

**PRECLINICAL EVALUATION OF SIDDHA POLYHERBAL FORMULATION
“PITHA KAMALAI CHOORNAM” FOR ITS HEPATO PROTECTIVE ACTIVITY IN
WISTAR ALBINO RATS**

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **Preclinical evaluation of Siddha Polyherbal formulation *Pitha Kamalai Choornam* for its Hepatoprotective activity in wistar albino rats** is a bonafide and genuine research work carried out by me under the guidance of **Lecturer Dr. R. Karolin Daisy Rani M.D(S)**, Post Graduate Department of *Gunapadam*, Govt. Siddha Medical College, Arumbakkam, Chennai-106 and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or other similar title.

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ABBREVIATIONS

Alb	Albumin
ALP	Alkaline phosphatase
ALT	Alanine transaminase
AST	Aspartate transaminase
ANOVA	Analysis Of Variance
CAT	Catalase
CCL ₄	Carbone tetrachloride
CCl ₄	Carbon tetra chloride
CMC	Carboxy Methyl Cellulose
CPCSEA	Committee for the purpose of control and supervision of experimental animals.
DC	Differential count
Dep.	Deposits
E	Eosinophil
ESR	Erythrocyte Sedimentation Rate
FPC	Few Pus Cells
FTIR	Fourier Transform Infrared Spectroscopy
GPx	Glutathione peroxidase
GSH	Reduced Glutathione
Hb	Haemoglobin
IAEC	Institutional Animal Ethical Committee

ICMR	Indian Council of Medical Research
ICP-OES	Inductively Coupled Plasma Optic Emission Spectroscopy
L	Lymphocyte
MDA	Malondialdehyde
OECD	Organisation for Economic Co-Operation & Development
P	Polymorphs
SC	Sangu Chunnam
SEM	Scanning Electron Microscope
SEM	Standard error mean
SGOT	Serum Glutamic Oxaloacetic Transeaminase
SGPT	Serum Glutamic Pyruvic Transaminase
SOD	Superoxide Di Mutase
TA	Total Protein
TB	Total Bilirubin
TBARS	Thiobarbituric acid reactive substances
TC	Total count
TP	Total Protein
UV	Ultra Violet
WHO	World Health Organization
XRD	X-Ray Diffraction studies

INTRODUCTION

Liver is one of the most important and massive visceral organ present in the substantial portion of abdomen. It is also known as "Hepar" made up of hepatocytes which carry out multiple metabolic processes essential for life ^[1]. Liver acts as both secretory and excretory organ and so is described as the central laboratory of the body as it participates in the maintenance of homeostasis by controlling all types of metabolism ^[2].

Liver is considered to be one of the most vital organ that function as a centre of metabolism of nutrients and also excretion of drugs, other xenobiotics from the body there by providing protection against foreign substances by detoxifying and eliminating them ^[3].

As Liver mounts an immune response to portal pathogens, clinical consequence of severe Liver are therefore wide ranging and can be life threatening either suddenly as a result of our whelming acute Liver injury or gradually at the end stage of chronic Liver diseases.

Jaundice which is the one of the symptom of Liver disease 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 ^[4].

World Health Organisation defined health in its broader since in 1948 constitution as "A state of complete physical, mental and social wellbeing and not merely the absence of disease of infirmity" ^[5].

According to the latest WHO data published in May 2014, Liver disease deaths in India reached 2,16,865 or 2.44% of total deaths. A sedentary life style, increased alcohol consumption and obesity are contributing towards making India the world capital of Liver diseases by 2025. Therefore Liver disease worldwide has been recognized as a major health burden ^[6].

Conventional drugs used in the treatment of Liver diseases or sometimes

inadequate and can have serious adverse effects. Although there are lot of drugs available in modern medicine throughout the world,

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.

The researches have been in continuous search for some effective therapy for restoring the Liver functions^[7]. A large number of medicinal plants exist in nature of which are yet to be explored and validated for their potency and medicinal value^[8].

Siddha is one of the oldest system of medicine practiced in India have been conceived by Siddhars or evolved souls who lived in the past. The word "Siddha" comes from "Siddhi" which means an object to be attained or perfection of heavenly bliss.

Siddha system of medicine is an ancient system of medicine found by Siddhars through their high intellectual, supernatural and spiritual powers. They lived for thousands of years and formulated many medicines for a healthy life. They strongly believed that healthy body is essential to attain the eternal life. Thus the great Siddhar Thirumoolar has written in his song as

“உடம்பார் அழியின் உயிரார் அழிவர்

திடம்பட மெய்ஞானம் சேரவும் மாட்டார்

உடம்பை வளர்க்கும் உபாயம் அறிந்தே

உடம்பை வளர்த்தேன் உயிர்வளர்த் தேனே^[9]”

- திருமூலர்

So the physical and mental wellbeing is more important and Siddhars have gifted them to be mankind to lead a healthy life. Healthy lifestyle includes all day to day activities and food habits.

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.

Siddhars were great doctors of medicine, philosophers, and men with deep knowledge.

Very basic concept of Siddhars is

“ உணவே மருந்து; மருந்தே உணவு ”

Food is being medicine and medicine is being food

Siddhars denotes the jaundice as *Kamalai* in their poetries. The drugs which were indicated for *Kamalai* (jaundice) explore the hepatoprotective activity in research.

Herbal drugs are most widely used than Allopathic drugs as Hepatoprotective because of their efficacy, better compatibility to maintain the normal functional status of Liver without side effects^[10]

A large number of medicinal plants have been tested and found to contain active principles with curative properties against a variety of diseases^[11]. Liver protective plants contains a variety of chemical constituents like phenols, coumarins, ligans, essential oil, monoterpenes, carotenoids, glycosides, flavonoids, organic acids, lipids, alkaloids and xanthenes^[12]. Recent experience has shown that plant drugs are relatively non-toxic, safe and even from serious side effects^[13].

Some of the most valuable plants used for the treatment of liver problems are *Phyllanthus amarus*, *Silybum marianum*, *Glycyrrhiza glabra*, *Curcuma longa*, *Terminalia chebula*, *Picrorhiza kurroa*^[14].

One of the simple forms of internal medicine in Siddha system is Choornam. Choornam are fine dry powders of drugs. The term Choornam may be applied to the powder of a single drug or a mixture of two or more drugs which are powdered separately prior to their being mixed to homogeneity. The Choornam should be very fine, amorphous and should be perfectly dry before the Choornam is prepared the process of purification as to be done.

Purification of raw drugs is a process aimed at both purification as well as concentration of the raw drug. It usually involves process like cleaning, frying, soaking and grinding with herbal juices until impurities are removed. No medicinal preparation is done without prior suddhi process. This process helps raw material or crude drugs to lose their undesirable or toxic effects and thereby giving better efficacy^[15].

One among the Choornam to treat liver disease is "*Pitha Kamalai Choornam*" which is taken from the compiling of siddha medicines written by one of the great siddhar "korakkar" from his "*Korakkar maruththuvam*"^[16].

Pitha kamalai choornam consist of *Piper nigrum*, *Cuminum cyminum*, *Curcuma longa*, *Feniculum vulgare*, *Zingiber officinale* which acts as Hepatoprotective and Anti oxidant activities.

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

Despite tremendous advances in modern medicine, hepatic disease remains a worldwide health problem, thus the search for new medicines is still ongoing. As numerous formulations of medicinal plants are used to treat Liver disorders in Siddha medicine is available, I therefore exhibit "*Pitha Kamalai Choornam*" which is a poly herbal combination of drugs to undergo various significant hepatoprotective study so as to govern by standards of safety and efficacy^[17].

2. AIM AND OBJECTIVE

Aim

The present investigation was aimed to validate the safety and efficacy of the Siddha herbal formulation '*PITHA KAMALAI CHOORNAM*' for its Hepatoprotective activities in Wistar albino rats and Anti-oxidant activity in *in vitro* study.

Objectives

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

- Collection of significant text from classical Siddha literature as well as Modern medical science literature that supported this study.
- Description of pharmacognostic features of the plants in this formulation including the taxonomic identification, collection, purification of raw materials etc.
- Preparation of the formulation according to the procedure described in Siddha literature.
- Standardization of the trial drug by means of physico-chemical analysis, phyto chemical analysis.
- Revealing the anions and cations present in the drugs through adjacent chemical analysis.
- Elucidation of the chemical structure, microscopical structure of the drugs by means of instrumental analysis.
- Evaluating the microbial lode, and activity of the trail drug.
- Interpreting the results of acute and repeated 28 days oral dose toxicity of *Pitha Kamalai Choornam* according to OECD guidelines 423 and 407.
- Detailing the study of pharmacological activities like CCL₄,induced hepato toxicity, Paracetamol induced hepato toxicity activity of the trial drug *Pitha Kamalai Choornam* in Wistar albino rats, and Antioxidant activity in *in vitro* study.

3. REVIEW OF LITERATURE

3.1 GUNAPADAM ASPECT

பித்த காமாலை சூரணம்

“பாரப்பா பித்தகா மாலைசூரணத்தைப்
 பாடுகிறேன் புலத்தியனே பண்பாய்க் கேளும்
 சீரப்பா திரிகடுகு வகைக்கு மைங்காய
 சீரகமிரண் டரையாம் மஞ்சளோடு ஓமம்
 சேரப்பா வகைக்குமுறை சீர கந்தான்
 பேருட முக் கழஞ்சிவையும் ஒன்றாய்க் கலந்து
 பாரப்பா எலுமிச்சஞ் சாற்றி லாட்டி
 கறையவைத்தே மூன்றுதினம் இப்படியே செய்யே!”

“செய்வதுதா னரைபலந்தா னப்பாய்த் தெல்லாம்
 திறமாக உலர்த்தியே தூளாக்கி வடித்து
 உய்வதற்கு யெட்டொன்று சீனிகலந் திட்டு
 ஒடுங்காத பித்தகா மாலைக்கு
 உய்யவெரு கடியாய் கொள்ளு கொள்ளு
 இருபோது மண்டலமுண் டனுபானங் கேளு
 நய்வதில்லைப் பழச்சாறு தேனிலே யருந்து
 நலமான யிஞ்சிதென்ற நீரிலு மருந்தே!”

“அருந்தினால் தீருகிற நோய்வகை யப்பா
 அறைகிறேன் பித்தகா மாலைவகை யாறும்
 பொருந்தவே சொல்லுகிறோம் வெள்ளைமஞ்சள் ரத்தம்
 பொல்லாத நீரோடும் வறட்சியிது வெல்லாம்
 தீராத லுதுகா மாலையோடு யிவைகள்
 தீர்ந்துபோம் பத்தியந்தான் சிறுகீரை யுப்பே!^[18] “

- கோரக்கர் மருத்துவம்

Following are the Ingredients mentioned in the song

1. Chukku (*Zingiber officinale*)
2. Milagu (*Piper nigrum*)
3. Thippili (*Piper longum*)
4. Seeragam (*Cuminum cyminum*)
5. Manjal (*Curcuma longa*)
6. Omam (*Trachyspermum ammi*)
7. Poondu (*Allium sativum*)
8. Perunjeeragam (*Foeniculum vulgare*)
9. Vendhayam (*Trigonella foenum-graecum*)

10. Perungayam (*Ferula asafoetida*)

11. Kaduku (*Brassica juncea*)

Associate Ingredient

Lemon juice (*Citrus lemon*)

Chukku (*Zingiber officinale*)

Synonyms : Arukkan, artharagam, ubakullam, ularndha inji, kadubathiram, sundi, nagaram, vidamoodia amirdham, verkombu.

Parts used : Dried root

Taste : Pungent

Character : Hot

Division : Pungent

Action : Stimulant, stomachic, carminative



General properties:

“தலைமந்தம் நெஞ்செரிப்பு தோடமேப் பம்மழலை
மூலம் இரைப்பிருமல் மூக்குநீர் - வாலகப
தோடமதி சாரந் தொடர்வாத குன்மநீர்த்
தோடம்ஆ மம்போக்குஞ் சுக்கு.”

- அகத்தியர் குணவாகடம்

Indications:

Chukku cures Indigestion, Cough, Bronchial asthma, Sinusitis, Anemia, Cold and Fever.

Medicinal uses:

- Chewing chukku in mouth cures dental pain.
- Swallowing chukku juice after chewing will cure tonsillitis.
- Chukku powder when taken along with cow's milk is a good appetiser and also cures **jaundice**.
- Decoction of chukku cures indigestion, vomiting, chronic fever^[19A]

Milagu (*Piper nigrum*)

Synonyms	:	Kari, kayam, kolagam, masham, thirangal, malayali.
Parts used	:	Seed, climbing stem
Taste	:	Bitter, pungent
Character	:	Hot
Division	:	Pungent
Action	:	Acrid Carminative Antiperiodic Antidote Anti vatha



General properties:

“சீதசுரம் பாண்டு சிலேத்மங் கிராணிகுன்மம்
வாதம் அருசிபித்தம் மாமூலம் - ஓதுசன்னி
யாசமபஸ் மாரம் அடன்மேகம் காசமிவை
நாசங் கறிமிளகினால்”

- அகத்தியர் குணவாகடம்.

Indications:

Milagu cures Cold and Fever, Peptic ulcer, Indigestion, **Jaundice**, Piles, Genital disease, Hemiplegia.

Medicinal uses:

- Black pepper is one among ‘ thirikadugam’
- Powder of black pepper acts as appetiser
- Black pepper oil cures vatha disease, head disease, ear disease
- Black pepper, perunjeeragam along with honey cures piles^[19B].

Thippili (*Piper longum*)

Synonyms	:	Aargadhi, Kaman, kudari, kolagam, koli, kozhaileruki, adhimarundhu.
Parts used	:	Fruit

Taste	:	Sour
Character	:	Heat
Division	:	Sour
Action	:	Stimulant Carminative Expectorant



General properties:

“ஈளை யிரும லிரைப்புப் பசப்பிணிகள்
மாள வொழியாமல் வாட்டுமே - யாளுமுறை
பாங்கா யறிந்துசெய்வீர் பண்டிதத்தைப் பண்டிதரே
வேங்கைவாய்ப் பான்கணை மெய்.”

- தேரன் வெண்பா

Indications:

Long pepper cures Cold, Cough, Bronchial asthma and Indigestion.

Medicinal uses:

- Long pepper given along with betal leaf juice and honey cures cold and fever
- Long pepper powder boiled in cow's milk when taken cures delirium
- Equal quantity of long pepper powder and chebulic powder, when taken on both morning and evening along with honey for three months cures Tuberculosis^[19C].

Omam (*Tachyspermum ammi*)

Synonyms	:	Asamodham, thippium
Parts used	:	Seed
Taste	:	Sour
Character	:	Heat
Division	:	Sour



Action : Stomachic
Antispasmodic
Carminative
Tonic
Antiseptic

General properties:

“சீதசுரங் காசஞ் செரியாமந் தம்பொருமல்
பேதியிரைச் சல்கடுப்பு பேராமம் - ஓதிருமல்
பல்லொடுபல் மூலம் பகமிவைநோ யென்செயுமோ?
சொல்லொடுபோம் ஓமமெனச் சொல்.”

- அகத்தியர் குணவாகடம்

Indications:

The bishops weed cures Cough, Asthma, Indigestion, Gastritis, Dysentery and Cholera

Medicinal uses:

- About 30-60ml of omatheenir cures indigestion, dysentery and cholera
- 1 to 3 drops of bishops weed oil acts as carminative
- About 34gms of bishops weed and black pepper along with jaggery, when given twice a day for 10 days cures stomach ache and dysentery.^[19D]

Seeragam (*Cuminum cyminum*)

Synonyms : Asai, seeri, pithanasini, posanakudori, methiyam

Parts used : Seed

Taste : Sour, sweet

Character : Cooling

Division : Sweet

Action : Carminative
Stomachic
Astringent



General properties:

“ வாயுவொடு நாசினோய் வன்பித்தஞ் சேராது
காயம் நெகிழாது கண்குளிருந் - தூயமலர்க்
காரளகப் பெண்மயிலே! கைகண்ட தித்தனையுஞ்
சீரகத்தை நீதினமுந் தின்.

- அகத்தியர் குணவாகடம்.

Indications:

Cumin seed cures Vatha diseases, Nasal diseases, balances pitha kutram, gives coolness to eyes.

Medicinal uses:

- Cumin seeds alone when powdered and taken with sugar cures cough.
- Cumin seeds soaked in *eclipta alba* juice, when powdered and about 4gm taken along with 2gm of sugar and dried ginger powder cures fever and **jaundice**.
- Powdered cumin seeds when taken with butter cures peptic ulcer.
- Add cumin seeds in 1400ml of heated gingely oil, and then cool it. This oil when taken for head bath cures headache, eye diseases, giddiness and vomiting.
- Cumin seeds are also one of the ingredients of panchadeepakini legiyam^[19E].

Perunjeeragam (*Foeniculum vulgare*)

Synonyms : Sombu, venseeragam

Parts used : Flower, seed, root

Taste : Aromatic, sweet and sour taste

Character : Heat

Division : Sour

Action : Carminative

Stomachic



General properties:

“ யோனிநோய் குன்மம் உருட்சைமந் தம்பொருமல்
பேனமுறு காசம் பீலிகமிரைப் - பீனஉரை
சேர்க்கின்ற வாதமுபோஞ் சீர்பெரிய சீரகத்தால்
மூக்குநோ யில்லை மொழி.”

- அகத்தியர் குணவாகடம்.

Indications:

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

Medicinal uses:

- Sombutheenir: 1 part of Anise seed is mixed with 20 parts of water and allowed to the process of distillation. Dose : 15ml to 20ml.
- Anise seed is slightly fried and powdered. About 2gms of this powder along with sugar cures uterine disorders, abdominal pain.
- Distilled water taken from anise seeds flower, when given for children cures indigestion, worms and loose stools ^[19F].

Manjal (*Curcuma longa*)

Synonyms : Arisanam, kansani, nisi, peetham

Parts used : Rhizome

Taste : Sour, bitter

Character : Heat

Division : Sour

Action : Aromatic
Antiseptic
Carminative
Stimulant
Hepato tonic



General properties:

“பொன்னிறமாம் மேனி புலானாற்ற மும்போகும்
மன்னு புருட வசியமாம் - பின்னியெழும்
வாந்திபித்த தோடமையம் வாதம்போந் தீபனமாங்
கூர்ந்தமஞ்ச ளின்கிழங்குக்கு. ”

- அகத்தியர் குணவாகடம்.

Indications:

Turmeric paste applied in the body gives colour and changes odour. It also serves as appetiser. It cures Headache, Sinusitis, Leucorrhea, Rhinitis, Insect bites and Wounds.

Medicinal uses:

- Turmeric powder cures wound when applied over it.
- Turmeric mixed along with cooked rice and applied over tumour, gets healed.
- Smoke of burnt turmeric cures sinusitis.
- Paste of Turmeric and neem leaves cures chicken pox boils when applied.
- Paste of Adathoda leaves and turmeric cures certain skin diseases^[19G].

Poondu (*Allium sativum*)

Synonyms : Lasunam, kayam, ulli, vellai poondu, velvenkayam.

Parts used : Bulb

Taste : Sour

Character : Heat

Division : Sour

Action : Carminative
Stomachic
Tonic
Stimulant
Expectorant



General properties:

“சன்னியொடு வாதந் தலைநோவு தாள்வலி
மன்னிவரு நீர்க்கோவை வன்சீதம் - அன்னமே!
உள்ளுள்ளி கண்பாய் உளைமுல ரோகமும்போம்
வெள்ளுள்ளி தன்னால் வெருண்டு ”.

- அகத்தியர் குணவாகடம்.

Indications:

Garlic cures all the three Vatha, Pitha, Kabha diseases. Also cures Cough, Gastritis, Piles and Dysentery.

Medicinal uses:

- Equal quantity of garlic, pepper and eclipta leaf when taken regularly cures indigestion.
- About 20-30 drops of garlic juice taken for 2-3 times a day cures cough, bronchial asthma and worms.
- Garlic also cures tonsillitis^[19H].

Vendhayam (*Trigonella foenum graecum*)

Synonyms : Mendhiyum, medhi, vendhai.

Parts used : Leaf, seed

Taste : Bitter

Character : Cooling

Division : Sour

Action : Diuretic
Refrigerant
Laxative
Demulcent
Tonic



General properties:

“பித்தவுதி ரம்போகும் பேராக் கணங்களும்போம்
அத்திசுரந் தாகம் அகலுங்காண் - தத்துமதி
வேக இருமலொடு வீரு கயம்தணியும்
போகமுறும் வெந்தயத்தைப் போற்று ”.
- அகத்தியர் குணவாகடம்.

Indications

Cures body heat, Fever, Dysentery, Leucorrhoea, Blood pressure, Thirst.

Medicinal uses:

- Soak fried and dried fenugreek seeds in water and when taken regularly cures indigestion, heat, fever, leucorrhoea and dysentery.
- 17gm of fenugreek seed cooked in rice and taken increases blood.
- Fried fenugreek seed taken along with wheat instead of coffee as beverages suppresses body heat^[19].

Perungayam (*Ferula asafoetida.Linn*)

Synonyms : Athiyagraham, ranam, ramadam, Gandhi, kayam,
sandhunasam, boodhanasam, valleegam.

Parts used : Resin

Taste : Bitter

Character : Heat

Division : Sour

Action : Carminative
Antispasmodic
Expectorant
Diuretic
Anthelmentic



General properties:

“தந்தவே தந்த மூலத்தெழும்பிணி
சருவகாளம் விருச்சிகங்கீடம்மா
மந்தம்வாதம் உதாவர்த்தம் அல்குல்நோய்
மார்பணங்கட்ட குன்மம்மகோதரம்
உந்துகெர்ப்பத்தின் வித்திரஞ்சுலைச்சூர்
உதிரப்பூச்சி சிலேத்துமத்துறும்வலி
வந்தமெய்க்கடுப் போடிவைமுற்றுமே
மாயுநாறுநற் காயங்கிடைக்கிளே ”.

- தேரன் குணவாகடம்.

