUSH guidelines

S.No	Tests
1	Description, Colour, Odour
2	Weight Variation
3	Disintegration Time (Not more than 15 minutes)
4	Identification TLC/ HPTLC/GLC
5	Assay
6	Test for heavy/toxic metals Mercury Arsenic Cadmium Lead
7	Microbial Contamination Total Bacterial count Total Fungal count
8	Test for specific pathogen E.coli Salmonella species Pseudomonas aeruginosa Streptococcus aureus
10	Test for aflatoxins B1, B2, G1, G2

Modern Aspect of the formulation:

Tablet (Pill)-*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. [58]

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 excipents 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 IP2007 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 excipents, no additional cover is applied on to 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.

E.g: 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 E.g: glyceryl trinitrate.

8. Tablets for other routes of administration:

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

Tablet Ingredients:

A tablet consists of active medicament with excipents which are in powder form are compressed or pressed into a solid dosage form. In addition to active ingredients, tablet contains a number of inert materials known as additives or excipents. [59]

- Diluent
- Binder and adhesive
- Disintegrents
- Lubricants and glidants
- Colouring agents
- Flavouring agents
- Sweetening agents

1. Diluent:

Diluents are fillers are used to make bulk of the tablet when the drug dosage itself was inadequate to produce the bulk these are used. Secondary reason is to provide better tablet properties such as improve cohesion, to permit use of direct compression manufacturing or to promote flow.

2.Binders and Adhesives: These materials are added either dry or wet form granules or to form cohesive compacts for directly compressed tablet. Ex: Acacia, tragacanth-solution for 10-25% concentration.

3. Disintegrents:

It added to the tablet formulations to facilitate its breaking or disintegration, when it contacts in water in the GIT. Example: Starch- 5-20% of tablet weight. **Super Disintegrents**: Swells up to ten-fold within 30 seconds when contact water. Example: Crosscarmellose- cross-linked cellulose.

4. Lubricant and Glidants:

Lubricants are planned to prevent adhesion of the tablet materials to the surface of dies and punches, reduce inter particle friction and may improve the rate of flow of the tablet granulation Example: Lubricants- Stearic acid, stearic acid salt - Stearic acid.

5. Coloring agent:

The use of colors and dyes in a tablet has three purposes:

- Masking off colour drugs
- Product Identification
- Production of more elegant product

All coloring agents must be approved and certified by FDA.

1. **Flavoring agents:**

   For chewable tablet- flavor oil is used

2. **Sweetening agents:**

   For chewable tablets: Sugar, mannitol. Saccharine (artificial):500 times sweeter than sucrose.

Advantages:

- These are very easy and handy to use.
- As these are unit dosage form fixed dose was administered.
- Modified drug release rate and duration of tablets be able to increase their therapeutic effect and increase the patient compliance by reducing the frequency of drug administration.
- These are cost-effective dosage forms when compared to other dosage forms
- The physical, microbial and chemical stability of tablet are superior to other dosage forms.

Disadvantages:

- The systemic availability of the drug depends on many physiological factors
- The onset of action was lesser when compared to Intra Venous route. (except sublingual tablets)
- Geriatric and children of small age cannot swallow the tablets easily.

3.4.PHARMACOLOGICAL REVIEW

I. ANTIDYSLIPIDMIC ACTIVITY

1.TRITON- INDUCED HYPERLIPIDEMIAPURPOSE AND RATIONALE

The systemic administration of the surfactant Triton to mice or rats results in a biphasic elevation of plasma cholesterol and triglycerides (Frzntzyed and hinkelman 1955; Garattini et al. 1958, 1961; Holmes 1964; tamasi et al. 1968).

PROCEDURE:

Male Sprague dawley or wistar weighing 200-350g are starved for 18 h and then injected intravenously with 200 mg\kg Triton WR 1399 (isooctyl-polyoxyethylene phenol). Serum cholesterol levels increase sharply 2- 3 times after 24h (phase1). The hypercholesterolemia decreases nearly to control levels within 24h (phase 2). The test drugs employed or the solvent for the controls are administrated simultaneously with the Triton injection or 22h thereafter.

Serum cholesterol analyse are made 6, 24, and 24h after Triton injection. The mechanism of the Triton induced hypercholesterolemia in phase 1 is thought to be due to increased hepatic synthesis of cholesterol through the ability of Triton too interfere with the uptake of plasma lipids by the tissues. Drugs interfering with cholesterol biosynthesis were shown to be active in phase 1, while drugs interfering with cholesterol excretion and metabolism were active in phase 2.

EVALUTION

Mean value standard deviation are calculated for each group and time interval and compared statistically with the controls.

CRITICAL ASSESSMENT OF THE METHOD

The method employing Triton hypercholesterolemia is rather simple and rapid for detection of the compounds interfering with the synthesis and excretion of cholesterol. Since the test is rather artificial, the results have to be validated by other methods.

2. CHOLESTEROL-DIET INDUCED ATHEROSCLEROSIS IN RABBITS AND OTHER SPECIES

PURPOSE AND RATIONALE

Rabbits are known to be susceptible to hypercholesterolemia and arteriosclerosis after excessive cholesterol feeding, therefore, this approach has been chosen by many authors to study the effect of potential ant arteriosclerotic drugs.

PROCEDURE

Several modifications of the protocol have been described. Usually, male rabbits from an inbred strain, eg, white New Zealand, at an age of 8-10 weeks are used. Body weight variation should be as low as possible. At the beginning of the experiment, blood is withdrawn from the marginal ear vein for determination of total cholesterol, total glycerides and blood sugar.

Groups of 10 animals are used for treatment with drugs or as controls. The rabbits are switched from commercial food to a diet supplemented with 0.3-2% cholesterol and kept on this regimen for a period of 10- 12 weeks. One group is kept on normal diet. During and at the end of the experiment the blood is taken for analysis. Usually, cholesterol and tri glyceride levels increase several- fold over the original values.

The animals are sacrificed and the thoracic aorta is removed, cleaned of the surrounding tissues, and longitudinally cut and opened for fixation with formaldehyde. The tissue is stained with oil red. The percentage of the intimal surface covered by the oil red positive lesions is calculated with a computerized planimeter. In animals fed normal diet, the aorta does not show any staining, whereas in cholesterol- fed rabbits the aorta shows severe atherogenic lesion

EVALUATION

Data are expressed as means standard deviation. Statistical evaluation is performed by Dunnett's or Schefft's test. A p- value of <0.05 is regarded as statistically significant.

HEREDITARY HYPERLIPEMIA IN RABBITS

Watanabe et al. (1977, 1980) described a strain of rabbits with hereditary hyperlipidaemia (WHLL rabbit) which has those observed in humans. This M1. induction of experiment atherosclerosis 1100chapter M. Anti-atherosclerosis 1100chapter M. Anti-atherosclerotic activity animal model is used as background for atherosclerosis research and target validation. Walsh et al. (1989) and Rubin et al.

(1991) integrated human apolipoprotein A-I gene in transgenic mice resulting in an increase of HDL levels.

Linton et al. (1993) described the development of transgenic mice expressing high levels of human apolipoprotein B100 which are considered to atherogenic.

Transgenic mice lacking apolipoprotein E showed severe hypercholesterolemia and atherosclerosis (plump et al. 1992; Zhang et al. 1992).

Overexpression of apolipoprotein E in transgenic mice reduced plasma cholesterol hypercholesterolemia and inhibited (Harada et al. 1996)

FRUCTOSE INDUCED HYPERTRIGLYCERIDEMIA IN RATS

PURPOSE AND RATIONALE

Rats switched from a diet low in carbohydrates and high in protein to high intake of inhibition of this phenomenon afterwards, the animal is offered 20 % fructose.

Male Sprague Dawley rats weighing 200- 250 g are fred over a period of one

PURPOSE

Male Sprague Dawley rats weighing 200- 250 g are fred over a period of one week a diet enriched in protein with reduced carbohydrates content eg, altromin C1 080 or C1 009.

Groups of 10 animals are treated for 3 days with the test compound or the standard (clofibrate 100mg/kg) or the vehicle (polythene glycol) by oral gavage. From the second to the third-party water is withheld for a period of 24h. immediatel Solution ad lithium for a period of 20h after the last application of the test compound, the animals are anesthetized with ether and 1.2 ml blood is withdrawn by retroorbital puncture. The blood is centrifuged for 2 min at 16000g. total glycerol is determined in the serum according to Richterlich and Lauber (1962)

EVALUTION

The average values of total glycerol of the treated groups are compared with the control groups using students ‘st- test

INHIBITION OF ISOLATED ENZYME HMG-COA REDUCTASE IN VITRO

PURPOSE AND RATIONALE

For screening purpose, studies on the inhibition of HMG-COA reductase obtained from rat liver microsomal fraction can be used (Avigan et al. 1975; Philipp and shapiro 1979).

PROCEDURE

The inhibitory activity of the test compound on HMG CoA reductase is estimated with soluble enzyme preparations obtained from the microsomal fraction of rat liver (Philipp and Shapiro 1979).

The enzyme reaction is carried out with 50 ul partially purified HMG CoA reductase in buffer containing 25 Mm Tris, 10M EDTA, and 10 mM dithiothreitol at ph. 7.5, 20 ul of 910 µM HMG- CoA solution containing 100 nCi (3-7KBq) of 14 C- HMG-CoA and 20 ul of ADPH regenerating system (5.2x 10 -3 M glucose 6- phosphate, dehydrogenase, 5.3 x 10-3 M NADP), with the actual concentration of 50 mM NADPH

The final incubation volume is 200ul.

The main reaction is preceded by 20 min preincubation with the NAPDH regenerating system at 37’c followed by 20 min incubation at 37’c of the completed samples with the

test compound or the standard and stopped by addition of 75 µl 2 N HCLO4. After 60 min at room temperature, the samples are cooled in an ice bath and neutralized by addition of 75 ul 3N potassium acetate. Supplementing the volume 500µl, the precipitate is centrifuged and 250ul of the clear supernatant are applied to a column (0.6 x 8.0 cm of BIORAD AG 1-X8 (100-200 mesh)

Mevalolactone is eluted with water discarding the first 750 µl and collecting the next 3500ul. Five hundred ul of the eluate are used for measurement in duplicate, mixed in vials with 10 ml Ouickscint(Zinsser) and measured in a liquid scintillation counter (Beckman). The assay is generally performed in triplicate. Lovastatin sodium is used as standard.

EVALUATION

The means values with and without inhibitors are compared for the calculation of inhibition. IC_{50} values are calculated,

EX-VIVO INHIBITION OF CHOLESTROLBIOSYTHESIS IN ISOLATED RAT LIVER SLICES PURPOSE AND RATIONALE

Inhibition of cholesterol biosynthesis in rat livers can be measured in an ex vivo assay after oral treatment with HMG-CoA reductase inhibitors by cholesterol synthesis from labelled sodium octanoate.

PROCEDURE

Male Sprague Dawley weighing 110_ 130 g are kept on a reverse light cycle (lights 3:00 p.m to 3.00 a.m.) for 14 days prior to use, through the period of adaptation, the rats have free access to a low cholesterol diet and tap water. On the day of the experiment, the test compounds are given orally between 9:00 and11:00 a.m. as suspension in 0.5% methylcellulose.

After one hour, the rats are sacrificed, the livers removed and transferred to chilled oxygenated Krebs-Ringer-bicarbonate buffer (p^H 7.4). the livers are then chopped into 0.8mm2 pieces using a McElwain tissue chopper (eg, Brinkmann Instr., Westbury, USA)

N and are suspended in the same buffer.

Aliquots of the suspension are pipetted, in triplicate, into culture tubes which contains ($_{14}$c) sodium octanoate (300uM/1,6.67 Ci/M). The assay volume is 1ml. The tubes are gassed with 95% O_2 /5% CO_2 for 10 s, stoppered with a serum cap, and incubated at 37^0 Celsius in a metabolic shaker at 150 oscillations/min for 90 min.

The reaction is stopped by addition of 1ml 15% KOH in ethanol. An aliquot of the mixture assayed for protein concentration. An internal standard [3 H] cholesterol (30 000 dpm) is added to determine recovery, which ranges from 70-80%.

The tubes are saponified at 75 degrees Celsius for 2 h and then extracted with 10 ml petroleum ether for 30 min. the lower aqueous phase is frozen in a dry ice/alcohol mixture, and the ether phase is removed, washed with 2ml glass- distilled water and then evaporated to dryness.

The cholesterol synthesised is separated by thin layer chromatography on plastic silica gel plates using chloroform as eluent, after visualisation with iodine, the cholesterol spots are cut out, and radioactivity quantitated by liquid scintillation counting.

EVALUTION

Results are expressed as percentage inhibition compared to vehicle -treated control values. Using various doses, ED_{50} values of inhibition can be calculated from dose response curves.[69]

HYPO GLYCEMIC ACTIVITY

STREPTOZOTOCIN- INDUCED DIABETES STZ (2- deoxy(3methyl 3 nitrosourea) 1-D – glucopyranose) is board- spectrum antibiotic, which is produced from Streptomyces achromogen. Rakieten et al. first described the diabetogenic property of STZ.

Mechanism of causing B cell damage

1) By processes of methylation
2) Free radical generation and
3) Nitric oxide production

PROCEDURE

STZ induces diabetes in almost all species of animals. Diabetogenic dose varies with species and the optimal dose required in various specious are: rats (50-60 mg/kg i.p. or i.v.), mice (175-200 mg/kg i.p or i.v) and dogs (15mg/kg for 3 days) the blood glucose level shows the same triphase response as seen in the alloxan treated animals, hyperglycaemia at 1h, followed by hypoglycaemia, which lasts for 6h, stable hyperglycaemia by 24-48h after STZ administration.

MODIFICATION

Multiple low dose of STZ also induces diabetes by causing immune mediated pancreatic insulitis in rats. It has also been shown to have diabetogenic effect on the golden hamsters when given i.p. at a dose of 50mg/kg. cyclosporin-A when given with STZ enhances its diabetogenic efficacy. STZ combined with complete Freud's adjuvant: each of CFA, incomplete Freud's adjuvant.

Mycobacterium butyricum (component of CFA), Listeria monocytogenes, or endotoxin administered 24h prior to STZ (25 mg/kg) and then repeated in the three subsequent weeks, all produce hyperglycaemias. Fasting for 48h (24h prior to and 24h subsequent to STZ injection) also produces hyperglycaemia. Neither four administrations of CFA nor of STZ alone result in persistent hyperglycaemia.

ADVANTAGES AND DISADVANTAGES

STZ has almost completely replaced alloxan for inducing diabetes because of:

1) Greater selectively towards β-cells
2) Lower mortality rate
3) Longer or irreversible diabetes induction

4) However, guinea pigs and rabbits are resistant to its diabetogenic action.

ALLLOXAN INDUCED DIABETES

Alloxan a cyclic urea analog, was the first agent in this category, which was reported to produce permanent diabetes in animal.

MECHANISM OF ACTION

The mechanism by which it induces diabetes is not very clear. Alloxan is highly reactive molecule that is readily reduced to diuleric acid, which is auto oxidised back to alloxan resulting in the production of free radicals. These free radicals damage the DNA of β cells and cause cell death. Second mechanism proposed for alloxan is its ability to react with protein SH groups, especially the membrane proteins like glucokinase on the b cells, finally resulting in cell necrosis. However, there are major species difference in response to alloxan.

CHEMICALLY INDUCED DIABETES

Chemically induced type 1 diabetes is most commonly used animal model of diabetes. Chemical agents which produce diabetes can be classified into three categories and include agents that

4) Specifically, damage b cells
5) Cause temporary inhibition production and/ or secretion
6) Diminish the metabolic efficacy of insulin in target tissues.
   In general, chemicals in the first category are of interest as they reproduce lesions resembling IDDM.

HORMONE INDUCED DIABETES MELLITUS

Dexamethasone, a long acting glucocorticoid is used to produce NIDDM, NIDDM form of diabetes is produced when dexamethasone is administered at a dose of 2-5 mg/kg i.p twice daily over a number of days in rats.

Besides rat models, other experimental models using guinea pigs and rabbts are also

reported for the study of diabetes using corticotrophin is used to stimulate adrenal cortex that results in hormonal balance causing steroid diabetes.

INSULIN ANTIBODIES-INDUCED DIABETES

Giving bovine insulin along with CFA to guinea pigs produces anti- insulin antibodies. Intravenous injection of 0.25-1 ml guinea pig anti-insulin serum rats induces a dose dependent increase in blood glucose levels up to 300 mg%. this unique effect to guinea pig anti-insulin serum is due to neutralization of endogenous insulin by the insulin antibodies.It persists as long as the antibodies are capable of reacting with insulin remaining in the circulation. Slow i.e. infusion or i.p. injection prolongs the effect for more than few hours. However, large doses and prolonged administration are accompanied by ketonemia, ketonuria, glycosuria and acidosis and are fatal to the animals. After lower doses the diabetic syndrome is reversible after a few hours.[70]

ANTI-OXIDANT ACTIVITY

DPPH RADICAL SCAVENGING ACTIVITY

The free radical scavenging activity of methanol extract was measured by 1,1 diphenyl -2- picryl -hydrazyl (DPPH) using the method of Blois (1958) 0.1 Mm solution of DPPH in methanol was prepared and 1ml of this solution was added to 3ml of various concentrations of methanol extract and reference compound (125,250,500, and 1000 µg / ml). After 30 min, absorbance was measured at 517nm. BHT was used as a reference material.

All the tests were performed in triplicate and the graph was plotted with mean value. The percentage of inhibition was calculated of reference compound (control) and samples.

% of inhibition= {(A blank/A sample)/A blank} multiplied by 100 by comparing the absorbance values Where “A blank” is the absorbance of control reaction (containing all reagents except the test sample) and A sample is the absorbance of the extracts/standard.

HYDROXYL RADICLE SCAVENGING ACTIVITY

The scavenging capacity for hydroxyl radical was measured according to the modified methods of Halliwell et al. (1987). Stock solution of EDTA (1mM), fecl3 (10ml), ascorbic acid (10 mM), deoxyribose (10 mM), were prepared in distilled deionized water.

The assay was performed by adding 0.1 ml EDTA, 0.01ml of Fecl3, 0.1 ml h2o2, 0.36ml of deoxyribose, 1.0 ml of the extract of the different concentration (125,250,500& 100 µg/ml) dissolved in distilled water, 0.33 ml of phosphate buffer (50mM, pH 7,9), 0.1ml of ascorbic acid in sequence.

The mixture was then incubated at 37degree C for 1 hour. 1.0 ml portion of the incubated mixture was mixed with 1.0 ml of 10 % TCA and 1.0 ml of 0.5 % TBA (in 0.025 M NaOH containing 0.025 % BHA) to develop thepink chromogen measured at 532 nm. The hydroxyl radical scavenging activity of the extract is reported as % inhibition of deoxyribose degrading is calculated by using the following equation.

Hydroxyl radical scavenging activity = {(A0- A1)/A0) *100} where, A0 is the absorbance of the control reaction and A1 is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the result were averaged

SUPEROXIDE RADICLE SCAVENGING ACTIVITY

Super oxide anion scavenging activity was measured according to the method of Roback and Gryglewski (1988) with some modifications. All the solution was prepared in 100 mM phosphate buffer (pH 7.4) 1 ml of reduced Nicotinamide adenine dinucleotide (NADH, 468 µm) 3 ml of the plant extract of different concentration (125, 250, 500&100 g/ml) were mixed.

The reaction was initiated by adding 100 ml of phenazine methosulphate (PMS 60 um). The reaction mixture was incubated at 25-degree C for 5 min, followed by measurement of the absorbance at 560 nm. The percentage inhibition was calculated by using the following equation superoxide radicle scavenging activiy: {(A0- A1)/A0) ***100}**

Superoxide radicle scavenging activity:

Where, A0 is absorbance of the control reaction and A1 is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged.

ANTIOXIDANT ACTIVITY BY ABTS ASSAY

The radical scavenging activity of the extract was also analysed by the 2, 2- amino - bis (3- ethylbenzothiazoline-6-sulfonic acid) assay (TEAC) the total antioxidant activity of the samples was measured by ABTS radicle cation decolourization assay according to the method of Re et al (1999).

ABTS was produced by reacting 7mM ABTS aqueous solution with 2.4 mM potassium persulfate. This mixture was kept at ambient temperature for 12- 16 hours. Prior to assay, this solution was diluted in ethanol (about 1.89 v/v) and equilibrated at 30-degree C to given an absorbance at 734 nm of 0.700 +0.02 or – 0.02.

After the addition of 1 ml of diluted ABTS solution to 10 µl of sample or Trolox standard (final concentration 0-15µM) in ethanol, absorbance was measured at 30- degree C exactly 30 minutes after initial mixing.

Appropriate solvent blank was also run. Triplicate analyses were made at each dilution of the standard and the percentage inhibition was evaluated at 734nm. The percentage inhibition was plotted against Trolox concentration.

REDUCING POWER ASSAY

Reducing power was estimated according to the method of Oyaizu (1986). An aliquot of sample and standard solution was prepared. MeOH (250µ l) that was mixed with 250 ul of sodium phosphate buffer (0.25 M, pH6.6) and 250 µl of 1% K3Fe (CN)6 which was incubated at 50-degree C for 20 min.

