HEPATOPROTECTIVE ACTIVITY OF "*SIRINGIPAERATHI CHOORANAM*" ON CCl_4 AND PARACETAMOL INDUCED HEPATOTOXICITY AND ANTI - OXIDANT ACTIVITY IN IN-VIVO AND IN-VITRO MODELS

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CHENNAI-106

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"Hepatoprotective activity of *SIRINGIPAERATHI CHOORANAM* on CCl_4 and Paracetamol induced Hepatotoxicity and Antioxidant activity in In-vivo and In-vitro models"** is a bonafide and genuine research work carried out by me under the guidance of **Dr.V.Velpandian, M.D (S), Ph.D.,** HOD, Post Graduate Department of *Gunapadam*, Govt. Siddha Medical College, Arumbakkam, Chennai-106 and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or other similar title.

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CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled **"Hepatoprotective activity of *SIRINGIPAERATHI CHOORANAM* on CCl_4 and Paracetamol induced Hepatotoxicity and Antioxidant activity in In-vivo and In-vitro models"** is submitted to the TamilNadu Dr. M. G. R. Medical University in partial fulfilment of the requirements for the award of degree of M.D (Siddha) is the bonafide and genuine research work done by **Dr.R.VIJAYANIRMALA.** Under my supervision and guidance and the dissertation has not formed the basis for the award of any Degree, Diploma, and Associate ship, Fellowship or other similar title.

Date: **Seal & Signature of the Guide**

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ENDORSEMENT BY THE HOD

PRINCIPAL OF THE INSTITUTION

This is to certify that the dissertation entitled **"Hepatoprotective activity of *SIRINGIPAERATHI CHOORANAM* on CCl_4 and Paracetamol induced Hepatotoxicity and Antioxidant activity in In-vivo and In-vitro models"** is a bonafide work carried out by **Dr.R.VIJAYANIRMALA** under the guidance of Dr.V.Velpandian**, M.D (S), Ph.D.,** Post Graduate Department of Gunapadam, Govt. Siddha Medical College, Arumbakkam, Chennai - 106.

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Place: Chennai	**Place:** Chennai

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ABBREVIATIONS

Alb	Albumin
ALP	Alkaline phosphatase
ALT	Alanine transaminase
ANOVA	Analysis of Variance
AST	Aspartate Transaminase
BDL	Bile Duct Ligation
BUN	Blood Urea Nitrogen
CAT	Catalase
CCL_4	Carbone tetrachloride
CD	Conjugated Dienes
CMC	Carboxy Methyl Cellulose
CPCSEA	Committee for the purpose of control and supervision of experimental animals.
DC	Differential count
Dep.	Deposits
DMN	Dimethlnitrosamine
DPPH assay	2,2 Diphenyl-1-1-Picrylhydrazyl
E	Eosinophil
E.coli	Escherichia Coli
ESR	Erythrocyte Sedimentation Rate
FLD	Fatty Liver Disease
FPC	Few Pus Cells
FRAP	Ferric Reducing Anti oxidant Power
FTIR	Fourier Transform Infrared Spectroscopy

GIT	Gastro Intestinal Tract
GOT	Glutamate Oxaloacetate Transaminase
GPx	Glutathione peroxidase
GSH	Reduced Glutathione
Hb	Haemoglobin
Hcl	Hydrocloric acid
HNO_3	Nitric acid
HP	Hydrogen Peroxide
HPTLC	Higher Performance Thin Layer Chromatography
H_2SO_4	Sulphuric acid
IAEC	Institutional Animal Ethical Committee
ICMR	Indian Council of Medical Research
ICP-OES	Inductively Coupled Plasma Optic Emission Spectroscopy
L	Lymphocyte
LP	Liquid Paraffin
MAO	Mono Amine Oxidase
MDA	Malondialdehyde
NASH	Non Alcoholic Steato Hepatitis
OECD	Organisation for Economic Co-Operation & Development
P	Polymorphs
PCV	Packed Cell Volume
PT	Prothrombin Time
RBC	Red Blood Cell
RNA	Ribonucleic acid

ROS	Reactive Oxygen Species
S.aureus	*Salmonella aureus*
SEM	Scanning Electron Microscope
SEM	Standard Error Mean
SGOT	Serum Glutamic Oxaloacetic Transeaminase(ALP)
SGPT	Serum Glutamic Pyruvic Transaminase(AST)
SOD	Superoxide Di Mutase
SPC	*Siringipaerathi Chooranam*
TAA	Thioacetamide
TA	Total Protein
TB	Total Bilirubin
TBARS	Thiobarbituric acid REACTIVE substances
TC	Total count
TCW	Tender Coconut Water
TLC	Thin Layer Chromatography
TP	Total Protein
UV	Ultra Violet
WBC	White Blood Cell
WHO	World Health Organization
XRF	X-Ray fluorescence spectroscopy

1. INTRODUCTION

"**Is life worth living? It all depends on the liver**."

-William James

In traditional system of medicine, the Siddha system of medicine has its own uniqueness to maintain the balance of the body and mind. It is a boon offered by the eminent powers called Siddhars; this system not only helps to cure the diseases but also protects the body mainly for longevity with complete freedom from illness and preventing ageing (Rejuvenation).

As the number of people, preferring natural health remedies and herbal health remedies are increasing day by day and Indian medical systems are gaining popularity all over the world nowadays, this is the best time to introduce Siddha system to the World.

Siddhars are the spiritual scientists, who established the Siddha science. Siddhars have more knowledge about the universe and its contents.

Agastiyar is considered as the foremost Siddhar and guru of Siddha cult. There are also 18 prime Siddhars who are the followers of the primordial guru. One of the concepts of Siddha is focused to *"Attamasiddhi",* which are the eight supernatural powers. Those who attained or achieved the supernatural powers are known as Siddhars [1].

The main goal of Siddha system of medicine is to satisfy the people with healthy and hygienic life. "*Pancha bootham*" (Five primordial elements) and *"Tridhosam"* (Three humors) forms the basis of Siddha. All the physiological function in the body is arbitrated by *"Tridhosam" ('Vatham', 'Pitham' and 'Kabam')*[2].

Siddha system has an enormous pharmacopoeia containing herbs, metals, minerals, animals and its derivatives are the core sources of Siddha medicine. It is classified into 64 varieties, which consists of 32 types of internal medicines and 32 types of external medicines. Under each classification thousands of formulae are mentioned by the Siddhars.

The drugs used in Siddha medicine are classified on the basis of five properties: *'Suvai'* (Taste), *'Gunam'* (Character), *'Veeryam'* (Potency), *'Pirivu'* (Class) and *'Mahimai'* (Action) [3].

The importance of health science and its cure are mentioned in the Classical Tamil sangam literature *''THIRUKKURAL''*. Saint *Thiruvalluvar* defines medicine as,

"உற்றான் அளவும் பிணியளவும் காலமும்
கற்றான் கருதிச் செயல்"

The learned physician should ascertain the condition of his patient; the nature of his disease, and the seasonal changes and then proceed with his treatment. [4]

Siddha system is primarily based on 96 principles *(Thathuvas)* that offers better solution to 4448 diseases supposed to be affecting mankind and everything have been portrayed in the literatures. Among the traditional system of medicine, Siddha system has enormous number of liver tonics and liver protective agents of herbals and mineral origin for the benefit of mankind.

Liver is the largest organ in a human body, situated in the right side of upper abdominal cavity. It plays an important role in Carbohydrate, Protein, Lipid, Bilirubin, Bile acid, Vitamin and Mineral, Hormone, Drug, Alcohol, and Cholesterol metabolism. The liver plays an astonishing array of vital functions in the maintenance, performance and regulating homeostasis of the body. The bile secreted by the liver plays an important role in digestion.

Liver synthesizes all the coagulation factors and requires vitamin K to activate some factors. Prothrombin time depends on factors I, II, V, VII and X, and it gets prolonged when the plasma concentration of anyone of these falls below 30 % of normal.

Prothrombin time (normal value 12 seconds) is prolonged in:

- Severe liver damage as in acute hepatitis e.g. viral hepatitis
- Prolonged biliary obstruction which reduces vitamin K absorption.

Therefore, maintenance of a healthy liver is essential for the overall well being of an individual [5].

Environmental pollutions, fast foods, drugs, alcohol and sedentary lifestyles are the causes of Liver diseases. As a result it depresses the immune systems, constant fatigue, obesity, sluggish digestive system are the main cause of health problems. Alcohol and many pharmaceutical drugs can affect the metabolism of the liver, and if this continues for long periods of time, health will be endangered. The most common diseases of liver includes Hepatitis, Alcohol related liver diseases, Liver tumor and Rey's syndrome [6].

In recent years, people widely suffered from liver diseases. One of the major causes of liver diseases is Jaundice which affects humans in all ages. In Siddha system of medicine, "Jaundice" is correlated with "Pitha Kamaalai" or "Manjal Kamaalai". As per the text Manjal Kamaalai is of 13 types [7].

Jaundice is a general condition that results from abnormal metabolism in retention of bilirubin. Jaundice causes a yellow discoloration of the skin, mucous membranes and sclera. The three principle types of Jaundice are pre hepatic, hepatic and post hepatic. Many of the liver diseases are accompanied by Jaundice caused by increased levels of bilirubin in the system [8].

Liver disease is one of the major causes of morbidity and mortality in public affecting humans of all ages.

According to WHO, about 46% of global diseases and 59% of the mortality is because of chronic diseases and almost 35 million people in the world die of Chronic Liver diseases. Liver disease rates are steadily increasing over the years [9].

According to the latest WHO data published in may 2014 Liver Disease deaths in India reached 216,865 or 2.44% of total deaths. The age adjusted Death Rate is 21.96 per 100,000 of population [10].

This report gives an alarm, to decrease the morbidity and mortality rate caused by hepatic complications; one of the most common problems in the world is hepatic diseases. If it is persists for long time the morbidity and mortality will be increased. So we have to make the most of the existing formulations or evaluating new treatments.

The Liver is quantitatively the most important site of drug metabolism. However many drugs are known to cause hepatic injury. Conventional and synthetic drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effect.

Steroids, vaccines and anti-viral drugs, have been used as therapies for liver diseases, they have potential adverse effect. Current medical treatment for liver diseases is ineffective, and therefore efforts are being made to seek new effective medications [11].

In latest research, Poly-herbal combination treatment is an alternative medication in market and active transporters by herbal phytochemical fractions may affect therapeutic outcomes of co-administered allopathic medicines due to changes in their pharmacokinetic profiles.

Combination of different plant extracts as polyherbal medication is a new approach in treatment of liver disease, for example Liv 52 Himalaya drug co. product), Livergen (Standard Pharmaceuticals), Tefroliv (TTK Pharma Pvt. Ltd), etc are generally used in India for hepatotoxicity treatment. Polyherbal formulation is used widely by different pharmaceuticals companies for treatment of liver toxicity [12].

Due to known side effects of approved pharmaceuticals, patients often turned to alternative medicine which is considered natural and healthy [13].

Many herbal preparations support or promote the process of healing or regeneration of liver cells with fewer side effects. The majority of plants are used for liver disorders [14].

Poly herbal formulations reported to have hepatoprotective activities that are available in Indian market which comprise about 100 Indian medicinal plants. [15]

Some of the most valuable plants used for the treatment of liver problems are *Phyllanthus amarus, Silybum marianum, Glycyrrhiza glabra, Curcuma longa, Picrorhiza kurroa* [16].

Siddhars have achieved supreme knowledge in herbal medicines and enlightened spiritually as well. In short, herbs are a natural and primary therapeutic remedy for liver diseases.

Though the liver has a marked regenerative capacity and a large functional reserve, hepatic failure may develop from severe acute and fulminant liver injury with massive necrosis of liver cells (acute hepatic failure) or from advanced chronic liver disease. Hence, herbal drugs have become popular and their use is widespread [17].

"**Modern medicine is a negation of health. It isn't organized to serve human health, but only itself as an institution. It makes more people sick than its heals."**

-Ivan Illich.

Modern medicines have a little to offer for alleviation of hepatic diseases, only the plant based preparations are employed for the treatment of liver disorders. Herbal medicines are the most cost effective form of traditional medicine.

In our traditional system of medicine Siddha system is a treasure which contains immense herbal formulations.

"**Nature is the healer of disease**."

-Hippocrates

A number of plants and formulations have been claimed to have hepatoprotective activity. Nearly 160 phytoconstituents from various plants have been claimed to possess liver protecting activity. In India, more than 87 plants are used, 33 are patented as proprietary ingredient plant formulations.

Now- a- days there are so many medicines have been used in the treatment of liver diseases, but there is a need for the drug which could be less toxic, cost effective and more potent. Among them, one of the polyherbal formulation mentioned in Siddha literature for hepatoprotective activity is "***Siringipaerathi Chooranam***" consists of *Piper longum, Cuminum cyminum, Syzygium aromaticum, Bambusa arundinacea and Cinnamomum tamala* which acts as a Hepatoprotective and Anti-oxidant activities[18].

Scientific validation is not available in the formulation. Even though the shelf life period of Chooranam is limited, its potency is too high. So I have preferred to choose the "Chooranam" form of medicine that will definitely act as a "HEPATOPROTECTIVE" to safeguard the people from liver damage. .

I hope the study effect of **"*Siringipaerathi Chooranam*"** for hepatoprotective activity will discover a choice of new drug for better health care of patient to deal with hepatic disorder.

2. AIM AND OBJECTIVES

Aim

The present investigation was aimed to validate the safety and efficacy of the Siddha polyherbal formulation *'SIRINGIPAERATHI CHOORANAM'* for its Hepatoprotective activity against CCL_4 and Paracetamol induced hepatotoxicity and Anti-oxidant activity in In-vivo and In-vitro models.

Objectives

The objectives of this work were done through the following steps.

- Collection of relevant literature from classical Siddha texts as well as Modern sciences that supported this study.
- Description of pharmacognostic features of the plant in this formulation including the taxonomic identification, collection, purification of plants etc.
- Preparation of the drug according to the procedure described in Siddha literature**.**
- Standardisation of the trial drug by means of physico-chemical analysis, phyto chemical analysis.
- Revealing the Anions and Cations present in the drugs through proximate Chemical analysis.
- Elucidation of the chemical structure, microscopical structure of the drugs by means of instrumental analysis.
- Interpreting the results of acute and repeated dose 28-day oral toxicity of *SIRINGIPAERATHI CHOORANAM* according to OECD guidelines 423 and 407.
- Detailing the study of pharmacological activities like CCL_4, Paracetamol induced hepatotoxicity and Anti-oxidant activity of the trial drug *SIRINGIPAERATHI CHOORANAM* in In-vivo and In-vitro models.

3. REVIEW OF LITERATURE

DRUG NAME: "*SIRINGIPAERATHI CHOORANAM"*

SIDDHA LITERATURE*: SARABENDRA VAIDHIYA MURAIGAL*

"துதிசெய்யிஞ்சி மேற்றோலைச் சீவிக் கொண்டு
துண்டுதுண் டாய்நறுக்கி நிழலினிலு லர்த்தி
பதினாறு பலம்பசுவின் நெய்யி லிஞ்சி
பக்குவமாய் வறுத்திடித்து மிளகு நன்றாய்
விதித்திடுவி ராகனெடை பனிரெண் டெட்டாம்
வேண்டியதிப் பிலிநிறைதிப் பிலியின் மூலம்
அதினான்கு காசெடைமேற் கொடியின் வேலி
யாகும்வ ராகனெடை பத்துந் தானே".

"பத்தெல்லா லேலமில வங்கப் பட்டை
பகர்மூங்கி லுப்போடு லவங்கப் பத்திரி
யுத்தமமாம் சந்தனம்வி லாமிச் சம்வேர்
உயர்சாதிக் காயெழில்சீ ரகங்கி ராம்பே
யித்தனையும் வகைக்கொருப லந்தான் கூட்டி
யிடித்துவடி கட்டியிஞ்சி முதல்தூட் கெல்லாம்
புத்தநற்சீ னியுமிரண்டு பாங்காய்ச் சேர்த்து
பொருந்தவுந்தா னேகலந்து கொண்டி டாயே".

“கொண்டிருவ ராகனெடை யிரண்டு போதும்

குணமான தேனில்நித்த முண்டே வந்தால்

பண்டிருந்த சுரம்மார்பில் வலிகி ராணி

பகராம்பற் பித்தங்கா மாலை வாந்தி

துண்டரிக்கப் பீனிசமும் வாசி யாகும்

தோகையிடப் பாகர்பதப் பூசை யாலே

எண்டிசையுங் கீர்த்திபெற்ற தரும மூர்த்தி

யெங்கள்சர பேந்திரமா லியம்பும் வாறே”.

-சரபேந்திரர் வைத்திய முறைகள்[19].

THE FOLLOWING ARE THE INGREDIENTS QUOTED IN THIS SONG

Inji	*- Zingiber officinalis*
Milagu	*- Piper nigrum*
Thippili	*- Piper longum*
Thipili moolam	*- Piper longum*
Lavanga pathiri	*- Cinnamomum tamala*
Elam	*- Elettaria cardamomum*
Kodiveli ver	*- Plumbago zeylanica*
Lavanga pattai	*- Cinnamomum zeylanicum*
Moongil uppu	*- Bambusa arundinaceae*
Sandhana thool	*- Santalum album*
Vilamichu-ver	*- Plectranthus vettiveroides*

Sathikkai - *Myristica fragrans*

Seeragam - *Cuminum cyminum*

Kirambu - *Syzygium aromaticum*

Sugar - *Saccharum officinarum*

Nei - Ghee

3.1. BOTANICAL ASPECT OF DRUGS

GINGER

Botanical name : *Zingiber officinale*

Family : Zingiberaceae

Part used : Rhizome

Scientific classification

Kingdom : Plantae

Class : Monochlamydeae

Order : Zingiberales

Family : Zingiberaceae

Genus : *Zingiber*

Species : *officinale*

Occurrence and distribution

Inji is the rhizome of *Zingiber officinale* (Family: Zingiberaceae), widely cultivated in India, rhizomes dug in January- February, buds and roots removed, soaked overnight in water, decorticated and sometimes treaded with lime and dried.

Description

Macroscopic:

Drug occurs as entire rhizome or in pieces, rhizome laterally compressed bearing flattish ovate, oblique branches on upper side, each having a depressed scar at its apex, pieces 5 to 15 cm. Long, 1.5 to 6.5 cm. Wide (usually 3 to 4 cm.) and 1 to 1.5 cm .thick, fracture short with projecting fibres, transversely cut surface shows a wide central stele having numerous greyish cut ends of fibres and yellow secreting cells; odour characteristic; taste pungent.

Microscopic:

Rhizome – Shows a few layered, irregularly arranged, tangentially elongated, brown cells of outer cork and 6 to 12 rows of thin-walled. Colourless, radically arranged cells of inner cork. secondary cortex consisting of hexagonal to polygonal, isodiametric, thin-walled, parenchymatous cells containing numerous circular to oval starch grains with characteristic striations and hilum at one end measuring 5 to 25 µm in dia., idioblasts containing large yellowish to brownish globules of oleo-resin; walls of oil cells suberised; numerous ckised,conjoint,collateral, cortical fibro-vascular bundles scattered throughout cortical zone, greater number occurring in inner cortical region, larger bundles consists of 2 to 7 vessels, small cells of sieve tube; pericycle single layered enclosing central stele; larger bundles found scattered throughout stele, composed of xylem, phloem, parenchyma and sheath of sclerenchyma.

Constituents

Volatile oil containing cineole, zingiberol, zingiberene, bisabolene and phellandrene, gingerdione,dihydrogingerol,dexahydrocurcumin and desmethyk hexahydrocurcumin, dehydrigingerdione.

Medicinal Uses:

- It is used to treat cold, cough, regurgitation, diseases due to heat, derangement of 3 humours, diarrhoea due to indigestion, pain in the joints & it increases appetite.
- Ginger is a rejuvenating herb, promotes eye sight, *kapham* diseases[20].

PEPPER

Botanical name : *Piper nigrum*

Family : Piperaceae

Part used : Dried fruits

Scientific classification

Kingdom : Plantae

Phylum : Magnoliophyta

Class : Magnoliopsida

Order : Piperales

Family : Piperaceae

Genus : *Piper*

Species : *nigrum*

Etymology

The word "Pepper" is derived from the Dravidian word for long pepper, pippali. Ancient Greek and Latin turned pippali into the Latin piper, referring both black pepper and long pepper. "Pepper" was used in a figurative sense to mean "spirit" or "energy" at least as far back as the 1840s, in the early 20th century, this was shortened to pep[21] .

Distribution

It is cultivated in moist parts of India cultivated from Konkan southwards, especially in North Konkan Kerala and also in Assam.

Description

Macroscopic Character

Fruits greyish-black to black, hard wrinkled, 0.4 to 0.5 cm. in diameter, aromatic, pungent in taste.

Microscopic Characters

Fruit consists of a thick pericarp enclosing a small embryo, pericarp consists of epicarp mesocarp and endocarp, epicarp composed of single layered tubular cells forming epidermis, mesocarp composed of band of tangentially elongated parenchymatous cells and tangentially elongated oil cells present in outer region and a few fibro vascular bundles, a single row of oil cells in the inner region of mesocarp, endocarp composed of a row of beaker shaped stone cells testa single layered, yellow coloured, thick walled sclerenchymatous cells, perisperm contains parenchymatous cells having a few oil globules and angular polyhedral cells, packed with starch grains measuring 5.5 to 11.0 in diamter, having 2 or 3 components and a few minute aleurone grains[20a].

Constituents

Piperine, piperettine, piperolein A,B, ascorbic acid, carotene, ß-alanine piperidine, serine, threonine, piperyline, arginine, serine, glutamic acid, cystine

Properties and uses

The fruit is pungent, hot, bitter, anthelmintic, alterative, useful in *kapham, vadham*, asthma, pains, urinary discharges, night blindness, increase biliousness, brings on sleep and epileptic fits. The fruit has a sharp, pungent, slightly bitter taste, carminative, aphrodisiac, purgative, alexipharmic.

It is also useful in inflammation, pain in the muscles, diseases of liver and spleen, eructations, leucoderma, lumbago, chronic fever, facilitates menstruation.

It is employed in cholera, weakness following fevers, vertigo, coma.

It's action as a stimulant is more evident on the mucous membranes of the rectum and urinary organs. Externally it is an effective rubefacient[22].

LONG PEPPER

Botanical name : *Piper longum*

Family : Piperaceae

Parts used : Dried spikes

Scientific Classification

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Magnolipsida
Order	:	Piperales
Family	:	Piperaceae
Genus	:	*Piper*
Species	:	*longum*

Distribution

It is cultivated in hotter parts of India from Central Himalayas to Assam upto hills of West Bengal and evergreen forests of Western Ghats as wild and also in North East and many parts of the South.

Description

Macroscopic Characters

Fruit greenish-black to lack, cylindrical, 2.5 to 5 cm. long and 0.4 to 1 cm. thick, consisting of minute sessile fruits, arranged around an axis, surface rough and composite, broken surface shows a central axis and 6 to 12 fruitlets arranged around an axis, taste pungent producing numbness on the tongue, odour aromatic.

Microscopic Characters

Catkin shows 6 to 12 fruits, each having an outer epidermal layer of irregular cells filled with deep brown content and covered externally with a thick cuticle, mesocarp consists of larger cells, irregular in shape and thin walled.

Outer layer of this zone composed of thin walled cells and colourless, most of the endocarp cells filled with starch grains, round to oval measuring 3 to 8 in diameter[22a].

Constituents

Piperine, piplartine, alkaloid, starch, resin, gum, fat, triaconane, dihydrostigmasterol, reducing sugars, glycosides

Medicinal uses

- The root and fruits are used in palsy, gout, lumbago. The fruit has a bitter, hot, sharp taste; carminative, tonic to the liver, stomachic, emmenagogue, abortifacient, aphrodisiac, haematinic.
- Long pepper in the form of powder is suspended in warm water and given to women after parturition to check haemorrhagic fever.
- Pellitorine, piplartine, piperine exert medicinal uses. The fruits as well roots are attributed with numerous medicinal uses and may be used for respiratory diseases and as an emmenagogue, abortifacient[23].

INDIAN CINNAMON

Botanical name : *Cinnamomum tamala*

Family : Lauraceae

Part used : Dried Leaves

Scientific Classification:

Kingdom : Plantae

Class : Magnoliopsida

Order : Laurales

Family : Lauraceae

Genus : *Cinnamomum*

Species : *tamala*

Distribution

Dried mature leaves, a small evergreen tree up to 7.5 m. high and occurs in tropical, sub-tropical Himalaya between 900 to 2300 m; often raised from seeds sown in nursery; leaves collected in dry weather from about ten year old plants during October to March.

