A STUDY ON SCIENTIFIC EVALUATION OF SIDDHA POLYHERBAL FORMULATION *"NANNARI MATHIRAI"* FOR HEPATOPROTECTIVE ACTIVITY ON CCL_4, ETHANOL INDUCED HEPATOTOXICITY AND ANTIOXIDANT ACTIVITY IN WISTAR ALBINO RATS.

The dissertation Submitted by

Dr.T. MONIKA

Reg. No: 321612106

Under the Guidance of

Dr.M.D.SARAVANADEVI, M.D.(S).,

Dissertation submitted to

THE TAMILNADU DR. M.G.R MEDICAL UNIVERSITY

CHENNAI-600032

In partial fulfilment of the requirements

For the award of the degree of

DOCTOR OF MEDICINE (SIDDHA)

BRANCH-II GUNAPADAM

POST GRADUATE DEPARTMENT OF GUNAPADAM

THE GOVERNMENT SIDDHA MEDICAL COLLEGE

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GOVT. SIDDHA MEDICAL COLLEGE,

CHENNAI-106

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **A Study on Scientific Evaluation of Siddha Polyherbal formulation "*NANNARI MATHIRAI*" for Hepatoprotective Activity on CCL_4, ETHANOL induced Hepatotoxicity and Antioxidant activity in Wistar Albino Rats** is a bonafide and genuine research work carried out by me under the guidance of **Dr. M.D. Saravana Devi, M.D (S),** Professor, 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.

Date :

Place : Chennai. Signature of candidate

Dr.T.MONIKA

GOVT. SIDDHA MEDICAL COLLEGE,

CHENNAI-106

CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled **A Study on Scientific Evaluation of Siddha Polyherbal formulation "*NANNARI MATHIRAI*" for Hepatoprotective activity on CCL_4, ETHANOL induced hepatotoxicity and Antioxidant activity in Wistar Albino Rats** 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.T.MONIKA.** Under my supervision and guidance and the dissertation has not formed the basis for the award of any Degree, Diploma, Associateship, Fellowship or other similar title.

Date:

Place:

Seal & Signature of the Guide

Dr.M.D.SARAVANADEVI.M.D(S).

GOVT. SIDDHA MEDICAL COLLEGE, ARUMBAKKAM,

CHENNAI-106

ENDORSEMENT BY THE HOD AND PRINCIPAL OF THE INSTITUTION

This is to certify that the dissertation entitled **A Study on Scientific Evaluation of Siddha Polyherbal formulation "*NANNARI MATHIRAI*" for Hepatoprotective activity on CCL_4, ETHANOL induced hepatotoxicity and Antioxidant activity in Wistar Albino Rats** is a bonafide work carried out by **Dr.T.MONIKA** under the guidance of **Dr. M.D. Saravana Devi, M.D(S),** Post Graduate Department of Gunapadam, Govt. Siddha Medical College, Chennai - 106.

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Place:

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Dr.R.Meenakumari.MD(S).

Signature of the Principal

Dr.R.Meenakumari.MD(S).

ACKNOWLEDGEMENT

First and foremost, I would like to thank the Almighty for his showers and grace and the strength and caliber he gave in handling and understanding the difficulties during the tenure of this work and enabled to complete this tough task.

I would like to acknowledge and extend my cordial credit to the following persons who have made the completion of this dissertation study fruitful.

I hereby pledge my sincere devotion and respect to all the Siddhars who guided me eternally and dynamically.

I express my sincere thanks to our Principal **Prof**. **Dr. R. Meena kumari, M.D(S)**, Govt. Siddha Medical College, Chennai for her permission to perform this study and also for her valuable ideas and support throughout the course of the study.

It is a genuine pleasure to express my deep sense of thanks and gratitude to my mentor and guide **Dr.M.D.Saravana Devi, M.D(S), Professor, Dept of PG Gunapadam,** Govt Siddha College, Chennai. Her dedication and keen interest above all his overwhelming attitude to help his students had been solely and mainly responsible for completing my work. His timely advice, meticulous scrutiny, scholarly advice and scientific approach have helped me to a very great extent to accomplish this dissertation work.

I feel intensely grateful to **Dr. R.Meena kumari, M.D(S), Head of Department, PG *Gunapadam*,** Govt. Siddha Medical College, Chennai, for her valuable guidance, suggestions for completion of my whole study.

I would like to express my special gratitude to **Dr. R. Karolin Daisy Rani, M.D(S), Lecturer, PG *Gunapadam*,** Govt. Siddha Medical College, Chennai, for her valuable guidance, suggestions for completion of my whole study.

I owe my special thanks and sincere gratitude to my advisor **Dr.V.Velpandian, M.D(S).,Ph.D.,** for his support towards my dissertation topic discussions and selection. His guidance helped me in all time of my research work.

I express sincere thanks to our **Former Principal and presently the Director General Prof. Dr. K. Kanakavalli, M.D (S)., Central Council For Research In Siddha, Ministry of AYUSH, Chennai, Govt.of India** for her guidance towards this study.

I wish to express my thanks to co-guide **Dr. A. Ganesan, M.D(S).**, Asst. Lecturer, Department of PG Gunapadam for his valuable ideas and suggestions to my study.

I would like to utilize this opportunity to thank our Dept staffs **Dr. K.Nalina Saraswathi, M.D(S), Dr. S. Shankar M.D(S)**, **Dr. K.Rajamma Devi Sorubarani M.D(S)** for their support and guidance.

I cordially register thanks to **Dr. Muralidaran, Ph.D**., C.L Baid Metha College of Pharmacy, Assistant Professor advanced Centre for research for helping in the pharmacological study and advanced research for his assistance in the toxicity studies.

I extended my gratitude to the animal **Ethical Committee Members** for their approval to do animal studies in pre-clinical studies.

I wish to express my profound gratitude to **Dr. R. Rajesh M.Phil, Ph.D.,** Director, Biogenix research center, Trivandrum, for his valuable work in Antioxidant activity.

I acknowledge my thanks to **Mr. Selvaraj, M.Sc, M.Phil,** HOD, Department of Bio-Chemistry, Govt. Siddha Medical College, Chennai.

I would like to acknowledge **Dr.N.Kabilan, MD(s),Ph.D, The TamilNadu Dr.M.G.R Medical University** for doing my physicochemical analysis.

I express my thanks to our Librarian **Mr.V.Dhandayuthapani, B.Com, M.Lib.Sc** and staffs for their kind co-operation for my study.

I am also thankful to **Mrs. H.M.Sudha Merlin, D.Pharm,** Pharmacist, Post Graduate Department of Gunapadam for her kind co-operation in purification and preparation of the trail drug for my study and successful completion of dissertation.

I would like to thank **Vice Chancellor, The TamilNadu Dr.M.G.R Medical University** for giving permission to carry out my dissertation work and to the Additional Chief Secretary and Commissioner of Indian Medicine and Homeopathy Department, Arumbakkam, Chennai-106, for giving consent to do the dissertation.

I would like my deepest thanks to my seniors for her valuable suggestions in my study. I should express my gratefulness to them for lending their helping hands whenever needed during the course of the study.

Although I wish to thank extends beyond the limits of this format, I would like to thank my friends, and well-wishers for their support and inspiration throughout the dissertation work.

Last but not least , I would like to pay high regards to all my family members, **Father Dr.N.Thillaiarasan, Mother Mrs.T.Malarkodi,** Sister **T.Abinaya and My Batchmates especially Dr.R.Tamilselvan, Dr.L.Ilavarasai and V.Ponnaiya** for their sincere encouragement and inspiration throughout my research work and lifting me uphill this phase of life. I owe everything to them. Besides this several people have knowingly and unknowingly helped me in the successful completion of this project.

ABBREVIATIONS

Alb	Albumin
ALP	Alkaline phosphatise
ALT	Alanine transaminase
AST	Aspartate transaminase
ANOVA	Analysis Of Variance
APAP	Acetyl-Para-AminoPhenol
BDL	Bile Duct Ligation
BHA	Butylated Hydroxy Anisole
BHT	Butylated Hydroxy Tolune
BUN	Blood Urea Nitrogen
CAT	Catalase
CCL_3	Trichloromethyl
CCL4	Carbon tetra chloride
CD	Conjugated Dienes
CMC	Carboxy Methyl Cellulose
CPCSEA	Committee for the purpose of control and supervision of experimental animals.
CYP2E1	CytochromeP4502E1
DC	Differential count
DDT	Dichloro-Diphenyl-Tricholoroethane
Dep.	Deposits
DMN	Dimethyl Nitrosamine
DMSO	Dimethyl sulfoxide
E	Eosinophil
EDS	Energy Dispersive X-ray Spectroscopy

ABBREVIATIONS

ERCP	Endoscopic Retrograde Cholangiopancreatography
ESR	Erythrocyte Sedimentation Rate
FLD	Fatty Liver Disease
FPC	Few Pus Cells
FTIR	Fourier Transform Infrared Spectroscopy
GIT	Gastro Intestinal Tract
GPx	Glutathione peroxidase
GSH	Glutathione
Hb	Haemoglobin
Hcl	Hydrochloric acid
HNO3	Nitric acid
HPLC	Higher Performance Liquid Chromatography
HSC	Hematopoietic Stem Cells
IAEC	Institutional Animal Ethical Committee
ICMR	Indian Council of Medical Research
ICP-OES	Inductively Coupled Plasma Optic Emission Spectroscopy
L	Lymphocyte
MAO inhibitors	Monoamine Oxidase inhibitors
MDA	Malondialdehyde
MHI	Muller Hinton Medium
NASH	Non Alcoholic Steato Hepatitis
NAPQI	N-Acetyl-P-Benzoquinoneimine
NM/NNM	Nannari Mathirai
OECD	Organisation for Economic Co-Operation & Development
P	Polymorphs

ABBREVIATIONS

PT	Prothrombin Time
RBC	Red Blood Cell
ROS	Reactive Oxygen Species
SEM	Scanning Electron Microscope
SEM	Standard error mean
SGOT	Serum Glutamic Oxaloacetic Transaminase
SGPT	Serum Glutamic Pyruvic Transaminase
SOD	Superoxide Di Mutase
TA	Total Protein
TAA	Thioacetamide
TB	Total Bilirubin
TBARS	Thiobar Bituric Acid Reactive Substances
TC	Total Count
TCA	TetraCos15enoic acid
TIBC	Totaliron Binding Capacity
TP	Total Protein
UV	Ultra Violet
WHO	World Health Organization
XRD	X-Ray Diffraction studies

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INTRODUCTION

1. INTRODUCTION

Siddha system of medicine is a holistic medical system which has been developed gradually during the consecutive era. The word **Siddha** has been derived from the word **Siddhi** which literally means **"Attaining perfection in life"** or even it may be termed as **"Heavenly Bliss"**[1]**.**Traditional medicine is the sum total of knowledge, skills and practices based on theories beliefs and experiences indigenous to different cultures that are used to maintain health as well as to prevent, diagnose, improve or treat physical and mental illness. "An Ounce of prevention is worth a pound of cure".

Siddha is widely practiced in the Tamil speaking areas of South India. Over the last several decades, the place for Modern allopathic system of medicine in many segments of Health care system are followed by worldwide it is because of its advantage of managing medical emergencies. Siddha system of medicine has ability to cure the root cause of disease and hence effective in treating severe chronic disease. It is a well-known fact that, it is safe, time tested, without any drastic adverse effects. Siddha pharmacology consist of 32 internal and 32 external medicines**.** [2]

Medicinal plants are naturally gifted with invaluable bioactive compounds which form the backbone of traditional medicines. The actions are due to the presence of phytochemical components like glycosides, tannins, alcohols, aldehydes, etc [3]. Those chemical components are not only for the discovery of therapeutic agents but are also an asset for the future genera. "**Siddha is simply the science of understanding your own unique self and being who you are meant to be".**

Siddha medicine preparations includes Herbs, Metals, Minerals, Animal by products [3]. However, no scientific data regarding the identity and effectiveness of these herbal products are available. Now this is a time to standardization and prove the efficacy of these medicines through universally accepted scientific parameters. Whatever the system of medicine we are taking first of all its toxic substances affect the liver. So, we must protect the Liver by giving Hepatoprotective drugs.

The Greek word for Liver *"HEPATO"* or *"HEPAR".* Liver is the Largest vital organ in our body. It weighs about 1400 grams in adults. The Liver's mass comprises One-fourth or One-fifth of a total adult body's weight and one-eighteenth of the body's

weight during Infancy. It is the heaviest internal organ and the Largest gland in the Human body. It is located in Right upper quadrant of the abdominal cavity, it rests just below the diaphragm, to the right of the stomach and overlies the Gallbladder [4].

The Liver performs the metabolic homeostasis of the body as well as biotransformation, detoxification and excretion of many endogenous and exogenous compounds, including Pharmaceutical and environmental chemicals. Therefore, maintenance of a healthy liver is essential for the overall well-being of an individual [5]. The Liver's highly specialised tissues regulate a wide variety of high-volume biochemical reactions, including the synthesis and breakdown of small and complex molecules, many of which are necessary for normal vital functions.

Liver also involved in the synthesis of fatty acids, lipoproteins, amino acids and plasma proteins which is known as albumin, fibrinogen and prothrombin. Liver also plays an important role in storage and metabolism of fat-soluble vitamins and irons. Water soluble vitamins, vitaminB12 are also stored in liver [6].

The liver is a metabolically active organ responsible for many vital life functions. The liver is thought to be responsible for up to 500 separate functions, usually in combination with other systems and organs [7]. It interacts with cardiovascular and immune system and it also serves as an excretory organ.

Behind the liver there is a small organ called the gallbladder, which function is to store bile produced by the liver and empty it into the small intestine, digestion and absorption. Bile pigment, cholesterol and drugs are excreted by liver. Liver also regulate endocrine functions of hormones. Various hormones like insulin, glucagon, growth hormone and GI hormones are degraded by liver. Kuffer cells are most important phagocytic cells which is present in liver. These Kuffer cells plays an important role in removing unwanted materials from the circulation. Endocytosis is the mechanism by which these materials are removed. Liver cells are called Hepatocytes.

Hepatocyte plays an important role in the metabolism of drugs and xenobiotics. For effective elimination, the drug and the metabolite must be made hydrophilic. Reabsorption of these metabolite by renal tubules is dependent on its hydrophobicity. Many drugs and metabolites are hydrophobic. The more hydrophobic

a substance is, the more likely it will be reabsorbed. The liver converts them into hydrophilic compounds so the metabolite will easily remove from the body[6a].

The liver is the vital organ and supports almost every other organ in the body. Because of its strategic location and multidimensional function, the liver is also prone to many diseases [8].

Liver diseases caused by variety of factors. Such as viruses, alcohol, drugs particularly paracetamol and drugs used to treat cancer. Fast food, environmental pollutants and sedentary life style also caused liver disease. If the liver become diseased its results loss of vital functions like depression of immune system, sluggish digestive system, obesity, fatigue which can cause significant damage to the body. The most common liver diseases are Hepatitis, Cirrhosis, Alcohol and drug related liver diseases,FLD,Liver tumour, Rey's syndrome [9]. In recent years, people widely suffered from liver diseases. In India, liver disease is the tenth most common cause of death.

According to the latest WHO data published in May 2014, Liver disease deaths in India reached 2,16,865 or 2.44% of total deaths. The age adjusted death rate is 21.96 per 1,00,000 of populations. Population ranks India#61in the world [10]. According to WHO, about 46% of global disease and 59% of the mortality is because of chronic diseases and almost 35millon people in the world die of chronic liver diseases. Liver diseases rate steadily increasing over the years [11].

The major sign of liver disease is Jaundice (Hyperbilirubinemia) which is known as yellowish discolouration of skin and mucous membrane [12]. In Siddha system of medicine, "Jaundice" is correlated with "*Pitha Kaamaalai"* or "*Manjal Kaamaalai*". As per the text *Maanjal Kaamaalai* is of 13 types [13]. Clinically Jaundice is divided into three types. They are Prehepatic, Hepatic and Post hepatic. Many of liver diseases are accompanied by Jaundice[12a]. A healthy liver is essential for the healthy life. So it is important to take care of liver in a healthy way. One of the natural and healthy way to take care of liver is Siddha system of medicine. In this medicine various polyherbal preparations are available to treat liver diseases.

Herbal drugs are significant source of hepatoprotective drugs. Mono and polyherbal preparations have been used in various liver disorders. According to one

estimate, more than 700 mono and poly-herbal preparations in the form of decoction, tincture, tablets and capsules from more than 100 plants are in clinical use[7b]. In the modern scenario, diseases are becoming drug-resistant and scientists are studying possible roles of plant-based drugs for screening lifesaving drugs. Treatment options for common liver diseases such as cirrhosis, fatty liver, and chronic hepatitis are problematic. The effectiveness of treatments such as interferon, colchicine, penicillamine, and corticosteroids are inconsistent at best and the incidence of side effects profound. it may also cause various other diseases. So, there is a need for safe and potent hepatoprotective drug to treat various liver disease.

In recent years synthetic drugs are showing more adverse effect, to overcome this problem researchers are trying to avoid this risk of those drugs. Whenever a drug is prescribed to a patient, they are facing risk of side effect, so long-term use of these drugs patient should be careful. But in herbal medicine the toxicity of herbal drugs is less when compared with the synthetic medicines[7c]. Due to known side effect, patient preferred to take alternative medicine which is natural and healthy way [14]. The process of healing and regeneration of liver cells are support and promote by herbal preparations with less side effects.

Several hundred plants have been examined for use in a wide variety of liver disorders. Just a handful has been fairly well researched. The latter category of plants includes: *Silybum marianum* (milk thistle), *Picrorhiza kurroa* (kutkin), *Curcuma longa* (turmeric), *Camellia sinensis* (green tea), *Chelidonium majus* (greater celandine), *Glycyrrhiza glabra* (licorice) and *Allium sativa* (garlic).

The *Hemidesmus indicus* is well known plant because of its medicinal property its easily reliable and commonly used plant all over the world. The World Health Organization (WHO) estimates that 80% of the world health populations presently use herbal medicines for some aspect of primary health care[7d]. Although various drugs are flooding in the market used as hepatoprotective agent. In that many of them having adverse effects. The author is interested to describe about *Nannari Matirai* for hepatoprotective activity(Kaamaalai-Jaundice). I think it will be more effective to treat liver disease.

Siddha system is a treasure which contains enormous herbal preparations. They are highly effective and devoid of adverse effects. For this reason, Siddha system

of medicine has been drawing global attention in recent times. Liver disorders are increasing day by day. So, there is a need and demand for effective, safe and accurate treatment of liver diseases. Therefore, it is not surprising that a considerable interest has been developed in search of potent hepatoprotective drug from herbal origin.

Based on above facts, one of the Polyherbal preparation mentioned in Siddha literature is *Nannari Mathirai*. I hope this Siddha medicine *Nannari Mathirai* will be effective drug for the cure of liver disease*(Jaundice-Kaamaalai).* So, I interested to validate the *Nannari Mathirai*, a unique herbal preparation for its hepato-protective activity.

This Siddha drug **"*Nannari Mathirai*"** is used for ***(Jaundice-Kaamaalai)***is yet remained unexplored for its exact chemical, pharmacological, toxicological profile in terms of scientific research. To fill these scientific lacunae, the present work will be undertaken to evaluate the chemical, toxicity profile and pharmacological potentials of *Nannari Mathirai* in various hepatotoxicity models.

AIM AND OBJECTIVES

2.AIM AND OBJECTIVES

AIM:

The present study was focused to validate the Hepatoprotective (Jaundice-Kaamaalai) of ***NANNARI MATHIRAI*** in experimental animals with CCL_4, Ethanol induced hepatotoxicity and Anti-oxidant activity.

OBJECTIVE:

The scientific study on *Nannari Mathirai* was carried out in the following stages.

- Collection of different texts in Siddha and modern literature relevant to this study.
- Description of pharmacognostic features of the plant in this formulation including the taxonomic identification, collection, purification of the plant etc.
- Preparation of the trail drug *Nannari Mathirai* as per classical Siddha text.
- Standardisation of the trial drug *Nannari Mathirai* by means of physiochemical analysis, phytochemical analysis.
- Evaluate Bio chemical analysis of the test drug to derive acidic and basic radicals.
- To estimate the presence of elements, functional groups and particle size through instrumental analysis of the trial drug. Evaluating the microbial load and activity of the trail drug.
- Evaluation of the Acute and 28 days epeated oral toxicity study of the test drug according to OECD guidelines.
- Evaluation of Pharmacological study of the drug through the following activities:

1) Hepatoprotective activity of CCL_4 induced Hepatotoxicity.

2) Hepatoprotective activity of Ethanol induced Hepatotoxicity.

3) Anti-oxidant property.

REVIEW OF LITERATURE

3.1 REVIEW OF LITERATURE

DRUG REVIEW:

The trial drug "***NANNARI MATHIRAI"*** was taken from the Siddha literature "***KANNUSWAMY PARAMBARAI VAITHIYAM***" for treating Kaamaalai (Jaundice).P.no:141,Edition year:1948.

The ingredients of the drugs are.

1. Nannari (*Hemidesmus indicus*.)
2. Seeragam *(Cuminum cyminum)*
3. Elam (*Elettaria cardamomum*)
4. Perunseeragam *(Foeniculum vulgare)*
5. Sevvagathi (*Sesbania grandiflora*)

3.1.1. GUNAPADAM ASPECT

1. NANNARI

Scientific name: *Hemidesmus indicus*.

Synonyms:

Angarimooli, Narunetti, Paathaalamooli, Kopaagu, Saaribam, Paarkodi, Neerundi, Kaananuchaari, Krishnavalli, Chaariyam.

Vernacular names:

Tam	: *Nannari*
Eng	: Indian sarasaparilla
Mal	: *Nannari*
Kan	: *Sugandha-palada*
Sans	: *Sariba*
Hindi	: *Magrabu*

Properties and action:

Suvai	: *Inippu* (Sweet)
Thanmai	: Cold potency
Pirivu	: *Inippu* (Sweet)

Action:

- Diaphoretic
- Alternative
- Tonic
- Demulcent

- Diuretic

Parts used: Root

General characters:

சலதோடம் பித்தமதி தாகம் உழலை

சலமேறு சீதமின்னார் தஞ்சூ- டுலகமதிற்

சொன்னமது மேகம் புண் சுரமிவையெ லாமொழிக்கும்

மென்மதுர நன்னாரி வேர்.

- - தேரையர் குணவாகடம்

Indications:

Hemidesmus indicus cures pitha diseases, insect bite, thirst, sinusitis, diabetes mellitus, syphilis.

Important preparations

- Nannari karpam,
- Nannari manappagu.

Medicinal uses:

- The root is soaked with 30ml of warm water and give it thrice a day cure indigestion and impotence.
- Root powder mixed with cow's milk cures oliguria. It also gives along with cumin seeds [15].

2.SEERAGAM

Scientific name:*Cuminum cyminum*

Synonyms:

Asai, Chiri, Upakumbapeesam, Narcheeri, Thutthasambalam, Piraththi-vika,Pithanasini, Bosanakudori, Meththiyam.

Vernacular names

Tam : *Asai,Nar cirakam,pittanacini,pocanakutari*

Eng : Cumin Seed, Cumin

Hindi : *Jira, Safed jira*

Kan : *Jirage, Bilejirege*

Mal : *Jeerakam*

Mar : *Pandhare jire*

Sans : *Svetajiraka, Ajaji, iraka.*

Tel : *Jilakaraka, Tella jilakara.*

Parts used: Seeds

Properties:

Suvai : karppu, sweet

Thanmai : Coolant

Pirivu : Inippu (Sweet)

Actions :

- Astringent,
- Stimulant,
- Stomachic,
- Carminative.

General character:

பித்தமெனு மந்திரியைப் பினனப் படுத்தியவன்
சத்துருவை யுந்துரந்து சாதித்து - மத்தனெனும்
ராசனயு மவென்று நண்பைப் பலப்படுத்தி
போசனகு டாரிசெயும் போர்.

தேரன் வெண்பா.[17]

Indication:

It enhances hunger and it induces digestion.

Important preparation:

- Seeraga karpam.
- Asai chooranam
- Panchadhebageni legiyam
- Mayiliragathi chooranam
- Asaithylam

Medicinal uses:

- It improves Gut motility and helps in digestion. It is used to increase urine flow to relieve bloating.
- It helps in preventing boils, rashes, pimples and excess toxic content.
- Cumin also increases the release of bile from the liver. Bile helps digest fats and certain nutrients in our gut. Add cumin seeds in 1400ml of heated gingely oil, and then cool it. This oil when taken for head bath cures headache, eye diseases, giddiness and vomiting.
- Cumin seeds are also one of the ingredients of Panchadeepakini Legiyam[18]

3.ELAM

Scientific name: ***Elettaria cardamomum*** –

Synonyms **:** Onchi, *Gorangum, Thudi.*

Vernacular names:

Tamil : *Elam*

English : Cardamom

Tel : *Elakulu*

Mal : *Elattari*

Kan : *Elakki*

Sansk : *Ela*

Hin : Elachi

Partsused **:** Seeds

Properties:

Suvai : *Kaarppu* (Pungent)

Thanmai : *Veppam* (Hot potency)

Pirivu : *Karppu* (Pungent)

Actions:

- Carminative
- Stomachic
- Expectorant
- Tonic.

General characters:

தொண்டை வாய்கவுள் தாலுகு தங்களில்
தோன்றும் நோயதி சாரம்பன் மேகத்தால்
உண்டை போலெழுங் கட்டி கிரிச்சரம்
உழலை வாந்தி சிலந்தி விஷஞ்சுரம்
பண்டை வெக்கை விதாகநோய் காசாமும்
பாழுஞ் சோமப் பிணிவிந்து நட்டமும்
அண்டை யீளைவன் பித்தம் இவைக்கெல்லாம்
ஆல மாங்கமழ் ஏல மருந்ததே

- பதார்த்த குண சிந்தாமணி.[19]

Indications:

It cures cough, diarrhoea, haemorrhoids, dyspepsia, vomiting, anal fissure.

Medicinal uses:

- Powdered form of seeds of cardamom, dried ginger, cloves, cumin seeds cure stomach ache, ulcer.
- Roasted seeds of cardamom, omam and cumin seeds then they are powdered and given to cure indigestion [20].

4. PERUNSEERAGAM

Scientific name : *Foeniculum vulgare*

Other name : Sombu, Venseeragam

Vernacular names:

Tamil	: Sombu
Eng	: Fennel
Tel	: Peddajilakarra
Mal	: Perunjirakam
Hind	: Saumph
Kan	: Badhesoppu

Parts used: Flower, Seeds, Root.

Properties and action :

Suvai : Aromatic

Thanmai : Veppam (Hot potency)

Pirivu : Karppu (Pungent)

Actions:

- Carminative
- Stomachic.

General characters:

யோனிநோய் குன்மம் உரூட்சைமந் தம்பொருமல்
பேனமுறு காசம் பீலிகமிரைப் - பீனெளரை
சேர்க்கின்ற வாதமுபோஞ் சீர்பெரிய சீரகத்தால்
மூக்குநோ யில்லை மொழி

- அகத்தியர் குணவாகடம்.

Indications:

Cures Uterine disorders, Abdominal pain, Fever, Indigestion, Dyspepsia, Cough, Liver diseases, Tonsillitis, Rhinitis.

Medicinal uses:

- Sombutheenir: 1 part of Anise seed is mixed with 20 parts of water and allowed to the process of distillation.
- Dose:15ml to 20 ml.
- Anise seed is slightly fried and powdered. About 2gms of this powder along with sugar cures uterine disorders, Abdominal pain.
- Distilled water taken from anise seeds flower, when given for children cures indigestion, Worms and loose stools[18a].
- Fennel seeds oil is used to relive cold, bronchitis and as a massage oil to cure joint pains. The decoction of seed is used to increase breast milk secretion in nursing mothers

5. SEVVAGATHI

Scientificname : *Sesbania grandiflora*

Synonmys:

Accham, Muni, Kareram.

Vernacular names:

Tamil : Agathi

Eng : Sesban

Tel : Avise

Mal : Agatti

Pers : Agti

Kan : Agashi

Parts used: Leaves, Flower, Bark, Root.

Properties:

Suvai : Bitter

Thanmai : Coolent

Pirivu : Karppu (Pungent)

Actions:

- Antidote
- Refirgerant
- Laxative
- Vermifuge.