After adding 250 µl of 10 % trichloroacetic acid, the mixture was centrifuged at 3750 rpm for10 min. the supernatant (100µl) was then taken out and immediately mixed with

100 µl of MeOH and 25µ l and 25µ l of 0.1 % ferric chloride. After 10 min incubation the absorbance against blank was taken at 700 nm. Ascorbic acid was taken as standard.[71]

3.5. LATERAL RESERCH

1.PURASU

Antihyperglycaemic activity

The antihyperglycaemic activity of the ethanolic extract of *Butea monosperma* was studied in glucose-loaded and alloxan-induced diabetic rats. Single dose treatment ofzzethanolic extract of *Butea monosperma* (200 mg/kg, p.o.) significantly improved glucose tolerance and caused reduction in blood glucose level in alloxan-induced diabetic rats.[72]

Antimicrobial activity

A new bioactive flavone glycoside was isolated from the methanol soluble fraction of the flowers of *B. monosperma* which was identified as 5,7-dihydroxy-3,6,4'-trimethoxyflavone-7-O-α -L-xylopyranosyl-(1→3)-O- **α** -Larabinopyranosyl (1→4)-O-β-D galacto pyranoside by several colour reactions, chemical degradations and spectral analysis. The compound showed antimicrobial activityagainst various fungal species. The in vitro antimicrobial efficiency of seed oil of *B. monosperma* was studied by the filter paper disk method against several human pathogenic bacteria and fungi. The oil showed a significant bactericidal and fungicidal effect.[72]

Antifungal activity

The petroleum and ethyl acetate extracts of the stem bark from *B. monosperma* displayed antifungal activity against *Cladosporium cladosporioides*. The active constituent of low polarity was isolated by bioassay-monitored chromatographic fractionation, and identified as (-)-medicarpin by comparison of physical data. The antifungal activity of (-)-medicarpin was found to be greater than that of Benlate, a standard fungicide, while (-)-medicarpin acetate also exhibited significant activity against *C. Cladosporiodes.*[72]

Chemopreventive activity

The chemopreventive effects of *B. monosperma* extract on hepatic carcinogenesis and on tumor promoter induced markers and oxidative stress in male wistar rats. Treatment

of male wistar rats for five consecutive days with 2-AAF i.p. induced significant hepatic toxicity, oxidative stress and hyperproliferation. Pretreatment of *B.monosperma* extract (100 and 200 mg/kg body weight) prevented oxidative stress by restoring the levels of antioxidant enzymes and also prevented toxicity at both doses.[72]

Anthelmintic activity

The seeds of *B. monosperma* administered as crude powder at doses of 1, 2 and 3 g/kg to sheep naturally infected with mixed species of gastrointestinal nematodes exhibited a dose and a time-dependent anthelmintic effect. The maximum reduction of 78.4% in eggs per gram of feces (EPG) was recorded on day 10 after treatment with 3 g/kg. Levamisole (7.5 mg/kg), a standard anthelmintic agent, exhibited 99.1% reduction in EPG (Iqbal *et. al*., 2006). The methanol extract of *B. monosperma* seeds, tested in vitro, showed significant anthelmintic activity.[72]

Antidiarrhoeal activity

The antidiarrhoeal potential of the ethanolic extract of stem bark of *B. monosperma* has been evaluated using several experimental models in wistar albino rats. The extract inhibited castor oil induced diarrhoea and PGE2 induced enteropooling in rats; it also reduced gastrointestinal motility after charcoal meal administration. The results obtained establish the efficacy and substantiate the use of this herbal remedy as a non-specific treatment for diarrhoea in folk medicine.[72]

Antiviral activity

A potential antiviral flavone glycoside has been isolated from the seeds of *B. monosperma* and its structure determined as 5,2' -dihydroxy- 3,6,7-trimethoxyflavone-5-O-β-Dxylopyranosyl-(1→4) O-β-D-glucopyranoside by various spectral analysis and chemical degradations.[72]

Anticonvulsant activity

The bioassay-guided fractionation of dried flowers of *B. monosperma* was carried out to isolate the active principle responsible for its anticonvulsant activity. Further studies are required to investigate its usefulness in the treatment of epilepsy.[72]

Anti-giardial and immuno-stimulatory activity

Pippali rasayana, an Ayurvedic herbal medicine, prepared from *Piper longum* and *B. monosperma*, and prescribed for the treatment of chronic dysentery and worm infestations was tested for antigiardial and immunostimulatory activity in mice, infected with *Giardia lamblia* trophozoites. It produced up to 98% recovery from the infection. The rasayana had no killing effect on the parasite in vitro. It induced significant activation of macrophages as evidenced by increased macrophage migration index (MMI) and phagocytic activity. Enhancement of host resistance could be one of the possible mechanisms contributing towards the recovery of animals from the giardial infection.[72]

Anti-implantation activity

Butin isolated from the seeds of *B. monosperma* and administered orally to adult female rats at the doses of 5, 10 and 20 mg/kg from day 1 to day 5 of pregnancy showed antiimplantation activity in 40%, 70% and 90% of the treated animals, respectively. At lower doses, there was a dose-dependent termination of pregnancy and reduction in the number of implantation sites. In ovariectomized young female rats, the butin exhibited estrogenic activity at comparable anti- conceptive doses, but was devoid of antiestrogenic activity. Butin is a weak estrogen in that a significant uterotrophic effect was discerned even at 1/20th the anticonceptive dose.[72]

Antihepatotoxic activity

An extract from the flowers of *B. monosperma*, a plant drug used in India for the treatment of liver disorders, showed significant activity in different models of liver damage, the extract was fractionated by solvent partitioning and HPLC. The antihepatotoxic principles isolated consisted of two known flavonoids, isobutrin (3, 4, 2', 4'-tetrahydroxychalcone-3, 4' diglucoside), and the less active butrin (7, 3', 4'-trihydroxyflavanone-7, 3'-diglucoside). For qualitative and quantitative analysis of isobutrin and butrin in extracts of *B. monosperma* flowers a HPLC system was developed.[72]

2.MILAGU

Anti-bacterial activity

Piper nigrum leaf and stem assisted green synthesis of silver nano-particles and evaluated its antibacterial activity against agricultural plant pathogens and observe that these silver nano-particles showed the excellent antibacterial activity against plant pathogens. experimented photochemical analysis and antibacterial activity of *Piper nigrum* against human pathogenic bacteria and noted that presence of alkaloids, tannins, flavonoids, cardiac and cardiac glycosides shows antibacterial properties against the *Staphylococcus aureus, Salmonella typhi, Escherichia coli* and *Proteus sp.*[73]

Antioxidant activity of black pepper

Piper species significantly inhibited the atherogenic diet induced increased lipid profile and alteration in antioxidant enzymes activities. regenerated tissue of *Piper nigrum* like callus, *in vitro* shoots, roots, in vitro plantlets, possesses antioxidants activity which is probably due to the presence of flavonoids and phenolic contents. Piper species significantly inhibited the atherogenic diet induced increased lipid profile and alteration in antioxidant enzymes activities.[73]

Anti-cancer activity of black pepper

Piper nigrum reduced lung cancer by modulating lipid peroxidation and through the activation anti oxidative protection enzyme.[73]

Digestive activity of black pepper

Administration of piperine or *P. nigrum* stimulate the liver to the secrete bile acids which in turn play key role in the absorption and digestion of fats.[73]

Antidepressant activity of black pepper

The brainderived neurotrophic factor protein and mRNA levels in the hippocampus were also significantly decreased in corticosterone-treated mice. corticosterone induced the behavioral and biochemical changes after treatment to animals with piperine. These results showed that piperine produces an antidepressant-like effect.[73]

Insecticidal properties

Piperine against insecticide resistant and susceptible strains of *Anopheles* malaria vector mosquitoes and observed that Black pepper and piperine mixtures caused high

mortality in the An. Gambiae complex strains, with black pepper proving significantly more toxic than piperine. It is concluded that black pepper shows potential as a larvicide for the control of certain malaria vector species.[73]

Antiplatelet activity

Toxic effect of piperine on aggression of platelet in experimental rabbit induced by different factors which activate platelet, by collagen and thrombin.[73]

Molluscicidal activity

The effect of piperine on the absorptive function of the intestine. *In vitro experiments* showed an increased rate of lipid peroxidation in the freshly isolated epithelial cells of rat jejunum. These results suggested that piperine may interact with the lipid environment to produce effect which leads to increased permeability of the intestinal cells.[73]

3.EUPHORBIA ANTIQUORUM

Antihyperglycemic and *in silico* aldose reductase inhibitory activity

The ethanol extract exhibited a drastic blood sugar level reduction after 1 hour of post treatment. This effect was significant compared to standard drug Glibenclamide. The petroleum ether extract and aqueous extract also showed a decrease in blood glucose but not in a significant manner. Whereas ethanol extract has showed significant antihyperglycemic activity on 8th hour of post treatment.[74]

Cancer

Latex of EA has inhibitory effects on several different cancer cell lines. EA induced apoptosis, which was characterized by morphological change, DNA fragmentation, increased sub-G1 population, and alterations in levels of apoptosis associated proteins.[74]

Cytotoxic activity

The latex can be effectively used along with chemotherapeutic drugs for cancer treatment, because the latex by itself is not cytotoxic and when given along with the standard chemotherapeutic drug etoposide, it was able to counteract the toxicity of the drug in normal chick embryo fibroblasts. The results also suggest its use in combination therapy along with chemotherapy to fight cancer.[74]

Hepatoprotective and antioxidant activity

Aqueous extract of the aerial parts of EA has been reported to have hepatoprotective and antioxidant activity. EA extract exerted significant antioxidant activity (at 20 μg, 40 μg, 60 μg, 80 μg and 100 μg/ml *in-vitro*) as evidenced by its reducing power, hydroxyl and superoxide anion radical scavenging activities. The *invitro* efficacy was reinforced by a significant dose dependent hepato-protection (at 125 mg/kg and 250 mg/kg dose) by decreasing the activity of serum enzymes, bilirubin, cholesterol, triglycerides and lipid peroxidation while it significantly increased the reduced Glutathione levels of tissue in a dose dependant manner. The hepatoprotective and antioxidant activities of the extract have been found to be comparable to standards Silymarin and Sodium metabisulphite respectively. The hepatoprotective property was attributed to the antioxidant potential and the antioxidant principles of the plant which justifies the claim of the native practitioner that the decoction of the plant is useful in treating jaundice.[74]

Insecticidal activity

The latex of EA contains insecticidal activity. It was tested against common and widely spread pest species of vegetables and rice and also against some of their natural enemies.

These insects or pests are considered to be more resistant to synthetic insecticides. Results have revealed that the latex of EA is an effective insecticidal agent because both crude extract as well as partially purified extract caused more than 50% mortality of tested aphid populations. The observed LC_{50} of *M. persicae* with EA latex extract (0.012%) was, however,greater than that with *Azadirachta* seed oil (0.0024%). EA latex extract was not effective against insects with thick cuticle covers. Although soft-bodied natural enemies like spiders are susceptible to the latex of EA, most common and important natural enemies like ladybird beetles are highly resistant.[74]

Anti-inflammatory and anti-arthritic activities

The anti-inflammatory and anti-arthritic potential has been associated with aqueous and alcoholic extracts of EA. It showed the presence of triterpenoids in the stem and diterpenoids in the latex. It was reported that the anti-inflammatory activity is a common

property of many triterpenoids. The anti-inflammatory effects of triterpenes have been attributed to various mechanisms including inhibition of lipoxygenase and cyclooxygenase activities. Triterpenoids present in the extracts of EA might be responsible for anti-inflammatory and anti-arthritic effects.[74]

4. CAESALPINA CRISTA

Antidiabetic / Hypoglycemic

Most of the plant from Caesalpinia species shows Antidiabetic and Hypoglycemic activity. The ethanolic extract (250mg/kg/day) lowered blood glucose level within 2 weeks in the alloxan diabetic albino rats confirming its hypoglycemic activity. β - sistosterol isolated from the stem bark was found to posses potent hypoglycemic activity when compared to other isolated compounds.

(1) The seed kernel of Caesalpinia bonducella has significant antidiabetic and hypoglycemic effects. Activity may be partly due to a positive effect on glycogen synthesis in the liver, skeletal muscle and heart muscle due to an insulin-like action of its constituents and partly due to stimulatory action on insulin release.

(2) The ethanolic and aqueous extracts showed significant blood sugar lowering effect of C.bonducella.

(3) The aqueous extract of C. bonducella seed shell showed very significant blood sugar lowering in glucose loaded STZ and alloxan diabetic models.[75]

Anthelmintic activity

Anthelmintic activity of Caesalpinia crista (L.) against trichostrongylid nematodes of sheep,study showed C. crista possess anthelmintic activity in vitro and in vivo, supporting its traditional use in Pakistan.[75]

Antimalarial activity

Most of the plant from Caesalpinia species shows antimalarial activity. The isolated diterpenes such as 44 cassane- and norcassanetype diterpenes. Most of the tested diterpenes showed antimalarial activity, norcaesalpinin E showed the most potent activity, more than the drug chloroquine.[75]

Antioxidant activity

Study showed the methanolic extract of Caesalpinia crista has potent antioxidant activity and ROS scavenging activity as well as iron chelating property. Ethyl acetate extract showed a maximum of 49% free radical scavenging activity at the end of 1 hr.[75]

Antifilarial

The Caesalpinia bonducella seed kernel extract and fractions showed microfilaricidal, macrofilaricidal and female-sterilizing efficacy against L. sigmodontin and microfilaricidal and female-sterilizing efficacy against B. malayi in animal models, suggesting a potential for its use in new antifilarial drug development.[75]

Anxiolytic Activity

The seed extract of C. bonducella showed a significant and dose dependant anxiolytic activity.[75]

Antitumor / Antioxidant Activity

Study of methanol extract of Caesalpinia bonducella showed significant antitumor and antioxidant activity in Erllich ascites carcinoma (EAC)-bearing mice.[75]

Analgesic Activity:

The flower extract of Caesalpinia bonducella showed significant antinociceptive effect in the inflammatory phase of formalin-induced pain and acetic- induced parietal pain.[75]

Analgesic / Antipyretic / Anti-Inflammatory

The seed oil of Caesalpinia bonducella could be a potential source of an anti-inflammatory, antipyretic and analgesic agent.[75]

Immunomodulatory

The aqueous extract of Caesalpinia bonducella seeds on cell mediated and humoral components of the immune system in rats produced an increase in hemagglutinating antibody titer and a change in delayed-type hypersensitivity suggesting that the extract could be a promising immunostimulatory agent.[75]

Anti-Amyloidogenic / Alzheimer's disease

Caesalpinia crista leaf aqueous extract has anti-amyloidogenic potential. Study showed aqueous extract of C. crista could inhibit the Abeta aggregration. From monomers and oligomers and able to disintegrate the preformed fibrils.[75]

Nootropic / Memory Enhancer

Dried seed kernels of Caesalpinia crista extract have a potential as a learning and memory enhancer. Results suggest C. crista can be beneficial in improving cognition in disorders like demential and other neurodegenerative disorders.[75]

5.FERULA ASAFOETIDA

Relaxant effect

F. asafoetida or its constituents may bind to muscarinic receptor of tracheal smooth muscle and put off the binding of methacholine to this receptor, it suggested the competitive antagonistic effect of F. asafoetida at muscarinic receptors.[76]

Neuroprotective effect

In vitro experiments showed that asafoetida is a nerve stimulant and its management in neuropathic mice exerted neuroprotecting effects through stimulating axonal regeneration and remyelination and decrement of lymphocyte infiltration[76].

Anti-cytotoxicity activity, anti-obesity and fat lowering effect

To evaluate general cytotoxicity, the brine shrimp (Artemia salina) was employed as a model assay system, it provided a suitable in-house pre-screening method. The methanol extracts of Ferula species and the oleo-gum resin of F. asafoetida exhibited cytotoxic effect with LC50 values in the range of 6e321 mg/mL and showed a dose-dependent cytotoxicity. The effect of F. asafoetida on weight gain, fat accumulation, liver steatosis and leptin level in type 2 diabetic rats. Two treatment groups received F. asafoetida oleo-gum resin at doses of 25 or 50 mg/kg. Administration of F. asafoetida extensively decreased body weight, abnormal fat and size of epididymal adipocyte compared to untreated rats. Serum leptin levels were considerably decreased in treated rats. The results revealed that F. asafoetida gum has potent anti-obesity activities, fat lowering

and can prevent liver steatosis. F. asafoetida gum can be a good candidate for the treatment of diabetes-induced obesity and hepatosteatosis.[76]

Spermatic, testicular histopathology and antagonistic effect

Evaluated the effectiveness of asafoetida on spermatic parameters, blood testosterone levels and testis tissue. The asafoetida significantly increased the number and viability of sperms. Asafoetida has shown a positive effect on spermatic parameters even though the histopathological effects on the testis were observed, particularly at high doses.[76]

Toxic effect

A case of methemoglobinemia has been registered after intake of asafoetida in a 5 week old black male infant. Hewas recovered by the treatment of intravenous methylene blue from onset of tachypnea, grunting and cyanosis. Large dose intake of asafoetida can lead to swelling of the mouth, digestive illness such as flatulence and diarrhea, anxiety and headache. The intake of asafoetida is prohibited during the pregnancy.[76]

6. **ACACIA CATECHU**

Anti-obesity activity

The bark of *Acacia catechu* Willd family Fabaceae, maintains healthy fat metabolism and reduces the conversion of carbohydrates to fats. In studies of rats fed on a diet containing cholesteryl oleate, betel nut extracts significantly lowered cholesterol and triglycerides.[77]

Hypoglycaemic Activity

In eastern traditional medicine *Acacia catechu* Willd is extensively used in management of diabetes in combinations with other medicinal plants. Polar as well as non-polar components of *Acacia catechu* Willd shown hypoglycaemic activity. Hypoglycemic activity of extract of *Khadira* (*Acacia catechu* Willd) is assumed to be due to the presence of flavonoids which also show inhibition of cyclooxygenase and regenerate β cells. In an experiment, ethyl acetate extract of *Acacia catechu* Willd at a concentration of 500mg/kg/day used for 7 days, significantly decreases blood glucose level of normal as

well as alloxan induced diabetic albino rats but it was not effective as that of standard drug. Studies show that myricetin, quercetin and catechin-gallate inhibit insulin stimulated glucose transporters in cells.[77]

Antibacterial Activity

Khadira (*Acacia catechu* Willd) heartwood extract is found to be an effective antibacterial agent. A study conducted in ethanolic and aqueous heartwood extract of *Khadira,* proved its efficacy as a potent anti-bacterial agent. Taxifolin present in heartwood of *Khadira* is found to be responsible for its antibacterial effect. In vitro, *Acacia catechu* Willd is reported to have broad spectrum antimicrobial and antifungal activity. Phytochemical studies of *Khadira* leaves shows the presence of alkaloids, carbohydrates, flavones, glycosides, phenolic compounds, saponins, steroids and tannins which may be responsible for its antimicrobial activity. Its Methanolic extract having antimicrobial activity against pathogenic as well as non-pathogenic bacteria e.g. Bacillus subtilis, Staphylococcus aureus, Salmonella typhi, Escherichia coli, Pseudomonas aeruginosa and Candida albicans. It is effective against gram positive as well as gram negative bacteria.[77]

Antifungal Activity

Ethanolic extract of Heartwood of *Acacia catechu* Willd was tested for antifungal (antimycotic) activity against Candida albicans, Aspergillus Niger, Aspergillus fumigates, Mucor spp and Penicilium marneffei. Disc diffusion technique was followed for screening antifungal activity. The discs were loaded with 50µl of ethanolic extracts at different concentrations [25ug/disc, 250ug/disc and 500ug/disc]. Positive controls used were fluconazole (10 mcg/disc) and amphotericin B (100 units/disc). After incubation at 28oC for 48 hours, the zone of inhibition was measured. The extract at different concentrations showed varying degree of antifungal activity against the micro organisms tested compared to standard. Assay was conducted to check antifungal activity of the aqueous and methanol extract of *Acacia catechu* Willd against fourteen human pathogenic fungi using agar disc diffusion method. The methanol extract of *Acacia catechu* Willd was established most promising, and found active against Candida, Dermatophytes and Aspergillus species therefore stressing the need to locate the active principle.[77]

Anti-microbial activity

In vitro *Khadira* (*Acacia catechu* Willd) is reported to have broad spectrum anti-microbial and antifungal activity. Phytochemical studies of *Acacia catechu* Willd leaves shows the presence of alkaloids, carbohydrates,flavones, glycosides, phenolic compounds, saponins,steroids and tannins which may be responsible for its anti-microbial activity.Its Methanolic extract of has Anti-microbial activity against pathogenic as well as nonpathogenic bacteria e.g Bacillus subtilis, Staphylococcus aureus, Salmonella typhi, Escherichia coli, Pseudomonas aeruginosa and Candida albicans. It is effective against gram positive as well as gram negative bacteria. It was found to be most effective against Staphylococcus aureus with about 20mm zone of inhibition at minimum bactericidal concentration (MBC) of the crude extract 1,000 l g/ml.Experiments shows that anti-microbial activity of *Khadira* (*Acacia catechu* Willd) depends on nature of solvent used for extraction, thus organic solvents used in extraction of leaves are most effective than any other.[77]

Anti-oxidant Activity

As the *Khadira* (*Acacia catechu* Willd) contain many potent flavonoids such as catechin present in this plant plays a vital role as anti-oxidant. Catechins and rutin are most important constituents which are free radical scavengers. Antioxidant principles of *Acacia catechu* Willd were analysed by Dot-blot assay & quantitative analysis by DPPH radical scavenging assay with ascorbic acid as standard.[77]

Anti-cancer Activity

The aqueous extract of *Acacia catechu* has effective on epithelial and stromal cell carcinoma in cell line studies.[77]

Antidiarrhoeal Activity

Ethyl acetate extract of *Khadira* (*Acacia catechu* Willd) has been found to posses highly significant antipyretic activity in respect of latent period of onset of diarrhoea, average number of stool passed and purging index.[77]

Hepato protective activity

Cyanidanol, an active principle of *Acacia catechu* Willd, was claimed to be effective in treating liver diseases.[77]

Anti-pyretic and Anti-inflammatory properties

The chief major active chemical components of *Khadira* (*Acacia catechu* Willd) are flavonoids which inhibit Cyclooxygenase and 5-Lipoxygenase and hence decrease inflammation. Mixed extract of Scutellaria baicalensis and *Acacia catechu* inhibit Prostaglandin E2 generation in human osteosarcoma cells which express COX-2, and leukotriene production is also inhibited in human cell lines, immortalized THP-1 monocyte and HT-29 colorectal adenocarcinoma. Baicalin from Scutellaria baicalensis and catechin from *Acacia catechu Willd* are responsible for dual inhibition of Cyclooxygenase and 5-Lipoxygenase.[44] Baicalin and catechin are found to in hibit COX1, COX2 and 5-LOX. Baicalin also downregulates the expression of cytokines and PGE2, nitric oxide formation, and neutrophil invasion in a carrageenan-induced paw edema model.[77]

Sore throat

Khadira (*Acacia catechu* Willd) is one of most important ingredients used in Paan which is also called as beetle leaf. People of different ages use it for healing of sore throat, because of its astringent and soothing effect.Tannins present in *Acacia catechu* Willd are responsible for this property. This is very common in Asia especially in central Asian countries like Pakistan and India and most common home remedy used for sore throat.[77]

Anti-secretory and Anti-ulcer Activity

Study was conducted on antisecretory and antiulcer activity of *Khadira* (*Acacia catechu* Willd) against indomethacin plus pyloric ligation induced gastric ulcers in rats. The results of the study suggested that *Khadira(Acacia catechu* Willd) causes an inhibitory effect on release of gastric hydrochloric acids and protects gastric mucosal damage due to presence of flavanoids and tannins in the plant extract.