Description

Macroscopic:

Leaves-12.5 to 20 cm. long, 5 to 7.5 cm. Wide at the centre, 3 converging nerves from base to apex, young leaves pink ; petiole 7.5 to 13 mm. Long ; margin entire, apex acute or acuminate , both surfaces smooth ; stomata paracytic; odour aromatic; taste slightly sweet, mucilaginous and aromatic.

Microscopic:

Petiole and midrib – Transverse sections of petiole and midrib show epidermis externally covered with cuticle , uniseriate, multicellular trsichomes present with 1 to 3 cells; oil cells present as single or groups ; isolated large stone cells, much lignified and showing striations, are found scattered; most of the parenchymatous cells of cortex show reddish-brown contents; pericycle represented by a few layers of sclerenchymatous cells; stele more or less planoconvex as in the midrib of leaf; xylem on upper and phloem on lower side consisting of usual elements, present.

Constituents

β – Caryophyllene,linalool,caryophyllene oxide, d – β-phellandrene, eugenol, α and β pinene, p-cymene, 3,4`,5,7 –tetrahydroxy flavones, 3,3`,4`,5,7 – O – pentahydroxy flavones, kaempferol – 3-O-glucopyranoside,kaempferol -3-O-sophoroside,quercetin 3-O-rutinoside.

Medicinal Uses

- It cures cough ,asthma ,vomiting ,mouth ulcer
- Lavanga oil cures body pain, tooth ache and headache[20b].

CARDAMOM

Botanical name : Elettaria cardamomum

Family : Zingiberaceae

Part used : Seeds

Scientific Classification:

Kingdom : Plantae

Class : Monochlamydeae

Order : Zingiberales

Family : Zingiberaceae

Genus : *Elettaria*

Species : *cardamomum*

Occurrence and distribution

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

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.

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 cure stomach ache, ulcer.
- Roast seeds of cardamom, omam and cumin seeds then they are powdered and given to cure indigestion[24].

LEAD WORT

Synonyms

Plumbago viscosa

Common names

Ceylon lead word, Doctor bush, Plumbago

Etymology

The generic name Plumbago is derived from the Latin plumbum, lead.

The specific epithet zeylanica is from the Latinized name for Sri Lanka (Ceylon).

Botanical name : *Plumbago zeylanica*

Family : Plumbaginaceae

Part used : Root

Scientific Classification:

Kingdom : Plantae

Class : Magnoliopsida

Order : Plumbaginales

Family : Plumbaginaceae

Genus : *Plumbago L.*

Species : *zeylanica*

Distribution

It is a perennial herb distributed widely in India, much cultivated widely in the peninsular regions, probably in Bengal, Malay, Peninsula, Ceylon, tropics of the world.

Description

Macroscopic Characters

Mostly perennial shrubs of about 60-120 cm in height. Leaves alternate, ovate, acute, glabrous, entire, short stalk, flowers white, bracteates, glandular and elongated spikes, 10-30 cm long. Seeds oblong shaped. Roots 30 cm or more in length, 6 mm or more in diameter as also as short stout pieces, including root stocks reddish to deep brown, bark thin and brown, internal structure striated, odour, disagreeable, taste, acrid[25].

Microscopic Characters

Transverse section of root shows outer most tissue of cork consisting of 5 -7 rows, secondary cortex consists of 2-3 rows of thin walled rectangular, light brown cells, starch grains. Secondary cortex followed by a wide zone of cortex is composed of large polygonal parenchymatous cells varying in size and shape, containing starch grains and some cells with yellow contents, fibres scattered singly or in groups consisting of usual elements and phloem fibres, similar to cortical zone, phloem fibres usually in groups of 2-5 or more but occasionally occurring singly, lignified with pointed ends and narrow lumen, cambium indistinct, xylem light yellow to whitish radically arranged, 1-6, seriate, radically elongated, starch grains, stone cells absent.

Constituents

Plumbagin, citranone, elliptone, ß-sitosterol-glucoside, bakuchiol, phenols, isoaffinetin, saponaretin, flavanoids, psorealen, iso-orientin.

Medicinal uses

- The root increases the digestive power, promotes the appetite and useful in dyspepsia, piles, anasarca, skin diseases.
- The active principle "Plumbagin" stimulates the contraction of the heart, intestine and the uterus. It stimulates the secretion of sweat, urine and bile[26].

CINNAMON BARK

Botanical name : *Cinnamomum zeylanicum*

Family : Lauraceae

Part used : Bark

Scientific Classification:

Kingdom : Plantae

Class : Magnoliopsida

Order : Laurales

Family : Lauraceae

Genus : *Cinnamomum*

Species : *verum*

Distribution

Dried inner stem bark, a moderate sized evergreen tree usually attaining a height of 6 to 7.5m; cultivated in the Western Ghats and adjoining hills; bark collected during April-July and October-December.

Description

Macroscopic:

Bark pieces about 0.5mm. thick, brittle, occurs as single or double, closely packed compound quills, up to a metre or more in length and up to about 1cm.in diameter,inner surface darker in colour, striated with longitudinally elongated .

Microscopic:

Transverse section of bark (devoid of cork and cortex) shows except at certain places pericyclic sclerenchyma, 3 or 4 rows of isodiametric cells, sometimes tangentially elongated.Inner and radial walls often being thicker than the Outer containing axially elongated secreting cells containing volatile oil or mucilage; phloem fibres with very thick walls, upto 30 µm in diameter, isolated or in short tangential rows; cortical parenchyma and medullary rays containing small starch grains.

Constituents

Cinnacassiol A,B and C, trans-cinnamic acid,cinnamaldehyde and eugenol.

Medicinal Uses:

- Powder cures vomiting, indigestion, stomach pain, diarrhoea.
- Bark powder 650 gm cures dysentery[20c].

THORNY BAMBOO

Synonyms

Bambusa bambos Druce

Botanical name : *Bambusa arundinaceae*

Family : Poales

Scientific Classification:

Kingdom : Plantae

Class : Monocotyledanae

Order : Poales

Family : Poaceae

Genus : *Bambusa*

Species : *arundinaceae*

Etymology

The etymology of the word BAMBOO comes from the wrong pronunciation of the Indian word MAMBU, which is the local name of a native species of the plant. Carl Linnaeus in his best known "Species plantarum" mentioned the "Arundo Bambos", and this name, then modified in BAMBUSA, has been officially adopted as identification of the family[21a].

Distribution

It is distributed throughout the world especially in Western ghats, Eastern Ghats, all over foot hills, Western plains in India.

Description

Shrubs or trees are usually large, stem sheaths are broad, the blade often triangular. Petiolate leaves. Spikelets -1 flowered usually arranged in a large leafy panicle or in paniculate spikes.Stamens 6 free, ovary oblong or ovoid with a hairy tip; styles short or long; stigma 2-3, grain oblong or linear oblong furrowed on one side; pericarp thin adherent to the seed[27].

Constituents

Protein, carbohydrates, aminoacids, minerals like potassium, calcium, manganese, zinc, copper, iron, thiamine, niacin, vitamins A,B, B_6.

Medicinal Uses

- The stem and leaves are acrid, sour, bitter, cooling, laxative and burning sensations, blood coagulopathies, biliousness, leucoderma, inflammations, strangury and piles.
- In North Lakhimpur forest division of Assam, very soft shoots of the plant are reported to be used for birth control[28].

SANDAL WOOD

Botanical name : *Santalam album*

Family : Santalaceae

Parts used: Heart Wood and oil

Scientific Classification:

Kingdom	:	Plantae
Class	:	Magnoliosida
Order	:	Santalales
Family	:	Santalaceae
Genus	:	*Santalum*
Species	:	*album*

Distribution

It is commonly found in comparatively dry regions of peninsular India from Vindhya mountains southwards, especially in Mysore & Tamilnadu, ascending to an altitude of 1200 m . It has also been found in dry districts of Gujarat, Konkan, Deccan, and Rajasthan, parts of Uttar Pradesh, Madhya Pradesh and Orissa.

Description

A small medium sized, ever green semi parasiti tree, with slender branches, sometimes reaching up to 18 m in height &2.4 m in girth. Bark reddish or grey or nearly black, rough with deep vertical cracks on old trees; leaves glabrous ,thin, elliptic-ovate or ovate-lanceolate,1.5-8 cm.x1.6-3.2 cm., some times larger; flowers straw – coloured, brownish purple, reddish purple or violet, unscented, in terminal ,axillary paniculate cymes; drupe globose or 3 cm diam.,purple-black, with hard, ribbed endocarp; seeds globose or ovoid.

Chemical Constituents

Sandalwood oil contains α & β santalol, α and β-santalenes, santenol, teresantalol, nor-tricycloekasantalal, exo-norbicycloekasantalal, isovaleraldehyde, santanone, teresantalicacid, trans-β-santalol, epi-cis-β-santalol, β-santalol, epi-β-santalane, cis-lanceol, cis-nuciferol, tropan alkaloidssantalbicacid, palmitcacid.

Principles constituents of sandal wood:

Santalols (up to 90%), fusanol santene, Santalic acid, terestanol, borneol, santalone.

Medicinal Uses

- It is used to treat derangement of 3 humors, internal heat, leucorrhoea, thirst, itching. It strengthens the body[29].

WHITE CUS CUS GRASS

Botanical name : *Plectranthus vettiveroides*

Family : *Lamiaceae*

Parts used : Root

Scientific Classification:

Kingdom : Plantae

Class : Magnoliosida

Order : Lamiales

Family : Lamiaceae

Genus : *Plectranthus*

Species : *vettiveroides*

Parts used: Aril part of root

Occurrence and Distribution:

Found wild in dry and Barren hills of sub tropical Himalayas including Kumaon and Nepal ascending to 2700m, in the Deccan, Peninsula, Gujarat and Bihar; cultivated in Baroda and Maharashtra.

Description

A perennial plant, branched, aromatic herb, about 30 to 62 cm height with thick root . Stem stout, villous or hispid . Flowers born in racemes, stout; Upper calyx, lip rounded – ovate, corolla pale blue; fruits nut lets.

Chemical Constituents:

- Allylroyleanone, barbatusin, 3β-hydroxy-3-deoxy-barbatusin, coleons E and F, cyclobutatusin, plectrin, plectrinonA, plectrinon B, brbatusol.
- 20-deoxocarnosol,coleonol D, coleonol E, coleonone, coleosol, α-copaene deoxycoleonol,β-bisabolene, bornylacetate, camphene.
- β-cymene, 3-decanone, β-clemene, cariocal, 6α-hydroxycarnosol.

Medicinal uses

- Aril: Spasmolytic, root: hypertensive,spasmolytic and given to children in constipation: decoction as tonic and in the treatment of worms, parts to allay. Burning festering boils: mixed with mustard oil, grounded root externally applied to eczema and skin disease.
- Forskolin,isolated from roots, is a bronchodilatator, cardiac tonic in the treatment of congestive heart failure, glaucomotherapy, anti-hypertensive, remedy for metastatic condition and thrombosis[30].

NUTMEG TREE

Botanical name	:	*Myristica fragrans*
Family	:	Myristicaceae
Part used	:	Mace (aril) and nutmeg(seeds)

Scientific Classification:

Kingdom	:	Plantae
Class	:	Magnoliopsida
Order	:	Magnoliales
Family	:	Myristicaceae

Genus : *Myristica*

Species : *fragrans*

Occurrence and Distribution:

A native of Moluccas, cultivated in many tropical countries. Grown in kerela , Karnataka, The Niligiris and West Bengal.

Description:

A dioecious are occasionally monoecious, evergreen, aromatically, usually 9 to 12 m height and often attaining a height of 20m or more. Blackgreyish-black, longitudionally fissured in old trees.

Leaves coriaceous,elliptic or oblong – lanceolate , upper Surface deep green and lower greyish, turning red greyish when ripe; veins on lower surface; petiole 0.61 to 1.27cm long. flowers greemy yellow, fruits globose or broadly pyiriform , 6 to 9cm long ,seed arillate albuminous, broadly ovoid.flowers appear before the rainy season and fruits appear later.

Macroscopic:

Aril –Aril of *M.fragans* is commonly known as mace,which constitutes the outermost third integument of the seed, covering the basal part of it by scarlet or pale yellow ribbon- like robes, laciniated, recticulate; lobes 3 or 4 cm long and 2 or 3 mm wide and about 1 mm thickness, strongly aromatic with a pleasant aromatic taste .

Seed – Ovoid, 2.2 to 3.2 cm length and 1.5 to 2.5 cm breadth ; the kernel is greyish brown externally and is marked with numerous minute dark reddish- brown points and lines; it is also reticulately marked with small furrows. The outer region is a thin layer of perisperm which grows inwards to the endosperm at the position of the external furrows.

Kernal of seeds (endosperm) rather soft bud firm , whitish and strongly aromatic with a warm slightly bitter taste.

Microscopic:

Aril –TS of the lobe of the aril shows epidermis consisting a single row of highly thick walled ,rectangular cells. The parenchyma cells are polygonal without intercellular spaces.

They contain carbohydrate reserve food material as irregular granules. The oil cells ate which are scattered among the parenchyma are mostly rounded in shape.

Seed – TS of the seed shows testa , perisperm , endosperm. Testa comprises of epidermis with large cells, several rows of thin- walled tangentially elongated cells filled with brown coloured material and occasional groups of sclerenchymatous fibers.

Chemical Constituents:

β-pinine,α-terpinene,terine,methyleugenol,myristicin,elemicin,trimyristin, dihydrodisoeugenol.

Medicinal uses

- Mace is beneficial in Asthma; combination with aromatics found useful in chronic bowel complaints.
- When roasted both nutmeg and mace are efficacious in diarrhoea, flatulence, colic and dyspepsia[30a].

CUMIN SEED

Botanical name : *Cuminum cyminum*

Family : Apiaceae

Part used : Seeds

Scientific Classification:

Kingdom : Plantae

Class : Asterids

Order	:	Apiales
Family	:	Apiaceae
Genus	:	*Cuminum*
Species	:	*cyminum*

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.

Description

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

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.

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.

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[22b].

CLOVES

Botanical name : *Syzygium aromaticum*

Family : Myrtaceae

Parts used : Dried flower buds

Scientific Classification:

Kingdom : Plantae

Class : Dicotyledons

Order : Myrtales

Family : Myrtaceae

Genus : *Syzygium*

Species : *aromaticum*

Occurrence and distribution

A tree cultivated in many parts of the world and extent in south India, Tamil Nadu and Kerala.

Description

A pyramidal or conical evergreen tree usually up to 12 meter in height with single main stem bearing obliquely oriented branches leaves simple, lanceolate, gland – dotted, fragrant. Flower buds greenish to pink, clustered at the ends of the branches, buds are measuring 10 to 17.5mm in length. Colour- Dark brown to black, four side hypanthium readily exuding oil when pressed; fruits fleshly, dark pink drupes seeds oblong, grooved on one side. Odour is strongly aromatic; taste is pungent. Aromatic followed by slight tingling of the tongue.

Chemical constituents:

Caryophylleneoxide, Eugenol, Acetophenone, Benzylsalicylate, Palustrol.

Medicinal uses

- Cloves (unopened flower buds): To relieve Flatulence, Gastric irritability, dyspepsia and to increase the flow of saliva.
- A wineglassful of hot water to which are added 5 grams of bruised cloves and 20 grams of bicarbonate of soda is taken before meals for indigestion[31].

SUGARCANE

Botanical name : *Saccharum officinarum-Sarkkarai*

Family : Poaceae

Part used : Root, Sugar, Juice

Scientific Classification:

Kingdom : Plantae

Class : Monocotyledons

Order : Poales

Family : Poaceae

Genus : *Saccharum*

Species : *officinarum*

Occurrence and distribution

Sugar cane is indigenous to tropical south and Southeast Asia.

Description of the plant

It is a tropical, perennial grass that forms lateral shoots at the base to produce multiple stems, typically three or four meters high and about 5 cm diameter and once harvested the stalk will regrow allowing the plant to live for between 8 to 12 years. The leaves of grow from the nodes of the stem, arranged in two rows on either side of the stem. The leaves are tubular and blades like, thicker in the centres than at the margins and encircle than the stem.

Medicinal uses

- It purifies the air present in chronic ill patients' room.
- Sugar syrup can be used as preservative and also it cures Sinusitis and Rhinitis[32].

3.2. GUNAPADAM ASPECT OF DRUGS

INJI (GINGER)

Metonymy

Allam ,Aartharagam, Aathiragam, Ilaakkottai, Narumaruppu madhil

Vernacular names:

Tamil	:	*Aartterakam, Allam, Narumaruppu matil*
English	:	Ginger
Tel	:	*Allamu, Allam*
Mal	:	*Inchi*
Kan	:	*Alla, Hasishunti*
Sansk	:	*Ardraka,Katybhadra,Srngavera*
Hin	:	*Adarakha*

Parts used : Rhizome

Properties and action

Suvai (Taste)	:	*Kaarppu* (Pungent)
Thanmai (Potency)	:	*Veppam* (Hot potency)
Pirivu (Bio-Transformation)	:	*Kaarppu* (Pungent)
Seigai (Actions)	:	Stimulant, Stomachic, Carminative, Digestive

General Character

"இஞ்சிக் கிழங்குக் கிருமல்யம் ஓக்காளம்
வஞ்சிக்குஞ் சன்னிசுரம் வன்பேதி-விஞ்சுகின்ற
சூலையறும் வாதம்போந் தூண்டாத தீபனமாம்
வேலையறுங் கண்ணாய்-விளம்பு".

"இஞ்சியின் குணமே தென்றி யல்புட னுரைக்கக் கேளீர்
அஞ்சிடுஞ் சன்னியெல்லா மகன்றிடும் பித்த தோடம்
நெஞ்சினி லிருமற் கோழை நெகிழ்ந்திடும் கபங்கள் தன்னை
மிஞ்சினி வருமோ வென்று விளம்பிடும் தேவ நூலே".

"மணிலாக் கோட்டையொன்று மாத்திரமே கற்பமென்ப
தெண்ணிக்கை யாகி யிருக்குமே-கண்ணுக்கு
நன்முறையாஞ் சீதளத்தை நாடாம லேயடிக்குந்
தின்முறைமை நீயறிந்து தின்".

- குணபாடம் மூலிகை வகுப்பு.

Medicinal Uses:

- It is used to treat cold, cough, regurgitation, diseases due to heat, derangement of 3 humours, diarrhoea due to indigestion, pain in the joints & it increases appetite.
- Ginger is a rejuvenating herb, promotes eye sight, *kapham* diseases[32a].

MILAGU (PEPPER)

Scientific name: *Piper nigrum - Milagu*

Other names:

Malayali,Masam, Sarumabandam, Kayam, Kalinai, Miriyal.

Vernacular names:

Tamil	:	*Milagu*
English	:	Black pepper
Tel	:	*Miriyalu*
Mal	:	*Kurumilagu*
Kan	:	*Menasu*
Sansk	:	*Maricha*
Hin	:	*Kali-mirch*

Parts used: Dried fruit

Properties and action

Suvai (Taste)	:	*Kaippu*(Bitter), *Kaarppu*(Pungent)
Thanmai (Potency)	:	*Veppam* (Hot potency)
Pirivu (Bio-Transformation)	:	*Kaarppu* (Pungent)
Seigai (Actions)	:	Carminative, Stimulant, Anti-vadha, Antidote.

General characters

"சீதசுரம் பாண்டு சிலேத்மங்க கிராணிகுன்மம்
வாதம் அருசிபித்தம் மாமூலம்- ஓதுசன்னி
யாசமபஸ் மாரம் அடன்மேகம் காசமிவை
நாசங் கறிமிள கினால்".

- அகத்தியர் குணவாகடம்[33]

Indications:

It cures Anaemia, gastric ulcer, giddiness, diarrhoea, dysentery, vomiting, anal fissure and Cataract.

Therapeutic uses:

- Dried unripe fruits are prescribed in cholera, dyspepsia, flatulence and various gastric ailments.
- Powder of black pepper used as tooth powder [34].

THIPPILI (LONG PEPPER)

Scientific name: *Piper longum – Thippili*

Other names:

Pippli, Kaman, Sowndi, Kanam, Saram, Koli, Ambu, Aathimarunthu, Kanai.

Vernacular names:

Tamil	:	*Thippili*
English	:	Long pepper
Tel	:	*Pippilu*
Mal	:	*Thippili*
Kan	:	*Hippili*
Sansk	:	*Pippali*
Hin	:	*Pipar*

Parts used : Dried fruit and Roots

Properties and action

Suvai (Taste)	:	*Kaarppu* (Pungent)
Thanmai (Potency)	:	*Veppam* (Hot potency)

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

Seigai (Actions) : Carminative, Stimulant, Expectorant,Antiseptic, Febrifuge.

General character

"கட்டி யெதிர்நின்று கடுநோயெல் லாம்பணியும்
திட்டி வினையகலும் தேகமெத்த-புட்டியாம்
மாமனுக்கு மாமனென மற்றவர்க்கு மற்றவனாங்
காமமெனுந் திப்பிலிக்கும் கை".

- தேரன் வெண்பா[35]

Indications:

It relief *kapham* related diseases and strength the body.

Therapeutic uses

- Long pepper powder with honey and betel leaf juice cures fever.
- Powdered form of long pepper seeds with ghee for aphrodisiac activity[32b].

LAVANGAPPATTIRI (INDIAN CINNAMON)

Scientific name: *Cinnamomum tamala –Lavangappattiri*

Other names : *Thamalappattiri*

Vernacular names:

Tamil : *Lavarikappattri*

English : Indian cinnamon

Tel : *Akupatri*

Mal : *Karuvapatta patram*

Kan : *Tamalpatra,Dalchini ele*

Sansk : *Tvaka patra,Varanga,Coca*

Hin : *Tejpatra*

Properties and action

Suvai (Taste) : *Kaarppu* (Pungent)

Thanmai (Potency) : *Veppam* (Hot potency)

Pirivu (Bio-Transformation) : *Karppu* (Pungent)

Seigai (Actions) : Appetiser, Carminative, Diaphoretic

General Character

"மேகசுரம் சீதசுரம் வெட்டைசுவா சங்காசம்

தாகபித்தம் வாந்திசர் வாசியநோய்- மேகத்தின்

கட்டியொடு தாதுநட்டங் கைப்பருசி போக்கிவிடும்

இட்டஇல வங்கத் திலை".

-அகத்தியர் குணவாகடம்[33a].

Medicinal Uses

- It cures cough ,asthma ,vomiting ,mouth ulcer
- Lavangam oil cures body pain, tooth ache and headache[20d].

ELAM (CARDAMOM)

Scientific name: *Elettaria cardamomum - Elam*

Other names:

Onchi, Gorangum, Thudi.

Vernacular names:

Tamil : *Elam*

English : Cardamom

Tel : *Elakulu*

Mal : *Elattari*

Kan : *Elakki*

Sansk : *Ela*

Hin : *Elachi*

Parts used : Seeds

Properties and action

Suvai (Taste) : *Kaarppu* (Pungent)

Thanmai (Potency) : *Veppam* (Hot potency)

Pirivu (Bio-Transformation) : *Karppu* (Pungent)

Seigai (Actions) : Carminative, Stomachic, Expectorant, Tonic.

General characters

"தொண்டை வாய்கவுள் தாலுகு தங்களில்
தோன்றும் நோயதி சாரம்பன் மேகத்தால்
உண்டை போல்எழுங் கட்டி கிரிச்சாரம்
உழலை வாந்தி சிலந்தி விஷஞ்சுரம்
பண்டை வெக்கை விதாகநோய் காசமும்
பாழுஞ் சோமப் பிணிவிந்து நட்டமும்
அண்டை யீளைவன் பித்தம் இவைக்கெல்லாம்
ஆல மாங்கமழ் ஏல மருந்ததே".

***- பதார்த்த குண சிந்தாமணி*[36]**

Indications:

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

Therapeutic uses:

- Powdered form of seeds of cardamom, dried ginger, cloves, cumin seeds cure stomach ache, ulcer.
- Roast seeds of cardamom, omam and cumin seeds then they are powdered and given to cure indigestion[37].