Indications:

Cures Gastritis, Indigestion, Uterine disorders, Ascites, Worms and Vatha diseases

Medicinal uses:

- Asafoetida given along with egg to cure dry cough.
- Equal quantity of aloe juice, pepper and asafoetida cures amenorrhoea.
- Asafoetida heated with gingely oil and used as ear drops cures ear ache.^[19]

Kaduku (*Brassica juncea*)

Synonyms : Iyavi

Parts used : Seed

Taste : Sour

Character : Heat

Division : Sour

Action : Emetic
Digestive
Stimulant
Rubefacient



General properties:

“இடிகாச நாசிச்சூ ரீளைகபம் பித்தங்
கடிவாத சீதங் கடுப்போ - டுடலிற்
படுகோட்டு நோயென்னும் பங்கிவைக ளைப்புண்
கடுகோட்டு மேன்மருந்து காண்”.

- அகத்தியர் குணவாகடம்

Indications

Cures Cough, Bronchial asthma, Pitha disease, Vatha disease Kabha disease and Ulcer in the body.

Medicinal uses :

- About 2gm of mustard seed pasted and given along with water induces vomiting.
- When pasted along with honey and given cures cough and Asthma.
- Mustard oil is slightly irritative and creates blisters in skin when applied^[19K].

Associate ingredient:

Elumichai (*Citrus lemon*)

Synonyms : Sambeeram

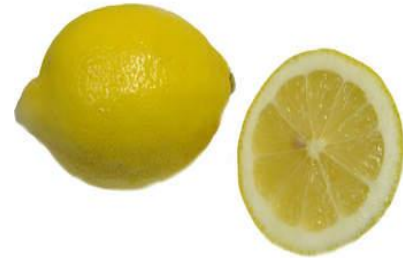
Parts used : Leaf, fruit, juice, oil

Taste : Pungent

Character : Heat

Division : Sour

Action : Refrigerant



General properties:

“ தீதெலு மிச்சங்காய் டேர்முத்தோ டத்தையுமுள்
வாதகப சூலையையும் மாகொடிய - சாதியெனுஞ்
சர்த்திகுன் மத்தையுமுள் தங்கமருந் திட்டதையும்
பித்தவெப்பை யுந்தணிக்கும் பேசு ”.

- அகத்தியர் குணவாகடம்.

Indications:

Balance all the three Vatha, Pitha, Kabha thodam. It also cures vomiting and peptic ulcer.

Medicinal uses :

- Lemon juice along with sugar cures nausea and vomiting.
- Lemon fruit when taken as kaya kalpam for 6 months prevents aging and also cures many diseases.
- Lemon juice and fried cumin seeds together cures vomiting and diarrhoea^[19L].

3.2. BOTANICAL ASPECT:

Zingiber officinale

Common name:

Chukku

Vernacular names:

- Hindi : Adarak
- Kan : Hasisunti, Ardraka
- Mal : Inci, Erukkilannu
- San : Ardrakam
- Tam : Chukku
- Tel : Allamu, Ardrakamu

Botanical classification:

- Kingdom : Plantae
- Division : Angiosperms
- Class : Monocotyledons
- Order : Epigynae
- Family : Zingiberaceae
- Genus : *Zingiber*
- Species : *Officinale*^[20A]

The plant description:

Distribution:

Cultivated throughout India and run wild in some places in the Western Ghats.

Description:

A slender, perennial rhizomatous herb leaves linear, sessile, glabrous. Flower yellowish. The rhizomes are white to yellowish brown in colour, irregularly branched, somewhat annulated and laterally flattened.

Parts used:

Rhizomes (raw as well as dry)

Chemical constituents:

Indian dry ginger contains an aromatic volatile oil of light yellow colour having a characteristic odour and containing camphene, phellandrene, zingiberine, uneol and borneol. Gingerol – an oleo resin other resins and starch^[24A]

Properties and uses:

- Dry ginger is acrid, thermogenic, emollient, appetiser, laxative, stomachic and carminative'
- It is useful in dropsy, otalgia, asthma, cough, colic, diarrhoea, flatulence, anorexia, dyspepsia, cholera, nausea, vomiting, elephantiasis and inflammation.
- Ginger with rock salt taken before meals, cleans the tongue and throat, increases the appetite and produces an agreeable sensation.
- Dry ginger is much used as a carminative adjunct along with black pepper and long pepper under the name Trikaduku.

Piper nigrum

Common name:

Milagu

Vernacular names:

- Eng : Black pepper
- Hind : Kalimark, mirc
- Kan : Ollimonasu, miri
- Mal : Kurumulaku
- San : Maricam
- Tam : Milaku
- Tel : Miriyalu^[20B]

Botanical classification:

- Kingdom : Plantae
- Division : Angiosperms
- Class : Dicotyledons
- Sub class : Monocotyledons
- Order : Microembryeae
- Family : Piperaceae
- Genus : *Piper*
- Species : *nigrum*

The plant description:

Distribution:

Throughout India in every green forest up to 1500m and also widely cultivated.

Description:

A branching and climbing perennial shrub that branches stoutly and roots are at the nodes. Leaves are entire and 12.5-17.5 long and 5.0-12.5cm wide. Fruits are globose or ovoid, bright red when ripe, seeds usually globose.

Parts used:

Fruits

Chemical constituents:

Phytochemical investigation on the plant revealed the presence of piperettine, piperolein A and piperolein B, trichostachinepiperlylin, feruperine, dihydroferuperine, N- trans feruloyltyramine, p- cymene, α – thujene, β - alanine, arginine, pipecolic acid, ascorbic acid, carotene.^[21A]

Properties and uses:

- The fruit has a sharp, pungent, slightly bitter taste, is a carminative, stamachic, aphrodisiac, purgative, alexipharmic.
- Dried fruits known as black pepper in market are used as aromatic, stimulant and stomachic.
- It is used in the treatment of dyspepsia, flatulence, malarial fever, **jaundice**, paraplegia and arthritis.
- They also find local application for sore throat, piles and skin diseases.
- It is used as antiperiodic in malarial fever and as an alterative in paraplegia and arthritic diseases.

Piper longum**Common name:**

Thippili

Vernacular names:

- Eng : Indian long pepper
- Hind : Pipli, papal
- Mal : Tippali
- San : Pippali, Tippili
- Tam : Pippili, Tippili
- Tel : Pippallu

Botanical classification:

- Kingdom : Plantae
- Division : Angiosperms
- Class : Dicotyledons

- Subclass : Monocotyledons
- Order : Microembryeae
- Family : Piperaceae
- Genus : *Piper*
- Species : *longum* ^[20C]

The plant description:**Distribution:**

Cultivated throughout India

Description:

A slender aromatic climber, root at the nodes, the branches are erect, subscaudent, swollen at the nodes. Leaves alternate, flowers in solitary spikes, fruit berries, small, red when ripe. The mature spikes collected and dried from the commercial form of thippili, roots are known as tippilimulam.

Chemical constituents:

The plant contains essential oil consisting of long chain hydrocarbons, mono and sesquiterpenes. Other constituents are piperine, pipartine, piperlongumine, piperlonguminine, and its dihydro- derivative, piperonaline, piperundecalidine, pipericide and guineensine, sesamin, β - sitostero ^[21B].

Properties and uses:

- The roots are bitter, thermogenic, tonic, diuretic, purgative, expectorant, anthelmintic, stomachic, digestive and emmenagogue.
- They are useful in vitiated conditions of vata, gout, lumbago, dyspepsia, apoplexy, stomachalgia and splenomegaly'
- They are useful in anorexia, flatulent, colic, asthma, bronchitis, hiccough, gastropathy, epilepsy, fever, gonorrhoea and haemorrhoids.
- It is an important ingredient in the preparation of medicated oil used externally in sciatica and paraplegia.
- Powdered long pepper mixed with honey is efficacious in cold, cough, asthma and hiccough.

Curcuma longa

Common name:

Manjal

Vernacular names:

- Eng : Turmeric
- Hind : Haldi, halda
- Kan : Arisina
- Mal : Mannal, paccamannal, varattumannal
- San : Haridra, varavarnini
- Tam : Mancal
- Tel : Pasupu

Botanical classification:

- Kingdom : Plantae
- Division : Angiosperms
- Class : Monocotyledons
- Order : Epigynae
- Family : Zingiberaceae
- Genus : *Curcuma*
- Species : *longa* ^[20D]

The plant description:

Distribution:

Cultivated throughout India

Description:

A perennial herb, 60-90cm in height, with a short stem and tufts of erect leaves, rhizome cylindrical, ovoid, orange coloured and branched. Leaves are simple, very large.

Parts used:

Rhizomes (dry as well as raw)

Chemical constituents:

An essential oil, resin, an alkaloid, curcumin, turmeric oil or turmerol. Turmeric oil contains phellandrene and an alcohol called turmerol. It also contains caproic acid with combined valeric acid [24B].

Properties and uses:

- The roots are bitter, thermogenic, tonic, diuretic, stomachic, digestive and emmenagogue, **hepatoprotective**.
- They are useful in vitiated conditions of vata, gout, lumbago, dyspepsia, apoplexy, stomachalgia and spleenopathy.
- They are useful in anorexia, bronchitis, hiccough, gastropathy, epilepsy, fever, gonorrhoea, haemorrhoids.
- It is used both internally and externally in skin diseases due to impurity of the blood.
- In small pox and chicken pox a coating of turmeric powder or thin paste is applied to facilitate the process of scabbing.

Cuminum cyminum

Common name:

Seeragam

Vernacular names:

- Eng : Cumin seeds
- Hin : Jira, saphedjira
- Kan : Jirige
- Mal : Jirakam
- San : Jiraka
- Tam : Jirakam
- Tel : Jilakarra

Botanical classification:

- Kingdom : Plantae
- Division : Angiosperms
- Class : Dicotyledons
- Sub class : Polypetalae
- Series : Calyciflorae
- Order : Umbellales
- Family : Umbelliferae
- Genus : *Cuminum*
- Species : *cuminum* ^[20E]

The plant description:

Distribution:

Cultivated throughout India

Description:

A small slender glabrous annual herb about 30cm in height with much branched angular or striated stem, leaves bluish green, two or three partite. Flowers are small white or rose coloured in compound umbels. Fruits are greyish tapering towards both ends.

Parts used:

Fruits

Chemical constituents:

Fatty oil, resin, mucilage, a valuable essential oil thymine contains cuminol or cumic aldehyde 56 p.c, a mixture of hydro carbons, cyme or cymol, terpene etc ^[22A].

Properties and uses:

- Seeds are carminative, aromatic, stomachic, stimulant and astringent.
- Cumin seeds are largely used as a condiment or spice in curries, pickles etc.

- They are medicinally useful in hoarseness of voice, dyspepsia and chronic diarrhoea and cures **jaundice**.
- Seeds are also cooling in effect and therefore form an ingredient of most prescriptions for gonorrhoea.
- Seeds mixed with lime juice are administered in cases of bilious nausea in pregnant females and after child birth, cumin seeds taken internally increases milk secretion.

Foeniculum vulgare

Common name:

Perunjeeragam

Vernacular names:

- Eng : Fennel
- Hind : Saumph
- Kan : Badhesoppu
- Mal : Perunjirakam
- Tam : Sompu
- Tel : Peddajilakarra

Botanical classification:

- Kingdom: Plantae
- Division : Angiosperms
- Class : Dicotyledons
- Sub class : Polypetalae
- Series : Calyciflorae
- Order : Umbellales
- Family : *Foeniculum*
- Species : *vulgare* ^[20F]

The plant description:

Distribution:

Cultivated throughout India

Description:

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

Parts used:

Fruits

Chemical constituents:

Ascorbic acid, riboflavin, α , β and γ tocopherol, β tocotrienol, choline, trigonelline, p- cymene, anethole, anisaldehyde, camphene, estragole, fenchone, fenchene, foeniculin, methylchavicol, chlorogenic, hydroxyl benzoic and hydroxyl cinnamic acids ^[22B].

Properties and uses:

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

Ferula asafoetida

Common name:

Perungayam

Vernacular names:

- Eng : Asafoetida
- Hind : Hing
- San : Hingu
- Kan : Hingu
- Mal : Kayam
- Tam : Perunkayam

Botanical name:

- Kingdom: Plantae
- Division : Angiosperms
- Class : Dicotyledons
- Sub class: Polypetalae
- Series : Calyciflorae
- Order : Umbellales
- Family : Umbelliferae
- Genus : *Ferula*
- Species : *asafoetida* ^[20G]

The plant description:

Distribution:

Cultivated in the north western part of India

Description:

The trees are attaining a height of 2-3m in length. Stem stout and much branched. Leaves are puberulous and minutely glandular or somewhat tomentose, the radical one is large and ternatisect, with segments oblong lanceolate and obtuse. Umbels on fleshy peduncles, the flower yellow. Flowers in april- may and fruits later.

Parts used:

Stem, leaves, root and gum resin.

Chemical constituents:

Luteolin and its glucopyranoside (fruits), seven S containing compounds in essential oil, α - pinene, phellandrene, butyl propenyldisulfide, a trisulfide, asaresinotannol, farnesiferol A, gummosin, kamolonol, mogoltadone, polyanthinin, polyanthin, umbelliferone, acetic acid. ^[22C]

Properties and uses:

- It is used as anti spasmodic, anthelmintic, aphrodisiac, carminative, diuretic, emmanogogue, expectorant, mild laxative and nervine tonic.

- It is useful in remedy for asthma, bronchitis, croup, flatulence, colic pain, spasmodic movement of the bowels and infantile convulsions.
- An important ingredient in compounding medicinal preparations, prescribed in diarrhoea, flatulence, habitual abortion, indigestion and **liver troubles**.
- Applied externally in ring worm.
- It is valuable remedy for hysteria and nervous disorders of women and children and in the advanced stages of whooping cough, pneumonia and bronchitis of children.

Trigonella foenum graecum

Common name:

Vendhayam

Vernacular names:

- Eng : Ferugreek
- Hind : Meti, mutti
- San : Methika, methi
- Mal : Uluva
- Tam : Ventayam
- Tel : Mentulu, mentikura

Botanical classification:

- Kingdom: Plantae
- Division : Angiosperm
- Class : Dicotyledons
- Sub class : Polypetalae
- Order : Thalamiflorae
- Series : Parietales
- Family : Cruciferae
- Genus : *Brassica*
- Species : *juncea* ^[20H]

The plant description:

Distribution:

Grown in Kashmir, Punjab and the upper gangetic plains and widely cultivated in many parts of India.

Description:

It is an erect aromatic annual herb of about 30-60 cm tall. Leaves pinnate, 3-foliolate, flowers axillary, 1 or 2, white or yellowish white, seeds 10-20 oblong with a deep groove across one corner, greenish brown. Flowers during January and fruits during march.

Parts used:

Seeds and leaves

Chemical constituents:

Young seeds mainly contain carbohydrates and sugar, mature seeds yield amino acids and fatty acids on hydrolysis, Carotenes, vitamins, saponins. fenugrin B and sapogenins such as diosgenin, gitogenin, neogitogenin, homoorientin, saponaretin and hipogenin, alkaloids trigonelline and choline.^[23]

Properties and uses:

- It is used as mucilaginous, demulcent, diuretic, tonic, carminative, emmanagogue, astringent, emmolient and aphrodisiac.
- It is useful in remedy for colic, flatulence, dysentery, diarrhoea, anorexia, cough, dropsy, **enlargement of liver** and spleen.
- Used to treat rheumatism, lymphatism, rickets, anaemia and diabetes.
- The alkaloids of fenugreek seeds stimulate the appetite by their action on the nervous system or reduce a diuretic or ureo-poetic effect

Trachyspermum ammi

Common name:

Omam

Vernacular name:

- Eng : Ajowan
- Hind : Ajowan
- San : Java
- Beng : Jowan
- Tamil : Omam

Botanical classification

- Kingdom : Plantae
- Division : Angiosperms
- Class : Dicotyledons
- Sub class : Polypetalae
- Order : Calyciferae
- Family : Umbelliferae
- Genus : *Trachyspermum*
- Species : *ammi*^[20]

The plant description:

Distribution:

Extensively cultivated over Punjab and West Bengal in India

Description:

It is a Pubescent or glabrescent herb. Root rusiform stem. Erect branched and leafy. Leaves are 2-3 pinnate, ultimate segments linear. Flowers are white, polygamous and sterile. Fruits are ovoid. Flowers and fruits from January to april.

Parts used:

Fruits, leaves and root.

Chemical constituents:

Camphene, careen, carvacrol, p-cymene, dipentene, myrcene, α and β pinenes, phenol, α and β phellandrene, γ terpinene, thymol, linoleic acid, oleic, palmitic,

petroselinic acids, resin acids, nicotinic acids, riboflavin, protein, sugars, tannins, flavones and sterol isolated from fruits. Phenolic glycosides from seed.^[22D]

Properties and uses:

- It is used as anti diarrhoeal, antiseptic, antispasmodic, carminative, stimulant, stomachic and tonic.
- Ajwan- ka – arak (aqueous extract) is a popular remedy for diarrhoea.
- It is beneficial in bronchitis, atonic, dyspepsia, flatulence, hysteria and sore throat.
- Oil is antiseptic, carminative and expectorant, efficacious in bronchial pneumonia other respiratory disorders.

Allium sativum

Common name:

Poondu

Vernacular names:

- Eng : Garlic
- Hind : Lasun
- Kan : Belluli
- Mal : Velluli
- San : Lasunah
- Tam : Vellaipuntu
- Tel : Velluli

Botanical classification:

- Kingdom : Plantae
- Division : Angiosperms
- Class : Monocotyledon
- Order : Thalamyflorae
- Series : Coronarieae
- Family : Lilliaceae
- Genus : *Allium*
- Species : *sativum*^[20J]

The plant description:

Distribution:

Cultivated throughout India

Description:

A scapigerous foetid perennial herb with underground compound bulbs covered over by outer white thin scales and with simple, smooth, round stem, surrounded at the bottom by tubular leaf sheath, leaves simple, long, flat, linear, flowers small, white in the rounded umbels mixed with small bulbils, the entire umbel enclosed in a teardrop shaped membranous spathe, flowers usually sterile.

Parts used:

Bulbs

Chemical constituents:

An acrid volatile oil which is the active principle, starch, mucilage, albumen, sugar etc, volatile essential oil obtained by distilling the bruised bulbs contained allyl, propyl disulphide and other organic sulphides or sulphur compounds. It is a clear dark brown or yellow colour of very intense garlic odour of repugnant taste ^[24C].

Properties and uses:

- The bulbs are acrid, bitter, sweet, astringent, salty, thermogenic, aperients, anodyne, aphrodisiac, anthelmintic, expectorant, febrifuge, diuretic, alexeteric, emmenagogue and tonic.
- They are useful in vitiated conditions of kapha and vata.
- It has a special influence over the bronchial and pulmonary secretions, useful in cases of dilated bronchi with fetid expectoration.
- It improves appetite, helps in gaining weight, and renders sleep regular.
- It acts as an emmenagogue it promotes the flood of menses, tonic, carminative and stimulant of the skin and kidneys.

Brassica juncea

Common name:

Kadugu

Vernacular names:

- Eng : Indian mustard
- Hind : Rayi
- Kan : Sassive
- Mal : Katuku
- San : Sarsapah
- Tam : Katugu
- Tel : Avalu

Botanical classification:

- Kingdom : Plantae
- Division : Angiosperm
- Class : Dicotyledons
- Sub class : Polypetalae
- Order : Thalamiflorae
- Series : Parietales
- Family : Cruciferae
- Genus : *Brassica*
- Species : *juncea* ^[20K]

The plant description:

Distribution:

Cultivated throughout India

Description:

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

Parts used:

Seeds, oil

Chemical constituents:

Seeds contain a bland fixed oil, sinapin, sulphocyanide, lecithin, mucilage [24D].

Properties and uses:

- The seeds are acrid, bitter, thermogenic, anodyne, anti inflammatory, carminative, digestive, anthelmintic, aperients, sudorific and tonic.
- They are useful in vitiated conditions of vata and kapha.
- It is used in dengue fever, abdominal colic, anorexia, dyspepsia, intestinal worms, flatulence, inflammations, and morbid state of the cerebrospinal system, skin diseases, splenomegaly and persistent vomiting.
- Mustard is used in larger doses as an emetic in cases of poisoning.
- It will also cause hyperdipsia, burning sensation and other disorders due to the vitiation of pitha.

Associate ingredient:

Citrus lemon

Common name:

Lemon

Vernacular names:

- English : Lime
- Beng. : Kagzilebu
- Guj. : Khata limbu
- Hindi : Kaghzi nimbu
- Kan. : Nimbe
- Mal. : Cherh-naragam
- Tam. : Elurmichai
- Tel. : Nimba.

Botanical classification:

- Kingdom : Plantae
- Division : Angiosperm

- Series : Disciflorae
- Family : Rutaceae
- Genus : *Citrus*
- Species : *Lemon* ^[20L]

The plant description:**Distribution:**

Cultivated all over India

Description:

An armed, straggling shrub or tree; leaves elliptic-oval, petiole winged flowers white or pink in lax racemes; hesperidium greenish yellow, round to oval, rind paper-like, aromatic, oil glands conspicuous; seeds oval, pointed.

Phytochemical constituents:

The major constituents of the distilled oil are d-limonene, α - and β - pinenes, γ -terpinolene, α -terpineol, β -bis-abolene, 1,4-cineol, terpinen-4-ol, and mycrene. Lemon juice contains citric acid, sugars, polysaccharides, Organic acids, Phosphoric and malic acids, lipids, carotenoid, vitamins and flavonoids.^[24 E]

Properties and uses:

- Lemon-juice prevents or restrains influenza, malaria and cold.
- Vitamin C in lemon strengthens the blood vessels and prevents internal haemorrhage. It is, therefore, extremely useful in high blood pressure, in which cerebro-vascular accidents commonly occur.
- Lemon juice is useful for people with heart problems. This is because of its high potassium content.
- It prevents vomiting and helps to **cure hepatitis**.

3.3 DISEASE REVIEW

3.3.1 Siddha Aspect Disease Review

Jaundice which is otherwise known as Kamila, kamalai, manjal kamalai is one among those diseases which occur due to derangement of pitha uyir thathu. This disease comprises of the yellow discolouration of eyes, tongue and whole body.

Aetiology:

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

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

Intake of variable food and other deeds stimulates *pttha thathu*, *pitha* increases in its strength, joins *kaba* and becomes *pitha kaba* factor. This factor spoils the spreading *vayu* (*paravu kal- Vyanan*) and prevents it from doing its normal work and thus it spoils the strength of blood. Because of this, liver gets affected and so the bile is not able to flow in its normal route as there is an obstruction.