Immunomodulatory Activity

Immuomodulatory activity of aqueous extract of *Acacia catechu* Willd after oral administration (5 mg/kg and 50 mg/kg). The effect was studied in neutrophil adhesion test, mice lethality test, carbon clearance assay, cyclophosphamide induced neutropenia, serum immunoglobulin levels and the heamagglutination test. *Acacia catechu* Willd extract showed an increase in the neutrophil adhesion to the nylon fibres produced a significant increase in the phagocytic index and a significant protection against cyclophosphamide induced neutropenia indicating its effect on cell mediated immunity.[77]

Wound healing

In Asia crushed bark of *Acacia catechu* Willd is used topical on wounds as it is potent wound healing medicinal plant. It has astringent effect and also cause precipitation of skin which makes it very good wound healing plant. Furthermore, it also exhibits antimicrobial property which prevents growth of microbes on wounds. This activity is due to presence of tannins and flavonoids in bark of *Acacia catechu* willd.[77]

4. MATERIALS AND METHODS

4.1. PREPARATION OF THE DRUG:

The trial drug Indhirani Maathirai was prepared as per classical Siddha literature sarabendra Siddha maruthuva sudar 4th edition written by Doctor M.Sowrirajan page number 336 published by the Saraswati Mahal library, Tanjore.

INGREDIENTS:

purasan ver (root of beautea monosperma) - 17.5 gram

Milagu (Piper nigrum) - 17.5 gram

Kalli ver (root of Euphorbia antiquorum) - 17.5 gram

Kalarchi ver (root of Caesalpinia bonduc) - 17.5 gram

Kadugu (Brassica juncea) - 17.5 Gram

Kambiccharam (potassium nitrate) - 17.5 gram

Perungayam (ferula asafoetida) - 17.5 gram

Karungali kudineer (Acacia catechu stem decoction) - Sufficient amount

COLLECTION OF THE DRUG :

The Pepper(Piper nigrum), Kadugu (Brassica juncea), Asafoeitida (Ferula asafoetida), Kambicharam (Potassium nitrate), Karungali Kattai (Acacia catachu stem) were bought from the Ramaswamy Chetty raw drug stores at Parrys Corner. Kalli ver (the root of euphorbia antiquorum), Poovarasan ver (the root of beautea monosperma) were collected from the village mukundaram in Gudiyatham. Kalarchi ver (the root of caesalpinia bonduc) was collected from the village Lakshmana Puram in Gudiyattam.

IDENTIFICATION AND AUTHENTICATION:

All drugs were identified and authenticated by botanist and experts of gunapadam department (pharmacology) in government Siddha Medical College, Chennai. The

identified product samples were preserved in the PG gunapadam laboratory for future references.

METHOD OF PURIFICATION:

Milagu,Kadugu,Perungayam are mildly roasted in a pan separately and allowed to cool.[78] Kalli ver, Kalarchi ver, Purasan ver were washed through water completely and allowed to dry.[79] Kambicharam is the Vediyuppu which was distilled 6 times.[80] The Vediyuppu was taken in a pot and dissolved with sufficient amount of water. The salt solution was filtered and placed in a stove and allowed to boil until the kuzhambu padham was attend. The pot was taken away from the stove and allowed to cool. After sometime the potassium nitrate crystals were formed the process was repeated 6 times. Finally got the potassium nitrate crystals formed like wire/rods.

METHODS OF PREPARATION:

All the above ingredients are crushed and powder by Iron mortar and sieved by th a cloth separately. Then the fine powder was collected. The wood of Karungali (Acacia catechu) was crushed in Iron mortar. The crushed pieces are collectod and add sufficient amount of water in a pan and subjected to boil and then filter. The karungali Kudi Neer was obtained. The fine powder was titrated (ground) with karungali kudineer by stone mortar up to attein the mezhugu padham. Then the karkam was collected and rolled into Payiralavu Maathirai and allowed to dry.

PRESERVATION:

Fully dried pills / tablets was preserved in a clean air tight glass container.

ADMINISTRATION OF THE DRUG :

Form of the medicine -Maathirai

Route of Administration -Enteral

Dose -Two tablets

Time of Administration - Two times a day

Adjuvant -Water

Indication -Adhi Thoola rogam

4.2. STANDARDIZATION OF INDHIRANI MAATHIRAI BY USING MODERN TECHNIQUES:

Standardization of drug helps to authenticate and determine its quality and efficiency. Standardization of herbal drug is rooted in qualitative and quantitative analysis by means of Physico chemical properties and instrumental analysis. The Physico-chemical analysis of Indhirani Maathirai was done. The chemical finger print was engaged by using modern analytical technique FTIR (Fourier Transform Infra-Red Spectroscopy). The particle size and qualitative analysis of chemical elements of Indhirani Maathirai were also assessed by Scanning Electron Microscope (SEM). The quantitative and qualitative analysis of chemical elements was carried out by using inductively X-Ray Diffraction Study (XRD).

4.2.1. ORGANOLEPTIC EVALUATION:

The organoleptic characters of the sample drug were evaluated. 1gm of the IM was taken and the colour, texture, particle size and other morphological characters were viewed by naked eye under natural light.[81]

4.2.2. PHYSIO-CHEMICAL INVESTIGATION:

Physio-chemical studies like total ash, water insoluble ash, acid Insoluble ash, loss on drying at 105°C and pH were done at, The Tamil Nadu Dr. M. G. R. Medical University, Guindy, Chennai.

1.pH value:

Potentiometrically pH value determined by a glass electrode and a suitable ph meter. The p^H of the IM was written in results column.

2.Loss on Drying:

An accurately weighed 2g of Indhirani maathirai formulation was taken in a tarred glass bottle. The crude drug was heated at 105^0C for 6 hours in an oven till a constant weight. The percentage moisture content of the sample was calculated with reference to the

shade dried material

3.Determination of Alcohol Soluble Extractive:

2.5 gm of air dried drugs, coarsely powdered was macerated with 50ml alcohol in closed flask for 24 hrs. with frequent shaking, it was filtered rapidly taking precaution against loss of alcohol. 10ml of filtrate was then evaporated in a tarred flat bottom shallow dish, dried at 100^0C and weighted. The percentage of alcohol soluble extractive was calculated with reference to dried drug.

4.Solubility:

A pinch of sample (IM) was taken in a dry test tube and to it 2ml of the solvent was added and shaken well for about a minute and the results are observed. The test was done for solvents like distilled water, ethanol, chloroform and results were observed individually.[82]

4.2.3. PRELIMINARY PHYTOCHEMICAL SCRENNING OF *INDHIRANI MAATHIRAI:*

The preliminary phytochemical screening test was carried out for the extract of Indhirani maathirai as per standard procedure.

1. DETECTION ALKALOIDS:

1. **Mayer's Test**: filtrat were treated with Mayer's reagent (potassium, mercuric iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.
2. **Wagnor's Test:** filtrates were treated with Wagner's reagent (iodine in potassium iodide) formation of brown or reddish precipitate indicates the presence of the alkaloids.
3. **Dragendroff's' Test**: filtrates were treated with Dragendroff's reagent (solution of potassium with bismuth iodide). Formation of red precipitate indicates the presence of alkaloids.
4. **Hager's Test**: filtrates were treated with Hager's reagent (saturated picric acid solution) presence of alkaloids conformed by the formation of yellow precipitate.

2. DETECTION OF CARBOHYDRATES:

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test the presence of carbohydrates.

1) **Molisch's Test:** To 2ml of a plant sample extract, two drops of alcoholic solution of alpha naphthol are added. The mixture is shaken well few drops of concentrated sulphuric acid slowly along the sides of the test tube. A violent ring indicates the presence of carbohydrates.
2) **Benedict's Test**: filtrates were treated with benedict's reagent and heated gently orange red precipitate indicates the presence of reducing agents.

3. DETECTION OF GLYCOSIDES:

Extracts were hydrolysed with dilute HCl and then subjected to the test of glycosides.

1) Modified Bontrager's Test: Extracts were treated with ferric chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose pink colour in ammonial layer indicates the presence of anthranol glycosides.

2) **Cardiac Glycoside (Keller-Killiani Test):**Extracts was shaken with distilled water (5ml). to this, glacial acetic acid (2ml) containing few drops of ferric chloride was added followed by sulphuric acid (1ml) along the side of the test tube. The formation of the brown ring at the interface gives positive indication for cardiac glycoside and a violet ring may appear below the brown ring.

4. DETECTION OF SAPONINS:

1**.Froth Test**: Extracts were diluted with distilled water to 20 ml and this was shaken graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

2**.Foam Tests**: 0. Gm of the extract was shaken with 2 ml of water if foam produced persists for ten minutes. It indicates the presences of saponins.

4. DETECTION OF PHYTOSTEROLS:

Salkowski's Test: extracts were treated with chloroform and filtered. The filtrates were treated with few drops of conc. sulphuric acid shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

6. DETECTION OF PHENOL FERRIC CHLORIDE TEST:

Extracts were extracted with 3- 4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of the phenols.

7. DETECTION OF TANNINS GELATIN TEST:

The extract is dissolved in 5ml distilled water and 2ml of 1% solution of Gelatin containing 10% NaCl is added to it. White precipitate indicates the presence of phenolic compounds.

8. DETECTION OF FLAVONOIDS:

1.Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

2.Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

9. DETECTION OF PROTEINS AND AMINOACIDS:

1.Xanthoproteic Test: The extracts were treated with few drops of concentrated Nitric acid. Formation of yellow colour indicates the presence of proteins.

2.Ninhydrin Test: To the extract ,0.25% w/v Ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

10. DETECTION OF DITERPENES COPPER ACETATE TEST:

Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

11. GUM AND MUCILAGE:

To 1ml of extract add 2.5 ml of absolute alcohol and stirring constantly. Then the precipitate was dried in air and examine for its swelling properties. Swelling was observed that will indicate presence of gum and mucilage.

12. Test for Fixed Oils and Fats:

Spot Test: A small quantity of extract is pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oils.

13.TEST FOR QUINONES:

Extract was treated with sodium hydroxide blue or red precipitate indicates the presence of Quinones.[83]

4.2.4. High Performance Liquid Chromatography (HPLC)

HPLC pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material. Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out of the column. In this study, the detection and quantitation were carried out using 515 HPLC pumps and 2489 UV/Visible detectors of Waters Company while the software used was Empower.

Two methods using different mobile phases were used for chromatographic separation of the research drugs – Method I (binary gradient method of Acetonitrile & 0.1% Phosphoric acid in Water) and Method II (binary gradient method of Methanol & 1:25 Acetic acid in Water). Results obtained during Method I have been discussed since better separation of compounds was observed during this analysis.

The chromatographic conditions for Method I are as given below:

Column : Symmetry C18, 5 µm, 4.6x250 mm Run Time : 30 minutes

Injection Volume : 20 µl

Wavelength(Dual) : 272nm&360nm

Solvent A : Acetonitrile

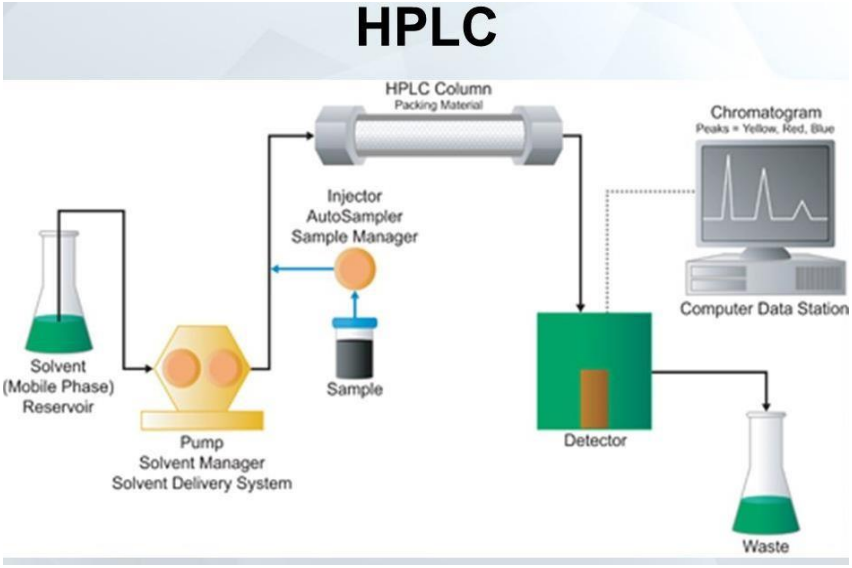
Solvent B : 0.1% Phosphoric acid in water

Flow rate : 1.0 ml/min.

Pump Mode : Gradient Processing Method :

Time (min)	%A	%B
0	15	85
12	25	75
20	25	75
22	15	85
30	15	85

Fig No:4.1 HPLC INSTRUMENT



4.2.5. BIO-CHEMICAL ANALYSIS

The bio-chemical analysis was done to identify the acid and basic radical present in the trial drug *Indhirani maathirai.*

Preparation of extract

5g of *Indhirani maathirai* was taken in a 250 ml clean beaker and 50 ml of distilled water was added, boiled well and allowed to cool and filtered in a 100 ml volumetric flask and made up to 100 ml with distilled water.

Preliminary basic and acidic radical studie

Test for basic radicals

1. **Test for Potassium**

To a pinch of the *Indhirani maathirai* 2 ml of Sodium nitrate and 2 ml of Cobalt nitrate solution in 30% glacial acetic acid was added and observed for the presence of yellow precipitate Test for Calcium

To 2 ml of Indhirani maathirai extract, 2 ml of 4% Ammonium oxide solution was added and observed for the formation of white precipitate**.**

2. **Test for Magnesium**

To 2ml of Indhirani maathirai extract, drops of Sodium hydroxide solution was added and watched for the appearance of white precipitate

3. **Test for Ammonium**

To 2ml of Indhirani maathirai extract few ml of Nessler's reagent and excess of Sodium hydroxide solution are added for the appearance of brown colour

4. **Test for Sodium**

Hydrochloric acid was added with a pinch of the Indhirani maathirai, made as paste and introduced into the blue flame of Bunsen burner and observed for the appearance of intense yellow colour.

5. **Test for Iron (Ferrous)**

The Indhirani maathirai extract was treated with Conc. HNO_3 and Ammonium thiocyanate and waited for the appearance of blood red colour**.**

6. **Test for Zinc**

To 2 ml of the Indhirani maathirai extract drops of Sodium hydroxide solution was added and observed for white precipitate formation.

7. **Test for Aluminium**

To the 2m1 of the Indhirani maathirai extract sodium hydroxide was added in drops and changes are noted.

8. Test for Lead

To 2 ml of Indhirani maathirai extract 2ml of Potassium iodide solution was added and noted for yellow coloured precipitate**.**

9. Test for Copper

a) A pinch of Indhirani maathirai was made into a paste with con. HCl in a watch glass and introduced into the non-luminous part of the flame and noted for blue colour appearance.

b) To 2 ml of Indhirani maathirai extract excess of Ammonia solution was added and observed for the appearance of blue coloured precipitate**.**

11.Test for Mercury

To 2m1 of the Indhirani maathirai extract sodium hydroxide solution was added and noted for yellow precipitate formation**.**

13.Test for Arsenic

To 2 ml of the Indhirani maathirai extract 2ml of Sodium hydroxide solution was added and observed for brown or red precipitate and noted.

TEST FOR ACID RADICALS

Test for Sulphate

To 2 ml of the Indhirani maathirai extract 5% of Barium chloride solution was added and observed for the appearance of white precipitate.

Test for Chloride

The *Indhirani maathirai* extract was treated with Silver nitrate solution and observed for the appearance of white precipitate.

Test for Phosphate

The Indhirani maathrai extract was treated with Ammonium molybdate and conc. HNO_3 and observed for the appearance of yellow precipitate.

Test for Carbonate

The Indhirani maathirai extract was treated with conc. HCl and observed for the appearance of effervescence

Test for Fluoride & Oxalate

To 2ml of Indhirani maathirai extract 2ml of dil. acetic acid and 2ml calcium chloride solution was added and heated and watched for cloudy appearance.

Test for Nitrate

To 1 gm of the Indhirani maathirai Copper turnings was added and again conc.H_2SO_4 was added, heated and the test tube was tilted vertically down and observed for any changes.[85]

4.2.6. Microbial load Availability of bacterial load

Enumeration of bacteria by plate count – agar plating technique. The plate count technique is one of the most routinely used procedures because of the enumeration of viable cells by this method.

Principle

This method was based on the principle that when a material containing bacterium was cultured, every viable bacterium develops into a visible colony on a nutrient agar medium. The numbers of colonies were the same as the number of organisms contained in the Indhirani maathirai.

Dilution

A small measured volume of Indhirani maathirai was mixed with a large volume of sterile water called the diluent or dilution blank. Dilution is usually made in multiples of ten. A single dilution is calculated as follows:

$$\text{Dilution} = \frac{\text{Volume of the sample}}{\text{Total volume of the sample and the diluents}}$$

Requirements

Sample or Bacterial suspension

9 ml dilution blanks (7)

Sterile petri dishes (12)

Sterile 1 ml pipettes (7)

Nutrient agar medium (200 ml)

Colony counter

Procedure

- Label the dilution blanks as 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}.
- Prepare the initial dilution by adding 1 ml of the Indhirani maathirai extract into a 9 ml dilution blank labelled 10^{-1} thus diluting the sample 10 times.
- Mix the contents by rolling the tube back and forth between hands to obtain uniform distribution of organisms.
- From the first dilution transfer 1 ml of the suspension while in motion, to the dilution blank 10^{-2} with a sterile and fresh 1 ml pipette diluting the original specimen to 100 times.
- From the 10^{-2} suspension, transfer 1 ml of suspension to 10^{-3} dilution blank with a fresh sterile pipette, thus diluting the original sample to 1000 times.
- Repeat this procedure till the sample has been diluted 10,000,000 times, every time using a fresh sterile pipette.
- From the appropriate dilutions transfer 1ml of suspension while in motion, with the respective pipettes, to sterile petri dishes. Three petri dishes are to be used for each dilution.
- Approximately 15 ml of the nutrient medium is added, melted and cooled to 45^0c, to each petri dish containing the diluted Indhirani maathirai extract. Mix the contents of each dish by rotating gently to distribute the cells throughout the medium.

- Allow the plates to solidify.
- Incubate these plates in an inverted position for 24-48 hours at 37^0c.

Observation

Observe all the plates for the appearance of bacterial colonies. Count the number of colonies in the plates.Calculate the number of bacteria per ml of the original suspension as follows:

$$\text{Organisms per millimetre} = \frac{\text{Number of colonies average (3repleates)}^{86}}{\text{Amount plated} \times \text{dilution}}$$

4.2.7. SOPHISTICATED INSTRUMENTAL ANALYSIS

FT IR - Fourier Transform Infra-red Spectroscopy

FTIR (Fourier Transform Infra-red Spectroscopy) was a sensitive technique particularly for identifying organic chemicals in a whole range of applications although it can also characterise some inorganics. Examples include paints, adhesives, resins, polymers, coatings and drugs. FTIR was an effective analytical instrument for detecting functional groups.

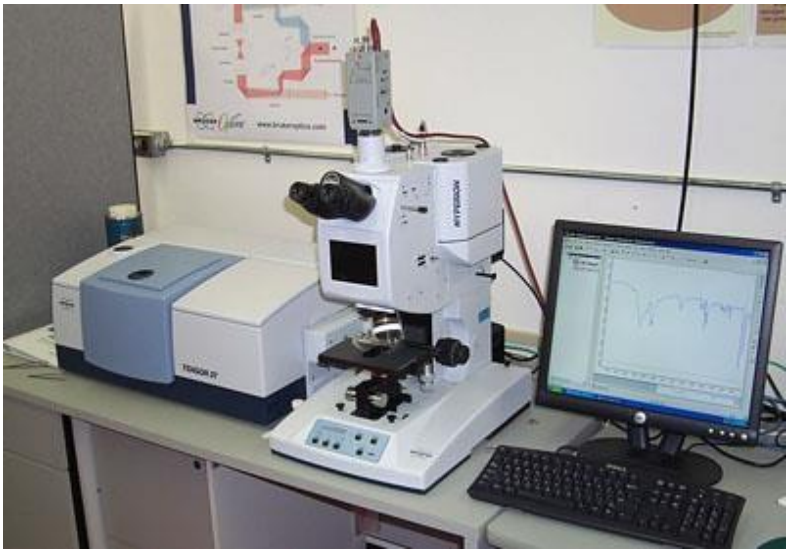


Fig No.4.3 FTIR MECHINE

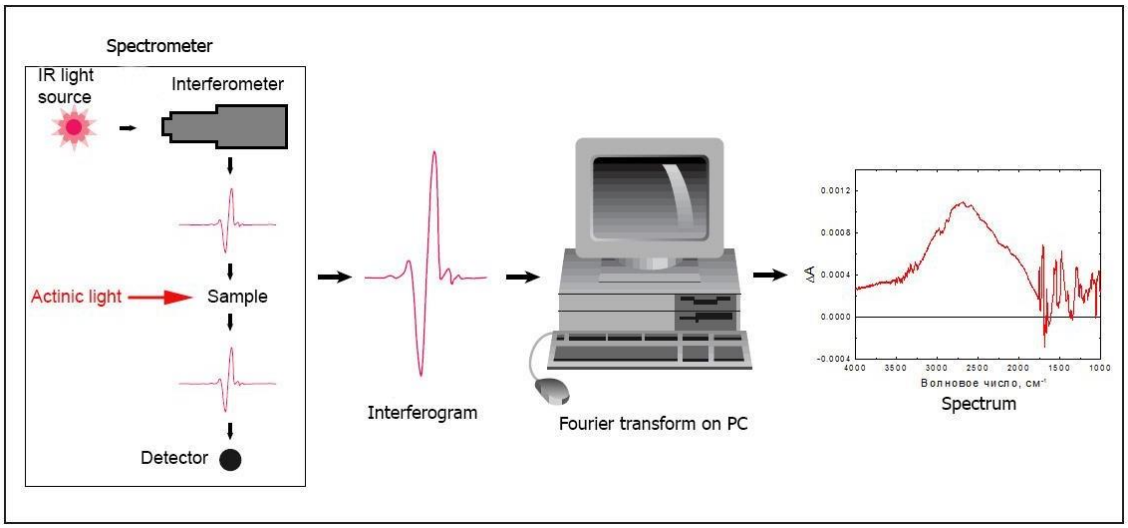


Fig No.4.4 FTIR MECHANISM

APPLICATIONS:

- Quantitative scans
- Qualitative scan solids, liquids, gases
- Organic samples,
- Inorganic samples
- Unknown identification
- Formulation Pharmaceuticals

Principle:

Spectrophotometric tests were commonly used in the Identification of chemical substances and quantification of polymorphic forms. The test procedures were applicable to substances that absorb IR radiation. The IR absorption spectrum of a substance compared with that obtained concomitantly for the corresponding reference standard / reference substance provide conclusive evidence of the identity of the substance being tested.