KODIVELI-VER (LEAD WORT)

Synonyms

Plumbago viscosa

Common names

Ceylon lead wort, Doctor bush, Plumbago **.**

Vernacular names

Tamil	:	Chittiri, Chittira
English	:	Leadwort
Tel	:	Chitramulamu
Mar	:	Chitramul
Ben	:	Chita
Sansk	:	Anala, Dahana, Pithi, Vahnisajnaka, Agni, Agnika, Jyothi.
Hin	:	Cheetha

Properties and action

Suvai (Taste)	:	Kaarppu (Pungent)
Thanmai (Potency)	:	Veppam (Hot potency)
Pirivu (Bio-Transformation)	:	Karppu (Pungent)
Seigai (Actions)	:	Anti-periodic, Diaphoretic.

Medicinal Uses:

- The root and root bark are bitter, hot, dry, stomachic, carminative, anthelmintic, alterative, cure intestinal troubles, dysentery, leucoderma, inflammation, piles, bronchitis, itching, diseases of liver, consumption, ascites, good in anaemia.
- The root has a sharp bitter taste, laxative, expectorant, stomachic, tonic, abortifacient, alexipharmic, good appetizer, useful in laryngitis, rheumatism, diseases of spleen, leucoderma, ring-worm, scabies.
- It acts as a powerful sudorific.
- The use of plumbago as a rubefacient, vesicant, local ecbolic, sudorific has a rational basis . The root appears to possess abortifacient and vesicant. properties. The drug is known to cause abortion and in Malaya even eating the leaves is said to cause abortion [23a].

LAVANGAPATTAI (CINNAMON BARK)

Scientific name: Cinnamomum zeylanicum-*Lavangapattai*

Other Names*:*

Karuvapattai

Vernacular Names:

Tamil	:	*Karuvapattai,Lavarikappatai*
English	:	Cinnamon bark
Tel	:	*Lavangapatta,Dalchini chekka*
Mal	:	*Karuvapatta,Illavarngathely*
Kan	:	*Dalchini Chakke*
Sansk	:	*Tvak,Darusita*
Hin	:	*Dalchini*
Parts Used	:	Bark

Properties and action

Suvai (Taste) : *Inippu*(Sweet) ,Karppu (Pungent)

Thanmai (Potency) : *Thatpum* (Cold Potency)

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

Seigai (Actions) : Aphrodisiac, Carminative, Stimulant

Indications:

- It cures peptic ulcer, asthma, indigestion.
- It cures flatulence ,dyspepsia, diarrhoea, dysentery, fever, nausea and vomiting.

Therapeutic Uses:

- Powder cures vomiting, indigestion, stomach pain, diarrhoea.
- Bark powder 650 gm cures dysentery[20e].

MOONGILUPPU (THORNY BAMBOO)

Synonyms

Bambusa bambos Druce

Common names

Golden bamboo, Buddha's belly bamboo, Thorny Bamboo, Bans, Bamboo

Vernacular Names:

Tamil : *Moongil, Mulla Veduru*

English : Thorny Bamboo

Mal : *Mula, Mola*

Kan : *Biduru, Gatte*

Mar : *Kate Bamboo*

Properties and Action

Suvai (Taste) : Astringent

Thanmai (Potency) : *Veppam* (Hot Potency)

Pirivu (Bio-Transformation) : *Karppu*(Pungent)

Seigai (Actions) : Stimulant, Tonic, Anthelmintic, Emmenagogue

Medicinal Uses

- The stem and leaves are acrid, sour, bitter, cooling, laxative and burning sensations, blood coagulopathies, biliousness, leucoderma, inflammations, strangury and piles.
- In North Lakhimpur forest division of Assam, very soft shoots of the plant are reported to be used for birth control[28a] .

*SANTHANATHOOL (*SANDAL WOOD)

Scientific name: *Santalum album-Chandanam*

Vernacular Names:

Tamil : *Chandanam*

English : Sandalwood

Tel : *Gandeapu-chkka*

Mal : *Chandana*

Kan : *Gadnhad chehke*

Sansk : *Shri-gandha chandanam*

Hin : *Chandan*

Parts used : Wood, oil

Properties and Action

Suvai (Taste) : Bitter, Mild Astringent

Thanmai (Potency) : *Thatpum*(Cold Potency)

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

Seigai (Actions) : Alterative, Diuretic, Diaphoretic, Stimulant, Astringent,Coolant.

General Character

"கோதில் சந்தனஞ் சீதோஷ்ணங் கொண்டிருக்கும்
வாதபித்தம் ஐயம் மனப்பிரமை-ஓதுசுரம்
மேகம் தனித்தாகம் வெப்பு சொறி யும்போக்கும்
ஆகந் தனக்குறுதி யாம்"

. **- அகத்தியர் குணவாகடம்**[33b]

Medicinal Uses:

- Decoction of sandal wood powder used to treat in Fever, Tachycardia and decreases the pulse rate.
- Also used to treat in various skin Diseases, Diarrhorea[32c].

***VILAMICHU-VER* (WHITE CUS CUS GRASS)**

Scientific name: *Plectranthus vettiveroides-Vilamichu-ver*

Vernacular Names:

Tamil : *Vilamichu-ver*

English : White cus cus grass

Mal : *Iruveli*

Sansk : *Hroeberam*

Properties and Action

Suvai (Taste) : *kaippu* (Bitter)

Thanmai (Potency) : Coolant

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

Seigai (Actions) : Refrigerent, Anti-pittha.

General character

“மேகம் விழியெரிச்சல் வீறிரத்த பித்தமொடு

தாகமத மூர்ச்சைபித்தந் தன்மயக்கம்-சோகஞ்

சிரநோய் இவையேகுஞ் செய்யவிலா மிச்சுக்

கெரிசுரமும் இல்லை யிசை”.

-குணபாடம் மூலிகை வகுப்பு.

Indications:

- It cures diabetes, burning sensation in eyes, hypertension, giddiness, dropsy, and headache[32d].

***SATHIKKAI* (NUTMEG TREE)**

Scientific name: *Myristica fragrans*

Other names:

Kullakai, Jathikai

Vernacular Names:

Tamil	:	*Jadikai*
English	:	Nutmeg tree
Tel	:	*Jajikai, Jadikai(seed), Apatri(aril).*
Mal	:	*Jarikaya, Jatikka(seed), Jatipattri(aril)*
Kan	:	*Jajikai, Jaikai(seed), Jaipatri(aril)*
Sansk	:	*Jatiphala,Jatipatri*
Hin	:	*Jaiphal, Javitri*

Properties and Action

Suvai (Taste) : *Thuvarpu* (Astringent), *Karppu* (Pungent)

Thanmai (Potency) : *Veppam* (Hot Potency)

Pirivu (Bio-Transformation) : *Karppu* (Pungent)

Seigai (Actions) : Stimulant, Carminative, Aromatic, Tonic.

General Character

"தாது நட்டம் பேதி சருவாசி யஞ்சிர நோய்

ஓதுசுவா சங்காசம் உட்கிரணி-வேதோ

டிலக்காய் வரும்பிணிபோம் ஏற்றமயல் பித்தங்

.குலக்கா யருந்துவர்க்குக் கூறு"

- குணபாடம் மூலிகை வகுப்பு.

Therapeutic Uses

- The powder made from nutmeg (0.4gm),dry ginger(0.4gm) and caraway (0.7gm) is a good carminative.
- The oil of nutmeg and mace is used in sprains , paralysis , Rheumatism[32e].

SEERAGAM (CUMIN SEED)

Scientific name:*Cuminum cyminum*-*Seeragam*

Other Names

Asai, Chiri, Upakumbapeesam, Narcheeri, Thutthasambalam, Piraththi-vika, Pitha nasini, 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*
Sansk	:	*Sveta jiraka, Ajaji, Jiraka, Ajajika*
Tel	:	*Jilakaraka, Tella jilakara*

Parts used : Seeds

Properties and Action

Suvai (Taste)	:	*karppu*, sweet
Thanmai (Potency)	:	Coolant
Pirivu (Bio-Transformation)	:	*Inippu*(Sweet)
Seigai (Actions)	:	Astringent, Stimulant, Stomachic, Carminative

General character

“வாந்தி யருசிகுன்மம் வாய்நோய்ப்பி லீகமிரைப்
பேற்திருமல் கல்லடைப்பி லாஞ்சனமும்- சேர்ந்தகம்மல்
ஆசனகு டாரியேனும் அந்தக் கிரகணியும்
போசனகு டாரியுண்ணப் போம்”.

- அகத்தியர் குணவாகடம்[33e]

“பித்தமெனு மந்திரியைப் பின்னப் படுத்தியவன்
சத்துருவைய் யுந்துரந்து சாதித்து- மத்தமெனும்
ராசனையு மீவென்று நண்பைப் பலப்படுத்திப்
போசனகு டாரிசெயும் போர்”.

- தேரன் வெண்பா[35a]

Medicinal uses

- Seeragam added with palm jaggery powder used for strengthens the body
- 1 gm Seeragam heated with 90 ml of gingely oil used for oil bath to reduce giddiness, head ache, vomiting and eye disease[38].

KIRAMBU (CLOVES)

Scientific name: Syzygium *aromaticum* - *Kirambu*

Other names:

Anjugam, Urkadam, Sosam, Thirali ,Varangam

Vernacular names:

Tam	:	*Kirambu*
Eng	:	Cloves, Clove tree
Hindi	:	*Long*
Kan	:	*Lavanga*
Mal	:	*karampu*
Sansk	:	*Lavangam*
Tel	:	*Lavangalu*

Parts used: Dried flower buds

Properties and action

Suvai (Taste)	:	*Kaarppu* (Pungent)
Thanmai (Potency)	:	*Veppam* (Hot potency)
Pirivu (Bio-Transformation)	:	*Kaarppu* (Pungent)
Seigai (Actions)	:	Carminative, Stimulant, Antispasmodic, Expectorant.

General characters

"பித்த மயக்கம் பேதியொடு வாந்தியும்போம்
சுத்தவிரத் தக்கடுப்புந் தோன்றுமோ - மெத்த
இலவங்கங் கொண்டவருக் கேற் சுகமாகும்
மலமங்கே கட்டுமென வாழ்த்து".

-அகத்தியர் குணவாகடம்[33c]

Indications:

It cures diarrhoea, vomiting, dysentery, Asthma, cough, flatulence.

Therapeutic uses

- Cloves (unopened flower buds): To relieve Flatulence, Gastric irritability, dyspepsia and to increase the flow of saliva.
- A wineglassful of hot water to which are added 5 grams of bruised cloves and 20 grams of bicarbonate of soda is taken before meals for indigestion[39].

KARUMBU (SUGARCANE)

Scientific name: ***Saccharum officinarum****-Karumubu*

Other names:

Punarpoosam, Ikku, Vaei

Vernacular Names

Tam	:	*Karumubu*
Eng	:	Sugar cane
Hindi	:	*Ukh-Ganna*
Kan	:	*Khabbu*
Mal	:	*Karinpu*
Sansk	:	*Ikshu*

Parts used : Juice, Sugar, Root

Properties and action

Suvai (Taste) : *Inippu* (Sweet)

Thanmai (Potency) : *Thatpam* (Clod potency)

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

Seigai (Actions) : Anti septic, Demulcent

General characters

"அருந்து மருந்திற் கனுபான மாகப்

பொருந்துமடல் வாந்திபித்தம் போக்கும் அருந்தருசி

நீக்கு மதிகபத்தை நீற்று மகிழ்ச்சியுண்

டாக்கு நறுஞ் சர்க்கரை".

- அகத்தியர் குணவாகடம் [33d]

Indications:

- It can be use the as adjuvant for several medicine
- It cures vomiting.

Therapeutic uses

- It cures chronic healing ulcer.
- Sugar along with honey wax can be used to treat Acne.
- Sugar can be used as an antidote for toxicity of Copper, Arsenic and Mercury[39a].

GHEE

Butter is cleaned and heated in a vessel. When it melts either the leaves of Moringa olifera are the bettle leaves are added and filtered immediately, since it is difficult to filter after cooling.

If Ghee is preserved in good vessel, it won't spoil upto 3 months. Cow's ghee is slightly yellowish in colour. Ghee should be consumed with food. Food without ghee is considered to be forbidden. It is good to consume the melted ghee and diluted butter milk. The following lines stress these points :

" நெய்யில்லா உண்டி பாழ்"

"நெய்யை உருக்கியும் மோரைப் பெருக்கியும்"

Among all the ghees, cow's is the best. If this is not available mixed ghee may be consumed.

Properties of mixed ghee:

- This will improve sperm production.
- It also stimulate appetite and controls *pitha dhosa* and *pitha fever*. In addition, it gives strength to the body.

Constituents:

Saturated, Transaturated, Monounsaturated, Polyunsaturated fats, Cholesterol, VitaminA, and VitaminE.

Medicinal Uses:

- Cow's ghee cures morbid thirst, vomiting, emaciation increased pitha, vatha diseases, gonorrhoea, hiccough, cough, abdominal pain, gastritis, intestinal disorders, fatigue, haemorrhoids and dryness, hypermotility of gut, weakness of bones.
- Ghee is good for eyes.
- It increases body weight and gives a golden complexion.
- It also cures the diseases of the eye, eyebrow ,forehead and head[40].

3.3 PHARMACEUTICAL REVIEW

CHOORANAM

Definition

Chooranam are fine dry powders of drugs. The term *"Chooranam"* may be applied to the powders of a single drug or a mixture of two or more drugs, which are powdered separately prior to their being mixed to homogeneity.

Method of preparation

Equipment required

1. The drug enumerated in the recipe in clean and dried state.
2. A mortar and pestle.
3. A fine sieve or fine cloth of close mesh.

Process of preparation

The drugs which are to be used in the preparations should be taken from recently collected material. Drugs which are aged by prolonged storage or changed in colour, taste and scent and those that are insects infected or attacked by fungi should be positively rejected.

However, drugs like Emboli fruits, Senna, Long pepper, Jaggery and cow's ghee are preferred from fairly aged stock, provided they are not infested with pests, deteriorated or spoiled or developed rancidity.

In general, the aromatic drugs are slightly fried in order to enhance their aroma and milling properties. Any extraneous material, organic or inorganic, should be removed from the drugs by close inspection.

The Chooranam should be as fine as to be called amorphous and should be never damp. The fineness of the sieve should be 100 mesh or still finer.

Purification of the prepared Chooranam

“தானென்ற சூரணத்தின் சுத்திக்கேளு
தப்பாதே சரக்கெல்லாஞ் சூரணித்து
நானென்ற வாவின் பாலாற் பிசைந்து
நலமான சட்டியிலே பாலைவிட்டு
வானென்ற சுத்தசலம் பாதிவிட்டு
வளமாக மேற்சீலை கோடு கட்டிப்
பானென்ற சூரணத்தைப் பிட்டுபோல் வைது
பதறாதே வெந்தெடுக்கச் சித்தியமே!”

- அகத்தியர் வைத்திய இரத்தினச்சுருக்கம்

The prepared Chooranam is mixed with the milk in half quantity milk and half quantity water is taken. The mouth of the pot is covered with a thin cloth material. Above this cloth the mixed Chooranam is placed. The pot is placed over the stove and heated.

“ஆமப்பா ரவியுலர்த்திப் பொடிதான் செய்து
அப்பனே சமனாய்ச் சர்க்கரையைச்சேர்த்து
நாமப்பா கொண்டு வர தோஷம் போச்சு
நன்றாக்ச் சுத்தி செய்யாச் சூரணந்தான்
தாமப்பா ரோகத்தை வெல்லா தப்பா
தளமான வியதி யெல்லாம் பாரிக்கும் பார்
வேமப்பா சுத்தி செய்து கொண்டாயனால்
வெகுசுறுக்காய் தீருமா வியாதி கேளு”

- அகத்தியர் வைத்தியஇரத்தினச்சுருக்கம்[41]

Then the Chooranam is placed in the sunlight and powdered. Equal amount of sugar is added and taken internally. All type of disease gets cured. If the drug is taken without purification the diseases does not cure. If taken after purification the disease cures easily.

Storage

The prepared Chooranam should be allowed to cool by spreading and mixing, prior to packing. They should be stored in tightly closed glass container, polythene or tin containers or cellophane bags and sealed. These bags should in turn be enclosed in card board boxes.

The Chooranam, to facilitate easy handling and to assure exact dosage of administration, could be pressed into tablets with the addition of a suitable binder.

These tablets could be packed in bottles or tubes made either of glass or packed in strip of metal foil or plastic sheets. The Chooranam is said to retain its potency for three months and then gradually deteriorate.

However, if properly packed and stored they keep good for a year [42].

In industry the tablets are made, counted and packed by electronic devices. According to AYUSH guidelines shelf life of Chooranam is one year [43].

Table: 1 ANALYTICAL SPECIFICATIONS OF CURNA/CHOORNAM

S.No	TESTS
1.	Description Macroscopic, Microscopic
2.	Loss on drying at 1050 C
3.	Total – ash
4.	Acid – insoluble ash
5.	Water-soluble extractive
6.	Alcohol – soluble extractive
7.	Particle size (80-100 mesh for Churna; 40-60 mesh for churna)
8.	Identifications, TLC/HPTLC-with marker (wherever possible)

9.	Test for heavy/Toxic metals
	Lead
	Cadmium
	Mercury
	Arsenic
10.	Microbial contamination
	Total bacterial count
	Total fungal count
11.	Test for specific Pathogen
	E. coli
	Salmonella spp.
	S.aureus
	Pseudomonas aeruginosa
12.	Pesticide residue
	Organochlorine pesticides
	Organophosphorus pesticides
	Pyrethroids
13	Test for Aflatoxins (B1,B2,G1,G2)

DISEASE REVIEW

3.4. SIDDHA ASPECT DISEASE REVIEW

JAUNDICE (*KAMALAI*)

This is one among those diseases which occur due to derangement of *Pittha uyir thathu. Pithha* disease, *Manjal kamalai, Kamalai,* and *Manjal* disease are the synonyms of this disease. In this disease, urine, eyes tongue and the body will get yellowish discolouration.

Aetiology:

Consuming more food which stimulates *Pittha thathu,* drinking unhygienic and impure water and seasonal changes cause this disease.

Yugi vaidhya chinthamani states that jaundice will occur when one consumes more *Pittha*- stimulating food and indulges in excessive sexual activity under conditions of severe anaemia.

Apart from this, obstruction of bile flow will cause jaundice. When there is obstruction in bile duct, the bile, instead of going to the intestine, returns and mixes with the blood.

Obstruction of bile flow will occur due to bile stone in the bile duct, worms, constriction in the inner walls of the bile duct and new growths.

Cancerous growth, abscess or tumours or lymph nodules which occur in neighbouring parts of the bile duct may also obstruct the bile flow in the bile duct.

The occurrence of any inflammation in the small branches of the bile duct may also cause obstruction.

Because of the toxicity of certain metallic substances like copper sulphate, lead, white arsenic and copper, inflammatory reaction will occur in the liver leading to the obstruction of bile flow.

There may be certain defects in RBCs by birth. Apart from this, on account of malaria and snake poison, the RBCs will get destroyed. In this condition also, jaundice will occur.

In cirrhosis of liver also causes this disease. Cirrhosis will occur in malaria and yellow fever and on account of certain toxic substances like alcohol and toddy and so in this condition also jaundice occur. When the blood *Thathu* increases in the body, jaundice will occur.

Premonitory symptoms

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

முற்குறிகுணங்கள்:

“பருகவே உள்ளங்கா லுள்ளங் கைகள்

பகர்முகங்கண் ணுடம்புமிக வெளுப்பு காணுங்

கருகவே கால்கைக ளோய்ச்ச லாகுங்

கனமாக நடுக்கியே இளைப்புண் டாக்குஞ்

சுருகவே மலந்தானும் வறண்டு கட்டுந்

தூயமுக மஞ்சளிட நிறம தாகும்

வெருகவே வீக்கமாய்க் களைப்புண்டாகும்

மிகக்காது மந்தந்தலை கனப்புண்டாகும்."

-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, constipated and hardened faeces, and yellowish discolouration of face, oedema, lassitude.[44].

Classification of the disease

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

1. *Vatha kamalai*
2. *Pittha kamalai*
3. *Kaba kamalai*
4. *Vatha kaba kamalai*
5. *Pittha kaba kamalai*
6. *Thirithathu kamalai*
7. *Perunkamalai*
8. *Uthu kamalai*
9. *Varattu kamalai*
10. *Azhagu kamalai*
11. *Sengamala kamalai*
12. *Kumba kamalai*
13. *Gunma kamalai*

Curable and Incurable

Among the 13 varieties, jaundice due to *Pittha* factor, severe jaundice, jaundice due to *Kaba* factor, jaundice with swelling, jaundice with dryness, jaundice due to *Vatha Kaba* factor and jaundice due to *Kaba Vatha* factor are curable easily. The other varieties are not easily curable.

நாடி நடை:

பண்பான பித்தத்தில் சேத்து மநாடி

கண்காது நயனமலம் நீரு மஞ்சள்.

(சதக நாடி)

சாறுமடா பித்தமந்த வாதத்தி லேறில்

தளஞ்செய்யும் பாண்டு காமாலை தானும்

(நாடி நூல்)

பித்தஜயக் கலப்பாலும், பித்தவாதக் கலப்பாலும்
மஞ்சள்நோய் உற்பத்தியாகும் என்பதாம்[45]

Treatment

Thanks to the variable intake of food and other deeds which stimulate *Pittha thathu, Pittha* increases in its strength, joins *Kaba* and becomes *Pittha 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 so the bile is not able to flow in its normal route as there is obstruction.

Hence the bile mixes with the blood and jaundice occurs. The *Vatha* factor gets affected and the disease occurs due to *Pittha Vatha* factor.

Apart from this, the other *Vayu* (gases) also get spoiled. Hence the duty of the doctor is to set right the altered *Kabam* and *Pittha 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 and diarrhoea

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

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

- Anthemides flower (*simai samanthi flower*)
- Buds of rose (*roja*)
- Grapes (*kodi munthirigai*)
- Bark, leaves and flower of purging cassia (*sarakkonrai*)
- Phyllanthus amarus (*kizhanelli*)
- Terminalia chebula (*kadukkai*)
- Terminalia bellerica (*thandrikkai*)
- Phyllanthus emblica (Indian gooseberry)
- Picrorhiza kurroa (*kadugurohini*)
- Root of Indian jalap (*sivathai*)
- Flower of hygrophila paniculata (*nirmulli* flower)
- Flower of neem (*veppam pu*)
- Tinospera cardifolia (*sinthil*)
- Tribulus terrestris (*nerunjil*)

A decoction of the above substances may be made and given for jaundice for laxative purpose.[44a]

LIVER DISEASES AND MEDICINAL PLANTS

Polyherbal formulations reputed to have hepatoprotective activity that is available in the Indian market which comprises about 100 medicinal plants [15b].

Andrographis paniculata

For centuries *Andrographis* has been important herb in the Asian healing system of Ayurveda, Unani and Traditional Chinese medicine. Traditionally herb has been used to potentiate immune system response to inflammation and infections, and as an Anti-inflammatory ,Anti – pyretic and a hepatoprotective. Andrographolide ,the active constituents isolated from the plant *Andrographis paniculata* showed a significant dose .

Phyllanthus amaris

Phyllanthus amaris has been researched for its effects on hepatitis and reported that 22 of 37 cases of Hepatitis B lost their "Carrier" status after using the herb for a month [46].

Boerhavia diffusa

An alcoholic extract of whole plant *Boerhavia diffusa* given orally exhibited hepatoprotective activity against experimentally induced CCL4 hepatotoxicity in rats and mice[47].

Swertia chirata

Mukherjee *et a*l.,(1997) reported that simulataneous treatment with S.chirata (in different doses,vix.,20,50, and 100 mg/kg body wt daily) and CCL4 caused improvement at both biochemical and histopathological parameters compared to that of CCL4 treatment alone but it was most effective when S.chirata was administered in a moderate dose (50 mg/kg body wt) [48].

Terminalia belerica

Compound I isolated from fraction TB5 of Terminalia belerica identified as 3,4,5-trihydroxy benzoic acid(gallic acid) and was evaluated for its hepatoprotective activity against CCL4-induced physiological and biochemical alterations in the liver.

Administration of compound led to significant reversal of majority of the altered parameters confirming the presence of hepatoprotective activity [49].

Cichorium intybus

Cichorium intybus is a popular Ayurvedic medicine for the treatment of liver diseases. It is commonly known as kasni and is part of polyherbal formulations used in the treatment of liver diseases. In mice, liver protection was observed at various doses of *Cichorium intybus* but optimimum protection was seen with a dose of 75mg/kg given 30 minutes after CCL4 intoxication. Kalantari and Rastmanesh (1985) in his preclinical studies showed that an alcoholic extract of *Cichorium intybus* was found to be effective against chlorpromazine-induced hepatic damage in adult albino rats. A bitter glucoside,Cichorin(C32H34O19) has been reported to be the active constituent of the herb.