General characters:

நாடிரற் பனிப்பீனும் நாசிக் கறைக்கதழ்வை
யோடித் துடைத்துள் ளொளியாற்றும் - பாடெத்
தவரகத்தி னுந்துணங்கைத் தண்டா மயிலே
துவரகத்திப் பூக்காண் டுதி.

- அகத்தியர் குணவாகடம்.

Indications:

It controls nasal bleeding and it excretes excessive heat from our body.

Medicinal uses:

- It can be used in detoxification of the body, if there is chronic toxicity due to improper dietary patterns over a period of time.
- The leaves prove to be helpful in worm infestations and bleeding disorders [21].

3.2.1 BOTANICAL ASPECT

1. HEMIDESMUS INDICUS

Scientific classification:

Kingdom : Plantae

Division : Magnoliophyta

Class : Magnoliopsida

Order : Gentianales

Family : Apocyanaceae

Genus : *Hemidesmus*

Species : *indicus*

The plant description:

It is semi erect and sometimes prostrate shrub with woody aromatic roots. The slender, numerous stems is terrate with thick nodes. The leaves are variable, opposite and short petioled. They are oblong, elliptic to linear lanceolate. The flowers are green on the outside and purplish inside. The stem and branches of the plant twine anticlockwise and the roots have a camphor like smell. Flowers may be greenish yellow to greenish purple on the outside and yellow to light purple on the inside. It has fused corolla that numbers twice that of the calyx.

Pistil is bicarpellary with free ovaries many ovuled and having distrint styles.

Distribution:

The species is distributed throughout the tropical and subtropical parts of India, especially in upper Gangetic plains, Bengal, Madhya Pradesh and South India.

Part used: Leaves, Flower, Bark, Seed, Roots.

Chemical constituents:

The root contains hexatriacontane, lupeol, octacosanoate, α-amyrin, β-amyrin, acetate, sitosterol, coumarino-lignoid-hemidesminine, hemidesmin 1 and hemidesmin 2, six pentacyclic triterpenes including two oleaneces and three ursenes.

Properties and uses:

- Root bark of this plant known as "Indian sarsaparilla" are prescribed in dyspepsia, loss of appetite, nutritional disorders, fever, skin diseases and ulceration especially those of syphilitic origin, constitutional syphilitis, chronic rheumatism and leucorrhoea.
- Hot infusion of root bark with milk and sugar is used as tonic especially for children in chronic cough and diarrhoea. [22]

2. CUMINUM CYMINUM

Scientific Classification:

Kingdom : Plantae

Class : Asterids

Order : Apiales

Family : Apiaceae

Genus : *Cuminum*

Species : *cyminum*[23]

The Plant Description:

A slender, annual, glabrous herb, leaves twice or thrice 3-partite, ultimate segments filiform, Flowers in compound umbels bracteole, bracts linear rigid. Calyx teeth small, subulate, unequal shaped. Fruit cylindric shape, tip narrowed, seeds somewhat dorsally compressed.

Distribution:

Extensively cultivated as a cold-season crop on the plains and as summer crop on the hills in Northern India, Himalayas, Punjab, Baluchistan, Kashmir, Kumarun, Garhwal and Chamb. The plant also imported from Persia and Asia minor.

Part used : Seeds

Macroscopic Characters:

Fruit, a cremocarp, often separated in to mericarps, brown with light coloured ridges, ellipsoidal, elongated, about 4 to 6 mm, high and 2 mm,wide.

Chemical Constituents

Cuminaldehyde, Cuminin,1,3 - β - menthadien -7-al, 1,4- β - menthadien -7-al, β-cymene, terpinene, β-pinene,7-1(O-β-D-galalacturonide)-4,5dihydroxy, glycosides, luteolin and apigenin. Fatty oil, resin, mucilage, a valuable essential oil thymine contains cuminol or cumic aldehyde 56 p.c, a mixture of hydro carbons, cyme or cymol, terpene etc (22a).

Medicinal uses

- Seeragam, Chukku, Elam and Nellimulli powder 1-part sugar ½ part mixed with and used for reduce pitham.
- Cumin seeds soaked in karisalai leaf juice then make it dried powder mixed with sugar and chukka powder used for the treatment of jaundice and vatha Disease[24a].

3. ELETTARIA CARDAMOMUM

Scientific Classification:

Kingdom	: Plantae
Class	: Monochlamydeae
Order	: Zingiberales
Family	: Zingiberaceae
Genus	: *Elettaria*
Species	: *cardamomum*

The Plant Description:

A tall herbaceous perennial, with branching subterranean roots, stock, from which arise a number of upright leafy shoot. It is 5-18 ft. height and bearing alternate, elliptical or lanceolate sheathing leaves. Flowers are borne in panicles and arising from the base of vegetative shoots.

Fruits are trilocular capsules, fusiform to ovoid in shape and pale green in yellow in colour, its containing 15-20 seeds. Cardamom seeds have pleasant aroma and pungent taste.

Distribution*:*

It is a native of the evergreen forest of south India, growing wild in the Western Ghats.

Parts used: Seeds.

Chemical Constituents:

The seeds contain Volatile oil, Cineol, Terpineol, Terpinene, Limonene and Sabinene.

Medicinal Uses

- Powdered form of seeds of cardamom, dried ginger, cloves, cumin seeds cures stomach ache, ulcer.
- Roast seeds of cardamom, omam and cumin seeds then they are powdered and given to cure indigestion [25].

4. FOENICULUM VULGARE

Scientific classification:

Kingdom	: Plantae
Division	: Angiosperms
Class	: Dicotyledons
Order	: Umbellale
Family	: Apiaceae
Genus	: *Foeniculum*
Species	: *vulgare*. [26]

The Plant descripition:

A stout, erect, glabrous aromatic herb upto 1.8m in height, leaves 3-4 times pinnate with very narrow linear or subulate segments. Flowers are small. Fruits are oblong, ellipsoid or cylindrical, straight or slightly curved greenish or yellowish brown.

Distribution:

Cultivated throughout India.

Parts used:

Flowers, Seed,Fruits.

Chemical constituents:

Ascorbic acid, riboflavin, a, ß and tocopherol, ß tocotrienol, choline, trigonelline,p-cymene, anethole, anisaldehyde, camphene, estragole, fenchone, fenchene, foeniculin, methylchavichol, chlorogenic, hydroxyl benzoic and hydroxyl cinnamic acids[27].

Medicinal uses:

- The fruits are sweet, acrid, bitter, emollient and refrigent.
- They are used as anthelmintic, aromatic, carminative, emmanogogue, stimulant and stomachic.
- It is beneficial in diseases related to chest and kidney.
- Juice is used to improve eyesight, hot infusion given in amenorrhoea.
- Oil from seeds is anodyne, diuretic, stimulant and vermicide.

5. SESBANIA GRANDIFLORA

Scientific classification:

Kingdom : Plantae
Division : Angiosperms
Class : Dicotyledons
Order : Fabales
Family : Fabaceae
Genus : *Sesbania*
Species : *grandiflora*

The Plant descripition :

It is a fast-growing tree, the leaves are regular and rounded and the flowers white, Red or pink. The fruits look like flat, long, thin green, beans. Leaves are 15-30 cm long with leaflets in 10-20 pairs or more and an odd one. Flowers are oblong, 1.5-10 cm long in lax, with two to four flower racemes.

Distribution:

South east Asia to Northern Australia, India, Sri lanka.

Parts used: Leaves, Flower, Bark, Root.

Chemical constituents:

Three isoflavanoids, isovestitol, medicarpin, sativaan along with another known compound, betulinic acid were isolated from the root of sesbania grandiflora.The structures of the isolated compoundswere characterised by means of spectroscopic techniques.(UV,IR,MS).Pharmaceuticals.2012 Aug;5(8):882-889.

Medicinal uses:

- The juice from the flowers is used to treat headache, headache, head congestion, or snuffy nose. As a snuff, the juice is supposed to clear the nasal sinuses. The root is used for inflammation.
- The bark is used in infusions for small pox. The powdered bark is used for ulcers of the mouth and alimentary canal.

3.2 DISEASE REVIEW

3.2.1 SIDDHA ASPECT DISEASE REVIEW:

(KAAMAALAI) JAUNDICE

Other names:

In *Siddha* literature *Kaamaalai* also known as *Manjal kaamaalai, Pitha kaamaalai, Kamala, Kamila.*

Kaamaalai is one among those diseases which occur due to derangement of pitha uyir thathu. In this disease eyes, tongue, urine and the whole body will get yellowish discolouration.

Aetiology:

Yugi vaidhya chinthamani states that jaundice will occur when one consumes more food which stimulates *pitha thathu,* drinking unhygienic and impure water, seasonal changes and also indulges in excessive sexual activity under the conditions of severe anaemia are the causes of this disease.

Premonitory symptoms:

In this disease, excessive salivation, nausea, bitterness of tongue, anorexia, indigestion, dryness of the body and shrinking of skin like a frog. After that, eyes, nail beds, face and skin and also urine become yellow in color.

“பருகவே உள்ளங்கா லுள்ளங் கைகள்
பகர்முகங்ண் ணுடம்புமிக வவளுப்பு காணுங்
கருகவே கால்கைக யோய்ச்ச லாகுங்
கனமாக நடுக்கியே இளைப்புண் டாக்குஞ்

சுருகவே மலந்தானும் வறண்டு கட்டுந்
தூயமுக மஞ்சளிட நிறம தாகும்
வெருகவே வீக்கமாய்க் களைப்புண்டாகும்
மிகக்காது மந்தந்ததலை கனப்புண்டோகும்."

-யூகிமுனி

Again, in this disease, palm, sole, face, eyes and the body will become pale; there would be severe fatigue in the extremities, shivering of the body, frequent dyspnoea, constipated and hardened feces, and yellowish discolouration of face, oedema, lassitude, deafness and heaviness of head [28].

Classification of the disease:

In *Siddha* literatures, it is classified in to 13 varieties. They are as follows;

- *Vatha kaamaalai*
- *Pittha kaamaalai*
- *Kaba kaamaalai*
- *Vatha kaba kaamaalai*
- *Pittha kaba kaamaalai*
- *Mukkutra kaamaalai*
- *Manjal kaamaalai*
- *Uthu kaamaalai*
- *Varal kaamaalai*
- *Azhagu kaamaalai*
- *Sengamala kaamaalai*
- *Kumba kaamaalai*
- *Gunma kaamaalai*

Among the 13 varieties of jaundice, seven types are curable and eight types are incurable. Curable types include

1.Udhu kaamaalai,

2.Varal kaamaalai,

3.Manjal kaamaalai,

4.Pitha kaamaalai,

5.Kabha kaamaalai,

6.Vatha kabha kaamaalai,

7.Pitha kabha kaamaalai.

The other varieties are not easily curable.

Treatment:

Intake of variable food and other deeds stimulates *pittha thathu*, *pitha* increases in its strength, joins *kaba* and becomes *pitha kaba* factor. This factor spoils the spreading *vayu (paravu kal- Vyanan)* and prevents it from doing its normal work and thus it spoils the strength of blood. Because of this, liver gets affected and also the bile unable to flow in its normal route as there is an obstruction.

Hence the bile mixes with the blood and jaundice occurs. The *vatha* factor gets affected and the disease occurs due to *pitha vatha* factor. Apart from this, the other *vayu* (gases) also get spoiled.

Hence the duty of the doctor is to set right the altered *pitha kabam* and *pitha vatham*, in order to make the bile flow in its normal route and to increase the strength of blood by suitable treatment. The altered spreading *vayu* (gas) and other *vayu* (gases) should be brought to normal and made to do the normal regular work. Then medicines for the disease should be given.

To induce vomiting:

Since vomiting is a symptom in this disease, it should not be induced though it is advocated in Siddha literatures.

To induce diarrhoea:

To stimulate normal and easy bowel movements, the following substances which have laxative action can be given:

- *Phyllanthus emblica* (*Indian gooseberry)*
- *Terminalia chebula* (*Kadukkai)*
- *Anthemides flower* (*Simai samanthi flower)*
- Buds of rose (Roja*)*
- Grapes (Kodi *munthrigai)*
- *Picorrhiza kurroa* (*Kadukurohini)*
- Bark, leaves and flower of purging *cassia* (*Sarakkondrai)*
- Root of Indian jalap (*Sivathai)*
- Flower of neem (*Veppam pu)*
- *Tinospora cordifolia* (*Senthil)*

A decoction of the above substances may be made and given for jaundice for

laxative purpose[28a].

Other medicines for vomiting and diarrhoea:

1.Malakudara oil:

The medicinal *malakkudara* oil in a dose of one teaspoonful with a small quantity of milk can be given at bed time. The next day morning faeces will be passed out easily.

2.Malakudara mezhugu:

Malalakudara wax (*mezhugu*) of the size of a fever nut (*kazhal kay-Caelpinia bonduc*) can be given at bet time. Easy motion will occur in the morning.

3.Thithippu bedi mezhugu or legiyam:

Sweet diarrhoea wax (*thithippu bedhi mezhugu*) or sweet diarrhoea *leghiyam* (*thithippu bedhi leghiyam*) should be taken at bed time as a size of Indian gooseberry. Easy to motion will occur in the morning. It may be given in suitable doses in the morning and evening.

4.Sanjivi tablets:

If the motion is not passed out properly by the above methods, 2 *Sanjivi* tablets given along with hot water can be given for children. Motion will be passed easily.

For adults, one among the following may be given in a dose of 2 tablets with hot water in the morning alone. Faeces will be passed easily. The medicines are *vajjirakandi* tablet, *attabairava* tablet, *suka viresana* tablet, *jivarathina* tablet, *virechana bhupathy* and *lavangathy* tablet. When sanjivi tablet along with leaf juice of *Euphorbia nivula* (*ilaikkalli*) is given, vomiting and diarrhoea will be induced. Vomiting for two or three times will occur. Diarrohoea will also occur. Along with vomit or faeces, the bile fluid will also come out.

5.Marukkarai kaai:

The unripened fruit of *Randia dumetorum* (*marukkarai kaai*) in its tender reddish form may be taken. It may be soaked in lime juice and leaf juice of *Euphorbia nivula*(*ilaikkalli*) for two days in each. Then it can be taken out and dried.

This can be ground and made into powder. ½ pinches can be given in the morning alone. Diarrhoea and vomiting will occur [29].

Medicines for Jaundice (Siddha Aspect):

1.*Karisalai chooranam* (Powder of *Eclipta alba*):

- Powder of dry leaves of *Eclipta alba* (*Karisalai*) 35 gm
- Powder of epicarp of *Terminalia chebula* (*Kadukkay*) 15gm
- Powder of pepper (*Milagu*) 10 gm
- Powder of the root of *Lowsonia alba* – henna plant (*Maruthonri*) 10 gm.

Mix the all the above ingredients and ground it in the mortar to make it as a fine powder. Take 2gm of the above, add 200mg of rusted iron *Chenduram* and take 2 times a day with buttermilk. Within 5 to 10 days, jaundice will get cured.

2.*Karisalai matthirai* (Tablet of *Eclipta alba*):

- *Eclipta alba* - one hand full
- Black cumin
- Long pepper
- Pepper (*Piper nigrum)*
- Garlic (*Allium sativam*)

Take each ingredient ¼ *palam*(8.5 gm) , Ground them all in the mortar and make tablets in the size of *Solanum torvum* (*Sundai*), dry them in shade and put them in a widemouthed bottle; pour good quality gingily oil and close it with a lid and put it in sunlight. Take each tablet twice a day. Jaundice along with oedema will get cured. Tamarind and salt should be avoided [(30)].

3.Jaundice powder (another process):

- Charred turmeric – one part
- Cubeb – fried, pounded and powdered – one part
- Cumin seeds – fried, pounded and powdered – one part
- Cane sugar powder – 4 parts
- Calx of gypsum – 1 part

Mix all the above five ingredients and make it as a powder. Jaundice will be cured when this powder is taken in doses of 10 to 15 *Kundri* two to three times a day when with cow's milk or goat's milk or honey or in orange juice. It can also use as adjuvant to any other calyx or *Chenduram* or any other medicines prescribed for jaundice. By this, anaemia, oedema and liver diseases will get cured [31].

4.*Kizha nelli ney* (Ghee of *Phyllanthus amarus*):

- Juice of *Phyllanthus amarus* 1.35 liter (one measure)
- Cow's ghee1.35 liter (one measure)
- Cubeb (*Valmilagu*)
- Nutmeg (*Myristica fragrances*) (*Jathikkay*)
- Cardamom (*Eletaria cardamom*) (*Elam*)

Each 17.5 gm (1/2 *palam*) is taken. All of them may be ground in a mortar with milk. Then this may be heated and processed for oil (*Thylam*) and filtered. 16 ml (the standard volume of a small spoon (*Uchikarandi*) may be consumed in the morning and evening.

Jaundice will be cured. Salt-free diet is essential[31a].

5.*Karisalainey* (Ghee of *Eclipta Alba)*

- Juiceof *Eclipta* 1.35 litre (1Measure),
- Cow's ghee 1.35 litre (one measure),
- *Thirikaduku* (dry ginger, pepper, long pepper) 35 gm (one *palam*),
- *Hyoscyamus niger*(*Kurosani omam*) 8.5 gm (1/4 *palam*),
- Cubeb (*Valmilagu*) 17.5 gm (½ *palam*).

Ghee may be prepared as per the literature. Mix the above two and take in doses of ¼ to ½ teaspoon two or three times a day with cow's milk or its buttermilk or goat's milk or its buttermilk.

It can be given with honey also or it can be taken separately. It can be used as an adjuvant for any other Calyx or *Chenduram*. It will give an excellent cure for spleen and liver enlargement also[31b].

6.*Ponnangani* (Ghee of *Alternanthera sessilis* for jaundice):

The root of *Alternanthera sessilis* may be collected and macerated on a stone slab. It is taken in elumichai alavu and be soaked into 4 liters of cow's milk and is allowed to mix with it. The next day, the butter from it may be taken out and consumed. Jaundice will be cured.

7.*Arunelli* (*Phyllanthus distichus* for jaundice):

Phyllanthus distiches in the size of the fruit of *Alexandrian laurel* (*punnai kay*) may be taken and macerated on a stone slab. This may be given along with ¼

of a measure of sour buttermilk for 3 days in the morning. Jaundice will be cured. Rice with goat's milk can be taken. Salt should be avoided.

8.*Nandiyavattai* (Powder of East Indian rose for jaundice):

Pericarp of the root of multiple-layered East Indian rose, pericarp of the root of Indian jalap which is cooked in milk, the outer part of *Terminalia chebula* – equal quantities of these 3 things may be dried and pounded in a stone mortar and the powder may be filtered by a muslin cloth. If this powder in a three-finger pinch is consumed with hot water, jaundice, predominant *Pittha* condition, and oedema can be cured.

9.*Kaiyanthakarai karkam* (Green paste of *Eclipta alba*):

Tender leaves of *Eclipta alba*, tender leaves of *Coldenia procumbens* (*Seruppadai*), turmeric and pepper- equal quantities of the above 4 substances may be taken and macerated on a stone slab.

A lime- sized paste may be consumed along with goat's urine. Jaundice and oedematous jaundice will be cured.

10.*Pirandai* (*Cissus quadrangularis* medicine for jaundice):

Tender leaves of *Cissus quandrangularis*, Pepper,*Acorus calamus*, dry ginger equal quantities of these ingredients may be taken and macerated on a stone slab. An areca nut sized ball of this paste may be covered in rice bran and consumed.

11.*Aridradhi churnam parpam* (Medicine of turmeric, etc. and calx for jaundice):

Turmeric (*Manjal*), pericarp of *Terminalia chebula* (*Kadukkay thol*), pericarp of *Terminalia belerica* (*Thandrikay thol*) and pericarp of Indian gooseberry (*Nelli mullai*), *Pircorzhiazha kurroa* (*Kadugurohini*), rock salt (*Induppu*) equal quatities of the above substances may be taken, dried and pounded in a stone mortar. One *Verukadi* (cat's foot print) quantity mixed with water may be consumed. Jaundice will be cured.

Leaves of *Pavonia zeylanica* (*Chitramutti*), bark of *Cassia fistula* (*Konrai*), *Syzygium cumini* (*Naval*), coriander leaves, purified iron powder, leaves of *Indigofera tinctoria*(*Avuri*) – equal quantities of the above substances may be taken and Calx of the above may be prepared. The Calx may be put in a mortar and ground with lime juice. The paste in the size of the *Solanum torvum(Sundaikkay*) may be consumed. Jaundice will be cured.

12.*Amanakkilai marundhu* (Leaves of castor plant for jaundice):

Tender leaves of castor plant (*Aamanakku kozhundhu*), tender leaves of *Trianthema protulacasturm* (*Saranai kozhundhu*), dry ginger and white onion- equal quantities of the above things may be taken and macerated on a stone slab. The paste may be mixed with buffalo curd and consumed. Jaundice will be cured.

13.*Arappodi marunthu* (Medicine of iron filings for jaundice):

Iron filings may be put soaked in the bark juice of *Terminalia arjuna* (*maruthu*) and allowed to absorb the juice. Then the iron filings may be taken out and dried in the sun. Then it is powdered. Take 40 *Terminalia chebula* fruits. Remove the seeds. Fry the epicarp portion in a vessel and make it charred, and then it is powdered. Equal quantities of this powder along with the iron powder may be consumed. Ascitis, anaemia and jaundice will be cured.

14.*Puvarasu kozhundhu ilai kudineer* (Decoction of tender leaves of *Thespesia populnea for* jaundice):

Put the iron pot on a stove. 8 gm of pepper may be put into the pot and fried. Make a powder of it keeping it in the pot itself. Then pour ½ measures of tender coconut water into the pot over the pepper. Let it be boiled in the pot. One handful of tender leaves of *Thespesia populnea* may be taken and squeezed to get juice. The juice may be poured into the pot when it starts boiling and the squeezed leaves may also be put in to the pot itself. Within a few minutes, the water portion in the pot will be reduced to 1/8 of a measure.

Take the pot away from the stove and make it cool. Filter the decoction. The decoction is to be consumed when it is slightly hot. If it is prepared and consumed for three times, jaundice will get cured immediately.

Saltiest and pungent diet should not be taken at the time of drinking this decoction for the whole day. Sweet substances are also to be reduced. Before consuming this medicine, the required food can be eaten [32].

The procedure for consuming this medicine:

If the first dose of medicine is taken at 6pm of the day. The second dose is to be taken at 6 am of the next day. The third dose is to be taken at 6pm on the second day. For the whole day, salt-free rice porridge alone should be taken.

The next day morning a little of cow's butter may be put on the head and after half an hour, bath should be taken in cold water.

Then the needed food can be taken. Those who suffer from this disease for a longer duration can consume 3 doses of this medicine with a break of one day. For children, dosage should be adjusted suitably according to their age, body type and strength [33].

15.Decoction for jaundice:

Flower of *Madhuca longifolia* (*Iluppai*), *Tinospera cordifolia* (*Sinthil*), neem petioles, petioles of *Adathoda vasica*, clearing nut, *Vettiver ziznaioides*- take equal quantities of the above and prepare a decoction. The decoction may be taken along with sugar, ghee and honey. Jaundice will get cured.

70 gm of *Sivanatha* powder along with cold water or honey or with the three fruit (three myrobalans-*kadu, tanri* and *nelli*) decoction may be consumed. Jaundice will get cured.

Diet for jaundice:

- Salt should be restricted according to the strength of the patient. Porridge without salt and tamarind is good. Twice boiled rice can be given.
- As stated in above, when the bile flow is obstructed in the bile duct, fat will not be digested as bile is not available for digestion. Ghee, butter, oil and all other fatty substances should be avoided until the disease is cured completely.
- Tender vegetables which are not fried with mustard and gingelly oil, green, fruits, butter milk and goat's milk can be taken in. Ginger paste can be added to diet to induce appetite. To the diet, cane juice, lime juice and ginger can be added.
- Smoking, tobacco chewing, and alcohol–like substances should be fully avoided. Rest is essential until the disease is completely cured [34].

Some of the medicinal plants to treat Liver diseases:

***Foeniculum vulgare*:**

Hepatoprotective activity of *Foeniculum vulgare* (Family of Umbelliferae) essential oil was studied using a carbon tetrachloride induced liver fibrosis model in

rats. The hepatotoxicity produced by chronic carbon tetrachloride administration was found to be inhibited by *Foeniculum vulgare* essential oil with evidence of decreased levels of serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and bilirubin.

***Indigofera tinctoria*:**

A bioactive fraction, indigtone (12.5100mg/kg p.o) characterized as trans-tetracos15enoic acid (TCA), obtained by fractionation of a petroleum ether extract of the aerial parts of *Indigofera tinctoria* (Family of Fabaceae), showed significant dose dependent hepatoprotective activity against paracetamol (200mg/kg i.p) and CCl4 (0.5ml/kg p.o mixed with liquid paraffin 1:1) induced liver injury in rats and mice. Pre-treatment reduced Hexobarbitone induced sleep time, and zoxazolamine induced paralysis time. Pre and post treatment reduced levels of transaminases, bilirubin, TG, LPO and restored the depleted GSH in serum.

***Rubia cordifolia*:**

Rubiadin isolated from *Rubia cordifolia* Linn, (Family of Rubiaceae) at a dose of 50, 100 and 200 mg/kg was administered orally once daily for 14 days in rats. The substantially elevated serum enzymatic activities of serum GOT, GPT, ALP and GGT; decreased activities of glutathione S transferase and glutathione reductase were restored towards normalization in dose dependent manner which were induce by CCl4 treatment in rats. It also significantly prevents the elevation of hepatic MDA formation and depletion of reduced GSH content in the liver.

Solanum nigrum:

The effects of *Solanum nigrum* (Family of Solanaceae) extract (SNE) was evaluated on thioacetamide (TAA) induced liver fibrosis in mice. Mice in the three TAA groups were treated daily with distilled water and SNE (0.2 or 1.0 g/kg) via gastrogavage throughout the experimental period. SNE reduced the hepatic hydroxyproline and αsmooth muscle actin protein levels in TAA treated mice. SNE inhibited TAA induced collagen (α1) (I), transforming growth factor-β1 (TGF-β1) and mRNA levels in the liver. Histological examination also confirmed that SNE reduced the degree of fibrosis caused by TAA treatment. Oral administration of SNE significantly reduces TAA induced hepatic fibrosis in mice, probably through the reduction of TGF-β1 secretion.

***Terminalia catappa*:**

Punicalagin and Punicalin isolated from the leaves of *Terminalia catappa* L. (Family of Combretaceae) reduced hepatitis by reducing levels of AST and ALT which increased by APAP administration in rats [35].

3.2.2. MODERN ASPECT OF DISEASE REVIEW:

Liver Diseases:

Definition

Liver diseases is any disturbances of liver functions that causes illness. The liver is responsible for many critical functions within the body and should it become diseased or injured, the loss of those functions can cause significant damage to the body.

Liver diseases also referred as hepatic disease. Liver diseases are a broad term recounting any number of diseases affecting the liver. Many are escorted by jaundice caused by increased levels of bilirubin in the system.

Liver disease may be classified as: -

- Hepatitis, inflammation of the liver, instigated mainly by various viruses but also by some poisons, autoimmunity or hereditary conditions.
- Cirrhosis is the foundation of fibrous tissue in the liver, replacing dead liver cells. The death of the liver cells can be affected by viral hepatitis, alcoholism or contact with other liver toxic chemicals.
- Haemochromatosis, a hereditary disease causing the accretion of iron in the body, eventually leading to liver damage [36].
- Cancer of the liver (Primary hepatocellular carcinoma or Cholangio carcinoma and metastatic cancers, usually from other Parts of the gastrointestinal tract).
- Wilson's disease, a hereditary disease which reasons the body to retain copper. Primary sclerosing cholangitis, an inflammatory disease of the bile duct, likely autoimmune in nature.
- Primary billiary cirrohisis, autoimmune disease of slight bile ducts.
- Budd-Chiari syndrome, complication of the hepatic vein.
- Gilbert's syndrome, a genetic syndrome of bilirubin metabolism, found in about 5% of the population.

- Glycogen storage disease type II, the build-up of glycogen causes liberal muscle weakness (Myopathy) throughout the body and touches various body tissues, particularly the heart, skeletal muscles, liver and nervous system [37].