Recording Infrared spectrum of a solid as a disc (as per USP ‹197K›) :

- Triturate about 1 to 2 mg of the substance to be examined with 300 to 400 mg, unless otherwise specified, of finely powdered and dried potassium bromide. If the substance is a hydrochloride it is preferable to use potassium chloride.
- Carefully grind the mixture and spread it uniformly in a suitable di

- Submit it to the pressure of about 800 mPa (8 tons/cm2).
- Examine the disc visually and if any lack of uniform transparency was observed, reject the disc and prepare again.
- Record the spectrum between 4000 to 650 cm-1 unless otherwise specified in individual standard test procedure.
- When sample and standard were measured for concordance, the transmittance obtained at the start of the scan range, should not deviate by more than 10% between them (For e.g. If the standard shows a transmittance of 75%, the sample transmittance can be between 65% and 85%).

FT-IR was the most advanced and the major advantage was its

- Speed
- Sensitivity
- Mechanical Simplicity
- Internally Calibrated [87]

ICPOES (INDUCTIVELY COUPLED PLASMA OPTIC EMISSION SPECTROMETRY)

Fig: 4.5 ICP-OES INSTRUMENT

Manufacturer: Perkin Elmer

Model: Optima 5300 DV ICP-OES Inductively Coupled Plasma Spectrometer (ICP)

Principle:

An aqueous sample was converted to aerosols via a nebulizer. The aerosols are transported to the inductively coupled plasma which was a high temperature zone (8,000–10,000ºC). The analysts are heated (excited) to different (atomic and/or ionic) states and produce characteristic optical emissions (lights). These releases are separated based on their respective wavelengths and their strengths are measured (spectrometry). The intensities are proportional to the concentrations of analyses in the aqueous sample. The quantification was an external multipoint linear standardization by comparing the emission intensity of an unknown sample with that of a standard sample. Multi-element calibration standard solutions are prepared from single- and multi element primary standard solutions. With respect to other kinds of analysis where chemical speciation was relevant (such as the concentration of ferrous iron or ferric iron), only total essential concentration was analysed by ICP-OES. [86]

Application:

The analysis of major and minor elements in solution IMM.

Objectives:

- Determine elemental concentrations of different metals.
- Learn principles and operation of the ICP-OES instrument
- Develop and put on a method for the ICP-OES sample analysis
- Enhance the instrumental conditions for the analysis of different elements
- probes the outer electronic structure of atoms

Mechanism:

In plasma emwassion spectroscopy (OES), a IMM solution was presented into the core of inductively coupled argon plasma (ICP), which generates temperature of approximately 8000°C. At this temperature all elements become thermally excited and emit

light at their characteristic wavelengths. This light was collected by the spectrometer and passes through a diffraction grating that serves to resolve the light into a spectrum of its essential wavelengths. Within the spectrometer, this deflected light was then collected by wavelength and amplified to yield an strength of measurement that can be converted to an elemental concentration by comparison with standardization values.[87]

The Inductively coupled plasma optical emission spectrometric (ICP-OES) analysis was done in SAIF, IIT MADRAS, and Chennai-36 using Perkin Elmer Optima 5300 DV.

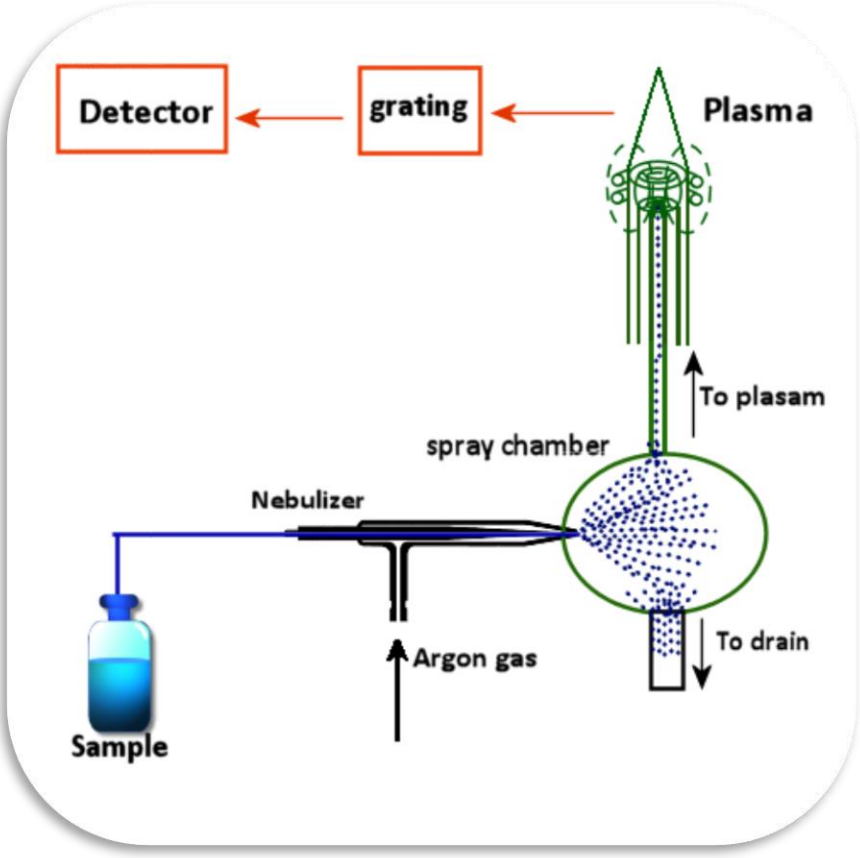


Fig No.4.6.1 ICP-OES MECHANISM

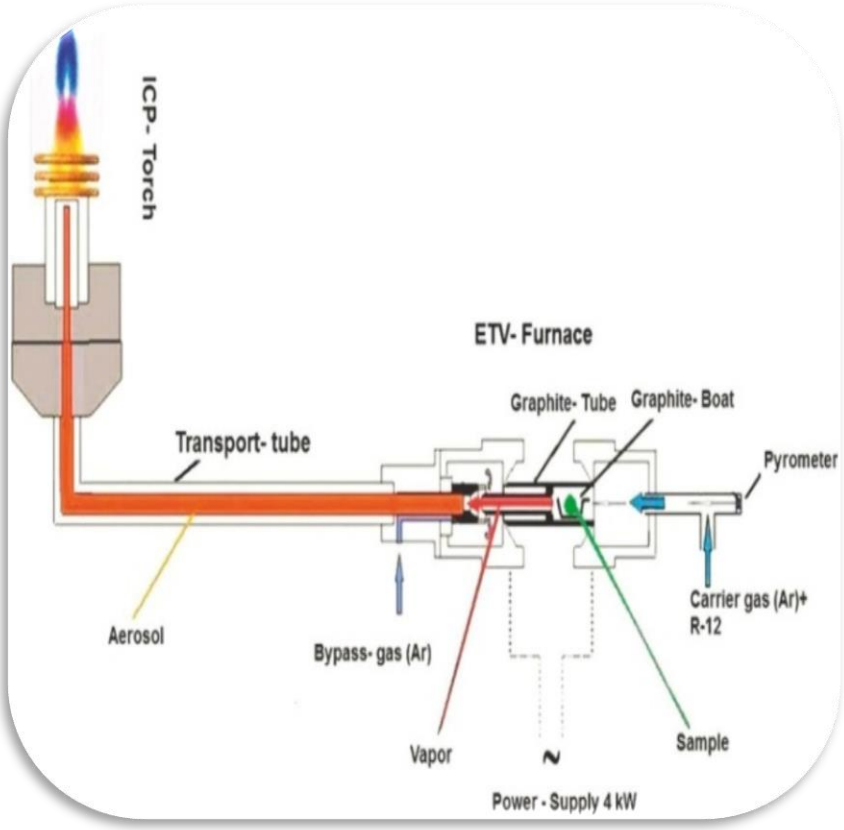


Fig No:4.6.2 ICP-OES MECHANISM

Sample preparation:

Inductively Coupled Plasma Spectroscopy techniques are the so-called "wet" sampling methods whereby IMM are introduced in liquid form for analysis.

100 mg IMM was occupied in a clean, dry test tube. To this, 3 ml Nitric acid was added and mixed well and allowed for few minutes untill the reactions were completed. And then, 25 ml of Refined water, was added to prepare digested solution. The digested IMM solution was shifted into plastic containers and labeled properly. It was completed in Bio-chemistry lab, Govt. Siddha Medical College, Chennai-106

SEM - Scanning Electron Microscope DEFINITION

Scanning Electron Microscopy (SEM), also known as SEM analysis or SEM microscopy, was used very effectively in microanalysis and failure analysis of solid inorganic materials. Scanning electron microscopy is performed at high magnifications,

generates high-resolution images and precisely measures very small features and objects.

SEM ANALYSIS APPLICATIONS

The signals generated during SEM analysis produce a two-dimensional image and reveal information about the sample including:

External morphology (texture)

- Chemical composition (when used with EDS) Orientation of materials making up the sample

The EDS component of the system is applied in conjunction with SEM analysis to:

- Determine elements in or on the surface of the sample for qualitative information
- Measure elemental composition for semi-quantitative results

Identify foreign substances that are not organic in nature and coatings on metalSEM Analysis with EDS – qualitative and semi-quantitative results Magnification – from 5x to 300,000x

Sample Size – up to 200 mm (7.87 in.) in diameter and 80 mm (3.14 in.) in height Materials analysed – solid inorganic materials including metals and minerals.

Fig No:4.7 SEM INSTRUMENT

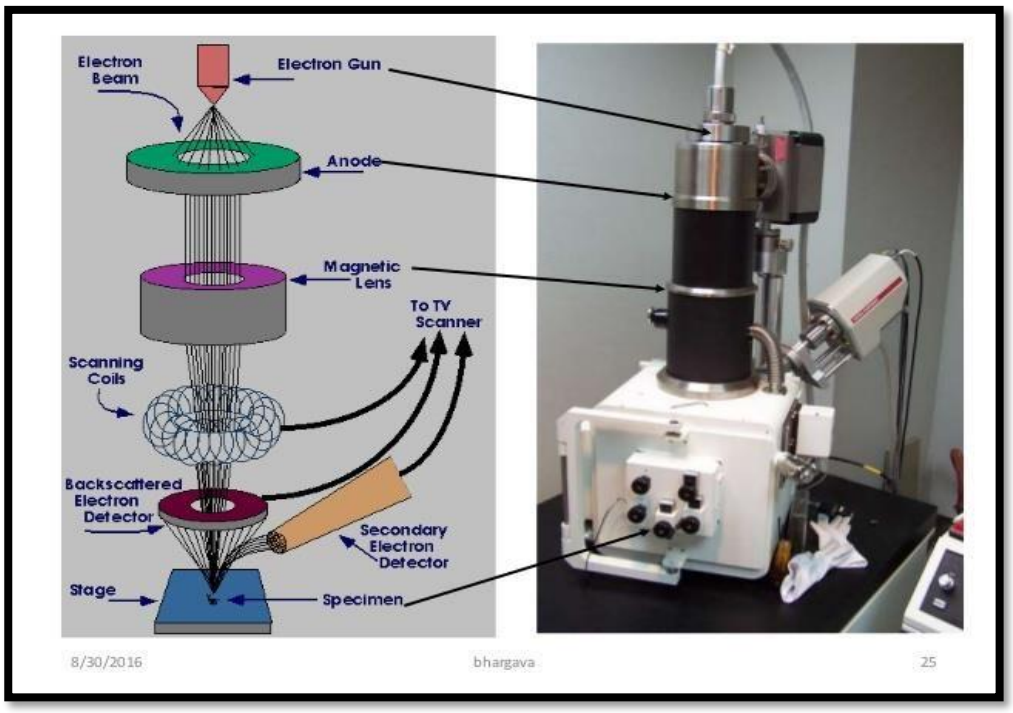


Fig No:4.8 SEM MECHANISM

THE SEM ANALYSIS PROCESS

Scanning Electron Microscopy uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens. In most SEM microscopy applications, data is collected over a selected area of the surface of the sample and a two-dimensional image is generated that displays spatial variations in properties including chemical characterization, texture and orientation of materials.

The SEM was also capable of performing analyses of selected point locations on the sample. This approach is especially useful in qualitatively or semi-quantitatively determining chemical compositions, crystalline structure and crystal orientations.

The EDS detector separates the characteristic X-rays of different elements into an energy spectrum and EDS system software is used to analyse the energy spectrum in order to determine the abundance of specific elements. A typical EDS spectrum is portrayed as a plot of X-ray counts vs. energy (in keV). Energy peaks correspond to the various elements in the sample.

Energy Dispersive X-ray Spectroscopy can be used to find the chemical composition of materials down to a spot size of a few microns and to create element composition maps over a much broader raster area. Together, these capabilities provide fundamental compositional information for a wide variety of materials, including polymers.

In scanning electron microscope high-energy electron beam was focused through a probe towards PP. Variety of signals was produced on interaction with the surface of the sample. This results in the emission of electrons or photons and it was collected by an appropriate detector.

The types of signal produced by a scanning electron microscope include:

- Secondary electrons
- back scattered electrons
- characteristic x-rays light
- specimen current
- Transmitted electrons.
- This gives the information about the sample and it includes external morphology, texture, its crystalline structure, chemical composition and it displays the shape of the sample.[88]

XRD - X-ray Powder Diffraction (XRD)

X-ray powder diffraction (XRD) is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions. The analysed material is finely ground, homogenized, and average bulk composition is determined.

DEFINITION

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 important to studies in geology, environmental science, material science and biology.

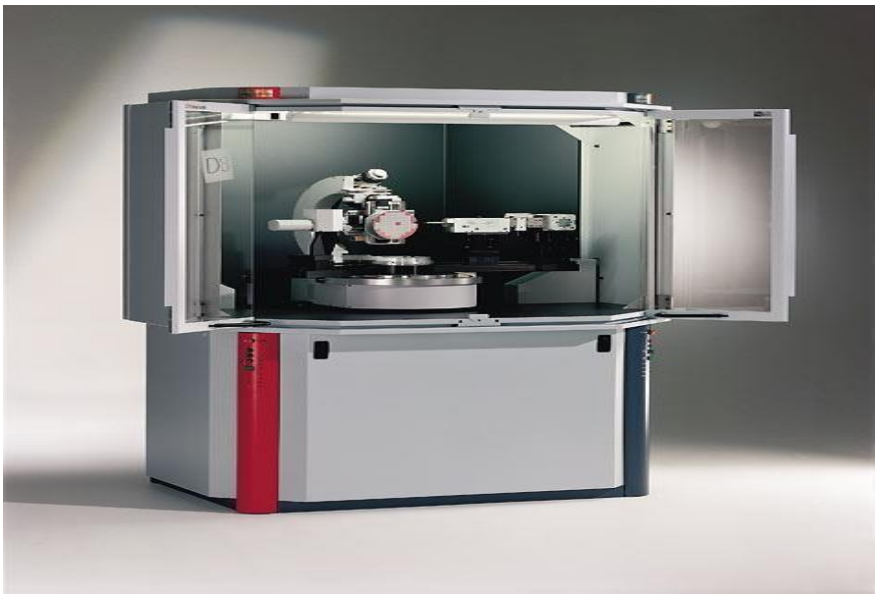


Fig No:4.9 XRD - X-ray Powder Diffraction

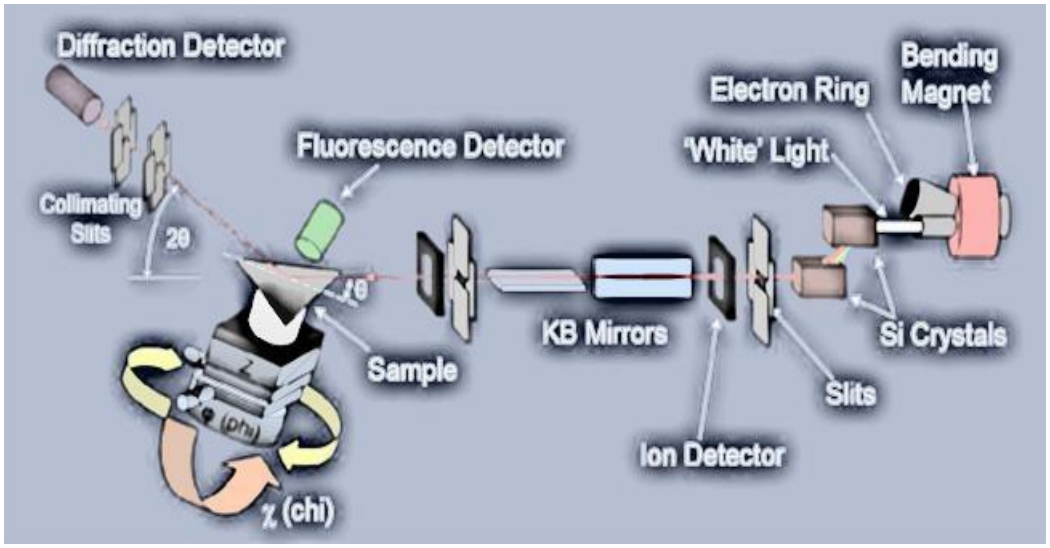


Fig No:4.10 XRD Mechine

APPLICATIONS:

- Characterization of crystalline materials
- Identification of fine-grained minerals such as clays and mixed layer clays that are difficult to determine optically
- Determination of unit cell dimensions.

With specialized techniques, XRD can be used to:

- Determine crystal structures using Rietveld refinement
- Determine of modal amounts of minerals (quantitative analysis)
- Characterize thin films samples by:
- determining lattice mismatch between film and substrate and to inferring stress and strain
- determining dislocation density and quality of the film by rocking curve measurements
- measuring super lattices in multi-layered epitaxial structures
- determining the thickness, roughness and density of the film using glancing incidence X-ray reflectivity measurements
- Make textural measurements, such as the orientation of grains, in a polycrystalline sample.

Strengths and Limitations of X-ray Powder Diffraction: Strengths:

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

Limitations:

- Homogeneous and 3single-phase material is best for identification of unknown
- Must have access to a standard reference file of inorganic compounds
- Requires tenths of a gram of material which must be ground into a powder
- For mixed materials, detection limit is ~ 2% of sample
- For unit cell determinations, indexing of patterns for non-isometric crystal systems is complicated.

Sample Collection and Preparation:

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

- Obtain a few tenths of a gram (or more) of the material, as pure as possible
- 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. Powder less than ~10 μm(or 200-mesh) in size is preferred
- Place into a sample holder or onto the sample surface.[89]

4.3. TOXICOLOGICAL STUDY

4.3.1. ACUTE ORAL TOXICITY STUDY OF *INDHIRANI MAATHIRAI(IM)*

(OECD GUIDELINE – 423)

Introduction:

- The acute toxic class method is a stepwise procedure with the use of 3 animals of a single sex per step.
- Depending on the mortality and/or the moribund status of the animals, on average 2-4 steps may be necessary to allow judgement on the acute toxicity of the test substance.
- This procedure is reproducible, uses very few animals and is able to rank substances in a similar manner to the other acute toxicity testing methods.

- The acute toxic class method is based on biometric evaluations with fixed doses, adequately separated to enable a substance to be ranked for classification purposes and hazard assessment.
- In principle, the method is not intended to allow the calculation of a precise LD50 but does allow for the determination of defined exposure ranges where lethality is expected since death of a proportion of the animals is still the major endpoint of this test.
- The method allows for the determination of an LD50 value only when at least two doses result in mortality higher than 0% and lower than 100%.
- The use of a selection of pre-defined doses, regardless of test substance, with classification explicitly tied to number of animals observed in different states improves the opportunity for laboratory to laboratory reporting consistency and repeatability.

Principle of the Test:

It is the principle of the test that based on a stepwise procedure with the use of a minimum number of animals per step, sufficient information is obtained on the acute toxicity of the test substance to enable its classification. The substance is administered orally to a group of experimental animals at one of the defined doses.

The substance is tested using a stepwise procedure, each step using three animals of a single sex. Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.

- no further testing is needed
- dosing of three additional animals, with the same dose
- dosing of three additional animals at the next higher or the next lower dose level. The method will enable a judgment with respect to classifying the test substance to one of a series of toxicity classes.

Methodology:

Selection of Animal Species

The preferred rodent species is the wistar albino rat, although other rodent species may be used. Healthy young adult animals are commonly used laboratory strains should be employed. Females should be nulliparous and non-pregnant. Each animal, at the **commencement of its dosing, should be between 6 to 8 weeks old and the weight (150-200gm) should fall in an interval within±20 % of the mean weight of any previously dosed animals.**

Housing and Feeding Conditions

The temperature in the experimental animal room should be 22ºC $\pm$ 3ºC. Although the relative humidity should be at least 30% and preferably not exceed 70% other than during room cleaning the aim should be 50-60%.

Lighting should be artificial, the sequence being 12 hours light, 12 hours dark. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water. Animals may be group-caged by dose, but the number of animals per cage must not interfere with clear observations of each animal.

Preparation of animals:

The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 7 days prior to dosing to allow for acclimatization to the laboratory conditions

Test Animals and Test Conditions:

Sexually mature Female Wistar albino rats (150-200gm) were obtained from TANUVAS, Madhavaram, Chennai. All the animals were kept under standard environmental condition (22±3°C). The animals had free access to water and standard pellet diet (Sai Meera foods, Bangalore).