Glychyrrhiza glabra

Glychyrrhiza glabra,commonly known as licorice contains triterpene saponin, known as glycyrrhizin, which has potential hepatoprotective activity. It belongs to a group of compounds known as sulfated polysaccharides.

Experimental hepatitis and cirrhosis studies on rats found that it can promote the regeneration of liver cells and at the same time inhibit fibrosis.

Glycyrrhizin can alleviate histological disorder due to inflammation and restore the liver structure and function from the damage due to CCL4.

The effects including: lowering the SGPT, reducing the degeneration and necrosis and recovering the glycogen and RNA of liver cells.

Effects of glycyrrhizin has been studied on free radical generation and lipid peroxidation in primary cultured rat hepatocytes.

Favourable results have been reported in children suffering from cytomegalovirus after treating with glycyrrhizin.

Curcuma longa

Curcuma longa turmeric has been found to protect animal livers from a variety of hepatotoxic substances, including CCL4, galactosamine,pentobarbital,1-chloro-2,4-dinitrobenzene, 7.4-hydroxy-nonenal and paracetamol .[50]

Other Medicines for Jaundice (Siddha Aspect)

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

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

Sweet diarrhoea wax (*Thithippu bedhi mezhugu*) or sweet diarrhoea *leghiyam* (*Thithippu bedhi leghiyam*) of the size of Indian gooseberry can be taken at bedtime. Easy to motion will occur in the morning. It may be given in suitable doses in the morning and evening.

If the motion is not passed properly by the above methods, 2 *sanjivi* tablets 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* tablets, *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.

The unripened fruit of *Randia dumetorum* (*Marukkarai kay*) 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 in 1 pinch can be given in the morning alone. Diarrhoea and vomiting will occur.

Tinospera cardifolia (*Sinthil*), *Eclipta alba* (*Karisalai*), *Phyllanthus amarus*(*Kizha nelli*), *Sivanar* neem, *Indigofera aspalathoides* (*Sivanar vembu*), *Terminalia chebula* (*Kadukkay*),Puncture vine(*Tribulas terrestris – nerunjil*), flower of purging cassia (*Cassia fistula- Sarakkonrai*) or its leaf or bark.

Any one of the above can be ground in raw greenish form and made into a green paste (*Karkam*). This can be given for jaundice.

This may be considered as a preventive and curative medicine for jaundice. With salt-free diet, goat's milk and rice may be eaten. The next day salt may be included in the diet[51].

Tablet of *Eclipta alba (Karisalai matthirai*)

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

Each ¼ *palam* (8.5 gm), Grind them all in the mortar and make tablets in the size of *Solanum torvum* (*Sundai*), dry them in shade and put them in a wide-mouthed bottle; pour good quality gingili oil and close it with a lid and put it in sunlight. One tablet each may be taken in the morning and evening. Jaundice along with oedema will get cured. Tamarind and salt should be avoided [52].

Ghee of *Phyllanthus amarus* (*Kizha nelli ney*)

- 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.

Ghee of *Eclipta alba (Karisalai ney)*

- Juice of *Eclipta* 1.35 litre (one measure),
- 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*)

May be taken and the ghee may be prepared as described 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 Calx or *Chenduram*. It will give an excellent cure for spleen and liver enlargement also.

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 and make a single powder of them.

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 used 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 [53].

Decoction of tender leaves of *Thespesia populnea* for jaundice (*Puvarasu kozhundhu ilai kudinir*)

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 [54].

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 [55].

Powder of East Indian rose (*Nandiyavattai*) 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.

Coconut medicine for jaundice:

A well – ripened coconut may be taken, its eyes may be opened and the coconut water may be poured into a vessel. The root of *Boerhavia diffusa* (*Mukkirattai*) may be rubbed with coconut water and a paste is made.

This paste must be inserted into the coconut through the opened eyes and then the eyes may be closed properly and a strong mud-sealing may be made over it and dried. This may be buried under the ground and kept for 3 days under the earth.

It may be taken out on the fourth day and the sealing may be removed. The coconut may be broken and the medicine inside the coconut may be divided into 3 equal parts. One part per day along with buffalo curd may be consumed. Fat free diet is essential.

Two medicines for jaundice:

The whole plant of *Solanum nigrum* (*Manathakkali*) or the whole plant of *Phyllanthus amarus* (*Kizhanelli*) may be macerated on a stone slab with water. A lime sized paste of it may be consumed with cow's buttermilk or curds.

Green paste of *Eclipta alba* (*Kaiyanthakarai karkam*)

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.

Medicine of *Acacia concinna* (*Sikaikkay*) for jaundice:

Four numbers of fresh fruit of Soapnut (*Acacia conenua*) may be collected and the seeds inside the fruit may be removed.

The seedless fruits may be macerated with water very well and it may be consumed mixing it with half a litre of cow's milk. If diarrhoea occurs, then it is good. This medicine may be consumed for 3 days.

Rice with cow's milk may be taken. Salt and tamarind should be avoided. Jaundice of any type will be cured.

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

The root of *Alternanthera sessilis* may be collected and macerated on a stone slab. It is taken in lime fruit size 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.

***Cissus quadrangularis* (*Pirandai*) 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.

Eye drops for jaundice:

Leaves of *Caesalpinia bouduc*, garlic and *Acorus calamus*- these three may be squeezed and put in a cloth.

The drops of it may be squeezed out from the cloth and dropped into both the eyes. As it is a pungent drug, only a small drop alone is to be poured into the eyes.

If there is swelling in the eyes, tender leaves of *Cynodon dactylon* (*Arugu*) may be macerated with cow's milk and the paste may be given for 6 days. Jaundice will be cured.

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

Turmeric (*Manjal*), pericarp of *Tchebula* (*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 ..

Leaves of castor plant for jaundice (*Amanakkilai marundhu*)

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.

Medicine of iron filings for jaundice (*Arappodi marunthu*)

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.

***Phyllanthus distichus* (*Arunelli*) 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.

Excreta milk of buffalo for jaundice (*Erumai sani pal*)

The excreta milk of buffalo and buffalo curd may be mixed together. Cumin seeds and onion in equal quantities may be macerated and added to the above.

The mixture may be again mixed with cow's milk and given in 6 to 7 equal doses. Jaundice will be cured.

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.

White variety of Indian jalap (*Vellai sivathai*), dry ginger (*Chukku*), root of *Burleria prionities* (*Semmulli ver*) make a powder of these and mix together and take with milk and sugar.

The whole plant of *Phyllanthus amarus* (*Kizhanelli*) 50 gm and dry ginger (*Chukku*) 10gm may be macereated and given or a decoction made out of them may be given.

Phyllanthus amarus (*Kizhanelli*), *Terminalia chebula* (*Kadukkay*- gall- nut)-a decoction may be prepared of the above and consumed along with sugar. Jaundice will get cured.

Root of *Alternanthes sessilis* (*Ponnankani*), root of *Indigofera tinctoria* (*Avuri*), *Terminalia chebula* (*Kadukkay*-gall- nut), tender leaves of white pumpkin (*Venpusani kozundhu*) any of the above may be macerated with goat's butter milk and given for jaundice. Jaundice will get cured.

Glycyrrhizha glabra (*Athimathuram*), sugar taken from *Tinospera cordifolia* (*Sinthil sarakkarai*), clearing nut (*Thetran kottai*), sandal (*Chandhanam*), all the above may be macerated with cold water and given for jaundice. Jaundice will get cured.

Pericarp of the dry fruit of Indian gooseberry (*Nelli thol*), dry ginger (*Chukku*), pepper (*Milagu*), long pepper (*Thippili*), turmeric (*Manjal*) take equal quantities of the above and make a Chooranam or powder. To the above powder, iron *Chenduram* may be added and consumed. It can be taken along with honey, ghee or sugar.

Make a green paste from the whole plant of *Phyllanthus amarus* (*Kizhanelli*) and take the paste in the size of the area nut. Add 300 mg of conch shell Calx to this and consume it in the morning alone as single dose with butter milk.

Take it for 3 or 5 days like this. Avoid salt. Jaundice will get cured.

Purified gypsum may be macerated with cow's urine and consumed. Jaundice with oedema will get cured.

Powder of *Eclipta alba* (*Karisalai churanam*):

- 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 and grind it in the mortar to make it 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.

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 aetiology, 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[56].

3.5.Disease Modern Aspect

Liver Diseases

Liver diseases are a broad term re-counting 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:-

1. Hepatitis, inflammation of the liver, instigated mainly by various viruses but also by some poisons, autoimmunity or hereditary conditions.

2. 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.

3. Haemochromatosis, a hereditary disease causing the accretion of iron in the body, eventually leading to liver damage[57].

4. Cancer of the liver (Primary hepatocellular carcinoma or Cholangio carcinoma and metastatic cancers, usually from other Parts of the gastrointestinal tract).

5. Wilson's disease, a hereditary disease which reasons the body to retain copper.

6. Primary sclerosing cholangitis, an inflammatory disease of the bile duct, likely autoimmune in nature.

7. Primary billiary cirrohisis, autoimmune disease of slight bile ducts.

8. Budd-Chiari syndrome, complication of the hepatic vein.

9. Gilbert's syndrome, a genetic syndrome of bilirubin metabolism, found in about 5% of the population.

10. 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.

Liver diseases are mainly caused by

1. Infections
2. Autoimmune disorder
3. Chemical agents (certain Antibiotics, Peroxidised oil, Aflatoxin, Carbontetra chloride, Chlorinated hydrocarbon, Paracetamol etc.
4. Excess consumption of alcohol.

Hepatitis

It is the infection and damage of liver particularly involving the hepatocytes. It is usually due various infective and toxic substances. The condition can be selflimiting, healing on its own, or can progress to scarring of the liver.

A group of viruses 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[58].

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 canicola, Taxoplasma gondii, Borrelia recurrentis, etc..

Toxic Agents

Chlorpromazine and other Phenothiazine derivatives, Monoamine oxidise inhibitors (MAO-inhibitors),Erythromycin,Tetracycline,INH,Rifampicin,Methyldopa, Chlorpropamide, Phenylbu-tazone,Indomethacin, Paracetamol, Thiouracil, Acetaminophen, Halothen, Alcohol, Carbon tetrachloride, etc[59].

Table : 2 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	

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. 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 [60], is probably spread by blood and sexual contact .

There is very little evidence that this virus causes hepatitis, as it does not appear to replicate primarily in the liver[61]. 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.

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[62].

Liver Cancer:

The liver is inclined to cancer induction by a variety of human made and naturally occurring chemicals. Chemical substance 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.

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.

1) 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 tetrachloride, Chloroform, Tetrachloroethane, Iodoform and elemental phosphorus.

2) Indirect: -These are more selective, and are antimetabolic and related compounds that produce hepatic hurt by interference with specific metabolic pathway or process.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[63] (Lynch and Price, 2007). 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 amount 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[64]

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[65].

More than 900 drugs have been concerned in causing liver injury [65] 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 all hospital admissions and 50% of all acute liver failures [66].

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[67] . They play an important role in the inflammation process after intoxication by Ethanol, Carbon tetrachloride.

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[68].

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

3.6.PHARMACOLOGICAL REVIEW

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: CCl_4 dimethylnitrosamine (DMN), galactosamine; bile duct epithehal cells: thioacetamide (TAA))

(2) Immunological-induced damage (heterogonous serum and experimental schistosomiasis).

(3) Biliary damage (common bile duct ligation (BDL) or occlusion)

(4) 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

(5), Fatty liver disease, in particular the 'malignant' inflammatory form non-alcoholic 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 [69]

The genetic lepton-deficient (ob/ob) or lepton- resistant (db/db) mice and the dietary methionine/ choline-deficient models are cast-off in the majority of published research. Progressive fibrosis was reported only in the methionine/choline-deficient models in 100% of the mice.

BDL and CCl_4 are the most widely used rodent models [70] 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[71].

Examples of knockouts with spontaneous formation of liver fibrosis are mdr2-/- mice[72] 1hx2-/- mice, and the mice models for NASH mentioned above[69a].

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 initiates lipid peroxidation [73].The damage to hepatocytes by CCl_4 is replicated by high plasma Alanine transaminase (ALT) and Aspartate transminase (AST) levels after CCl_4 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 (1) acute damage (72 hours after a single injection of CCI_4) with HSC activation (2) early and establish fibrosis (4-6 week of twice weekly CCl4 dosing), (3) early cirrhosis (8 week of twice weekly CCl_4dosing) and (4) advanced micronodular cirrhosis (12 week of twice weekly CCl_4dosing). In addition for each of these models (5) 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 indosages 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 biliarycirrhosis, such as extrhepatic 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[74].

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 close 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 Cytochroom 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[75]. 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 sis 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 my 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 Nycoeenz 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 or 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
- A combined digestion with collagenase / pronase, after which HSC were separated from other liver nonparenchymal cells by centrifugation over density gradients similar to threat procedure.

The first method will yield a combination of various (myo) fibroblastic cells including HSC and myofibroblsts. 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 acting.

Liver Slice System

A second in vitro test system which was recently developed to assess effects of ant fibrotic 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 (CCl4)-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 CCl4 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, CCl4 intoxicated rats treated with TCW retained almost normal levels of these constituents. Decreased activities of antioxidant enzymes in CCl4 intoxicated rats and their reversal of antioxidant enzyme activities in TCW treated rats, shows the effectiveness of TCW in combating CCl4 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 CCl4 intoxicated rats .

3.7.LATERAL RESEARCH

Plumbago zeylanica

The root of *Plumbago zeylanica* and its constituents are cradited with potential therapeutic properties and including Anti-artherogenic, Cardiotonic, Hepatoprotective and Neuro protective properties.

Anti-oxidant effects of the aqueous /alcoholic extracts of root corresponding to medicinal preparations and the active ingredients plumbagin, were studied methods used included : Ferric reducing /Anti – Oxidant power(FRAP) radical scavenging DPPH and 2,2`-azobis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS), lipid peroxidation in rat liver mitochondria induced by different agents and estimating phenolic and flavanoid content[76].

Zingiber officinale

The main pharmacological actions of ginger (*Zingiber officinale*) and compounds isolated there from include immuno-modulatory, Anti-tumerogenic, Anti-inflammatory, Anti-appoptotic, Anti-hyperglycemic, Antilipidemic and Anti- emetic. Ginger is a strong Anti-oxidant substance and may either mitigatic or prevents generation of free radicals.

Santalum album

Over 100 constituents have been identified in *Santalum album* (Sandalwood oil) with major constituent being α-Santalol. Sandal wood oil was mutagenic in spore Rec assay and was found to have Anti-Carcinogenic, Anti-viral and bactericidal activity [78].

Myristica fragrans

The effect of the hydro alcoholic extracts of fruits of *Myristica fragrans* was investigated on chlorpromazine - induced glucose and triglyceride elevation in male swiss albino mice [79].

Syzygium aromaticum

Syzygium aromaticum (cloves) having Anti-septic, Anti-bacterial, Anti-fungal and Anti-viral properties. One of the main constituents of clove oil (Eugenol) exhibits broad Anti-microbial activities against Gram - positive and Gram-negative and acid-fact bacteria as well as fungi. Cloves are well known also for their Anti-emetic and Carminative properties [80].

Elettaria cardamomum

Elettaria cardamomum compares the effect of different extraction solvent used (chloroform, methanol, ethanol, diethyl ether) and Anti-oxidant, Anti- microbial activities of the essential oil oleoresins(*Elettaria cardamomum*) of seeds and pods[81].

Bambusa arundinaceae

Antiarthritic activity of bamboo in treating rheumatoid arthritis using CFA induced arthritis animal model was investigated. The methanolic extract of bamboo significantly decreased the bone erosion, Spleen enlargement, rheumatoid factor at a dose of 100, 200, 300mg/kg compared to the control group [82].

Piper nigrum

The extracts of black pepper were evaluated for antibacterial activity by Disc diffusion method. The results indicate inhibition on the growth of gram positive bacilli like Staphylococcus aureus, Bacillus cereus, Staphylococcus faecalis and gram negative bacilli like Pseudomonas aeruginosa, Salmonella typhi and E.coli[83].

Earlier Studies on Hepatoprotective

Hydro alcoholic extract of tubers of Momordica tuberosa was subjected to preliminary phytochemical screening and assessed for in vitro and in vivo antioxidant and hepatoprotective action against CCl4 induced liver damage in rats.

Pre-treatment with 70% ethanolic extract of M. tuberosa reversed CCl4 induced elevation of levels of serum biomarkers to near normal levels, suggesting that the tubers of M. tuberosa possess hepatoprotective property and this property may be attributed to the antioxidant property of the plant [84].

Hepatoprotective and antioxidant effects of tender coconut water (TCW) were examined in carbon tetrachloride (CCl4)-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 CCl4 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.

On the other hand, CCl4 intoxicated rats treated with TCW retained almost normal levels of these constituents. Decreased activities of antioxidant enzymes in CCl4 intoxicated rats and their reversal of antioxidant enzyme activities in TCW treated rats, shows the effectiveness of TCW in combating CCl4 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 CCl4 intoxicated rats [85].

Laboratory Tests

- ✓ Abdominal ultrasound
- ✓ Autoimmune blood markers
- ✓ Hepatitis virus serologists
- ✓ Liver function tests
- ✓ Liver biopsy to check for liver destruction
- ✓ 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) 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 events 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 [86].

4. MATERIALS AND METHODS

Drug selection

In this dissertation purified and prepared ***"Siringipaerathi Chooranam"*** was taken as a trial drug for Hepatoprotective activity from the Siddha literature **"*Sarabendra Vaidhiya Muraigal". Soolai, Moola, Kusta, Pitharoga Muraigal***, page no: 194-195.

Table: 3 Ingredients

NAME OF DRUGS	BOTANICAL NAME	QUANTITY
Inji	*Zingiber officinalis*	560 gm (16 palam)
Milagu	*Piper nigrum*	50.4 gm (12 varahan)
Thippili	*Piper longum*	33.6gm(8 varahan)
Thipili moolam	*Piper longum*	16.8 gm(4 varahan)
Lavanga pathiri	*Cinnamomum tamala*	35 gm(1 palam)
Elam	*Elettaria cardamomum*	42 gm (10 varahan)
Kodiveli ver	*Plumbago zeylanica*	42 gm (10 varahan)
Lavanga pattai	*Cinnamomum zeylanicum*	35 gm (1 palam)
Moongil uppu	*Bambusa arundinaceae*	35 gm (1 palam)
Sandhana thool	*Santalum album*	35 gm (1 palam)
Vilamichu-ver	*Plectranthus vettiveroides*	35 gm (1 palam)
Sathikkai	*Myristica fragrans*	35 gm (1 palam)
Seeragam	*Cuminum cyminum*	35 gm (1 palam)
Kirambu	*Syzygium aromaticum*	35 gm (1 palam)
Sugar	*Saccharum officinarum*	Equal quantity
Nei	English Name : Ghee	Sufficient 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

All the plant materials were identified and authenticated by the Botanists and Gunapadam experts in Government Siddha Medical College, Arumbakkam, and Chennai-106. The specimen sample of all the herbs have been preserved in PG *Gunapadam* department individually for future reference.

Purification of the drugs

All the drugs mentioned here were purified as per the Siddha literature [87].

Inji - Outer skin of ginger was peeled off.

Milagu - It was soaked in sour buttermilk for 3 hours and allowed to dry

Thippili - Soaked in lemon juice and allowed to dry.

Thippilimoolam - Remove the nodules and dried.

Lavangapathiri - Dried in sun light.

Elam - Roasted in the pan and outer skin was removed.

Kodiveli-ver - The root was cleaned with a white cloth.

Lavangapattai - Dried in sun light.

Sandhana kattai - The skin was peeled off to get purified and powdered

Vilamichu-ver - The root was cleaned with a white cloth.

Sathikkai - Cleaned and cut into small pieces and dried.

Seeragam - Clean the dust particles and allowed it to dry.

Kirambu - Flower buds were removed.

4.1. Preparation of the Drug

Procedure

In order to obtain the purified form of ginger, the upper skin of ginger was peeled off and then sliced into small pieces. The sliced pieces were dried in sunshade for two days. After complete drying 560 grams of dried ginger was taken and fried well in ghee and then powered.

50.4 grams of Purified Pepper, 33.6 grams of *Thippili,* 16.8 grams of *Thipplimoolam*, 42 grams of *Kodiveli-ver*, 35 grams of *Moongil uppu, Lavangapathiri, Sandhana thool, Vilamichu-ver, Lavanga Pattai, Adhikari, Seeragam, Kirambu* were taken and powered separately then mixed together with processed ginger powder.

Finally, the mixture was ground well which favors the homogenous preparation. Then the mixture powder was sieved through the thin clean white cloth. After that twice the weight of sugar was added to the mixture and again it was ground well.

Finally, the end product was obtained, which was kept in an air tight container and labeled as "***Siringipaerathi Chooranam*" (*SPC*).**

Purification of the Chooranam: steaming process (*Pittaviyal murai*)

The *"**Siringipaerathi Chooranam"*** was purified by *pittaviyal* method (steam cooking in milk) as per Siddha classical literature. A mud pot was taken and it was half filled by milk and mixed with equal quantity of pure water. The mouth of the pot was sealed by a cloth. This chooranam was placed over a clean dry cloth and tied firmly around the mouth of mud pot. The gap between mud pots was tied with a wet cloth to avoid evopration.The mud pot was kept on fire and boiled until the cow's milk reduced in the lower pot.

The same drug was later dried and powdered then sieved again. It was used for the further study [88].

Storage of the drug

The prepared test drug was stored in a clean, air tight glass container.

Administration of the drug

Form of the medicine	: *Chooranam*
Route of Administration	: Enteral
Dose	: 2 gm twice a day depending on the severity
Adjuvant	: honey

Indication:

Kamaalai, *Marbuvali, Kirani, Suram, Vaanthi, Peenisam.*

Fig: 1 Ingredients of *Siringipaerathi Chooranam*

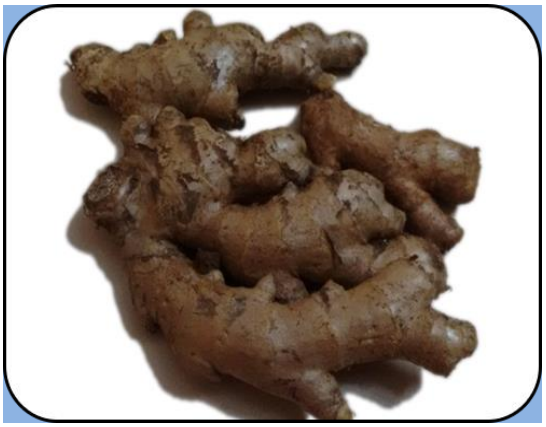


Fig: 1.1 *Zingiber officinalis*

Fig: 1.2 *Zingiber officinalis (cut into pieces)*

Fig: 1.3 *Fried Zingiber officinalis*

Fig: 1.4 Piper *nigrum*

Fig: 1.5 ***Piper longum***

Fig: 1.6 ***Piper longum***

Fig: 1.7 ***Cinnamomum tamala***

Fig: 1.8 ***Elettaria cardamomum***

Fig: 1.9 ***Plumbago zeylanica***

Fig: 1.10 ***Cinnamomum zeylanicum***

Fig: 1.11 ***Bambusa arundinaceae***

Fig: 1.12 ***Santalum album***

Fig: 1.13 ***Plectranthus vettiveroides***

Fig: 1.14 ***Myristica fragrans***

Fig: 1.15 ***Cuminum cyminum***

Fig: 1.16 ***Syzygium aromaticum***

Fig: 1.17 Ghee

Final Product (*Siringipaerathi Chooranam*)

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 analysis 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. Thus standardization brings the efficacy and potency of the drug.

4.2.1. Organoleptic character

The organoleptic characters of the sample drug were evaluated. 1gm of the test drug was taken and the color, texture, particle size and other morphology were viewed by naked eye under sunlight. Then the result was noted.

4.2.2 PHYSICOCHEMICAL ANALYSIS

Physicochemical studies of the trial drug have been done [89].

Determination of Ash Values

Total Ash

3g of the test drug was accurately weighed and incinerated in a crucible dish at a temperature not exceeding 450^{o}C until it was free from carbon. It was then cooled and weighed. The % w/w of ash with reference to the air-dried powder was calculated.