Hence the bile mixes with the blood and jaundice occurs. The *vatha* factor gets affected and the disease occurs due to *pitha vatha* factor. Apart from this, the other *vayu* (gases) also get spoiled. Hence the duty of the doctor is to set right the altered *pitha kabam* and *pitha vatham*, in order to make the bile flow in its normal route and to increase the strength of blood by suitable treatment. The altered spreading *vayu* (gas) and other *vayu* (gases) should be brought to normal and made to do the normal regular work. Then medicines for the disease should be given.

Premonitory symptoms

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

“பருகவே உள்ளங்கா லுள்ளங் கைகள்
பகர்முகங்ண் ணுடம்புமிக வெளுப்பு காணுங்
கருகவே கால்கைக ளோய்ச்ச லாகுங்
கனமாக நடுக்கியே இளைப்புண் டாக்குஞ்
சுருகவே மலந்தானும் வறண்டு கட்டுந்
தூயமுக மஞ்சளித நிறம தாகும்
வெருகவே வீக்கமாய்க் களைப்புண்டாகும்
மிகக்காது மந்தந்தலை கனப்புண்டாகும்.”

-யுகிமுனி

Again, in this disease, palm, sole, face, eyes and the body will become pale; there would be severe fatigue in the extremities, shivering of the body, frequent dyspnoea, constipated and hardened faeces, and yellowish discolouration of face, oedema, lassitude, deafness and heaviness of head. [25]

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. *Mukutra kamalai*
7. *Manjal kamalai*
8. *Uthu kamalai*
9. *Varal kamalai*
10. *Azhagu kamalai*
11. *Sengamala kamalai*
12. *Kumba kamalai*
13. *Gunma kamalai*

Curable and Incurable

Among the 13 varieties, jaundice, seven are curable and eight types are incurable. Curable types include udhu kamalai, varal kamalai, manjal kamalai, pitha kamalai, kabha kamalai, vatha kabha kamalai, pitha kabha kamalai. The other varieties are not easily curable. [26]

Treatment for jaundice (Siddha aspect)

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:

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

A decoction of the above substances may be made and given for jaundice for laxative purpose. [27A]

Other medicines

Malakudara oil

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

Malakudara mezhugu

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

Thithippu bedi mezhugu

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.

Sanjivi tablets

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* tablet, *suka viresana* tablet, *jivarathina* tablet, *virechana bhupathy* and *lavangathy* tablet. When sanjivi tablet along with leaf juice of *Euphorbia nivula* (*ilaikkalli*) is given, vomiting and diarrhoea will be induced. Vomiting for two or three times will occur. Diarrohoea will also occur. Along with vomit or faeces, the bile fluid will also come out.

Marukkarai kaai

The unripened fruit of *Randia dumetorum* (*marukkarai kaai*) in its tender reddish form may be taken. It may be soaked in lime juice and leaf juice of *Euphorbia nivula*(*ilaikkalli*) for two days in each. Then it can be taken out and dried. This can be ground and made into powder. ½ pinches can be given in the morning alone. Diarrhoea and vomiting will occur. ^[27B]

Medicines for Jaundice

Kizha nelli ney:

- Juice of *Phyllanthus amarus* 1.35 liter (one measure)
- Cow's ghee 1.35 liter (one measure)

- *Piper cubeba* (*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. [28A]

Karisalai ney:

- Juice of *Eclipta* 1.35 litre (one measure),
- Cow's ghee 1.35 litre (one measure),
- *Thirikaduku* (dry ginger, pepper, long pepper) each 35 gm (one *palam*),
- *Hyoscyamus niger* (*kurosani omam*) 8.5 gm (1/4 *palam*),
- Cubeb (*valmilagu*) 17.5 gm (1/2 *palam*)

The above drugs are taken and the ghee may be prepared as described mix the above two and take in doses of 1/4 to 1/2 teaspoon two or three times a day with cow's milk or buttermilk or goat's milk or buttermilk.

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

Karisalai matthirai:

- *Eclipta alba* - one handful
- Black cumin
- Long pepper
- Pepper
- Garlic

Each 1/4 *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. Dry jaundice along with oedema will get cured. Tamarind and salt should be avoided ^[28B].

Kaiyanthakarai karkam:

Tender leaves of *Eclipta alba*, tender leaves of *Coldenia procumbens* (*seruppada*), turmeric and pepper- equal quantities of the above 4 substances may be taken and macerated on a stone mortar. A lime- sized paste may be consumed along with goat's urine. Jaundice and oedematous jaundice will be cured

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 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.

Ponnangani for dry type of jaundice:

The root of *Alternanthera sessilis* may be collected and macerated on a stone mortar. 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 with dryness will be cured.

Pirandai for jaundice:

Tender leaves of *Cissus quadrangularis*, *Piper nigrum*, *Acorus calamus*, *Zingiber officinale* – equal quantities of these ingredients may be taken and macerated on a stone mortar. An areca nut sized ball of this paste may be covered in rice bran and consumed.

Aridradhi Choornam:

Turmeric (*manjal*), pericarp of *Terminalia chebula* (*kadukkay thol*), pericarp of *Terminalia belerica* (*thandrikay thol*) and pericarp of Indian gooseberry (*nelli mulli*), *Pircorzhiazha kurroa* (*kadugurohini*), rock salt (*induppu*) equal quantities of the above

substances may be taken, dried and pounded in a stone mortar. One *verukadi* (cat's foot print) quantity is mixed with water and may be consumed. Jaundice will be cured.

Amanakkilai marundhu:

Tender leaves of castor plant (*amanakku 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 mortar. The paste may be mixed with buffalo curd and consumed. Jaundice will be cured ^[28C].

Arunelli for jaundice:

Phyllanthus distiches in the size of the fruit of *Alexandrian laurel* (*punnai kay*) may be taken and macerated on a stone mortar. 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 ^[28D].

Karisalai Choornam:

- 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 *Lawsonia 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

Jaundice powder (another process)

- Charred turmeric – one part
- Cubebs – 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* (1.3g to 1.9g) two to three times a day with cow's milk or goat's milk or honey or in orange juice. It can also used

as adjuvant to any other *chenduram* or any other medicines prescribed for jaundice. By this, anaemia, oedema and liver diseases will get cured [29].

***Nandiyavattam* for jaundice:**

Pericarp of the root of multiple-layered *Tabernaemontana divaricata*, 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 is consumed in a three finger pinch with hot water, jaundice, predominant *pitha* condition, and oedema can be cured.

Coconut medicine for jaundice:

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

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

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

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 ^[30].

Liver disease and herbal plants

Phyllanthus amarus

Hepatoprotective effects of aqueous extract from *Phyllanthus amarus* is evaluated on ethanol-induced hepato-toxic rats. Hepatic injury were studied in in-vitro model where *Phyllanthus amarus* increased the 3- [4,5-dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide (MTT) reduction assay and decreased the release of aspartate transaminase (AST) and alanine transaminase (ALT) in rat primary cultured hepatocytes treated with ethanol. The results revealed that treatment of rats with *Phyllanthus amarus* extract orally brought cell recovery in ethanol-induced liver injury by bringing the levels of Aspartate transaminase (AST), Alanine transaminase (ALT), High-sensitivity human thyroglobulin (HTG) and Tumor necrosis factor (TNF- α) to normal. Histopathological study confirmed the beneficial effect of *Phyllanthus amarus* with its potential antioxidant activity ^[31].

Eclipta prostrata

Therapeutic efficacy of *Eclipta prostrata* was evaluated using male Wistar rats and it was found that the curative effect of *Eclipta prostrata* was comparable to that of the standard drug Silymarin. The optimum effective dosage was found to be 200mg of ethanolic extract per kg body weight of the experimental animals used. In this study, the plant *Eclipta prostrata* was found to produce significant liver protection. The drug was standardized pharmacognostically and the optimum dosage was found to be 200 mg/kg body weight of the animal. Technically and economically feasible protocol was developed for optimization of the secondary metabolite of *Eclipta prostrata*.^[32]

Curcuma longa

Curcumin possesses hepatoprotective and choleric properties. Curcumin has been demonstrated in-vivo to prevent lipid peroxidation from diverse agents such as carbon tetrachloride, and Aflatoxin from *Aspergillus parasiticus*. In animal models, curcumin proved to be a potent choleric, increasing bile output by almost 100% in one study. ^[33]

Cuminum cyminum

Aqueous ethanolic extract of dried seeds of *Cuminum cyminum* revealed hepatoprotective activity in Nimesulide intoxicated albino rats. *Cuminum cyminum* extract reduced the level of liver markers ALP, SGOT, SGPT and TB. *Cuminum cyminum* extract at a dose level of 200 mg/kg, significantly ($p < 0.001$) reduced the level of all elevated parameters as compared to other doses. *Cuminum cyminum* extract in 300 mg/kg showed better reduction in the level of all the parameters as compared to the dose of 100 mg/kg but less than dose of 200 mg/kg^[34].

Terminalia chebula

Ethanolic extract of *Terminalia chebula* fruit showed strong hepatoprotective activity. It also showed similar property against anti-tuberculosis drug Rifampicin, Isoniazid and Pyrazinamide (combination) induced toxicity. This is because of its prominent anti-oxidative and membrane stabilizing activities. Protective effects of aqueous extract of *Terminalia chebula* fruit on the tert-butyl hydroperoxide-induced oxidative injury was observed in cultured rat primary hepatocytes and rat liver models^[35].

3.3.2 Modern aspect of diseases:

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^[36].

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 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.

Causes for Liver diseases

1. Infections
2. Autoimmune disorder
3. Chemical agents (certain antibiotics, peroxidised oil, aflatoxin, carbon tetra 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 to various infective and toxic substances. The condition can be self limiting, healing on its own, or can progress to scarring of the liver. A group of viruses had known as the hepatitis viruses' origin most cases of liver damage worldwide. Hepatitis can also be due to toxins (notably alcohol), other infections or from autoimmune process. ^[37]

Viral Hepatitis:

Viral hepatitis is the cause of most cases of acute hepatitis. Types include Hepatitis A, Hepatitis B, Hepatitis C, Hepatitis B with D, Hepatitis E, Hepatitis F virus (existence unknown), and Hepatitis G or GBV-C. Hepatitis A or infectious jaundice is affected by a picornavirus transmitted by the fecaloral route.

It causes an acute form of hepatitis and does not have a chronic stage. Hepatitis B is caused by a hepadnavirus, which can cause 500,000 to 1,200,000 deaths per year worldwide due to the complications of chronic hepatitis, cirrhosis, and hepatocellular carcinoma. Hepatitis C (originally "non-A non-B hepatitis") is caused by a virus with an RNA genome that is a member of the Flaviviridae family.

Hepatitis C may lead to a chronic form of hepatitis, culminating in cirrhosis. Hepatitis D is caused by hepatitis delta agent, which is alike to a viroid as it can only propagate in the presence of the Hepatitis B virus. Hepatitis E produces symptoms similar to hepatitis A. Hepatitis F virus is a hypothetical virus linked to hepatitis. Several hepatitis F virus candidates emerged in the 1990s; none of these reports have been substantiated. Another potential viral cause of hepatitis, initially identified as hepatitis G virus^[38] 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^[39]. 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.

Infective Agents

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

Toxic Agents

Chlorpromazine and other Phenothiazine derivatives, Monoamine oxidase inhibitors (MAO-inhibitors), Erythromycin, Tetracycline, INH, Rifampicin, Methyl

dopa, Chlorpropamide, Phenylbutazone, Indomethacin, Paracetamol, Thiouracil, Acetaminophen, Halothane, Alcohol, Carbon tetrachloride, etc.^[40]

Table: 1. List of Hepatotoxic therapeutic agents and chemicals:

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

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^[41].

Liver Cancer:

The liver is inclined to cancer induction by a variety of human made and naturally occurring chemicals. Chemical substances include, aflatoxin B, cycasin, and safrole etc among human made substance are DDT, carbon tetrachloride, chloroform, thioacetamide. Studies in experimental animals designate quite clearly that development

of cancer of the liver is associated with the number of obvious non-malignant lesions appearing prior to the occurrence of neoplastic malignancy.

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.

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 tetra chloride, 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.

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 ^[42]. 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^[43].

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^[44].

More than 900 drugs have been concerned in causing liver injury, and it is the most common reason for a drug to be withdrawn from the market. Drug persuaded liver injury is responsible for 5% of hospital admissions and 50% of all acute liver failures^[45].

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^[46]. They play an important role in the inflammation process after intoxication by ethanol, carbon tetrachloride or carrageenan.

These radicals and the reactive species resultant from them react with cell membrane, induce lipid peroxidation and are responsible for various deleterious belongings in cells and tissues where they are generated. ROS induce alterations and loss of structural/functional architecture in the cell, leading directly to cytotoxicity and/or indirectly to genotoxicity, with numerous serious anomalies favouring disharmony and diseases^[47].

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

3.4 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: CCl₄ dimethylnitrosamine (DMN), galactosamine; bile duct epithelial cells: thioacetamide (TAA)),

(2) immunological-induced damage (heterogenous 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 the genetic leptin-deficient (ob/ob) or leptin-resistant (db/db) mice.

The dietary methionine/ choline-deficient models are cast-off in the majority of published research.^[48] Progressive fibrosis was reported only in the methionine/choline-deficient models in 100% of the mice.

BDL and CCl₄ are the most widely used rodent models^[49] in liver fibrosis research to assess the effectiveness 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^[50].

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 for rats are now applied

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

Acute and Chronic Models with Carbon Tetrachloride (CCl₄)

CCl₄ 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 CCl₄ by cytochrome P450, which results in the formation of trichloromethyl radical in these cells and this free radical initiates lipid peroxidation^[51].

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

The CCl₄ 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 after CCl₄ administration > 20%.

In animal models CCl₄ 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 CCl₄ that are administered.

The models for CCl₄ that are used in liver fibrosis research, are (1) acute damage (72 hours after a single injection of CCl₄) with HSC activation (2) early and establish fibrosis (4-6 week of twice weekly CCl₄ dosing), (3) early cirrhosis (8 week of twice weekly CCl₄ dosing) and (4) advanced micronodular cirrhosis (12 week of twice weekly CCl₄ dosing).

In addition for each of these models (5) spontaneous recovery from fibrosis can be studied after cessation of dosing of CCl₄. 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.

CCl₄ is administered to the animals via intraperitoneal, subcutaneous or oral administration or by inhalation. For intraperitoneal injections, CCl₄ 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/l in drinking water are used and started 1 week before the initial exposure to CCl₄.

In case of inhalation of CCl₄ 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 CCl₄ 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 extrahepatic biliary atresia and primary sclerosing 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 ^[52].

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

After administration of DMN, hemorrhagic necrosis is evident in centrilobular 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 anti-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 myofibroblasts, and this transformation process is related with cellular activation, proliferation and matrix production resembling cellular activities that also happen in vivo.

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

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

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

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

The yield of HSC after collagenase / pronase digestion and Nycodenz separation is about 20-40 x 10⁶ cells per rat liver.

The yield of HSC attained from a mouse liver is much smaller and to isolate and purify proper amounts of HSC, about 5 mice have to be used at the same time in one total isolation (Geerts, personal communication)

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

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

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

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

Liver Slice System

A second in vitro test system which was recently developed to assess effects of anti 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 ^[54].

Exams and Tests Physical Examination

- Nutritional assessment
- Yellowing of the sclera is usually the first detectable sign of jaundice.
- Darkening of urine
- Skin examination for icterus
- Stigmata of chronic liver disease
- Abdominal examination
- Enflamed and tender liver
- Fluid in the abdomen (ascites) that can become infested
- Blood tests
- These may initially include
- Complete blood count – TC ,DC, ESR, Cholesterol
- Liver function test
- In women, a pregnancy test may be obtained.
- Urine analysis: Urine analysis for bile salts and bile pigments

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, 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 ^[55]).

3.5 LATERAL RESEARCH

Piper nigrum

➤ **Anti asthmatic activity:**

Oral administration of piperine in different proportion to mice suppressed and reduced the infiltration of eosinophil, hyper responsiveness and inflammation due to the suppression of the production of histamine, interleukin-5, immunoglobulin E and interleukin-4^[56].

➤ **Anti bacterial activity:**

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 *Escherichia coli*^[57].

➤ **Antidiarrhoeal property**

The antidiarrhoeal activity was done against castor oil induced diarrhoea in mice. In such induced situation piperine sequentially prevent the small intestine fluid accumulation.^[58]

Curcuma longa

➤ **Anti inflammatory activity:**

The laboratory studies have identified a number of different molecules involved in inflammation that are inhibited by curcumin including phospholipase, lipoxygenase, cyclooxygenase, thromboxane, prostaglandins. The human studies have found some evidence of anti inflammatory activity of curcumin.^[59]

Zingiber officinale

➤ **Anti oxidant:**

The CO₂ extract from ginger has high polyphenol content. It manifested a very good scavenging DPPH and reduced its reducing capacity. It also showed antioxidant activity comparable with that of BHT^[60].

➤ **Antihypertensive activity**

Ghayur has reported that the crude extract of ginger induces the Ca^{2+} channel-Blocking (CCB) activity that lowers the blood pressure which ultimately reduces the hypertension in the patients ^[61].

Cuminum cyminum

➤ **Anti bacterial:**

Essential oil of *Cuminum cyminum* contains p-mentha-1, cuminaldehyde, terpene, pinene which shows a Anti bacterial activity against clavibacter, curtobacterium, rhodococcus, erucinia, xanthomonas, agro bacterium which are responsible for plant or cultivated disease worldwide.^[62]

➤ **Nephroprotective activity:**

The cumin seeds extracts and the coriander leaf extract incobination shows nephroprotective activity in profenofos induced neprotoxicity in swiss albino mice. The biochemical assessment shows the nephroprotective activity of cumin and coriander. In comparison to control group mice group creatinine, urea, uric acid were increased in profenofos treated group and profenofos control group. But in cumin, and coriander treated group it shows declination in the level of creatinine & urea level ^[63].

Ferula asafoetida

➤ **Anti ulcer:**

This herb comes under the WHO recognised plant drugs. Asafoetida is very useful in the digestion process. Many researchers proved its efficacy in gastric irritation and acid peptic disease ^[64].

Trigonella foenum graecum

➤ **Anti plasmodic activity:**

Some of the alkaloids and tannins like phenolic compounds from *Trigonella foenum graecum* showed potential antiplasmodial properties against in vitro culture of chloroquine sensitive and resistant P. Falciparum^[65].

Piper longum**➤ Anti cancer activity:**

The alcohol extract of *Piper longum* and piperine inhibits solid tumour development in mice induced with Dalton's lymphoma ascites cells and increases the life span of mice ^[66].

➤ ACE inhibition and Anti-oxidant activity

Hydro alcoholic extract of *Piper longum* showed ACE inhibitor activity in a concentration dependent manner, in liberation of hippuric acid from HHL catalyzed by the ACE method. Particularly Piperine extract showed most active. The anti-oxidant activity of piperine extracts showed maximum anti-oxidant activity by the scavenging effect on the stable DPPH free radical activity method^[67].

Allium sativum**➤ Anti diabetic effect:**

S- allyl cystiene sulphoxide (SACS) a sulphur containing amino acid of garlic which is the precursor of allicin and garlic oil, has been found to show significant anti diabetic effects in alloxan diabetic rats.^[68]

3.6 PHARMACEUTICAL REVIEW***Choornam*****Definition**

Choornam is a fine powder of drugs. The "Choornam" 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 well 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 storages or changed in colour, taste and scent, and those that are insects infested or attacked by fungi should be positively rejected. However drugs like Embelia fruits, Senna, Long Pepper, Jaggery and cows ghee are preferred from fairly aged stock, provided they are not infested with pests, deteriorated or spoiled or developed acidity.

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 so fine to be called amorphous and should never be 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 வாவின்பாலாற் பிசைந்து

நலமான சட்டியிலே பாலைவிட்டு

வானென்ற சுத்தசலம் பாதிவிட்டு

வளமாக மேற்சீலை கோடு கட்டிப்

பானென்ற சூரணத்தைப் பிட்டுபோல் வைது

பதறாதே வெந்தெடுக்கச் சித்தியமே!”

-அகஸ்தியர் வைத்திய இரத்தினச்சுருக்கம்

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

“ஆமப்பா ரவியுலர்த்திப் பொடி தான்செய்து
அப்பனே சமனாய்ச் சர்க்கரையைச்சேர்த்து
நாமப்பா கொண்டு வரதோஷம் போச்சு
நன்றாகச் சுத்திசெய்யாச் சூரணந்தான்
தாமப்பா ரோகத்தை வெல்லா தப்பா
தளமான வியதியெல்லாம் பாரிக்கும்பார்
வேமப்பா சுத்திசெய்து கொண்டாயனால்
வெகுசுறுக்காய் தீருமா வியாதிகேளு”^[69]

-அகஸ்தியர் வைத்திய இரத்தினச்சுருக்கம்

Then the Chooranam is placed in the sunlight and powdered. Equal amount of sugar is added and taken internally to cure all diseases. If the drug is taken without purification the disease 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 stopper glass, polythene or tin containers, or in polythene or cellophane bags and sealed. These bags should in turn be enclosed in cardboard boxes.

The Chooranam to facilitate easy handling and to assure exact dosage administration, could be pressed into tablets, could be packed in bottles or tubes made either of glass or plastic or packed in strip of metal foil or plastic sheets.

In industry the tablets are made, counted & packed by electronic devices.

Then Chooranam is said to retain its potency for three months and then gradually deteriorate. However if properly packed & stored they keep good for a year.

According to AYUSH guidelines shelf life of Chooranam is one year ^[70].

Table: 2. ANALYTICAL SPECIFICATIONS OF CURNA/CHOORNAM

Sl. 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)

4. MATERIALS AND METHODS

Materials:

In this dissertation the preparation drug '*Pitha Kamalai Choornam*' is taken as a trial drug for hepatoprotective, from *Korakkar Maruththuvam* Published by *Siddha Maruththuva Nool Veliyeettu Pirivu, Indian Medicine and Homeopathy*, Chennai-600106, Page No: 126-127.