Preparation for Acute Toxicity Studies

Rats were deprived of food overnight (but not water 16-18 h) prior to administration of the, ***Indhirani Maathirai (IM)***

The principles of laboratory animal care were followed and the Institutional Animal

Ethical Committee approved the use of the animals and the study design.

IAEC approved Number: 03/321/PO/Re/S/01/CPCSEA Dated:12/10/2018

Test Substance	: *INDHIRANI MAATHIRAI* (IM)
Animal Source	: TANUVAS, Madhavaram, Chennai.
Animals	: Wister Albino Rats (Female-3+3)
Age	**:** 6-8 weeks
Body Weight on Day 0	**:** 200-250gm.
Acclimatization	**:** Seven days prior to dosing.
Veterinary examination	**:** Prior and at the end of the acclimatization period.
Identification of animals	**:** By cage number, animal number and individual marking by using Picric acid
Number of animals	: 3 Female/group,
Route of administration	**:** Oral
Diet	**:** Pellet feed supplied by Sai Meera foods Pvt Ltd, Bangalore
Water	**:** Aqua guard portable water in polypropylene bottles.
Housing & Environment	**:** The animals were housed in Polypropylene cages provided with bedding of husk.
Housing temperature	**:** between 22ºC $\pm$ 3ºC.
Relative humidity	**:** between 30% and 70%
Air changes	**:** 10 to 15 per hour and
Dark and light cycle	**:** 12**:**12 hours.

Duration of the study **:** 14 Days

Administration of Doses:

INDHIRANI MAATHIRAI **(IM)** was suspended in water and administered to the groups of wistar albino rats in a single oral dose by gavage using a feeding needle. The control group received an equal volume of the vehicle.

Animals were fasted 12 hours prior to dosing. Following the period of fasting, the animals were weighed and then the test substance was administered. Three Female animals are used for each group. The dose level of 20, 40, 60 and 200 mg/kg body weight was administered stepwise. After the substance has been administered, food was withheld for a further 3-4 hours. The principle of laboratory animal care was followed.

Observations were made and recorded systematically and continuously as per the guideline after substance administration.

The visual observations included skin changes, mobility, aggressively, sensitivity to sound and pain, as well as respiratory movements. Finally, the number of survivors was noted after 24 hrs and these animals were then monitored for a further 14 days and observations made daily. The toxicological effect was assessed on the basis of mortality.[90]

Limit test

The limit test is primarily used in situations where the experimenter has information indicating that the test material is likely to be nontoxic, i.e., having toxicity only above regulatory limit doses.

A limit test at one dose level of 200 mg/kg body weight was carried out with three animals per step. The test substance-related mortality was not produced in animals, so further testing at the next lower level need not be carried out.

Observations:

Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days, except where they need to be removed from the study and humanely killed for animal welfare reasons or are found dead.

It should be determined by the toxic reactions, time of onset and length of recovery

period, and may thus be extended when considered necessary. The times at which signs of toxicity appear and disappear are important, especially if there is a tendency for toxic signs to be delayed. All observations are systematically recorded with individual records being maintained for each animal.

a.Mortality

Animals were observed intensively at 0.5, 2.0, 4.0, 6.0, 12.0, 24.0- and 48.0-hours following drug administration on day 1 of the experiment and daily twicethereafter for 14 days.

b.Body weight

Individual weight of animals was determined before the test substance was administered and weights will be recorded at day 1, 7, and 14 of the study. Weight changes were calculated and recorded. At the end of the test, surviving animals were weighed and humanly killed.

c. Cage-side observation

These include changes in skin and fur, eyes and mucous membranes and also respiratory, circulatory, autonomic and central nervous systems, somato motor activity and behavioral patterns. Attention should be directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma.

d. Gross necropsy

All animals (including those which die during the test period are removed from the study) will be subjected to gross necropsy. Gross necropsy includes examination of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents, brain, eye, thymus, lungs, heart, spleen, liver, kidneys, adrenals, testes and uterus of all animals.

Histopathology

Microscopic examination will be carried out in organs to show the evidence of any toxicity in gross pathology.

Data and reporting

All data were summarized in tabular form, (Table-1-4) showing for each test group the number of animals used, the number of animals displaying signs of toxicity, the number of animals found dead during the test, description of toxic symptoms, weight changes, food and water intake.

Test substance and Vehicle

In order to ensure the uniformity in drug distribution in the medium the suspension was made by mixing *Indhirani maathirai with 2% CMC* and it was found suitable for dose accuracy.

Justification for choice of vehicle

The vehicle selected as per the standard guideline was pharmacologically inert and easy to employ for new drug development and evaluation technique.[91]

4.3.2. REPEATED DOSE 28-DAY ORAL TOXICITY (407) STUDY OF *INDHIRANI MAATHIRAI* (IM)

Test Substance	**:** ***INDHIRANI MAATHIRAI*** **(IM)**
Animal Source	**:** TANUVAS, Madhavaram, Chennai.
Animals	**:** Wistar Albino Rats (Male -24, and Female-24)
Age	**:** 6-8 weeks
Body Weight	**:** 200-250gm.
Acclimatization	**:** Seven days prior to dose.
Veterinary examination	**:** Prior and at the end of the acclimatization period.
Identification of animals	**:** By cage number, animal number a n d individual marking by using Picric acid
Diet	**:** Pelletfeedsuppliedby Sai Meera Foods Pvt Ltd,Bangalore

Water : Aqua guard portable water in polypropylene bottles.

Housing&Environment : The animals were housed in Polypropylene cages provided with bedding of husk

Housing temperature : between 22ºC±3ºC.

Relative humidity : between 30% and 70%,

Air changes : 10 to 15 per hour

Dark and light cycle : 12:12 hours.

Duration of the study : 28 Days.

Justification for Dose Selection:

The results of acute toxicity studies in Wistar albino rats indicated that *Indhirani maathirai* was non-toxic and no behavioural changes was observed up to the dose level of 200 mg/kg body weight. On the basis of body surface area ratio between rat and human, the doses selected.

As, per OECD guideline three dose levels were selected for the study. They are low dose (5X), high dose (10X). X is calculated by multiplying the acute toxicity dose 200mg) and the body surface area of the rat (0.018), 5X dose is (10mg/kg), 10X dose is (20mg/kg) The oral route was selected for use because oral route is considered to be a proposed therapeutic route.

Preparation and Administration of Dose:

Indhirani Maathirai suspended in with water, it was administered to animals at the dose levels of 1X, 5X, 10X. The test substance suspensions were freshly prepared every two days once for 28 days. The control animals were administered vehicle only. The drug was administered orally by using oral gavage once daily for 28 consecutive days.

Methodology

Randomization, Numbering and Grouping of Animals:

80 Wistar Albino Rats (40M + 40F) were selected and divided into 4 groups. Each group consist of 20 animals (Male -10 and Female-10). First group treated as a control and other three group were treated with test drug (low, high) for 28 days. Animals were allowed acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. Each animal was marked with picric acid. The females were nulliparous and non-pregnant.

Observations:

Experimental animals were kept under observation throughout the course of study for the following:

Body Weight:

Weight of each rat was recorded on day 0, at weekly intervals throughout the course of study. From the data, group mean body weights and percent body weight gain were calculated.

Food and water Consumption:

Food and water consumed per animal was calculated for control and the treated dose groups.

Clinical signs:

All animals were observed daily for clinical signs. The time of onset, intensity and duration of these symptoms, if any, were recorded.

Mortality:

All animals were observed twice daily for mortality during entire course of study.

Functional Observations:

At the end of the 4^{th} week exposure, 'sensory reactivity' to graded stimuli of different types (auditory, visual and proprioceptive stimuli), 'motor reactivity' and 'grip strength' were assessed.

Laboratory Investigations:

Following laboratory investigations were carried out on day 29 in animals fasted over-night. Blood samples were collected from orbital sinus using sodium heparin (200IU/ml) for Biochemistry and potassium EDTA (1.5 mg/ml) for Hematology as anticoagulant. Blood samples were centrifuged at 3000 r.p.m. for 10 minutes.

Hematological Investigations

Blood samples of control and experimental rats was analyzed for hemoglobin content, total red blood corpuscles (RBC), white blood corpuscles (WBC) count and packed cell volume (PCV).

Biochemical Investigations

Serum was used for the estimation of biochemical parameters. Samples of control and experimental rats were analyzed for protein, bilirubin, urea, BUN, creatinine, triglyceride, cholesterol and glucose levels was carried using standard methods.

Activities of glutamate oxaloacetate transaminase/Aspartate aminotransferase (GOT/AST), glutamate pyruvate transaminase/ Alanine amino transferase (GPT/ALT) and alkaline phosphatase were estimated as per the colorimetric procedure.

Necropsy:

All the animals were sacrificed by excessive anesthesia on day 29. Necropsy of all animals was carried out.

Histopathology

Histopathological investigation of the vital organs was done. The organ pieces (5-6µm thick) of the highest dose level of 300 mg/kg were preserved and were fixed in 10% formalin for 24 hours and washed in running water for 24 hours.

Samples were dehydrated in an auto technique and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the “L” moulds. It was followed by microtome and the slides

were stained with Hematoxylin-eosin. The organs included heart, kidneys, liver, ovary, pancreas, brain, spleen and stomach, of the animals were preserved they were subjected to Histopathological examination.

Statistical analysis:

Findings such as body weight changes, water and food consumption, hematology and blood chemistry were subjected to One -way ANOVA followed by Dunnett's multi comparison test using a computer software program– GRAPH PAD VERSION-3 version.[93]

4.4. PHARMACOLOGICAL ACTIVITY

4.4.1. ANTI – DYSLIPIDEMIC ACTIVITY

TRITON WR 1339 INDUCED HYPERLIPIDEMIA FOR *INDHIRANI MAATHIRAI*

Experimental protocol

Total number of groups	4
Number of animals / groups	6
Sex	: Both
Strain	: Wistar albino rats
Body Weight	: 200 – 250 g

Surfactant administration

Surfactant	: 10% Triton WR 1339
Route of administration	: intravenously
Vehicle	: Saline

Test drug administration

Vehicle	: Water
Route of administration	: Oral
Drug dose	: IM - 100 mg/kg b. wt.

Procedure

Wistar rats weighing 200–350 g was starved for 18 h and then injected intravenously with 10% Triton WR 1 339 (isooctyl-polyoxyethylene phenol).

Phase I: Serum cholesterol levels increase sharply 2–3 times after 24 h.

Phase II:The hypercholesterolemia decreases nearly to control levels within the next 24 h.

The test drugs employed or the solvent for the controls are administered simultaneously with the Triton injection or 22 h thereafter. Serum cholesterol analyses are made 6, 24, and 48 h after Triton injection.

Mechanism

The mechanism of the Triton induced hypercholesterolemia in phase I was thought to be due to increased hepatic synthesis of cholesterol through the ability of Triton to interfere with the uptake of plasma lipids by the tissues. Drugs interfering with cholesterol biosynthesis were shown to be active in phase I, while drugs interfering with cholesterol excretion and metabolism were active in phase II.

Experimental design

The animals were divided into four groups with six animals in each group.

Table No:4.1. Experimental design

Groups	**Treatment**
Group I	Normal Control
Group II	Diabetic control- STZ (55 mg/kg)
Group III	Diabetic control- glibenclamide (5 mg/kg)
Group IV	Diabetic control- *Indhirani maathirai* 40mg/kg
Group V	Diabetic control- *Indhirani maathirai* 60mg/kg

All the animals after 72 hours of triton injection (i.e. after inducing hyper lipidemia) the respective treatment was continued for 7 days.

Collection of blood

On the 8th day the blood was collected by retro orbital sinus puncture, under mild ether anaesthesia. The collected samples were centrifuged for 10 minutes. Then serum samples were collected and it is used for various biochemical experiments. Then animals were sacrificed and collected the liver.

Biochemical analysis

- Total cholesterol

- High-density lipoprotein
- Low-density lipoprotein
- Very low-density lipoprotein
- Triglycerides [93]

4.4.2. Hypoglycemic activity of *Indhirani maathirai (IM)*

Screening the drug *Indhirani maathirai* against Streptozotocin (STZ) induced Diabetes in Wistar albino Rats.

Experimental Animals

The animals were divided into 5 groups each constituting 6 rats. Group I was normal rats, Group II were STZ (55 mg/kg b.w., i.p) induced diabetic rats. Group III STZ (55 mg/kg b.w., i.p) induced diabetic rats were treated with Glibenclamide 5mg/kg b.w/p.o Group IV STZ (55 mg/kg b.wt., i.p) induced diabetic rats were treated with Infhirani maathirai 40mg/kg b.w/ p.o Group V STZ (55 mg/kg b.w., i.p) induced diabetic rats were treated with Infhirani maathirai 60mg/kg b w/p.o for 28 days.

Methodology Induction of Diabetes

Diabetes was induced in male Wistar albino rats aged 2–3 months (180–200 g body weight) by intraperitoneal administration of STZ (single dose of 55 mg/kg b.w) dissolved in freshly prepared 0.01 M citrate buffer, pH 4.5.

After injection the animals had food and water ad libitum and were given 5% glucose in their drinking water for the first 24 hours to counter any initial hypoglycemia. The development of diabetes was confirmed after 72 hours of the Streptozotocin injection. After 72 hrs of STZ injection under mild anaesthesia the blood was withdrawn from the tip of the tail of each rat and the blood glucose level was analysed. Animals with more than 250 mg/dl was considered as diabetic.

Fasting blood glucose levels was measured before the administration of extracts. The blood glucose levels were checked on 0th, 7th, 14th, and 21st day of the treatment period. Blood was collected from snipping of the rat tail. Blood glucose levels were measured

Experimental Design

Diabetic rats were divided into four groups with six animals in each group.

Table No:4.2. Experimental design

Groups	Treatment
Group I	Normal Control
Group II	Diabetic control- STZ (55 mg/kg)
Group III	Diabetic control- glibenclamide (5 mg/kg)
Group IV	Diabetic control- Indhirani maathirai 40mg/kg
Group V	Diabetic control- Indhirani maathirai 60mg/kg

<All the experimental rats were fasted overnight and the blood was withdrawn through puncturing retro orbital sinus on the 5^{th} day,15^{th} day and 20^{th} day of post induction period to determine blood glucose level by GOD-POD kit method. The change in the body weight was observed throughout the treatment period in experimental animals.

Statistical Analysis

All the values were expressed as Mean ± S.D the difference between control and treatment groups were tested for significance using ANOVA followed by Dunnett's test<0.05 were considered significant.[94]

4.4.3. ANTI-OXIDANT ACTIVITY

DPPH RADICAL SCAVENGING ASSAY

The radical scavenging activity of different extracts was determined by using DPPH assay according to Chang et al. The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517 nm. Ascorbic acid (10mg/ml DMSO) was used as reference.

PRINCIPLE

1, 1-diphenyl-2-picryl hydrazyl is a stable free radical with pink colour which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity. The scavenging reaction between (DPPH) and an antioxidant (H-

A) can be written as,

DPPH + [H-A] →DPPH-H + (A)

Antioxidants react with DPPH and reduce it to DPPH-H and as consequence the absorbance decreases. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

REAGENT PREPARATION

0.1mM DPPH solution was prepared by dissolving 4mg of DPPH in 100ml of ethanol.

PROCEDURE

Different volumes of extracts 1.25µl - 20µl (12.5 - 200µg/ml) from a stock concentration 10mg/ml were made up to a final volume of 20µl with DMSO and 1.48ml DPPH (0.1mM) solution was added. A control without the test compound, but an equivalent amount of distilled water was taken. The reaction mixture incubated in dark condition at room temperature for 20 minutes. After 20 minutes, the absorbance of the mixture was read at 517nm. 3ml of DPPH was taken as control.[95]

CALCULATION

$$\% \text{ inhibition} = \frac{control - test}{control} X100$$

Ingrediants of indhirani maathirai

PURASU VER

KALLI VER

KALARCHI VER

MILAGU

KADUGU

PERUNGAYAM

KARUNKALI

VEDIYUPPU

Preparation of drug

5. RESULTS AND DISCUSSION

There are various modern scientific studies have been carried out to bring the potency, efficacy and safety of the drug ***Inthirani Maathirai***.

This study includes:

- Literary collections
- Organoleptic characters
- Physicochemical analysis
- Phytochemical analysis
- Instrumental analysis
- Toxicological study
- Pharmacological study

The drug ***Inthirani Maathirai*** has been selected for Anti-dyslipidemic activity.

- Literary collections about the drug from various text books were done. Siddha literatures related to the drug bring the evidence and importance of its utility in treating the dyslipidemia.
- The drug review brings the identification, morphological description of the plants and drugs, chemical constiduents and effectiveness of the ingredients present in the drug for anti-dyslipidemic, anti-diabetic and anti-oxident property. Also gives the medicinal uses of the ingredients.
- Siddha and modern aspect of the drug explains about clinical features and treatment aspect of Dyslipidaemia and also explains adverse effect of drugs used in modern aspect.
- Pharmaceutical review describes about the purification, preparation, administration of trial drug and explains about its shelf-life properties.
- The pharmacological review explains about the methodology of Anti-dyslipidemic Activity, Hypoglycaemic Activity and Anti- oxidant activity of the drugs used.

Standardization of The Test Drug *Inthirani Maathirai*

The efficacy and potency of the trial drug was exhibited through standardization. It was done by analysing the drug with several modern scientific studies. Physicochemical, phytochemical studies and biochemical analysis were done and the results were tabulated. Toxicological and pharmacological results of the drug were derived. Its result has been tabulated and interpretations were done, thus it gave a comprehensive justification, which brings the efficacy of the trial drug.

Organoleptic Character

The following organoleptic characters have been noted in ***Inthirani Maathirai***

Table No.5.1 Results of Organoleptic characters

Colour	Sandish Brown
Odour	Pleasant
Taste	Astringent, milld Pungent
Texture	Pills or Tablets
Size	Payiralavu Maathirai (42mg)

PHYSICOCHEMICAL ANALYSIS

Table No.5.2. Results of Physicochemical analysis

S.NO	PARAMETER	RESULT
1	pH	4.9
2	Total ash	12.8%
3	Water soluble ash	3.96%
4	Acid soluble ash	2.47%
5	Loss on drying (at 105°C)	1%
6	Water soluble extractive	18.4 %
7	Alcohol soluble extractive	6%

8	Solubility		
	I	Distilled water	Soluble
	II	NaOH	Soluble
	III	Hcl	Soluble

Interpretation

The Physico chemical results of the drug reveals the pH, total ash, water soluble ash, acid insoluble ash, loss on drying, water soluble extractive, alcohol soluble extractive and solubility of the trial drug.

pH:

P^H value reveals the hydrogen ion concentration. It is the measure of the acidic or alkaline nature. If the p^H of the drug 7.0, it is neutral, above 7.0, it is alkaline and below 7is acidic.

Abioavailability and effectiveness. The result concludes that the oral bioavailability of the drug *Indhirani Maathirai* is very high.[96]

ASH VALUES:

Ash content of the drug is generally taken to be the residue remaining after incineration at 650-700^0 Celsius. It usually represents the inorganic salts naturally occurring in the drug and adhering to it.

Depending upon the concentration and type of minerals existing, the quality of the drug was determined.

The total ash value of *Indhirani Maathirai* is 12.87% which indicates the purity of the drug.

Acid Insoluble Ash

Acid insoluble ash value of the drug *Indhirani Maathirai* is less than 2.47% this indicates the good quality of the drug.

Water soluble ash

Water soluble ash value for *Indhirani Maathirai* is 3.96% which reveals that the sample drug has easy facilitation of diffusion and osmosis mechanism.

Loss on Drying

The moisture content of a sample was determined by Loss on drying. The moisture content of the drug reveals the stability and better shelf life. Loss on drying value of the trial drug *Indhirani Maathirai* is 1% at 105°C. The less value of moisture content could prevent bacterial, fungal or yeast growth **Solubility**

Solubility is the basic requirement for the absorption of the drug in GIT.The poor solubility and low permeability are the causes of low oral availability. Here the water-soluble nature of *Indhirani Maathirai* is 18.4% This nature might be helpful for the better absorption. The sample drug also soluble in Hydrochloric acid, Sodium hydroxide.[97]

PHYTO CHEMICAL ANALYSIS

Table No:5.3 Results of Phytochemical Analysis

S.NO	Phytochemicals	Test name	H2O Extract
1.	Alkaloids	Mayer's Test	- ve
2.	Carbohydrates	Molisch's Test	- ve
3.	Glycosides	Modified Bontrager's Test	- ve
4.	Saponin	Froth Test	+ ve
5.	Phenols	Ferric chloride Test	- ve
6.	Tannins	Gelatin Test	- ve
7.	Flavonoids	Alkaline reagent Test	+ ve
8.	Proteins	Xanthoprotein Test	- ve
9.	Amino acids	Ninhydrin Test	- ve

10.	Diterpenes	Copper acetate Test	+ ve
11.	Gum & Mucilage	Extract + Alcohol	+ ve
12.	Quinones	NAOH+Extract	- ve
13.	Fat & Fixed Oil	Spot Test	- ve

Interpretation:

All the plants have different Phyto chemical constituents with them. These constituents have the definite physiological action on the human body.

The Phytochemical screening of *Indhirani Maathirai* shows the presence of Saponin, Phenol, Tannins, Flavonoids, Diterpenes, Quinones Gums and Mucilage.

Flavonoids:

Flavonoids are poly phenolic molecules containing 15 carbon atoms and are soluble in water. They provide health benefits through cell signalling path way and antioxidant effects.[98]

Flavonoids have the anti-oxidant properties, due to this character it shows the major influence on vascular system. They have the anti-atherosclerotic effect, exhibited by the oxygen radicals which oxidise LDL, injures the endothelial wall and there by promotes anti-atherosclerotic changes.[99]

Saponins:

Saponins are a diverse group of chemicals, which derive their name from their ability to form soap-like foams in aqueous solutions. Saponins are the metabolic glycosides present in many plants.[100]

Saponins have the cholesterol lowering activity. It lowers the cholesterol level by blocking the absorption of cholesterol by the body and increases the excretion of the cholesterol. [101]

Diterpenes

Diterpenes are structurally diverse class of C_{20} natural compounds widely distributed in nature. They are derived from Geranylgeraniol pyrophosphate. Naturally occurring diterpenes exert several activities such as anti-spasmodic, anti- inflammatory.