Water Soluble Ash

The total ash was obtained as the above method for preparation of total ash. The ash was boiled with 25ml of water for 5mins. The insoluble ashes were collected using filter paper. It was then washed with hot water and transferred to the silica crucible. It was then ignited for 15minutes at temperature not exceeding 450^{o}C. For determination of weight of the water soluble ash the silica crucible and residue were weighed until constant weight was attained. The weight of the water soluble ash was determined by subtracting the weight of insoluble ash from the weight of total ash.

Acid insoluble Ash

The total ash was obtained as the above method for preparation of total ash. The ash was boiled for 5minutes with 25ml 10% Hcl. The insoluble ashes were collected using filter paper and washed with hot water. It was then transferred to the silica crucible and ignited for 15minutes at temperature not exceeding 450^{o}C. The silica crucible and residue were weighed until constant weight was attained.

Determination of Extractive Value

Alcohol Soluble Extractive Value

3g of test drug powder was weighed and macerated with 100ml of ethanol in a closed container for 24 hours. The resulting solution was shaken continuously for 6 hours. It was then allowed to stand and soak for 18 hours.

The solution was filtered and evaporated of the filtrate in a flat bottomed shallow dish and dried at 105^{o}C. Then the content was cooled and weighed.

Water soluble Extractive value

3g of test drug powder was weighed and macerated with chloroform and water, respectively, at 80^{o}C for 24 hrs. The resulting solution was shaken continuously for 6 hours and allowed to stand and soak for 24hrs then filtered. The solution from both chloroform and water respectively was filtered and evaporated of the filtrate in a flat bottomed shallow dish. It was dried at 105^{o}C then cooled and weighed.

Loss on Drying

The powdered drug was taken and dried in the oven at 100- 105°C to constant weight. The result was noted.

Physical characterization

Solubility: A little of the sample was shaken well with distilled water. **.** A little of the sample was shaken well with con Hcl and Con $H2SO_4$. Sparingly soluble character indicates the presence of Silicate.

pH value: Potentiometrically pH value was determined by a glass electrode and a suitable pH meter.

Action on heat: A small amount of the sample was taken in a dry test tube and heated gently. If there was a strong white fumes evolving it indicates the presence of Carbonate.

Flame test: A small amount of the sample was made into a paste with con.Hcl in a watch glass. It was then introduced into non-luminous part of the Bunsen flame. Appearance of bluish green flame indicates the presence of Copper.

Ash Test: A filter paper was soaked into a mixture of sample and cobalt nitrate solution. It was then introduced into the Bunsen flame and ignited. Appearance of yellow colour flame indicates the presence of Sodium.

4.2.3. PHYTOCHEMICAL ANALYSIS

The Phytochemical screening of the extract gives general idea regarding the nature of chemical constituents present in the crude drug. The phytochemical tests were done as the method illustrated [90].

Test for Alkaloids

A small portion of solvent free extracts were stirred separately with few drops of dilute hydrochloric acid and filtered & tested carefully with various alkaloidal reagents.

Mayer's reagent - Cream precipitate

Dragendroff's reagent - Orange brown precipitate

Hager's reagent - Yellow precipitate

Wagner's reagent - Reddish brown precipitate

Test for Carbohydrates and Reducing Sugars

The minimum amount of extracts were dissolved in 5ml of distilled water & filtered. The filtrate was subjected to test for carbohydrates & glycosides.

a) Molisch's test

The filtrate 1 ml was treated with 2-3 drops of 1% alcoholic alpha naphthol & 2ml concentrated sulphuric acid was added along the sides of test tube. Violet ring was observed at the junction of 2 layers which showed the presence of carbohydrate.

b) Benedict's test

The filtrate 1 ml was treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

c) Fehling's test

The filtrate 1 ml was treated with equal volume of Fehling's solution A and B and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Test for Glycosides

The extract was hydrolyzed with dil. HCl and subjected to test for glycosides.

a) Modified Borntrager's test

To the hydrolysate extract, 1 ml of Ferric chloride solution was added and immersed in boiling water for about 5 min. The mixture was cooled and extracted with equal volume of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose pink colour in the ammoniacal layer indicates the presence of Anthranol glycosides.

b) Legal's test

The hydrolysate extract was treated with Sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of Cardiac glycosides.

Test for Saponins

The extract 0.5 ml was shaken with 5 ml distilled water. The presence of saponins was indicated by formation of copious lather.

Test for Tannins

Gelatin test

To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

Test for Phenolic compounds

To 0.5 ml of extract, 1 ml of alcoholic ferric chloride solution was added. Formation of bluish green or bluish black indicates the presence of Phenolic compounds.

Test for Phytosterols

Ferric chloride – acetic acid test

1 ml of extract was treated with 1 ml of chloroform and then, 2 ml of ferric chloride acetic acid reagent was added followed by 1 ml of conc. sulphuric acid. Appearance of reddish pink colour shows the presence of Phytosterols.

Test for Diterpenes

Copper acetate test

1 ml of extract was dissolved in water and treated with 3-4 drops of Copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

Test for Triterpenes

Salkowski's test

1 ml of extract was treated with 1 ml of chloroform followed by 1 ml of conc. sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour shows the presence of Triterpenes.

Test for Flavonoids

a) Alkaline reagent test

To 1 ml of extract, 1 ml of 10% sodium hydroxide solution was added. Formation of dark yellow colour indicates the presence of flavonoids.

b) Lead acetate test

To 1 ml of extract, 3-4 drops of 10% lead acetate solution was added. Formation of yellow precipitate indicates the presence of flavonoids.

c) Ferric chloride test

To 1 ml of extract, 3-4 drops of ferric chloride solution was added. Formation of dark green colour indicates the presence of flavonoids.

d) Shinoda test

To 1 ml of extract, few mg of magnesium turnings was added followed by few drops of conc. hydrochloric acid and boiled for five minutes in a boiling water bath. Formation of red colour indicates the presence of flavonoids.

Test for Proteins and Free Amino Acids

a) Xanthoproteic test

To 1 ml of extract, 3-4 drops of conc. nitric acid was added. Formation of yellow precipitate indicates the presence of proteins.

b) Million's test

To 0.5 ml of extract, 2.5 ml of Million's reagent was added. Formation of white precipitate and the precipitate warmed indicates the presence of proteins.

c) Biuret test

To 0.5 ml of extract, 2.5 ml of diluted Biuret reagent was added. Appearance of purple colour or brick red precipitate showed the presence of proteins and free amino acids.

Test for Quinones

Sodium hydroxide test

To 0.5 ml of extract, 1 ml of 10% sodium hydroxide was added. Appearance of blue or green or red colour shows the presence of quinones.

TLC/ HPTLC finger print studies

HPTLC finger printing was carried out as per the reference. [91]

Preparation of spray reagent-vanillin-sulphuric acid reagent

Vanillin (1g) was dissolved in ice cold ethanol (95ml). Add to 5ml of cooled concentrated sulphuric acid. Ice was added and stirred well. The solution was stored in refrigerator.

Chromatographic conditions

Instrument : CAMAG (Switzerland).

Sample Applicator : Camag Linomat - IV applicator with N_2 gas flow.

Photo documentation System : Digi store - 2 documentation system with Win Cat & video scan software.

Scanner : Camag HPTLC scanner - 3 (030618), Win Cats - IV.

Development Chamber : Camag HPTLC 10X10, 10 X 20 twin trough linear development chamber.

Quantity applied : 5, 10 µl for extracts and 5 µl for standards

Stationary phase	: Aluminium plate pre-coated with silica gel 60 (E. Merck)
Plate thickness	: 0.2 mm.
Mobile Phase	: For Chloroform extract - Toluene: Ethyl acetate (9:1) and ethanol extract - Toluene: Ethyl acetate (1:1).
Scanning wavelength	: 254 nm
Laboratory condition	: 26 ± 5^{o}C and 53 % relative humidity

The plate was developed up to a height of 8 cm, air dried, spots were observed under the UV light at 254 and 366 nm. Finally the plates were derivatized using vanillin-sulphuric acid reagent heated at 105° till colour spots appeared.

4.2.4. BIO-CHEMICAL ANALYSIS

Preliminary Basic and Acidic radical studies

Preparation of extract

10g of sample was taken in a 250 ml of clean beaker and 50 ml of distilled water was added to it. Then it was boiled well for about 10 mins. Then it was allowed to cool and filtered in a 100 ml volumetric flask and made up to 100 ml with distilled water. This preparation was used for the qualitative analysis of acidic/ basic radicals and biochemical constituents in it.

Test for Basic radicals

1. Test for Potassium

To a pinch of the *SPC* 2 ml of sodium nitrate and 2 ml of cobalt nitrate solution in 30% glacial acetic acid was added and observed for the presence of yellow precipitate.

2. Test for Calcium

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

3. Test for Magnesium:

To 2ml of *SPC* extract, drops of sodium hydroxide solution was added and watched for the appearance of white precipitate.

4. Test for Ammonium:

To 2ml of *SPC* extract few ml of Nessler's reagent and excess of sodium hydroxide solution are added for the appearance of brown colour.

5. Test for Sodium

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

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

The *SPC* extract was treated with Conc. HNO_3 and ammonium thiocyanate and waited for the appearance of blood red colour**.**

7. Test for Zinc

To 2 ml of the *SPC* extract drops of sodium hydroxide solution was added and observed for white precipitate formation.

8. Test for Aluminium

To the 2m1 of the *SPC* extract sodium hydroxide was added in drops and changes are noted for white precipitate formation.

9. Test for Lead

To 2 ml of *SPC* extract 2ml of potassium iodide solution was added and noted for yellow colored precipitate**.**

10. Test for Copper

a. A pinch of *SPC* was made into a paste with con. Hcl in a watch glass and introduced into the non-luminous part of the flame and noted for blue color appearance.

b. To 2 ml of *SPC* extract excess of ammonia solution was added and observed for the appearance of blue coloured precipitate**.**

11. Test for Mercury

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

12. Test for Arsenic

To 2 ml of the *SPC* extract 2ml of sodium hydroxide solution was added and brown or red precipitate formation was noted.

Test for acid radicals

1. Test for Sulphate

To 2 ml of the *SPC* extract 5% of barium chloride solution was added and observed for the appearance of white precipitate.

2. Test for Chloride

The *SPC* extract was treated with silver nitrate solution and observed for the appearance of white precipitate.

3. **Test for Phosphate**

The *SPC* extract was treated with ammonium molybdate and conc. HNO_3 and observed for the appearance of yellow precipitate.

4. **Test for Carbonate**

The *SPC* extract was treated with conc.Hcl and observed forth appearance of effervescence.

5. Test for Fluoride & Oxalate:

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

6. Test for Nitrate:

To 1 gm of the *SPC,* copper turnings was added and again conc.H_2SO_4 was added, heated and the test tube was tilted vertically down and observed for yellowish red color.

4.2.5. AVAILABILITY OF BACTERIAL LOAD:

Enumeration of bacteria by plate count – Agar plating technique

The plate count technique was one of the most routinely used procedures because of the enumeration of viable cells by this method. [92]

Principle:

This method is based on the principle that when material containing bacteria are cultured, every viable bacterium develops into a visible colony on a nutrient agar medium. The number of colonies therefore is the same as the number of organisms contained in the sample.

Dilution:

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

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

Requirements:

- Sample or Bacterial suspension
- 9 ml dilution blanks (7)
- Sterile petri dishes (12)
- Sterile 1 ml pipettes(7)
- Nutrient agar medium (200 ml)
- Colony counter

Procedure:

1. Label the dilution blanks as 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7}.
2. Prepare the initial dilution by adding 1 ml of the sample into a 9 ml dilution blank labeled 10^{-1} thus diluting the original sample 10 times.

3. Mix the contents by rolling the tube back and forth between hands to obtain uniform distribution of organisms.
4. From the first dilution transfer 1 ml of the suspension while in motion, to the dilution blank 10^{-2} with a sterile and fresh 1 ml pipette diluting the original specimen to 100 times.
5. From the 10^{-2} suspension, transfer 1 ml of suspension to 10^{-3} dilution blank with a fresh sterile pipette, thus diluting the original sample to 1000 times.
6. Repeat this procedure till the original sample has been diluted 10,000,000 times using every time a fresh sterile pipette.
7. From the appropriate dilutions transfer 1ml of suspension while in motion, with the respective pipettes, to sterile petri dishes. Three petri dishes are used for each dilution.
8. Add approximately 15 ml of the nutrient medium, melted and cooled to 45^0c, to each petri dish containing the diluted sample. Mix the contents of each dish by rotating gently to distribute the cells throughout the medium.
9. Allow the plates to solidity.
10. Incubate these plates in an inverted position for 24-48 hours at 37^0c.

Observation:

Observe all the plates for the appearance of bacterial colonies. Count the number of colonies in the plates.

Calculate the number of bacteria per ml of the original suspension as follows:

$$\text{Organisms per millimeter} = \frac{\text{Number of colonies (average of 3 replates)}}{\text{Amount of plated} \times \text{dilution}}$$

4.2.6. SOPHISTICATED INSTRUMENTAL ANALYSIS

FT-IR (Fourier Transform Infra-Red)

Fig: 2 FTIR (Fourier Transform Infrared Spectroscopy)

Fig: 2.1 FTIR-INSTRUMENT

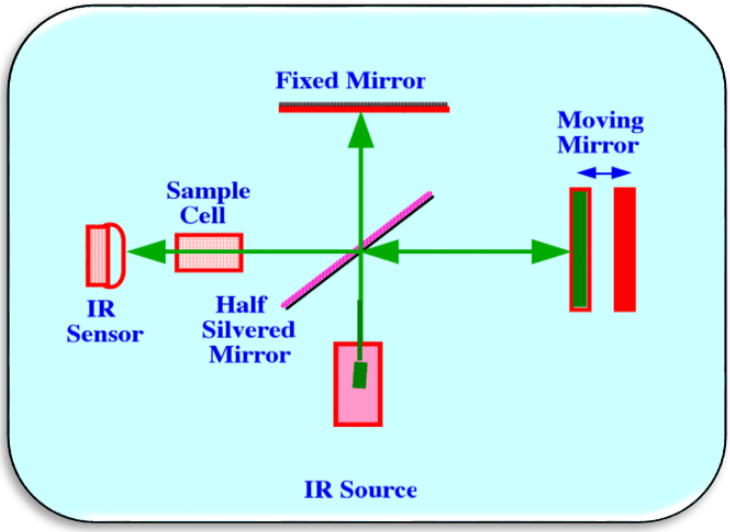


Fig: 2.2 FTIR-MECHANISMS

Model	**: Spectrum one: FT-IR Spectrometer**
Scan Range	**: MIR 450-4000 cm-1**
Resolution	**: 1.0 cm-1**
Sample required	**: 50 mg, solid or liquid.**

It is the preferred method of infrared spectroscopy. FT-IR is an important and more advanced technique. It is used to identify the functional group, to determine the quality and consistency of the sample material and can determine the amount of compounds present in the sample. It is an excellent tool for quantitative analysis. [93]

In FT-IR infrared is passed from a source through a sample. This infrared is absorbed by the sample according to the chemical properties and some are transmitted. The spectrum that appears denotes the molecular absorption and transmission. It forms the molecular fingerprint of the sample. Like the finger print there is no two unique molecular structures producing the same infrared spectrum. It is recorded as the wavelength and the peaks seen in the spectrum indicates the amount of material present.

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

- Speed
- Sensitivity
- Mechanical Simplicity
- Internally Calibrated

Fig: 3 SEM (SCANNING ELECTRON MICROSCOPE)

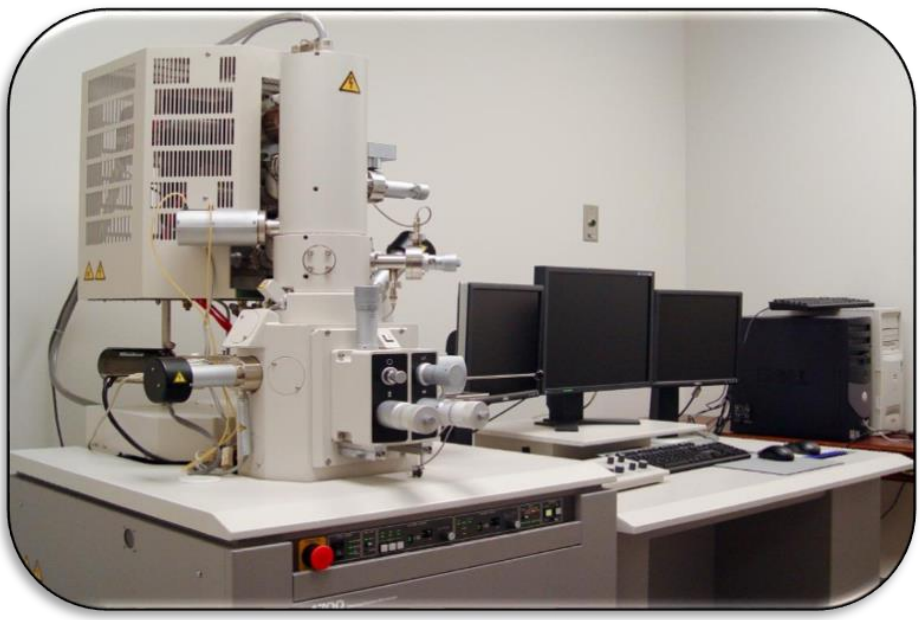


Fig: 3.1 SEM INSTRUMENT

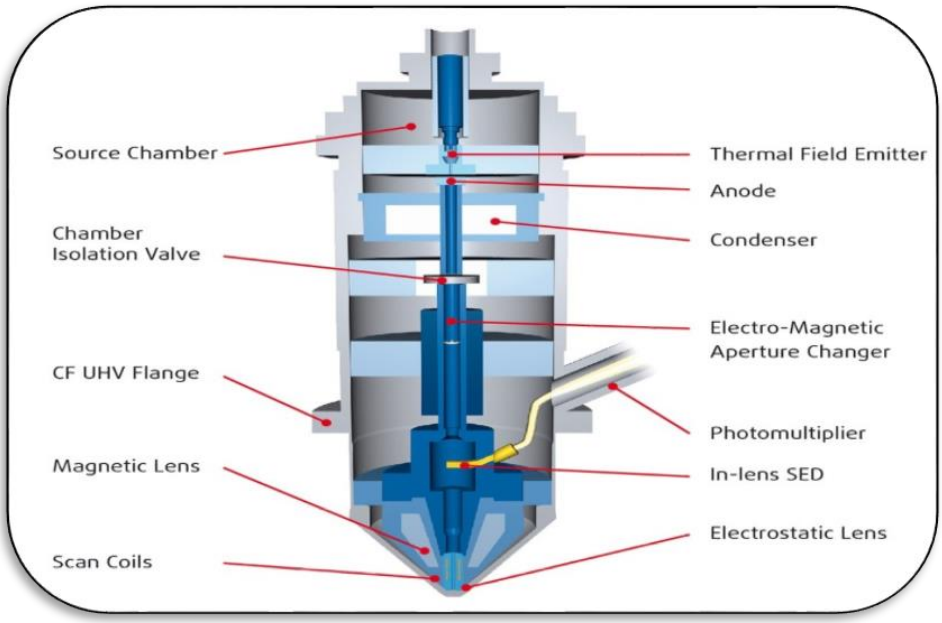


Fig: 3.2 SEM MECHANISM

In scanning electron microscope high-energy electron beam was focused through a probe towards the sample material. Variety of signals was produced on interaction with the surface of the sample. This results in the emission of electrons or photons and it is 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 [94].

Fig: 4 ICP-OES (INDUCTIVELY COUPLED PLASMA OPTIC EMISSION SPECTROMETRY)

Fig: 4.1 ICP-OES ANALYSER (Perkin Elmer Optima 5300 DV)

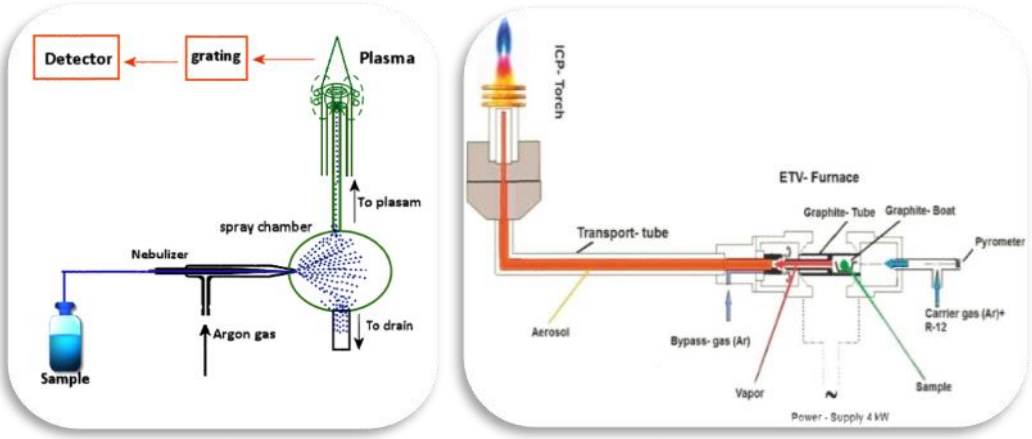


Fig: 4.2 Mechanism of ICP-OES analyzer

Manufacturer: Perkin Elmer

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

Principle:

An aqueous sample is converted to aerosols via a nebulizer. The aerosols are transported to the inductively coupled plasma which is 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 is 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 is relevant (such as the concentration of ferrous iron or ferric iron), only total essential concentration is analyzed by ICP-OES.

Application:

The analysis of major and minor elements in solution samples.

Objectives:

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

Mechanism:

In plasma emission spectroscopy (OES), a sample solution is 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 is 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 is 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.

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 [95].

Sample preparation:

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

100 mg *"Siringipaerathi Chooranam"* was occupied in a clean, dry test tube. To this, 3 ml Nitric acid was added and mixed well and allowedfor few minutes untill the reactions were completed. And then, 25 ml of Refined water, was added to prepare digested solution.

The digested sample solution was shifted into plastic containers and labeled properly. It was completed in Bio-chemistry lab, Govt. Siddha Medical College, Chennai-106.

4.3. TOXICOLOGICAL STUDIES

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%.

4.3.1. ACUTE ORAL TOXICITY – OECD GUIDELINES - 423

Acute toxicity study was carried out as per OECD guideline. (Organization for Economic Co - operation and Development, Guideline-423[96].

The experimental protocol was approved by the institutional ethical committee (IAEC) under CPCSEA (approval no: IAEC/XLIV/31/CLBMCP/2014.

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

Studied carried out at three female rats under fasting condition, signs of toxicity was 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 was rat, although other rodent species may be used.

Healthy young adult animals of commonly used laboratory strain Swiss albino rat were obtained from Animal house of king's institute, Guindy, Chennai. Female 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. The studies were conducted in the animal house of C.L.Baid Metha College of pharmacy, Duraipakkam, Chennai.

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.

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

"Siringipaerathi Chooranam" 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 gavages 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 h prior to the administration of the test suspension.

Finally, the number of survivors was noted after 24 h 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 hrs

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 hrs.
- Special attention: First 1-4 hrs after administration of drug, and
- 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 and reversibility, 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 ***"Siringipaerathi Chooranam"*** *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 [97].

4.3.2. REPEATED DOSE 28 DAYS ORAL TOXICITY STUDY OF *"SIRINGIPAERATHI CHOORANAM"* ON RATS – (OECD-407 guidelines) [98].

Justification for Dose Selection

The results of acute toxicity studies in Wistar albino rats indicated that *"Siringipaerathi Chooranam"* 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, 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.

Preparation and administration of dose

"Siringipaerathi Chooranam" at three doses respectively was suspended in 2 ml of 2% CMC in distilled water. It was administered to animals at the dose levels of 100, 200 and 400 mg/kg. The test substance suspensions were freshly prepared every day for 28 days. The control animals were administered vehicle only. Administration was by oral (gavages), once daily for 28 consecutive days.

METHODOLOGY

Randomization, Numbering and Grouping of Animals

Ten rats (Five Male and Five Female) were in each group randomly divided into four groups for dosing up to 28 days. Animals were allowed acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. Each animal was fur marked with picric acid. The females were nulliparous and non-pregnant.

OBSERVATIONS

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

Body Weight*:* Weight of each rat was recorded on day 0, at weekly intervals throughout the course of study and at termination to calculate relative organ weights. From the data, group mean body weights and percent body weight gain were calculated.