Causes for Liver diseases:

Liver disease can be caused by a variety of factors. Causes include:

Congenital birth defects, or abnormalities of the liver present at birth metabolic disorders, or defects in basic body processes Viral or bacterial infections, Alcohol or poisoning by toxins, Certain medications that is toxic to the liver. Nutritional deficiencies, Trauma, or injury[37]

Symptom of liver diseases includes:

- Symptoms may begin slowly and slowly get worse. They may also begin suddenly and be severe from the start. Early symptoms may be mild and include:
- Breath with a musty or sweet odor, Change in sleep patterns, Changes in thinking,Confusion that is mild, Forgetfulness, Mental fogginess, Personality or mood changes, Poor concentration, Poor judgment, Worsening of handwriting or loss of other small hand movements.

More severe symptoms may include:

- Abnormal movements or shaking of hands or arms, Agitation, excitement, or seizures (occur rarely), Disorientation, Drowsiness or confusion, Strange behavior or severe personality changes, Slurred speech, Slowed or sluggish movement, people with hepatic encephalopathy can become unconscious, unresponsive, and possibly enter a coma.
- A rare but severe form of the liver infection called acute fulminant hepatitis causes liver failure. Symptoms of liver failure include:
- An enlarged and tender liver, Enlarged spleen, Susceptibility to bleeding, Encephalopathy which is a disorder that affects how the brain functions, Changes in mental status or level of consciousness, Ascites which is an accumulation of fluid inside the abdomen, Edema or swelling under the skin, Aplastic anemia a condition in which the bone marrow cannot make blood cells[38a].

Hepatitis:

It is the infection and damage of liver particularly involving the hepatocytes. It is usually due to various infective and toxic substances. The condition can be self-limiting, healing on its own, or can progress to scarring of the liver. A group of viruses had known as the hepatitis viruses' origin most cases of liver damage worldwide. Hepatitis can also be due to toxins (notably alcohol), other infections or from autoimmune process[37a].

Viral Hepatitis:

- Viral hepatitis is the cause of most cases of acute hepatitis. Types include Hepatitis A, Hepatitis B, Hepatitis C, Hepatitis B with D, Hepatitis E, Hepatitis F virus (existence unknown), and Hepatitis G or GBV-C.
- Hepatitis A or infectious jaundice is affected by a picornavirus transmitted by the fecaloral route. It causes an acute form of hepatitis and does not have a chronic stage.
- Hepatitis B is caused by a hepadnavirus, which can cause 500,000 to 1,200,000 deaths per year worldwide due to the complications of chronic hepatitis, cirrhosis, and hepatocellular carcinoma. Hepatitis C (originally "non-A non-B hepatitis") is caused by a virus with an RNA genome that is a member of the Flaviviridae family.
- Hepatitis C may lead to a chronic form of hepatitis, culminating in cirrhosis.
- Hepatitis D is caused by hepatitis delta agent, which is alike to a viroid as it can only propagate in the presence of the Hepatitis B virus.
- Hepatitis E produces symptoms similar to hepatitis A.
- Hepatitis F virus is a hypothetical virus linked to hepatitis. Several hepatitis F virus candidates emerged in the 1990s; none of these reports have been substantiated.
- Another potential viral cause of hepatitis, initially identified as hepatitis G virus is probably spread by blood and sexual contact [43]. There is very little evidence that this virus causes hepatitis, as it does not appear to replicate primarily in the liver.It is now classified as GB virus C.

In addition to the hepatitis viruses, other viruses can also cause hepatitis, including cytomegalovirus, Epstein-Barr virus, yellow fever, etc. Non-viral infection like Toxoplasma, Leptospira and Q fever also causes hepatitis [38].

Fatty Liver:

- Fatty liver, also known as fatty liver disease (FLD), Steatorrhoeic hepatosis or Steatosis hepatitis, is a reversible condition where outsized vacuoles of triglyceride collect in liver cells via the process of Steatosis. Normal liver may cover as much as 5% of its weight as fat. Lipiotic liver may contain as much as 50% of its weight as fat, most of being triglycerides.
- Severe fatty liver is sometimes accompanied by inflammation, a situation that is mentioned to as Steatohepatitis. The progression to cirrhosis may be influenced by the amount of fat and degree of Steatohepatitis and by a variety of other informing factors.

Cirrhosis:

Cirrhosis can be defined as a chronic disease condition giving morphological alteration of the lobular structure characterized by destruction and regeneration of the parenchyma cells and increased connective tissue. Major morphological changes induce granular or nodular appearance and are characterized by the presence of septate or collagen throughout the liver [39].

Infective Agents:

These are mainly viruses like, Type A and Type B, Non – A, Non – B, Delta agent, virus of yellow fever, Epstin – Barr virus, cytomegalovirus, virus of Herpes simplex, Rubella, Marburg agent and others like *Leptospira icterohaemorrhagiae*, *Leptospira canicola*, *Taxoplasma gondii*, *Borrelia recurrentis*, etc

Toxic Agents:

Chlorpromazine and other Phenothiazine derivatives, Monoamine oxidase inhibitors (MAO-inhibitors), Erythromycin, Tetracycline, INH, Rifampicin, Methyldopa, Chlorpropamide, Phenylbu-tazone, Indomethacin, Paracetamol, Thiouracil, Acetaminophen, Halothen, Alcohol, Carbon tetrachloride, etc [40].

Tab 1. ***List of Hepatotoxic therapeutic agents and chemicals*****:**

	Therapeutic agents	**Chemicals**
Allopurinol	Methotrexate	Alcohol
Amiodarone	Nicotinic acid	Arsenic
Azathioprine	Nitrofurantoin	Carbon tetrachloride
Carbamazepine	Paracetamol	Chloroform
Chlorpromazine	Phenelzine	Copper
Chloroform	Phenytoin	
Ciglitazone	Pravastation	
Cimetidine	Quinidine	
Dantrolene	Rifampicin	
Erythromycin	Salicylates	
Galactosamine	Simvastatin	
Halothane	Sodium valproate	
Iproniazid	Sulphonamides	
Isoniazid	Tetracyclines	
Ketoconazole	Ethanol	

Liver Cancer:

The liver is inclined to cancer induction by a variety of human made and naturally occurring chemicals. Chemical substances include, aflatoxin B, cycasin, and safrole etc among human made substance are DDT, carbon tetrachloride,

chloroform, thioacetamide. Studies in experimental animals designate quite clearly that development of cancer of the liver is associated with the number of obvious non-malignant lesions appearing prior to the occurrence of neoplastic malignancy.

Diagnosis:

- Many further tests may also be used to support the diagnosis. These include blood test such as, Liver function tests, which are blood tests that check a wide variety of liver enzymes and by products.
- A complete blood count (CBC), which looks at the type and number of blood cells in the body.
- Abdominal X-rays.
- Ultrasounds, to show size of abdominal organs and the presence of masses.
- An upper GI study, which can detect abnormalities in the esophagus caused by liver disease.
- Liver scans with radio tagged substances to show changes in the liver structure.ERCP, or endoscopic retrograde cholangiopancreatography. A thin tube called an endoscope is used to view various structures in and around the liver.
- Abdominal CT scan or abdominal MRI, which provide more information about the liver structure and function.

Diagnosis of Drug-Related Hepatotoxicity:

There is no single test, including liver biopsy that can be used to diagnose drug-related Hepatotoxicity. Other causes of liver injury must first be considered with the use of a combination of serologic tests, imaging studies, and clues from the patient history. CT denotes computed tomography, MRI magnetic resonance imaging, MRCP magnetic resonance cholangiopancreatography, ERCP endoscopic retro grade cholangiopancreatography, AST aspartate aminotransferase, ALT alanine aminotransferase, TIBC totaliron-binding capacity, and A1AT alpha1-antitrypsin[38b].

Hepatotoxicity:

Hepatotoxicity implies chemical-driven liver damage. The liver plays a Central role in transforming and clearing chemicals and is disposed to the toxicity from these agents. Certain medicinal agents when taken in overdoses and sometimes

even when introduced within therapeutic ranges may injure the organ. Other chemical agents such as those used in laboratories and industries, and natural chemicals (e.g. microcystins) can also induce hepatotoxicity.

Chemicals that cause liver injury are called hepatotoxins. The human body identifies almost all drugs as foreign substances (i.e. Xenobiotics) and subjects them to various chemical processes, (i.e. metabolism) to make them suitable for elimination. This involves chemical transformations like reduction in fat solubility and alteration in biological activity.

Although almost all tissue in the body have some ability to metabolize chemicals, smooth endoplasmic reticulum in liver is the principal "metabolic clearing house" for both endogenous chemicals (e.g., cholesterol, steroid hormones, fatty acids, and proteins), and exogenous substances (e.g. drugs). The central role played by liver in the clearance and transformation of chemicals also kinds it susceptible to drug induced injury.

The mechanism of hepatotoxicity in liver can be labelled by two methods:

Direct: - This group comprises the products (or their metabolic products) that produce direct injury to the plasma membrane, endoplasmic reticulum and other organelles of the hepatocytes. Direct hepatotoxicity may be exemplified as non-selective destruction of the structural basis of hepatocyte metabolism.

Some of the direct hepatotoxins comprise carbon tetra chloride, chloroform, tetrachloroethane, iodoform and elemental phosphorus.

Indirect: -These are more selective, and are antimetabolic and related compounds that produce hepatic hurt by interference with specific metabolic pathway.

- The hepatic damage produced by indirect hepatotoxins may be mainly cytotoxicity expressed as necrosis or mainly cholestatic expressed as arrested bile flow with or without bile duct injury.
- A group of enzymes located in the endoplasmic reticulum, recognized as cytochrome P-450, is the most important family of metabolizing enzymes in the liver. Cytochrome P-450 is the terminal oxidase component of an electron transport chain.
- It is not a single enzyme, but rather covers of a family of closely related 50 isoforms, six of them metabolize 90% of drugs [41]. There is a remarkable diversity of individual P-450 gene products and this heterogeneity allows the

liver to perform oxidation on a vast array of chemicals (including almost all drugs). Due to its unique metabolism and close relationship with the gastrointestinal tract, the liver is subject to injury from drugs and other substances. About 75% of blood coming to the liver arrives directly from gastrointestinal organs and then spleen via portal veins which carry drugs and xenobiotics in concentrated form. Several mechanisms are accountable for either inducing hepatic injury or worsening the damage process.

- Many chemicals damage mitochondria, an intracellular organelle that produces energy. Its dysfunction releases extreme number of oxidants which in turn injures hepatic cells. Activation of some enzymes in the cytochrome P-450 system such as CYP2E1 also chief to oxidative stress injury to hepatocyte and bile duct cells lead to accumulation of bile acid inside liver [42].
- This promotes further liver damage. Non-parenchymal cells such as Kupffer cells, fat storing stellate cells and leukocytes (i.e. neutrophil and monocyte) also have role in the mechanism [43].
- More than 900 drugs have been concerned in causing liver injury, and it is the most common reason for a drug to be withdrawn from the market. Drug persuaded liver injury is responsible for 5% of hospital admissions and 50% of all acute liver failures [44].
- The liver produces large quantities of oxygen free radicals in the course of detoxifying xenobiotic and toxic substances.
- Reactive oxygen species (ROS) has been exposed to be linked to liver diseases, such as hepatitis, cirrhosis, portal hypertension, viral contagions and other liver pathological conditions [45]. They play an important role in the inflammation process after intoxication by ethanol, carbon tetrachloride or carrageenan.
- These radicals and the reactive species resultant from them react with cell membrane, induce lipid peroxidation and are responsible for various deleterious belongings in cells and tissues where they are generated. ROS induce alterations and loss of structural/functional architecture in the cell, leading directly to cytotoxicity and/or indirectly to genotoxicity, with numerous serious anomalies favouring disharmony and diseases [46].

- ❖ Hepatic injury caused by chemicals, drugs, and virus is a well-known toxicological problem to be occupied care of by various therapeutic measures.

3.3PHARMACEUTICAL REVIEW:

MATHIRAI:

Some of the raw drugs are ground well by adding juices of leaves, ginger, breast-milk,lemon juice, or some decoctions and made into small pills according to its dose and dried.(47)

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,Urandai,Vadagam.*

Equipment required:

1. Mortar and pestle
2. Trays and dishes for handling powders and pastes of the drugs and for making the pills or tablets.
3. Vessels and spoons for preparation of and the handling of decoctions and juices.

Purification of drugs in Siddha system (*suddhi seithal)* **:**

- ❖ 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.

Rules of tablet preparation:

- All the metals, minerals and poisonous organic drugs should be purified as per the rules of Siddha system of medicines.
- 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. The compounded drugs should be ground in a mortar for the prescribed period, with the addition of prescribed juices and decoctions.
- If any of the preparation of pills, if mercury and sulfur are the key ingredients then the 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. The individual drugs should be separately weighed after being powdered and then taken in the ratio prescribed in the preparation.

Preparation of *Mathirai* in Manufacturing Units :

- In manufacturing unit, *Chooranam* is compressed into tablets. Binders like Gum acacia, lubricants like liquid paraffin and disintegrators like Talcum powder are used for preparing tablets. *Chooranam* is first prepared according to the above procedures. Then the ingredients are mixed with in the form of granules before compressing as tablets.
- They must be mixed with some adhesive substances or binders such as gum acacia. To prevent the sticking of the tablets to the punches and dyes a lubricant like liquid paraffin is added. If the tablet is to dissolve quickly, a disintegrator like talcum powder is added [48]
- The pills should be always dried in a warm, dry shady Place and never kept in the sun, because volatile matters in the pills are easily lost and Phyto chemical break down of active principle are faster in sunlight of the tropics.

Storage:

- Almost all the maathirai is contain highly active ingredients (or) poisonous drugs, (or) metallic compounds etc., hence they should be stored in well stoppered glass vials, with relevant labels and instructions.
- The adjuvants recommended for each medicine are beneficial in giving good treatment results.
- If the maathirais lose their natural shape, color, smell, taste etc., it is not advisable to consume them. If properly stored, they can be used for for one year. [49]

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.

Tab 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
9	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. (50)

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. [51]

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

1. Diluents:

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- crosslinked 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.

6.Flavoring agents:

For chewable tablet- flavor oil are used

7.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

CCl_4 induced toxicity:

Carbon tetrachloride has been widely used to study liver damage used by free radicals. CCl_4 toxicity is initiated by the bioactivation of CCl4 to CCl3 (tricloromethyl free radicals) by the enzymes CYP2E1, CYP2B1, CYP2B2, CYP3A chiefly by CYP2E1. The formed CCl_3 rapidly reacts with molecular oxygen to form CCl3O2 (peroxytricloromethyl free radicals). Both CCl_3 and CCl3O2 are highly reactive, they covalently bind to macromolecules such as proteins, lipids and nucleic acids and react with polyunsaturated fatty acids and form a series of self-propagating chain reactions known as “propagation of lipid peroxidation” which may lead to damage of endoplasmic reticulum and cell membrane which may lead to necrosis.

Paracetamol induced liver toxicity:

Paracetamol is a non-steroidal anti-inflammatory drug which is available as OTC (over the counter) drug. The caution of this acetaminophen (Paracetamol) is its active metabolite is injury to liver (i.e) leads to liver damage. Normal dose of the drug- 4000mg per day (Maximum) (Franciscus A;2012) 2000-3000mg per day is mostly recommended. The active metabolite of acetaminophen is N-acetyl p-benzoquinoneimine(NAPQI)This NAPQI is toxic to the liver cells. Mostly the 90% of acetaminophen is metabolized by glucuronide and sulfate conjucation and then excreted in the urine. 5-10% is metabolized by cytochrome P450, mainly by CYP2E1 which produces NAPQI.

Alcohol induced toxicity:

Alcohol is metabolized by liver. This process produces a number of potentially dangerous byproduct. Alcohol is converted to acetaldehyde by the enzyme Alcohol dehydrogenase (ADH). The formed acetaldehyde is highly toxic. Normally the enzyme Aldehyde dehydrogenase (ALDH) rapidly

oxidizes acetaldehyde to acetate. Both these enzymes ADH and ALDH are also involved in metabolism of vitamin A. Apart from ADH, the enzyme CYP2E1 (microsomal ethanol oxidizing system; MEOS) is also involved in metabolizing alcohol. MEOS plays a major role when blood ethanol levels are high. CYP2E1 produces a toxic byproduct *N*-acetyl-*p*- benzo-quinone imine (NAPQI) which is responsible for damaging the hepatic protein[38c].

Models of Liver Fibrosis:

Several approaches to induce fibrosis in animals are designated and these models can be divided according to their stimulus from inciting injury. Liver fibrosis models are connected with (1) Toxic damage (hepatocytes: CCl4 dimethylnitrosamine (DMN), galactosamine; bile duct epithehal cells: thioacetamide (TAA), Immunological-induced damage (heterogonous serum and experimental schistosomiasis), Biliary damage (common bile duct ligation (BDL) or occlusion), Alcohol-induced damage (baboon ethanol diet or Tsukamoto / French model in rats). Nowadays, fibrosis-related models are established that have their origin in fatty liver disease, Fatty liver disease, in particular the 'malignant' inflammatory form nonalcoholic steatohepatitis (NASH), can increase to liver fibrosis and cirrhosis.

It is strongly associated with obesity and diabetes, two modern health problems in Western countries. Of the existing animal models for fatty liver disease, as reviewed by the genetic leptondeficient (ob/ob) or lepton- resistant (db/db) mice [52].

The dietary methionine/ choline-deficient models are cast-off in the majority of published research. Progressive fibrosis was reported only in themethionine/choline-deficient models in 100% of the mice.

BDL and CCl_4 are the most widely used rodent models [53] in liver fibrosis research to assess the effectively of experimental drugs on the pathogenesis, since these models represent features of human pathogenesis. Therefore, these models are the best categorized with respect to histological, biochemical, cell and molecular changes connected with the development of fibrosis.

In the past years, there is a tendency in fibrosis - related research to shift from rat to mice models, and most of the models originally described ferrates

are now applied in mice. Moreover, new testing models arise due to the development of transgenic or knock-out mice models, which were developed to elucidate the pathogenesis and common pathways in liver fibrosis. Examples of knockouts with spontaneous formation of liver fibrosis are mdr2-/- mice 1hx2-/- mice, and the mice models for NASH mentioned above [54].

Acute and Chronic Models with Carbon Tetrachloride (CCl_4)

CCl_4 intoxication results in hepatocyte necrosis and apoptosis with damage predominantly in zone III (around central vein) of the liver. The mechanism behind this hepatocyte damage is the activation of CCl_4 by cytochrome P450, which results in the formation of trichioromethyl radical in these cells and this free radical initiate lipid peroxidation [55].

The damage to hepatocytes by CCl_4 is replicated by high plasma alanine transaminase (ALT) and aspartate transminase (AST) levels after CCl4 administration, CCl_4 causes also fatty changes in the hepatocytes. This initial damage is followed by hepatic stellate cell activation and tissue fibrosis.

The CCl_4 model is related with tremendous inflammation, a feature that is also often seen in livers of patients with liver fibrosis. Disadvantages of this model are the variations obtained in disease induction in the animals and the relatively high rate of mortality alter CCl_4 administration > 20%.

In animal models CCl_4 treatment is used to get different stages of the fibrotic process, ranging from early damage and HSC activation until advanced cirrhosis. The fibrotic stage obtained in the rodents depends on the number of injections of CCl_4 that are administered.

The models for CCI_4 that are used in liver fibrosis research, are Acute damage (72 hours after a single injection of CCI_4) with HSC activation

Early and establish fibrosis (4-6 week of twice weekly CCl_4 dosing),

Early cirrhosis (8 week of twice weekly CCl_4 dosing)

Advanced micronodular cirrhosis (12 week of twice weekly CCl_4 dosing). In addition, for each of these models, Spontaneous recovery from fibrosis can be studied after cessation of dosing of CCl_4.This latter model is a valuable model to determine drug induced acceleration of recovery from established fibrosis after removal of the inciting stimulus.

This is similar to treatment situations in patients with liver fibrosis in case their inciting stimulus can be eradicated for instance after alcohol abstinence or after antiviral therapy beside hepatitis virus infections.

CCl_4 is administered to the animals via intraperitoneal, subcutaneous or oral administration or by inhalation. For intraperitoneal injections, CCl_4 is diluted in olive oil and given in dosages of 0.5 - 1.0 ml / kg to rats and mice. Often supplementation of phenobarbital in drinking water (resulting in induction of hepatocyte cytochrome P450) is used to get more reproducible fibrosis improvement and to accelerate the speed of fibrosis development. Usually, phenobarbital concentrations of 0.3 - 0.4 g/I in drinking water are used and started I week before the initial exposure to CCl_4.

In case of inhalation of CCl_4 the animals are placed in an inhalation chamber twice a week with a progressively increasing exposure time (1.5 min). Also, with this procedure, supplementary phenobarbital in drinking water is added. To reduce early toxicity and mortality, some research groups vary with the dose of CCL_4 in time. In these cases, gradually growing dosages in the first weeks are administered to the rats.

Bile Duct Ligation (BDL):

The second well-studied experimental animal model of liver fibrosis is the bile duct ligation model. This model corresponds with the human pathology of biliary cirrhosis, such as extrahepatic biliary atresia and primary sclersoing cholangitis. Ligation of the bile duct causes acute epithelial impairment and the detergent action of the subsequently released bile salts in the liver is likely associated with the solubilization of plasma membranes and hepatocyte cell death.

This latter is envisaged by elevated ALT and AST levels in plasma, in particular proximately after ligation (first week). Characteristics of obstruction of the bile are the appearance of bile products, such as bilirubin into the blood circulation, which causes jaundice in these animals [56].

The initial damage is followed by a massive expansion of the bile duct epithelial cells and periductal my fibroblasts, which can be referred to as portal expansion (stage 1) in total this results in marked liver enlargement, which can be up to twice the weight as compared to normal.

Then, bile duct epithelial cells and my fibroblasts in the portal tract are increasingly expanding which results in a gradual remodelling of the liver architecture by linking adjacent portal tracts (biliary cirrhosis stage IV).

To ligate the bile duct, the abdomen of the rat is opened under general anesthesia (preferably N2O/O2/halothane inhalation to agree quick recovery from narcosis) to identify the common bile duct. The bile duct turns from the helium of the liver, where the hepatic ducts meet, through the pancreas, into the lower end of the duodenum. Of note, threat has no gall bladder in contrast to other rodents.

Three ligatures are located and tied around the bile duct; two closes to the liver and one close to the duodenum. The first ligatures will prevent formation of a reservoir of bile outside the liver. After tight closure, the bile duct is cut between the second and third ligation in order to prevent restoration of the bile flow by bile duct formation around the ligature. Subsequently, the abdomen is closed over and analgesics can be given to the rats.

We use a local anaesthetic compound (Marcaine which contains bupivacaine), but also systemic acting analgesics are sometimes administered (e.g. Temgesic (containing buprenorphine). For mice, the procedure is a little bit more complicated because a mouse possesses a gall bladder, and consideration should be [paid to tightly ligate the whole duct, in general more than three ligatures are needed, to prevent rupture of the bladder and subsequent problems.

Already in the first days after ligation, proliferation of bile duct epithelial cells, activation and proliferation of HSC and my fibroblasts, and deposition of extra cellular matrix can be detected microscopically starting in the portal areas of the liver (zone 3). After one week, a fibrous expansion of the portal areas is visible and after about 10-14 days, portal- portal bridging is visible.

Three to four weeks after ligation, these rates develop advanced cirrhosis characterized by extensive proliferation of the bile ducts, around which the activated and transformed HSC are detectable (Markers: a - smooth muscle action and PDGF beta receptor) and around which the interstitial collagens (types I and III) are deposited.

A major advantage of the BDL model is the relatively fast development of fibrosis (within 3 weeks) in rats. Furthermore, the model is quite reproducible, and the mortality due to the ligation procedure in rats is low (<10%). Disadvantages of

the BDL models are the limited inflammation associated with this type of fibrosis development and the excessive expansion of bile duct epithelial cells.

Another drawback with regard to drug screening is that the BDL-induced disease is difficult to reverse with experimental drugs, and a reason for this may be because the initiating stimulus (ligation of the bile duct) remains present during treatment periods and causes continuous damage as subsequent fibrosis that troubles the potential treatment effects.

Dimethylnitrosamine (DMN):

DMN induces liver damage leading to fibrosis and cirrhosis. Characteristic for this model is that ongoing administration of this toxic compound finally leads to the development of hepatocellular carcinoma in rodents.

DMN induces liver injury by starting damage to the hepatocyte. It is metabolized primarily in hepatocytes by Cytochrome P450 (isotype 2E1) to more toxic compounds with formation of reactive oxygen species in hepatocytes and sub-sequent this will lead to lipid peroxidation. In difference to the hepatotoxin CC14, DMN administration does not cause fatty changes, steatosis in the hepatocytes [53]. To induce the fibrosis, DMN (10 microliter/kg body wt., i.p) is given 3 days a week for 3 weeks to rats.

After administration of DMN, hemorrhagic necrosis is evident in centrolobular part (zone III) of the liver. Incomplete septa appear after 7 days and micronodular cirrhosis is developed after 3 weeks of treatment with DMN. Increased numbers of HSC and my fibroblasts are found in the formed septa. Influx of inflammatory cells, mainly lymphocytes, is noted early in DMN- induced liver injury. Advantages of this model are that the disease induction is quite reproducible in the animals, and this model is associated with a prominent inflammatory reaction. Furthermore, this model can be used to study the transition from cirrhosis to hepatocellular carcinoma, and the effect of drugs on this process.

HSC in Culture (In Vitro System):

HSC are key players in fibrosis and these cells predominantly orchestrate the development of the disease. To evaluate the ant fibrotic efficacy of experimental drugs, these primary cultured cells are useful in assessing specific effects on HSC activities. In particular, the primary isolated HSC are valuable in

drug research, because in vitro they spontaneously transform into my fibroblasts, and this transformation process is related with cellular activation proliferation and matrix production resembling cellular activities that also happen in vivo.

This transformation does not occur in the various HSC cell lines that are also used in literature. Proximately after isolation they signify a inactive stage, e.g. as present in the normal healthy liver, with vitamin A droplets as their main characteristic. During culture on plastic for about 10-14 days a cell with fibroblast - like features is attained. This transformed cell displays different cellular activities as compared to the original isolated one.

The procedure to isolate HSC is well described by various fibrosis research groups Briefly, HSC are isolated from livers of normal rats weighing at least 500g in order to achieve a good separation from the other hepatic cells.

The liver is digested with pronase, collagenase and DNase by in situ perfusion. Pronase is essential in the isolation, yet it affects the viability of other hepatic cells (i.e. hepatocytes) and therefore this procedure can only be used isolate HSC from the liver.

After several centrifuge steps, the cells suspension is subjected to a Nycodenz gradient to gather the HSC on top of the Nycodenz layer. The separation is based on the low density of the HSC as compared to other liver cells, as a consequence of their high cellular lipid content.Instead of Nycodenz, also other compounds are used e.g Stractan, Metrizamide, or Percoll, to separate the HSC from the other cells by density gradients.

The yield of HSC after collagenase / pronase digestion and Nycodenz separation is about 20-40 x 10E6 cells per rat liver. The yield of HSC attained from a mouse liver is much smaller and to isolate and purify proper amounts of HSC, about 5 mice have to be used at the same time in one total isolation (Geerts, personal communication)

The purity after isolation can be established by phase contrast microscopy or by staining of the cells with markers for hepatic cell types. The isolated cells are cultured in DMEM containing 10% FCS 100 U/ml penicillin, and 100 ug/ml streptomycin. After 10-14 days in culture, the cells exhibition an activated phenotype as assessed by light microscopy and acquires the presence of alpha-smooth muscle action.

Additionally, it is also conceivable to isolate HSC from human livers. Often (parts of) human livers are used that are unbecoming for transplantation are derived from tumor-free parts of the human liver and separated after partial hepatectomy.

Roughly, two methods are used to isolate human stellate cells out-growth of the cells by culturing small pieces of the livers in medium and a combined digestion with collagenase / pronase, after which HSC were separated from other liver non - parenchymal cells by centrifugation over density gradients similar to threat procedure. Of note, the first method will yield a combination of various (myo) fibroblastic cells including HSC and myofibroblasts.

