HEPATOPROTECTIVE ACTIVITY OF *"MAAVILINGAPATTAI CHOORANAM"* ON CCL_4, PARACETAMOL AND ETHANOL INDUCED HEPATOTOXICITY IN IN-VIVO MODELS.

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CHENNAI-106

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"Hepatoprotective activity of *MAAVILINGAPATTAI CHOORANAM* on CCl_4, Paracetamol and Ethanol Induced Hepatotoxicity in In-vivo models"** is a bonafide and genuine research work carried out by me under the guidance of **Dr.R. Karolin Daisy Rani, M.D (S),** Lecturer, 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
ANOVA	- Analysis of Variance
AST	- Aspartate Transaminase
BDL	- Bile Duct Ligation
BUN	- Blood Urea Nitrogen
CAT	- Catalase
CCL_4	- Carbone tetrachloride
CD	- Conjugated Dienes
CMC	- Carboxy Methyl Cellulose
CPCSEA	- Committee for the purpose of control and supervision Of experimental animals.
DC	- Differential count
Dep	- Deposits
DMN	- Dimethlnitrosamine
E	- Eosinophil
E.coli	- Escherichia Coli
ESR	- Erythrocyte Sedimentation Rate
FLD	- Fatty Liver Disease
FPC	- Few Pus Cells
FRAP	- Ferric Reducing Anti oxidant Power

FTIR	- Fourier Transform Infrared Spectroscopy
GIT	- Gastro Intestinal Tract
GOT	- Glutamate Oxaloacetate Transaminase
GPx	- Glutathione peroxidase
GSH	- Reduced Glutathione
Hb	- Haemoglobin
Hcl	- Hydrocloric acid
HNO3	- Nitric acid
HP	- Hydrogen Peroxide
HPLC	- Higher Performance Liquid Chromatography
H2SO4	- Sulphuric acid
IAEC	- Institutional Animal Ethical Committee
ICMR	- Indian Council of Medical Research
ICP-MS	- Inductively Coupled Plasma Mass Spectrometry
L	- Lymphocyte
LP	- Liquid Paraffin
MAO	- Mono Amine Oxidase
MDA	- Malondialdehyde
NASH	- Non Alcoholic Steato Hepatitis
OECD	- Organisation for Economic Co-Operation & Development
P	- Polymorphs
PCV	- Packed Cell Volume

PT	- Prothrombin Time
RBC	- Red Blood Cell
RNA	- Ribonucleic acid
ROS	- Reactive Oxygen Species
S.aureus	- Salmonella aureus
SEM	- Scanning Electron Microscope
SEM	- Standard Error Mean
SGOT	- Serum Glutamic Oxaloacetic Transeaminase(ALP)
SGPT	- Serum Glutamic Pyruvic Transaminase(AST)
SOD	- Superoxide Di Mutase
MPC	- Maavilingapattai Chooranam
TAA	- Thioacetamide
TA	- Total Protein
TB	- Total Bilirubin
TBARS	- Thiobarbituric acid REACTIVE substances
TC	- Total count
TCW	- Tender Coconut Water
TP	- Total Protein
UV	- Ultra Violet
WBC	- White Blood Cell
WHO	- World Health Organization
XRD	- X-Ray Powder Diffraction

1. INTRODUCTION

“Life love the liver of it.”

-Maya Angelou.

Siddha system of medicine is one of the ancient system of medicine in India. The term siddha is derived from the word *‘Siddhi’*. *Siddhi* means attainment of perfection, accomplished or achievement. The unique nature of this system is its continuous service to humanity[1].

In *Siddha* system, there were eight supernatural powers are called “*Attamasiddhi”*. Those who attained the supernatural powers are called *Siddhars*. *Siddhars* gained the supreme wisdom and overall immortality. Through this spiritual knowledge, they wrote scriptures on all aspect of life, from arts to science and truth of life to miracle cure for diseases[2].

Siddhars have the concept that a healthy soul can only be developed by a healthy body. Saint *Thirumoolar* said that,

உடம்பார் அழியில் உயிரார் அழிவர்

திடம்பட மெய்ஞானம் சேரவும் மாட்டார்

உடம்பை வளர்க்கும் உபாயம் அறிந்தே

உடம்பை வளர்த்தேன் உயிர் வளர்தேனே.

-திருமந்திரம்.[3]

The art of bestowing immortality is a unique feature of *Siddha* medicine which is termed as “*Kayakalpa”*.

Kaya means body, *Kalpa* means stone like, together meaning keeping the body as strong as a stone. *Kayakalpa* therapy is based on prevention of diseases. It also promote the health of the body and prevent aging process[1a].

According to *Siddha* system, the physiological components of the human beings are classified as *Vaatham* (air), *Pitham* (fire) and *Kabam* (earth and water). When the normal equilibrium of this three humors were disturbed, disease is caused[4].

The treatment in *Siddha* medicine is aimed at keeping the three humors in equilibrium and maintenance of seven elements such as *saaram* (plasma), *cheneer* (blood), *oon* (muscle), *kozhuppu* (fatty tissue), *enbu* (bone), *moolai* (bone marrow), s*ukkilam* (semen) or *suronitham* (ovum)[1b].