Ingredients:

1.	Chukku	-	Dried ginger (<i>Zingiber officinale</i>)	-15 gm
2.	Milagu	-	Black pepper (<i>Piper nigrum</i>)	-15 gm
3.	Thippili	-	Long pepper (<i>Piper longum</i>)	-15 gm
4.	Kadugu	-	Indian mustard (<i>Brassica juncea</i>)	-15 gm
5.	Vellai poondu	-	Garlic (<i>Allium sativum</i>)	-15 gm
6.	Perungayam	-	Asafoetida (<i>Ferula asafoetida</i>)	-15 gm
7.	Vendhayam	-	Fenugreek (<i>Trigonella foenum graecum</i>)	-15 gm
8.	Seeragam	-	Cumin seeds (<i>Cuminum cyminum</i>)	-15 gm
9.	Perunjeeragam	-	Anise seeds (<i>Foeniculum vulgare</i>)	-15 gm
10.	Manjal	-	Turmeric (<i>Curcuma longa</i>)	-15 gm
11.	Omam	-	Bishops weed (<i>Tachyspermum ammi</i>)	-15 gm

Other Ingredient:

Elumichai charu – Lemon juice (*Citrus lemon*)

Collection of the Drug:

All raw materials were collected from Ramasamy Pillai shop, Parris corner, Chennai, Tamilnadu.

Identification and Authentication of the Drug:

All the raw materials were identified and authenticated by the Botanist and Professor in Gunapdam Department 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.

(Ref No: GSMC/ PGGM/0096-107/2014-2017)

Purification of the Drug:

All the drugs mentioned here were purified as per the Siddha literature.^[71]

- Milagu, Thippili, Kadugu, Vendhayam, Narseeragam, Perunjeeragam were cleaned well without any dust and impurities.
- Outer skin of Chukku is removed.
- Outer skin of Poondu is removed
- Impurities from Manjal is removed
- Perungayam is roasted.

4.1 PREPARATION OF THE TRIAL DRUG *PITHA KAMALAI CHOORNAM*:

Procedure:

The above mentioned purified drugs were slightly roasted, finely powdered separately and mixed well, and then the mixture was ground with the lemon juice for few hours and again allowed to dry and powdered. Then the Choornam is purified by pittaviyal method as per Siddha literature and again, dried, sieved through thin clean white cloth and collected in a air tight container. Finally Pitha Kamalai Choornam is labelled as PKC.



Powdering



Sieving



Pitha Kamalai Choornam

Purification of the Choornam:

The “*Pitha Kamalai Choornam*” 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^[72].

Storage of the Drug:

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

Administration of the Drug:

Form of the medicine:	Choornam
Route	: Enteral
Dose	: 2 -3 gms twice a day
Adjuvant	: Lemon juice, honey, ginger juice.
Indication	: Jaundice

4.2 STANDARDIZATION OF THE DRUG

Standardisation 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. The standardization brings the efficacy and potency of the drug.

4.2.1 Organoleptic characters:

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

4.2.2 Physicochemical analysis:

Physicochemical studies of the trial drug have been done according to the AYUSH guidelines^[73].

Determination of the Ash Values:

Total ash

3g of the test drug was accurately weighed and incinerated in a crucible dish at a temperature not exceeding 450⁰ 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 5 minutes. 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 15 minutes at temperature not exceeding 450⁰ 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 is 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 5 minutes 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 15 minutes at temperature not exceeding 450⁰ C. The silica crucible and residue were weighed until constant weight is 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 is then allowed to stand and soak for 18 hours. The solution is filtered and evaporated of the filtrate in a flat bottomed shallow dish and dried at 105⁰ 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 80⁰ C for 24 hours. The resulting solution was shaken continuously for 6 hours and allowed to stand and soak for 24 hours 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 105⁰C then cooled and weighed.

Loss on Drying

The powdered drug was taken and dried in the oven at 100 – 105⁰C to constant weight. The result was noted.

Physical characterisation:

Solubility: a. A little of the sample was shaken well with distilled water.

b. A little of the sample was shaken well with Con HCl and Con H₂SO₄.

Springly 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 is then introduced into non-luminous part of the Bunsen flame. A 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. All these results were tabulated.

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^[74].

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 1ml 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 1ml 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 minutes. 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 Phytosterol

Ferric chloride – acetic acid test

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

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 is 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 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.

4.2.4 TLC/ HPTLC finger print studies

HPTLC finger printing was carried out as per the reference.^[75]

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 and 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°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.

4.2.5 Bio-chemical analysis

Methodology for chemical analysis

Preparation of extract

5gm of KVNC was weighed accurately and placed in a 250ml clean beaker and added with 50ml of distilled water. Then it was boiled well for about 20 minutes. Then it was cooled and filtered in a 1000ml volumetric flask and made up to 100ml distilled water.

Table.No:3. Test for basic radicals

PROCEDURE	OBSERVATION	INFERENCE
Test for Potassium: A pinch of sample is treated with 2ml of sodium nitrate solution and then treated with 2ml of cobalt nitrate in 30% of glacial acetic acid.	Formation of Yellow colour precipitate	Presence of Potassium
Test for Calcium: Taken 2 ml of extract in a clean test tube. Then acetic acid and potassium chromate solution were added	No Yellow precipitate	Presence of Calcium
Test for Magnesium: 2ml of extract was taken in a clean test tube, few drops of Magneson reagent was added in drops.	Formation of Blue colour precipitate	Presence of Magnesium
Test for Ammonium: 2ml of extract was taken in a test tube and added few ml of Nessler's reagent.	Appearance of Brown colour	Presence of Ammonium

MATERIALS AND METHODS

PROCEDURE	OBSERVATION	INFERENCE
Test for Sodium: 2 pinches of <i>Pitha kamalai Choornam</i> was mixed with HCl and made it into paste. And introduced into the blue flame of Bunsen burner.	Appearance of intense Yellow colour	Presence of Sodium
Test for Iron (Ferrous): 2ml of extract was taken in a clean dried test tube and conc. HNO_3 and ammonium thiocyanate were added.	Appearance of Blood red colour	Presence of Ferrous iron
Test for Zinc: 2 ml of the extract was taken in a test tube and Potassium ferro cyanide solution was added.	Formation of White colour precipitate	Presence of Zinc
Test for Aluminium: To the 2ml of the extract was taken in a test tube sodium hydroxide drops were added to it.	White precipitate obtained	Presence of Aluminium
Test for Lead: 2 ml of extract was taken in a test tube and added with 2ml of potassium iodide solution	Formation of yellow colour precipitate	Presence of Lead
Test for Copper: To a small portion of a extract dilute hydrochloric acid was added and then hydrogen sulphide gas is passed through the solution.	Black precipitate	Presence of Copper
Test for Mercury: 2ml of the extract was taken in a test tube and treated With 2ml of sodium hydroxide solution.	Formation of Yellow precipitate	Presence of Mercury
Test for Arsenic: 2ml of the extract was taken in a test tube and treated with 2ml of sodium hydroxide solution.	Formation of brownish red precipitate	Presence of Arsenic

Results were tabulated in Table no: 10

Table.No:4. Test for acidic radical

PROCEDURE	OBSERVATION	INFERENCE
<p>Test for Sulphate: 2 ml of the extract was taken in clean, dry test tube and 5 % barium chloride solution was added to it.</p>	Formation of white precipitate	Presence of Sulphate
<p>Test for Chloride: The extract was taken in a test tube and then treated with Silver nitrate solution.</p>	Formation of White precipitate	Presence of Chloride
<p>Test for Phosphate: The extract was taken in a test tube and treated with ammonium molybdate and conc. HNO₃.</p>	Formation of Yellow precipitate	Presence of Phosphate
<p>Test for Carbonate : The substance was taken in a clean dry test tube and then treated with Conc. HCl.</p>	Formation of Effervescence	Presence of Carbonate
<p>Test for fluoride & oxalate: 2ml of extract was taken in a test tube and added with 2ml of dil. acetic acid, 2ml calcium chloride solution and then heated.</p>	Formation of cloudy appearance	Presence of Fluoride & Oxalate
<p>Test for Nitrate: 1gm of the <i>Pitha kamalai Choornam</i> was heated with copper turnings and concentrated H₂SO₄ and observed the test tube vertically down.</p>	Characteristic changes	Presence of Nitrate

Results were tabulated in Table no: 11

4.2.6. Availability of the bacterial load:**Enumeration of bacteria by plate count – Agar plating technique**

The plate count technique was one of the most routinely used procedure because of the enumeration of viable cells by this method^[76].

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 diluent or dilution blank. Dilution is usually made in multiples of ten. A single dilution was calculated as follows:

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

Requirements:

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

Procedure:

- Label the dilution blanks as 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , 10^{-7} .
- Prepare the initial dilution by adding 1 ml of the sample into a 9 ml dilution blank labeled 10^{-1} thus diluting the original sample 10 times.
- Mix the contents by rolling the tube back and forth between hands to obtain uniform distribution of organisms.

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

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:

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

4.2.7. Sophisticated instrumental analysis

FT-IR (Fourier Transform Infra-Red)

A. FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)



Figure no: 3.1 FTIR Spectroscopy analyser

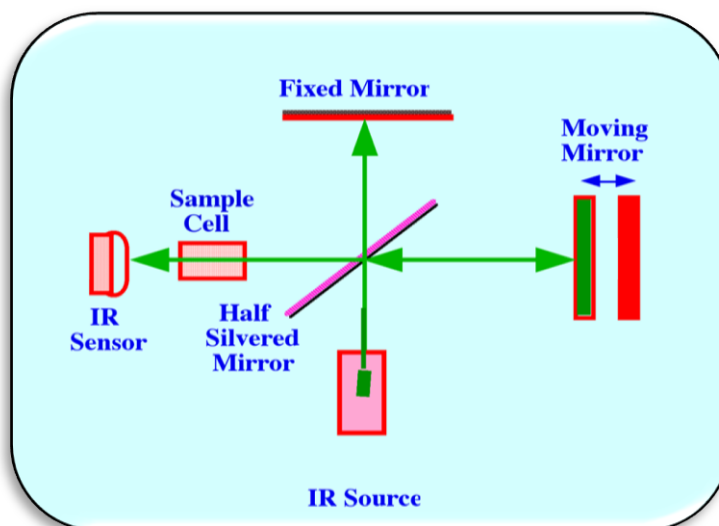


Fig: 3.2 FTIR-MECHANISMS

Instrument details:

Model : SHIMADZU FT-IR Spectrometer
 Scan Range : MIR 450-4000 cm⁻¹

Resolution : 1.0 cm⁻¹
Sample required : 50 mg, solid or liquid.

Fourier transform - Infra red (FTIR) spectroscopy study was carried out in IIT Campus Chennai, Tamil Nadu by using SHIMADZU FTIR Spectrometer.

Sample Preparation

A small amount (2-4mg) of *Pitha kamalai Choornam* sample was mixed with 7 drops of distilled water. After dissolution, the solution was taken in a small test tube and transfers this solution with a pipet onto the IR plates. The KBr plates must be methodically cleaned after this process to prevent contamination of next concentration sample.

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. ^[77]

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

SEM (SCANNING ELECTRON MICROSCOPE)

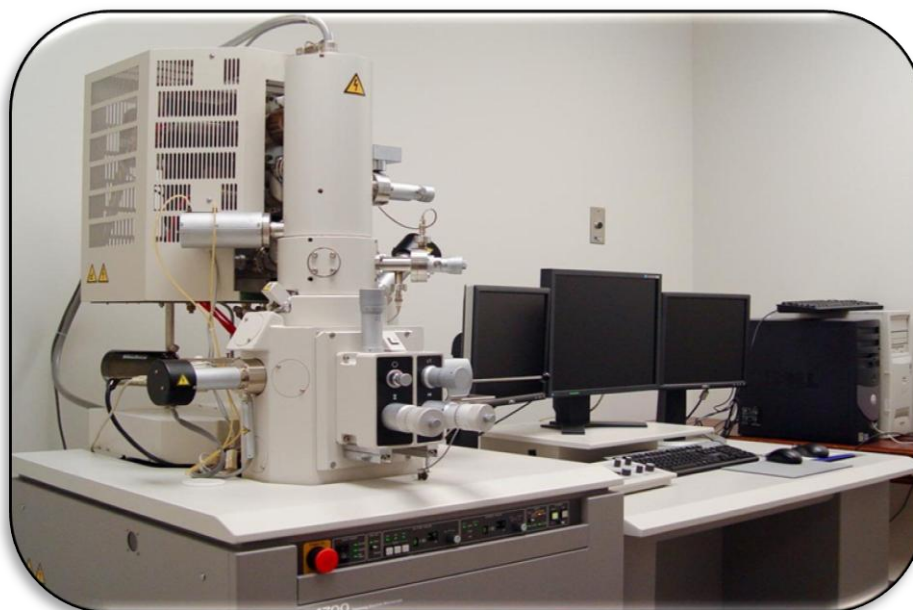


Fig: 4.1 SEM INSTRUMENT

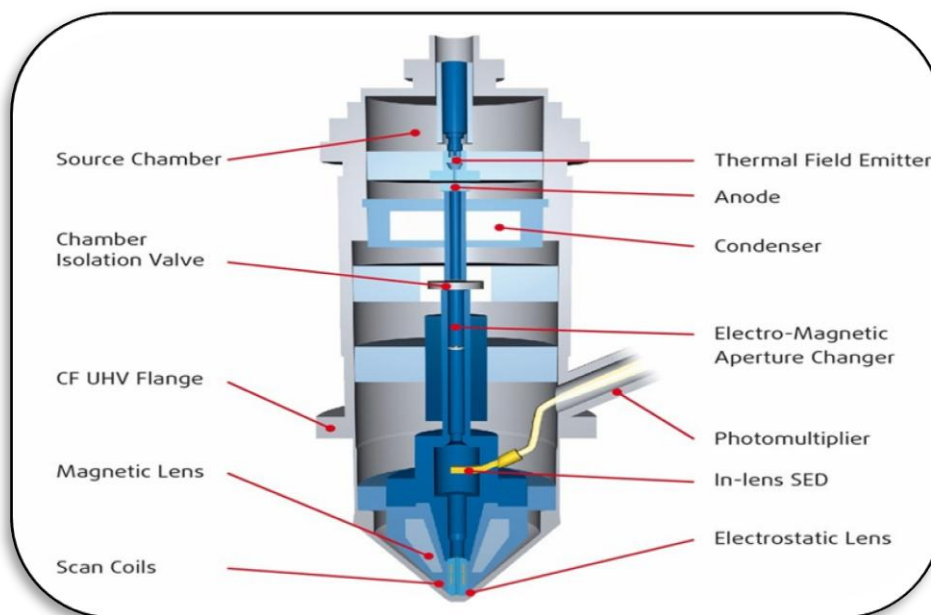


Fig: 4.2 SEM MECHANISMS

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 a 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^[78].

XRD (X-RAY POWDER DIFFRACTION)

Definition

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

Applications:

- Characterization of crystalline materials^[79]
- Identification of fine-grained minerals such as clays and mixed layer clays that are difficult to determine optically
- Determination of unit cell dimensions.
With specialized techniques, XRD can be used to:
 - Determine crystal structures using Rietveld refinement
 - Determine of modal amounts of minerals (quantitative analysis)
 - Characterize thin films samples by:
 - determining lattice mismatch between film and substrate and to inferring stress and strain
 - determining dislocation density and quality of the film by rocking curve measurements
 - measuring super lattices in multilayered epitaxial structures

- determining the thickness, roughness and density of the film using glancing incidence X-ray reflectivity measurements
- Make textural measurements, such as the orientation of grains, in apolycrystalline sample.

Strengths and Limitations of X-ray Powder Diffraction

Strengths

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

Limitations

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

Sample Collection and Preparation

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

- Obtain a few tenths of a gram (or more) of the material, as pure as possible
- Grind the sample to a fine powder, typically in a fluid to minimize inducing extra strain (surface energy) that can offset peak positions, and to randomize orientation.
- Powder less than ~10 μm (or 200-mesh) in size is preferred place into a sample holder or onto the sample surface.

ICPOES (INDUCTIVELY COUPLED PLASMA OPTIC EMISSION SPECTROSCOPY)

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 (8,000–10,000°C). The analytes 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 analysed by ICP-OES.

Application:

It is 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^[80].

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 *Pitha kamalai Choornam* was occupied in a clean, dry test tube. To this, 3 ml Nitric acid was added and mixed well and allowed for few minutes until 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

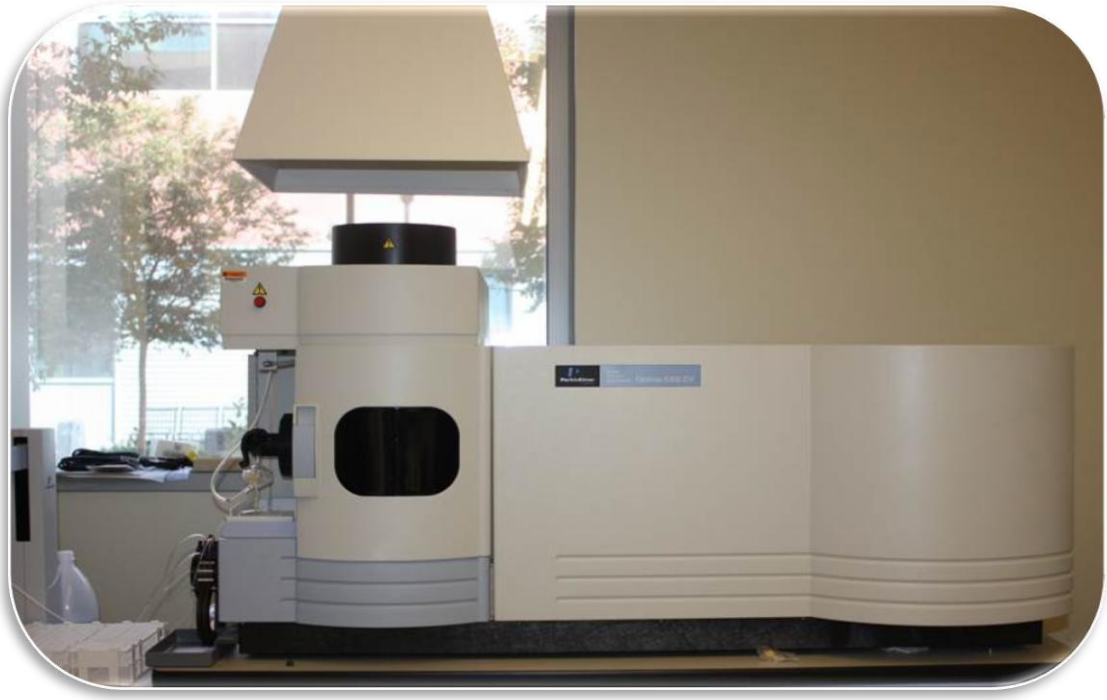


Fig: 5.1 ICP-OES ANALYSER (Perkin Elmer Optima 5300 DV)

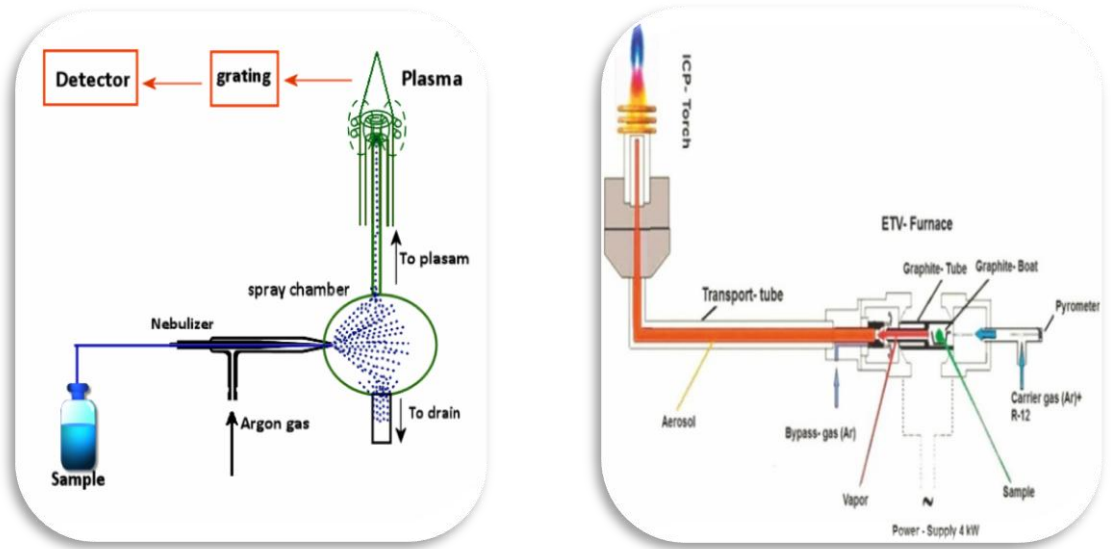


Fig 5.2 Mechanism of ICP-OES analyser

4.3. TOXICOLOGICAL STUDIES

4.3.1. Acute oral toxicity – OECD guidelines - 423

Introduction:

The acute toxic class method is a stepwise procedure with the use of 3 animals of a single sex per step. Depending on the mortality and/or the moribund status of the animals, on average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance. Morbid animals or animals obviously in pain or showing signs of severe and enduring distress shall be humanely killed, and are considered in the interpretation of the test results in the same way as animals that died on test. The method allows for the determination of an LD50 value only when at least two doses result in mortality higher than 0% and lower than 100%.

Acute toxicity study was carried out as per OECD guideline. (Organization for Economic Co - operation and Development, Guideline - 423^[81]).

The experimental protocol was approved by the institutional ethical committee (IAEC) under CPCSEA (approval no: **IAEC/XLV111/12/CLBMCP/2016**)

Animal: Healthy Wistar albino female rat weighing 200–220 gm Studied carried out at three female rat 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 $\pm 20\%$ 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 22°C ($+3^{\circ}\text{C}$). Although the relative humidity should be at least 30% and preferably not exceed 70% other than during room cleaning the aim should be 50-60%. Lighting should be artificial, the sequence being 12 hours light, 12 hours dark. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water. Animals may be 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

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

It was given in a single oral dose by gavage using a feeding needle. Animals were fasted prior to dosing. Following the period of fasting, the animals were weighed and then

the test substance was administered. After the substance has been administered, food was withheld for a further 3-4 hours. The principle of laboratory animal care was followed. Observations were made and recorded systematically and continuously observed as per the guideline after substance administration.