These cells are afterward cultured in DMEM< supplemented with 5% Fetal Calf Serum and 5% G Human Serum. The fibroblastic nature of the cells can be microscopically evaluated, and tested for the expression of a smooth muscle action.

Liver Slice System:

A second in vitro test system which was recently developed to assess effects of antifibrotic drugs is the liver slice preparation. Drug studies with tissue slices (8mm diameter, 250 un thickness that is about 10-12 cell-layers thick) comprising stellate cells in their natural environment that uphold there in vivo cellular functional and anatomic relationships, may provide additional information about the hepatocellular specificity of the experimental drug and their effects on all hepatic cells.

Hepatoprotective and antioxidant effects of tender coconut water (TCW) were examined in carbon tetrachloride (CCl_4)-intoxicated female rats.

Liver damage was showed by the increased levels of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and decreased levels of serum proteins and by histopathological studies in CCl_4 intoxicated rats.

Augmented lipid peroxidation was presented by elevated levels of thiobarbituric acid reactive substance (TBARS) viz, malondialdehyde (MDA), hydroperoxides (HP) and conjugated dienes (CD), and also by significant reduction in antioxidant enzymes activities, such as superoxide dismutase (SOD), catalase (CAT) and also reduced glutathione (GSH) content in liver.

Darkening of urine on the other hand, CCl_4 intoxicated rats treated with TCW retained almost normal levels of these constituents. Decreased activities of

antioxidant enzymes in CCl_4 intoxicated rats and their reversal of antioxidant enzyme activities in TCW treated rats, shows the effectiveness of TCW in combating CCl_4 induced oxidative stress.

Hepatoprotective outcome of TCW is also evidenced from the histopathological studies of liver, which did not show any fatty infiltration or necrosis, as observed in CCl_4 intoxicated rats [57].

Exams and Tests Physical Examination:

1. Nutritional assessment
2. Yellowing of the sclera is usually the first detectable sign of jaundice.
3. Darkening of urine
4. Skin examination for icterus
5. Stigmata of chronic liver disease
6. Abdominal examination
7. Inflammed and tender liver
8. Fluid in the abdomen (ascites) that can become infested
9. Blood tests
10. These may initially include
11. Complete blood count – TC, DC, ESR, Cholesterol
12. Liver function test
13. In women, a pregnancy test may be obtained.
14. Urine analysis: Urine analysis for bile salts and bile pigments.

Laboratory Tests:

1. Abdominal ultrasound
2. Autoimmune blood markers
3. Hepatitis virus serologists
4. Liver function tests
5. Liver biopsy to check for liver destruction
6. Paracentesis if fluid is in abdomen Tests for Liver Function.

Bilirubin:

Bilirubin is one of the most important factors indicative of hepatitis. It is a red-yellow pigment that is normally metabolized in the liver and then defecated in the urine.

In patients with hepatitis, the liver cannot process bilirubin, and blood levels of this substance rise. High levels of bilirubin cause the yellowish skin tone known as jaundice.

Liver Enzymes (Aminotransferases):

Enzymes known as aminotransferases, including aspartate (AST) and alanine (ALT), are free when the liver is damaged. Measurements of these enzymes, particularly ALT, are the least expensive and most non-invasive tests for determining sternness of the underlying liver disease and monitoring treatment effectiveness. Enzyme levels vary, however, and are not always an accurate indicator of disease activity.

Alkaline Phosphatase (ALP):

High ALP levels can indicate bile duct blockage.

GGT (gamma glut amyl transpeptidase):

GGT is often elevated in those who use alcohol or other liver-toxic substances to excess.

Serum Albumin:

Serum albumin measures protein in the blood (low levels indicate poor liver function). Total protein, Serum total protein, protein in the blood (low levels indicate poor liver function).

Prothrombin Time (PT):

The PT test measures in seconds the time it takes for blood clots to form (the longer it takes the greater the risk for bleeding [58].

3.5 LATERAL RESEARCH

HEMIDESMUS INDICUS :

Anti-microbial activity:

The Antimicrobial activity *of H.indicus* was evaluated against pathogenic bacteria Stahylocococcus aures, Pseudomonas and Klebsiella in an in vitro condition.The aqueous extract inhibits the growth of bacteria with minimal inhibitory concentration ranging from 0.04 mg to 0.08mg.Thus it has significant antibacterial activity against Pathogenic bacteria.(61)

In vitro antioxidant and antithrombotic activity:

The methanolic extract of *H.indicus* roots was found to inhibit lipid peroxidation and and scavenge hydroxyl and superoxide radicals in vitro. The amount required for 50% inhibition of lipid peroxide formation was 217.5µg/ml. The extract also inhibited ADP-induced platelet aggregation in vitro [59].

***Wound healing activity*:**

Coarsely powdered shade dried roots of *H.indicus* was compactly packed in a Soxhlet extractor with methanol as a solvent and it was heated (60-80ºC) for 24 h and the final product obtained was dried. The ointment made from the methanolic extract of *H.indicus* displayed significant wound healing activity.(60)

CUMINUM CYMINUM

Hepato protective activity:

Protective effect of *Cuminum cyminum* on Profenofos induced Liver toxicity. Cumin seeds have anti- carcinogenic properties. In one study, it was observed that cumin prevents the development of stomach or liver tumours in laboratory animals.This cancer protective effect may be due to cumin's potent free radicle scavenging abilities and its effect to enhance liver detoxification enzymes.(62)

Hypolipidemic activity and Anti-diabetic activity:

Oral administration of 0.25g Kg diabetic rats resulted in significant reduction in blood glucose and an increase in total haemoglobin and glycosylated haemoglobin. It also prevented a decrease in body weight. C.cyminum treatment also resulted in significant reduction in plasma and tissue cholesterol, free fatty acids and triglycerides. (63)

Anti-bacterial activity:

Essential oil of *Cuminum cyminum* contains p-mentha-1, cuminaldehyde, terpene, pinene which shows a Anti-bacterial activity against clavibacter, curtobacterium, rhodococcus, erucinia, xanthomonas, agro bacterium which are responsible for plant or cultivated disease world-wide. (64)

Nephroprotective activity:

The cumin seeds extract and the coriander leaf extract incobination shows nephroprotective activity in profenofos induced neprotoxicity in swiss albino mice. The biochemical assessment shows the nephroprotective activity of cumin and coriander. In comparison to control group mice group creatinine, urea, uric

acid was increased in profenofos treated group and profenofos control group. But in cumin, and coriander treated group it shows declination in the level of creatinine & urea level. (65)

***ELETTARIA CARDAMOMUM*:**

Gastroprotective activity of Cardamom:

A crude methanolic extract, essential oil, Petroleum ether soluble and insoluable fractions of methanolic extract were studied in rats at doses of 100-500, 12.5-50, 12.5-150 and 450 mg/kg, respectively for their ability to inhibit the gastric lesions induced by aspirin, ethanol and pylorous ligature. (66)

Anti-ulcerogenic activity* of *Elettaria cardamomum

The gastro protective action of petroleum ether soluble fractions and essential oils of *E.cardamomum* is due to increase in gastric motility and it has inhibitory effect in over production of some products of 5-lipoxygenase pathway.(67)

Anti-convulsant activity:

The methanolic extract of *E.cardamomum* against chemically (pentylentetrazole) and electrically (maximal electric shock) induced seizures in mice.Various pharmacological activities Anti -inflammatory, Analgesic, Anti -oxidant, Anti- microbial effects.(68)

Antimicrobial activity:

The n-hexane seed of the cardamom seed was found to exhibit rather broad antimicrobial activity against S.mutans, Propinobacterium acenes,Pityrosporum ovale, Trichophyton mentagrophytes.Isao Kubo, Masaki Himejima, Antimicrobial activity of flavor compenents of Cardamom *Elettaria cardmomum*.(69)

FOENICULUM VULGARE:

Antioxidant activity:

Antioxidant activity of ethanol and water extracts of FS was determined by the thiocyanate method, the extraction yields and gallic acid equivalents.The inhibition of lipid peroxidation in percent of water and ethanol extracts of FS, a-tocopherol, BHA and BHT. Ethanol and water extracts of FS exhibited effective antioxidant activity at all concentrations. The effects of various amounts of water and ethanol extracts of FS (50–250 mg) on peroxidation of linoleic acid emulsion. Determination of in vitro antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. (70)

Anti-bacterial activity:

Fennel is used to treat many bacterial, fungal, viral, and mycobacterial infectious diseases (31). Fennel has antibacterial activity due to compounds such as, linoleic acid, undecanal, 1, 3-benzenediol, oleic acid and 2,4-undecadienal. Fennel has 5-hydroxy-furanocoumarin which has important role antibacterial activity of this plant.

Antifungal activity:

Antifungal activity of fennel essence on Sclerotinia sclerotiorum was investigated. The antifungal effect of this plant against Sclerotinia sclerotiorum observed based on survival of the microorganisms [(70a)].

A study demonstrated that nitric oxide production in peritoneal macrophages which were treated with fennel extract at a concentration of 10 mg/ml significantly increased. Also, the production of reactive oxygen species compared to the control group increased.

Gastro-protective activity:

It has been shown that fennel plant has significant protective effect on gastrointestinal disorders. It was shown that the use of fennel oil emulsions eliminated colic in 65% of treated infants which was considerably better than the control group. [(71)]

SESBANIA GRANDIFLORA

Hepatoprotective activity:

The hepatoprotective activity of ethanolic and aqueous extractof *sesbania grandiflora* (Linn) flower in ccl4 induced hepatotoxicity models in rats was investigated. The ethanolic and aqueous extract of *sesbania grandiflora* (Linn) flower was screened for hepatoprotective activity in ccl4 induced rats for its dose 200mg/kg bw. The ethanolic and aqueous extract of *s.grandiflora* (Linn) flower significantly ($p<0.001$) decreased the biochemical parameters (SGOT, SGPT, ALP,TP, and TB). Silymarin (25mg/kg), a known hepatoprotective drug used for comparison exhibited significant activity $p<0.001$). The extract did not shown any mortality upto a dose of 2000g/kg body weight. These findings suggested that the ethanolic and aqueous extract of *sesbania grandiflora* (Linn) flower 500mg/kg was effective in bringing out functional improvement of hepatocytes. The healing effect of this extract was also confirmed by histological observations.

The ethanolic extract at doses of 250 and 500mg/kg,p.o and aqueous extractat doses500mg/kg, p.o of *sesbania grandiflora* (Linn) flower had significant effect on the liver of ccl4 induced hepatotoxicity animal model.[72a]

Antioxidant and cardioprotective effect:

Sesbania grandiflora was evaluated for the cardio protective effects against cigarette smoke-induced oxidative damage in rats. Adults male wistar-kyoto rats were exposed to cigarette smoke for a period of 90 days and consequently treated with *s.grandiflora* aqueous suspension (SGAS, 1000mg/kg body weight per day orally)for a period of 3 weeks. The results suggested that chronic cigarette smoke exposure increases the oxidative stress, thereby disquieting the cardiac defence system and *s grandiflora* protects the heart from the oxidative damage through its antioxidant potential. [72b]

Antiurolithiatic activity:

The leaf juice of *s.grandiflora* was evaluated for median lethal dose, gross behavioural changes,antiurolithiatic and antioxidant activities. The antiurolithiatic activity wasevaluated by a calculi-producing diet model, using gentamicin (subcutaneously) and 5%ammonium oxalate in rat feed to induce calcium oxalate-type stones. The leaf juice of *s.gradiflora* was safe orally and exhibited no gross behavioural changes except for an increase in urination. The leaf juice of *s.grandiflora* showed significant antiurolithiatic activity against calcium oxalate-type stones and also exhibited antioxidant properties.[72c]

MATERIALS AND METHODS

4. MATERIALS AND METHODS

DRUG SELECTION:

This present study, the Herbal formulation ***"NANNARI MATHIRAI"*** [73] was taken as the compound drug preparation for ***Kaamaalai (Jauncide)***mentioned in the classical Siddha literature ***"Kannuswamy parambarai vaithiyam"*** written by *Kannuswamy* pillai, published by Thirumagal Vilasa acchagam, Chennai, pg.no:141, Edition year:1948.

INGREDIENTS:

Table 3: Ingredients of *Nannari mathirai:*

NAME OF DRUGS	BOTANICAL NAME	QUANTITY
Nannari	*Hemidesmus indicus*	1 Thola (12 grams)
Seeragam	*Cuminum cyminum*	1 Thola (12 grams)
Elam	*Elettaria cardamomum*	1 Thola (12 grams)
Perunseeragam	*Foeniculum vulgare*	2 Thola (24 grams)
Sevvagathi	Sesbania grandiflora	Requid quantity

Collection of the Plant materials:

All the raw materials were bought from the Ramasamy Mudhaliyar Store, Parry's corner, Chennai.

Identification and Authentication of the drug:

The raw materials were identified and authenticated by the experts of *Gunapadam,* Government Siddha Medical College, Arumbakkam, Chennai- 106. The specimen sample of each raw material has been kept in the PG *Gunapadam* department individually for future reference.

Purification of the drugs:

Purification process was done as per classical *Siddha* literature [74].

Table 4: Purification of drugs:

Raw Drug	Method of Purification
Nannari	The root was cleaned with a white cloth. Finally, washed and dried.
Elam	Roasted in the Pan and outer skin are removed.
Seeragam	Cleaned well without any dust impurities and slightly roasted in pan.
Perunjeeragam	Cleaned well without any dust impurities and slightly roasted in pan.
Sevvagathi	The Fresh flowers is washed in the running water.

4.1. PREPARATION OF THE TRAIL DRUG NANNARI MATHIRAI:

Procedure:

All the ingredients such as *Root bark of Nannari, Seeragam, Elam*, were purified and dried in the shade until complete evaporation of the moisture content and Required quantity of fresh Sevvagathi flower juice was taken. Except Sevvagathi flower juice all other ingredients are taken and powered separately. Then all the powder was mixed together.

Finally, the mixture was ground well which favours the homogenous preparation. Then the mixture of the powder was sieved through the thin clean white cloth. After that by adding required quantity of Nannari root bark juice and Sevvagathi juice then ground it well and make it as a karkam. Finally make it as a Pill for 130mg.Finally, the end product was obtained, which was kept in an air tight container and labeled as ***"Nannari Mathirai" (NM)***.

Storage of the Drug:

The prepared test drug was stored in a clean, air tight glass container.The contents were inspected frequently to avoid moisture and insects.

Administration of the Drug:

Form of the medicine	: Mathirai
Route of administration	: Enteral
Dose	: 130mg.1 tab twice a day
Adjuvant	: Water.
Indication	: Kaamaalai (Jaundice).

Fig 1: INGREDIENTS OF NANNARI MATHIRAI:

1.1 HEMIDESMUS INDICUS

Nannari

1.2 ELETTARIA CARDAMOMUM

Elam

1.3 CUMINUM CYMINUM

Seeragam

1.4 FOENICULUM VULGARE

Perunjeeragam

1.5 SESBINIA GRANDIFLORA

Sevvagathi flower

FIG 2. PREPARATION OF NANNARI MATHIRAI

2.1 POWDERING

2.2 SIEVING

2.3 CHOORANAM

2.4 *GROUNDING THE NANNARI ROOT BARK*

2.5 *ROOT BARK JUICE OF NANNARI*

2.6 GROUNDING OF SEVVAGATHI FLOWER

2.7 JUICE OF SEVVAGATHI FLOWER

2.8 Grounding the Powder

2.9 Final Product ***(NANNARI MATHIRAI)***

4.2. STANDARDIZATION OF THE DRUG:

Standardization of the drug brings the validation to be used as a medicine by subjecting the drug to many analyses and determining its quality and effectiveness. Standardization includes many studies such as its organoleptic properties, physical characteristics and phytochemical properties and also to assess the active principles and elements present in the drug.

Method of standardization:

Techniques Involved in Standardization of Compound Drugs:

- ❖ Macroscopic Methods
- ❖ Microscopic Methods
- ❖ Physical Methods
- ❖ Chemical Methods
- ❖ Biological Methods

4.2.1. ORGANOLEPTIC CHARACTER:[75]

The organoleptic characters of the sample were evaluated which include evaluation of the formulation by its colour, odour, taste, texture etc. Ten tablets were taken into watch glasses and positioned against white back ground in white tube light. Its colour was observed by naked eye and results are noted.

Colour:

A sample of Mathirai were taken in watch glasses and placed against white back ground in white tube light. The *Mathirai* were observed for its color by naked eye.

Odour:

Ten numbers of tablets were smelled individually.*Mathirai* were smelled, the time intermission between two smelling was kept 2 minutes to nullify the effect of previous smelling.

Taste:

A sample of about *Mathirai* was tasted and the taste was reported.

Size:

The diameter of ten tablets was measured by Vernier caliper. The mean value of diameter was noted.

4.2.2 PHYSIOCHEMICAL ANALYSIS OF NANNARI MATHIRAI

Physicochemical- studies of the trial drug have been done according to WHO guidelines. Physico chemical studies like total ash, water soluble ash, acid Insoluble ash, water and alcohol soluble extract, loss on drying at 105°C and pH were done at, Dr. MGR University, Chennai. [76]

1.Solubility Test:

A pinch of sample (*NM*) was taken in a dry test tube and to it 2 ml 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 the results are observed individually.

2.pH value:

Potentiometrically, pH value is determined by a glass electrode and a suitable pH meter. The pH of the ***Nannari mathirai*** was written in results column

3. Loss on Drying:

An accurately weighed 1g of ***Nannari mathirai*** 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.

4. **Determination of total ash:**

Weighed accurately 2g of ***Nannari mathirai*** was added in crucible at a temperature 600^0C in a muffle furnace till carbon free ash was obtained. It was calculated with reference to the air-dried drug.

5.Determination of acid insoluble ash:

Ash above obtained, was boiled for 5min with 25ml of 1M Hydrochloric acid and filtered using an ash less filter paper. Insoluble matter retained on filter paper was washed with hot water and filter paper was burnt to a constant weight in a muffle furnace. The percentage of acid insoluble as was calculated with reference to the air-dried drug.

6.Determination of water-soluble ash:

Total ash 1g was boiled for 5min with 25ml water and insoluble matter collected on an ash less filter paper was washed with hot water and ignited for 15min at a temperature not exceeding 450^0C in a muffle furnace. The amount of soluble ash is determined by drying the filtrate.

7.Determination of water-soluble Extractive:

5gm of air-dried drug, coarsely powered ***Nannari mathirai*** was macerated with 100ml of distilled water in a closed flask for twenty-four hours, shaking frequently. The Solution was filtered and 25 ml of filtrated was evaporated in a tarred flat bottom shallow dish, further dried at 100^0 C and weighted. The percentage of water-soluble extractive was calculated with reference to the air-dried drugs.

8.Determination of alcohol soluble extractive:

1 gm. of air-dried drugs, coarsely powdered ***Nannari mathirai*** was macerated with 20 ml. 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 air dried drug.

Tablet Disintegration test

Each *Nannari mathirai* was placed in each of the six tubes of the basket present in the disintegration apparatus. The apparatus was operated by using water as the immersion fluid maintained at 35-39 °C. At the end of 30 min, the basket is lifted from the fluid and the state of the tablet is observed. The disintegration time of *Nannari mathirai* was recorded [(78)].

All the results were noted and tabulated in Table No.5

Weight variation test

It was carried out to make sure that, each number of tablets contains the proper amount of drug. The test was carried out by weighing the 20 tablets individually using analytical balance, then the average weight was calculated and comparing the individual tablet weights to the average [(79)].

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

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

Table No. 5. Weight variation limits of Tablets (IP)

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

4.2.3 PHYTOCHEMICAL SCREENING:

The preliminary phytochemical screening test was carried out for each extract of ***Nannari mathirai*** as per the standard procedure [77].

1. Detection of alkaloids:

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

a) Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow colored precipitate indicates the presence of alkaloids.

2. Detection of carbohydrates:

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

a) Molisch's Test: To 2 ml of plant sample extract, two drops of alcoholic solution of α- naphthol are added. The mixture is shaken well and few drops of concentrated sulphuric acid is added slowly along the sides of test tube. A violet ring indicates the presence of carbohydrates.

3. Detection of glycosides:

Extracts were hydrolyzed with dil. HCl, and then subjected to test for glycosides.

a) Modified Borntrager'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 color in the ammonical layer indicates the presence of anthranol glycosides.

4. Detection of saponins:

a) Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

5. Detection of phenols Ferric Chloride Test:

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

6. Detection of tannins Gelatin Test:

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

7. Detection of Flavonoids:

a) Alkaline Reagent Test: Extracts were treated with few drops of sodiummhydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

8. Detection of proteins:

a) Xanthoprotein Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow color indicates the presence of proteins.

9. Detection of aminoacids:

a) Ninhydrin Test: To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue color 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 color indicates the presence of diterpenes.

11. Gum and Mucilage:

To 1ml of extract add 2.5ml 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 Quinones

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

13. Test for Fixed oils and Fats:

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

4.2.4.BIO-CHEMICAL ANALYSIS [80]:

Methodology for chemical analysis

The bio-chemical analysis was done to identify the acid and basic radicals present in the sample.

Preparation of extract:

5g of *NM* 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 studies:

Table 6: Test for basic radicals of Nannari mathirai

PROCEDURE	OBSERVATION	INFERENCE
Test for Potassium: A pinch of sample is treated with 2ml of sodium nitrate solution and then treated with 2ml of cobalt nitrate in 30% of glacial acetic acid.	Formation of Yellow Colour precipitate	Presence of Potassium
Test for Calcium: Taken2 ml of extract in a clean Test tube.Then acetic acid and otassium chromate solution were added.	No Yellow precipitate	Presence of Calcium
Test for Magnesium: 2ml of extract was taken in a clean test tube, few drops of Magnason reagent was added in drops.	Appearance of Brown colour	Presence of Ammonium
Test for Sodium:2 pinches of NM were mixed with HCl and made it into paste. And introduced into the blue flame of Bunsen burner.	Appearance of intense Yellow colour	Presence of Sodium

Test for Iron (Ferrous): 2ml of extract was taken in a clean dried test tube and conc. HNO_3 and ammonium thiocyanate were added.	Appearance of Blood red colour	Presence of Ferrous iron
Test for Zinc: 2 ml of the extract was taken in a test tube and Potassium ferro cyanide solution was added.	Formation of White colour precipitate	Presence of Zinc
Test for Aluminium: To the 2m1of the extract was taken in a test tube sodium hydroxide drops were added to it.	White precipitate Obtained	Presence of Alumini um
Test for Lead: 2 ml of extract was taken in a test tube and added with 2ml of potassium iodide solution	Formation of yellow colour precipitate	Presence of Lead
Test for Copper: To a small portion of a extract dilutehydrochloric acid was added and then hydrogen sulphide gas is passed through the solution.	Formation Black Precipitate	Presence of Copper
Test for Mercury:2m1 of the extract was taken in a test tube and treated with 2ml of sodium hydroxide solution.	Formation of Yellow Precipitate	Presence of Mercury
Test for Arsenic: 2m1 of the extract was taken in a test tube and treated with 2ml of sodium hydroxide solution.	Formation of brownish red Precipitate	Presence of Arsenic

Table.No:7. Test for acidic radical for Nannari mathirai

PROCEDURE	OBSERVATION	INFERANCE
Test for Sulphate: 2 ml of the extract was taken in clean, dry test tube and 5 % barium chloride solution was added to it.	Formation of white Precipitate	Presence of Sulphate
Test for Chloride: The extract was taken in a test tube and then treated with Silver nitrate solution.	Formation of White colour precipitate	Presence of Chloride
Test for Phosphate: The extract was taken in a test tube and treated with ammonium molybdate and conc. HNO_3.	Formation of Yellow colour precipitate	Presence of Phosphate
Test for Carbonate: The substance was taken in a clean dry test tube and then treated with Conc. HCl.	Formation of Effervescence	Presence of Carbonate
Test for fluoride &oxalate: 2ml of extract was taken in a test tube and added with 2ml of dil. acetic acid, 2ml calcium chloride solution and then heated.	Formation of cloudy Appearance	Presence of Fluoride & Oxalate
Test for Nitrate: 1gm of the *NM* was heated with copper turnings and conc H_2SO4 and observed the test tube vertically down.	Characteristic changes	Presence of Nitrate.

4.2.5 ANTIMICROBIAL ACTIVITY [81]

AGAR- WELL DIFFUSION METHOD

Principle

The antimicrobials present in the samples are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in **millimeters**.

Materials required

1.Muller Hinton Agar Medium (1 L)

The medium was prepared by dissolving 33.8 g of the commercially available Muller Hinton Agar Medium (MHI Agar Media) in 1000ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petriplates (25-30ml/plate) while still molten.

2. Nutrient broth (1L)

One litre of nutrient broth was prepared by dissolving 13 g of commercially available nutrient medium (HI Media) in 1000ml distilled water and boiled to dissolve the medium completely. The medium was dispensed as desired and sterilized by autoclaving at 15 lbs pressure (121ºC) for 15 minutes.

3. Streptomycin (standard antibacterial agent, concentration: 10mg / ml)

4. Culture of test organisms; growth of culture adjusted according to McFarland Standard, 0.5%

1. *E. coli* (ATCC 25922)
2. *Staphylococcus aureus* (ATCC 25923)
3. *Pseudomonas aeroginosa* (ATCC 27853)
4. *Klebsiella pneumoniae* (ATCC 13883)

Procedure

Petriplates containing 20ml Muller Hinton Agar Medium were seeded with bacterial culture of *E. coli, Pseudomonas aeroginosa, Klebsiella pneumoniae a*nd *Staphylococcus aureus* (growth of culture adjusted according to McFarland Standard, 0.5%). Wells of approximately 10mm was bored using a well cutter and different concentrations of sample such as 250µg/mL, 500µg/mL and 1000µg/mL were added.

The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993). Streptomycin was used as a positive control.

ANTIFUNGAL ACTIVITY

AGAR- WELL DIFFUSION METHOD

Principle

In order to access the biological significance and ability of the sample, the antifungal activity was determined by Agar well diffusion method. The antifungals present in the samples are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in **millimeters**.

Materials required

1.Potato Dextrose Agar Medium (1 L)

The medium was prepared by dissolving 39 g of the commercially available Potato Dextrose Agar Medium (HiMedia) in 1000ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petriplates (25-30ml/plate) while still molten.

3. Clotrimazole (standard antifungal agent, concentration: 10mg / ml)

4. Culture of test organisms; growth of culture adjusted according to McFarland Standard, 0.5%

- *Aspergillus niger* (ATCC 16404)

Procedure

Potato Dextrose agar plates were prepared and overnight grown species of fungus, *Aspergillus niger* were swabbed. Wells of approximately 10mm was bored using a well cutter and samples of different concentrations such as 250µg/mL, 500µg/mL and 1000µg/mL were added. The zone of inhibition was measured after overnight incubation at room temperature and compared with that of standard antimycotic (Clotrimazole) (NCCLS, 1993).

4.2.6 INSTRUMENTAL ANALYSIS

SOPHISTICATED INSTRUMENTAL ANALYSIS:

HPLC - High Performance Liquid Chromatography (HPLC)[82]:

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. It relies on 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)	: 272 nm & 360 nm
Solvent A	: Acetonitrile
Solvent B	: 0.1% Phosphoric acid in water
Flow rate	: 1.0 ml/min.
Pump Mode	: Gradient

FT IR - Fourier Transform Infra-red Spectroscopy [83]

FTIR (Fourier Transform Infra-red Spectroscopy) is 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 is an effective analytical instrument for detecting functional groups.