Proper diet, medicine and a disciplined regimen of life are advised for a healthy living and to restore equilibrium of humors in diseased condition. Saint *Thiruvalluvar* said that,

உற்றவன் தீர்ப்பான் மருந்துழைச் செல்வானென்

றப்பனாற் கூற்றே மருந்து.

-திருக்குறள்.

He explains four requisites of successful treatment. These are the patient, the attendant, physician and medicine. When the physician is well-qualified and the other agents possess the necessary qualities, even severe diseases can be cured easily, according to these concepts[1c].

Hepatoprotection or Anti-Hepatotoxicity means the ability to prevent damage to the liver[5].

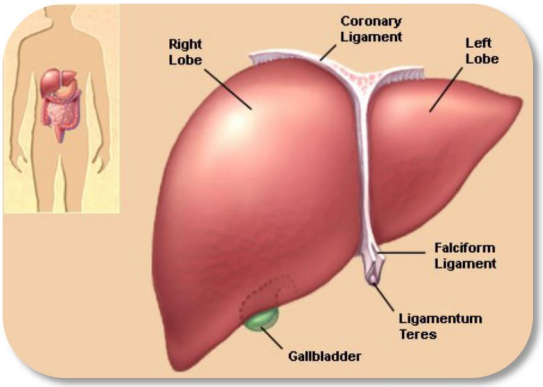


Fig 1.1

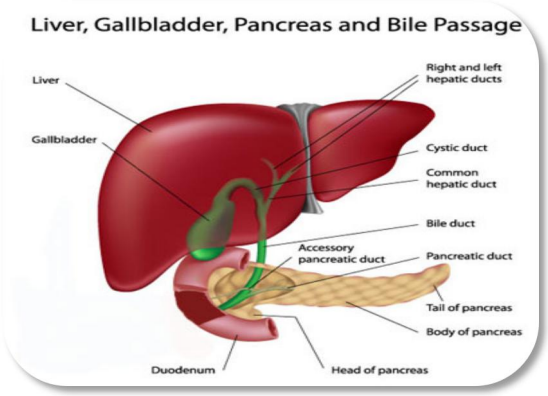


Fig 1.2

Liver is the largest internal organ weighing about 1400grams in adults. It constitute 2.5% of total body weight. Liver plays an important role in carbohydrate, protein and lipid metabolism. In carbohydrate metabolism maintaining blood glucose level is the important function of liver. In lipid metabolism it regulates the circulating blood lipids.

Liver also involved in the synthesis of fatty acids, lipoproteins, amino acids and plasma proteins which is known as albumin, fibrinogen and prothrombin. Liver also plays an important role in storage and metabolism of fat soluble vitamins and irons. In water soluble vitamins, Vitamin B_{12} are also stored in liver[6].

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

Behind the liver there is a small organ called the gallbladder, which function is to store bile produced by the liver and empty it into the small intestine to aid digestion and absorption[7a]. Bile pigment, cholesterol and drugs are excreted by liver. Liver also regulate endocrine functions of hormones. Various hormones like insulin, glucagon, growth hormone and GI hormones are degraded by liver.

Kuffer cells are most important phagocytic cells which is present in liver. These kuffer cells plays an important role in removing unwanted materials from the circulation. Endocytosis is the mechanism by which these materials are removed.

Liver cells are called Hepatocytes. Heaptocyte plays an important role in the metabolism of drugs and xenobiotics. For effective elimination, the drug and the metabolite must be made hydrophilic. Reabsorption of these metabolite by renal tubules is dependent on its hydrophobicity. Many drugs and metabolites are hydrophobic. The more hydrophobic a substance is, the more likely it will be reabsorbed. The liver converts them into hydrophilic compounds so the metabolite will easily removed from the body[6a].

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

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

In recent years, people widely suffered from liver diseases. In India, liver disease is the tenth most common cause of death.

According to the latest WHO data published in may 2014, Liver disease deaths in India reached 2,16,865 or 2.44% of total deaths. The age adjusted death rate is 21.96 per 1,00,000 of populations. Population ranks India #61 in the world[10].

According to WHO, about 46% of global disease and 59% of the mortality is because of chronic diseases and almost 35millon people in the world die of chronic liver diseases. Liver diseases rate steadily increasing over the years[11].

The major sign of liver disease is Jaundice (Hyperbilirubinemia) which is known as yellowish discolourisation of skin and mucous membrane[12]. In *Siddha* system of medicine, "Jaundice" is correlated with "*Pitha Kamaalai*" or "*Manjal Kamaalai*". As per the text *ManjalKamaalai* is of 13 types.[13] Clinically Jaundice is divided into three types. They are Pre hepatic, Hepatic and Post hepatic. Many of liver diseases are accompanied by Jaundice[12a].

A healthy liver is essential for the healthy life. So it is important to take care of liver in a healthy way. One of the natural and healthy way to take care of liver is *Siddha* system of medicine. In this medicine various polyherbal preparations are available to treat liver diseases.

Herbal drugs are significant source of hepatoprotective drugs. Mono and poly-herbal preparations have been used in various liver disorders. According to one estimate, more than 700 mono and poly-herbal preparations in the form of decoction, tincture, tablets and capsules from more than 100 plants are in clinical use[7b].