Diterpenes derived from plants has been used as medication for reducing glucose and lipid level. They also possess anti-oxidant activity.[102]

Gum & Mucilage:

Guar gum was significantly effective in comparison with glibenclamide in the treatment of hyperlipidemia and hyperglycemia in diabetes rats. Therefore, it may be suggested as a reliable fiber in diabetic regimes in diabetic patients.[103]

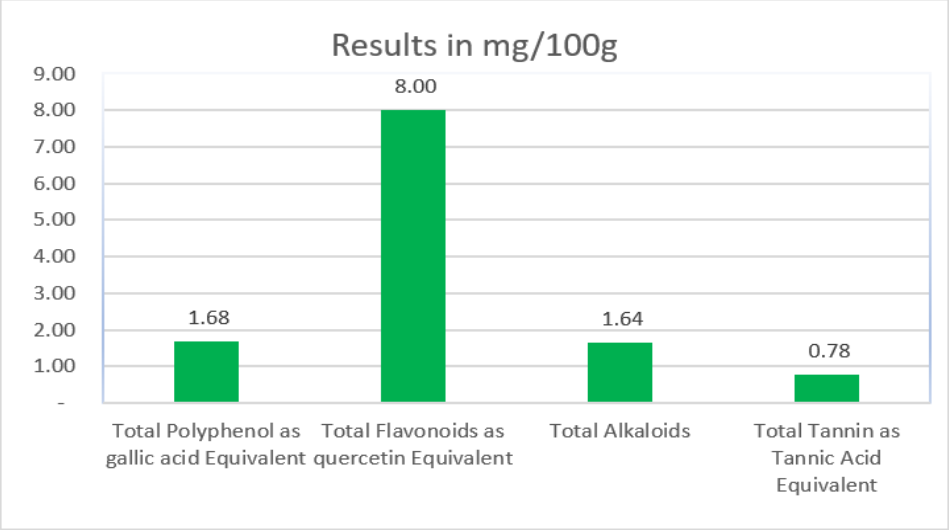
HPLC ANALYSIS

HPLC is a technique in analytical chemistry which is used to separate the components in a mixture, to identify each component, and to quantify each component

Table No:5.4 HPLC Analysis report of *Indhirani Maathirai*

S.no	PARAMETERS	UNITS	RESULTS
1.	Total Polyphenol as gallic acid Equivalent	mg/100g	1.68
2.	Total flavonoids as quercetin Equivalent	mg/100g	8
3.	Total Alkaloids	mg/100g	1.64
4.	Total Tannin as Tannic Acid Equivalent	mg/100g	0.78

Chart. No:5.1 HPLC Analysis Results of *Indhirani Maathirai*



INTERPRETATION

This HPLC study results, indicating the presence of phenolic compounds such as Gallic Acid, Quercetin, Alkaloids and Tannic acid were found in the trial drug *Indhirani Maathirai.*

Flavonoids as Quercetin

Flavonoids are group of plant metabolites, having anti-hyperlipidaemic, anti-hyperglycaemic and anti-cancerous activities.[104] Flavonoids of all sub class have proven to have hypoglycaemic properties by the actions like

1.enhancing insulin secretion via regeneration of pancreatic β cells. 2.enhancing insulin mediated glucose uptake by target cells.

3.inhibitng aldose reductase enzyme.

4.increasing ca^{2+} uptake. [105]

High intake of dietary quercetin decreases plasma total cholesterol and increases HDL cholesterol.

Consumption of quercetin reduce thiobarbituric acid reactive substance levels and elevated activities of superoxide dismutase, catalase and glutathione peroxidase in the liver. Thus, quercetin could be effective in improves hypoglycaemic, dyslipidemic and

antioxidant status in type 2 diabetes.[106]

Thus, the flavonoids enhance the anti-dyslipidemic, hypoglycaemic and anti-oxidant activities.

BIOCHEMICAL ANALYSIS

Table No: 5.5Results of Basic radical's studies

S.no	Parameter	Result
1	Test for Potassium	Present
2	Test for Calcium	Present
3.	Test for Sodium	Present
4.	Zinc	Present
5.	Aluminium	Present

Interpretation

The basic radical test shows the presence of potassium, calcium zinc and aluminium.

Potassium:

The potassium rich diet has significant reduction in cholesterol ester deposition in atherosclerosis

Calcium:

Increased intake of calcium produces marked decrease in serum lipids.Calcium can bind with bile acids& fatty acids in the gut, leading to mal absorption of fat from the digestive tract. Thus, calcium intake has a positive effect on serum lipids. The calcium content of the trial drug will support to reduce dyslipidemia.

Sodium:

Hypertriglyceridemia may be a factor contributingto abnormal proximal sodium handling and sodium retention.[107]

Zinc

Zinc improved the effectiveness of OHA and may be beneficial in decreasing blood glucose, TG, urinary albumin excretion and inflammation in diabetic nephropathy patients and thus reducing the risk of complications.

Zinc supplementation significantly reduced total cholesterol, LDL cholesterol and triglycerides. In addition to that, Zinc supplementation in non-healthy patients demonstrated a significant elevation of HDL cholesterol. Therefore, it may have the potential to reduce the incidence of atherosclerosis related morbidity and mortality especially in non-healthy patients who are at risk of atherosclerosis.[108]

Aluminium:

Aluminium mediated drug reactions participate the lipid metabolism and corrects hyperlipidemia.[109]

Table No:5.6 Results of Acid Radicals

S.NO	Parameter	Result
1.	Sulphate	Present
2.	Phosphate	Present
3.	Nitrate	Present

Interpretation

The acidic radicals test shows the presence of Sulphate and Nitrate.

Sulphate:

Sulphate ions attached with oxidised form of cholesterol and it is highly protective against fatty streaks and atherosclerosis.[110]

Nitrate:

Dietary nitrate improves glucose tolerance and lipid profile in an animal model

REF Existence of these radicals will help the trial drug for its therapeutic effects.[111]

Phosphate:

The dyslipidemia was accompanied with significantly elevated serum phosphate level. On the other hand, no significant difference was evident in the serum lipid or phosphate concentrations of subclinical hypothyroid patients compared to euthyroid subjects. A significantly reduced serum phosphate level was shown in hyperthyroid patients with unaltered serum lipid levels. Significant correlations were evident between TSH and T4 levels as independent parameters and the serum concentrations of triglyceride, cholesterol and phosphate. The results indicate in hypothyroidism that a secondary hyperphosphatemia may aggravate myocardial and arterial abnormalities induced by the secondary hyperlipidemia, which may need correction.[112]

MICROBIAL LOAD

Table No.5.7 Bacterial and fungal dilutions

MICROBES	DILUTION	RESULT
Bacteria	**10^{-4}**	**5**
Bacteria	**10^{-6}**	**2**
Fungi	**10^{-2}**	**1**
Fungi	**10^{-3}**	**Nil**

Interpretation

- The bacterial load availability in the IM has been performed by Agar plate technique. As IM was made from plant materials it is more susceptible to to contamination. The contamination of herbal drugs by microorganism not only cause bio deterioration but also reduces the effectiveness of drug. The toxin produced by microbes makes herbal drugs unfit for human
- consumption because the contaminated drug may develop various disease instead of disease being cured.

- The contamination of IM has been examined by bacterial and fungal load.
- Total bacterial load in 10^{-4} dilution is 5 and 10^{-6} dilution is 2.
- Total fungal load in 10^{-2} dilution is 1 and 10^{-3} dilution is nil.

This result shows the presence of bacterial and fungal load in the trial drug *(IM)*. Load of bacteria and fungi are within the limits of WHO norms

Fig.No.5.1 FTIR- FOURIER TRANSFORM INFRARED SPECTROSCOPY

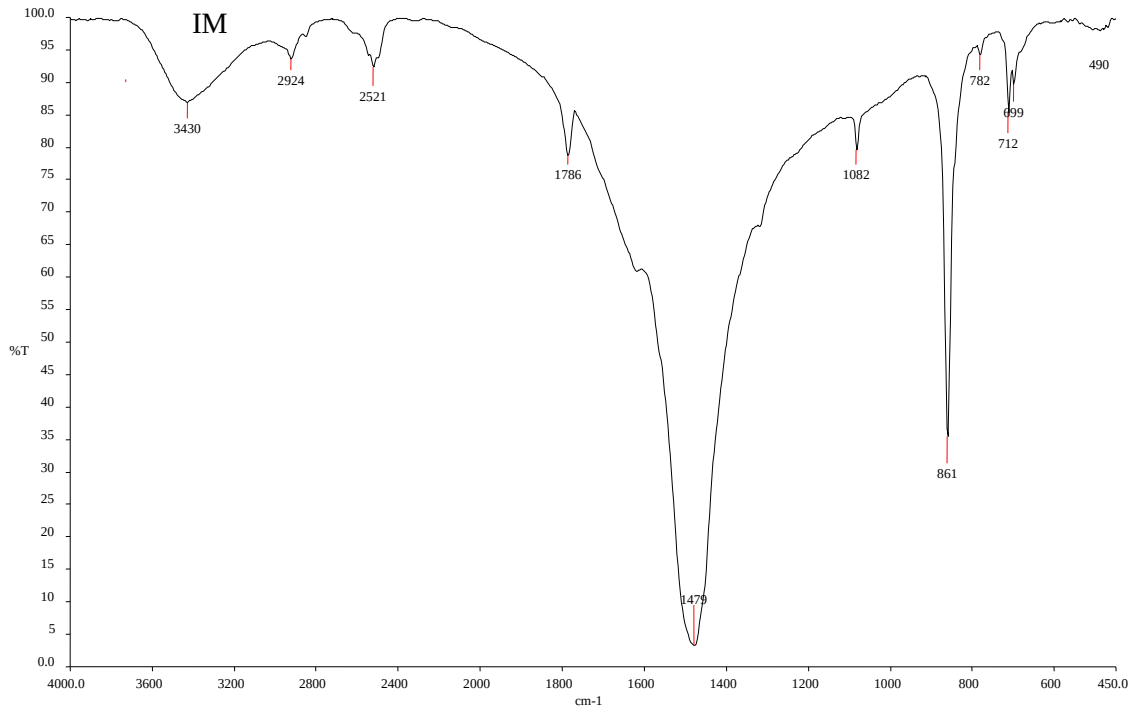


Table No:5.8 Result of FTIR Spectrum

S.NO	PEAK VALUE	INTENSITY	STRETCH	FUNCTIONAL GROUP
1.	3430	Strong	O-H Stretch	Alcohol
2.	3430	Medium	N-H Stretch	Aliphatic primary amine
3.	2924	Strong	O-H Stretch	Carboxylic acids
4.	2924	Weak	O-H Stretch	Alcohol
5.	2924	Strong	N-H Stretch	Amine salt
6.	2924	Medium	C-H Stretch	Alkane
7.	2521	Weak	S-H Stretch	Thiol
8.	1786	Weak	C-H Bond	Aromatic compound (over tone)
9.	1786	Strong	C=O Stretch	Anhydride
10.	1786	Strong	C=O Stretch	Acid halide
11.	1786	Strong	C=O Stretch	Conjucated acid halide
12.	1479	Medium	C-H Bond	Alkane
13.	1089	Strong	C-F Stretch	Fluoro compound
14.	1089	Strong	C-O Stretch	Alkyl aryl ether
15.	1089	Strong	C-O Stretch	Vinyl ether

16.	1089	Strong	C-O Stretch	Aliphatic ether
17.	1089	Strong	C-O Stretch	Secodary Alcohol
18.	861	Strong	C=C Bond	Alkene
19.	782	Medium	C=C Bond	Alkene (tri substituted)
20.	712	Strong	C=C Bond	Alkene (di substituted)
21.	699	Strong	C=C Bond	Alkene (di substituted)
22.	699	Strong	C-Br Stretch	Halo compound
23.	490	Strong	C-I Stretch	Halide

Interpretation

FTIR instrumental analysis was done for the test drug and it was identified that there were 11 peaks values. They are the functional groups existing in the trial drug ***Indhirani Maathirai***.

The table shows the presence of alkanes, alkyl halides, alkenes, amines, alcohol, carboxylic acid, thiol,aromatic compouds, anhydrides, fluro compound,ether, ketone groups which represents the peak value. Existence of this these functional groups will intensify the trial drug's property.

Carboxylic Acid

Carboxylic acid is present in the trial drug in the form of per-hydro fluorene which is a peroxime proliferator activated receptor. It is a nontoxic one, because of having an effect of regulating PPAR, is useful as hypoglycemic and hypolipidemic and preventing remedy for metabolic disorders.[113]

Ether

In this trial drug the aromatic ether is present as cyclo heptane. This cycloheptane useful in the treatment of various metabolic disorder such as diabetes mellitus, dyslipidemia.[114]

Alkane

In the trial drug the alkanes are present in the form of tridecane, which is found in lime oil. It presents in all the spices. The spices having the anti-oxidant property which helpful in reducing the oxidative stress in dyslipidemic conditions.[115]

ICP-OES-Inductively Coupled Plasma Mass Spectrometry

It is a technique used to analyse trace levels of a wide range of inorganic elements.it identifies and quantifies the metals and inorganic elements.

Table no.5.9 ICP-OES Interpretation

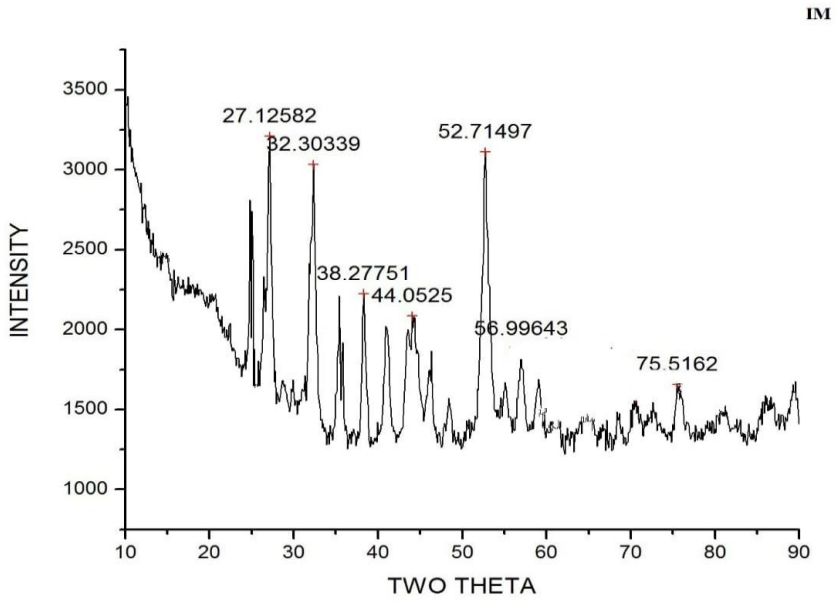
S.NO	ELEMENTS	DETECTED LEVELS
1.	Arsenic	BDL (LOQ 0.1)
2	Cadmium	BDL (LOQ 0.1)
3.	Mercury	BDL (LOQ 0.1)
4.	Lead	BDL (LOQ 0.1)

Interpretation:

The ICP-OES study results of the trial drug show below detectable values of Arsenic, Cadmium, Mercury, Lead. The absence of these heavy metals ensures the quality of the drug and the safety of the drug. Thus, the long-term use of the trial drug will not be harmful to human beings.

XRD (X-Ray diffraction)

Fig No: 5.2 XRD



Interpretation

Dependent on the route of synthesis, the crystalline structure, the size and the shape of the particles of the drug are present and also it determines the efficacy of a drug. The micro particles may enhance bio absorption of the drug.

In the XRD analysis results of *IM* have the major diffraction peaks. They are identified and concluded in the range of 27-75 nm is association with organic molecules. These molecule plays an important role in making the drug to biocompatible and nontoxic at therapeutic doses. Other elements present in *IM* act as an added supplement and helps in increasing the efficacy of the formulation.

SCANNING ELECTRON MICROSCOPE

In addition, the particle size and chemical elements were assessed by Scanning Electron Microscope SEM is one of the most widely used instruments in research areas.

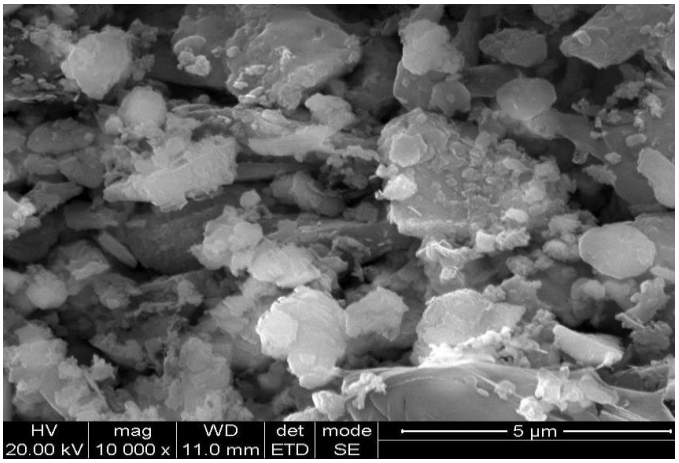


Fig. No:5.3 SEM IMAGE 5µM

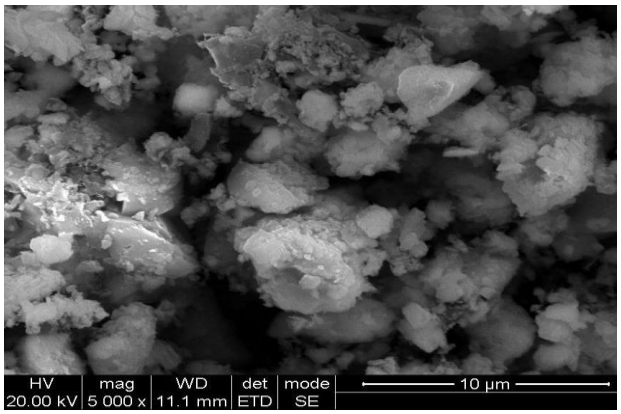


Fig. No:5.4 SEM IMAGE 10µM

The SEM image is done by 500X magnification via 10µm aperture shows maximum depth focused.

Interpretation:

Biodegradable microparticles have been used frequently as drug delivery vehicles due to its grand bioavailability, better encapsulation, control release and less toxic properties. The advantages of microparticles are

The test drug *Indhirani Maathirai* contains micro particles.

Micro particles present in the drug results in a better bioavailability and facilitates absorption.

The particles of micro size show that the drug may easily enter the cells at the molecular level to treat the disease rapidly and increase the therapeutic effect.

Micro particles are defined as particulate dispersion or solid particles with a size in the range of 100-1000nm in diameter.

- The above SEM studies of microscopic resolution showed objects of sizes ranging from 5-10 microns.
- Size and surface of micro particles can be easily manipulated to achieve both passive and active drug targeting.
- They control and sustain, the release of drug during the transportation and at the site of localization, alters drug distribution in the body and subsequent clearance of the drug so as to achieve increased drug therapeutic efficacy thereby bio-availability and reduced side effects [104]
- Hence *Indhirani Maathirai* which is prepared biologically contains microparticles to enhance the pharmacological action in the target site.

TOXICOLOGICAL STUDY RESULTS OF *Indhirani Maathirai*

As per the OECD guidelines, toxicological studies were very essential in animal models in order to establish the safety and efficiency of a new drug. Toxicological studies help to make conclusion whether a novel drug should be approved for clinical use or not.

Toxicity produces the negative, lethal and adverse effects of drugs.
Some chemical elements present in plants, which may produce weakness
in general well- being and mortality.

To avoid the adverse effects, the herbal formulation of *Indhirani Maathirai* has been engaged in to toxicity studies.

Acute Oral Toxicity Study *of Indhirani Maathirai*

Wistar albino rat was treated with the test drug *Indhirani Maathirai* of single dose of 200mg/kg in water as suspension.

The LD50 value of the drug was determined by acute toxicity study to determine the immediate toxic effect, single dose of drug given in large quantity.

This study was conducted as per the OECD guidelines. The result of acute toxicity of

Indhirani Maathirai has been tabulated below.

Table No: 5.10 Observations in Behavioural Signs of Acute Oral Toxicity

S.No	Group CONTROL	Observation	SL	Group TEST GROUP	Obsevation
1.	Body weight	Normal	1	Body weight	Normally increased
2.	Assessments of posture	Normal	2	Assessments of posture	Normal
3.	Signs of convulsion limb paralysis	Normal	3	Signs of convulsion limb paralysis	Normal
4.	Body tone	Normal	4	Body tone	Normal
5.	Lacrimation	Normal	5	Lacrimation	Normal
6.	Salaivation	Normal	6	Salaivation	Normal
7.	Change in skin color	No significant color change	7	Change in skin color	No significant Color change
8.	Piloerection	Normal	8	Piloerection	Normal
9.	Defecation	Normal	9	Defecation	Normal
10.	Sensitivity response	Normal	10	Sensitivity Response	Normal
11.	Locomotion	Normal	11	Locomotion	Normal
12.	Muscle gripness	Normal	12	Muscle gripness	Normal
13.	Rearing	Mild	13	Rearing	Mild
14.	Urination	Normal	14	Urination	Normal

Table No:5.11 Dose finding experiment and its behavioural signs of toxicity for *Indhirani Maathirai*

Dose 200mg/kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
A1 20mg/kg	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A2 40mg/kg	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A3 60mg/kg	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-

1.Alertness 2. Aggressiveness 3. Pile erection 4. Grooming 5. Gripping 6.Touch Response 7. Decreased Motor Activity 8. Tremors 9. Convulsions 10. Muscle Spasm, 11.Catatonia 12. Muscle relaxant 13. Hypnosis 14. Analgesia 15. Lacrimation 16. Exophthalmos 17. Diarrhoea 18. Writhing 19. Respiration 20. Mortality.

(+ Present, - Absent)

Interpretation

- The Acute toxicity result shows no mortality rate up to 200mg/kg.
- The rats did not reveal any observable signs of central nervous system and mortality. It does not show any changes in alertness, grooming and touch response. The behavioural changes are normal.
- This dose level did not produce signs of toxicity, behavioural changes, and mortality in the test groups as compared to the controls when observed during 14 days of the acute toxicity experimental period. Hence the test drug *Indhirani Maathirai* is a safe herbal drug and can be used for continued administration.