Fig no:3 FTIR INSTRUMENT

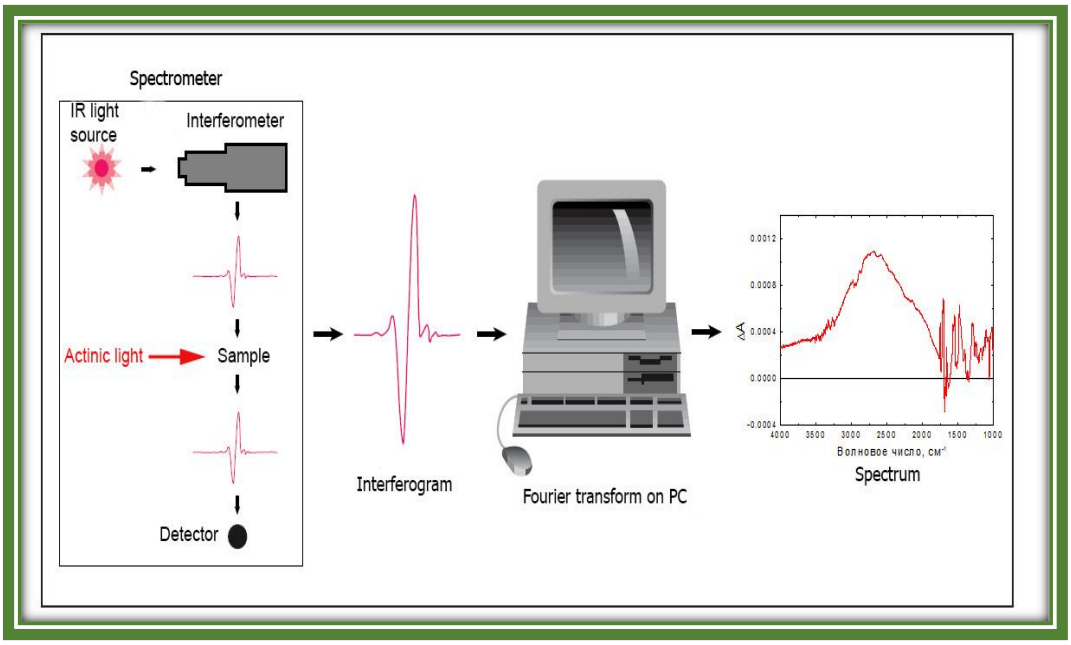


Fig no:3.1 FTIR MECHANISM

Principle:

Spectrophotometric tests are commonly used in the Identification of chemical substances and quantification of polymorphic forms. The test procedures are 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 die.
- Submit it to the pressure of about 800 mPa (8 tons/ cm^2).
- Examine the disc visually and if any lack of uniform transparency is 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 are measured for concordance, the transmittance obtained at the start of the scan range, should not deviate by more than 10% between them (For eg. If the standard shows a transmittance of 75%, the sample transmittance can be between 65% and 85%).

APPLICATIONS:

- Quantative scans
- Qualitative scan solids, liquids, gasses
- Organic samples, inorganic samples
- Unknown identification
- Impurities screening
- Formulation
- Pharmaceuticals

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

- Speed
- Sensitivity
- Mechanical Simplicity
- InternallyCalibrated

ICPOES (INDUCTIVELY COUPLED PLASMA OPTIC EMISSION SPECTROMETRY)

Fig: 4.1 ICP-OES INSTRUMENT

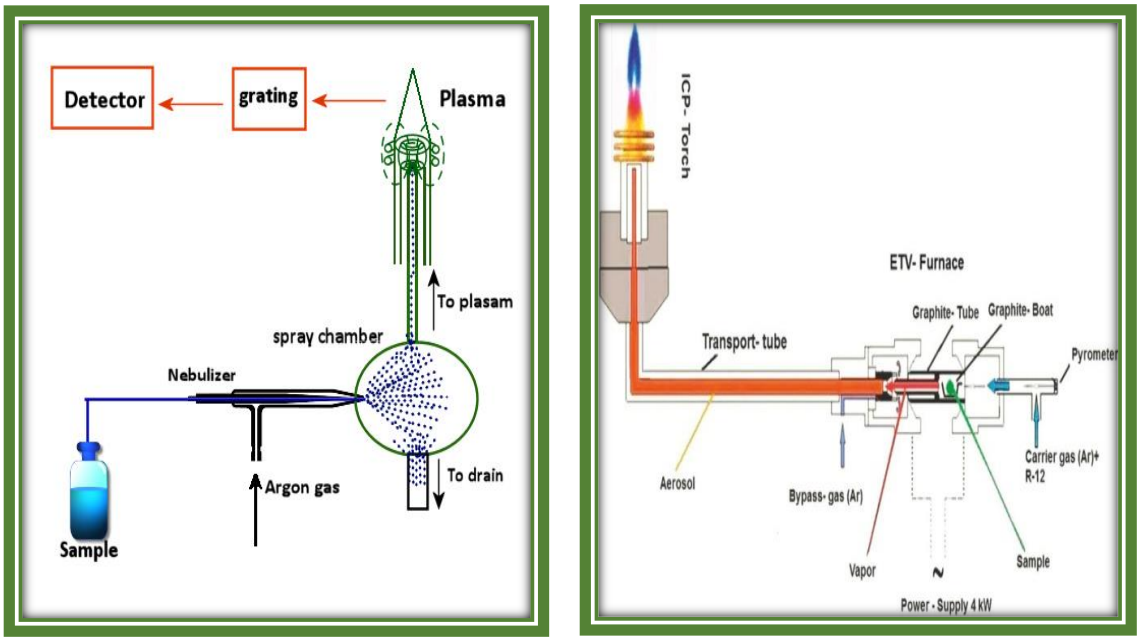


Fig:4.2 ICP-OES MECHANISM

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. [84]

Mechanism:

In plasma emwassion spectroscopy (OES), a VKM 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.[93]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.

Application:

The analysis of major and minor elements in solution NM.

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

SEM - SCANNING ELECTRON MICROSCOPE [85]

DEFINITION

Scanning Electron Microscopy (SEM), also known as SEM analysis or SEM microscopy, is 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) and 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 metal
- SEM 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: 5.1 SEM INSTRUMENT

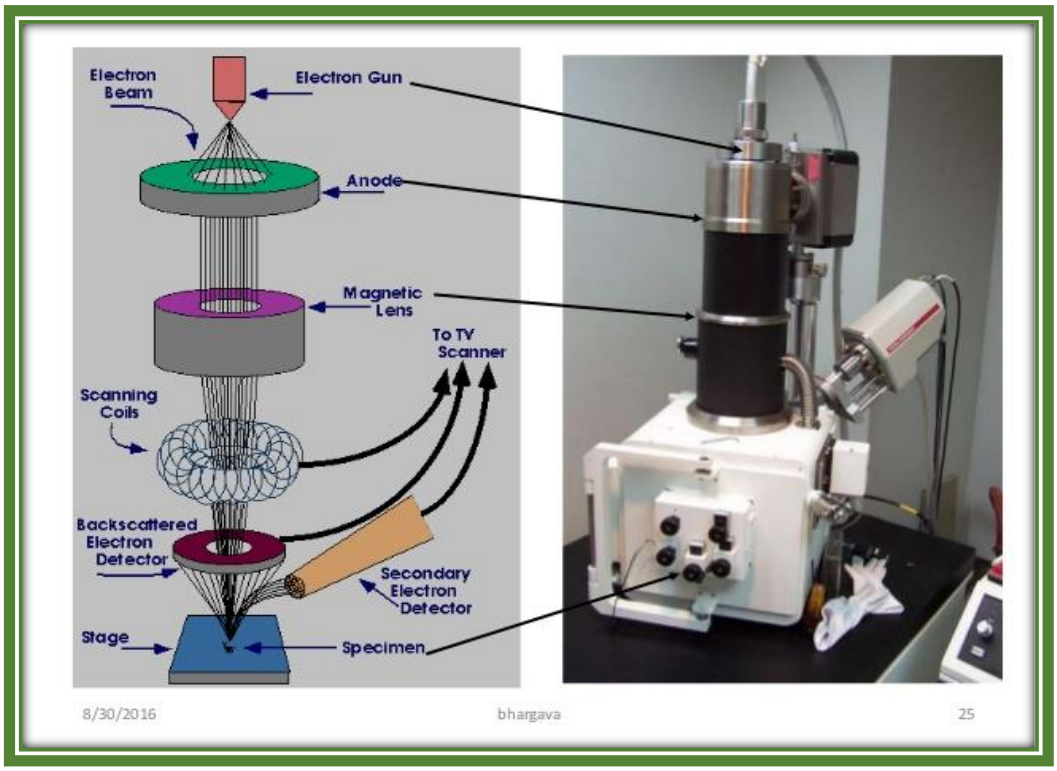


Fig no: 5.2 SEM MECHANISMS

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 is 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.

XRD - X-ray Powder Diffraction (XRD) [86]

X-ray powder diffraction (XRD) is a rapid analytical technique primarily used for phase identification of a crystalline material the analysed material is finely ground, homogenized, and average bulk composition is determined. (e.g. Determination of unknown solids is important to studies in geology, environmental science, material science and biology.

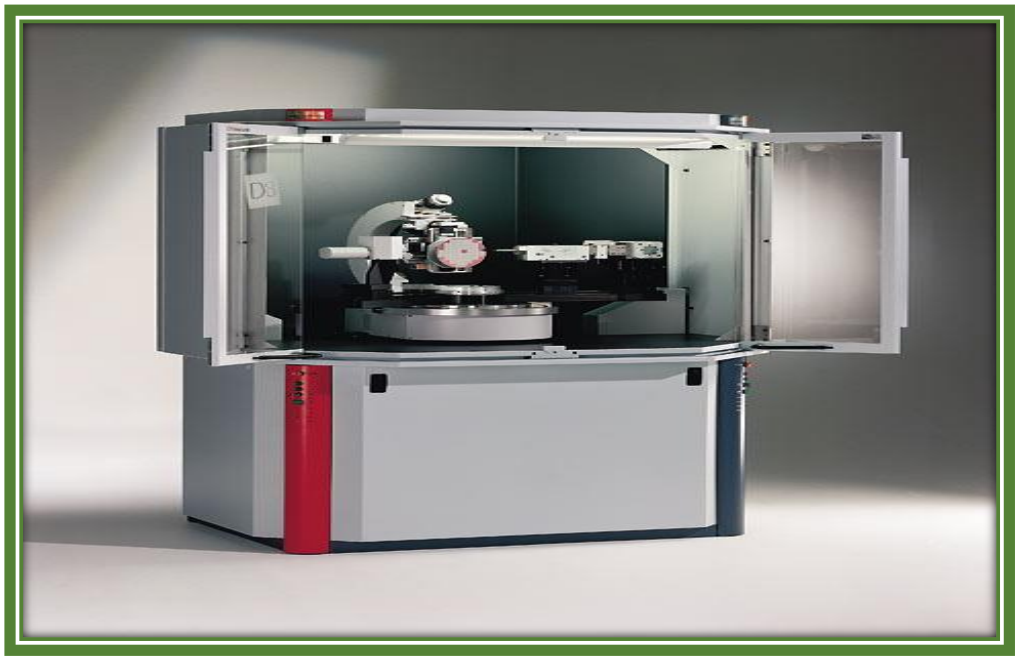


Fig no: 6.1 XRD - X-ray Powder Diffraction

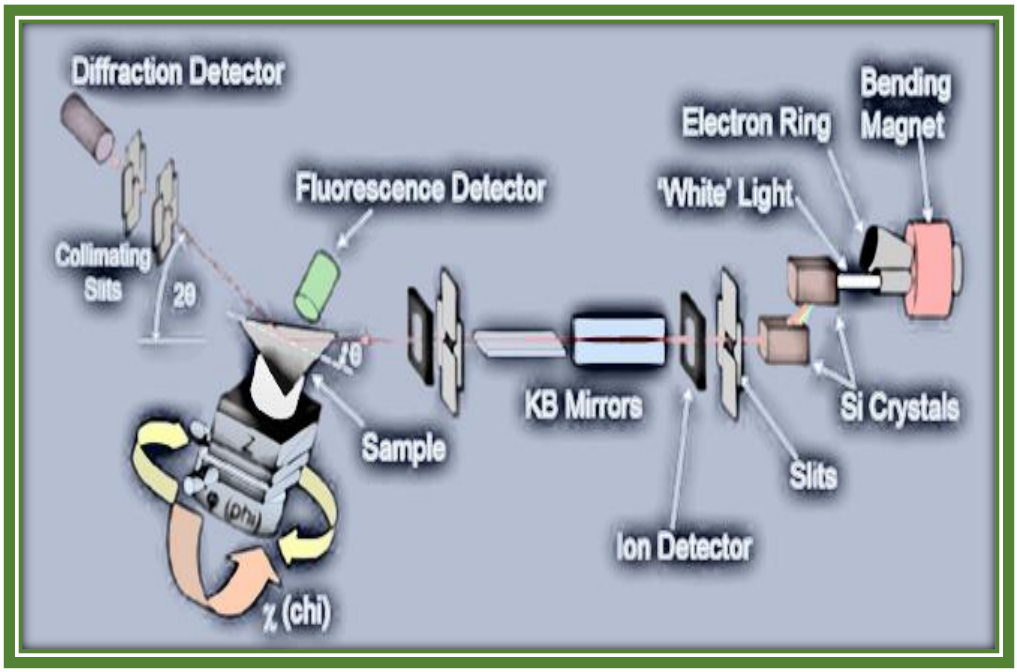


Fig no: 6.2 XRD Mechanism

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 multilayered 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 single-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 ɜm (or 200-mesh) in size is preferred.
- Place into a sample holder or onto the sample surface.

4.3. TOXICOLOGICAL STUDIES:

4.3.1 ACUTE ORAL TOXICITY – OECD GUIDELINES - 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 judgment on the acute toxicity of the test substance. Morbid animals or animals obviously in pain or showing signs of severe and enduring distress shall be humanely killed, and are considered in the interpretation of the test results in the same way as animals that died on 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%.

Acute toxicity study was carried out as per OECD guideline (Organization for Economic Co - operation and Development, Guideline-423. [87]

The experimental protocol was approved by the institutional ethical committee (IAEC) under CPCSEA (approval IAEC no**: 06/321/PO/Re/S/01/CPCSEA dated12/10/2018**

Animal: Healthy wistar albino female rat weighing 200–220 gm

Studied carried out at three female rats under fasting condition, signs of toxicity were observed for every one hour for first 24 hours and every day for about 14 days from the beginning of the study.

PRINCIPLE:

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 rat, although other rodent species may be used. Healthy young adult animals of commonly used laboratory strain Swiss albino are used. Females should be nulliparous and non-pregnant. Each animal at the commencement of its dosing should be between 8 and 12 weeks old and its weight should fall in an interval within±20 % of the mean weight of the animals.

Housing and feeding conditions:

The temperature in the experimental animal room should be 22ºC (+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 hrs light, 12 hrs dark. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water. Animals may be grouped and tagged by dose, but the number of animals per cage must not interfere with clear observations of each animal.

Test Animals and Test Conditions:

Sexually mature Female Wistar albino rats (200-220gm) 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 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.

Experiment Procedure:

Administration of doses

"Nannari mathirai" prepared as per the classical Siddha literature was suspended in 2% CMC with uniform mixing and was administered to the groups of Wistar albino rats.

It was given in a single oral dose by gavage using a feeding needle. Animals were fasted prior to dosing. Following the period of fasting, the animals were weighed and then the test substance was administered. 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 observed as per the guideline after substance administration.

The visual observations included skin changes, mobility, and aggressiveness, sensitivity to sound and pain, as well as respiratory movements. They were deprived of food, but not water 16–18 hours prior to the administration of the test suspension. Finally, the number of survivors was noted after 24 hours and these animals were then maintained for a further 14 days and observations made daily. The toxicological effect was assessed on the basis of mortality.

Number of animals and dose levels

Since this test drug has been under practice for long time and likely to be non-toxic, a limit test at one dose level of 2000 mg/kg body weight will be carried out with 6 animals (3 animals per step).

Duration of Study: 48 hours

Evaluation: 14 Days

Limit test

The limit test was 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 2000 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

The animals were observed individually after dosing at least once during the first 30mins and periodically during the first 24 hours.

Special attention: First 1-4 hours after administration of drug.

It was observed daily thereafter for a total of 14 days, except when they needed to be removed from the study and killed humanely for animal welfare reasons or are found dead.

a. Mortality

Animals will be observed intensively at 0.5, 2.0, 4.0, 6.0, 12.0, 24.0 and 48.0 hour following drug administration on day 1 of the experiment and daily twice thereafter for 14 days.

b. Body weight

Body weights will be recorded at day: -1, day 1, 2, 7 and 14 of the study

c. Cage-side observation

These include changes in skin and fur, eyes and mucous membranes and also respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behaviour 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 the data were summarised in tabular form showing the animals used, number of animals displaying signs of toxicity, the number animals found dead during the test or

killed for humane reasons, a description and the time course of toxic effects andreversibility, and necroscopic findings.

Test substance and Vehicle

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

Justification for choice of vehicle

The vehicle selected as per the standard guideline is pharmacologically inert and easy to employ for new drug development and evaluation technique [88]

4.3.2 REPEATED DOSE 28-DAY ORAL TOXICITY STUDY OF *NANNARI MATHIRAI* (OECD GUIDELINE - 407)

Test Substance	**:** *NANNARI MATHIRAI* (NM)
Animal Source	**:** TANUVAS,Madhavaram, Chennai.
Animals	**:** Wister Albino Rats (Male -40, and Female-40)
Age	**:** 6-8 weeks
Body Weight	**:** 150-220gm.
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.
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 + 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:

As per OECD guideline three dose levels were selected for the study. They are low dose (X), high dose (2X). X is calculated from the acute toxicity dose(2000mg) and the body surface area of the rat. i.e X dose is 200 mg/animal, 2X dose is 400 mg/animal. The results of acute toxicity studies in Wistar albino rats indicated that *"Nannari mathirai"* was non-toxic and no behavioral changes was observed up to the dose level of 2000 mg/kg body weight. On the basis of body surface area ratio between rat and human, the doses selected for the study were100mg/kg (Low dose), 200 mg/kg (Mid dose) and 400 mg/kg (High dose) body weight. The oral route was selected for use because oral route is considered to be a proposed therapeutic route.

Preparation and administration of dose

"Nannari mathirai" at three doses respectively was suspended in 2 ml of 2% CMC (Carboxymethyl cellulose) in distilled water. It was administered to animals at the dose levels of 100 mg/kg, 200 mg/kg and 400 mg/kg. The test substance suspensions were freshly prepared every day for 28 days. The control animals were administered vehicle (Water) only. Administration was by 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, mid and 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.

Table 8

Groups	No of Rats
Group I Vehicle control	20(10male, 10female)
Group II NM - low dose (100mg)	20(10male, 10female)
Group III NM -Mid dose (200 mg)	20(10male, 10female)
Group IV NM- High dose (400 mg)	20 (10male,10female)

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 1,15,28 at biweekly intervals throughout the course of study.

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.

Functional Observations:

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

Mortality:

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

Laboratory Investigations:

Following laboratory investigations were carried out on day 28 in animals fasted over-night. Blood samples were collected from orbital sinus using sodium heparin (200IU/ml) for Bio chemistry and potassium EDTA (1.5 mg/ml) for Hematology as anticoagulant. Blood samples were centrifuged at 3000 r.p.m. for 10 minutes. On 28th day of the experiment, 24hours urine samples were collected

by placing the animals in the metabolic cage with free access to tap water but no feed was given.

The urine was free from fecal contamination. Toluene was used as a preservative while collecting the sample. The sediments present in the urine were removed by centrifugation and the collected urine was used for biochemical estimations.

On 29th day, the animals were fasted for approximately 18 hours, then slightly anesthetized with ether and blood samples were collected from the retro-orbital plexus into two tubes: one with EDTA for immediate analysis of haematological parameters, the other without any anticoagulant and was centrifuged at 4000 rpm at 4 °C for 10 minutes to obtain the serum. Serum was stored at 20 °C until analyzed for biochemical parameters.

Haematological Investigations:

Blood samples of control and experimental rats was analyzed for haemoglobin 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.

Urine analysis:

Urine samples were collected on end of treatment for estimation of normal parameters. The estimations were performed using appropriate methodology.

Necropsy:

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

liver, kidneys, spleen, brain, heart, and lungs were recorded. The relative organ weight of each animal was then calculated as follows;

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of animal on sacrifice day (g)}} \times 100$$

Histopathology:

Control and highest dose group animals will be initially subjected to histopathological investigations. If any abnormality found in the highest dose group than the low, then the mid dose group will also be examined. Organs will be collected from all animals and preserved in 10% buffered neutral formalin for 24 h and washed in running water for 24 h. The organ sliced 5 or 6µm sections and were dehydrated in an auto technicon 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 Haematoxylin-eosin.

Statistical analysis:

Findings such as clinical signs of intoxication, body weight changes, food consumption, hematology and blood chemistry were subjected to One-way ANOVA followed by Dunnet test using a statistics software Graph pad version 7.

4.4. PHARMACOLOGICAL STUDIES:

4.4.1 HEPATOPROTECTIVE STUDY OF *NANNARI MATHIRAI* IN CCL_4 INDUCED HEPATOTOXICITY IN WISTAR ALBINO RAT MODEL:

Experimental design:

Animals were divided into 5 groups of 6 rats each. Group I animals served as control and received liquid paraffin (LP) subcutaneously at the dose of 3 ml/kg body weight of each animal. Group II animals received CCl_4+ LP (for 14 days) at the dose 1 ml CCl_4/kg body weight, in a suspension of double the volume of LP (which served as vehicle) subcutaneously at lower abdomen **1** on every 14 days of the treatment 2. Group III and IV animals received subcutaneous administration of CCl_4+ LP. They also received test drugs orally at the dose of 100, 200 mg/kg body weight respectively as a suspension of water. Group VI received in addition to CCl_4

suspension, silymarin (100 mg/kg body weight) daily. Silymarin was used as a standard reference drug 3.

The animals were kept starved overnight on 14th day of experiment. On the next day the animals were sacrificed by decapitation, and the blood was collected by cutting the jugular vein. The liver and kidney in each case were dissected out, blotted of blood, washed in saline and stored in a freezer. Liver were used for various biochemical estimations.

Biochemical parameters studied

The activities of serum glutamate pyruvate transaminase, and serum glutamate oxaloacetate transaminase was estimated using standard methods. Estimation of serum ALP, serum bilirubin and electrolytes were also carried out to assess the acute hepatic damage caused by CCL_4.

Statistical analysis

The data obtained from the study were subjected to statistical analysis by one way ANOVA followed by Dunnet„t" test, and results were expressed in terms of Mean±SEM values. Statistical analysis was performed using INSTAT- V3 Software programme [89].

4.4.2. HEPATOPROTECTIVE ACTIVITY OF *"NANNARI MATHIRAI"* AGAINST ETHANOL INDUCED HEPATOTOXICITY IN WISTAR ALBINO RAT MODEL:

Experimental design:

The animals were divided into five groups, six animals in each group. Animals in Group 1 were treated with Vehicle only twice a day P.O for 25 days served as Normal Control Group.Group 2 animals were treated with 40% ethanol 3.76 gm/kg twice a day P.O for 25 days which served as Positive Control Group.

Others animals were pre-treated twice daily with NM 100 and NM 200 mg & Silymarin 100 mg/kg P.O for 25 days, 1 hour before Ethanol administration. At the termination day, animals were anaesthetized using anesthetic ether and blood collected from retro orbital puncture. The level of AST, ALT, TP and ALP and BN were estimated as per the standard procedures described by manufacturer using serum kit.

Biochemical parameters studied:

The activities of serum glutamate pyruvate transaminase, and serum glutamate oxaloacetate transaminase was estimated using standard methods. Estimation of

serum ALP, serum bilirubin and electrolytes were also carried out to assess the acute hepatic damage caused by Ethanol.

Statistical analysis:

The data obtained from the study were subjected to statistical analysis by one way ANOVA followed by Dunnett t-test, and results were expressed in terms of Mean±SEM values. Statistical analysis was performed using INSTAT- V3 Software programme [90].

4.4.3 ANTIOXIDANT ACTIVITY OF NANNARI MATHIRAI [91]

DPPH radical scavenging assay (2, 2-diphenyl -1-picrylhydrazyl)

The radical scavenging activity of different extracts was determined by using DPPH assay according to Chang et al [2001]. 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,

$$DPPH + [H\text{-}A] \rightarrow DPPH\text{-}H + (A)$$

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 methanol.

Procedure

Different concentrations of sample such as 12.5µg/mL- 200µg/mL from a stock concentration of 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

Calculation

Percentage of inhibition = $\frac{control-test}{control} X100$

Reults were noted and tabulated in Table No.35 and Graph 8.

RESULTS AND DISCUSSION

5. RESULTS AND DISCUSSION

The well-known Traditional medicine ***NANNARI MATHIRAI*** had been subjected to various studies and standardization to establish the works of *Siddhars* to be true. The study includes

- Literary collections,
- Organoleptic character,
- Physicochemical analysis,
- Phytochemical analysis,
- Instrumental analysis,
- Toxicological study and
- Pharmacological study.

The drug "***NANNARI MATHIRAI"*** has been selected for the treatment of ***KAAMAALAI (JAUNDICE)*** in reference with the text ***"KANNUSWMY PRAMBARAI VAITHIYAM".***

Discussion on review of literature:

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 ***Kaamaalai (*** **Jaundice).**

Botanical aspect explains the identification, description, active principle and medicinal uses of the plants.

Gunapadam review brings the effectiveness of the drug in treating ***Kaamaalai (*** **Jaundice).**

Pharmaceutical review describes about the *Mathirai* and its properties.

The pharmacological review explains about the methodology of Hepatoprotective Activity and the drugs used for this study.

Modern and *Siddha* aspect of the disease was also reviewed.

Standardization of the test drug:

Standardisation of the drug is more essential to derive the efficacy and the potency of the drug by analysing it by various studies. Following are the results of physicochemical and phytochemical analysis. Physical characterisation and estimation of basic and acidic radicals have been done and tabulated.

Toxicological results of the drug and pharmacological activity of the drug was derived. Its result has been tabulated and the interpretation was made below. Thus, it

gives a complete justification to bring the effectiveness of the trial drug *Nannari Mathirai.*

The extensive review on botanical aspect gave information about the microscopical characters, macroscopical characters, medicinal uses, constituents and the importance of the herbs in detail. Most of the herbs included in the formulation are hepatoprotective in activity. The studies strongly supported the fact through these results. They are discussed below

Nannari is indicated for curing Jaundice (*Kaamaalai*).

Elam is used in the treatment of biliousness.

Seeragam is indicated for the treatment of Liver diseases. It also known to improving the fuctions of liver.

Perunjeragam is indicated for curing Jaundice (*Kaamaalai*).

Sevvagathi flower has an action of tonic to Liver.

Discussion on pharmacological aspect:

The pharmacological aspect of the drug says about their mode of action and the side effects which were used worldwide since ancient times.The current pharmacological methods available for carrying out the Hepatoprotective studies were explained clearly and the suitable In-vivo models carry out the activities were discussed.

Result from the pharmacological study denotes the effects of *Nannari ,Mathirai* showed the promising effects in treating liver damage. Moreover, the increased levels of the serum enzymes were significantly decreased by the treatment with *Nannari Mathirai* implying that the drug prevents the liver damage.

Discussion on Pharmaceutical review:

This review explained the preparation of *Mathirai* in detail including the purification of raw drugs, methods of manufacturing of *Mathirai* and the *Siddha* parameters for the standardization of analyzing *Mathirai.*

The powdered drugs were filtered through the white cloth so as to reduce the size of the particle in turn which enhances the bio-availability.The shelf life of the drug is improved by proper purification methods and preservation.

Discussion on Materials and Methods:

The preparation of the drug was done carefully so as to achieve the highest potency. *Mathirai* are fine, dry powders of drugs. The term *Mathirai* may be applied to the powder of single drug or a mixture of two or more drugs.

On purification, the weight of the *Mathirai* is different from the exact value but not from the mean value when calculated.

The *Mathirai* were also subjected to *Siddha* parameters of the testing like,

- *Mathirai* tends to be amorphous,
- It should be never damp,
- The fineness of the sieve should be 100 mesh or still finer.

The standardization of the drugs was achieved through various procedures like analyzing the organoleptic characters, physico-chemical characters, elements present in the drug and the results and discussion of standardization parameters is described below.

Organoleptic characters:

Table: 9. Organoleptic characters of *Nannari mathirai:*

Colour	Dark brown
Odour	Pleasant
Taste	Sweet
Texture	Powder in integrated form
Particle size	Completely pass through sieve no 88 when powdered

Table 10. Physicochemical Analysis of NM:

S.no	Parameters	Percentage
1	PH	5.45%
2	Loss on drying	2.88%
3	Total ash value	9.5%
4	Acid insoluble ash	6%
5	Water soluble ash	3%
6	Water soluble extraction	16%

7	Alcohol soluble extraction	8%
8	Solubility	
	Distilled water	Soluble
	Chloroform	Soluble
	Ethanol	Soluble

The physicochemical analysis of the drug (*NM)* result reveals pH, Loss on drying, Total ash value, Acid insoluble ash and Water-soluble ash. The interpretation of the result was given below.