The visual observations included skin changes, mobility, and aggressiveness, sensitivity to sound and pain, as well as respiratory movements. They were deprived of food, but not water 16–18 hours prior to the administration of the test suspension.

Finally, the number of survivors was noted after 24 hours and these animals were then maintained for a further 14 days and observations made daily. The toxicological effect was assessed on the basis of mortality.

Number of animals and dose levels

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

Duration of Study	:	48 hours
Evaluation	:	14 Days

Limit test

The limit test was primarily used in situations where the experimenter has information indicating that the test material is likely to be nontoxic, i.e., having toxicity only above regulatory limit doses. A limit test at one dose level of 2000 mg/kg body weight was carried out with three animals per step. The test substance-related mortality was not produced in animals, so further testing at the next lower level need not be carried out.

Observations

- The animals were observed individually after dosing at least once during the first 30mins and periodically during the first 24 hours.
- Special attention: First 1-4 hours after administration of drug.
- It was observed daily thereafter for a total of 14 days, except when they needed to be removed from the study and killed humanely for animal welfare reasons or are found dead.

a. Mortality

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

b. Body weight

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

c. Cage-side observation

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

d. Gross necropsy

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

Histopathology

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

Data and reporting

All the data were summarised in tabular form showing the animals used, number of animals displaying signs of toxicity, the number animals found dead during the test or killed for humane reasons, a description and the time course of toxic effects 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 "*Pitha kamalai Choornam*" 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^[82].

4.3.2. Repeated dose 28 days oral toxicity study of “*pitha kamalai Choornam*” on rats – (oecd-407 guidelines)^[83].

Justification for Dose Selection

The results of acute toxicity studies in Wistar albino rats indicated that “*Pitha kamalai Choornam*” 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 were 100mg/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

“*Pitha kamalai Choornam*” 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 (gavage), 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, 24hours urine samples were collected by placing the animals in the metabolic cage with free access to tap water but no feed was given.

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

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

Haematological Investigations:

Blood samples of control and experimental rats was analyzed for 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;

Absolute organ weight (g)

Relative organ weight = _____ ×100

Body weight of animal on sacrifice day (g)

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 hours and washed in running water for 24 hours. Samples were dehydrated in an auto technique and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by

the “L” moulds. It was followed by microtome and the slides were stained with 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^[84].

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 multicomparison test using a computer software programme GRAPH PAD INSTAT-3 version.

4.4. PHARMACOLOGICAL STUDIES

4.4.1. Hepatoprotective activity of “*Pitha kamalai Choornam*” (*Pkc*) in CCl_4 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 CCl_4 + LP (for 14 days) at the dose 1 ml CCl_4 /kg body weight, in a suspension of double the volume of LP (which served as vehicle) subcutaneously at lower abdomen on every 14 days of the treatment .

Group III and IV animals received subcutaneous administration of CCl_4 + LP. They also received test drugs orally at the dose of 100, 200 mg/kg body weight respectively as a suspension of water. Group VI received in addition to CCl_4 suspension, silymarin (100 mg/kg body weight) daily. Silymarin was used as a standard reference drug.

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

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 Dunnett's test, and results were expressed in terms of Mean±SEM values. Statistical analysis was performed using INSTAT- V3 Software programme^[85].

4.4.2. Experimental design for hepatoprotective activity of “*Pitha Kamalai Choornam*” against paracetamol induced hepatotoxicity in rat's model.

Paracetamol induced hepatotoxicity in rats model was used for evaluation of hepato-protective activity for the *Pitha Kamalai Choornam*. 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 5th day. Groups IV- V, received *Pitha Kamalai Choornam* (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 minutes; 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 CCL₄.

Statistical analysis

The data obtained from the study were subjected to statistical analysis by one way ANOVA followed by Dunnett‘t’ test, and results were expressed in terms of Mean±SEM values. Statistical analysis was performed using INSTAT- V3 Software programme^[86].

4.4.3. ANTIOXIDANT ACTIVITY OF “PITHA KAMALAI CHOORNAM”**DPPH ASSAY (2, 2-diphenyl -1-picrylhydrazyl)**

The radical scavenging activity of different extracts was determined by using DPPH assay^[87] 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, $DPPH + [H-A] \rightarrow DPPH-H + (A)$.

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

Reagent Preparation

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

Procedure

Different volumes of 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

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

Control

RESULTS AND DISCUSSION

The well known Traditional medicine *Pitha Kamalai Choornam* had been subjected to various studies and standardization to establish the works of *Siddhars* to be true. Literary collections, Physicochemical Studies, Elemental analysis, Bio-chemical analysis, Toxicity studies, and Pharmacological studies are done to prove the Hepatoprotective activity of *Pitha Kamalai Choornam*. Literary collections about the drug from various text books give hope about its activity. The studies strongly supported the fact through these results. They are discussed below.

Discussion on review of literature

The extensive review on botanical aspect gave information about the microscopical, macroscopical, medicinal uses, constituents and the importance of the herbs in folklore and the details of the herbs in detail. Most of the herbs included in the formulation exerted Hepatoprotective action.

The review of the herbs in the texts depicted through the songs of *Siddhars* some 2000 years ago, strengthened the facts mentioned in the modern botanical aspect. *Siddhars* explained the medicinal properties of the plants through taste and most of the drugs included in the formulation have acrid taste and comes under the category of heat provoking medicines.

- 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

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

Toxicological results of the drug and pharmacological activity of the drug were derived. Its result has been tabulated and interpretation was made below. Thus it is to give a complete justification to bring the effectiveness of the trial drug *Pitha Kamalai Choornam*.

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 results of these studies have strongly proved the fact. They are discussed below.

- *Milagu* is indicated for diseases of Liver
- *Thippili* has action of Liver tonic
- *Seeragam* is indicated for curing Jaundice
- *Manjal* is a proven drug for curing jaundice
- *Chukku* is a herb used for biliousness
- *Perunjeeragam* is indicated for curing Jaundice
- *Vendhayam* is used in the treatment of biliousness
- *Poondur* is known to have liver tonic action

Discussion on pharmacological aspect

- The pharmacological aspect of the drug says about the mode of action, 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 animal for carrying out the activities were discussed to be Wistar strain of albino rats.
- They are also more sensitive compared to other animals and hence they were chosen for the study.

- Results from the pharmacological studies denote the effects of *Pitha Kamalai Choornam* showed promising effects in treating liver damage. Moreover, the increased levels of the serum enzymes were significantly decreased by the treatment with *Pitha Kamalai Choornam* implying that the drug prohibited the liver damage.

Discussion on Pharmaceutical review

- This review explained the preparation of *Choornam* in detail including the purification of raw drugs, methods of manufacturing *Choornam* and the Siddha parameters for the standardization of analyzing *Choornam*.
- The purification of drugs like chukku by the removal of skin of ginger is justified by the fact that the proteolytic enzymes are removed.
- Roasting of aromatic substances may result in certain chemical changes and increase in antioxidants.
- The powdered drugs were filtered through the white cloth so as to reduce the size of the particle in turn which enhances the bio-availability.
- The shelf life of the drug is improved by proper purification methods and preservation.

Discussion on Materials and Methods

- The preparation of the drug was done carefully so as to achieve the highest potency. *Choornam* are fine dry powders of drugs. The term *Choornam* may be applied to the powders of single drug or a mixture of two or more drugs.
- The impregnation with lemon juice is to enrich the therapeutic potentiality of the drug.
- The lemon juice not only contains citric acid but also many minerals like sodium, potassium and pantothenic acid which helps in the chemical detoxification and purification.
- Also the citric acid forms ligand with the toxic substances so that it weakens the bonds and disassociates them.
- On purification (pittaviyal), the weight of the *Choornam* is different from the exact value but not from the mean value when calculated.

- The *Choornam* were also subjected to Siddha parameters of the testing like, *Choornam* tends to be amorphous, It should be never damp, The fitness of the sieve should be 100 mesh or still finer.
- The standardization of the drugs was achieved through various procedures like analyzing the organoleptic characters, physico-chemical characters, elements present in the drug and the results and discussion of standardization parameters is described below.

Organoleptic character

Pitha Kamalai Choornam showed the following characters.

Table: 5. Organoleptic characters

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

Table: 6. Physicochemical Analysis

S.NO	Parameter	Result
1	Ph	5.63
2	Ash(%)	4.98
3	Acid Insoluble ash (%)	0.597
4	Water soluble ash	2.02
5	Loss on drying(at 105 ⁰ C)	8.74
6	Alcohol soluble extractive	36.60
7	Solubility	Positive

	I. Distilled water	Soluble
	II. Benzene	Soluble
	III. Chloroform	Soluble
	IV. Carbontetrachloride	Soluble
	V. Xylene	Soluble
	VI. Petroleum ether	Soluble
	VII. Propylene ether	Non Soluble
8	Action on heat	Negative
9	Flame test	Negative
10	Ash test	Negative

Interpretation

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

➤ **pH:**

It is a measure of hydrogen ion concentration; it is the measure of the acidic or alkaline nature. 7.0 is neutral, above 7.0 is alkaline and below is acidic. The pH of the drug *Pitha Kamalai Choornam* is 5.63 which is weak acidic in nature. Acidic drug is essential for its bioavailability and effectiveness. Acidic drugs are better absorbed in stomach^[88].

➤ **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 of plant material indicated the amount of minerals and earthy materials present in the drug. The total ash value of *Pitha Kamalai Choornam* is 4.98 which determine the absence of inorganic content.

➤ **Water soluble ash:**

Water-soluble ash is the part of the total ash content, which is soluble in water. It is 2.02 for PKC

➤ **Moisture (Loss on drying):**

The total of volatile content and moisture present in the drug was established in loss on drying. Moisture content of the drug reveals the stability and its shelf-life. High moisture content can adversely affect the active ingredient of the drug. Thus low moisture content could get maximum stability and better shelf life. Loss on drying of PKC is 8.74^[89].

Phytochemical analysis

Table: 7. Phytochemicals screening test

SNO	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, to protect against diseases. The phytochemical analysis reveals the presence of Alkaloids, Glycosides, Phenol, Triterpenes, Flavanoids and Quinones^[90].

Alkaloids

- Alkaloids possess antispasmodic, analgesic, bactericidal effects.
- Alkaloids are the active principles producing many essential effects in protecting the body ^[91].

Phenols

- Phenol groups are the essential part of many anti-oxidant compounds
- They possess rich Anti-Oxidant property and protect body from oxidative stress.
- Effective anti-hyperglycaemic agent ^[92].

Flavonoids

- It is the most important group of polyphenolic compounds in plants.
- Flavonoids have potent Anti-Oxidant activity and it is its important function.
- 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.
- It also possesses anti-microbial activity which is confirmed by the various anti-microbial assays.
- Flavanoids are immunomodulator^[93].

Phytosterols

- Phytosterols are plant sterols, phytosterols have anti-inflammatory effect, phytosterols reduce oxidative stress
- Phytosterols have an anti-oxidant property
- Various bioactivities of phenolic compounds are responsible for their chemopreventive properties ^[94].

Triperpenes

- The Triterpenes are the best immunomodulator and have anti-oxidant property.
- Triterpenes suppress the inflammatory response and has Anti microbial activity.
- It serves as Anti bacterial agent ^[95].

Carbohydrates

- Carbohydrates are important in the storage of glucose.
- Carbohydrates play important role in homeostasis of glucose and fatty acids in liver^[96].

Protein and amino acids

- Proteins are very useful in the liver regeneration and energy production.
- They boost glutathione production to protect the liver.
- Protein is an amalgamation of amino acids. It is an important component of every cell in the body. Body uses protein to build and repair tissues^[97].

TLC/HPTLC Analysis of chloroform extract:**HPTLC analysis**

Chloroform extract was applied in TLC aluminium 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, 254nm and visible light (Vanillin- Sulphuric acid).

TLC Photo documentation : *Pitha kamalai chooranam*

Stationary Phase - Silica Gel 60 F₂₅₄

Mobile Phase – Toluene: Ethyl acetate: Formic Acid (9:2:0.2 v/v/v)

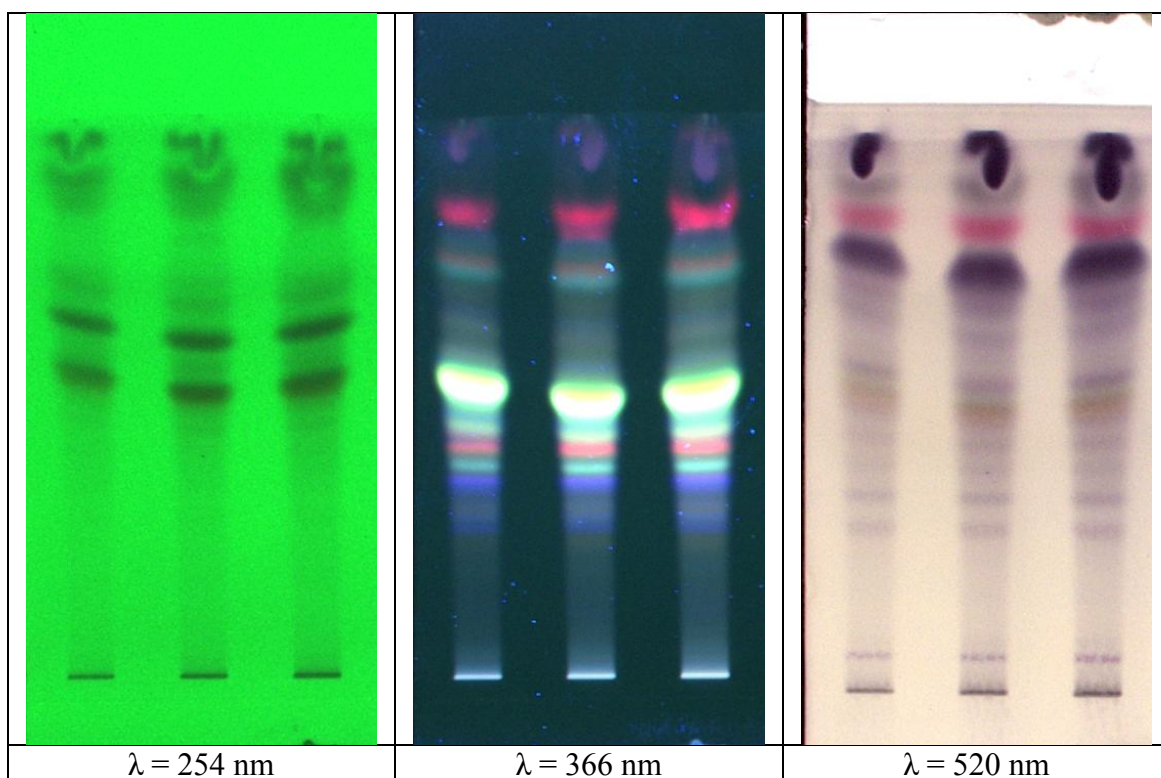


Fig 6: TLC Photo documentation

Table: 8. Rf Values for the chloroform extract

Rf	Colour	Rf	Colour	Rf	Colour
0.51	Dark	0.27	Blue	0.06	Violet
0.60	Dark	0.35	Blue	0.28	Voilet
0.67	Light	0.37	Green	0.33	Voilet
		0.41	Orange	0.38	Light violet
		0.44	White	0.43	Light violet
		0.50	Yellow	0.48	Yellow
		0.71	Green	0.52	Light violet
		0.74	Orange	0.60	Light violet
		0.81	Pink	0.65	Blue
				0.72	Pink

Under UV 254nm, it shows 3 major spots at Rf 0.51, 0.60, 0.67 major compounds are found.

Under UV 366nm, it shows 9 major spots at Rf 0.27, 0.35, 0.37, 0.41, 0.44,0.50, 0.71, 0.74, 0.81 major compounds are found.

UV Under 520nm, it shows 10 major spots at Rf 0.06, 0.28, 0.33, 0.38, 0.43, 0.48, 0.52, 0.60, 0.65.,0.72 major compounds are found.

HPTLC finger print analysis for chloroform extract

The finger print chromatogram was recorded at 366 nm. It showed 10 peaks of which peaks at R_f and were the major peaks and others were moderately smaller peaks. $\lambda = 366\text{nm}$

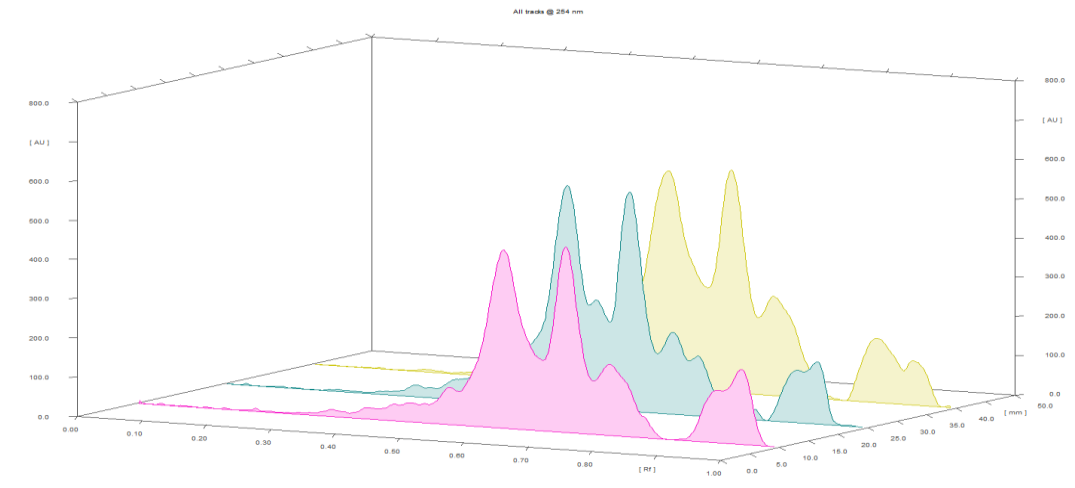


Fig 7: 3 D Chromatogram of 254 nm

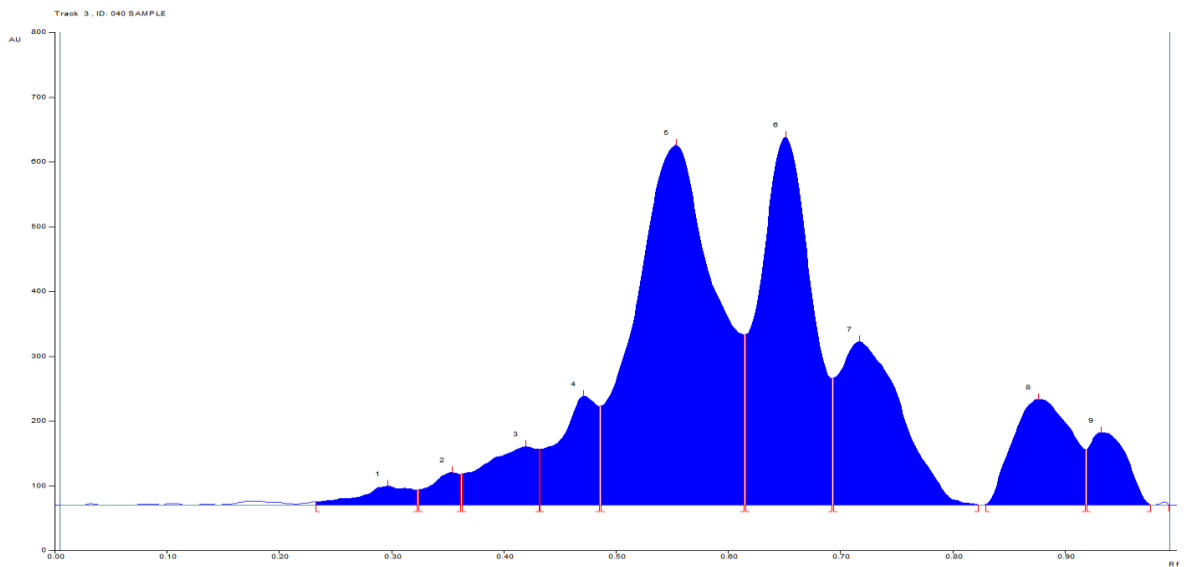


Fig 8: HPTLC Chromatogram of chloroform extract scanning at 254 nm

Table: 9. Peak Table@254 nm:

Track 3, ID: 040 SAMPLE

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.23 Rf	4.8 AU	0.30 Rf	29.3 AU	1.48 %	0.32 Rf	22.8 AU	1093.2 AU	1.28 %
2	0.32 Rf	23.2 AU	0.35 Rf	50.1 AU	2.52 %	0.36 Rf	47.6 AU	1040.7 AU	1.22 %
3	0.36 Rf	47.8 AU	0.42 Rf	90.0 AU	4.53 %	0.43 Rf	86.0 AU	3534.5 AU	4.13 %
4	0.43 Rf	86.1 AU	0.47 Rf	167.5 AU	8.43 %	0.49 Rf	52.2 AU	4822.1 AU	5.64 %
5	0.49 Rf	152.7 AU	0.55 Rf	555.4 AU	27.95 %	0.61 Rf	62.6 AU	32448.1 AU	37.95 %
6	0.62 Rf	263.1 AU	0.65 Rf	567.5 AU	28.56 %	0.69 Rf	95.1 AU	21242.9 AU	24.85 %
7	0.69 Rf	195.7 AU	0.72 Rf	252.0 AU	12.68 %	0.82 Rf	0.2 AU	11432.6 AU	13.37 %
8	0.83 Rf	0.6 AU	0.88 Rf	163.2 AU	8.21 %	0.92 Rf	85.0 AU	6876.4 AU	8.04 %
9	0.92 Rf	85.6 AU	0.93 Rf	112.0 AU	5.64 %	0.98 Rf	0.2 AU	3004.0 AU	3.51 %

Interpretation

- The quantitative analysis of compounds present in the PKC has been performed by HPTLC. The method may be applied to identify the PKC from other manufacturing process. It provides the identification of constituents, determination of impurities and quantitative determination of active substance present in PKC.^[98]
- The Rf value of the PKC supports the better standardization of the drug.
- The present study revealed that PKC showed best results in Toluene: Ethyl Acetatae (9:2) Solvent system. After scanning and visualizing the plates in absorbance mode at 254nm, 366nm and 520nm. Best results were shown at visible light range.
- TLC plate showed different colour phytoconstituents of chloroform extract of PKC. The bands revealed presence of two green, three blue and two yellow, two orange, two pink, three violet bands showing the presence of alkaloids, glycosides, phenols, triterpenes, flavonoids and quinines.
- The results from HPTLC finger print scanned for chloroform extract of PKC. There are thirteen polyvalent phytoconstituents and corresponding ascending order of Rf values start from 0.02 to 0.92 in which highest concentration of the phytoconstituents was found to be 28.96% and 27.95% with its corresponding Rf value found to be 0.02 and 0.92 respectively.