In the modern scenario, diseases are becoming drug-resistant and scientists are studying possible roles of plant based drugs for screening life saving drugs. Treatment options for common liver diseases such as cirrhosis, fatty liver, and chronic hepatitis are problematic. The effectiveness of treatments such as interferon, colchicine, penicillamine, and corticosteroids are inconsistent at best and the incidence of side-effects profound. it may also cause various other diseases. So there is a need for safe and potent hepatoprotective drug to treat various liver disease.

In recent years synthetic drugs are showing more adverse effect, to overcome this problem researchers are trying to avoid this risk of those drugs. Whenever a drug is prescribed to a patient they are facing risk of side effect, so long term use of these drugs patient should be careful. But in herbal medicine the toxicity of herbal drugs is less when compared with the synthetic medicines[7c].

Due to known side effect, patient preferred to take alternative medicine which is natural and healthy way[14]. The process of healing and regeneration of liver cells are support and promote by herbal preparations with less side effects.

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

The World Health Organization (WHO) estimates that 80% of the world health populations presently use herbal medicines for some aspect of primary health care[7d].

Although various drugs are flooding in the market used as hepatoprotective agent. In that many of them having adverse effects. *Siddha* system is a treasure which contains enormous herbal preparations. They are highly effective and devoid of adverse effects. For this reason *Siddha* system of medicine has been drawing global attention in recent times.

Liver disorders are increasing day by day. So there is a need and demand for effective, safe and accurate treatment of liver diseases.

Therefore it is not surprising that a considerable interest has been developed in search of potent hepatoprotective drug from herbal origin.

Based on above facts, One of the polyherbal preparation mentioned in *Siddha* literature is *Maavilingapattai Chooranam.* I hope this *Siddha* medicine *Maavilingapattai Chooranam* will be effective drug for the cure of liver disease. So, I interested to validate the *Maavilingapattai Chooranam,* a unique herbal preparation for its hepato-protective activity.

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

2.AIM AND OBJECTIVES

AIM:

The present study was focused to validate the hepatoprotective potentials of *Maavilingapattai Chooranam* in experimental animals with CCL_4, Paracetamol and Ethanol induced hepato toxicity.

OBJECTIVE:

The scientific study on *Maavilingapattai Chooranam* was carried out in the following stages.

- Collection of various *Siddha* and modern literature relevant to this study.
- Description of pharmacognostic features of the plant in this formulation including the taxonomic identification, collection, purification of the plant etc.
- Preparation of *Maavilingapattai Chooranam* as per classical *Siddha* text.
- Standardisation of the trial drug *Maavilingapattai chooranam* by means of physio chemical analysis, phyto chemical analysis.
- Evaluate bio chemical analysis of the test drug to derive acidic and basic radicals.
- To estimate the presence of elements, functional groups and particle size through instrumental analysis of the trial drug.
- Evaluation of the acute and 28 days repeated oral toxicity study of the test drug according to OECD guidelines.
- Evaluation of pharmacological study of the drug through the following activities:
    1) Hepatoprotective activity of CCL_4 induced hepatotoxicity.
    2) Hepatoprotective activity of Paracetamol induced hepatotoxicity.
    3) Hepatoprotective activity of Ethanol induced hepatotoxicity.

3. REVIEW OF LITERATURE

INGREDIENTS OF MAAVILINGAPATTAI CHOORANAM:

1.	*Iruveli*	*- Vetiveria zizanioides*
2.	*Vilamichu*	*- Plectranthus vetttiveroides*
3.	*Chiru kurinchan*	*- Gymnema sylvestre*
4.	*Poonai vanangi*	*- Acalypha indica*
5.	*Kozhunji*	*- Tephrosia purpurea*
6.	*Koovilam*	*- Aegle marmelos*
7.	*Pathiri*	*- Stereospermum colais*
8.	*Thulasi*	*- Ocimum sanctum*
9.	*Musumuskkai*	*- Mukia maderaspatana*
10.	*Musuttai*	*- Rivea ornata*
11.	*Vila*	*- Limonia acidissima*
12.	*Nannari*	*- Hemidesmus indicus*
13.	*Kurundhotti*	*- Sida rhombifolia*
14.	*Ashwagandhi*	*- Withania somnifera*
15.	*Parangichakkai*	*- Smilax china*
16.	*Maavilingapattai*	*- Crateva magna*
17.	*Seeni sarkarai*	*- Saccharam officinaram*

DRUG REVIEW

3.1.GUNAPADAM ASPECT OF THE DRUGS:

1. Iruveli

Scientific name: *Vetiveria zizanioides*

Synonyms: *Vettiver, Vizhal ver, Viranam, Guruver.*

Vernacular names:

Tam : *Vettiver*

Eng : Cuscus grass

Tel : *Avvuuru-goddi-veru*

Mal : *Vetti-veru*

Kan : *Lavancha*

Sansk : *Usheera, veeranam*

Hindi : *Balah*

Parts used : Roots

Properties and action:

- ✓ *Suvai* (Taste) : *Inippu* (sweet)
- ✓ *Thanmai* (Potency) : *Thatpam* (cold potency)
- ✓ *Pirivu* (Bio transformation) : *Inippu* (sweet)
- ✓ *Seigai* (Actions) : Tonic, Antispasmodic, Diuretic, Emmenagogue.