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

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

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

Laboratory Investigations: Following laboratory investigations were carried out on day 29 in animal's fasted over-night. Blood samples were collected from orbital sinus using sodium heparin (200IU/ml) for Blood chemistry and potassium EDTA (1.5 mg/ml) for Haematology as anticoagulant. Blood samples were centrifuged at 3000 r.p.m. for 10 minutes. On 28th day of the experiment, 24h 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 h, and 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 hemoglobin content, total red blood corpuscles (RBC), white blood corpuscles (WBC) count and packed cell volume (PCV).

Biochemical Investigations: Serum was used for the estimation of biochemical parameters. Samples of control and experimental rats were analyzed for protein, bilirubin, urea, BUN, Creatinine, triglyceride, cholesterol and glucose levels was carried using standard methods. Activities of glutamate oxaloacetate transaminase/ Aspartate aminotransferase (GOT/AST), glutamate pyruvate transaminase/ Alanine amino transferase (GPT/ALT) and alkaline phosphatase were estimated as per the colorimetric procedure.

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 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: Histopathological investigation of the vital organs was done. The organ pieces (3-5µm thick) of the highest dose level of 400 mg/kg were preserved and were fixed in 10% formalin for 24 h and washed in running water for 24 h.

Samples were dehydrated in an auto technique and then cleared in benzene to remove absolute alcohol.

Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the "L" moulds. It was followed by microtome and the slides were stained with Haematoxylin-eosin. The organs included heart, kidneys, liver, ovary, pancreas, brain, spleen and stomach, of the animals were preserved they were subjected to histopathological examination [99].

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's multicomparision test using a computer software programme GRAPH PAD INSTAT-3 version.

4.4. PHARMACOLOGICAL STUDIES

4.4.1. HEPATO PROTECTIVE ACTIVITY OF "*SIRINGIPAERATHI CHOORANAM" (SPC)* IN CCL4 INDUCED HEPATOTOXICITY RATS

Experimental design:

Animals were divided into six 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 CCl4+ LP (for 14 days) at the dose 1 ml CCl4/kg body weight, in a suspension of double the volume of LP (which served as vehicle) subcutaneously at lower abdomen on every 14 days of the treatment .

Group III and IV animals received subcutaneous administration of CCl4+ 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 CCl4 suspension, Silymarin (100 mg/kg body weight) daily. Silymarin was used as a standard reference drug.

The animals were kept starved overnight on 14^{th} 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, kidney and serum were used for various biochemical estimations [100].

Biochemical parameters studied

The activities of serum glutamate pyruvate transaminase, and serum glutamate oxaloacetate transaminase were estimated using standard methods. Estimation of serum ALP, serum bilirubin and electrolytes were also carried out to assess the acute hepatic damage caused by CCL4.

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.

4.4.2. EXPERIMENTAL DESIGN FOR HEPATOPROTECTIVE ACTIVITY OF *"SIRINGIPAERATHI CHOORANAM"* AGAINST PARACETAMOL INDUCED HEPATOTOXICITY IN RATS MODEL

Experimental design:

Paracetamol induced hepatotoxicity in rats model was used for evaluation of hepato-protective activity for the *Siringipaerathi Chooranam*. Animals were divided into five groups, each group containing five animals.

Group I (normal) received distilled water or 2% CMC for 14 days. Group II (Control) received paracetamol 1ml/kg, i. p. 1:1 dilution with coconut oil on 5th day. Group III received standard marketed drug Silymarin (25mg/kg per day, p.o.) for 14 days and paracetamol induction on 5^{th} day. Groups IV- V, received *Siringipaerathi Chooranam* (5mg/kg and 10mg/kg p.o) for 14 days and paracetamol induction on 5th day.

After 14 days of experimental period blood sample had been collected individually for all the animals by retro-orbital puncture method and the blood was allowed to clot for 30 min; serum was separated by centrifuging and was used for various parameter estimations.

Later all the animals were sacrificed by cervical dislocation, liver samples were collected and the individual weights of the livers were estimated. For histopathological study, liver tissue was quickly removed after autopsy and fixed in 10% formalin in saline.

Biochemical parameters studied

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

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. [101]

4.4.3. ANTIOXIDANT ACTIVITY OF *"SIRINGIPAERATHI CHOORANAM"*

DPPH ASSAY (2, 2-diphenyl -1-picrylhydrazyl)

The radical scavenging activity of different extracts was determined by using DPPH assay [102]. 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 red 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 ethanol.

Procedure

Different volumes of plant extracts were made up to 40µl with DMSO and 2.96ml DPPH (0.1mM) solution was added. 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

$$\% \text{ inhibition} = \frac{\text{control-test}}{\text{Control}} \times 100$$

5. RESULTS AND DISCUSSION

Many studies have been carried out to bring the efficacy and potency of the drug *Siringipaerathi Chooranam*. The study includes literary collections, organoleptic character, physicochemical and phytochemical analysis, instrumental analysis, toxicological study and pharmacological study.

The study ***Siringipaerathi Chooranam*** has been selected for Hepatoprotective activity in reference with the text "***SARABENDRA VAIDHIYA MURAIGAL***".

- Literary collections about the drug from various text books were done. Siddha literatures related to the drug bring the evidence and importance of its utility in treating the 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 Jaundice.
- Pharmaceutical review describes about the Chooranam and its properties.
- The pharmacological review explains about the methodology of Hepatoprotective Activity and the drugs used.
- Modern and Siddha aspect of the disease was also reviewed.

STANDARDIZATION OF THE TEST DRUG

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

Toxicological results of the drug and pharmacological activity of the drug are derived. Its result has been tabulated and interpretation is made below. Thus, it is to give a complete justification, to bring the effectiveness of the trial drug *Siringipaerathi Chooranam.*

The extensive review on botanical aspect gave information about the microscopical, macroscopical, 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:

- *Milagu* is indicated for diseases of Liver, Spleen.
- *Thippili* has an action of tonic to Liver.
- *Moongil uppu* is used in the treatment of biliousness.
- *Seeragam* is indicated for curing Jaundice and also used for strengthening of body.
- *Inji* is a rejuvenating herb used for biliousness
- *Kodiveli ver* is indicated for the treatment of Liver diseases. Tonic in action.
- *Lavanga pattai* is indicated for biliousness.

Discussion on pharmacological aspect

The pharmacological aspect of the drug says about their mode of action and side effects which were used worldwide since ancient times. The current pharmacological methods available for carrying out the Hepatoprotective activity studies were explained clearly and the suitable In-vivo model and Anti-oxidant in In-vitro models carrying out the activities were discussed. Result from the pharmacological study denotes the effects of *Siringipaerathi Chooranam (SPC)* showed promising effects in treating liver damage. Moreover, the increased levels of the serum enzymes were significantly decreased by the treatment with *Siringipaerathi Chooranam* implying that the drug prohibited the liver damage.

Discussion on Pharmaceutical review

This review explained the preparation of *Chooranam* in detail including the purification of raw drugs, methods of manufacturing *Chooranam* and the Siddha parameters for the standardization of analyzing *Chooranam.* 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. *Chooranam* are fine dry powders of drugs. The term *Chooranam* may be applied to the powders of single drug or a mixture of two or more drugs.

On purification (pittaviyal), the weight of the *Chooranam* is differed from the exact value but not from the mean value when calculated.

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

- *Chooranam* tends to be amorphous.
- It should be never damp.
- The fineness of the sieve should be100 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 CHARACTER

The following characters have been noted in *Siringipaerathi Chooranam*.

Table: 4. Organoleptic characters

Colour	Brown
Odour	Pleasant
Taste	Bitter
Texture	Fine powder
Particle size	Completely pass through sieve no 92

Table: 5. Physicochemical Analyses

S.No	**Parameter**	**Result**
1.	pH	6.4
2.	Ash (%)	13.23
3.	Acid Insoluble ash (%)	0.79
4.	Water soluble ash (%)	5.79
5.	Loss on drying (%)	9.26
6.	Solubility	Positive
7.	Action on heat	Negative
8.	Flame test	Negative
9.	Ash test	Negative

Interpretation

The physicochemical analysis of the drug result reveals the pH, Moisture, Solubility, Water soluble ash, Ash and Acid insoluble ash.

- **pH:**

  pH is a measure of hydrogen ion concentration; it is the measure of the acidic or alkaline nature. 7.0 is a neutral, above 7.0 is an alkaline and below are acidic. The pH of the drug *Siringipaerathi Chooranam* is 6.4 which are weak acidic in nature. Acidic drug is essential for its bioavailability and effectiveness. Acidic drugs are better absorbed in stomach. [103]

- **Ash**:

  Ash constitutes the inorganic residues obtained after complete combustion of a drug. Thus Ash value is a validity parameter describe and to assess the degree of purity of a given drug. Total ash value will determine the amount of minerals and earthy materials present in the drug. The total ash value of *SPC* is 13.23 % which determines the absence of inorganic content.

- **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 is 0.79 for *SPC*.

- **Water soluble ash:**

  Water-soluble ash is the part of the total ash content, which is soluble in water. It is 5.79 for *SPC.*

- **Moisture (Loss on drying)**:
  - The moisture present in the drug was established in loss on drying.
  - The 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 *SPC* is 9.26% [104].

PHYTOCHEMICAL ANALYSIS

Table: 6. Phytochemicals screening test

Phytochemicals	Test	Result
1. Alkaloids	Mayer's test	Present
2. Carbohydrates	Molisch's test	Present
3. Glycosides	Modified Borntrager's test	Absent
4. Saponins	Froth test	Absent
5. Phenols	Alcoholic Ferric chloride test	Present
6. Phytosterols	Ferric chloride acetic acid test	Absent
7. Triterpenes	Salkowski's test	Present
8. Flavanoids	Alkaline reagent test	Present
9. Proteins and amino acids	Xanthoproteic test	Present
10. Quinones	Sodium hydroxide test	Absent

Interpretation:

Phytochemicals are natural bioactive compounds, found in plants and fibres, which act as a defense system against diseases and more accurately protect against diseases. The phytochemical analysis reveals that the presence of Alkaloids, glycosides, phenol, Triterpenes, Flavanoids and Quinones [105].

Alkaloids

- Alkaloids possess antispasmodic, analgesic, bactericidal effects.
- Alkaloids are the active principles producing many essential effects in protecting the body [106].

Carbohydrates

- Carbohydrates play important role in storage of glucose
- Carbohydrates play important role in homeostasis of glucose and fatty acids in liver [107].

Phenols

- They possess rich Anti-Oxidant property and protect body from oxidative stress.
- Phenol groups are the essential part of many anti-oxidant compounds.
- It is an Anaesthetic or pain reliever [108].

Phytosterols

- Phytosterols are plant sterols, Phytosterols have anti-inflammatory effect, Phytosterols reduce oxidative stress
- Various bioactivities of Phenolic compounds are responsible for their chemo preventive properties
- Phytosterols have an anti-oxidant property [109].

Triterpenes

- Suppress the inflammatory response.
- The Triterpenes are the best immunomodulator and have anti-oxidant property.
- Anti microbial activity.
- Anti bacterial agent [110].

Flavonoids

- It is the most important group of polyphenol compounds in plants.
- Flavonoids are a group of plants metabolites which provide health benefits through cell signaling pathways and antioxidant effects.
- 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 [111].

Protein and amino acids

- Proteins and amino acids helps in liver regeneration and energy production
- Boosts glutathione production to protect the liver

- Increases satiety to promote weight loss and reduce fat accumulation in 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 [112].

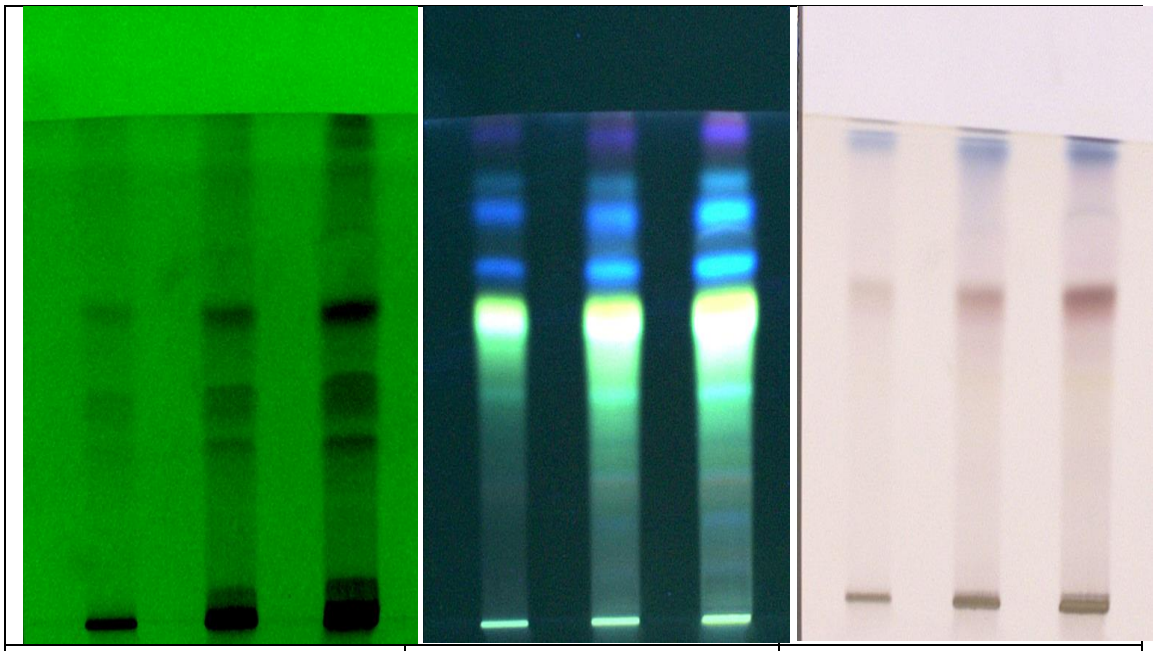
A synergistic effect of all these flavonoids, alkaloids, carbohydrates, phenols, Phytosterols**,** Triterpenes increases the potency of the drug against hepatic damage.

TLC/HPTLC analysis of chloroform extract

HPTLC analysis

Chloroform extract was applied in TLC aluminum sheet silica gel 60(E. MERCK) and plate was developed using the solvent system Toluene: Ethyl acetate (9:1). After development, the plate is allowed to dry in air and examined under UV - 254nm, 366 nm and Visible light (Vanillin - Sulphuric acid).

Fig 5.1 HPTLC Chloroform extracts Photos



λ 254 nm	λ 366 nm	White light

Table: 7. Rf Values for the chloroform extract

Color	R_f value(s)	Color	R_f value(s)	Color	R_f value(s)
Green	0.08	Green	0.12	Red	0.60
Green	0.35	Pale green	0.46	Violet blue	0.90
Green	0.47	Fluorescent yellow	0.59	Magenta blue	0.95
Green	0.62	Blue	0.70	Magenta blue	0.98
		Blue	0.82		
		Magenta blue	0.88		
		Violet	0.98		

HPTLC finger print analysis for chloroform extract

The finger print chromatogram was recorded at 366 nm. It showed 8 peaks of which peaks at Rf. and were the major peaks and others were moderately smaller peaks.

λ= 366 nm

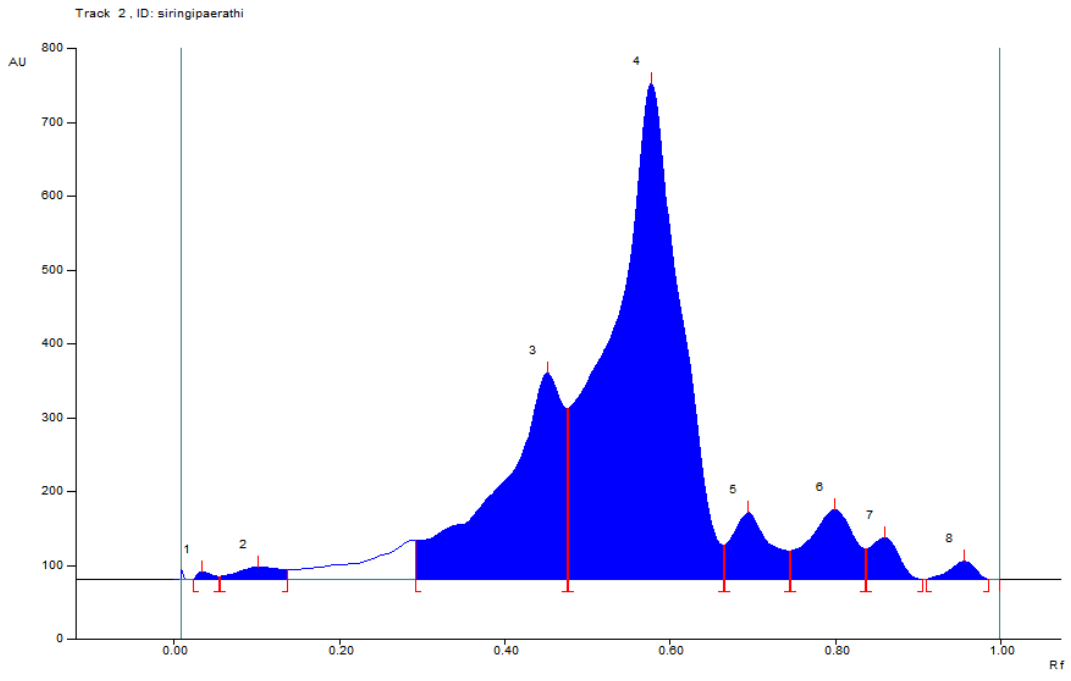


Fig 5.2 HPTLC finger print for chloroform extract

Table: 8.Chloroform extracts - Rf values in HPTLC finger print

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	0.4 AU	0.03 Rf	10.4 AU	0.84 %	0.06 Rf	3.9 AU	143.0 AU	0.20 %
2	0.06 Rf	4.0 AU	0.10 Rf	16.8 AU	1.35 %	0.14 Rf	13.4 AU	677.5 AU	0.96 %
3	0.29 Rf	52.4 AU	0.45 Rf	279.5 AU	22.47 %	0.48 Rf	31.7 AU	16844.8 AU	23.90 %
4	0.48 Rf	232.2 AU	0.58 Rf	671.0 AU	53.95 %	0.67 Rf	46.7 AU	43492.0 AU	61.71 %
5	0.67 Rf	47.1 AU	0.70 Rf	90.2 AU	7.25 %	0.75 Rf	38.2 AU	3275.3 AU	4.65 %
6	0.75 Rf	38.5 AU	0.80 Rf	94.7 AU	7.61 %	0.84 Rf	41.4 AU	3963.5 AU	5.62 %
7	0.84 Rf	41.5 AU	0.86 Rf	56.8 AU	4.57 %	0.91 Rf	0.0 AU	1507.4 AU	2.14 %
8	0.91 Rf	0.1 AU	0.96 Rf	24.4 AU	1.96 %	0.99 Rf	0.0 AU	573.5 AU	0.81 %

Interpretation:

- The quantitative analysis of compounds present in the *SPC* has been performed by HPTLC. The method may be applied to identify the *SPC* from other manufacturing process. It provides the identification of constituents, determination of impurities and quantitative determination of active substance present in the *SPC*. They provide the identification of constituents, determination of impurities and quantitative determination of active substance present in the *SPC*.[113]
- The Rf value of the *SPC* supports the better standardization of the drug.
- The present study revealed that *SPC* showed best results in Toluene: Ethyl acetate (9:1). Solvent system. After scanning and visualizing the plates in absorbance mode at 254nm, 366 nm and visible light range, best results were shown at visible light range.
- TLC plate showed different colour phyto constituents of chloroform extract of *SPC*. The bands revealed presence of six green, two blue, and one fluorescent yellow, bands showing the presence of alkaloids, glycosides, phenols, Triterpenes, flavonoids and quinones.
- The results from HPTLC finger print scanned for chloroform extract of *SPC*. There are thirteen polyvalent phyto constituents and corresponding ascending order of Rf values start from 0.02 to 0.91 in which highest concentrations of the phyto constituents was found to be 53.95% and 22.47 % with its corresponding Rf value were found to be 0.02 and 0.91 respectively.

Table: 9. Results of basic radicals studies

S.NO	Parameter	Observation	Result
1	Test for Potassium	Formation of yellow colour precipitate	Positive
2	Test for Calcium	Formation of white colour precipitate	Negative
3	Test For Magnesium .	Formation of white colour precipitate	Positive
4	Test For Ammonium .	Appearance of brown colour	Negative
5	Test For Sodium	Appearance of intense yellow colour	Negative
6	Test for Iron (Ferrous)	Appearance of blood red colour	Positive
7	Test For Zinc	Formation of white colour precipitate	Positive
8	Test For Aluminium	Characteristic changes	Negative
9	Test For Lead	Formation of yellow colour precipitate	Negative
10	Test for Copper	Formation of blue colour precipitate	Negative
11	Test For Mercury	Formation of yellow colour precipitate	Negative
12	Test for Arsenic	Formation of brownish red precipitate	Negative

Interpretation

The sample contains Potassium, Magnesium, Iron, Phosphorus, and Zinc. These trace quantities of minerals along may play an important role in the functioning of various enzymes in biological systems and have immunomodulatory functions and thus influence the susceptibility to the course and the outcome of a variety of viral infections.

Potassium

- Potassium is important for maintaining the integrity of cell membranes and functions as a vital electrolyte.
- Potassium is absorbed through the small intestine; severe lack of potassium can disrupt liver function.
- If potassium level falls below 30% to 40% are prone to liver disease [114].

Magnesium

- It enhances immune system.
- Depletion of Magnesium level leads to Cirrhosis, Fatty liver syndrome,
- Thus magnesium is essential for liver to prevent Liver diseases.[115]

Iron

- Needed for energy metabolism.
- It is crucial for oxygen transport, energy production, and cellular growth and proliferation.

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.
- Liver as a powerful antioxidant.
- Deficiency of zinc leads to malabsorption syndrome , Cirrhosis of liver.[116]

The basic radical test shows the presence of **Potassium, Magnesium, Iron and Zinc** absence of heavy metals such as lead, arsenic and mercury.

Table: 10. Results of acid radical studies

S.NO	Parameter	Observation	Result
1	Test for Sulphate	Formation of white precipitate	Positive
2	Test for Chloride	Formation of white precipitate	Negative
3	Test for Phosphate	Formation of yellow precipitate	Negative
4	Test for Carbonate	Formation of effervescence	Negative
5	Test for fluoride &oxalate	Formation of cloudy appearance	Negative
6	Test For Nitrate	Characteristic changes	Negative

Interpretation:

The acidic radicals test shows the presence of sulphate.

Availability of bacterial and fungal load in *Siringipaerathi Chooranam*

Table: 11. Bacterial and fungal dilutions

MICROBES	DILUTION	RESULT
BACTERIA	**10^{-4}**	**7**
BACTERIA	**10^{-6}**	**4**
FUNGI	**10^{-2}**	**3**
FUNGI	**10^{-3}**	**4**

Interpretation:

- The availability of bacterial load in the *SPC* has been performed by Agar plate technique.
- *SPC* is an herbal drug which are prepare from plant material they are prone to contamination. The contamination of herbal drugs by micro organism not only cause bio deterioration but also reduces the efficacy of drugs.
- The toxin produces by microbes makes herbal drugs unfit for human consumption because the contaminated drug may develop unwanted disease instead of disease being cured.
- The contamination of *SPC* has been examined by bacterial and fungal load.
- Total bacterial load in 10^{-4} dilution is 7 and 10^{-6} dilution is 4.
- Total fungal load in 10^{-2} dilution is 3 and10^{-3} dilution is 4.

Here, the result shows presence of bacterial and fungal load in the trial drug *(SPC).*They present within the normal limits [117].