Interpretation:

1. pH:

It is a measure of hydrogen ion concentration. It is the measure of the acidic or alkaline nature. 7.0 is neutral, above 7.0 is alkaline and below is acidic.

The pH of the drug *Nannari Mathirai* is 5.45 which is acidic in nature. Acidic drug is essential for its bioavailability and effectiveness. Acidic drugs are better absorbed in stomach.[92]

2. Moisture (Loss on drying):

The total amount of volatile content and moisture present in the drug was established in loss on drying. Moisture content of the drug reveals the stability and its shelf-life.

High moisture content can adversely affect the active ingredient of the drug. Thus, low moisture content could get maximum stability and better shelf life. Loss on drying of *Nannari Mathirai* is 2.88.[93]

3. Total Ash:

Ash constitutes are the inorganic residues obtained after complete combustion of a drug. Thus, Ash value is a validity parameter to describe and to assess the degree of purity of a given drug. Total ash value of plant material indicated the amount of minerals and earthy materials present in the drug. The total ash value of *Nannari Mathirai is* 9.5 % which determine the absence of inorganic content.

5. Acid insoluble ash:

The acid insoluble ash value of the drug denotes the amount of siliceous matter present in the plant. The quality of the drug is better if the acid insoluble value is low. Acid insoluable ash value of *Nannari Mathirai* is 6 %.

6. Water soluble ash:

Water-soluble ash is the part of the total ash content, which is soluble in water. Decreased water soluble ash value indicates easy facilitation of diffusion and osmosis mechanism. Water soluble ash value of *Nannari Mathirai* is 3%.

7. Solubility:

Solubility is the major factor that controls the bioavailability of a drug substance. It is useful to determine the form of drug and processing of its dosage form.

The most frequent causes of low oral bioavailability are attributed to poor solubility and low permeability.[94]

NM is soluble in major solvents and sparingly soluble in some solvents proves that its efficiency of solubility in the stomach indirectly, increasing the bio availability.

Weight variation test :

Weight variation was carried out to ensure that, each of tablets contains the proper amount of drug. The test was carried out by weighing the 20 tablets individually using analytical balance, then calculating the average weight, and comparing the individual tablet weights to the average. The percentage of weight variation is calculated by using the following formula. The result of weight variation test was tabulated in Table No11.

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

Table No 11: Uniformity weight variation test result of *Nannari Mathirai:*

S.NO	Weight of each mathirai(mg)	%of weight variation	Maximum weight variation within ± 7.5%	Maximum weight variation within ± 15.0%
1	129	5.444 %	Yes	Yes
2	137	4.666 %	Yes	Yes
3	132	2.123 %	Yes	Yes
4	131	-0.987 %	Yes	Yes
5	134	7.874 %	Yes	Yes
6	143	6.189 %	Yes	Yes
7	127	-5.690 %	Yes	Yes
8	122	1.900 %	Yes	Yes
9	134	-8.654 %	Yes	Yes
10	128	5.777 %	Yes	Yes
11	144	-2.763 %	Yes	Yes
12	138	0.076 %	Yes	Yes
13	130	3.132 %	Yes	Yes
14	126	-5.849 %	Yes	Yes
15	139	1.904 %	Yes	Yes
16	122	3.896 %	Yes	Yes
17	123	1.768 %	Yes	Yes
18	120	-3.765 %	Yes	Yes
19	143	-1.872 %	Yes	Yes
20	127	4.660 %	Yes	Yes
Average: 130.66 mg				

Average weight of the *Mathirai* was noted as 129.855g. Out of 20 tablets tested, 19 tablets of them lies within ±7.5% weight variation (1 tab above the limit) and all 20 tab lies within ±15% weight variation.

- According to the limits of weight test cited in the Indian pharmacopoeia, *Nannari Mathirai* passed the Uniformity weight test.
- The uniformity test resembles unformal distribution of this tablet helps good absorption and distribution.
- Hence all the tablets passed the weight variation test.

Table no 12. Traditional test for pill:

SL.NO	Character	Inference
1.	Non sticky on rolling	+
2.	No cracks over the surface after drying	+
3.	Shall be rolled uniformly over the plane surface	+
4.	Shining surface	+

Phytochemical analysis:

Table no :13. The phytochemical *Nannari Mathirai* result were given below:

S.No.	Phytochemicals	Test Name	H2O Extract
1	Alkaloids	Mayer's Test	+ve
2	Saponins	Foam Test	+ve
3	Phenols	Ferric Chloride Test	+ve
4	Flavonoids	Alkaline Reagent Test	+ve
5	Proteins	Xanthoprotein Test	+ve
6	Amino acids	Ninhydrin Test	+ve
7	Diterpenes	Copper Acetate Test	+ve

8	Gum & Mucilage	Extract + Alcohol	+ve
9	Quinones	NAOH + Extract	+ve

The above stated phytochemical properties for the given sample certified to be present.

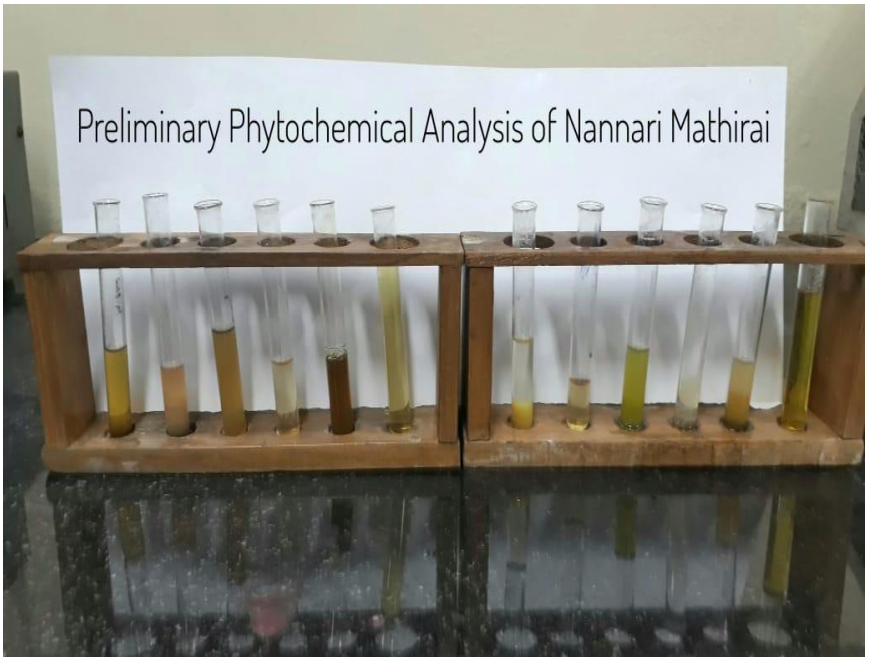


Fig 7: Phytochemical analysis of *Nannari Mathirai*

The phytochemical analysis of the drug (*NM*) result reveals Alkaloids, Saponins, Phenols, Flavanoids, Proteins, Amino acids, Diterpenes, Gum & Mucilage and Quinones. The interpretation of the result was given below.

Interpretation:

Alkaloids:

- Alkaloids possess antispasmodic, analgesic, bactericidal effects.
- Alkaloids are the active principles producing many essential effects in protecting the body [95].

Saponins:

- Saponins include, supporting kuffer cells in the liver and encouraging normal detoxification.
- In the digestive tract, saponins produce an emulsification of fat-soluble molecules. Saponins bind with bile acids and helps to eliminate them from the body, preventing cholesterol from being reabsorbed.

- Saponins can boost the immune system, have an antioxidant effect and may even support bone strength.[96]

Phenols:

- Phenols possess rich anti-oxidant property and protect the body from oxidative stress.
- Phenols inhibit the LDL cholesterol levels.
- Phenols reduces cell death and it regulate glucose metabolism.
- Phenols increase the vasodilation of blood vessels to promote circulation. It is a Effective anti-hyperglycaemic agent. [97]

Flavanoides:

- It is the most important group of polyphenolic compounds in plants.
- Flavonoids can exert their anti-oxidant activity by scavenging the free radicals, by chelating metal ions or by inhibiting enzymatic systems responsible for free radical generation.
- Flavanoids are immunomodulator. It also possesses anti-microbial activity which is confirmed by the various anti-microbial assays.[98]

Protein and amino acids:

- Proteins are very useful in the liver regeneration and energy production.
- They boost glutathione production to protect the liver.
- Protein is an amalgamation of amino acids. It is an important component of every cell in the body. Body uses protein to build and repair tissues [99].

Diterpenes:

- Diterpenes has an anti-oxidant effect. Diterpenes helps to cure hpertension. It also has tumour inhibitory properties as well as a stimulating effect on the immune system.
- It is used widely as a stomachic.[100]

Gum & Mucilage:

- It is used as a bulk laxative.Gum and mucilage are used for their demulcent properties for cough suppression.[101]

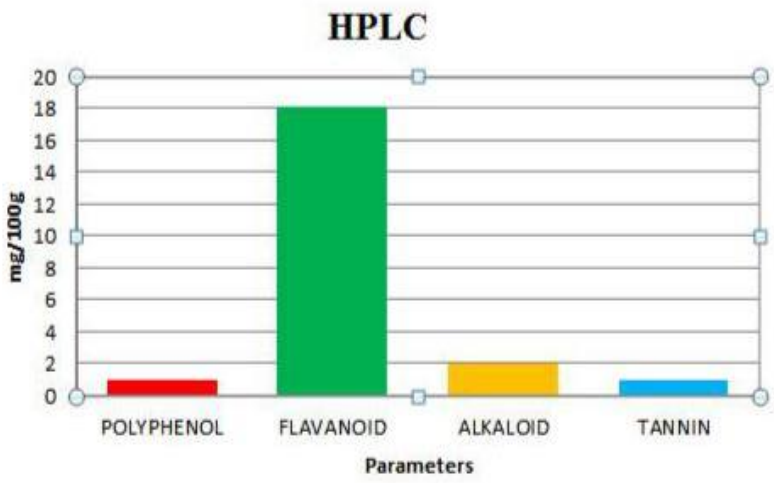
High Performance Liquid Chromatography (HPLC):

- HPLC analysis were done. HPLC analysis performed with *Nannari Mathirai* revealed the presence of following compounds:

Table:14. HPLC analysis for Nannari mathirai:

S.no	PARAMETERS	METHODS	UNITS	RESULTS
1.	Total polyphenol gallic acid equivalent	Indian Pharmacopoeia 2014	Mg/10g	0.34
2.	Total flavonoids as quercetin equivalent	TNTH/STP/FOOD/110	Mg/100g	17.20
3.	Total alkaloids	TNTH/STP/FOOD/426	Mg/100g	1.56
4.	Total tannin as tannic acid equivalent	AOAC20thEdn.2012, 955.35	Mg/100g	0.89

HPLC analysis reveals the presence of polyphenols, Flavanoids, Alkaloids and Tannins



Graph 1

Interpretation:

- Polyphenols are the member of very large family of plant derived compounds which had the anti lipidogenic effect. This is mainly due to reduced fatty acid and triglycerol synthesis, increased in fatty acid oxidation and reduction of oxidative stress and inflammation.

- Beneficial effects of polyphenols in the prevention and treatment of liver steatosis have been reported.
- Polyphenols are biomolecules which produce hepatoprotective effects which reduce the liver fat accumulation, mainly by reducing lipogenesis and by increasing fatty acid oxidation and decrease oxidative stress and inflammation are the main factors responsible for liver damage [102].
- Flavanoids a group of plant compounds which have the beneficial effects against Non-Alcoholic Fatty Liver Disease.
- Flavanoids prevent Hepatosteatosis by increasing fatty acid oxidation in liver. They can also reduce caloric intake and decrease body weight and fat deposition in viseral tissues.
- Flavanoids are the unique antioxidant. It also corrects dislipidemia and blood pressure [103].
- Tannins and alkaloids contain anti-oxidant effect which produce many essential effects in protecting the body.

BIOCHEMICAL ANALYSIS

Table: 15. Results of basic radicals' studies on *Nannari Mathirai*:

S.no	Parameter	Observation	Result
1	Test for Potassium	Yellow colour Precipitate	Positive
2	Test for Magnesium	White colour precipitate	Positive
3	Test for Iron (Ferrous)	Blood red colour	Positive
4	Test for Zinc	Formation of white Precipitate	Positive

The basic radical test reveals the presence of Potassium, Magnesium, Iron, Zinc. The interpretation of the result was given below.

Interpretation:

Presence of these traces of minerals play an important role in the functioning of various enzymes in biological system and also have immunomodulatory function and hence the susceptibility to the course and the variety of viral infections.

Potassium:

- Potassium levels may be an indicator of impending liver problems. Potassium is absorbed through the small intestine. Severe lack of potassium can disturb the liver function and if potassium level falls below 30% to 40% causes Non-Alcoholic Fatty Liver Diseases.
- Potassium is important for maintaining the integrity of cell membranes and functions as a vital electrolyte.[104]

Magnesium:

- Magnesium is essential for liver to prevent liver diseases. It enhances immune system. Depletion of magnesium levels leads to Cirrhosis and Fatty liver syndrome.
- It also helps to regulate blood glucose levels and aid in the production of energy and protein [105].

Iron:

- Iron is an essential micronutrient that is a critical component of oxygen transport proteins (Haemoglobin & Myoglobin). Chronic iron deficiency results in decreased haemoglobin production and anaemia which may result chronic liver diseases.
- Iron is essential for oxygen transport, energy production, other cellular growth and proliferation.Iron is an essential element for blood production and also needed for energy metabolism.[106]

Zinc:

- The liver plays a central role in zinc homeostasis. Zinc is a trace mineral that is essential to the normal functioning of the immune system.
- Zinc is essential for many metabolic and enzymatic functions. In Liver it act as powerful antioxidant.Deficiency of zinc leads to malabsorption syndrome and Cirrhosis of liver[107].

Table: 16. Test for acid radical studies on *Nannari Mathirai*:

S.no	Parameter	Observation	Result
1	Test for Sulphate	Formation of white precipitate	Positive

Interpretation:

The acidic radicals test reveals the presence of Sulphate. Presence of Sulphate is essential for liver protection by reducing increased serum enzymes of liver.

MICROBIAL LOAD:

Principle:

The antimicrobials present in the samples are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in **millimeters**.

GRAM NEGATIVE

Tab no 17.1 Organism: *E. coli*

Sample	**Concentration (µg/mL)**	**Zone of inhibition (mm)**
***Nannari Mathirai* (NM)**	Streptomycin (100µg)	26
	250	14
	500	16
	1000	16

14mm-Low sensitive,15mm- Moderate,16mm-Highly sensitive.

Tab no 17.2 Organism: *Klebsiella pneumoniae*

Sample	**Concentration (µg/mL)**	**Zone of inhibition (mm)**
***Nannari Mathirai* (NM)**	Streptomycin (100µg)	25
	250	16
	500	17
	1000	19

14mm-Low sensitive,15mm- Moderate,16mm-Highly sensitive.

Tab no: 17.3 Organism: *Pseudomonas aeruginosa*

Sample	Concentration (µg/mL)	Zone of inhibition (mm)
***Nannari Mathirai* (NM)**	Streptomycin (100µg)	30
	250	15
	500	16
	1000	17

14mm-Low sensitive,15mm- Moderate,16mm-Highly sensitive.

GRAM POSITIVE

Tab no 17.4 Organism: *Staphylococcus aureus*

Sample	Concentration (µg/mL)	Zone of inhibition (mm)
***Nannari Mathirai* (NM)**	Streptomycin (100µg)	26
	250	16
	500	19
	1000	20

Note: Concentration of stock 10mg/mL DMSO

14mm-Low sensitive,15mm- Moderate,16mm-Highly sensitive.

ANTIFUNGAL ACTIVITY

Tab no 17.5 Organism: *Aspergillus niger*

Sample	Concentration (µg/mL)	Zone of inhibition (mm)
***Nannari Mathirai* (NM)**	Clotrimazole(100µg)	37
	250	18
	500	20
	1000	24

14mm-Low sensitive, 15mm- Moderate,16mm-Highly sensitive.

INTERPRETATION:

Tab no 17.6 Microbial activity Results:

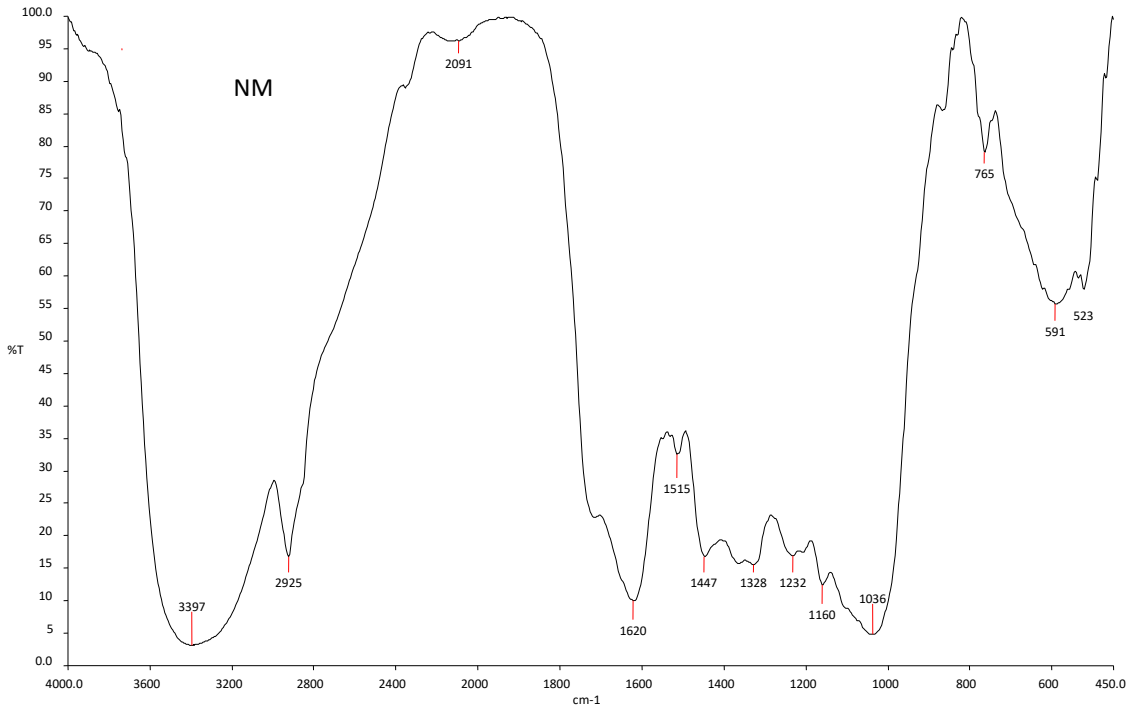
S.no	Name of the Microbes	Zone of Inhibition
1.	Escherchia coli (Highly sensitive)	500(µg/ml)
2.	Klebsiella pneumoniae (Highly sensitive)	250(µg/ml)
3.	Pseudomonas aeruginosa (Highly sensitive)	500(µg/ml)
4.	Staphylococcus aureus (Highly sensitive)	250(µg/ml)
5.	Aspergillus niger (Highly sensitive)	250(µg/ml)

Discussion:

The development of resistance against the presently available antibiotics arises the necessity of rediscovery of new anti-bacterial and anti-fungal agents in traditional systems of medicine. Different dosages of test drug against the microbes in antimicrobial activity of *NM* was compared with Standard drug Streptomycin and Clotrimazole (100µg)/ml disc for the following pathogens, they are *Escherchia coli*, *Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus, Aspergillus niger*. The results represent *NM* potentially inhibit the growth of all above organism in 250µl, 500µl and 1000µl / disc. 14 mm – Low sensitive, 15 mm – Moderate, above 16 mm – Highly sensitive. The findings reveal that the Siddha drug *NM have* anti-microbial potency against bacterial and fungal pathogens which is used in the treatment of diseases.

INSTRUMENTAL ANALYSIS:

FTIR (Fourier Transform Infrared) Spectrum Analysis: FT-IR is an effective analytical tool for identification of an unknown sample screening and profiling samples. The sample is irradiated by a broad spectrum of infrared light and after Fourier transformation the level of absorbance at a particular frequency is plotted. The resulting spectrum is characteristic of the organic molecules present in the sample.



Graph:2 FT-IR analysis for Nannari Mathirai

Table 18: FT-IR INTERPRETATION:

Absorption peak cm-1	Stretch	Functional group
3399	N–H stretch	1°, 2° amines, amides
2925	C–H stretch	Alkanes
2091	-C=N Stretch	Alkanes
1620	–C=C– stretch	Alkanes
1515	N–O asymmetric stretch	nitro compounds
1447	C–H bond	Alkanes
1328	N–O symmetric stretch	nitro compounds
1232	C–N stretch	aliphatic amines
1160	C–N stretch	aliphatic amines
1036	C–N stretch	aliphatic amines
765	C–H “oop”	Aromatics
591	C–Br stretch	alkyl halides
523	C–Br stretch	alkyl halides

The above table shows the presence of alkanes, amide, alkyl halides, nitro compounds, aromatics, aliphatic amines which represents the peak value. The interpretation of the result was given below.

Interpretation:

FTIR instrumental analysis was done. The test drug (*NM*) was identified to have 13 peaks. They are the functional groups present in the trial drug *Nannari Mathirai.*

Aromatic amines have anti-oxidant property. In liver aromatic amines involves metabolic activation and also involved in detoxification. [108]

Nitro compounds has higher potential towards inhibitory activity against microorganisms.

Amides enhance the drug effect against the hepatic disease. It also acts on the neurotransmitters; it is involved in the protein synthesis.[109]

Alkanes have little biological activity; it protects against Microorganism.

Alkyl halides involved in free radical scavenging activity and also used in the treatment of cancer.

Aliphatic compounds possess diverse biological activities, for example, hepatoprotective activity, antioxidant and antidepressant activities [110].

SEM (SCANNING ELECTRON MICROSCOPE):

The Scanning Electron Microscope uses a focused beam of high energy electrons to generate a variety of signals at the surface of solid specimens. The signals that derive from electron-sample interactions reveal information about the sample including external morphology, chemical composition, crystalline structure and orientation of materials making up the sample.

Fig 8 SEM 5µm (SCANNING ELECTRON MICROSCOPE)

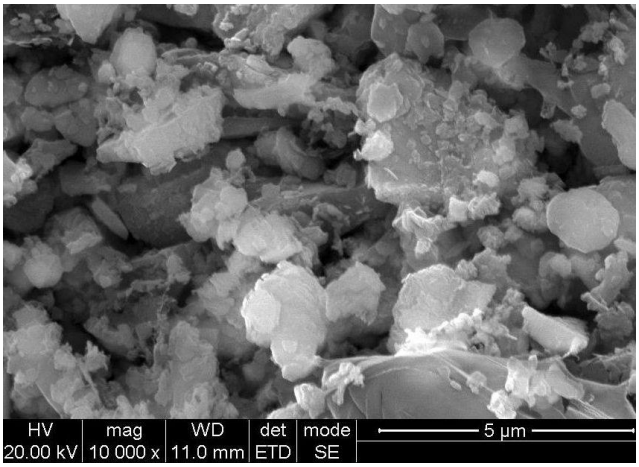
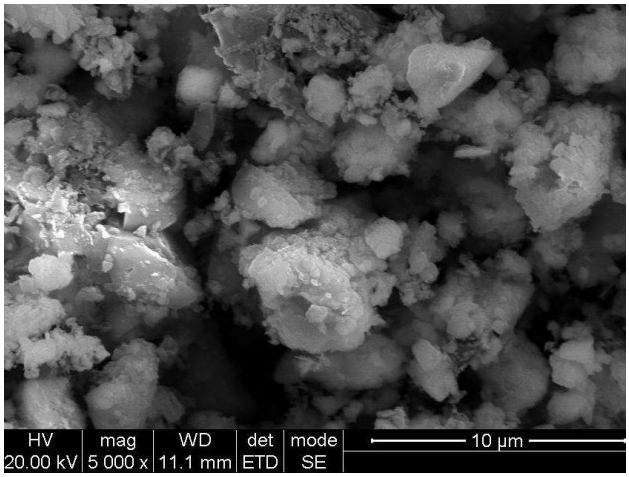


Fig 8.1 SEM 10µm (SCANNING ELECTRON MICROSCOPE)



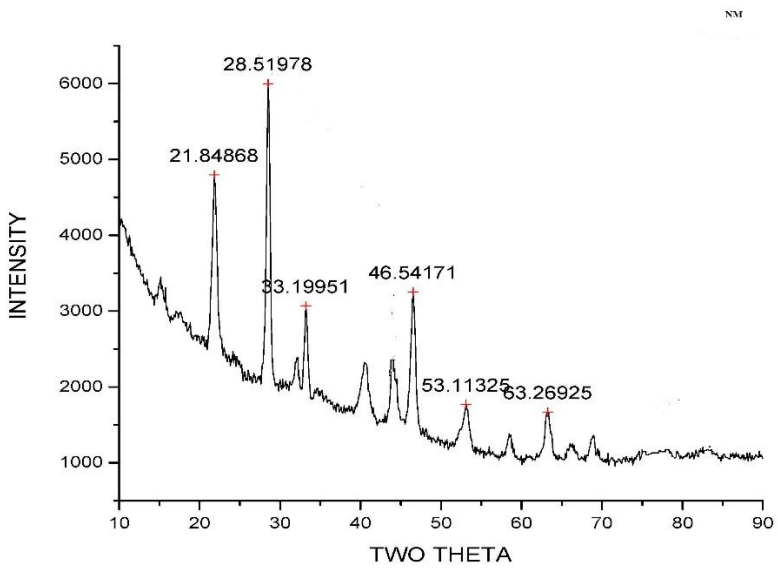
The SEM image is done by 500X magnification via 10µm aperture shows maximum depth focused.

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 *Nannari Mathirai* 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 1-3 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 [111]
- Hence *Nannari Mathirai which* is prepared biologically contains nanoparticles to enhance the pharmacological action in the target site.

XRD (X-Ray Diffraction studies) :

X-Ray Diffraction allows one to asceertain the molecular structure of a crystalline material by diffracting X-rays through the sample.



GRAPH:3 XRD for NM

Interpretation :

The crystalline structure, the size and shape of the particles are highly dependent on the route of synthesis and highlight the efficacy of the drug. The nano particles may enhance bio absorption of the drug.

XRD pattern of *Nannari Mathirai* shows the good crystallinity after calcinations process. The major diffraction peaks range from (21.84 to 63.26) are identified after XRD analysis *NM* concluded that Nano crystalline range (21-46nm) is association with organic molecules probably plays an important role in making it biocompatible and nontoxic at therapeutic doses. Other elements present in *NM* act as additional supplement and possibly helps in increase the efficacy of the formulation [112].

ICPOES (Inductively Coupled Plasma Optical Emission Spectrometry):

ICPOES is a technique in which the composition of elements in samples can be determined using plasma and a spectrometer.

Table 19: ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry) results of *Nannari Mathirai* :

S.NO	Elements	Wavelength	Detected levels
1	Aluminium (Al)	396.152	BDL
2	Arsenic (As)	188.979	BDL
-3	Calcium (Ca)	31.150	31.150 mg/L
4	Cadmium (Cd)	228.802	BDL
5	Copper (Cu)	327.393	BDL
6	Iron (Fe)	238.204	01.554mg/L
7	Mercury (Hg)	253.652	BDL
8	Pottasium (K)	766.491	03.171 mg/L
9	Magnesium (Mg)	285.213	01.804 mg/L
10	Sodium (Na)	589.592	24.150mg/L
11	Nickle (Ni)	231.604	BDL
12	Lead (Pb)	220.353	BDL
13	Phosporous (P)	213.617	99.307 mg/L
14	Zinc (Zn)	206.200	01.206 mg/L

Interpretation:

The above results indicate that the trial drug is extremely safe as it contains heavy metals within specified limits. The presence of Ca (31.150 mg/l), Fe (01.554 mg/l), K (03.171 mg/dl), Na(24.150mg/l), P(99.307 mg/dl) and Zn(01.206mg/l) has physiologically important. In Nannari Mathirai, the heavy metals like Al, As, Cd, Cu, Hg, Pb, and Ni were below detectable level. This reveals the safety of the drug. From the above results the heavy metals are observed with in permissible limits. Hence the safety of the drug is ensured.