General characters:

பித்தவி தாகம் சகிகா மிலங்கறைப் பித்தமனற்
றத்திடு குட்டஞ் சிரநோய் களமடி தாதுநட்ட
மத்தம னற்புண் டனப்புண்வன் மூர்ச்சை வரிவிழிநோய்
வித்தர மேகத்தின் கட்டியும் போம் வெட்டி வேரினுக்கே.

- அகத்தியர் குணவாகடம்.

Indications:

Vettiver cures jaundice, hypertension, fever, impotence, abscess or ulcer in the breast and venereal abscess.

Medicinal uses:

1) Decoction of *vetiver* 30-60ml use to treat intestinal related diseases.
2) Root extract is used for headache and toothache[15].

2. Vilamichu- ver

Scientific name: *Plectranthus vettiveroides*

Vernacular names:

Tam : *Vilamichu-ver*

Eng : white cuscus grass

Mal : *Iruveli*

Sans : *Hroeberam*

Parts used : Roots

Properties and action:

- ✓ *Suvai* (Taste) : *Kaippu* (Bitter)
- ✓ *Thanmai* (Potency) : *Thatpam* (Cold potency)
- ✓ *Pirivu* (Bio transformation) : *Inippu* (Sweet)
- ✓ *Seigai* (Actions) : Refrigerant, Anti pittha.

General charactrs:

மேகம் விழியெரிச்சல் வீறிரத்த பித்தமொடு
தாகமத மூர்ச்சைபித்தந் தன்மயக்கம்-சோகஞ்
சிரநோய் இவையேகுஞ் செய்யவிலா மிச்சுக்
கெரிசுரமும் இல்லை இசை.

-அகத்தியர் குணவாகடம்.

Indications:

Vilamichu cures diabetes mellitus, hypertension, dropsy, head ache, bilious fever, burning sensation in the eyes and mental delusion.

Medicinal uses:

1) While taking vilamichu oil bath everyweek it cures head ache, burning sensation in the eyes, bilious fever, mental delusion[15a].

3. Chiru-kurinchan

Scientific name: *Gymnema sylvestre*

Synonyms: *Kurinchan*

Vernacular names:

Tam : *Chiru-kurinchan*

Eng : Periploca of the woods

Tel : *Poda-patra, Putla-podra*

Mal : *Cheru kurinja*

Sans : *Sarpadarushtrik, Meshasri*

Parts used : Leaves, Root.

Properties and action:

- ✓ *Suvai* (Taste) : *Kaippu*(Bitter)
- ✓ *Thanmai* (Potency) : Hot potency
- ✓ *Pirivu* (Bio transformation) : *Kaarppu*(Pungent)
- ✓ *Seigai* (Actions) : Stomachic, Astringent, Emetic.

General characters:

சிறுகுறிஞ்சான் வேர்விடத்தைத் தீர்க்கும் அனிலத்
துறுசுரங்கள் வாதம் ஒழிக்குந் - தெறிபாணக்
கண்ணாய்! இருமல்முதற் காசசுவா சந்தணிக்கும்
விண்ணா டருக்கிதனை விள்.

-அகத்தியர் குணவாகடம்.

Indications:

Gymnema sylvestre root cures poisonous bites, vatha fever, cough and bronchial asthma.

Medicinal uses:

1) Decoction of *Chiru kurinchan* leaves cures cough and fever.
2) Chewing of the leaves depressed the taste of the tongue. So the tongue doesn't know the sweet and bitter taste.
3) Root of this plant used both internally and externally. Externally root powder dusted over the area of snake bite or decoction of this root gives internally cures snake bite[15b].

4. Kuppai-meni

Scientific name: *Acalypha indica*

Synonyms: *Arimangchari, Poonaivanangi, Meni*

Vernacular names:

Tam : *Kuppai meni*

Eng : Indian acalypha, Cat's struggle

Tel : *Kuppi-chettu*

Mal : *Kuppigida*

Sans : *Arittamanjarie*

Hindi : *Kuppi*

Parts used : Whole plant

Properties and action:

- ✓ *Suvai* (Taste) : *Kaippu*(Bitter), *Kaarppu*(Pungent)
- ✓ *Thanmai* (Potency) : Hot potency

- ✓ *Pirivu* (bio transformation) : *Kaarppu*(Pungent)
- ✓ *Seigai* (Action) : Anodyne, Emmenogogue, Diuretic,Cathartic, Emetic.

General characters:

தந்தமூ லப்பிணிதீத் தந்திடுபுண் சர்வவிடம்
உந்துகுன்மம் வாதம் உதிரமூ- லந்தினவு
சூலஞ்சு வாசம் தொடர்பீ சங்கபம்போம்
ஞாலங்கொள் மேனியத னால்.

- தேரன் குணவாகடம்.

Indications:

Acalypha indica leaves are used to cure gum related diseases, burns, stomach ache, piles, itching, bronchial asthma and sinusitis.