INSTRUMENTAL ANALYSIS

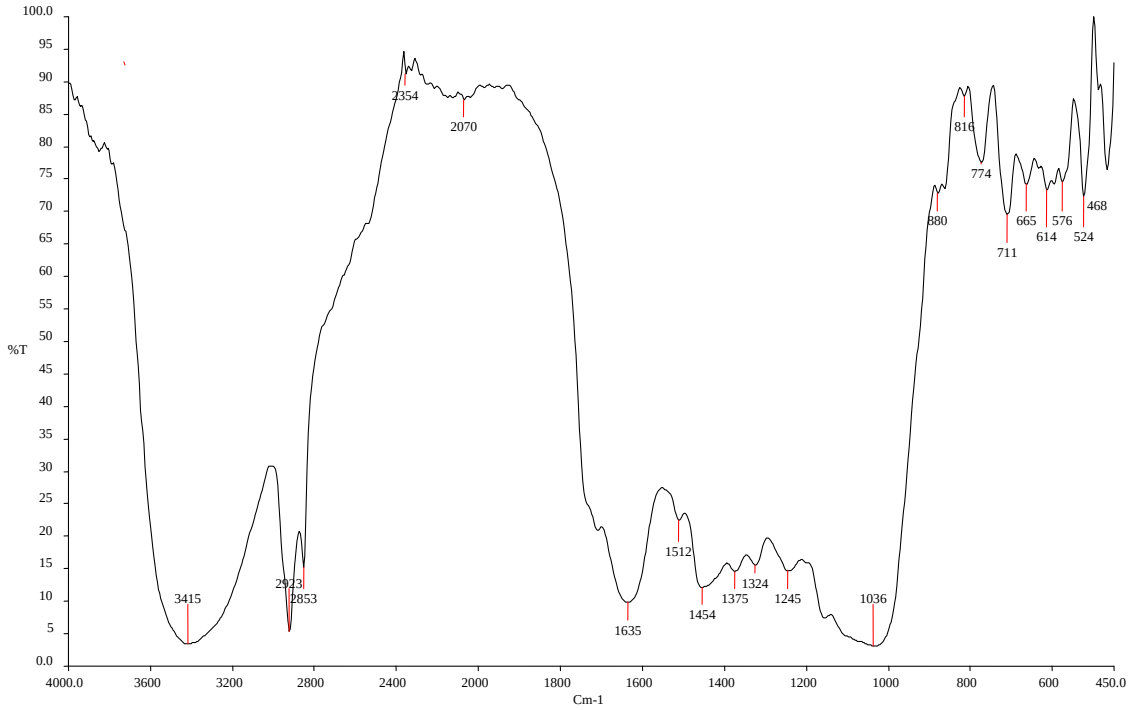


Fig: 6 FT-IR (Fourier Transform InfraRed spectroscopy)

FTIR

Siringipaerathi Chooranam.

Table: 12. FT-IR

Absorption peak cm^{-1}	Stretch	Functional group
3415	O-H Stretch, bonded N-H Stretch	Alcohol Amine
2923	C-H Stretch O-H Stretch	Alkane Acid
2853	C-H Stretch	Alkane

	O-H Stretch	Acid
1635	N-H Bending C=C Stretch	Amide Alkane
1512	C=C Stretch C-Br Stretch	Aromatic Alkyl Halide
1454	C=C Stretch -C-H Bending	Aromatic Alkane
1375	-C-H Bending C-F Stretch N-O Stretch	Alkane Alkyl Halide Nitro
1324	C-F Stretch	Nitro
1245	C=O Stretch C-N Stretch C-O Stretch	Acid Amine Ether
1036	C-O Stretch C=O Stretch	Ether Ester
880	==C-H Bending	Alkene
816	==C-H Bending	Alkene
774	C-Cl Stretch	Alkyl Halide
711	C-Cl Stretch ==C-H Bending	Alkyl Halide Alkene
665	C-Cl Stretch	Alkyl Halide
614	C-Cl Stretch	Alkyl Halide
576	C-Br Stretch	Alkyl Halide
524	C-Br Stretch	Alkyl Halide
468	C-Br Stretch	Alkyl Halide

Interpretation

FTIR instrumental analysis was done. The test drug was identified to have 15 peaks. They are the functional groups present in the trial drug *Siringipaerathi Chooranam*. The above table shows the presence of amide, phenols, alkanes, alkyl halide, acid, aromatic, ester, ether and alcohol groups which represents the peak value.

- OH group has higher potential towards inhibitory activity against microorganisms.
- Phenols possess highly Anti-Oxidant property which enhances the drug effect against the disease.
- Amines enhance the drug effect against the disease [118].

SEM (SCANNING ELECTRON MICROSCOPE)

The above SEM studies of microscopic resolution of 1.00kx and examining surface area of 800x800µm^2, showed objects of sizes ranging from 265nm to 409nm.The surface of the sample grains is uniformly arranged in agglomerates. They are micro particles ranging from 265nm, 276nm, 303nm, 409nm.

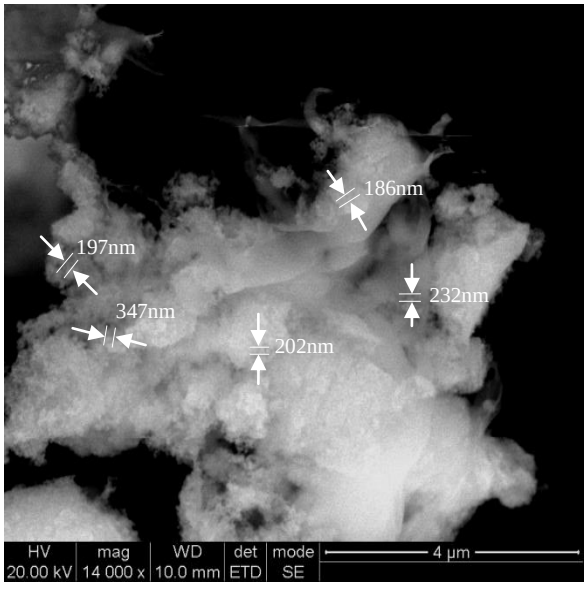


Fig: 7.1 SEM (Scanning Electron Microscope)

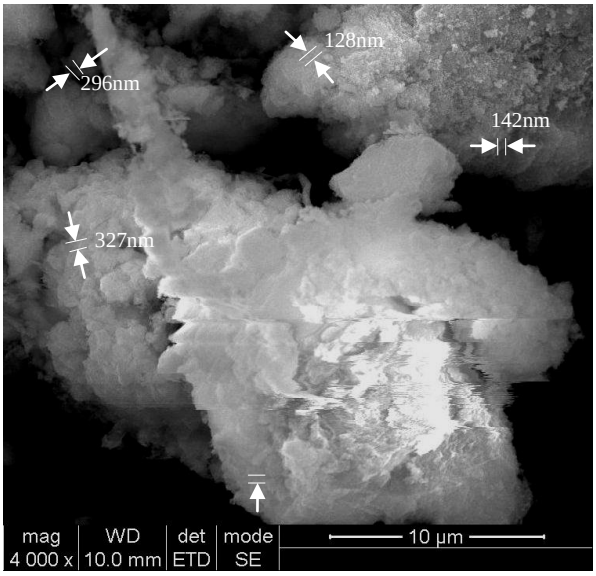


Fig: 7.2 SEM (Scanning Electron Microscope)

Interpretation for SEM

- Micro particles are defined as particulate dispersion or solid particles with a size in the range of 100-1000nm in diameter.
- 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, alter 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 [120].

Hence *Siringipaerathi Chooranam* which is prepared biologically contains nanoparticles to enhance the pharmacological action in the target site.

Table: 13. ICP-OES RESULTS OF *SIRINGIPAERATHI CHOORANAM*

S. No	Elements	Detected levels
1.	Arsenic	BDL
2.	Calcium	54.120 mg/L
3.	Cadmium	BDL
4.	Iron	12.300 mg/L
5.	Mercury	BDL
6.	Potassium	60.821 mg/L
7.	Sodium	03.110 mg/L
8.	Nickel	BDL
9.	Lead	BDL
10.	Phosphorus	08.541 mg/L
11.	Sulphur	BDL

The toxic metals and the permissible limits

Heavy metals	WHO & FDA limits
Arsenic (As)	3ppm
Mercury (Hg)	1ppm
Lead (Pb)	10ppm
Cadmium (Cd)	0.30ppm

Discussion:

- The above results indicate that the trial drug is extremely safe as it contains heavy metals within specified limits.
- The presence of Ca(54.120 mg/l), Fe(12.300 mg/l), K(60.821 mg/dl), Na(0.3110 mg/l), P(8.541 mg/dl) has physiologically important. In *Siringipaerathi Chooranam*, the heavy metals like As, Cd, Hg, Pb,S 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

Table: 14. Dose finding experiment and its behavioral Signs of Toxicity for *Siringipaerathi Chooranam*

Observation done:

Group	Day
Body weight	Increased
Assessments of posture	Normal
Signs of Convulsion Limb paralysis	Absence (-)
Body tone	Normal
Lacrimation	Absence
Salivation	Absence
Change in skin color	No significant colour change
Piloerection	Normal

Group	Day
Defecation	Normal
Sensitivity response	Normal
Locomotion	Normal
Muscle gripness	Normal
Rearing	Mild
Ur	No

Table: 15. Dose finding experiment and its behavioral Signs of Toxicity for *Siringipaerathi Chooranam*

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

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

Interpretation:

In the acute toxicity study, the rats were treated with different concentration of *Siringipaerathi Chooranam* from the range of 5mg/kg to 2000mg/kg which did not produce signs of toxicity, behavioral changes, and mortality in the test groups as compared to the controls when observed during 14 days of the acute toxicity experimental period. These results showed that a single oral dose of the extract showed no mortality of these rats even under higher dosage levels indicating the high margin of safety of this extract. In acute toxicity test the *Siringipaerathi Chooranam* was found to be non-toxic at the dose level of 2000mg/ kg body weight.

Weight gain of rats

Table: 16 Body weight (g) changes of rats when exposed to *SPC*

Dose (mg/kg/day)	***Days***				
	0	**7**	**14**	**21**	**28**
Control	120.59±0.92	122.79±0.87	123.52±1.18	127.24±1.12	131.25±1.05
100	121.53±0.93	124.14±0.58	127.24±1.15	128.92±1.40	132.23±1.05
200	122.65±0.91	127.83±0.90	128.23±1.15	130.59±1.59	134.05±0.98

*Values are expressed as mean ±S.E.M; N=3; *P<0.05, **P<0.01, ***P<0.001 vs control.*

Interpretation

The total body weight of the animals was weighed on 1st, 7th, 14th, 21st, 28th day and is shown in the table. It was found that the test drug produced significant weight gain than control, with administration of the drug.

Similarly the test drug at all dose levels induced weight gain and we could see longer the duration of administration of drug higher was the weight gain.

Results of organ weight in rats

Table. : 17 .Effect of *Siringipaerathi Chooranam* on organ weight in rats

Organ	**Control**	**100 mg/kg**	**200 mg/kg**
Liver (g)	3.07±0.20	4.41±0.32	4.72±0.32
Heart (g)	0.32±0.04	0.37±0.01	0.42±0.02
Lung (g)	0.28±0.05	0.32±0.01	0.38±0.01
Spleen (g)	0.74±0.07	0.68±0.17	0.78±0.08
Brain (g)	0.37±0.05	0.47±0.02	0.54±0.03
Kidney (g)	0.76±0.05	0.89±0.01	0.91±0.02

*Values are expressed as mean ±S.E.M; N=3; *P<0.05, **P<0.01, ***P<0.001 vs control.*

Interpretation

There is a slight significant change in the organ weight of the rats treated with different doses of test drug and the control.

Results of Haematological parameters

Table: 18 Effect of *Siringipaerathi Chooranam* on Haematological parameters in rats

Parameter	Control	100mg/kg	200 mg/kg
RBC(x 10^6/mm^3)	8.29±0.43	8.27±0.44	8.26±0.44
PCV (%)	49.66±0.77	47.98±1.04	47.20±0.91
Hb (%)	15.13±0.39	14.83±0.40	15.03±0.39
WBC(x 10^3/mm^3)	11.75±0.85	11.73±0.85	11.74±0.85
Neutrophils (%)	23.29±0.73	21.94±1.03	22.96±0.49
Eosinophills (%)	4.10±0.23	3.8±0.25	3.97±0.23
Lymphocyte (%)	85.5±0.46	83.7±0.72	84.87±0.32
Platelets(x 10^3/mm^3)	425.73±1.35	423.43±1.47	425.03±1.26

*Values are expressed as mean ± S.E.M; N=3; *P<0.05, **P<0.01, ***P<0.001 vs control.*

Interpretation

The haematological investigation results of the rats conducted on 28th day after the repeated dose of the drug revealed the values of different parameters. There is a slight variation in the values of RBC count values in the dose group of 100 and 200 when compared with that of the control. The increase and decrease in the values obtained were all within the normal biological and laboratory limits.

Results of Biochemical Parameters

Table.No:19 Effect of *Siringipaerathi Chooranam* on biochemical parameters in rats

Parameters	Control	100 mg/kg	200 mg/kg
Protein (g/dl)	8.58 ± 0.68	7.56±0.61	6.76±0.44
Albumin (g/dl)	5.34 ± 0.40	5.29±0.44	3.5±0.54
BUN (mg/dl)	22.06 ± 1.55	22.72±1.9	25.53±1.8
Urea (mg/dl)	64.24 ± 3.11	66.7±5.3	69.2±2.9
Creatinine (mg/dl)	0.85 ± 0.07	0.6±0.24	0.71±0.25
Total Cholesterol (mg/dl)	93.21 ± 1.16	92.17±1.13	91.53±1.35
Triglycerides (mg/dl)	52.58 ± 1.56	52.16±1.3	52.93±1.7
Glucose (mg/dl)	108.63 ± 0.81	107.97±1.12	109.4±0.51
Total Bilirubin (mg/dl)	0.205 ± 0.04	0.16±0.08	0.10±0.03
SGOT (U/L)	73 ± 2.4	72.67±1.64	65.52±2.4
SGPT(U/L)	28.4 ± 1.2	25.77±0.64	23.99±0.70
Alkaline phosphatase(U/L)	102.4 ± 3.6	101.3±1.5	91.33±4.26

*Values are expressed as mean ± S.E.M; N=3; *P<0.05, **P<0.01, ***P<0.001 vs control.*

Interpretation

The biochemical investigations were conducted on 28th day and the results are produced. The results revealed that there is a slight significant change in the values of different parameters with that of the control. All the values were within the normal biological and laboratory limits.

Results of Urine parameters

Table.No:20 Effect of *Siringipaerathi Chooranam* on Urine parameters in rats

Parameters	Control	100 mg/kg	200 mg/kg
Colour	Yellow	Yellow	Yellow
Transparency	Clear	Clear	Clear
Specific gravity	1.01	1.02	1.02
PH	7.2	7.4	7.3
Protein	Nil	Nil	Nil
Glucose	Nil	Nil	Nil
Bilirubin	-ve	-ve	-ve
Ketones	-ve	-ve	-ve
Blood	Absent	Absent	Absent
RBCs	Nil	Nil	Nil
Epithelial cells	Nil	Nil	Nil
Casts	Nil	Nil	Nil

Interpretation

Urine analysis data of control group and the test groups of animals taken on 28th day showed no abnormal results.

The above results showed that all parameters remained within normal limits.

Interpretation

The above slides show the histopathology studies of sub-acute toxicity. There is no toxicological abnormality seen in the vital organs after administration of the test drug *Siringipaerathi Chooranam*. Thus the safety of the drug is revealed so that it can be administered for long time without any side effects.

PHARMACOLOGICAL RESULT:

CCL4 INDUCED HEPATOTOXICITY

Table: 21 Effects of Serum Enzymes on *SPC*

Group	Treatment	SGPT U/L	SGOT U/L
1	Control	24.185 ±1.62	28.95 ±1.46
2	CCL4 + LP	102.8 ±1.25	167.84 ±2.94
3	Standard (Silymarin – 100 mg)	30.95 ±2.34	43.8 ±2.35**
4	CCL4 + low dose *SPC* – 100 mg	68 ±2.51	70.76 ±1.43
5	CCL4 + high dose *SPC* – 200 mg	34.53 ±3.38*	40.05 ±1.72*

*Values are expressed as mean ±S.E.M; N=6; *P<0.05, **P<0.01, ***P<0.001 vs control.*

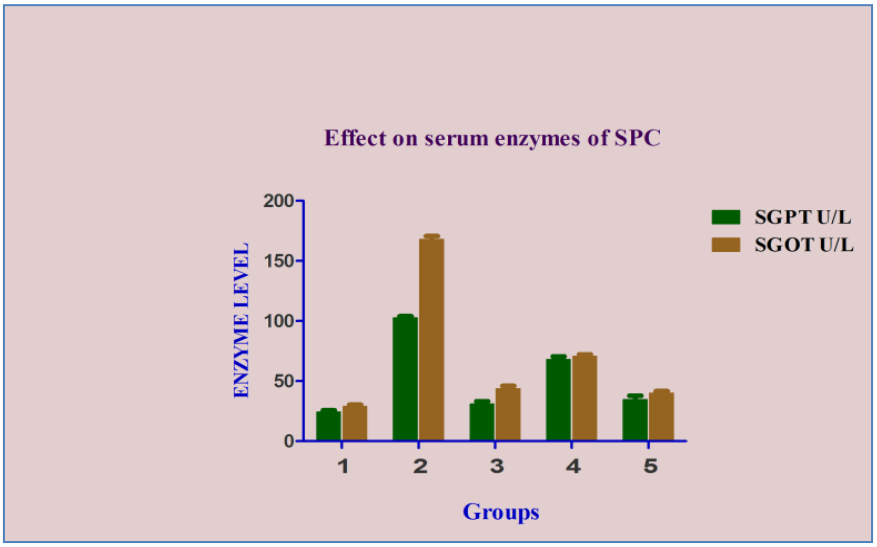


Chart: 1 Effect of Serum Enzymes on *SPC*

Table: 22. Effect of Serum Enzyme (ALP) on *SPC*

Group	Treatment	ALP (mg/dl)
1	Control	39.86 ±3.46
2	CCL4 + LP	89.33 ±0.97
3	Standard (Silymarin -100mg)	47.81 ±4.69**
4	CCL4 + low dose *SPC* – 100 mg	76.05 ±2.96*
5	CCL4 + high dose *SPC* – 200 mg	54.31 ±3.84*

*Values are expressed as mean ±S.E.M; N=6; *P<0.05, **P<0.01, ***P<0.001 vs control.*

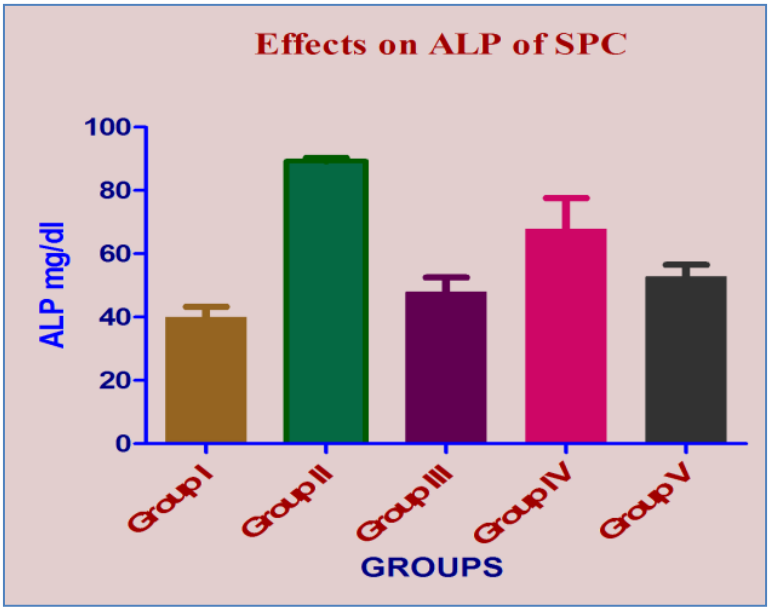


Chart: 2. Effect of Serum Enzyme (ALP) on *SPC*

Table: 23. Effects of Bilirubin Levels on *SPC*

Group	Treatment	Total Bilirubin mg/dl	Direct Bilirubin mg/dl
1	Control	0.253 ±0.003	0.17 ±0.003
2	CCL4 + LP	1.5 ±0.1	1.53 ±0.01
3	Standard (Silymarin – 100mg)	0.44 ±0.007**	0.24 ±0.004*
4	CCL4 + low dose *SPC* – 100 mg	1.25 ±0.015	1.21 ±0.040
5	CCL4 + high dose *SPC* – 200 mg	0.52 ±0.002*	0.20 ±0.018*

*Values are expressed as mean ± S.E.M; N=6; *P<0.05, **P<0.01, ***P<0.001 vs control.*

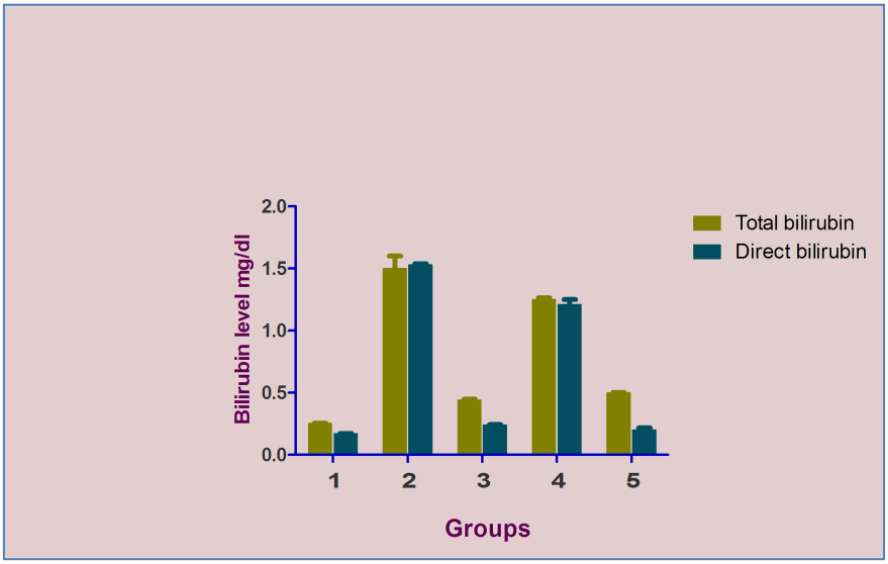


Chart: 3. Effects of Bilirubin Levels on *SPC*

- Group I: Control rat showing normal central vein and normal hepatocytes
- Group II: Showing dilated central vein and hepatocytes with degeneration
- Group III: Liver tissue of rats treated with *SPC* at 100mg/kg showing mild degree of necrosis (N) with normal cells (C)
- Group IV: Central vein showing normal hepatocytes with regenerating hepatocytes and mild inflammation in the portal area
- Group V: Photomicrograph of liver tissue treated with Silymarin showing normal hepatocytes, portal vein (V) and portal artery.

Interpretation

Discussion on hepatoprotective activity against CCl_4 induced hepatotoxicity in rats.

- The present studies were performed to assess the hepatoprotective activity in rats against Carbon tetrachloride as hepatotoxins. Clinical signs in rats that received CCl_4 (Group 2) included dullness and loss of appetite.
- The serum enzymes like SGOT, SGPT, ALP, Direct Bilirubin and Total Bilirubin treated animals were significantly reduced by seven days pretreatment of *SPC* at two dose levels 100mg/kg and 200mg/kg, when compared with CCl_4 treated control. No significant clinical abnormalities in other groups.
- Changes in the serum constituents In Table: 21,22,23 the activities of serum SGPT, SGOT and ALP and Total Bilirubin and Direct Bilirubin in the *SPC* treated groups (Tables:21,22,23) and the Silymarin group, were significantly decrease when compared to CCl_4 treated group and almost near the normal value when compared to the negative control.
- The changes associated with Carbon tetrachloride induced liver damage of the present study appeared similar to the acute viral hepatitis. In CCl_4 **i**nduced hepatotoxicity, the administration of the toxicant CCl_4 showed a distinct rise in the levels of serum marker enzymes namely SGOT, SGPT, ALP, Total Bilirubin and Direct Bilirubin and as shown in Table: 21, 22, and 23.

- A number of reports indicates that overdose of carbon tetrachloride can produce centrizonal hemorrhagic hepatic necrosis in humans and experimental animals.
- Carbon tetrachloride is biotransformed by the cytochrome P-450 system to produce the trichloromethyl free radical, which in turn covalently binds to cell membranes and organelles to elicit lipid peroxidation, disturb Ca 2+ homeostasis and finally result in cell death.
- The drug treatment was carried out at two dose levels 100 and 200mg/kg, both of which along with the standard treated group showed a significant reduction in the elevated enzyme levels.
- These data suggests a dose dependent hepatoprotective activity of *SPC*. The present studies were performed to assess the hepatoprotective activity in rats against CCL4 as hepatotoxins to prove its claims in clinical practice against liver disorders.
- Reduction in the levels of SGPT, SGOT, and ALP towards the normal value is an indication of regeneration process. The protective effect exhibited by *SPC* at dose level of 200 mg/kg was comparable with the standard drug. The *SPC* treatments significantly reversed the levels of ALP.
- These findings suggested the *SPC* administered has significantly neutralized the toxic effects of Carbon tetrachloride and helped in regeneration of hepatocytes.
- Estimating the activities of serum marker enzymes, like SGPT,SGOT, ALP, Bilirubin can make the assessment of liver function when liver cell plasma membrane is damaged, a variety of enzyme normally located in the cytosol are released into the blood stream.
- Their estimation in the serum is a useful quantitative marker of the extent and type of hepato cellular damage. The tendency of these enzymes to return to near normally in *SPC* administered group is a clear manifestation of anti hepatotoxic effects. Reduction in the levels of SGOT, SGPT and ALP towards the normal value is an indication of regeneration process.
- Reduction in ALP levels with concurrent depletion of raised bilirubin levels suggests the stability of the biliary function during injury with Carbon tetrachloride.