Table: 10. Results of basic radicals studies

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

Interpretation

The basic radical test shows the presence of Potassium, Magnesium, Iron, Zinc. Presence of these traces of minerals play an important role in the functioning of various enzymes in biological system and also have immunomodulatory function and hence the susceptibility to the course and the variety of viral infections.

Potassium

- Potassium is a vital electrolyte and is important for maintaining the integrity of cell membranes.
- Potassium is absorbed through the small intestine. Severe lack of potassium can disturb liver function and if potassium level falls below 30% to 40% causes liver disease^[99].

Magnesium

- Magnesium is essential for liver to prevent liver diseases. It enhances immune system.
- Depletion of magnesium levels leads to Cirrhosis, Fatty liver syndrome^[100].

Iron

- It is essential for oxygen transport, energy production, other cellular growth and proliferation.

- Iron is an essential element for blood production and also needed for energy metabolism.
- Liver performs three main functions in iron homeostasis.

Zinc

- Liver plays a central role in zinc homeostasis.
- Zinc is actually present within all bodily tissue and needed for healthy cell division.
- It acts like an Antioxidant within the body.
- Zinc also has a big impact on hormonal balance^[101].

Table: 11. Test for acid radical studies

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

Interpretation:

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

Availability of bacterial and fungal load in *Pitha Kamalai Choornam***Table: 12. Bacterial and fungal dilutions**

MICROBES	DILUTION	RESULT
BACTERIA	10^{-4}	6
BACTERIA	10^{-6}	5
FUNGI	10^{-2}	2
FUNGI	10^{-3}	3

Interpretation:

- The availability of bacterial load in the PKC has been performed by Agar plate techniques.

- As PKC is made from plant material it is more prone to contamination. The contamination of herbal drugs by microorganism not only cause bio deterioration but also reduces the efficacy of drugs.
- The toxin produced 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 PKC has been examined by bacterial and fungal load.
- Total bacterial load in 10^{-4} dilution is 6 and 10^{-6} dilution 5.
- Total fungal load in 10^{-2} dilution is 2 and 10^{-3} dilution is 3.

This result shows the presence of bacterial and fungal load in the trial drug (PKC). They are within the normal limits ^[102].

INSTRUMENTAL ANALYSIS

FTIR Spectrum Analysis

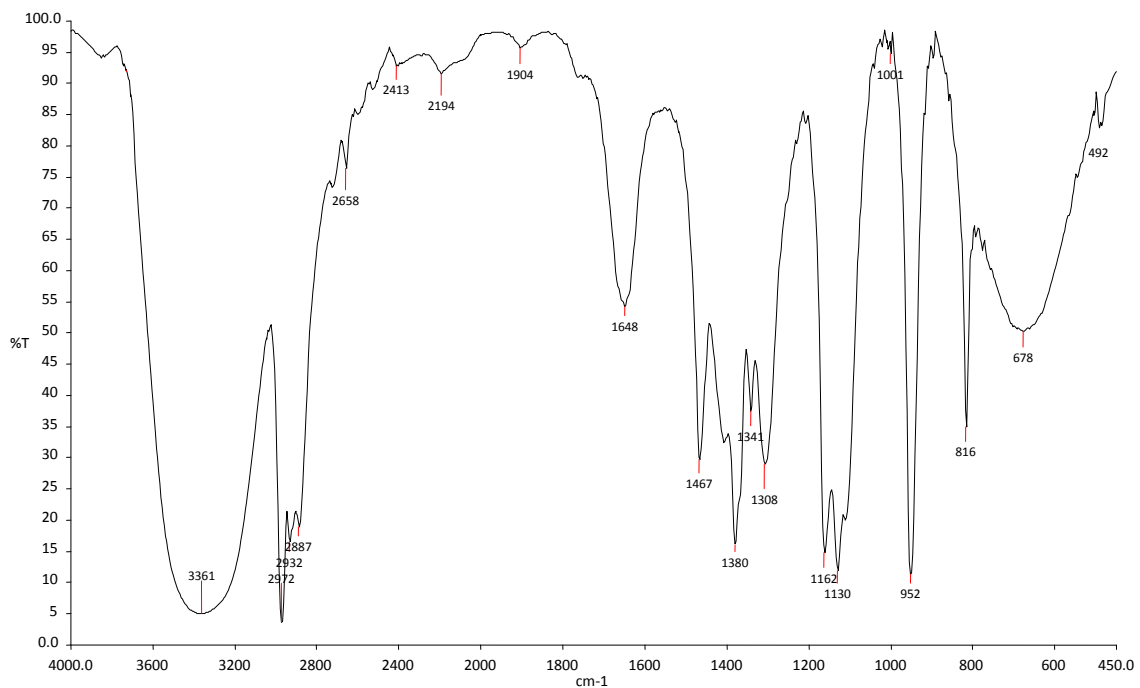


Fig 9: FTIR Spectrum analysis

Table 13: FT-IR INTERPRETATION

Absorption peak cm^{-1}	Stretch	Functional group
3361	O-H Stretch, Strong, Alcohol broad N-H Stretch	Alcohol Amine
2972	C-H Stretch, Strong	Alkane
2932	C-H stretch, Strong	Alkane
2887	C-H Stretch, Strong	Alkane
2658	O-H Stretch, Strong , very broad	Carboxylic acids
2413	-C \equiv C-Stretch, variable, not present in symmetrical alkynes	Alkynes
2194	-C \equiv C-Stretch, variable, not present in symmetrical alkynes	Alkynes
1904	C=O Strtch, Strong	Amide
1648	C=O Stretch, Strong -C=C- Stretch	Amide Alkenes
1380	C-F Stretch	Alkyl halide
1467	C=C Stretch, medium- weak	Aromatic
1341	C-F Stretch N-O, Symmetric Stretch	Alkyl halide Nitro compounds
1308	C-F Stretch C-N, Stretch	Alkyl halide Aromatic amines

Absorption peak cm^{-1}	Stretch	Functional group
1162	C-N, Stretch, Medium-Weak C-H Wag(-CH ₂)	Amine Alkyl halide
1130	C-N, Stretch, Medium-Weak C-O, Stretch, Strong	Amine Ether, Carboxylic acids, esters, ethers
1001	C-O, Stretch, Strong	Ether, Carboxylic acids, esters, ethers
952	=C-H bending	Alkene
816	=C-H bending	Alkene
678	=C-H bending	Alkene
492	C-Br Stretch	Alkyl halide

Interpretation

FTIR instrumental analysis was done. The test drug was identified to have 20 peaks. They are the functional groups present in the trial drug *Pitha Kamalai Chooranam*.

The above table shows the presence of phenols, alkane, acid, alkynes, amide, aromatic, alkyl halide, ether, alcohol and alkene 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^[103].

SEM: (SCANNING ELECTRON MICROSCOPE)

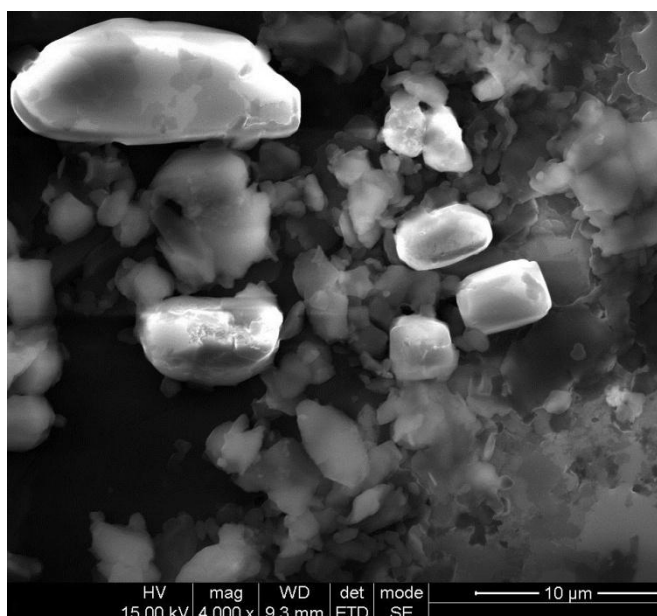
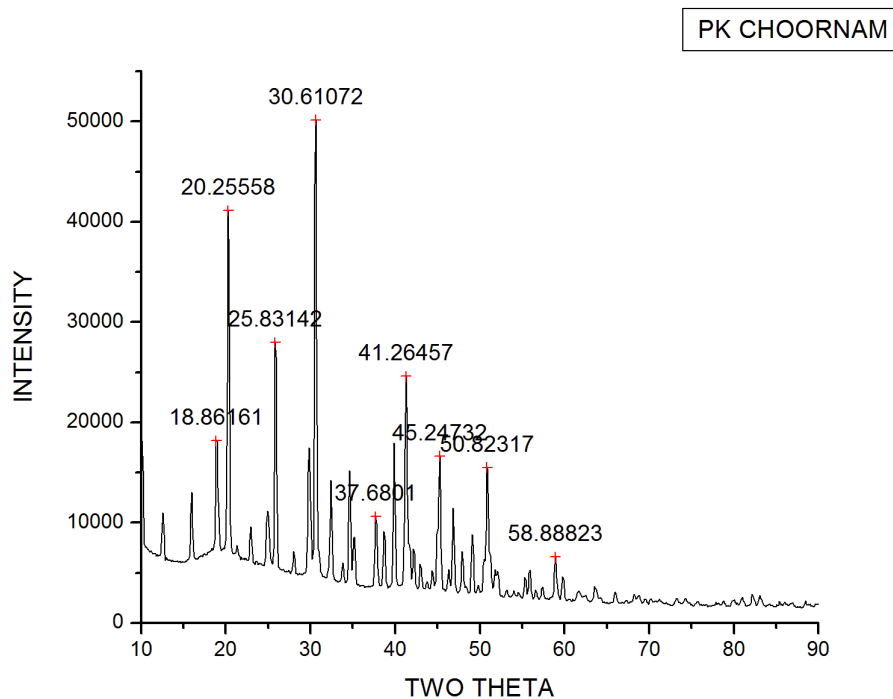


Fig: 10. SEM (SCANNING ELECTRON MICROSCOPE)

Interpretation for SEM

- Micro particles are defined as particulate dispersion or solid particles with a size in the range of 100-1000nm in diameter.
- The above SEM studies of microscopic resolution showed objects of sizes ranging from 3μm - 5μm.
- Size and surface of micro particles can be easily manipulated to achieve both passive and active drug targeting.
- They control and sustain, the release of drug during the transportation and at the site of localization, alters drug distribution in the body and subsequent clearance of the drug so as to achieve increased drug therapeutic efficacy thereby bio-availability and reduced side effects^[104]
- Hence *Pitha Kamalai Choornam* which is prepared biologically contains nanoparticles to enhance the pharmacological action in the target site.

XRD (X-Ray Diffraction studies)**Fig 11: XRD****Interpretation**

The structure, the size and shape of the particles are highly dependent on the route of synthesis and high lights the efficacy of the drug. The micro particles may enhance bio absorption of the drug.

The major diffraction peaks are identified after XRD analysis PKC concluded the range 20-50nm in association with organic molecules propably plays an important role in making it biocompatible and nontoxic at therapeutic doses. Other elements present in PKC act as additional supplement and possibly helps in increase the efficacy of the formulation.

Table 14: ICP-OES results of *Pitha Kamalai Choornam*

S.NO	Elements	Detected levels
1.	Aluminium	BDL
2.	Arsenic	BDL
3.	Calcium	01.450
4.	Cadmium	BDL
5.	Copper	BDL
6.	Mercury	BDL
7.	Potassium	44.801
8.	Magnesium	01.304
9.	Sodium	34.300
10.	Nickel	BDL
11.	Lead	BDL
12.	Phosphorus	18.301

Discussion:

- The above results indicate that the trial drug is extremely safe as it contains heavy metals within specified limits.
- The presence of Ca(01.450 mg/l), K(44.801 mg/l), Mg(01.304 mg/l), Na(34.300 mg/l), P(18.301 mg/l) is physiologically important. In *Pitha Kamalai Choornam*, 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

Acute oral toxicity study of *Pitha Kamalai Choornam* – OECD 423

Dose finding experiment and its behavioural Signs of acute oral Toxicity

Table: 15. Observation done:

S.NO	Group CONTROL	Observation	S.NO	Group TEST GROUP	Observation
1	Body weight	Normal	1	Body weight	Normally increased
2	Assessments of posture	Normal	2	Assessments of posture	Normal
3	Signs of Convulsion Limb paralysis	Normal	3	Signs of Convulsion	Absence of sign (-)
4	Body tone	Normal	4	Body tone	Normal
5	Lacrimation	Normal	5	Lacrimation	Absence
6	Salivation	Normal	6	Salivation	Absence
7	Change in skin color	No significant colour change	7	Change in skin colour	No significant color change
8	Piloerection	Normal	8	Piloerection	Normal
9	Defecation	Normal	9	Defecation	Normal
10	Sensitivity response	Normal	10	Sensitivity response	Normal
11	Locomotion	Normal	11	Locomotion	Normal
12	Muscle gripness	Normal	12	Muscle gripness	Normal
13	Rearing	Mild	13	Rearing	Mild
14	Urination	Normal	14	Urination	Normal

Table: 16. (Observational study Results)

No	Dose mg/kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1.	Control	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2.	2000mg	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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

Table: 17. Body weight of wistar albino rats group exposed to *Pitha Kamalai Choornam*

DOSE	DAYS		
	1	7	14
CONTROL	320.2±42.30	322.4 ± 60.10	323.6 ±52.10
HIGH DOSE	302.4± 1.21	302 ± 2.04	304.2 ± 2.10
P value (p)*	NS	NS	NS

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

Table: 18. Water intake (ml/day) of Wistar albino rats group exposed to *Pitha Kamalai Choornam*

DOSE	DAYS		
	1	6	14
CONTROL	58 ± 1.02	58±9.20	59.4±1.04
HIGH DOSE	59.4±2.20	59.8±3.40	59.9±6.24
P value (p)*	NS	NS	NS

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

Table: 19. Food intake (gm/day) of Wistar albino rats group exposed to *Pitha Kamalai Choornam*

DOSE	DAYS		
	1	7	14
CONTROL	61.04±2.62	62.2±4.76	64.3±6.26
High DOSE	69.4±4.23	70.4±6.22	71.6±4.18
P value (p)*	NS	NS	NS

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

Interpretation of Acute toxicity studies

- The acute oral toxicity potentials of *Pitha kamalai Choornam* in female Wistar albino rats were studied effectively.
- In the sighting study, the test substance was administered in sequential manner to one animal each at 2000 mg kg⁻¹ body weight followed by two animals at 2000 mg kg⁻¹ body weight.
- According to OECD guidelines, for acute oral toxicity LD₅₀ dose of 2000mg/kg of the drug is found to be safe.

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

Repeated dose 28-day oral toxicity of *Pitha Kamalai Choornam* OECD – 407

The results of acute toxicity studies in Wistar albino rats indicated that *Pitha kamalai Choornam* was non-toxic and no behavioural changes was observed up to the dose level of 2000 mg/kg body weight. On the basis of body surface area ratio between rat and human, the doses selected for the study were 200 mg/kg and 400 mg/kg body weight. The oral route was selected for use because oral route is considered to be a proposed therapeutic route. Then Sub acute oral 28 days repeated dose toxicity study was done and the results were tabulated below.

Weight gain of rats

Table: 20. Body weight of wistar albino rats group exposed to *Pitha Kamalai Choornam*

DOSE	DAYS				
	1	7	14	21	28
CONTROL	235.2±18.46	236.5 ± 35.10	236.6 ± 45.60	238.7± 56.16	238.4±66.15
LOW DOSE	248.2 ± 65.24	250.7 ± 66.28	254.6± 55.34	256 ±56.34	256.8±35.36
MID DOSE	252.4± 18.34	253.3 ± 16.24	253.4± 14.12	255.2 ±15.20	256.4±54.10
HIGH DOSE	261.6± 62.24	261.4±42.22	262.4 ± 52.24	263 ± 54.28	264 ± 74.60
P value (p)*	NS	NS	NS	NS	NS

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

Table: 21. Water intake (ml/day) of Wistar albino rats group exposed to *Pitha Kamalai Choornam*

DOSE	DAYS				
	1	6	14	21	28
CONTROL	60.1 ± 8.72	60±1.52	60.2±1.40	61±1.32	61.4±1.62
LOW DOSE	65.1±1.21	65.6±4.22	66.6±1.02	65.2±2.06	66.4±1.20
MID DOSE	62.1±1.02	62.3±1.21	62.1±2.62	63.4±4.32	63.4±1.64
HIGH DOSE	64.1±1.81	64.2±1.32	64.4±1.14	64.6±1.62	65.8±2.02
P value (p)*	NS	NS	NS	NS	NS

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

Table: 22. Food intake (gm/day) of Wistar albino rats group exposed to *Pitha Kamalai Choornam*

DOSE	DAYS				
	2	7	23	22	28
CONTROL	34±4.14	34.2±6.12	34.3±2.18	34.2±1.14	34±5.62
LOW DOSE	36.3±1.64	36.3±1.51	36.2±1.51	36.5±1.62	36.5±1.22
MID DOSE	34.1±2.12	34.2±3.50	34.2±2.14	34.2±2.16	35.2±1.64
HIGH DOSE	32.4±1.62	32.1±1.64	32.6±2.36	32.6±1.20	36.4±2.32
P value (p)*	NS	NS	NS	NS	NS

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

Interpretation of weight, water and food intake

- The overall percent of body weight gain in rats treated with the drug was found to be normal and showing a steady increase in weight indicating that

the test animals were in a healthy condition during the 28 days of observation period.

- There is no significant change in water intake by the animals during the period of study.
- The weight increase of the animals showed that the intake of food by the animals was good during the period of 28 days study.

Table: 23. Haematological parameters of Wistar albino rats group exposed to *Pitha Kamalai Choornam*

Category	Control	Low dose	Mid dose	High dose
Haemoglobin(g/dl)	13.4±0.71	13.30±0.14	13.4±0.13	13.72±0.13
Total WBC (×10 ³ l)	09.41±0.22	09.32±0.22	09.34±0.22	09.30±1.10
Neutrophils (%)	21.13±0.60	21.02±0.52	22.11±1.42	22.02±2.71
lymphocyte (%)	82.10±1.26	82.12±1.42	83.10±2.44	83.20±2.54
Monocyte (%)	1.1±0.03	1.1±0.01	1.2±0.04	1.1±0.03
Eosinophil (%)	0.8±0.03	0.8±0.04	0.9±0.05	0.9±0.08
Platelets cells10 ³ /μl	900.17±3.18	902.11±4.62	902.11±2.20	902.22±2.64
Total RBC 10 ⁶ /μl	9.32±0.11	9.47±0.33	9.50±0.64	9.60±0.46
PCV%	48.10±0.2	48.62±5.30	48.8±4.70	48.4±.71
MCHC g/Dl	36.5±1.61	36.2±1.51	36.8±1.30	36.13±1.60
MCV fL(μm ³)	58.2±2.02	58.2±1.80	58.7±1.10	59.7±1.30

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

Interpretation

- The haematological parameters of animals were done. The results of 28 days oral toxicity study was tabulated above.
- The Blood investigations of RBC, WBC, Hb, Platelets and ESR are normal that is within the limits.
- The differential count, PCV, MCV showed no significant changes.
- Thus the trial drug *Pitha Kamalai Choornam* was good and safe drug for oral administration

Table: 24. Biochemical Parameters of Wistar albino rats group exposed to *Pitha Kamalai Choornam*

BIOCHEMICAL PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
GLUCOSE (R) (mg/dl)	138.10±2.02	138.12±2.1 0	138.9±12.06	138.12±5.25
T.CHOLESTEROL (mg/dl)	140.14± 5.10	140.15±5.2 0	140.40±1.68	139.21±1.10
TRIGLYCERIDES (mg/dl)	74.15±1.82	74.11±1.32	74.15±1.22	73.16±1.21
LDL	78.6±2.13	78.7±2.05	78.10±1.03	77.40±01.32
VLDL	14.2±1.52	14.20±2.41	14.02±1.32	14.04±12.15
HDL	28.12±4.32	28.32±2.50	28.46±1.20	29.51±1.23
Ratio 1(T.CHO/HDL)	3.73±1.16	3.72±1.80	3.73±1.32	3.74±2.33
Ratio 2(LDL/HDL)	1.92±1.22	1.92±1.20	1.93±2.20	1.94±06.02
Albumin(g/dL)	6.21±0.22	6.22±0.52	6.4±7.20	6.55±6.48

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

Interpretation

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

Table: 26. Liver Function Test of of Wistar albino rats group exposed to PKC

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
T BILIRUBIN (mg/dl).	0.08±0.01	0.08±0.03	0.07±0.03	0.07±0.01
SGOT/AST(U/L)	64.11±1.53	62.12±0.22	60.24±1.54	58.74±1.53
SGPT/ALT(U/L)	79.21±1.02	77.34±1.04	71.44±1.16	66.38±0.21
ALP(U/L)	137.11±2.21	137±2.20	132±1.24	130.03±6.02
T.PROTEIN(g/dL)	7.2.40±0.14	7.4±0.41	7.4±0.60	7.5±0.61

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

Interpretation

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

Table: 25. Renal function test of of Wistar albino rats group exposed to Pitha Kamalai Choornam

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
UREA (mg/dl)	14.50±0.29	14.50±0.29	14.46±1.18	14.42±1.22
CREATININE(mg/dl)	0.42±0.02	0.41±0.04	0.43±0.03	0.44±0.09
BUN(mg/dL)	19.1±0.02	19.10±0.34	19.6±0.42	19.26±1.02
URIC ACID(mg/dl)	4.02±0.04	4.06±0.21	4.4±0.12	4.20±0.10

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

Interpretation

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

Table: 27. Urine Analysis

Parameters	Control	PKC 200mg/kg	PKC 400mg/kg
Transparency	Clear	Clear	Clear
Specific gravity	1.010	1.010	1.010
PH	6.4	6.8	6.5
Protein	Nil	Nil	Nil
Glucose	Nil	Nil	Nil
Bilirubin	-ve	-ve	-ve
Ketones	-ve	-ve	-ve
Blood	Absent	Absent	Absent
Pus cells	Absent	Absent	Absent
RBC	Nil	Nil	Nil
Epithelial cells	Nil	Nil	Nil
Casts	Nil	Nil	Nil
Others	Nil	Nil	Nil
Colour	Yellow	Yellow	Yellow

Interpretation of urine analysis

The urine analysis of the animals showed within the normal limits.