TOXICITY STUDY RESULTS:

Wistar albino rat was treated with the test drug *Nannari Mathirai* of single dose of 200mg/kg. This study was conducted as per the OECD guidelines. The result of acute toxicity of *Nannari Mathirai* has been tabulated below.

Acute oral toxicity study of *Nannari Mathirai* – OECD 423 Dose finding experiment and its behavioural Signs of acute oral Toxicity:

Administration of doses

"Nannari mathirai" prepared as per the classical Siddha literature was suspended in 2% CMC with uniform mixing and was administered to the groups of Wistar albino rats.

Table: 20. Observation done:

Group	Observation (control group)	Observation (Test group)
Body weight	Normal	Normally Increased
Assessments of posture	Normal	Normal
Signs of Convulsion Limb paralysis	Normal	Absence (-)
Body tone	Normal	Normal
Lacrimation	Normal	Absence
Salivation	Normal	Absence
Change in skin color	Normal	No significant colour change
Piloerection	Normal	Normal
Defecation	Normal	Normal
Sensitivity response	Normal	Normal
Locomotion	Normal	Normal
Muscle gripness	Normal	Normal
Rearing	Normal	Mild
Urination	Normal	Normal

Table: 21. (Observational study Results):

Dose mg/kg	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Control	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-
2000 mg	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-

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. Musclerelaxant 13. Hypnosis 14. Analgesia15.Lacrimation 16. Exophthalmos 17. Diarrhoea 18. Writhing 19 Respiration 20. Mortality.

Table 22 Body weight of wistar albino rats group exposed to *NANNARI MATHIRAI*:

DOSE (mg/kg/day)	DAYS		
	1	7	14
Control	280.2±42.30	281.4 ± 64.12	282.6 ±26.18
400(High dose)	270.4± 21.24	271 ± 3.64	271.4 ± 2
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 23 Water intake (ml/day) of Wistar albino rats group exposed to *NANNARI MATHIRAI:*

DOSE (mg/kg/day)	DAYS		
	1	7	14
Control	61 ± 1.12	62±2.22	63.9±1.14
400(High dose)	58.2±1.1	58±1.14	59.20±24
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 24: Food intake (gm/day) of Wistar albino rats group exposed to *NANNARI MATHIRAI :*

DOSE (mg/kg/day)	DAYS		
	1	7	14
Control	56.24±2.22	56.2±7.42	58.4±3.46
400(High dose)	59.6±1.63	59.6±2.62	60.1±5.38
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).*

Interpretation of Acute toxicity studies:

- The acute oral toxicity potentials of *Nannari mathirai* in female Wistar albino rats were studied effectively.In the sighting study, the test substance was administered in sequential manner to one animal each at 2000 mg kg-1 body weight followed by two animals at 2000 mg kg-1 body weight.
- According to OECD guidelines, for acute oral toxicity LD50 dose of 2000mg/kg of the drug is found to be safe.
- From the maximum tolerable dose 2000mg/kg of *Nannari Mathirai* 1/5th or 1/10th of the dose was considered as therapeutic dose for further study.
- The treated animals were observed for mortality, untoward clinical/toxic signs, and alterations in body weight gain and necropsy findings during the study.
- The treated animals survived throughout the study period and did not reveal any treatment related major abnormal clinical signs at the test dose levels.
- Morphological characters like changes in skin, eyes, fur, nose appeared normal.
- The rats did not reveal any observable signs of central nervous system.
- The rats showed signs of alertness, grooming and touch response at the dose level of 2000mg/kg of body weight.
- The overall percentage of body weight gain in rats treated with the drug every weekly was found to be normal indicating that the test animals were in a healthy condition during the days of observation period.
- The weight gain of the animal was showed in Table. The changes in water and food intake recorded and it did not show any distinct deviations.
- On necropsy, no abnormalities were observed. In conclusion, acute oral toxicity testing of screened drug did not produce any treatment-related adverse effects.
- This indicates that the dosages administered were below toxic level and proves the safety of the drug.
- Hence the test drug *Nannari Mathirai* is a safe herbal drug and can be used for long time administration.

Repeated dose 28-day oral toxicity of *Nannari Mathirai* OECD – 407

The results of acute toxicity studies in Wistar albino rats indicated that *Nannari Mathirai* was non-toxic and no behavioural changes was observed up to the dose level of 2000 mg/kg body weight. On the basis of body surface area ratio between rat and human, the doses selected for the study were100mg/kg, 200 mg/kg and 400 mg/kg body weight. The oral route was selected for use because oral route is

considered to be a proposed therapeutic route. Then Sub acute oral 28 days repeated dose toxicity study was done and the results were tabulated below.

Table 25 : Body weight (g) changes of rats exposed to *NANNARI MATHIRAI:*

Dose (mg/Kg/day)	Days				
	1	7	14	21	28
Control	220.37±3.21	240.14±4.09	281.21±2.17	272.21±5.11	331.32±1.89
100 (low dose)	220.28±3.21	220.21±3.25	240.17±2.71	242.12±3.41	262.22±3.54
200 (mid dose)	230.22±2.45	232.45 ±.65	253.25±3.42	263.25±2.14	292.25±2.34
400 (high dose)	210.12 ±.45	232.65±1.75	248.48±3.25	253.45±2.34	283.45 ±.25
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)*

Table: 26. Water intake (ml/day) of Wistar albino rats group exposed to *NANNARI MATHIRAI*:

Dose (mg/Kg/day)	**Days**				
	1	**6**	**14**	**21**	**28**
Control	60.2 ± 1.21	60.6±6.12	62.2±4.10	62±4.12	64.6±1.32
100 (low dose)	62.1±1.10	62.6±2.42	62.9±1.72	63.2±6.86	64.4±1.54
200 (mid dose)	58.1±1.26	58.3±3.21	59.1±6.41	59.4±1.72	59.4±1.82
400 (high dose)	54.1±1.41	54.2±1.42	54.4±1.44	54.6±1.52	55.8±2.82
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)*

Table: 27. Food intake (ml/day) of Wistar albino rats group exposed to *NANNARI MATHIRAI:*

DOSE (mg/Kg/day)	DAYS				
	1	7	14	22	28
CONTROL	36±4.12	36.2±3.12	37.3±2.84	37.2±1.41	38±2.43
100(low dose)	38.2±1.41	38.3±1.13	38.1±1.21	39.5±1.23	39.5±1.26
200 (mid dose)	35.1±3.32	35.2±3.04	35.2±2.42	36.2±2.61	37.2±1.42
400 (high dose)	37.1±1.32	37.1±1.41	37.6±2.62	38.2±1.10	39.6±3.42
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.*

Interpretation of weight, water and food intake

- The overall percent of body weight gain in rats treated with the drug was found to be normal and showing a steady increase in weight indicating that the test animals were in a healthy condition during the 28 days of observation period.
- There is no significant change in water intake by the animals during the period of study.
- The weight increase of the animals showed that the intake of food by the animals was good during the period of 28 days study.

Table 28: Effect of *NANNARI MATHIRAI* on Haematological parameters:

Parameters	Control	100 mg/kg	200 mg/kg	400 mg/kg	P value
Total cholesterol(mg/dl)	111.53±13.17	110± 18.2	108.6±7.8	105.4± 22.8	NS
Triglycerides (mg/dl)	97.56± 14.5	94.3± 11.2	86.2± 15.4	85.6±9.8	NS
Glucose (mg/dl)	113.4± 12.2	97.2± 9.6	83.4± 10.2	80.5± 13.4	NS
LDL	78.6±2.13	78.7±2.05	78.10±1.03	77.40±01.32	NS
VLDL	14.2±1.52	13.20±2.41	14.02±1.32	14.04±12.15	NS
HDL	28.12±4.32	28.32±2.50	28.46±1.20	29.51±1.23	NS
Albumin (g/dl)	6.21±0.22	6.22±0.52	6.4±7.20	6.55±6.48	NS

*Values are expressed as mean S.E.M (Dunnett's test). *P<0.05, **P<0.01,***P<0.001 vs control; N=10.*

LDL-Low Density Lipoprotein, VLDL-Very Low Density Lipoprotein, HDL-High Density Lipoprotein.

Interpretation

The biochemical parameters are within the normal range. This shows that the trial drug shows safe and non toxic effects on general body metabolism.

Table 29: Effect of *NANNARI MATHIRAI* in Liver Function test:

Parameters	Control	100 mg/kg	200 mg/kg	400 mg/kg
Protein (mg/dl)	6.33 ± 0.3	6.72 ± 1.4	6.4± 2.4	6.6± 0.8
Total Bilirubin (mg/dl)	0.9 ± 0.08	0.62 ± 0.04	0.72± 0.08	0.83± 0.12
SGOT (U/L)	86.5 ± 5.0	85.4 ± 8.2	83.2 ± 4.6	80.3± 13.3
SGPT(U/L)	46.5 ± 6.2	43.4± 7.3	42.4± 5.8	40.4 ± 16.2
Alkaline phosphatase(U/L)	48.6 ±7.2	52.6 ± 13.4	42.8 ± 1.2	56.3± 3.6

*Values are expressed as mean S.E.M (Dunnett's test). *P<0.05, **P<0.01,***P<0.001 vs control; N=10.*

SGOT-Serum Glutamic-Oxaloacetic Transaminase,SGPT-Serum Glutamate-Pyruvate Transminase,ALP-Alkaline phosphatase.

Interpretation

- The total bilirubin, bilirubin direct and bilirubin indirect showed that normal range.
- Thus the liver function test of *NM* shows normal in this 28 day repeated oral toxicity.

Table 30: Effect of *NANNARI MATHIRAI* In Renal function test:

Parameters	Control	100 mg/kg	200mg/kg	400mg/kg
BUN (mg/dl)	21.33±4.6	22.4±1.6	22.3±1.2	24.2±2.4
Urea (mg/dl)	63± 4.3	63±2.2	62±5.2	61.4±3.8
Creatinine(mg/dl)	0.53±0.03	0.62±0.04	0.55±0.007	0.63±0.04
Uric acid (mg/dl)	4.02±0..04	4.06±0.21	4.4±0.13	4.20±0.10

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

BUN-Blood Urea Nitrogen.

Interpretation:

The renal function test of the animals shows the normal limits thus the trial drug was safe and not produce any nephro toxicity, thus it suggests that the trial drug Nannari mathirai was safe for long term administration.

DISCUSSION:

Observations:

Overall observations were similar in both sex rats.

Clinical signs of toxicity:

No clinical signs of toxicity were observed

No mortality was observed after 28 days repeated dose administration of *Nannari mathirai.*

All animals survived to study termination period.

Body weight:

No significant alterations were observed in body weight.

Food and water consumption:

No significant alterations were observed.

Physiological activities:

No changes in the general behaviour.

Blood analysis:

Hematology:

No treatment related effects were observed.However there is a slight variations in the result but they were in the permissible limit.

Biological parameters:

There is a slight difference has been noted but it is normal within the normal limit.No treatment related effects were observed.

Histological examination:

Histological examination of organs did not show any pathological changes.

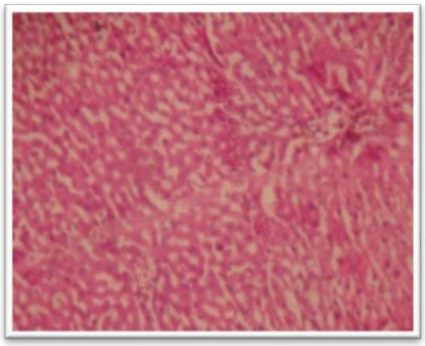
Discussion:

The acute and repeated 28 days oral toxicity studies of *Nannari mathirai* did not produce any toxicity signs in wistar albino rats. Daily administration of *Nannari mathirai* at different doses 100mg/kg,200mg/kg, 400mg/kg for 28 days was tolerated by the rats without any mortality and morbidity, indicates the drug tolerance. Hence the polyherbal formulation of *Nannari mathirai* can be considered to be safe drug for prolonged duration use as revealed by toxicological studies.

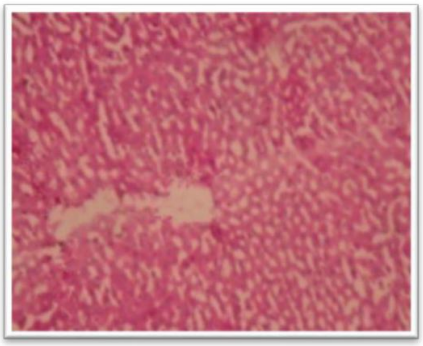
HISTOPATHOLOGY OF REPEATED ORAL TOXICITY STUDIES:

Fig:9

LIVER

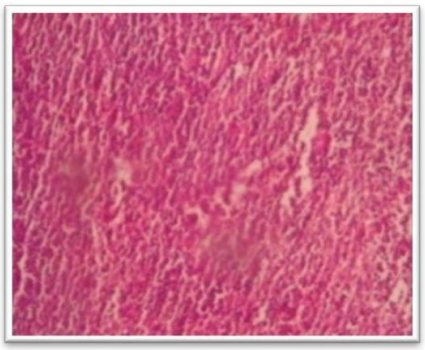


CONTROL

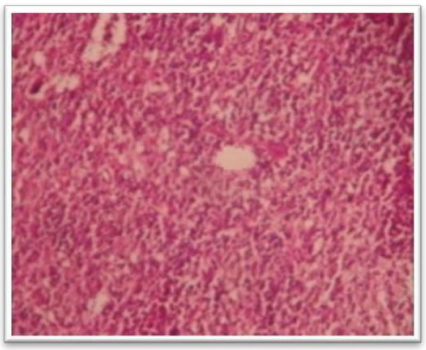


HIGH DOSE

SPLEEN

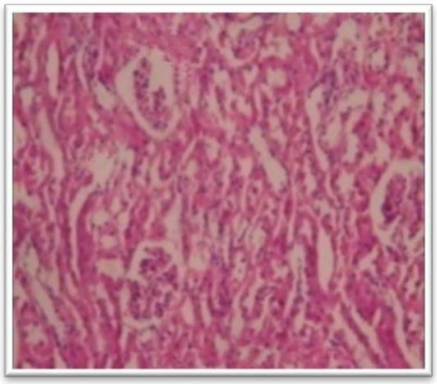


CONTROL

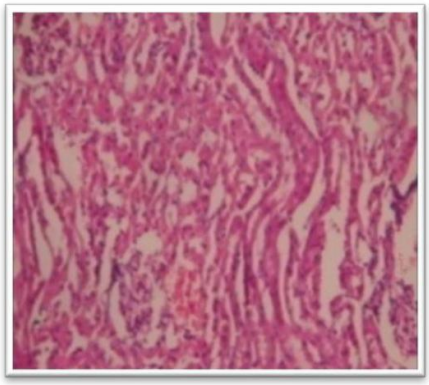


HIGH DOSE

KIDNEY



CONTROL



HIGH DOSE

Interpretation:

From the histopathological examination, the slides of animal organs did not reveal abnormalities.

From the acute and repeated oral toxicity studies the drug produced some significant changes. But the values were found within normal limits. So, the drug *Nannari Mathirai* was non-toxic and safe.

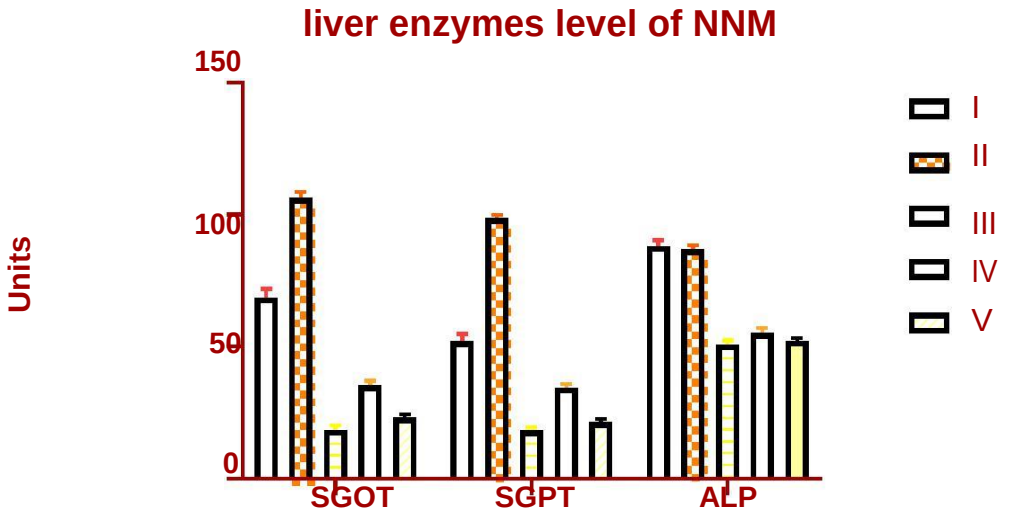
Thus, the safety of the drug is revealed so that it can be administered for long time without side effects.

PHARMACOLOGICAL ACTIVITIES:

Tab 31. EFFECT OF *NANNARI MATHIRAI* ON LIVER INJURY INDUCED BY CCL_4 IN WISTAR ALBINO RATS:

Group	Treatment	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)
I	Normal control	68.50±3.33	52.07±2.77	88.11±2.18
II	Normal saline+CCL_4	106.4± 2.26	98.64± 1.35	86.9±1.57
III	Silymarin + CCL4	18.62± 0.45**	18.28± 0.87**	50.87±0.48**
IV	NM 100mg + CCL4	35.36± 1.7**	34.45± 0.54**	55.18± 1.92**
V	NM 200mg + CCL4	23± 0.07**	21.43± 1.10**	52± 0.08**

*Values are expressed as mean ± S.E.M followed by Dunnett test. *P < 0.05, **P < 0.01, ***P < 0.001 vs control. (n - 6);*



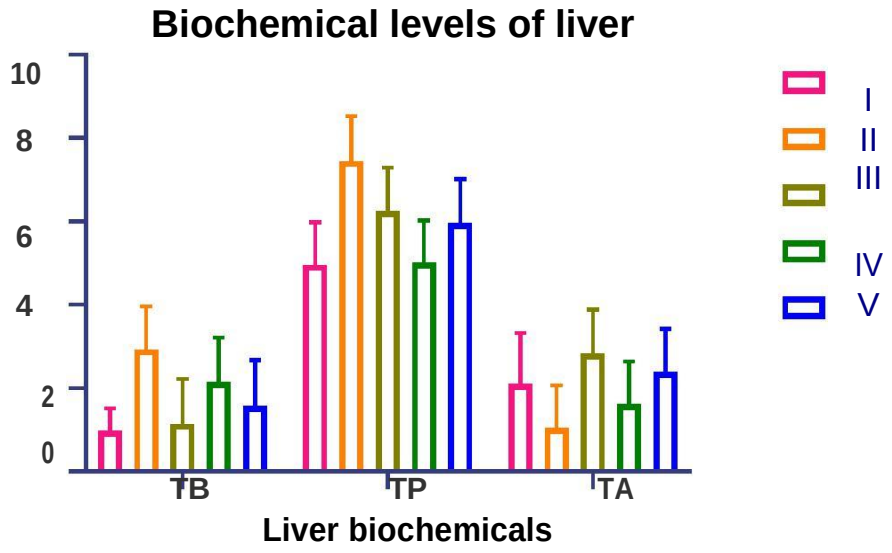
GRAPH4:

SGOT-Serum Glutamic-Oxaloacetic Transaminase,SGPT-Serum Glutamate-Pyruvate Transminase,ALP-Alkaline phosphatase.

Table no 32: Effect of total Protein and Bilirubin with CCL_4 IN RAT

Group	**Treatment**	TB (mg/100ml)	TP (mg/dl)	TA (mg/dl)
I	Control	0.98± 0.54	4.96±1.02	2.11±0.22
II	CCL_4	2.94 ± 0.02	7.45 ± 0.07**	1.05± 0.02
III	Silymarin	1.15 ± 0.07**	6.25 ± 0.04**	2.84± 0.05**
IV	NM100mg	2.16 ± 0.05**	5.01 ± 0.02**	1.63 ± 0.01**
V	NM200mg	1.59 ± 0.09**	5.96± 0.06**	2.40 ± 0.03**

*Values are expressed as mean ± S.E.M followed by Dunnett test. *P < 0.05,*



GRAPH 5:

Total Bilurubin, TP-Total Protein, TA-Total Albumin

HISTOPATHOLOGY SLIDES OF NM ON CCL_4 INDUCED HEPATOTOXICITY:

Group I: Control rat showing normal central vein and normal hepatocytes.

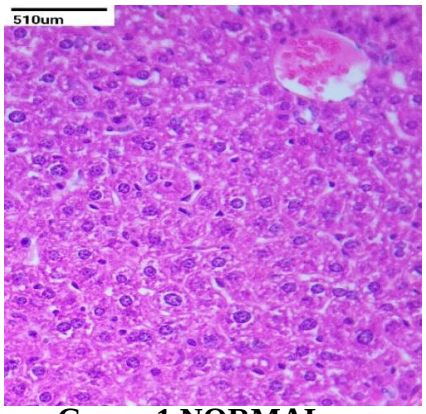
Group II: Showing dilated central vein and hepatocytes with degeneration.

Group III: Photo micrograph of liver tissue treated with silymarin showing normal hepatocytes, portal vein (V), portal Artery

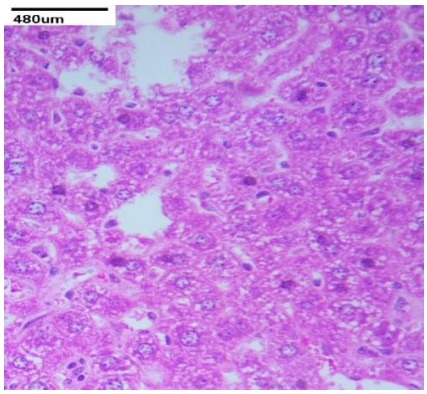
Group IV: Liver tissue of rats treated with *NM* 100 mg /kg showing mild degree of necrosis (N) with normal cells (C).

Group V: Liver tissue of rats treated with *NM* 200 mg /kg Central vein showing normal hepatocytes with regenerating hepatocytes and mild inflammation in the portal area.

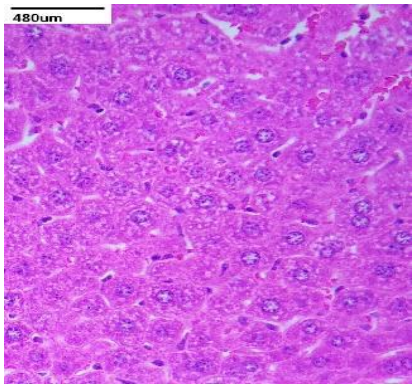
Fig 10



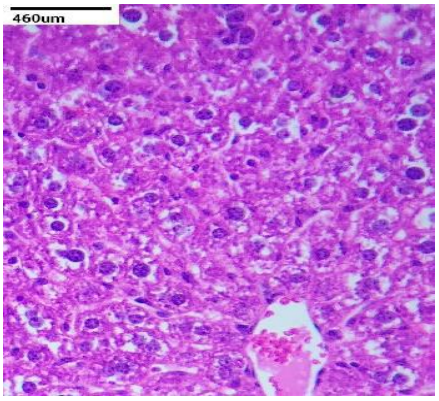
Group 1 NORMAL



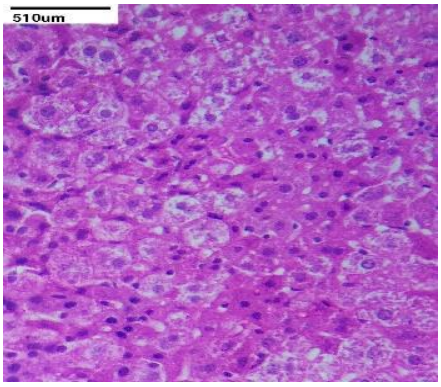
Group 2 CCL_4



Group 3 Silymarin



Group 4 NM 100mg



Group 5 NM200mg

Interpretation

- The serum marker enzymes SGOT, SGPT and Total Proteins were exceedingly susceptible to hepatotoxins. They assist as markers of inflammation of liver cells or death of some cells due to liver damage and oxidative stress, which stimulate the release of amino transferases from hepatocytes into the blood.
- The changes associated with Carbon tetrachloride induced liver damage of the present study appeared similar to the acute viral hepatitis.
- In CCl_4 induced hepatotoxicity, the administration of the toxicant CCl_4 showed a distinct rise in the levels of serum marker enzymes namely SGOT, SGPT and Total protein as shown in the Table. 29
- The protective effects of *Nannari Mathirai* and silymarin on the effect of liver serum marker enzymes are exposed in Table 32.
- The serum enzymes like SGOT, SGPT and Total Protein of treated animals were significantly reduced by seven days pre-treatment of *Nannari Mathirai* at three dose levels 100 mg/kg and 200mg/kg when compared with CCl_4 treated control. No significant clinical abnormalities in other groups.
- This study reveals that increase in the activity of the serum enzymes SGPT, SGOT and Total Proteins were detected in wister Albino rats treated with normal saline and CCl_4 (Group 1 and 2). However, the activities of these serum enzymes were significantly ($P < 0.01$) lower in rats treated with *Nannari Mathirai* (Group 3 and 4) than in Group 1 wister Albino rats.
- Reduction in the levels of AST, ALT, ALP and Total protein toward the normal value is an indication of regeneration process. The protective effect exhibited by NM at dose level of 200 mg/kg was comparable with the standard drug.
- These findings suggested that the administration of *Nannari Mathirai* has significantly neutralized the toxic effects of Carbon tetrachloride and helped in regeneration of hepatocytes.
- This present study confirmed that CCl_4 induced a marked rise in oxidative stress and cellular degeneration in wister Albino rat's liver. Both the doses of *Nannari Mathirai* treatment (100 and 200 mg/kg body wt.) significantly improved the effect of CCl_4 induced oxidative stress damage and reduced the expression of inflammatory and apoptotic proteins. As a result this protective effect of *Nannari Mathirai* could be correlated directly to its antioxidant property.

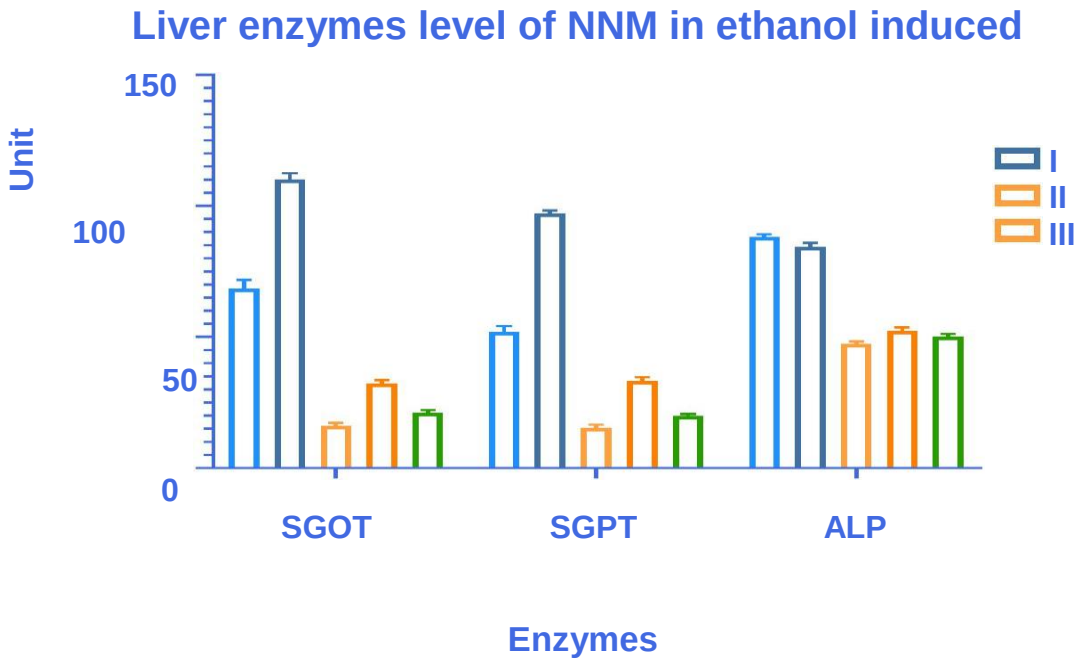
- Therefore the reduction in the activity of these enzymes may result in a number of deleteriousn effects by administration of *Nannari Mathirai* increased the activities against CCl_4 induced liver damage in rats to prevent the accumulation of excessive fats and protected the Liver [113].
- This was further confirmed by histopathological injuries.