- Therefore the reduction in the activity of these enzymes may result in a number of deleterious effects Administration of *SPC* increased the activities against CCL4-induced liver damage in rats to prevent the accumulation of excessive fats and protected the liver [121].
- This was further confirmed by histopathological injuries in Fig 9

PARACETAMOL INDUCED HEPATOTOXICITY

Table: 24. Serum Enzymes Value (AST, ALT and ALP)

Group	Treatment	AST(U/ml)	ALT(U/ml)	ALP (mg/dl)
A	Positive Control	39.29 ±0.72	35.45 ±1.16	32.2 ± 0.86
B	Negative Control (Paracetamol)	141.48 ±1.09	172.86 ±1.73	98 ± 0.70
C	Standard(Silymarin)	52.37 ±1.98**	62.80 ±1.15**	35 ± 0.70**
D	*SPC* – 100 mg	66.58 ±1.38*	88.75 ±1.85*	57.4 ± 1.077*
E	*SPC* – 200 mg	52.29 ±1.39*	58.27 ± 3.97	48.8 ± 0.86*

*Values are expressed as mean ± S.E.M; N=5; *P<0.05, **P<0.01, ***P<0.001 vs control.*

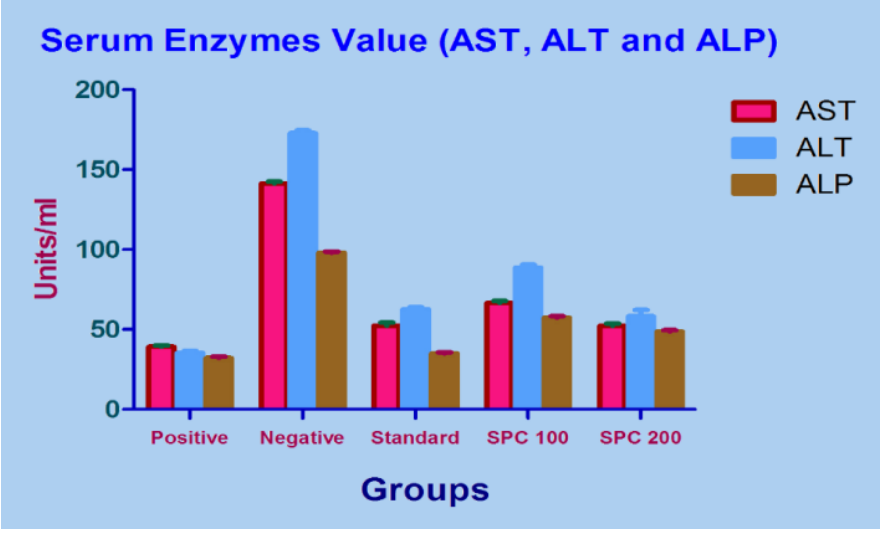


Chart: 4. Serum Enzymes Value (AST, ALT and ALP)

Table: 25. Effect of *SPC* on Total Cholesterol

Group	Treatment	Total Cholesterol (mg/dl)
A	Positive Control	150.4 ±6.62
B	Toxicant Control (Paracetamol -1.25 ml/kg)	338.8 ±10.04
C	Standard(Silymarin -100 mg/kg)	167 ±4.82**
D	*SPC* – 100 mg	270 ±7.38*
E	*SPC* – 200 mg	203.6 ±5.38*

*Values are expressed as mean ± S.E.M; N=5; *P<0.05, **P<0.01, ***P<0.001 vs control.*

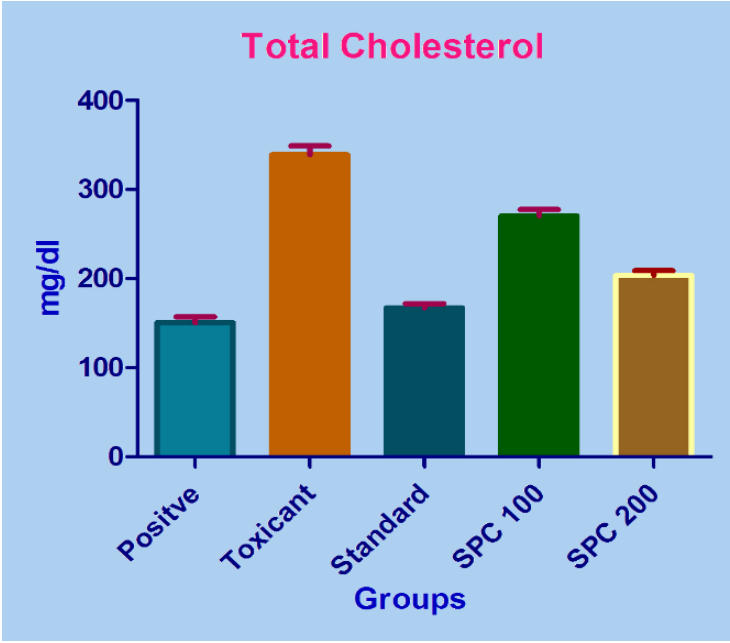


Chart: 5. Effect of *SPC* on Total Cholesterol

Table: 26. Effect of *SPC* on Triglycerides

Group	Treatment	Triglycerides (mg/dl)
A	Positive Control	0.65 ±0.046
B	Toxicant Control (Paracetamol -1.25 ml/kg)	2.564 ±0.076
C	Standard(Silymarin -100 mg/kg)	0.848 ±0.044*
D	*SPC* – 100 mg	1.99 ±0.035**
E	*SPC* – 200 mg	0.664 ±0.241*

*Values are expressed as mean ±S.E.M; N=5; *P<0.05, **P<0.01, ***P<0.001 vs control.*

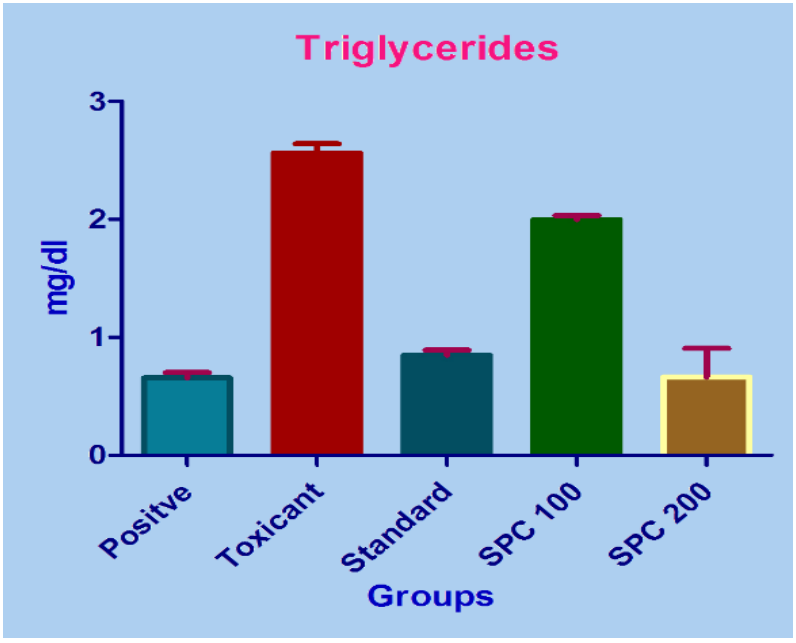


Chart: 6. Effect of *SPC* on Triglycerides

Table: 27. Effect of *SPC* on Liver volume and weight

Group	Treatment	Liver volume (ml)	Liver weight (g)
A	Positive Control	2.748 ±0.067	4.58 ±0.61
B	Negative Control (Paracetamol -1.25 ml/kg)	4.744 ±0.094	5.76 ±0.56
C	Standard (Silymarin-100mg/kg)	3.244 ±0.129	3.96 ±0.60
D	*SPC* – 100 mg	3.826 ±0.085*	3.91 ±0.52*
E	*SPC* – 200 mg	3.8 ±0.230*	4.2 ±0.36

*Values are expressed as mean ± S.E.M; N=5; *P<0.05, **P<0.01, ***P<0.001 vs control.*

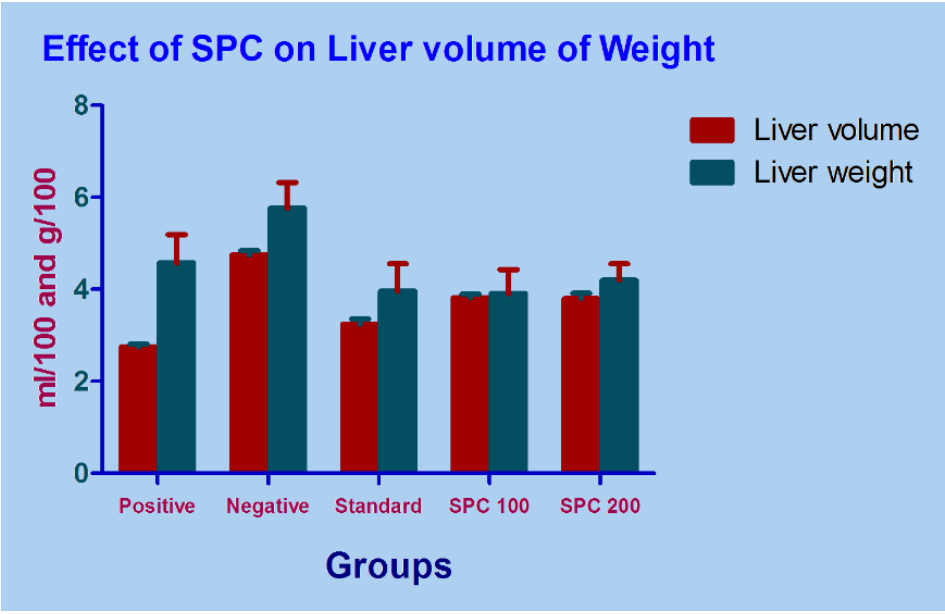


Chart: 7. Effect of *SPC* on Liver volume and weight

Table: 28. Effect of *SPC* on duration of sleep and onset of time

Group	Treatment	Duration of sleep(sec)	Onset of time(min)
A	Positive Control	96 ±0.707	175.4 ±1.88
B	Negative Control (Paracetamol -1.25 ml/kg)	225.8 ±1.88	59.8 ±0.86
C	Standard (Silymarin-100mg/kg)	120 ±1.14**	153 ±1.30
D	*SPC* – 100 mg	173.4 ±1.72*	98 ±1.224
E	*SPC* – 200 mg	125.2 ±0.86*	129.4 ±1.08

*Values are expressed as mean ±S.E.M; N=5; *P<0.05, **P<0.01, ***P<0.001 vs control.*

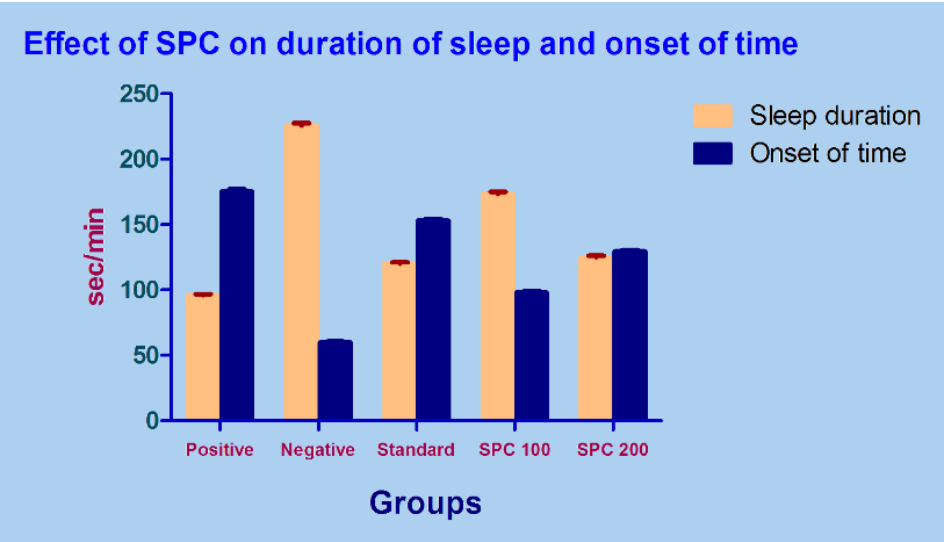


Chart: 8. Effect of *SPC* on duration of sleep and onset of time

Table: 29. Effect of *SPC* on Total Protein and Bilirubin

Group	Treatment	Total Protein (g/dl)	Bilirubin (mg/dl)
A	Positive Control	6.07 ±0.27	0.238 ±0.008
B	Toxicant Control (Paracetamol – 1.25 ml/kg)	3.49 ±0.29	2.202 ±0.02
C	Standard(Silymarin-100 mg/kg)	6.16 ±0.32**	0.408 ±0.012**
D	*SPC* – 100 mg	6.21 ±0.19	1.488 ±0.01
E	*SPC* – 200 mg	6.96 ±0.25*	0.634 ±0.017*

*Values are expressed as mean ±S.E.M; N=5; *P<0.05, **P<0.01, ***P<0.001 vs control.*

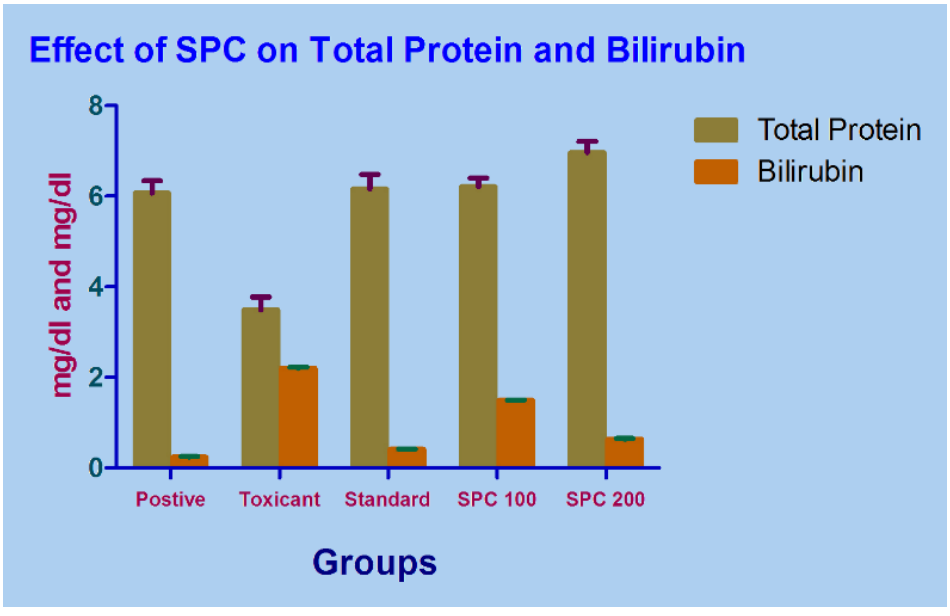


Chart: 9. Effects of *SPC* on Total Protein and Bilirubin

Interpretation

Effects of *Siringipaerathi Chooranam* on ALT, AST, and ALP, Total Protein, Bilirubin, Total Cholesterol, Triglycerides levels

Paracetamol induced hepatotoxicity is the generally used screening method for testing the hepatoprotective nature of drugs. The hepatic damage increases the level of serum marker enzymes like AST, ALT, ALP and Bilirubin. This indicates the cellular damage as well as loss of the functional integrity of cell membrane in liver.

In paracetamol treated rats the levels of serum marker enzymes (AST, ALT, ALP and Bilirubin) elevated significantly. Moreover, the increased levels of the serum enzymes were significantly decreased Total Cholesterol, Triglycerides significantly decreased and liver volume and liver weight significantly increased. Duration of sleep significantly increased and onset of time siginificantly decreased by the treatment with *Siringipaerathi Chooranam* at 100 mg/kg p.o and 200 mg/kg p.o, implying that the drug prohibited the liver damage.

The *Siringipaerathi Chooranam* treatment confirmed dose dependent activity, *Siringipaerathi Chooranam* at 200 mg/kg p.o revealed good result than 100mg/kg p.o [122]. This was further confirmed by histopathological injuries in Fig 10.

ANTI-OXIDANT ACTIVITY

RESULT AND DISCUSSION

Table: 30 DPPH Assay of *Siringipaerathi Chooranam*

Sample concentration (µg/ml)	Absorbance		Percentage of Inhibition	
	Drug	Standard	Drug	Standard
Control	0.5271	0.312	-	-
1.25	0.4262	0.278	19.14248	40.89
2.50	0.3704	0.202	29.7287	51.25
5	0.2277	0.084	56.80137	74.07
10	0.1835	0.052	65.18687	83.33
20	0.1375	0.034	73.91387	89.62

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

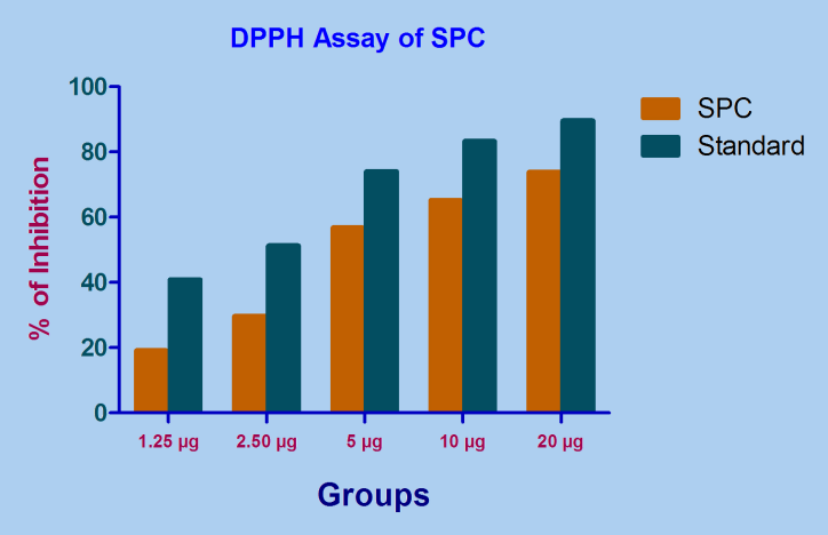


Chart: 10. DPPH Assay of *Siringipaerathi Chooranam*

DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of *SPC* extract. The antioxidant molecules can quench DPPH free radicals by providing hydrogen atom or by electron donation and a colorless stable molecule 1, 1 diphenyl-2- picrylhydrazil is formed and as a result of which the absorbance at 517nm of the solution is decreased. In the present study, the *SPC* extract was analyzed was able to decolorize DPPH and the free radical scavenging activity was expressed as the percentage decrease in absorbance.

In the present study, the extract of *SPC* was found to possess concentration dependent scavenging activity on DPPH radicals. The values of DPPH free radical scavenging activity of the *SPC* extract was given in (Table: 29). The extract of *SPC* showed the highest DPPH scavenging activity (81.71%) at 20µg/ml and the lowest percentage of inhibition (28.11%) at 1.25µg/ml. Ascorbic acid (Standard) showed highest percentage of inhibition (89.62%) at 20µg/ml and the lowest percentage of inhibition (40.89%) at 1.25µg/ml.

This indicated that % of inhibition increased with increase in concentration of both the standard and *SPC* extract. The *SPC* extract has more or less equal DPPH scavenging [123].

6. CONCLUSION

Liver diseases are the most common health problem in the world. The Liver is quantitatively the most important site of drug metabolism. However many drugs are known to cause hepatic injury. Conventional and synthetic drugs 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.

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.

In order to overcome this difficulty a novel attempt has been made to standardize the Siddha drug ***Siringipaerathi Chooranam*** for its hepatoprotective properties by using analytical, preclinical studies.

The drug ***Siringipaerathi Chooranam*** was selected from the Siddha literature ***SARABENDRA VAIDHYA MURAIGAL*** to validate the safety and its efficacy of CCL4 and Paracetamol induced hepatotoxicity and Anti-oxidant activity.

The ingredients of the test drug was identified and authenticated by Siddha experts. The drug was prepared as per the procedure and subjected to various studies to reveal its potency and effectiveness against the disease.

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 *Siringipaerathi Chooranam* was 6.4. This is weak acidic in nature, 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 flavonoids, phenols, Carbohydrates, Phytosterols, Triterpenes, Proteins, Amino acids and alkaloids. Biochemical analysis showed the presence of zinc, magnesium, potassium, calcium 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

Instrumental analysis:

Based on the results, *Siringipaerathi Chooranam* 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 the physiologically important of minerals like Na, K, Fe ,P, Ca. In *Siringipaerathi Chooranam,* the heavy metals like As, Cd, Pb and trace element like Ni were below detectable level. This reveals the safety of the drug

The FTIR results showed the presence of O-H Stretching and bend, OH group has higher potential towards inhibitory activity against microorganisms. Phenols possess highly Anti-Oxidant property which enhances the drug effect against the disease

The SEM picture shown the presence of nanoparticle of size 100-1000 nm in the drug *Siringipaerathi Chooranam.* Further, the study shows that *Siringipaerathi Chooranam* is a kind of nanomedicine 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 *Siringipaerathi Chooranam* may be absorbed in the upper part of GIT.

Toxicity studies:

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

Toxicological study of both acute and sub-acute toxicity study were 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 *Siringipaerathi Chooranam* treated over a period of 28 days (OECD 407). So, it can be concluded that the *Siringipaerathi Chooranam* can be prescribed for therapeutic use in human with the dosage recommendations of up to 100mg/kg body weight p.o.

Antimicrobial activity:

The *Siringipaerathi Chooranam* showed a 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 (*SPC*).They present within the normal limits.

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

- CCL_4 induced Hepato toxicity
- Paracetamol induced Hepato toxicity
- Anti-Oxidant Activity

Hepatoprotective activity against CCl_4, Paracetamol:

The present study showed that *Siringipaerathi Chooranam* produce protective against the hepatotoxicity induced by CCl_4, Paracetamol.

The hepatoprotective role of *Siringipaerathi Chooranam* might be due to its chemical constituent. Hence *Siringipaerathi Chooranam* may be act as prophylactic as well as curative drug in treating hepato toxic conditions. Further studies needs to isolate the active constituents and mechanism of action. Thus the author validates *Siringipaerathi Chooranam* as a new hepato-protective drug which is cost effective and without any side effect.

Anti-oxidant

In the present study, the extract of *SPC* was found to possess concentration dependent scavenging activity on DPPH radicals .The *SPC* extract has more or less equal DPPH scavenging, when compared to standard (Ascorbic acid), it was concluded that the *SPC* extract has a marked anti-oxidant activity at higher concentration.

7. FUTURE SCOPE

The trial drug *Siringipaerathi Chooranam* has its own potency of hepatoprotective activity in animal model which has been established in this study. However, the mechanism of action by which *Siringipaerathi Chooranam* 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.

8. SUMMARY

The Siddha Medical System is a renowned medical system belonging to Tamil speaking regions. Although our Indigenous systems of Medicine utilize raw materials from plant is unique to Siddha Medical system.

- The test drug *Siringipaerathi Chooranam* was selected from the Siddha literature ***SARABENDRA VAIDHYA MURAIGAL*** for its hepatoprotective activity**.** 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 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 present in 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 and anti-oxidant activity of *Siringipaerathi Chooranam* in animal model Wistar albino rats and In-vitro studies.
- Results and discussion gives 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 *Siringipaaerathi Chooranam* has impressive Hepatoprotective activity, which exemplifies the intelligence of the Siddha literature to reach globally for the welfare of mankind.
- Thus the herbal formulation *Siringipaerathi Chooranam* is validated for its safety and efficacy for treating jaundice and it would be a great drug of choice.

The final discussion and conclusion chapters analyzed the dissertation. The conclusion chapter also provides a discussion of the verification and validity of the research results carried out. The most vital part of some experience of the findings in the dissertation is also discussed and thereafter invites the reader to further studies and future research possibilities.

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