Table No:5.12 Body weight of wistar albino rats in acute group exposed to *Indhirani Maathirai (IM)*

DOSE	DAYS		
	1	7	14
CONTROL	218.4±1.24	216.4±1.14	214.2±1.42
HIGH DOSE 200mg/kg	230.2±1.22	232.4±1.44	236.4±3.37
P value (p)*	NS	NS	NS

*N.S- Not Significant, **(p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One-way ANOVA followed by Dunnett's test)*

Table No:5.13 (Water intake (ml/day) of Wistar albino rats group exposed to *INDHIRANI MAATHIRAI (IM)*

DOSE	DAYS		
	1	7	14
CONTROL	1.4±4.34	72.2±3.14	74.4±4.13
HIGH DOSE 200mg/kg	72.4±3.33	71.53±1.08	52.4±3.20
P value (p)*	NS	NS	NS

*N.S- Not Significant, **(p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One-way ANOVA followed by Dunnett's test)*

Table No:5.14 Food intake (gm/day) of Wistar albino rats group exposed to *INDHIRANI MAATHIRAI(IM)*

DOSE	DAYS		
	1	7	14
CONTROL	66.12±4.24	68.6±1.22	60.6±2.76
High DOSE 60mg/kg	61.2±1.64	63.3±3.13	64.2±3.1

*N.S- Not Significant, **(p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One-way ANOVA followed by Dunnett's test.*

Interpretation

The result of the body weight of rats exposed to control and the trial drug of *Indhirani Maathirai 200*mg exhibited overall non-significant changes throughout the dosing period of 14 days. This indicates that the tested animals were in the healthy condition during the 14 days observation period.

The quantity of water and food intake taken by the animals from the group and the control is comparably normal.

The results exhibit that the trial drug did not produce any adverse effects up to the dose level of 200 mg/kg body weight.

RESULTS OF REPEATED DOSE 28- DAY ORAL TOXIC STUDY OF *INDHIRANI MAATHIRAI (IM)*

TableNo:5.15Body weight of wistar albino rats group exposed to *INDHIRANI MAATHIRAI(IM)*

DOSE	**DAYS**				
	1	7	14	21	28
CONTROL	281.2±1.64	282.2 ± 1.04	283.4 ± 1.40	284.4± 1.40	285.2 ± 1.10
LOW DOSE 20mg/kg	281.2 ± 1.25	282.4 ± 1.19	283.3±1 .21	284 ±1.20	285.2± 1.10
MID DOSE 40mg/kg	281.1±1.27	282.3±2.10	283.8±2.71	284.3±3.20	285.1±1.50
HIGH DOSE 60mg/kg	281.2± 1.41	282.4±2.17	283.2 ± 1.64	284.2 ± 1.18	285 ± 2.30
P value (p)*	NS	NS	NS	NS	NS

*NS- Not Significant, **(p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One-way ANOVA followed by Dunnett's test)*

TableNo:5.16 Water intake (ml/day) of Wistar albino rats group exposed to *INDHIRANI MAATHIRAI (IM)*

DOSE	DAYS				
	1	7	14	21	28
CONTROL	61.5 ± 8.95	61±6.23	58.5±6.23	59±8.196	61.5±3.96
LOW DOSE	72.3±3.41	74.4±1.62	76.4±1.46	75.3±1.04	79.4±1.04
MID DOSE	67.2±1.15	67.2±2.40	69.2±2.45	67.6±1.28	69.2±2.40
HIGH DOSE	72.1±1.42	74.2±1.14	75.7±1.14	75.2±1.03	73.2±1.55
P value (p)*	NS	NS	NS	NS	NS

*N.S- Not Significant, **(p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One-way ANOVA followed by Dunnett's test)*

TableNo:5.17 Food intake (gm/day) of Wistar albino rats group exposed to *INDHIRANI MAATHIRAI (IM)*

DOSE	DAYS				
	1	7	14	21	28
CONTROL	37±5.37	38.5±3.22	39.5±3.37	38.5±3.37	37±3.12
LOW DOSE	62.6±1.18	64.3±1.62	64.4±1.20	65.4±1.10	66.6±1.42
MID DOSE	64.8±4.34	65.8±1.14	66.4±1.14	67.4±1.32	67.8±2.49
HIGH DOSE	65.2±1.04	65.2±1.04	69.2±1.46	69.2±1.34	69.1±1.52
P value (p)*	NS	NS	NS	NS	NS

*N.S- Not Significant, **(p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One-way ANOVA followed by Dunnett's test)*

TableNo:5.18 Haematological parameters of Wistar albino rats group exposed to *INDHIRANI MAATHIRAI (IM)*

Parameter	Control	20 mg/kg	40 mg/kg	60 mg/kg
RBC (x 10^6/mm^3)	7.51 ± 0.16	7.46±0.26	7.69±0.46	7.3±0.43
PCV (%)	48.2 ± 1.3	46.4±3.2	48.46±4.2	44.6±2.6
Hb (%)	15.6 ± 0.19	15.3±0.8	15.6±0.4	15.2±1.7
WBC (x 10^3/mm^3)	10.12 ± 1.2	10.4±1.2	10.2±2.3	10.6±0.89
Neutrophils (%)	22 ± 4	24.3±1.6	19.3±0.67	23.6±1.34
Mononuclear cells (%)	76 ± 2	72.4±2.2	77.6±1.8	73.14±3.2
Eosinophils(%)	2.4 ± 0.6	2.4±0.12	2.12±0.16	3.2±0.46
Platelets (x 10^3/mm^3)	423.2 ± 48.8	433.4±34.3	412.6±23.46	422.68±48.2

*N.S- Not Significant, **(p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One-way ANOVA followed by Dunnett's test)*

TableNo:5.19 Biochemical Parameters of Wistar albino rats group exposed to *INDHIRANI MAATHIRAI (IM)*

Parameters	Control	20 mg/kg	40 mg/kg	60mg/kg
Albumin (g/dl)	4.8 ± 0.6	4.4±0.48	4.42±0.6	4.82±0.8
Total Cholesterol(mg/dl)	91.24 ± 1.35	101.34±1.62	97.56±2.46	96.2±5.8
Triglycerides (mg/dl)	50.15 ± 3.21	52.6±2.8	54.3±3.4	51.5±4.6
Glucose (mg/dl)	110.16 ±8.62	114.6±3.4	93.6±11.2	97.2±8.2
Sodium (mEq/L)	138.12 ± 3.14	129.2±4.3	124.2±4.6	132.6±2.3
Potassium (mEq/L)	7.2 ± 1.34	5.4±0.8	5.6±0.4	6.7±0.6

*NS- Not Significant, **(p > 0.01), * (p >0.05), n = 10 values are mean ± S.D (One-way ANOVA followed by Dunnett's test)*

TableNo:5.20 Renal function test of of Wistar albino rats group exposed to *INDHIRANI MAATHIRAI(IM)*

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
BUN (mg/dl)	19.2 ± 1.2	20.6±1.4	21.2±2.1	20.4±0.78
Urea (mg/dl)	64.24 ± 3.11	64.3±2.6	62.6±4.	59.6±3.14
Creatinine (mg/dl)	0.82 ± 0.16	0.84±0.02	0.82±0.04	0.73±0.24

*NS- Not Significant, **(p > 0.01), * (p >0.05), n = 10 values are mean ± S.D (One-way ANOVA followed by Dunnett's test)*

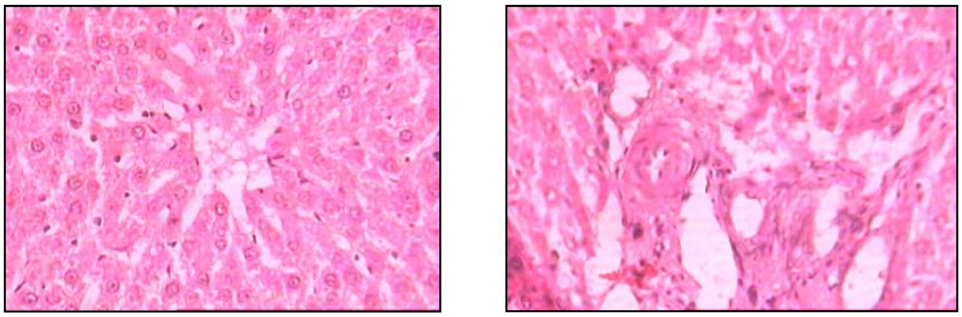
TableNo:5.21 Liver Function Test of Wistar albino rats group exposed to *INDHIRANI MAATHIRAIAM (IM)*

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
Total Bilirubin (mg/dl)	0.205 ± 0.04	0.214±0.06	0.31±0.04	0.36±0.04
SGOT (U/L)	73 ± 2.4	76.4±2.4	69.2±5.6	73.6±3.2
SGPT(U/L)	28.4 ± 1.2	27.6±4.2	28.25±4.3	29.8±2.2
Alkaline phosphatase(U/L)	102.4 ± 3.6	95.4±4.3	97.6±3.2	103.4±11.2
Protein (g/dl)	8.62 ± 1.3	8.2±1.6	8.6±0.85	8.4±1.16

*NS- Not Significant, **(p > 0.01), * (p >0.05), n = 10 values are mean ± S.D (One- way ANOVA followed by Dunnett's test)*

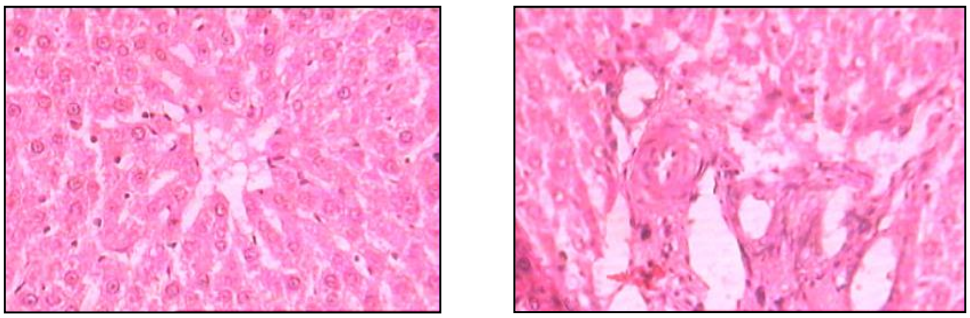
FIGURE:5.4 HISTOPATHOLOGICAL SLIDES OF IRM

NORMAL **HIGH DOSE**



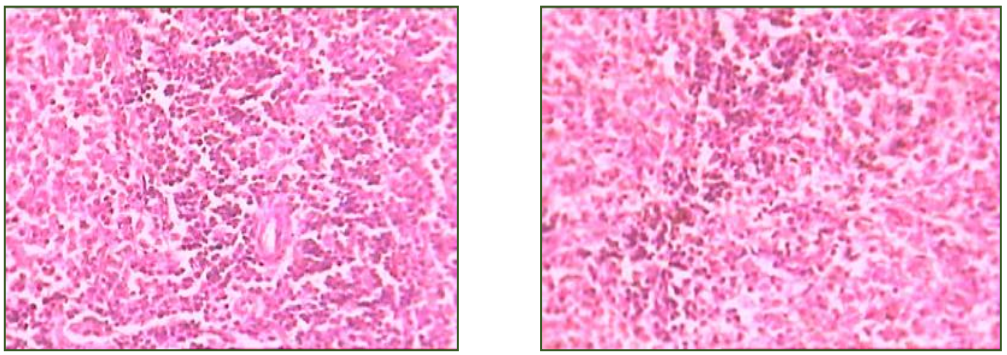
KIDNEY

NORMAL **HIGH DOSE**



LIVER

NORMAL **HIGH DOSE**



SPLEEN

Results Observations

Overall observations were similar in both male and female rats.

Clinical signs of toxicity

No clinical signs of toxicity were observed.

Mortality

No mortality was observed after 28 days repeated dose administration of *IM.* All animals survived to study termination period.

Body weight

Body weights has slight variations when compared to their initial weight. No significant alterations were observed in body weight.

Food and water consumption

No effect of treatment was noted.

Physiological activities

No changes in the general behaviour

Blood analysis

a. **Haematology**

   No treatment related effects were observed.

b. **Biological parameters**

   No treatment related effects were observed.

c. **Histological examination**

   Histological examination of organs did not show any pathological changes.

Discussion

- The acute and repeated 28 days oral toxicity studies *of Indhirani Maathirai* did not produced any toxicity signs in wistar albino rats. Daily administration of *IM* at different doses 20 mg/kg, 40mg/kg, 60mg/kg for 28 days was tolerated by the rats without any mortality and morbidity, indicates the drug tolerance.
- Hence the herbomineral formulation of *IM* was considered to be safe drug for long- term use, as revealed by toxicological studies.

PHARMACOLOGICAL ACTIVITY

Pharmacological activity explained the efficacy of the drug. Each activity is carried in animal model and the corresponding results were tabulated. These results reveal the potency of the test drug *Indhirani Maathirai* and its effectiveness in treating the diseases.

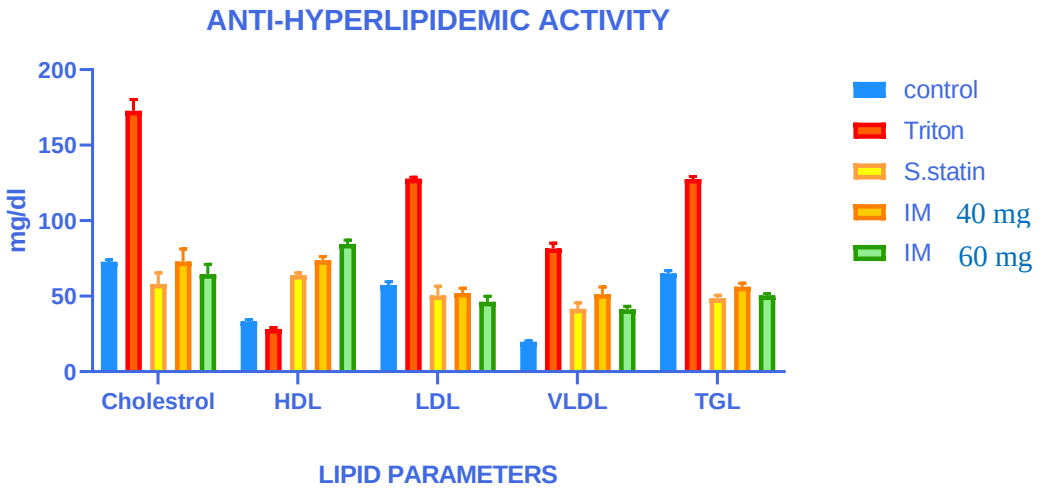
ANTI-HYPERLIPIDEMIC ACTIVITY RESULTS

Table: 5.22 Effect of *INDIRANI MATHIRAI* on lipid profile of Triton induced hyperlipidemia in rats

Lipid parameters	Group-I Normal control	Group-II Triton control rats	Group-III Hyperlipidemic + Simvastatin	Group-IV Hyperlipidemic + IM 40mg	Group-V Hyperlipidemic + IM60mg
Cholesterol(mg/dl)	72.80±1.20	172.81±7.43***	58.13±7.3**	72.90±8.4**	64±6.4**
HDL (mg/dl)	33.23±1.25	28.30±0.97**	63.90±1.59**	73.80±2.49**	84.63±2.52**
LDL (mg/dl)	57.43±2.2	127.80±0.90**	50.40±6.10**	52.10±3.20**	46.21±3.7**
VLDL (mg/dl)	19.74±0.60	81.50±3.55***	41.50±4.082**	51.25±4.87**	41.22±2.11**
TGL (mg/dl)	65.02± 1.88	127.50±1.70***	48.50±2.04**	56.25±2.40**	50.42±1.10**

*Values were expressed as Mean±SEM, n=6, Hyperlipidemic control was compared with normal control rats - Values are statistically significant at +P<0.05, ++P<0.01, +++P<0.001 Experimental groups(III & IV) were compared with hyperlipidemic control rats - Values are statistically significant at *P<0.05, **P<0.01, ***P<0.001*

Chart no:5.2 Anti-Hyperlipidemic activity of IM



INTERPRETATION

Anti-dyslipidemic activity of the test drug *Indhirani Maathirai* was conducted by Triton WR 1339 induced hyperlipidemia in animal model Wistar albino rats. In this study the triton induced group shows the levels of total cholesterol, triglycerides, LDL, VLDL which are significantly increased when compared to control groups. While HDL groups were significantly decreased in level when compared to control groups.

Triton WR-1339 (tyloxapol) is a non-ionic surfactant being widely used to explore possible mechanism of lipid lowering drugs. It causes drastic increase in serum TG and TC level due to increase in 3 hydroxy, 3 methyl glutaryl Co A (HMG Co A) reductase activity and by inhibition of lipoprotein lipase responsible for hydrolysis of plasma lipids.[116]

The test drug *Indhirani Maathirai* of 100mg/kg b. wt. showed significant changes in the lipid level. There is a significant decrease in the level of LDL, VLDL, TGL. There is a significant increase in the HDL also noted. The standard drug Lovastatin also showed the significant decrease in the LDL, VLDL, TG. But there is no significant increase in the HDL. This outcome of the trial drug may be due to the presence of flavonoids, saponins, diterpenes, gums and mucilage.

Disorders of lipid metabolism are associated with increased oxidative stress and over production of oxygen free radicals. Free radicals implicated in aetiology of several life style disorders such as atherosclerosis, stoke, diabetes and cancer.[117]

Consuming plant phytosterol containing products reduces LDL levels, greatly in individual with high baseline LDL level compared with those with normal to borderline baseline LDL levels[118]

The presence of flavonoids, alkaloids and polyphenols in the test drug reveals it has the anti-oxidant property, which will reduce lipid peroxidation[119]

The lipid lowering drugs such as Fibrates, Statins, bile acid sequestrants does not possess anti-oxidant property. Therefore, a drug having dual property of anti-dyslipidemic as well as anti-oxidant property activate from the natural product is the most preferred one. The trial drug *Indhirani Maathirai* may achieve the requirement.

Hypoglycaemic activity of *Indhirani Maathirai*

Fasting blood glucose levels was measured before the administration of extracts. The blood glucose levels were checked on 0th, 7th, 14th, and 21st day of the treatment period. Blood was collected from snipping of the rat tail. Blood glucose levels were measured and body weight was taken on day 0 and 28th day.

Table no 5.23 Animal groups and its dosage

Groups	**Treatment**
Group I	Normal Control
Group II	Diabetic control- STZ (55 mg/kg)
Group III	Diabetic control- glibenclamide (5 mg/kg)
Group IV	Diabetic control- *Indhirani Maathirai* 40mg/kg
Group V	Diabetic control- *Indhirani Maathirai* 60mg/kg

Table no 5.24: Effect of *INDIRANI MATHIRAI* on blood sugar level in Streptozotocin induced diabetic rats

Groups	Treatments	Blood glucose level (mg/dl) on post induction days			
		Initial	**5th day**	**10th day**	**15th day**
I	Normal control	70.50 ±9.23	66.45± 9.56	68.00 ±7.45	64.25 ±5.24
II	Diabetic Control	261.27±1.73***	270.36±1.59***	264.75±1.24***	259.80±1.10***
III	Diabetic rats + Glibenclamide	264.50± 1.70	252.86± 3.63	140.67±3.20*	71.54±3.65**
IV	Diabetic rats + IM-40mg/kg	272.34±3.46	266.45± 2.65	164.28±2.70*	148.68±3.38**
V	Diabetic rats + IM-60mg/kg	284.29±2.34	274±2.42	172.18±1.54*	116.20±2.20**

*Control rat - Values are statistically significant at *P<0.05, **P<0.01,***P<0.001. Experimental groups(III & IV) were compared with diabetic control rats - Values are statistically significant at *P<0.05, **P<0.01, ***P<0.001*

Chart no 5.3 Blood glucose level of IM

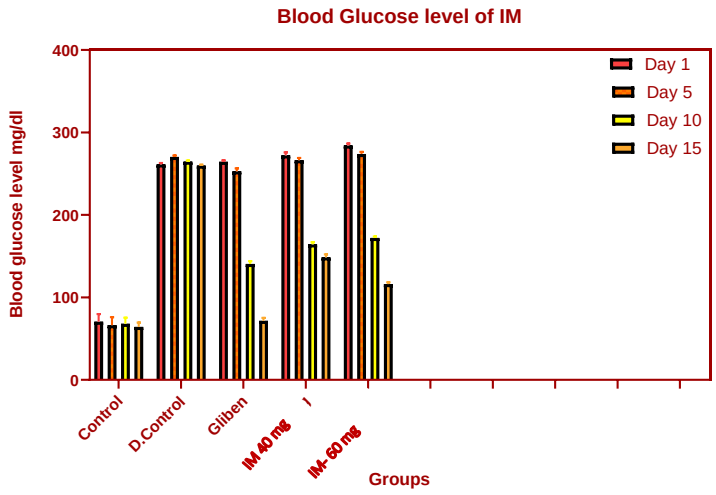


Table no 5.25: Effect of *INDIRANI MATHIRAI* on body weight in Streptozotocin induced diabetic rats

Group No.	Treatment	Body weight (g) on post induction days			
		Initial	**5th day**	**15th day**	**20th day**
I	Normal control	166.25±2.52	168.89±3.21	169.47±2.58	170.45±2.45
II	Diabetic Control	164.49±2.78	162.80± 3.34	135.80±2.10++	124.30±2.33++
III	Diabetic rats + Glibenclamide	161.40±3.26	164.10 ± 2.77	169.8±3.26*	177.30 ± 2.37**
IV	Diabetic rats + IM-40mg/kg	166.40±5.78	170.60 ± 4.23	174.80 ± 4.86*	176.40± 5.89**
V	Diabetic rats + IM-60mg/kg	220.32±5.21	200.31±2.72	204.71±2.91*	214.31±1.82**

*Control rat - Values are statistically significant at *P<0.05, **P<0.01,***P<0.001. Experimental groups (III & IV) were compared with diabetic control rats - Values are statistically significant at *P<0.05, **P<0.*