Medicinal uses:

1) Leaves ground along with salt applied over the skin cures scabies.
2) Leaf juice cures head ache. Leaf powder cures bed sore.
3) Decoction of the leaves drink with salt cures constipation.
4) Paste of acalypha root along with water cures rat poisoning[15c].

5. Kozhunji

Scientific name: *Tephrosia purpurea*

Synonyms: *Kollukkaivelai, Kaai velai*

Vernacular names:

Tam : *Kollukkaivelai*

Eng : Purple tephrosia or Wild indigo

Tel : *Vempali, Pampara chettu*

Mal : *Kozhinnila*

Kan : *Kaggi*

Sans : *Puleehashtree*

Hindi : *Sarphaoka*

Parts used : Leaves, Roots, Seeds, Root bark.

Properties and action:

- ✓ *Suvai* (Taste) : *Kaarppu* (Pungent)
- ✓ *Thanmai* (Potency) : Hot potency
- ✓ *Pirivu* (Biotransformation) : *Kaarppu* (Pungent)
- ✓ *Seigai* (Actions) : Nutritive, Expectorant, Diuretic, Deobstruent, Anthelmintic.

General properties:

வாதமிக்க தென்பார்க்கும் வாய்வறட்சி யென்பாக்கும்
மீதுதந்த மூலநோ யென்பார்க்கும்- ஓதமுற்ற
கொள்ளுக்கா தாரகபம் தோற்றியதென் பார்க்குமொரு
கொள்ளுக்காய் வேளைதனைக் கூறு.

-அகத்தியர் குணவாகடம்.

Indications:

Tephrosia purpurea cures dryness of the tongue, drooling of salaiva and dental diseases.

Medicinal uses:

1) *Tephrosia purpurea* root ground with turmeric and cow's milk applied externally cures lymphadenopathy.
2) Take 1part of this plant root, 2part of pepper- make it as a decoction cures fever, hepatomegaly and splenomegaly[15d].

6. Vilvam

Scientific name: *Aegle marmelos*

Synonyms: *Kusaabhi, Koovilam, Koovilai, Sivaththurumam, Ninmali, Maathuram.*

Vernacular names:

Tam : *Vilvam*

Eng : Bael tree, Holy fruit tree

Te : *Bilvamu*

Mal : *Kuvalam*

Kan : *Beta*

Sans : *Bilva*

Hindi : *Bel*

Parts used **:** Leaves, Flower, Fruit, Root, Resin, Root bark.

Properties and action:

- ✓ *Suvai* (Taste) : *Thuvarppu*(Astringent), *Kaippu* (Bitter)
- ✓ *Thanmai* (Potency) : Cold potency
- ✓ *Pirivu* (Bio transformation) : *Kaarppu*(Pungent)
- ✓ *Seigai* (Actions) : Diaphoretic, Digestive, Stomachic, Laxative.

General characters:

வில்வத்தின் வேருக்கு வீறுகுன்ம வாயுகபம்
சொல்வொணா பித்தந் தொடர்சோபை- வலகப
தாகசுரம் நீரேற்றஞ் சந்தியோடு மெய்வலியும்
வேகமொடு நீங்குமே.

-அகத்தியர் குணவாகடம்.

Indications:

Aegle marmelos cures tastelessness, loss of appetite, hiccup, vomiting, diarrhoea, dysentry.

Medicinal uses:

1) Root of *Aegle marmelos, Pavonia zeylanica, Zingiber officinalae* make it as a decoction and mixed it with honey which cures vomiting and balances vatha, pitha, kabha.
2) Leaf juice mixed with pepper powder cures dropsy and **jaundice**.
3) Leaf juice mixed with honey cures rhinitis and fever[15e].

7. Pathiri

Scientific name: *Stereospermum colais*

Synonyms: *Kanni, Paadalimaram, Paadalam, Punkaali.*

Vernacular names:

Tam : *Pathiri*

Eng : Trumpet flower, Yellow snake flower

Tel : *Kaligottu*

Mal : *Pathiri*

Sans : *Patala*

Hindi : *Peader*

Parts used **:** Leaves, Flower, Bark, Root.

Properties and action:

- ✓ *Suvai* (Taste) : *Thuvarppu*(Astringent)
- ✓ *Thanmai* (Potency) : Cold potency
- ✓ *Pirivu* (Biotransformation) : *Inippu*(Sweet)

✓ *Seigai* (Action) : Diuretic, Febrifuge

Gereral characters:

பாதிரியின் வேர்குணந்தான் பார்க்கில் மதுமேகம்
ஓதுசுரப் பானுழலை யோடிவிடும் - மாதேகேள்!
கண்ணெறிவு காதெரிவு கையெரிவு காலெரிவும்
நிண்ணயமாய்ப் போகும் நிசம்.

- அகத்தியர் குணவாகடம்.

Indications:

Stereospermum colais cures diabetes mellitus, eczema, peripheral neuritis, abscess, haemorrhoides, scabies, indigestion.