HEPATOPROTECTIVE ACTIVITY OF *NANNARI MATHIRAI* IN ETHANOL INDUCED HEPATOTOXICITY IN RATS:

Table no: 33 Effect of *NANNARI MATHIRAI* on liver injury induced by ethanol in rats

Group	Treatment	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)
I	Saline	68.50±3.33	52.07±2.77	88.11±2.18
II	Ethanol	110.15±2.36	97.2±1.14	84.48±1.43
III	Ethanol+Silymarin	16.25±1.04**	15.47±0.006**	47.38±0.25**
IV	Ethanol+NM100	32.38±1.11**	33.26±1.58**	52.43±1.33**
V	NM200	21±0.13**	20.0±0.62**	50.20±0.04**

*Values are expressed as mean ± S.E.M followed by Dunnett test. *P < 0.05, **P < 0.01, ***P < 0.001 vs control. (n - 6);*



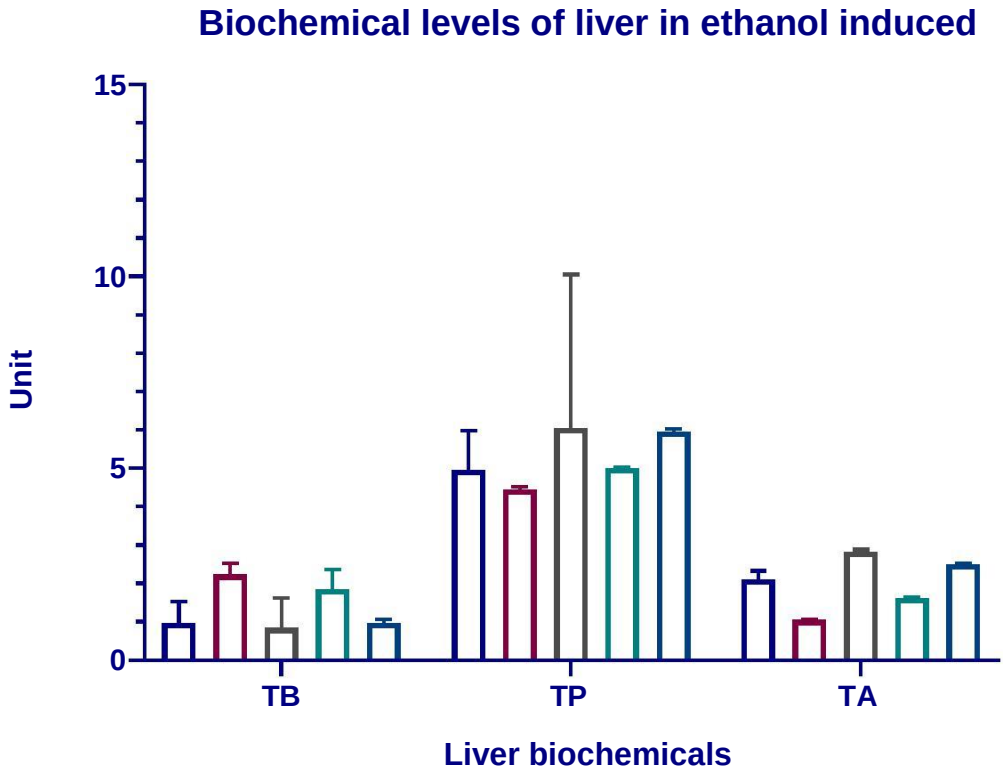
GRAPH 6

SGOT-Serum Glutamic-Oxaloacetic Transaminase,SGPT-Serum Glutamate-Pyruvate Transminase,ALP-Alkaline phosphatase.

Table 34 Bio chemical parameters in ethanol induced hepatotoxicity in rats on *NANNARI MATHIRAI*:

Group	Treatment	TB (mg/100ml)	TP (mg/dl)	TA (g%)
I	Control	0.98± 0.54	4.96±1.02	2.11±0.22
II	Ethanol	2.24 0.02	4.45 0.07	1.05 0.02
III	Silymarin	0.85 0.07**	6.05 0.04**	2.84 0.05**
IV	NM 100mg	1.86 0.05**	5.01 0.02**	1.63 0.01**
V	NM 200mg	0.97 0.09**	4.96 0.06**	1.50 0.03**

*Values are expressed as mean ± S.E.M followed by Dunnett test. *P < 0.05, **P < 0.01, ***P <0.001 vs control. (n - 6);*



GRAPH 7

TB: Total Bilirubin, TP: Total Protein and TA: Total Albumin

HISTOPATHOLOGY SLIDES OF *NANNARI MATHIRAI* ON ETHANOL INDUCED HEPATOTOXICITY:

Group I: Control rat showing normal liver tissue.

Group II: Liver tissue of rat administered alcohol showing periportal necrosis with the presence of multinucleated hepatocytes and dilated central vein.

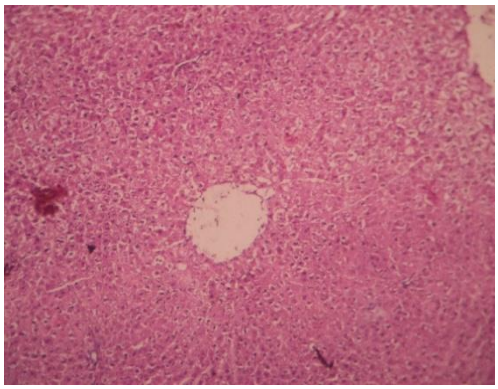
Group III: Photo micrograph of liver tissue treated with silymarin showing normal hepatocytes, portal vein (V), portal Artery

Group IV: Liver tissue of rats treated with *NM* at 100 mg/kg showing apparently normal liver with few scattered hepatocytes.

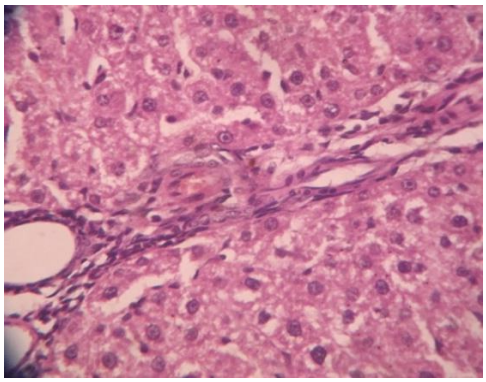
Group V: Liver tissue of rats treated with **NM** 200 mg/kg showing normal hepatocytes with regenerating hepatocytes and mild inflammation.

HISTOPATHOLOGY SLIDES OF *NANNARI MATHIRAI* ON ETHANOL INDUCED HEPATOTOXICITY:

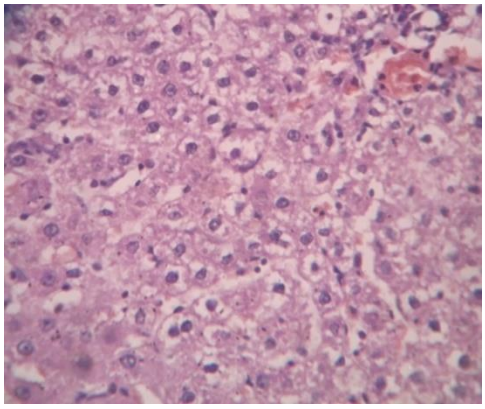
Fig 11



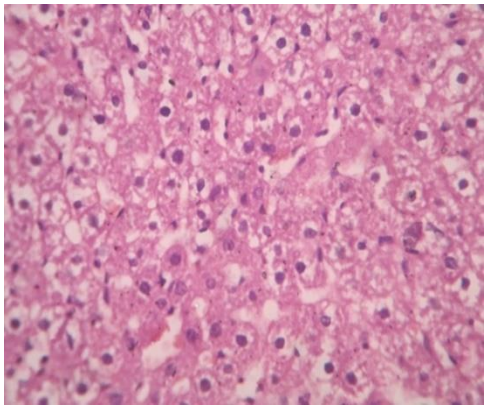
CONTROL



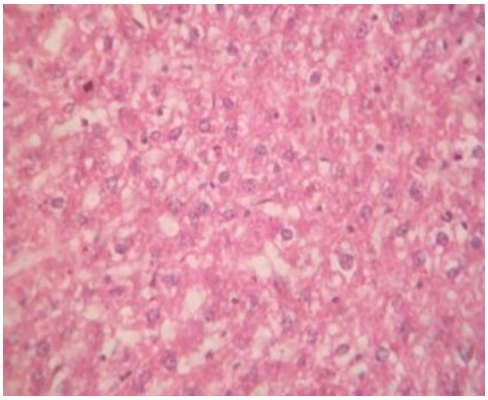
ETHANOL



SILYMARIN



LOW DOSE(100 mg/kg)



HIGH DOSE(200mg/kg)

Interpretation:

- In the present study ethanol was used to induce Hepatotoxicity.
- Ethanol produces a constellation of dose related deleterious effects in the liver. Both acute and chronic ethanol administration cause enhanced formation of cytokines, especially TNF-alpha by hepatic Kupffer cells, which have a significant role in liver injury.
- Elevated levels of SGOT, SGPT and ALP are indications of hepatocellular injury.
- In Ethanol treated rats the levels of serum marker enzymes (SGOT, SGPTand Bilirubin) elevated significantly. Moreover, the increased levels of the serum enzymes were significantly decreased by the treatment with *Nannari Mathirai* at 100 mg/kg and 200 mg/kg implying that the drug prevent the liver damage.
- The treatment *Nannari Mathirai* confirmed dose dependent activity, at dose level 200 mg/kg revealed good result than 100mg/kg.
- This was further confirmed by histopathological slides in Fig 11.

RESULTS OF ANTI-OXIDANT ACTIVITY OF *NANNARI MATHIRAI:*

Due to various factors of the fast moving life style like fast foods, change in daily routines, the level of oxidants or reactive oxygen species are increased in the body. It has been established that oxidative stress is the major causative factor in the induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immune suppression, neurodegenerative diseases and others. The free radicals are scavenged from the body by various mechanisms to enable good functioning of the organs.

The scavenging activity for free radicals, DPPH has widely used to evaluate the anti-oxidant activity of natural product from plant and natural sources. In this *in vitro* study of anti-oxidant activity of *Nannari Mathirai* DPPH was used as a free radical.

The preliminary phytochemical screening of *Nannari Mathirai shows* flavonoids, phenols, quinones, glycosides, terpenoids, steroid, and alkaloids followed by other accessions.

The presence of flavonoids, phenols, quinones were given a strong support to do the Qualitative analysis of antioxidant activity of *Nannari Mathirai.*

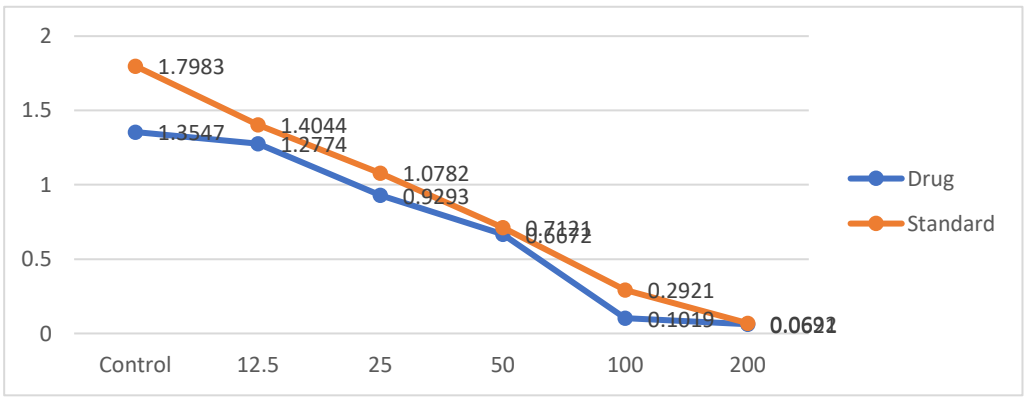
Qualitative analysis of antioxidant activity of *NM* extract:

The antioxidant activity of *Nannari Mathirai* extracts was determined by standard method. 50 µl of *Nannari Mathirai* extracts was taken in the micro titer plate. 100 µl of 0.1% methanolic 1,1-diphenyl- 2-picrylhydrazyl (DPPH) was added over the samples and incubated for 30minutes in dark condition. The samples were then observed for discolouration. The *Nannari Mathirai* extracts showed the colour changes from purple to yellow; it denotes the strong positive of the antioxidant activity. The antioxidant positive samples were subjected for further quantitative analysis. extracts showed the colour changes from purple to yellow; it denotes the strong positive of the antioxidant activity. The antioxidant positive samples were subjected for further quantitative analysis. extracts showed the colour changes from purple to yellow; it denotes the strong positive of the antioxidant activity. The antioxidant positive samples were subjected for further quantitative analysis.

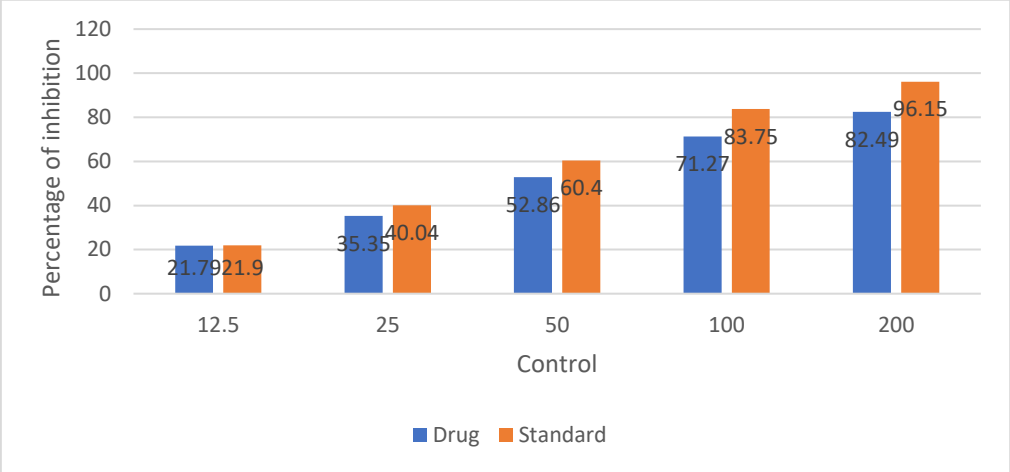
Table: 35. DPPH Assay of *Nannari Mathirai:*

Sample concentration(µg/ml)	Absorbance		Percentage of inhibition	
	Drug	Standard	Drug	Standard
Control	1.3547	1.7983	-	0.00
12.5	1.2774	1.4044	21.79	21.90
25	0.9293	1.0782	35.35	40.04
50	0.6672	0.7121	52.86	60.40
100	0.1019	0.2921	71.27	83.75
200	0.0621	0.0692	82.49	96.15

DPPH ASSAY ON NM



Graph 8



Graph 9:

Interpretation:

In the present study, the extract of *Nananri Mathirai* was found to possess concentration dependent scavenging activity on DPPH radicals. The values of DPPH free radical scavenging activity of the extract *Nananri Mathirai* was given.

The extract of *Nananri Mathirai* showed the highest DPPH scavenging activity (82.49%) at 200µg/ml and the lowest percentage of inhibition (21.79%) 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.

This indicated that % of inhibition with increase with increase in concentration of both the standard and *Nananri Mathirai* extract. The *Nananri Mathirai* extract has more or less equal DPPH scavenging activity [114].

CONCLUSION

6.CONCLUSION:

Siddha system of medicine believes that herbal formulation is one of the effective medicines for chronic diseases as is have no side effects. Herbal formulations are gaining popularity worldwide due to its nanomedicine form, increased bioavailability, minimal side effect, longer shelf life period and need less dosage.

The main obstacle in *Siddha* system of medicine in modern medical practice is the lack of scientific and clinical data and a better understanding of the efficacy and safety of herbal, metal and mineral drugs.

Liver diseases are the one of the most common health problems in the world wide. The Liver is quantitatively important site for drug metabolism. However, many drugs are known to cause hepatic injury. Conventional and synthetic drugs are used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effect. In the absence of a reliable liver protective drug in modern medicine there are a number of medicinal preparations from *Siddha* system of medicine recommended for the treatment of liver disorders.

In order to overcome this difficulty a novel attempt has been made to standardize the *Siddha* drug "***NANNARI MATHIRAI"*** for its Hepatoprotective properties by using analytical, preclinical studies.

The drug "***NANNARI MATHIRAI"*** was selected from the *Siddha* literature ***KANNUSWAMY PRAMBARAI VAITHIYAM*** to validate the safety and its efficacy of CCL_4, Ethanol induced hepatotoxicity and Antioxidant activity in Wistar albino rats.

The Raw drugs was authenticated by the Botanist and by the Gunapadam experts, GSMC, Chennai.

Actions of the drug profile:

Nannari is indicated for curing Jaundice (*Kaamaalai*).

Elam is used in the treatment of biliousness.

Seeragam is indicated for the treatment of Liver diseases. It also known to improving the fuctions of liver.

Perunjeragam is indicated for curing Jaundice (*Kaamaalai*).

Sevvagathi flower has an action of tonic to Liver.

Various analysis such as physicochemical, phytochemical, biochemical analysis, instrumental analysis was made. From the above analysis we came to know the presence of active ingredients responsible for its activity.

Physico-chemical analysis:

The pH of *Nannari Mathirai* was 5.45%. This is acidic in nature. Acidic drug is essential for its bioavailability and effectiveness. Acidic drugs are easily absorbed in upper part of stomach. Hence, the drug should not produce any harmful effect to the mucus membrane of the GI tract.

Phytochemical analysis:

Phytochemical analysis showed the presence of Carbohydrates, Glycosides, Saponins, Phenols, Flavanoides, Diterpenes, Gum & Mucilage.

Biochemical analysis:

Biochemical analysis showed the presence of Potassium, Magnesium, Iron, Zinc and Sulphate. Thus, from these results we come to know the effectiveness of the drug is due to the presence of these constituents and it has a synergistic effect in acting against the disease.

Phytochemicals are natural bioactive compound, found in plants and fibres, which act as a defense system against diseases and more accurately to protect against diseases.

HPLC analysis performed with *Nannari Mathirai* revealed the pressence of Polyphenols, Flavanoides, Alkaloides and Tannins.

Polyphenols are biomolecules which produce hepatoprotective effects. Mainly because of they reduce liver fat accumulation, mainly by reducing lipogenesis and by increasing fatty acid oxidation and decrease oxidative stress and inflammation, the main factors responsible for liver damage. Flavanoides are the unique antioxidant, which have the beneficial effects against Liver Disease.

Antimicrobial activity:

The showed a *Nannari Mathirai* broad-spectrum antimicrobial activity contrary to all the microorganisms. In the study reveals that the presence of bacterial and fungal load in the trial drug (*NM*). They present within the normal limits.

Instrumental analysis:

Based on the results, *Nannari Mathirai* is preferably non-toxic to human in its therapeutic dose. The standardization of the drug was evaluated by chemical characterization with heavy metal analysis, functional group analysis, elemental analysis and determination of particle size by ICP-OES, FTIR, and SEM respectively.

ICP-OES reveals that in *Nannari Mathirai*, the heavy metals like Al, As, Cd, Cu, Hg, Pb, and Ni were below detectable level. This reveals the safety of the drug.

The major diffraction peaks are identified after XRD analysis. XRD pattern of the trail drug *Nannari Mathirai* shows some good crystanility.

The SEM picture shown the presence of microparticle of size 100-1000 nm in the drug *Nannari Mathirai*. Further, the study shows that is a kind of micromedicine which favours the advantages of bio availability, better absorption and non-toxic with minimal dose level.

Pharmacokinetic aspect:

The acid medicines were absorbed in acid medium. That is the *Nannari Mathirai* may be absorbed in the upper part of GI tract.

Toxicity studies:

From the acute toxicity study as per OECD guideline 423, it was concluded that the test drug *Nannari Mathirai* is a safest drug. No mortality was obtained.

Toxicological study of both acute and sub-acute toxicity study was carried out in animal model Wistar albino rat according to the OECD guidelines. The test drug showed no acute toxicity as there was no mortality seen. The sub-acute toxicity after the repeated dose of 28 days was done. The mortality, functional observations, haematological and biochemical investigations were made. There was no significant change seen in the normal values. Thus, the toxicological study of the test drug greatly establishes the safety and gives the justification for long time administration.

In Conclusion, no toxic effect was observed up to 200mg/kg of *Nannari Mathirai* treated over a period of 28 days (OECD 407).

Pharmacological activities:

The pharmacological study was carried out in the animal model Wistar albino rats. Three activities were seen in the drug *Nannari Mathirai*. The Activities were,

CCL_4 induced Hepatotoxicity,

Ethanol induced Hepatotoxicity and

Anti-oxidant activity.

Hepatoprotective activity against CCL_4, Ethanol induced Hepatotoxicity and Antioxidant activity:

The present study showed that *Nannari Mathirai* produce protective against the hepatotoxicity induced by CCL_4 and Ethanol. The Hepatoprotective role of *Nannari Mathirai* might be due to its chemical constituent. Hence *Nannari Mathirai* may be act as prophylactic as well as curative drug in treating hepato toxic conditions. Further studies need to isolate the active constituents and mechanism of action. Thus, the author validates *Nannari Mathirai* as a new Hepato-protective drug which is cost effective and without any side effect.

Thus, by scrutinizing all the above-mentioned factors it is concluded that the test drug ***NANNARI MATHIRAI*** is a new ***Hepato-protective (KAAMAALAI-JAUNDICE)*** drug which is cost effective and without any side effect as obtained.

SUMMARY

7. SUMMARY

The test drug ***Nannari mathirai*** was selected from the *Siddha* literature ***Kannuswamy prambarai vaithiyam*** written by ***Kannuswamy pillai*** for its hepatoprotective activity (Kaamaalai-Jaundice).The dissertation started with an introduction explaining about the *Siddha* concept, prevalence of jaundice and role of the test drug in treating hepatic diseases.

The review of literature strengthened the positive facts of possessing the Hepatoprotective activity by each of the single drug included in the formulation.

The pharmacological review possessed all the information regarding the exertion of action of the drugs, available drugs in the market, their adverse effects.

The test drug *Nannari mathirai* was prepared properly by the given procedure. All the ingredients were identified and authenticated by the experts.

Review of literature in various categories was carried out. *Siddha* aspect, botanical aspect and pharmaceutical review disclosed about the drug and the disease.

Pharmacological review was done to establish the methodologies. The drug was subjected to analysis such as physicochemical, phytochemical, biochemical and also, instrumental analysis which provided the key ingredients presenting the drug thus it accounts the efficacy of the drug.

Toxicological study was made according to OECD guidelines comprising both Acute and sub-acute toxicity study. It showed the safety of the drug which attributes its utility in long time administration.

Pharmacological study was done. It revealed the Hepatoprotective activity of *Nannari mathirai* in animal model Wistar albino rats.

Results and discussion give the necessary justifications to prove the potency of the drug. Conclusion gives a compiled form of the study and explains the synergistic effect of all the key ingredients and activities that supports the study.This current analysis authenticates that ***Nannari mathirai*** has impressive Hepatoprotective activity (Kaamaalai-Jaundice) which exemplifies the intelligence of the *Siddha* literature to reach globally for the welfare of mankind. Thus, the herbal formulation ***Nannari mathirai*** is validated for its safety and efficacy for treating jaundice and it would be a great drug of choice.

FUTURE SCOPE

8. FUTURE SCOPE

The Herbal formulation ***"Nannari mathirai"*** was taken as the compound drug preparation for Hepatoprotective activity (***Kaamaalai-Jaundice)*** mentioned in the classical *Siddha* literature ***"Kannuswamy prambarai vaithiyam"*** written by ***Kannuswamy pillai .*** The mechanism of action by which ***Nannari mathirai*** produced its effect on hepatoprotective activity in experimental animals need to be validated in a scientific manner using specific experimental animal models and also multi-center clinical trials are required to understand the exact molecular mechanisms of action. So, it could be used worldwide for hepatoprotective action.

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BIBILOGRAPHY

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ANNEXURE

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ISO 9001 : 2008
Reg. No : RQ91/3038

Affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai.
Approved by Pharmacy Council of India, New Delhi, and
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 "A STUDY ON THE SAFTEY AND THERAPEUTIC OF SIDDHA POLY-HERBAL FORMULATION ***"NANNARI MATHIRAI"*** IN CCL4, ETHANOL INDUCED HEPATOTOXIC MODELS IN RATS" has been approved by the 53rd IAEC.

IAEC no: 06/321/PO/Re/S/01/CPCSEA dated 12/10/2018

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

Dr.P.Muralidharan
(Member Secretary)

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

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

This Certificate is awarded to Dr/~~Mr~~/~~Mrs~~.....T. MONIKA.....

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

"RESEARCH METHODOLOGY & BIOSTATISTICS"

*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.

Dr.N.KABILAN, M.D.(S),Ph.D.,
PROF & HEAD DEPT.OF SIDDHA

Prof.**Dr.T.BALASUBRAMANIAN,** M.D.,D.L.O.,
REGISTRAR

Prof.**Dr.S.GEETHALAKSHMI,** M.D., Ph.D.,
VICE CHANCELLOR

SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

CHENNAI – 600 119

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.~~ T. MONIKA

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

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
SIDDHA REGIONAL RESEARCH INSTITUTE
Poojappura, Thiruvananthapuram, Kerala

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. Monika T., GSMC, Chennai has ~~participated~~/presented a paper entitled A Review on Social and Preventive Aspects in Siddha System 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

सिद्ध क्षेत्रीय अनुसंधान संस्थान SIDDHA REGIONAL RESEARCH INSTITUTE RM & PHISSM 2018

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

IN COLLABORATION WITH

MINISTRY OF AYUSH Govt OF INDIA

सत्यमेव जयते

NORTHERN PROVINCIAL COUNCIL

1ST INTERNATIONAL CONFERENCE & EXHIBITION ON SIDDHA MEDICINE -2018

23RD - 27TH AT UNIT OF SIDDHA MEDICINE
UNIVERSITY OF JAFFNA.
SRI LANKA

CERTIFICATE

THIS IS TO CERTIFY THAT Prof / Dr. / Mr. / Ms. .....Monika. T..... PARTICIPATED / PRESENTED A PAPER ON .....Scientific evaluation of Lingakattu..... IN THE ABOVE CONFERENCE HELD ON 26TH & 27TH FEBRUARY 2018.

.....

Prof . R. VIGNESWARAN
VICE CHANCELLOR
UNIVERSITY OF JAFFNA

.....

Shri. A. NATRAJAN
CONSULATE GENERAL,
HIGH COMMISSION OF INDIA
JAFFNA

CLINICAL RESEARCH & HOSPITALS (P) LTD

National Conference on

HERBAL MEDICINE AND ETHNOPHARMACOLOGY

Date: 06.04.2017; Venue: TICEL Biopark.

This is to certify that ~~Ms./Mr.~~/Dr. T. MONIKA *from* DEPT. OF PHARMACOLOGY, GOVT. SIDDHA MEDICAL COLLEGE, CHENNAI

*attended the National Conference on "**Herbal Medicine and Ethnopharmacology**" conducted in V.S. Clinical Research & Hospitals (P) Ltd., Chennai, Tamil Nadu. He/She presented a paper/poster in the topic* EVIDENCE BASED REVIEW OF MEDICINAL PLANTS

T. Mathangi

Dr. T. Mathangi
Scientist & Coordinator

Dr. L. Lokoranjan
Chairman & Managing Director

GOVT. STANLEY MEDICAL COLLEGE, CHENNAI-1

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DEPARTMENT OF PHARMACOLOGY

&

THE TAMILNADU DR.M.G.R MEDICAL UNIVERSITY

CERTIFICATE

This is to certify that Dr / Mrs / Miss / Mr. T. MONIKA

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.~~ T. MONIKA

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