Chart no:5.4 Body Weight Changes Hypolipidemic Activity of *IM*

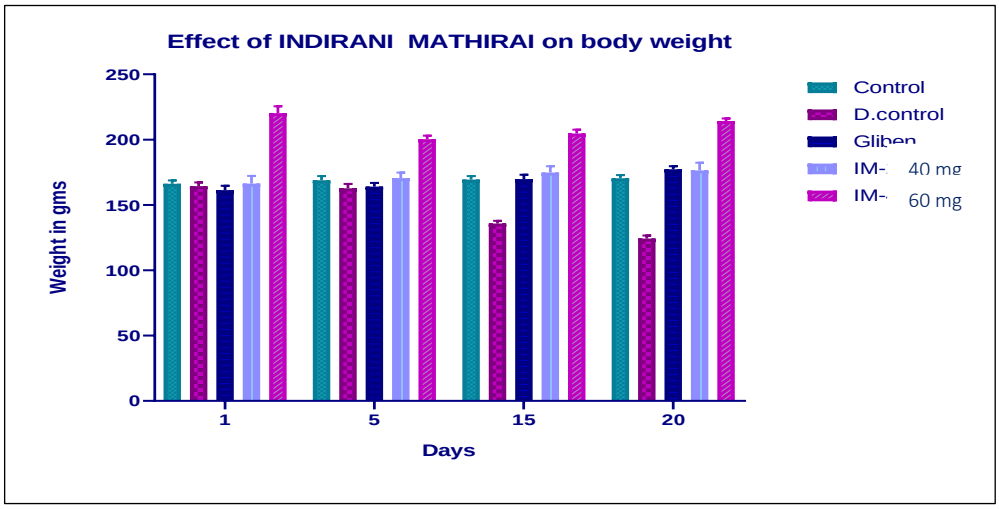
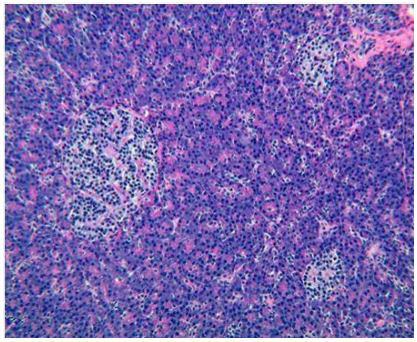
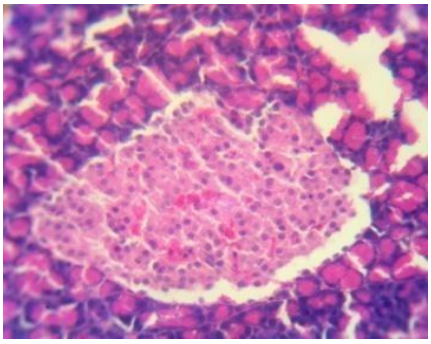


Fig no 5.5: Histopathology of Pancreas

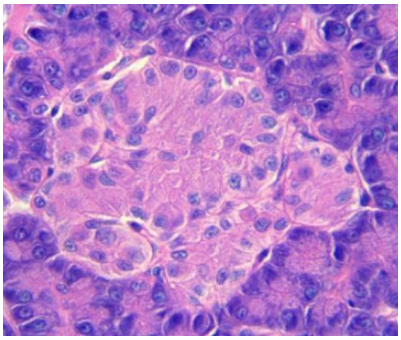
GROUP-I



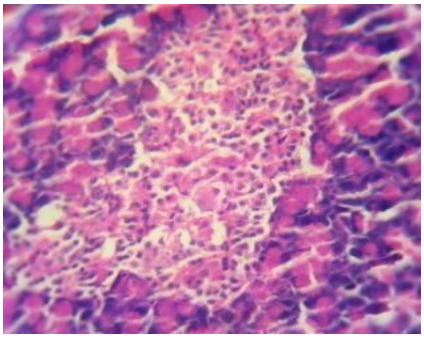
GROUP- II



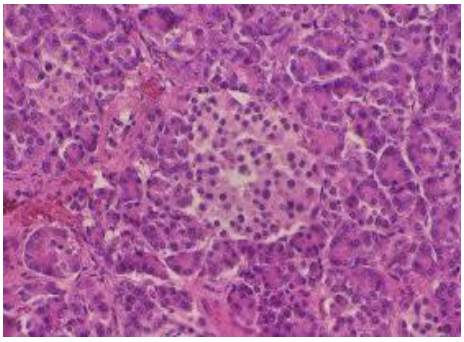
GROUP – III



GROUP IV



GROUP V



Interpretation Hypoglycemic activity

The hypoglycemic Activity of the test drug *Indhirani Maathirai* has been estimated in the streptozotocin induced diabetes in Wistar albino rat.

Administration of the streptozotocin effectively induces diabetes mellitus in the animal model which is known by the increased glucose level.

STZ, slightly cytotoxic agent of pancreatic beta cells, selectively destroys the pancreatic insulin secreting beta cells, thus leaving less active cells and resulting in diabetes mellitus. Thus, it is widely used to induce diabetes in animal models. It also interferes with cellular metabolic oxidative mechanisms [120]

Oral administration of the test drug *Indhirani Maathirai* taken in the dose of 20mg/kg showed significant decrease in the sugar level.

The possible mechanism by which the test drug *Indhirani Maathirai* brings about a decrease in the blood sugar may be by potentiation of the insulin effect of plasma by increasing either the pancreatic secretion of insulin from β-cells of the islets of Langerhans or its release.

Body weight changes

Body weight of the animal models in this study was also observed. There is decrease in the body weight of the animal treated with the control. Whereas the animal treated with *Indhirani Maathirai* shows a significant improvement in the body weight. Thus, the trial drug not only reduced the sugar level but also sustains the body weight in Diabetes Mellitus.

Histopathology slides of pancreas did not exhibit any pathological changes when compared to the control group.

Discussion on anti dyslipidemic and hypoglycemic effects of *Indhirani Maathirai*

Anti-dyslipidemic activity of the test drug *Indhirani Maathirai* was conducted by Triton WR 1339 induced hyperlipidemia in animal model Wistar albino rats. The results were tabulated above.

The test drug *Indhirani Maathirai* of 200mg/kg b. wt showed significant changes in the lipid level. There is a significant decrease in the level of LDL, VLDL, TGL. There is a significant increase in the HDL also noted. The standard drug also showed the significant decrease in the LDL, VLDL, TG. But there is no significant increase in the HDL.

In dyslipidemic conditions, hyperlipidemia is always accompanied by hyperglycemia representing risk factor for coronary heart disease and other complications. Hyperlipidemia may lead to insulin resistance. The enzymes used in biosynthesis of cholesterol was inhibited by insulin. Therefore, deficiency of insulin results in the increased level of LDL, VLDL, TG, cholesterol and decrease in HDL. Hyperlipidemia produces further vascular complications and increases the severity of diabetes.

The drug showed significant decrease in the LDL which is almost equal to the standard and marked increase in the HDL than the standard drug. Hence this anti-dyslipidemic activity supports the hypoglycemic activity of the drug *Indhirani Maathirai*, in giving an effective treatment as a whole.

ANTIOXIDANT ACTIVITY: (IN VITRO STUDY)

Antioxidants are substances that can prevent or slow down the damage to cells caused by free radicals, unstable molecules that the body produces as a reaction to environmental and other pressures. They are sometimes called "free-radical scavengers."

Antioxidants support to deactivate free radicals in our body and they thought to enhances overall health.

The cell damage caused by free radicals known as oxidative stress. Antioxidants can protect the cell against this oxidative stress.

Free radicals and their actions:

- Free radical is a molecular species, which has the capable of independent existence that contains an unpaired electron in an atomic orbital. The presence of an unpaired electron results in certain common properties that are shared by most radicals.
- Radicals are weakly attracted to a magnetic field and are said to be paramagnetic. Many radicals are highly reactive and can either donate an electron to or extract an electron from other molecules, therefore behaving as oxidants. As a result of this high reactivity most radicals have a very short half- life (10^{-6} seconds or less) in biological systems, although some species may survive for much longer.
- Oxidative stress has been linked to heart disease, cancer, arthritis, stroke, respiratory diseases, immune deficiency, emphysema, Parkinson's disease, and other inflammatory or ischemic conditions.[121]

Antioxidant Action in Dyslipidemia

In dyslipidemia, there is an elevated concentration of malondialdehyde, which is an end product of poly unsaturated fatty acid peroxidation. The increased level of malondialdehyde will produce excess of free radicals and leads to lipid peroxidation and cell oxidative injury. Free radicals adversely alter lipids, proteins and trigger number of diseases.

A balance between free radicals and anti-oxidant is necessary for proper physiological functions of the body Thus, anti-oxidant reduces lipid peroxidation rate and restore the anti-oxidant's capacity, which possibly prevent or delay the development of dyslipidemia.[122]

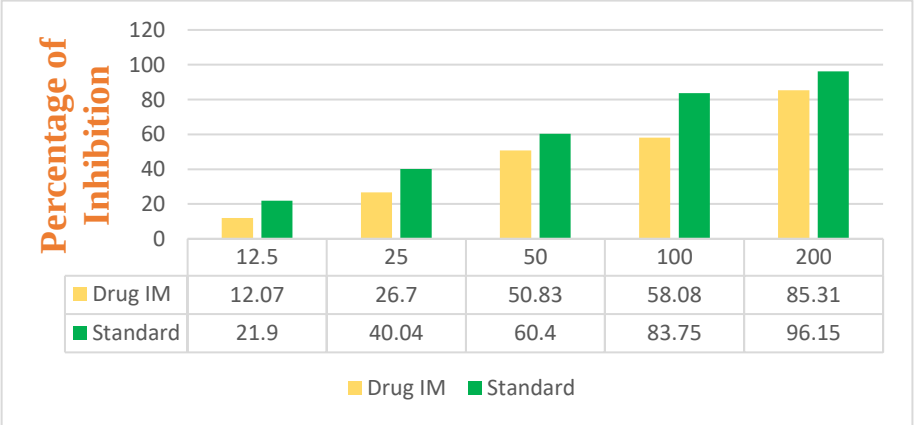
Result:

Table no. 5.26 DPPH assay on *Indhirani Maathirai*

Sample concentration (µg/ml)	Absorbance		Percentage of Inhibition	
	Drug	Standard	Drug IM	Standard Ascorbic acid
Control	0.3547	1.7983	0	0.00
12.5	0.3119	1.4044	12.07	21.90
25	0.2600	1.0782	26.70	40.04
50	0.1744	0.7121	50.83	60.40
100	0.1487	0.2921	58.08	83.75
200	0.0521	0.0692	85.31	96.15

**µg/ml: microgram per millilitre. Drug: IM (1.25-20µg/µl). Standard: Ascorbic acid (10mg/ml DMSO)*

Chart No:5 DPPH assay on *Indhirani Maathirai*



Discussion on Antioxidant activity in DPPH assay:

The values of DPPH free radical scavenging activity of the *IM* extract was given in (Table) expressed in the percentage. The extract of *IM* showed the highest DPPH scavenging activity (75.64%) at 200µg/ml and the lowest percentage of inhibition (12.80%) at 12.5µg/ml. Ascorbic acid (Standard) showed highest percentage of inhibition (96.15%) at 200µg/ml and the lowest percentage of inhibition (21.90%) at 12.5µg/ml. So, the trial drug has antioxidant property and it will supportive to the dyslipidemia condition.

6. CONCLUSION

The drug *Indhirani Maathirai* was selected to validate the safety and its efficacy for Anti-Dyslipidemic Activity in animal model (Wistar albino rats).

The ingredients of the drug were identified and authenticated by Gunapadam experts. The drug was prepared as per classical Siddha literary procedure and subjected to various studies to reveal the potency and efficacy of the drug.

The Organoleptic character and physico chemical studies were made to standardization of the drug *Indhirani Maathirai.* From the above studies, the *Indhirani Maathirai* was standardised as per AYUSH guidelines.

The analysis of biochemical, instrumental was made to know the presence of active ingredients in the drug which is responsible for its activity.

Here, the biochemical analysis showed the presence of Potassium, calcium, sodium, zinc, sulphate, nitrate by its synergistic effect, the drug as activity against the dyslipidemic condition.

In instrumental analysis, FTIR showed the peak values represents the functional groups responsible for its activity. SEM picture explained the particle size of the drug. In ICP-MS described about the absence of heavy metals and its permissible limits which showed the safety of the drug. The XRD results reveals the organic molecules present in the trial drug.

Toxicity studies revealed about the acute and sub-acute toxicity effect of the *Indhirani Maathirai* in rat models. The drug showed no toxicity and mortality in both acute and sub-acute toxicity.

According to OECD guidelines, the haematological, biochemical parameters were investigated. There were no significant changes in the functional behaviour and in the normal values. Thus, it was established the safety of the drug while administrated for prolonged time.

Pharmacological studies were done on the rat model for Anti-dyslipidemic, hypoglycaemic activity and in vitro study for Anti- oxidant activity.

In Dyslipidaemia the main pathophysiology is the increased oxidative stress which results in the tissue damage and it is the main reason for other complications. The anti-oxidant property of this drug is mainly due to the presence of phytochemicals and other active ingredients which involve in scavenging the free radicals and prevents tissue damage and other complications.

In Anti dyslipidemic activity, showed significant decreased LDL, VLDL, TGL, TC levels and marked increased HDL level in the triton WR-1339 induced dyslipidemic in Wistar albino rat models.

In Dyslipidemic conditions, hyperlipidaemia is always accompanied with the hyperglycaemia. The increase in level of LDL, VLDL, TGL, cholesterol and decrease in HDL will lead to insulin resistance. Dyslipidaemia produces further vascular complications and increases the severity of Diabetes. The drug showed significant decrease in the LDL and marked increase in the HDL than the standard drug.

In hypoglycaemic activity, there was significant decrease in blood glucose level and slightly increased body weight in the Streptozotocin induced Wistar albino rats.

In Anti-oxidant activity, there was significant effect of drug when compare to standard in DPPH assay.

Thus, by surviving all the above factors, it is concluded that the drug *Indhirani Maathirai* is safe and potent drug with Anti-dyslipidemic and hypoglycaemic activity with rich Anti-oxidant activity. This will support the treatment and management of dyslipidaemia and its complications. In treating, the dyslipidemic condition with this drug it has a synergistic effect of controls on lipid profile, blood sugar level and also reduces the oxidative stress and gives a complete treatment for dyslipidemic condition and its complications

7. SUMMARY

The herbal drug *Indhirani Maathirai* was taken as a test drug for Dyslipidaemia from the classical Siddha literature Sarabenthirar Siddha Maruthuva Sudar, 4th edition was edited by Dr.M.Sowrirajan and it was exposed to validate the safety and its efficacy for treating Anti-dyslipidemic, Hypoglycemic and anti-oxidant activity in animal model. The trial drug was subjected to various studies through which the efficacy of the drug is proved.

The preparation of trial drug was standardized by physico chemical and bio chemical analysis.

The bio chemical analysis of the drug shows the presence of zinc, calcium. phosphate. These elements are responsible for anti-dyslipidaemic activity. HPLC analysis disclose the percentage of elements presence in the drug

FTIR analysis revealed the presence of C=O groups, -C=C group, which indicates functional groups present in the sample. XRD Interprets the molecular structure of the sample.

SEM analysis showed the size of the drug in micro particles which denotes that the trial drug could have potent drug delivery. ICP-MS study reveals safety of the drug.

The acute and sub-acute toxicological studies proved that the drug is nontoxic and safe.

The pharmacological studies conducted by in vivo method revealed that the drug has effective results in Anti-dyslipidaemic, Hypoglycaemic and anti-oxidant activity.

8. FUTURE SCOPE

The trial drug ***Indhirani Maathirai*** was taken from the classical Siddha literature Sarabenthirar Siddha Maruthuva Sudar, 4th edition was edited by Dr.M.Sowrirajan. Its validation for Anti-dyslipidaemic, Hypoglycaemic and anti-oxidant activity was completed at preclinical level. The results showed the assurance of Anti-Dyslipidaemic activity against hyperlipidaemia. Clinical trials are required to understand the exact molecular mechanism of action and it would be a safer drug for Dyslipidaemia in worldwide.

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Government Siddha Medical College

Arumbakkam, Chennai – 600 106

CERTIFICATE

Certified that the samples submitted for identification by **Dr.P.Gnanavel** PG Scholar, Department of *Gunapadam,* Government Siddha Medical College, Arumbakkam, Chennai-600 106, were identified as:

Ingredients of *Indhirani maathirai*:

1. *Butea monosperma (Purasan ver)*
2. *Euphorbia antiquorum (Kalli ver)*
3. *Caesalpinia crista (Kalarchi ver)*
4. *Brassica juncea (Kadugu)*
5. *Ferula asafoeitida (Perungayam)*
6. *Piper nigrum (Milagu)*
7. *Acacia catechu (Karungali kattai)*
8. *Potassium nitrate (Vediyuppu)*

Date:

Place: Chennai **PG Department of Gunapadam**

The Tamil Nadu Dr.M.G.R.Medical University

69, Anna Salai, Guindy, Chennai - 600 032.

This Certificate is awarded to Dr/~~Mr~~/~~Mrs~~.....P. GNANAVEL.....

For participating as ~~Resource~~ ~~Person~~ / Delegate in the Twenty Fourth Workshop on

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For ***AYUSH Post Graduates & Researchers***

Organized by the Department of Siddha

The Tamil Nadu Dr. M.G.R. Medical University From 24th to 28th April 2017.

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All India Council for Technical Education, New Delhi

L. Uday Metha
Secretary & Correspondent

Dr. Grace Rathnam, M.Pharm, Ph.D
Principal

APPROVAL CERTIFICATE

This is to certify that the project titled "THE SCIENTIFIC VALIDATION OF ANTI-DYSLIPIDEMIC, HYPOGLYCEMIC AND ANTI-OXIDANT ACTIVITIES OF SIDDHA HERBO MINERAL FORMULATION "***INDHIRANI MATHIRAI***" IN ANIMAL MODEL" has been approved by the 53rd IAEC.

IAEC no: 03/321/PO/Re/S/01/CPCSEA dated 12/10/2018

C.L. Baid Metha College of Pharmacy ★ CHENNAI-97 ★ Thoraipakkam

Dr.P.Muralidharan

(Member Secretary)

SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

CHENNAI – 600 119

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CENTRE FOR LABORATORY ANIMAL TECHNOLOGY AND RESEARCH

(CPCSEA Approved)

WORKSHOP ON

TOXICOLOGICAL PROFILING AND ASSESSMENT OF TOXICITY OF DRUGS ON LAB ANIMALS

CERTIFICATE

This is to certify that Dr./~~Mr./Ms.~~ P. GNANAVEL

of Govt. Siddha Medical College, Chennai has participated in the two-day workshop on "**TOXICOLOGICAL PROFILING AND ASSESSMENT OF TOXICITY OF DRUGS ON LAB ANIMALS**" organized by the Centre for Laboratory Animal Technology and Research, Sathyabama Institute of Science and Technology, Chennai during 31st January – 1st February 2018.

B. Sheela Rani

Chair Person & Coordinator
Dr. B. SHEELA RANI
Director (Research)

Convener
Dr. R. SELVARAJ
Scientist In-charge

NATIONAL SEMINAR ON

"RESEARCH METHODOLOGY AND PUBLIC HEALTH INITIATIVE THROUGH SIDDHA SYSTEM OF MEDICINE"

(RM & PHISSM – 2018)

6TH & 7TH APRIL 2018

प्रमाण पत्र
CERTIFICATE

सिद्ध क्षेत्रीय अनुसन्धान संस्थान
पूजप्पुरा, तिरुवनंतपुरम, केरल
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केन्द्रीय सिद्ध अनुसन्धान परिषद्
(आयुष मंत्रालय, भारत सरकार)
CENTRAL COUNCIL FOR RESEARCH IN SIDDHA
Ministry of AYUSH, Govt. of India

This is to certify that Dr./Shri/Smt. Gnanavel P. GSMC, Chennai has participated/~~presented~~ a paper entitled.....

..... in the National Seminar on "Research Methodology and Public Health Initiative through Siddha System of Medicine" (RM & PHISSM – 2018) organized by Siddha Regional Research Institute, Thiruvananthapuram on 6th & 7th April 2018 at Dr. M R DAS Convention Centre, Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, Kerala.

डॉ. ए. कनगराजन / Dr. A. Kanagarajan
Organizing Secretary and Convenor

RM & PHISSM 2018

प्रो.डॉ.आर.एस. रामस्वामी / Prof. Dr. R. S. Ramaswamy
Director General, CCRS

GOVT. STANLEY MEDICAL COLLEGE, CHENNAI-1

STAN MEU & IEC

DEPARTMENT OF PHARMACOLOGY

&

THE TAMILNADU DR.M.G.R MEDICAL UNIVERSITY

CERTIFICATE

This is to certify that Dr / Mrs / Miss / Mr. P. GNANAVEL

has Participated in the CME on **"GOOD CLINICAL PRACTICE AND CLINICAL TRIALS"** conducted by the Department of Pharmacology, Govt. Stanley Medical College, Chennai on **04.07.2018 & 05.07.2018.**

Accredited by the Centre for Accreditation, The Tamilnadu Dr.MGR Medical University.

and has been awarded **10 credit points** under **Category II.**

DEAN
Govt. Stanley Medical College,
Chennai – 1.

Head of the Department
Department of Pharmacology
Govt. Stanley Medical College

GOVERNMENT SIDDHA MEDICAL COLLEGE

Arumbakkam, Chennai, 600106

This certificate is awarded to Dr./~~Mr./Ms.~~ .....P. GNANAVEL.....

for participating as a ~~resource person~~ / delegate in the seminar on

"Orientation to research Methods"

Organised by Suzhumunai Scientific forum Government Siddha Medical College on 22 March 2018

Dr. P. Manickam
Scientist E
(ICMR) National Institute of Epidemiology

Dr. K. Kanakavalli
Principal
Govt. Siddha Medical College

One day National Seminar on

"ROLE OF NEURO INFLAMMATORY MEDIATORS IN NEURODEGENERATIVE CONDITIONS IN DRUG DISCOVERY"

Conducted by

Department of Pharmacology
K.K.COLLEGE OF PHARMACY

Gerugambakkam, Chennai-600128.

Co Sponsored by

The Tamil Nadu Dr. MGR Medical University, Chennai

CERTIFICATE

This is to Certify that Dr/ Mr/Ms. P. GNANAVEL has Participated as Resourse person / Chair person / Delegate in the Seminar in ***"Role of neuro inflammatory mediators in neurodegenerative conditions in drug discovery"*** on 24th January 2018

Dr.R.PRAKASH
Organizing Secretary

Dr.V.VAIDHYALINGAM
Director

Dr.A.MEENA
Convenor