Medicinal uses:

1) Flower decoction cures venereal diseases and fever. While taking it with honey it cures hiccup.
2) 1 part of root bark soaked with 10 part of water about 15-30ml cures stomach ache, cough and dropsy.
3) Root decoction or root powder used to treat diabetes mellitus, eczema, peripheral neuritis, abscess, haemorrhoides, scabies and indigestion[15f].

8. Thulasi

Scientific name: *Ocimum sanctum*

Synonyms: *Ari, Ramathulasi, Krishnathulasi, Thiruthulaai, Thulavu, Kullai, Vanam, Viruntham, Thuzhasi, Maalalangal.*

Vernacular names:

Tam : *Thulasi*

Eng : Holy basil, Sacred basil

Tel : *Thulasi*

Mal : *Thulasi*

Sans : *Thulasi*

Hindi : *Thulasi*

Parts used : Leaves. Seeds, Root.

Properties and action:

- ✓ *Suvai* (Taste) : *Kaarppu* (Pungent)
- ✓ *Thanmai* (Potency) : Hot potency
- ✓ *Pirivu* (biotransformation) : *Kaarppu* (Pungent)
- ✓ *Seigai* (Actions) : Stimulant, Expectorant, Diaphoretic, Dimulcent.

General characters:

போலாட்டி வித்துதிரப் புண்பீனி சப்பிணியை
மேலாட்டி வித்து விளங்குமே - சால
வருந்து முடற்குவெப்பு வாராமெ லோட்டும்
பிருந்தமைப்ப யித்தியத்தைப் பேய்.

- தேரன் யமக வெண்பா.

Indications:

Ocimum sanctum cures sinusities and fever. It also reduces body heat.

Medicinal uses:

1) Leaves ground with water and then applied on bad boils.
2) Leaf juice powderd into the ear is a first rate remedy for earache. It also cures chronic fever, haemorrhage, dysentry and dyspepsia.
3) Leaf juice is mixed with small amount of ginger is given for colic in children and one thola is mixed with quarter thola of black pepper is given in catarrhal fever and in the cold stages of intermittent fever[15g].

9. Musumuskkai

Scientific name: *Mukia maderaspatana*

Synonyms: *Ayileyam, Irukurangin kai, Mosumosukkai.*

Vernacular names:

Tam : *Musumusukkai*

Eng : Rough bryony

Tel : *Musumusukaya*

Hindi : *Bilavi*

Mal : *Chitrati*

Parts used : Leaves, Root.

Properties and action:

- ✓ *Suvai* (Taste) : *Thuvarppu* (Astringent)
- ✓ *Thanmai* (Potency) : Hot potency
- ✓ *Pirivu* (Biotransformation) : *Kaarppu* (Pungent)
- ✓ *Seigai* (Actions) : Expectorant, Astringent.

General characters:

கந்தம் பரவு களிச்சளியும் புன்விடமு
மந்தம் பெருவிடமும் வாந்திகளும் - அந்தம்
பெருகுரங்குக் கிச்சூடும் பித்தமுமி ருக்கா
திருகுரங்குக் கைவேருக் கே.

- அகத்தியர் குணவாகடம்.

Indications:

Mukia maderaspatana cures impotence, indigestion, vomiting, ulcer and cardiac diseases.

Medicinal uses:

1) Leaf juice mixed with purified ox gall or ox bile cures impotence, indigestion, vomiting, ulcer and cardiac diseases.

2) Leaf decoction or powder cures cardiac diseases and vomiting[15h].

10. Musuttai

Scientific name: *Rivea ornata*

Synonyms: *Panjchi*

Vernacular name:

Tam : *Musuttai*

Parts used : Whole plant

Properties and action:

- ✓ *Suvai* (Taste) : *Kaippu* (Bitter), *Kaarppu* (Pungent)
- ✓ *Thanmai* (Potency) : Hot potency
- ✓ *Pirivu* (Biotransformation) : *Kaarppu* (Pungent)
- ✓ *Seigai* (Action) : Germicide, Anti-vatha, Laxative.

General characters:

மாதே! முசுட்டையது வாதமொடு ஐயத்தைத்
தீதே புரிநீரைத் தீர்க்குங்காண் - வேதனைசெய்
வன்மலத்தைத் தள்ளும் வறட்சி சொறிசிரங்கைச்
சன்மமறப் போக்கிவிடுஞ் சாற்று.

- அகத்தியர் குணவாகடம்.

Indications:

Rivea ornata cures leucorrhoea, scabies, eczema and constipation. It also balance vatha and kabha.

Medicinal uses:

1) Its root ground and mixed with gingely oil used to treat scabies and skin diseases.

2) Leaf decoction or powder cures leucorrhoea, scabies, eczema and burning micturation[15i].

11. Vila

Scientific name: *Limonia acidissima*

Synonyms: *Kadippagai, Kapiththam, vilavu. Vellil*

Vernacular names:

Tam : *Vila*

Eng : Wood-apple tree, Elephant apple, Curd fruit, Monkey fruit.

Tel : *Velaga*

Mal : *Vilav*

Kan : *Baelada*

Sans : *Kapitha*

Hindi : *Kavitha, Dharkahth-kaveet*

Parts used **:** Leaves, Fruit, Bark, Resin.