Interpretation

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

PHARMACOLOGY ACTIVITY

Pharmacological activity study result ccl₄ induced hepatotoxicity

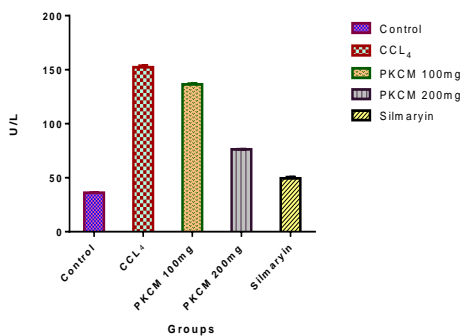
Table: 28. Level of Serum Enzymes value (AST, ALT, and ALP) with PKC

Groups	Treatment	ASTU/liter	ALT U/liter	ALP U/liter	GGT U/liter
Group 1	Normal	36.16±0.09	27.10±1.35	85.21±2.98	4.12±1.45
Group 2	CCL ₄ + LP	152.21 ±1.54*	193.58±1.00*	176.92±1.9	12.20±1.42*
Group 3	CCL ₄ +Low dose(PKC) 100mg/kg	136.42±0.64*	162.12±1.12	184.16±2.16	7.11±1.10*
Group 4	CCL ₄ +Highdose(PKC) 200mg/kg	76.21±0.16	45.76±2.17	97.12±2.15	4.97±1.01*
Group 5	CCL ₄ +Silymarin100 mg/kg	49.38±1.24*	30.17±0.68	91.62±1.83	4.16±2.11*

Statistical analysis ANOVA followed by Dunnett t-test.

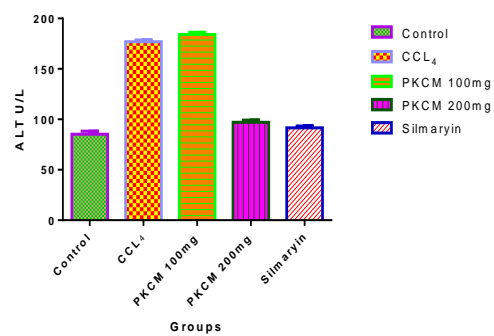
* P < 0.05; ** P < 0.01; *** P < 0.001 as compared with group I, a group II

AST level of PKCM treatment on CCL₄ induced toxicity



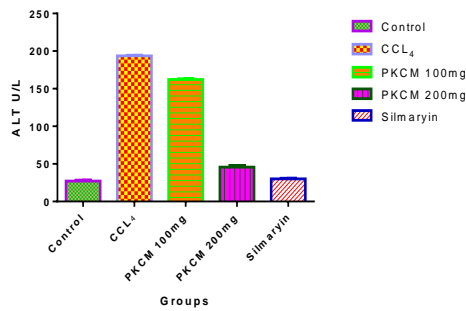
Graph 1a

Level of ALP on treatment with PKCM in CCL₄ induced toxicity



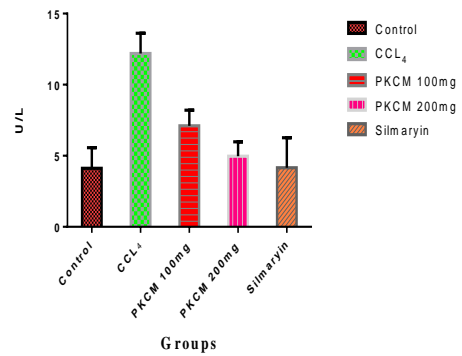
Graph 1b

Level of ALT on treatment with PKCM in CCL4 induced toxicity



Graph 1c

Level of GGT on treatment with PKCM in CCL4 induced toxicity



Graph 1d

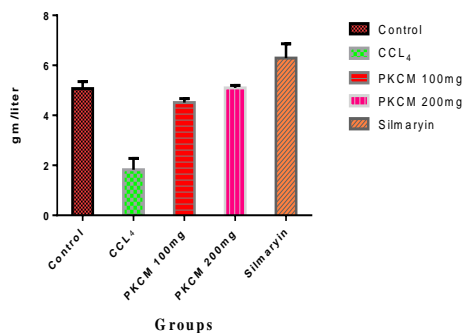
Table: 29. Effect of Total Protein and Bilirubin with PKC

Groups	Treatment	Total Protein(g/dl)	Bilirubin(mg/dl)
Group 1	Normal	5.07±0.28	0.27±0.01
Group 2	CCL ₄ + LP	1.82 ±0.46	1.7±0.07
Group 3	CCL ₄ +Low dose(PKC) 100mg/kg	4.51±0.16	1.35±0.01
Group 4	CCL ₄ +Highdose(PKC) 200mg/kg	5.10±0.10*	0.75±0.01*
Group 5	CCL ₄ +Silymarin100mg/kg	6.29±0.57**	0.47±0.01**

Statistical analysis ANOVA followed by Dunnett t-test.

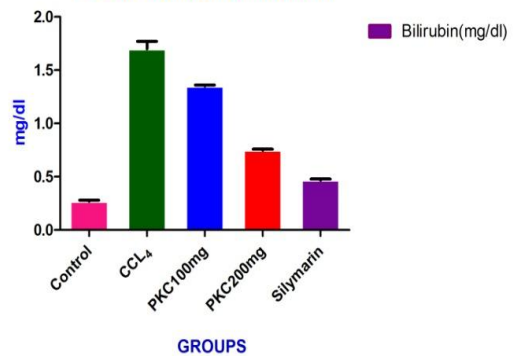
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ as compared with group I, a group II

Level of Total protein on treatment with PKCM in CCL4 induced toxicity



Graph 2a

Level of Bilirubin with PKCM



Graph 2b

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 PKC at 100 mg /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: Photo micrograph of liver tissue treated with silymarin showing normal hepatocytes , portal vein (V), portal Artery.

Interpretation

- The serum marker enzymes, AST, ALT, ALP, GGT and Total Proteins were exceedingly susceptible to hepatotoxins. They assist as markers of inflammation of liver cells or death of some cells due to liver damage and oxidative stress, which stimulate the release of amino transferases from hepatocytes into the blood.
- The changes associated with Carbon tetrachloride induced liver damage of the present study appeared similar to the acute viral hepatitis.
- In CCl₄ induced hepatotoxicity, the administration of the toxicant CCl₄ showed a distinct rise in the levels of serum marker enzymes namely AST, ALT, ALP, GGT and Total protein as shown in the Table.
- The protective effects of *Pitha Kamalai Choornam* and silymarin on the effect of liver serum marker enzymes are exposed in Table.
- The serum enzymes like AST, ALT, ALP, GGT, and Total Protein of treated animals were significantly reduced by seven days pretreatment of *Pitha Kamalai Choornam* at two dose levels 100mg/kg and 200mg/kg, when compared with CCl₄ treated control. No significant clinical abnormalities in other groups.
- This study reveals that increase in the activity of the serum enzymes AST. ALT, ALP, GGT and Total Proteins were detected in wister Albino rats treated with normal saline and CCl₄ (Group 1 and 2). However, the activities of these serum enzymes were significantly ($P < 0.01$) lower in rats treated with *Pitha Kamalai Choornam* (Group 3 and 4) than in Group 1 wister Albino rats.
- Reduction in the levels of AST, ALT, ALP, GGT and Total protein toward the normal value is an indication of regeneration process. The protective effect

exhibited by PKC at dose level of 200 mg/kg was comparable with the standard drug.

- These findings suggested that the administration of PKC has significantly neutralized the toxic effects of Carbon tetrachloride and helped in regeneration of hepatocytes.
- This present study confirmed that CCl₄ induced a marked rise in oxidative stress and cellular degeneration in wister Albino rat's liver. Both the doses of *Pitha Kamalai Choornam* treatment (100 and 200 mg/kg body wt.) significantly improved the effect of CCl₄ induced oxidative stress damage and reduced the expression of inflammatory and apoptotic proteins. As a result this protective effect of *Pitha Kamalai Choornam* could be correlated directly to its antioxidant property.
- Therefore the reduction in the activity of these enzymes may result in a number of deleterious effects by administration of PKC increased the activities against CCl₄ induced liver damage in rats to prevent the accumulation of excessive fats and protected the Liver^[105].
- This was further confirmed by histopathological injuries.

Effect of *Pitha Kamalai Choornam* on liver injury induced by paracetamol in wister albino rat

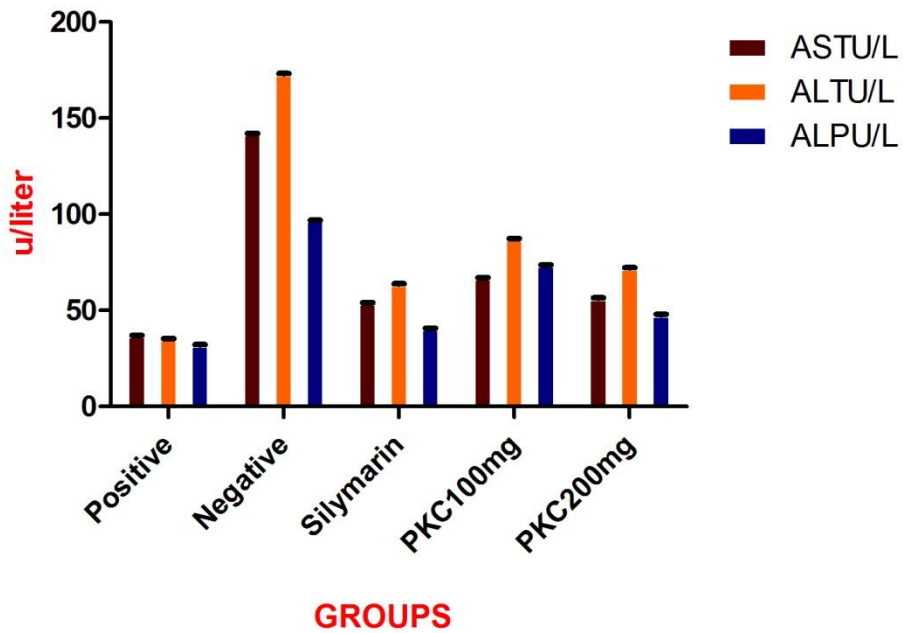
Table: 30. Effect of Serum enzymes with PKC

Groups	Treatment	ASTU/liter	ALT U/liter	ALP U/liter
Group 1	Positive Control	36.96±0.13	35.18±0.29	32.11±0.14
Group 2	Negative Control Paracetamol – 1.25ml/kg	142 ±0.12	173.07±0.24	96.85±0.12
Group 3	Standard (Silymarin -100mg/kg)	53.94±0.14 ^{**}	63.71±0.17 ^{**}	40.58±0.21 ^{**}
Group 4	PKC – 100 mg	66.98±0.13 [*]	87.30±0.18	73.52±0.18 [*]
Group 5	PKC – 200 mg	56.44±0.16 ^{**}	72.05±0.25 [*]	47.83±0.20 ^{**}

Statistical analysis ANOVA followed by Dunnett t-test.

* *P* < 0.05; ** *P* < 0.01; *** *P* < 0.001 as compared with group I, a group II

Effect of PKCM on serum enzymes



Graph 3

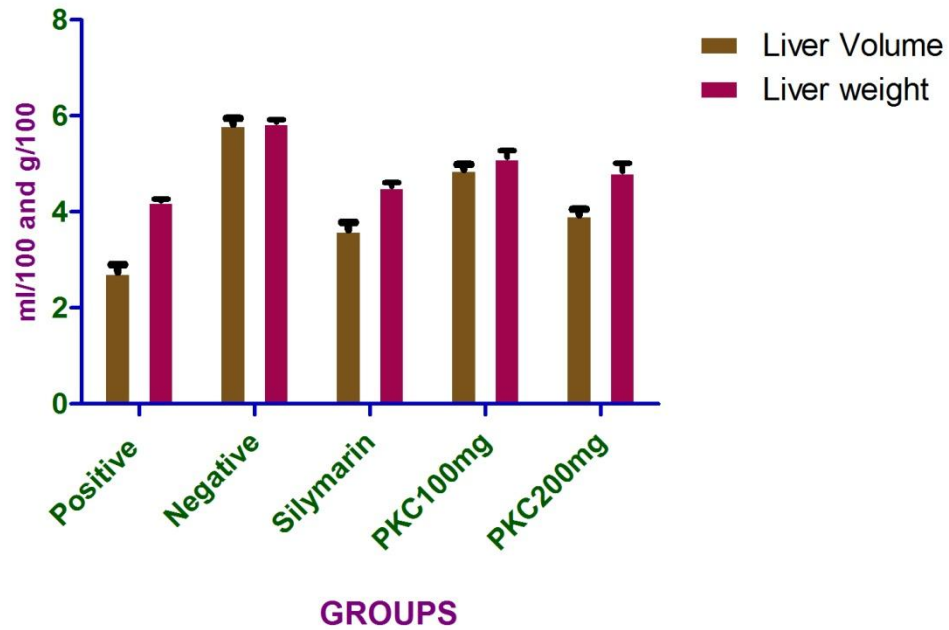
Table: 31. Effect of Liver Volume and Liver Weight with PKC

Group	Treatment	Liver Volume(ml)	Liver Weight(g)
A	Positive Control	2.73±0.15	4.23±0.04
B	Negative Control Paracetamol – 1.25ml/kg	5.83±0.12	5.87±0.05
C	Standard (Silymarin -100mg/kg)	3.63±0.15 ^{***}	4.53±0.08 ^{***}
D	PKC – 100 mg	4.89±0.10 [*]	5.14±0.14 [*]
E	PKC – 200 mg	3.94±0.12 [*]	4.84±0.18 [*]

Statistical analysis ANOVA followed by Dunnett t-test.

* P < 0.05; ** P < 0.01; *** P < 0.001 as compared with group I, a group II

Effect of PKCM on Liver volume & weight



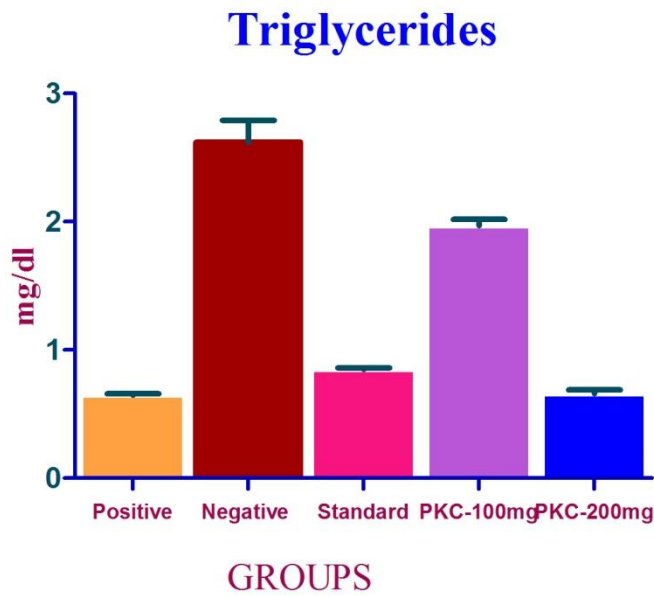
Graph: 4

Table: 32. Effect of Triglycerides with PKC

Group	Treatment	Triglycerides (mg/dl)
A	Positive Control	0.65±0.01
B	Toxicant Control(Paracetamol-1.25ml/kg)	2.621±0.17
C	Standard(Silymarin-100mg/kg)	0.850±0.01 ^{***}
D	PKC – 100 mg	1.97±0.05 ^{**}
E	PKC – 200mg	0.905±0.03 ^{**}

Statistical analysis ANOVA followed by Dunnett t-test.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ as compared with group I, a group II



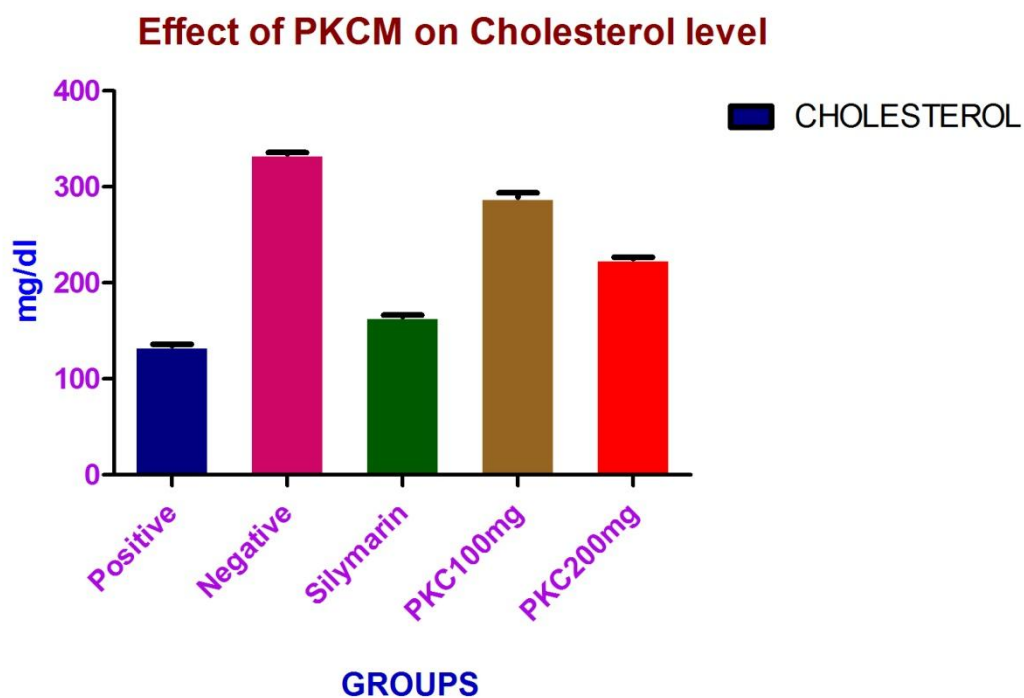
Graph: 5

Table: 33. Effect of Cholesterol with PKC

Group	Treatment	Cholesterol (mg/dl)
A	Positive Control	134.8±1.30
B	Toxicant Control(Paracetamol-1.25ml/kg)	334.6±1.28
C	Standard(Silymarin-100mg/kg)	165.2±1.14 ^{***}
D	PKC – 100 mg	289.5±4.35 [*]
E	PKC – 200mg	225.6±1.25 ^{**}

Statistical analysis ANOVA followed by Dunnett t-test.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ as compared with group I, a group II



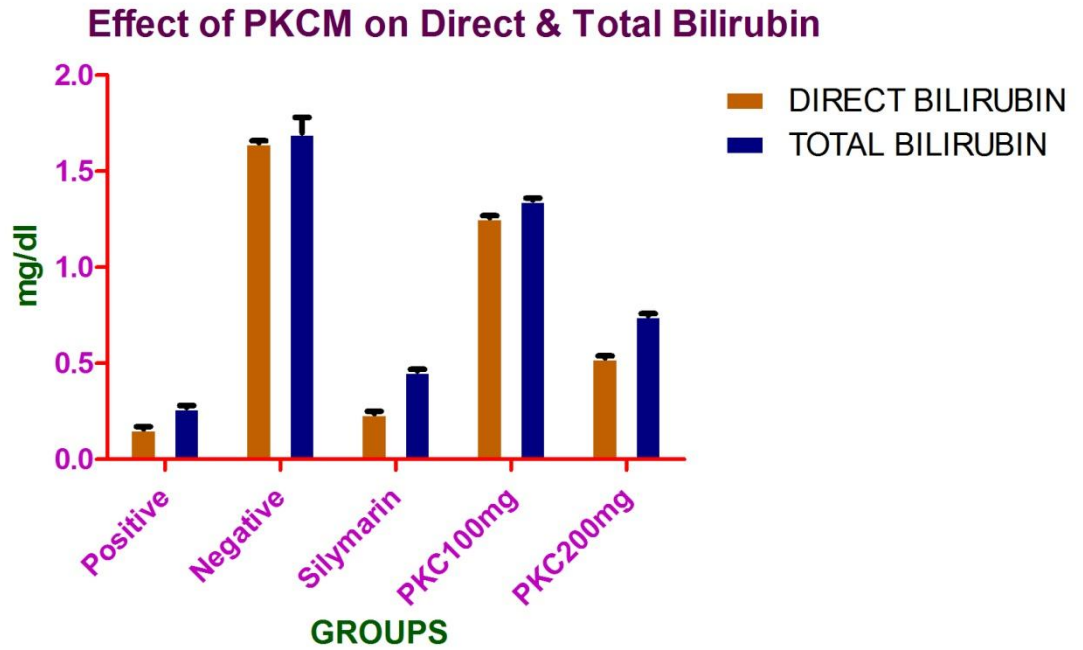
Graph: 6

Table: 34. Effect of Direct Bilirubin and Total Bilirubin with PKC

Group	Treatment	Direct Bilirubin mg/dl	Total Bilirubin mg/dl
A	Positive Control	0.16±0.01	0.27±0.01
B	Negative Control Paracetamol – 1.25ml/kg	1.65±0.01	1.7±0.08
C	Standard (Silymarin -100mg/kg)	0.24±0.01 ^{***}	0.46±0.01 ^{***}
D	PKC – 100 mg	1.26±0.01 [*]	1.35±0.01 [*]
E	PKC – 200 mg	0.53±0.01 ^{**}	0.75±0.01 ^{**}

Statistical analysis ANOVA followed by Dunnett t-test.