Properties and action:

- ✓ *Suvai* (Taste) : *Thuvarppu* (Astringent)
- ✓ *Thanmai* (potency) : Hot potency
- ✓ *Pirivu* (biotransformation) : *Inippu* (Sweet)
- ✓ *Seigai* (Action) : Aromatic, Demulcent, Carminative, Astringent, Refrigerant,.

General characters:

மன்னன் முதல்மூவர் மாறுபா டில்லாம
லுன்ன நினைத்த துதவுதலாற் - பன்னிச்
செபித்த வுடலாண்மை சேர்வித் திடலாடற்
கபித்தமவிழ் தக்கற்ப கம்.

- தேரன் வெண்பா.

Indications:

Limonia acidissima balance vatha, pitha and kaba. It also strengthen's our body.

Medicinal uses:

1) Leaves are purgative and sudorific and are used in snake bite.

2) The bark and leaves of Limonia acidissima are used for vitiated conditions of vatha and pitha.

3) Leaves are astringent and carminative, good for vomiting, indigestion, hiccup and dysentry. The leaves have hepatoprotective activity.

4) The gum is demulcent and constipating, and is useful in diarrhoea, dysentery, gastropathy, haemorrhoids and diabetes[15j].

12. Nannari

Scientific name: *Hemidesmus indicus*

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

Vernacular names:

Tam : *Nannari*

Eng : Indian sarasaparilla

Mal : *Nannari*

Kan : *Sugandha-palada*

Sans : *Sariba*

Hindi : *Magrabu*

Parts used : Root

Properties and action:

- ✓ *Suvai* (Taste) : *Inippu* (Sweet)
- ✓ *Thanmai* (Potency) : Cold potency
- ✓ *Pirivu* (Biotransformation) : *Inippu* (Sweet)
- ✓ *Seigai* (Action) : Alternative, Tonic, Demulcent, Diuretic, Diaphoretic.

General characters:

சலதோடம் பித்தமதி தாகம் உழலை
சலமேறு சீதமின்னார் தஞ்சூ-டுலகமதிற்
சொன்னமது மேகம் புண் சுரமிவையெ லாமொழிக்கும்
மென்மதுர நன்னாரி வேர்.

-தேரன் குணவாகடம்.

Indications:

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

Medicinal uses:

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

13. Kurunthotti

Scientific name: *Sida rhombifolia*

Synonyms: *Cittamutti, Kurunthotti, Yanaikkuruntotti, Mayirmanikkam, Velaippacai.*

Vernacular names:

Tam : *Kurunthotti*

Eng : Arrow leaf sida, Indian hemp

Mal : *Vankurunthotti*

Sans : *Athibala*

Hindi : *Barela, Lalbarela*

Ben : *Svetabrela, Svetabala, Pitaberela, Pitabala*

Parts used **:** Whole plant

Properties and action:

- ✓ *Suvai* (Taste) : *Thuvarppu* (Astringent)
- ✓ *Thanmai* (Potency) : Cold potency
- ✓ *Pirivu* (Biotransformation) : *Inippu* (Sweet)
- ✓ *Seigai* (Action) : Emollient

Medicinal uses:

1) The pounded leaves are used to relieve swelling. The fruits are used to relieve head ache.
2) Stem's mucilage is useful in calculous troubles and as a febrifuge with pepper.
3) Mucilage are also used in scorpion sting.
4) Root of this plant are used in the treatment of rheumatism[16].

14. Ashwagandhi

Scientific name: *Withania somnifera*

Synonyms: *Amukkrak-kizhangu, Amukkiri, Amukkuravi, Amukkuravu, Amukkinanagizhangu, Ashwagandham, Ashwaganthi, Ashuvam, Irulichchevi, Kidichchevi, Varahakarni.*

Vernacular names:

Tam : *Amukkurak-kizhangu*

Eng : Winter cherry

Mal : *Amukkuram*

Kan : *Sogade-beru*

Urdu : *Asgandh*

Tel : *Penneru-gadda*

Sans : *Aswagandha*

Arb : *Habdul kaknaje-hindi*

Pers : *Tukhma kaknaje-hindi*

Parts used **:** Leaves, Seeds, Root.

Properties and action:

- ✓ *Suvai* (Taste) : *Kaippu* (Bitter)
- ✓ *Thanmai* (Potency) : Hot potency
- ✓ *Pirivu* (Biotransformation) : *Kaarppu* (Pungent)
- ✓ *Seigai* (Action) : Febrifuge, Diuretic, Alternative, Aphrodisiac, Deobstruent, Tonic, Sedative.

General characters:

கொஞ்சந் துவர்ப்பாங் கொடியகயம் சூலையரி
மிஞ்சுகரப் பான்பாண்டு வெப்பதப்பு - விஞ்சி
முசுவுறு தோடமும்போ மோகம்அன லுண்டாம்
அசுவகந் திக்கென் றறி.

-அகத்தியர் குணவாகடம்.

Indications:

Root cures eczema, anemia, dropsy and also kabha diseases. It also cures loss of appetite.

Medicinal uses:

1) Root powder mixed with ghee and honey in equal parts is recommened for impotence or senile debility.
2) For leucorrhoea, ashwagandha powder 45grams and sugar candy 1 thola is given in cow's milk, morning and evening till cure is obtained.
3) The fresh green root of ashwagandha is mixed with cow's urine or with water used to treat scrofulous and other glandular swelling[15].