** P < 0.05; ** P < 0.01; *** P < 0.001 as compared with group I, a group II*



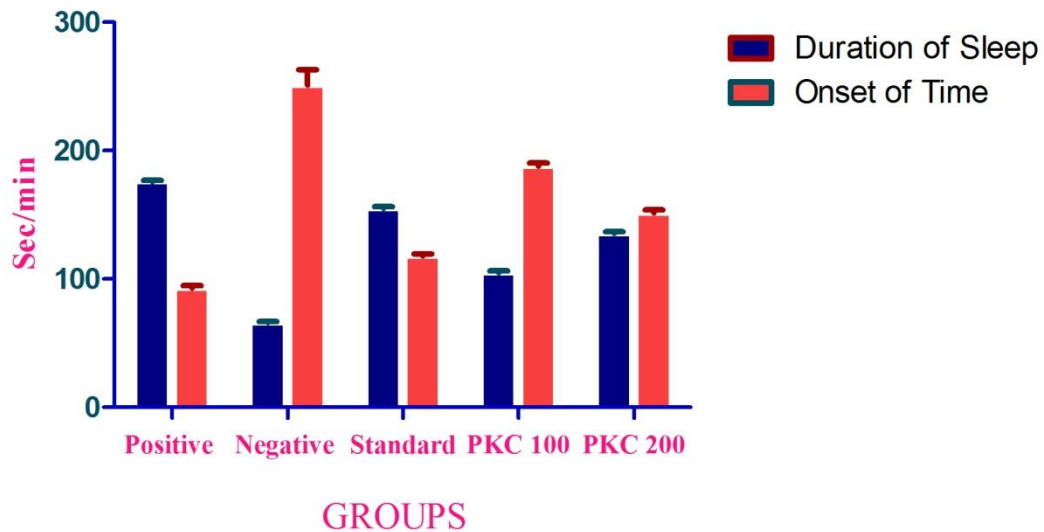
Graph: 7

Table: 35. Effect of Direct Duration of Sleep and Onset of Time with PKC

Group	Treatment	Duration of Sleep (sec)	Onset of Time (min)
A	Positive Control	175.8±0.95	93.2±1.56
B	Negative Control Paracetamol – 1.25ml/kg	66±0.96	251±12.13
C	Standard (Silymarin -100mg/kg)	155.2±1.07**	118.2±1.47**
D	PKC – 100 mg	105±1.32	188±2.39
E	PKC – 200 mg	135.5±1.34*	151.6±2.3*

Statistical analysis ANOVA followed by Dunnett t-test.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ as compared with group I, a group II



Graph: 8

HISTOPATHOLOGY OF PKC

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 PKC at 100 mg /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: Photo micrograph of liver tissue treated with silymarin showing normal hepatocytes, portal vein (V), portal Artery.

Interpretation

- Paracetamol induced hepatotoxicity is the generally used screening method for testing the hepato protective nature of drugs. The hepatic damage increases the level of serum marker enzymes like ALT, AST,

ALP. This indicates the cellular damage as well as loss of the functional integrity of cell membrane in liver.

- The test was also conducted to compare the Liver Volume and liver weight of Paracetamol induced rat and the rat with effect of *Pitha Kamalai Choornam*.
- In paracetamol treated Wister albino rat the levels of serum marker enzymes (ALT, AST, and ALP) elevated significantly and Triglycerides level. These increased levels of serum levels were depicted in Table.
- Owing to damage of hepatocytes the cell necrosis occurs. The increased production of serum enzymes in blood was related with central/submissive necrosis of liver which causes severe hepatic injury.
- There are no significant changes in liver weight in rats with the effect of *Pitha Kamalai Choornam* when compared to the standard drug.
- The hepatoprotective effect of *Pitha Kamalai Choornam* may be because of the antioxidant activity. The *in vitro* antioxidant activity of *Pitha Kamalai Choornam* such as GPx, GSH, CAT, SOD increase suggest the ability of *Pitha Kamalai Choornam* to reduce biological oxidative stress.
- Moreover, the increased levels of the serum enzymes were significantly decreased by the treatment with *Pitha Kamalai Choornam* at 100 mg/kg and 200 mg/kg, implying that the drug prohibited the liver damage.
- The *Pitha Kamalai Choornam* treatment confirmed dose dependent activity, *Pitha Kamalai Choornam* at 200 mg/kg revealed good result than 100mg/kg which is given in Table 25 for the determined levels of various serum enzymes.
- This was further confirmed by decreased amount of histopathological slides.

RESULTS OF ANTI-OXIDANT ACTIVITY OF *PITHA KAMALAI CHOORANAM*

Due to various factors of the fast moving life style like fast foods, change in daily routines, the level of oxidants or reactive oxygen species are increased in the body. It has been established that oxidative stress is the major causative factor in the induction

of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immune suppression, neurodegenerative diseases and others. The free radicals are scavenged from the body by various mechanisms to enable good functioning of the organs.

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

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

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

Qualitative analysis of antioxidant activity of PKC extract:

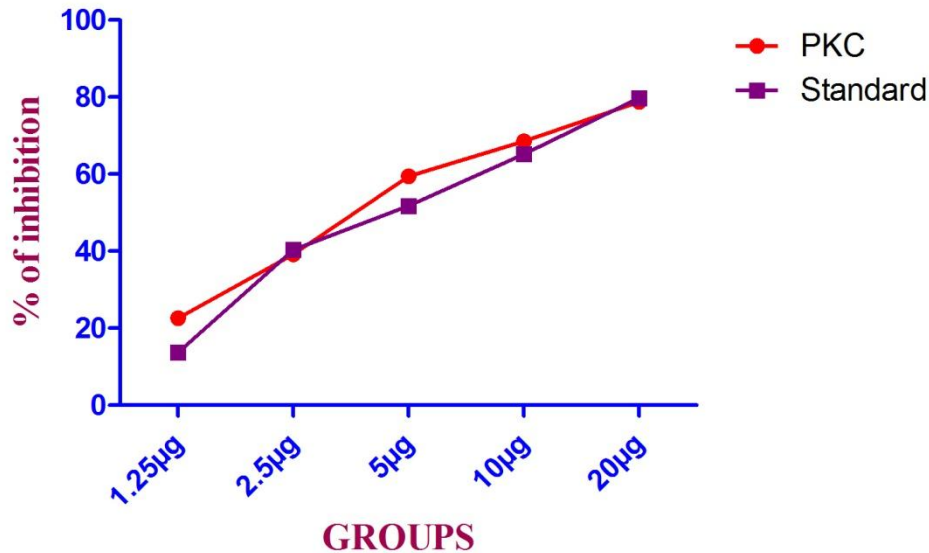
The antioxidant activity of *PKC* extracts was determined by standard method. 50 µl of *PKC* extracts was taken in the micro titer plate. 100 µl of 0.1% methanolic 1,1-diphenyl- 2-picrylhydrazyl (DPPH) was added over the samples and incubated for 30 minutes in dark condition. The samples were then observed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

Table: 36. DPPH Assay of *Pitha Kamalai Choornam*

Sample concentration(µg/ml)	Absorbance		Percentage of Inhibition	
	Drug	Standard	Drug	Standard
Control	0.5341	0.322	-	-
1.25	0.4132	0.278	22.6363	13.67
2.50	0.3246	0.192	39.2249	40.37
5	0.2164	0.165	59.4833	51.76
10	0.1682	0.112	68.5078	65.22
20	0.1242	0.094	76.7460	79.81

µg/ml: microgram per millilitre. Drug: *PKC* (5-20µg/µl). Standard: Ascorbic acid (10mg/ml DMSO)

DPPH Assay of PKC



Graph: 9

Interpretation

In the present study, the extract of PKC was found to possess concentration dependent scavenging activity on DPPH radicals. The values of DPPH free radical scavenging activity of the PKC extract was given in the (Table:), The extract of PKC showed the highest DPPH scavenging activity (76.75%) at 20µg/ml and the lowest percentage of inhibition (22.63%) at 1.25µg/ml. Ascorbic acid (Standard) showed highest percentage of inhibition (79.81%) at 20µg/ml and the lowest percentage of inhibition (13.65%) at 1.25µg/ml.

This indicated that % of inhibition with increase with increase in concentration of both the standard and PKC extract. The PKC extract has more or less equal DPPH scavenging activity ^[106].

CONCLUSION

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

Pitha Kamalai Choornam, a traditional Siddha herbo-mineral drug was prepared as per the procedures mentioned in Siddha literature. The chemical fingerprint was engaged by using modern analytical techniques like Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) and Fourier Transform Infra-Red Spectroscopy (FTIR). In addition, the particle size and qualitative analysis of chemical elements of *Pitha Kamalai Choornam* were also assessed by Scanning Electron Microscope (SEM).

The qualitative and quantitative elemental analysis of *Pitha Kamalai Choornam* was carried out by X-Ray Diffraction Study (XRD). The instrumental analysis report reveals the heavy metals like Hg, As, Cd, Pb and trace element like Ni were not detectable. It proves the safety of the drug.

The acute toxicity study exposes that the LD₅₀ of *Pitha Kamalai Choornam* is more than 2000mg/kg, body weight in Wistar Albino rat. 28-day repeated oral toxicity demonstrates that there was no Observed Adverse Effect Level of *Pitha Kamalai Choornam*.

The pharmacological analysis showed that the drug has got significant Hepatoprotective efficacy. And also the *Pitha Kamalai Choornam* has potent antioxidant activity.

In conclusion, the safety and efficacy of the *Pitha Kamalai Choornam* proves that it has remarkable medicinal value against the disease *Kamalai*.

SUMMARY

The trial drug *Pitha Kamalai Choornam* was selected from the text “*Korakkur Maruththuvam*” for the validation of safety, efficacy and its potency in hepatoprotective effect.

The raw drugs were collected from the Ramasamy pillai shops, at Paris corner, Chennai. The raw drugs were authenticated by the Botanist and Proffesor in Gunapdam Department in Government Siddha Medical College, Arumbakkam, and Chennai-106. .

Various collections of Siddha and Modern science about the ingredients of the drug supported the fact of hepatoprotective activity.

The shelf life of *Choornam* is mentioned in Siddha literature as 3 months. But the recent report has been published by the AYUSH and mentioned that the shelf life of *Choornam* is only 1 years.

Physico-chemical analysis

The pH of *Pitha Kamalai Choornam* was 5.63 which is acidic in nature. Acidic drugs are essential for Bioavailability and effectiveness. Acididic drugs are better absorbed in stomach. Hence, the drug does not produce any harmful effect to the mucus membrane of the GI tract.

Choornam is one of the basic medicines in Siddha system. The medicines on this order have fine particle size and low moisture content. The fine particle size enhances the pharmacokinetic actions and the low moisture content indicates the longer shelf life period of the drug.

Instrumental analysis:

Based on the results, *Pitha Kamalai Choornam* 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, XRD and SEM respectively.

ICP-OES reveals high concentration of K in *Pitha Kamalai Choornam* (44.801 mg/l). It also has physiologically important minerals like Na, Mg, P and Ca. In *Pitha Kamalai Choornam* the heavy metals like As, Cd, Pb and trace element like Ni were not detectable level. This reveals the safety of the drug.

The FTIR results showed the presence of O-H Stretching and bend, C-H Stretching and bend, C=O Stretching as functional groups. The shift of C=O stretching frequency indicates a bounding of the calcium and oxygen nanoparticles through this group.

XRD pattern of the trail drug *Pitha Kamalai Choornam* shows some good crystallinity. The major diffraction peaks are identified after XRD analysis. The crystalline may be due to the presence of the ingredients of the *Pitha kamalai choornam*.

The SEM picture shown the presence of nanoparticle of size 1 μ m – 500 nm in the drug *Pitha Kamalai Choornam*. Further, the study shows that *Pitha Kamalai Choornam* is a kind of nanomedicine which favours the advantages of bio availability, better absorption and non toxic with minimal dose level.

The Physico chemical analysis shows the presence of Calcium, phosphorus, sodium, magnesium which is physiologically important. In *Pitha Kamalai Choornam* the heavy metals like Arsenic, Cadmium, and Mercury were not detectable. This reveals the safety of the drug.

Pharmacokinetic aspect:

The weak acidic medicines were absorbed in alkaline medium. That is the *Pitha Kamalai Choornam* may be absorbed in small intestine.

Toxicity studies:

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

In Conclusion, no toxic effect was observed up to 400mg/kg of *Pitha Kamalai Choornam* treated over a period of 28 days (OECD 407). So, it can be concluded that

the *Pitha Kamalai Choornam* can be prescribed for therapeutic use in human with the dosage recommendations of up to 100mg/kg body weight p.o.

Hepatoprotective activity against CCl₄ and Paracetamol:

The present study showed that *Pitha Kamalai Choornam* produce protective action against the hepatotoxicity induced by CCl₄ and paracetamol. The hepatoprotective role of *Pitha Kamalai Choornam* might be due to its chemical constituent. *Pitha Kamalai Choornam* produces antioxidant activity so this mechanism suggesting that the *Pitha Kamalai Choornam* may be useful to prevent the oxidative stress induced damage in liver. Hence *Pitha Kamalai Choornam* may be act as prophylactic as well as curative drug in treating hepato toxic conditions. Further studies needs to isolate the active constituents and also to evaluate the exact mechanism of action.

Thus the author validated *Pitha Kamalai Choornam* as a new hepato-protective drug which is cost effective and without any side effects.

FUTURE SCOPE

Trial drug for the study *PITHA KAMALAI CHOORNAM*, was taken from the classic Siddha Literature *Korakkar Maruththuvam*, by *Siddha Maruththuva Nool Veliyeetu Pirivu*, *Indian Medicine and Homeopathy Department, Chennai 106*. Its validation for its Hepatoprotective and Anti Oxidant action were completed at preliminary level. The result enhanced and assured its Hepatoprotective property against CCL₄ and Paracetamol induced Hepatotoxicity. More specific experiments on animal models and also clinical trials are required to understand the exact molecular mechanism of action. So it could be used worldwide in safe treatment as Hepatoprotective.

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This Certificate is awarded to Dr/Mr/Mrs... *S. Sumithra*

for participating as ~~Resource Person~~ / Delegate in the Eighteenth Workshop on

“ RESEARCH METHODOLOGY & BIostatISTICS ”

FOR AYUSH POST GRADUATES & RESEARCHERS

Organized by the Department of Siddha

The Tamil Nadu Dr. M.G.R. Medical University from 20th to 24th July 2015.


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सिद्ध केंद्रीय अनुसन्धान संस्थान,

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20.1.2017

CERTIFICATE

Name of the student: Dr. S. Sumithra, III year PG student, Gunapadam, Government Siddha Medical College, Arumbakkam, Chennai-600 106.

Name of the sample: Pithakamalai Chooranam

Name of the Experiment	I	II	Mean
Loss on drying(at 105°C)	8.62 %	8.86 %	8.74 %
Total ash	4.88 %	5.07 %	4.98 %
Water soluble ash	2.04 %	2.00 %	2.02 %
Acid insoluble ash	0.498 %	0.696 %	0.597 %
Water soluble extractive	35.6 %	33.0 %	34.3 %
Alcohol soluble extractive	35.0 %	38.20 %	36.60 %
pH value (10%)	5.63	5.64	5.63
TLC/HPTLC	Report Enclosed		

(R. Shakila)

Research Officer (Chemistry) & Head,
Department of Chemistry

P. Elankani
(Dr. P. Elankani)

Research Officer (Scientist II) (Siddha)
for Assistant Director (Siddha) I/c



C.L.BAID METHA COLLEGE OF PHARMACY

(An ISO 9001-2000 certified institute)

Jyothi Nagar, Old Mahabalipuram Road

Thoraipakkam, Chennai – 600 097

CERTIFICATE

This is to certify that the project entitled, **Toxicological and Pharmacological study** on **PITHA KAMALAI CHOORANAM & SATTHICHAARANAI CHOORANAM** (*Trianthena decandra*) in rats submitted in partial fulfilment for the degree of **M.D. (siddha)** was carried out at C.L. Baid Metha college of Pharmacy, Chennai-97, in the Department of Pharmacology during the academic year of 2016-2017. It has been approved by the **IAEC No: IAEC/XLVIII/12/CLBMCP/2016**


Dr.P.Muralidharan

IAEC Member Secretary

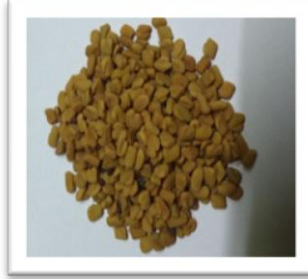
**C.L. BAID METHA COLLEGE OF PHARMACY,
THORAIPAKKAM, CHENNAI - 600 097.**



INGREDIENTS



Black Pepper



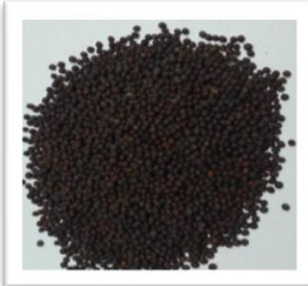
Fenugreek Seeds



Bishops Weed



Cumin Seeds



Indian mustard



Anise seeds



Turmeric



Garlic



Dried Ginge



Long Pepper

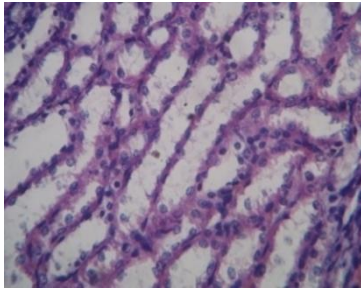


Asafoetida

HISTOPATHOLOGY: SUBACUTE ORAL TOXICITY STUDY

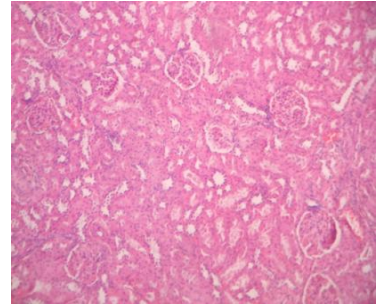
Control group:

Kidney

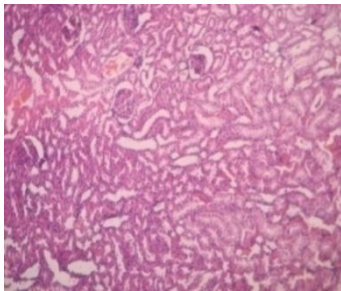


High Dose:

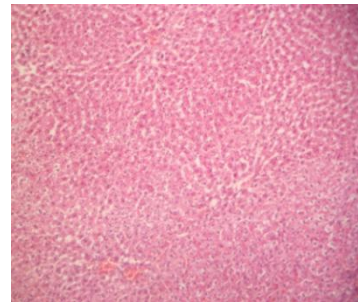
Kidney



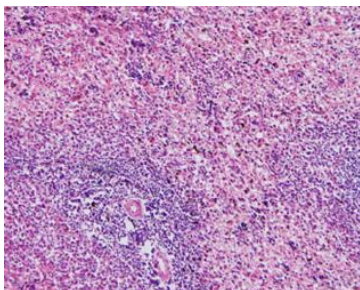
Liver



Liver



Spleen



Spleen

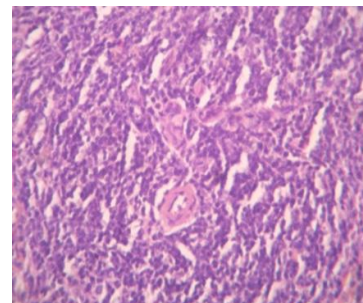


Figure: 12 Histopathological slides

**HISTOPATHOLOGICAL SLIDES OF HEPATOPROTECTIVE ACTIVITY OF
PITHA KAMALAI CHOORNAM AGAINST CCL_4 INDUCED
HEPATOTOXICITY**

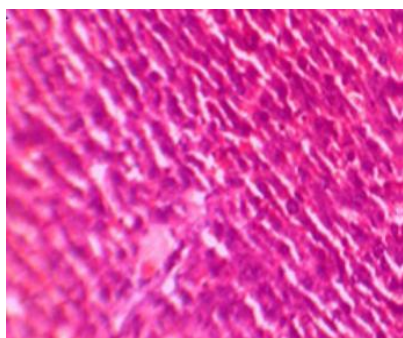


Fig 13a: Group I Control

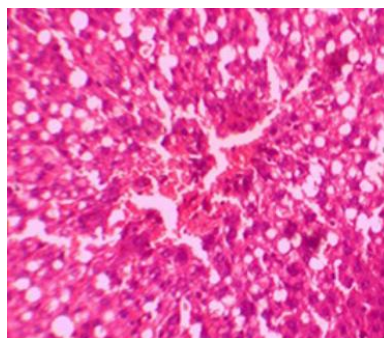


Fig 13b: Group II. CCL_4 treated

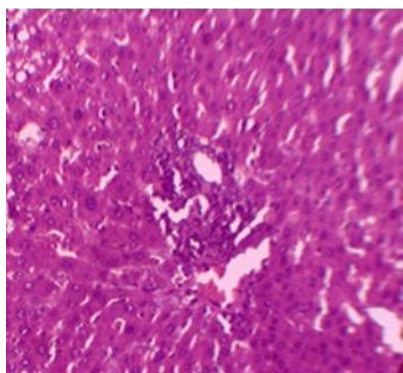


Fig 13c: Group III. CCL_4 +PKC100mg

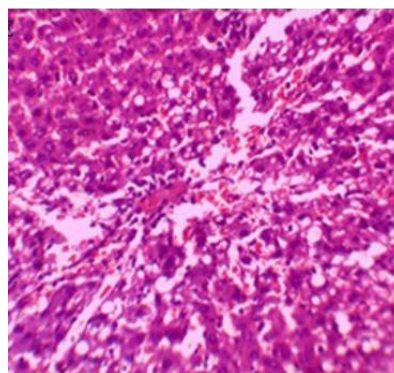


Fig 13d: Group IV CCL_4 +PKC200mg

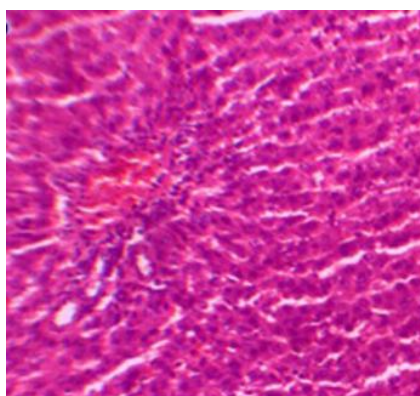


Fig 13e Group V CCL_4 +Silymarin