15. Parangichakkai

Scientific name: *Smilax china*

Synonyms: *Parankippattai, Mathusmikam, Mathusmigi, Chinappattai.*

Vernacular names:

Tam : *Parankippattai*

Eng : China root

Tel : *Pirangi-chekka*

Mal : *Pavu*

Sans : *Madushuhi*

Hindi : *Chobchini*

Parts used : Root

Properties and action:

- ✓ *Seigai* (Taste) : *Inippu* (Sweet)
- ✓ *Thanmai* (Potency) : Cold potency
- ✓ *Pirivu* (Biotransformation) : *Inippu* (Sweet)
- ✓ *Seigai* (Action) : Alternative, Antisyphilitic, Aphrodisiac, Depurative.

General characters:

தாகம் பலவாதந் தாதுநட்டம் புண்பிளவை
மேகங் கடிகிரந்தி வீழ்மூலந்-தேகமுடன்
குட்டை பகந்தமேற் கொள்வமனம் போம்பறங்கிப்
பட்டையினை யுச்சரித்துப் பார்.

-தேரையர் குணவாகடம்.

Indications:

Root cures thirst, vatha related diseases, diabetes mellitus, scabies, haemorrhoide, rheumatoid arthritis, syphilitic ulcer and also cures impotence.

Medicinal uses:

1) Root improves agni, cleans urine and stools.
2) Root cures flatulence, epilepsy, insanity, syphilis, colic, pain in the body and skin diseases[15m].

16. Maavilingappattai

Scientific name: *Crataeva magna*

Synonyms: *Maavilangu, Kumaaragam, Varani*

Vernacular name:

Tam : *Maavilangu*

Eng : Three leaved caper

Tel : *Urumathi*

Mal : *Nirvala*

Hindi : *Banah*

Sans : *Pashungandha, Asmarighna*

Kan : *Narumbele*

Parts used **:** Leaves, Bark, Root.

Properties and action:

- ✓ *Suvai* (Taste) : *Kaippu* (Bitter)
- ✓ *Thanmai* (Potency) : Hot potency
- ✓ *Pirivu* (Biotransformation) : *Kaarppu* (Pungent)
- ✓ *Seigai* (Action) : Stomachic, Febrifuge, Tonic, Rubefacient, Laxative, Lithontriptic.

General characters:

சுரங்கடியின் றோடந் தொலையாத வாதம்
உரம்பெறு விடங்க ளொழியும் - சுரமுங்
கருமா வடுவயிலுங் கண்டஞ்சுங் கண்ணாய்
ஒருமாவி லிங்குக் குரை.

- அகத்தியர் குணபாடம்.

மாவிலங்கப் பட்டையினால் வாதமொடு சன்னிகளும்
பரவுகின்ற கல்லடைப்பும் பாறுமே.

- அகத்தியர் குணபாடம்.

Indictions:

Crateva magna is used to treat fever, insect bite, snake bite and vatha diseases.

Bark cures vatha diseases, renal stones(urolithiasis) and it also balance vatha, pitha, kaba.

Medicinal uses:

1) Leaf juice cures fever and indigestion.
2) The extracts of bark or root bark is used to cure vatha diseases.
3) The leaf of *Crataeva magna* is applied externally for the treatment of piles and the juice is taken internally cures bleeding piles[15n].

17. Seeni sarkarai

Scientific name: *Saccharum officinaram*

Synonyms: *Punarpoosam, Ikku, vaei.*

Vernacular name:

Tam : *Karumbu*

Eng : Sugarcane, Noble cane.

Tel : *Cheruku*

Mal : *Karinpa*

Kan : *Khabbu*

Sans : *Ikshu, Rasalah*

Arab : *Qasabus-sakar*

Pers : *Nai-shakar*

Hindi : *Ukh-ganna*

Parts used **:** Juice, Sugar, Root.

Properties and action:

- ✓ *Suvai* (Taste) : *Inippu* (Sweet)
- ✓ *Thanmai* (Potency) : Cold potency
- ✓ *Pirivu* (Biotransformation) : *Inippu* (Sweet)
- ✓ *Seigai* (Action) : Antiseptic, Demulcent.

General characters:

சீனிச் சர்க்கைக்குத் தீராத வன்சுரமுங்
கூனிக்கும் வாதத்தின் கூட்டுறவும் - ஏனிற்கும்
வாந்தி யொடுகிருமி மாறாத விக்கலுமே
போந்திசையை விட்டுப் புரண்டு.

- அகத்தியர் குணபாடம்.

Indictions:

Seeni sarkarai cures vata fever, vata related diseases, vomiting and hiccup.

Medicinal uses:

1) Sugar syrup can be used as preservative and also it cures Sinusitis and Rhinitis[15o].

3.2.BOTANICAL ASPECT OF THE DRUGS:

1. Vetiveria zizanioides

Common name: *Vettiver*

Scientific classification:

- ➢ Kingdom : Plantae
- ➢ Division : Magnoliophyta
- ➢ Class : Liliopsida
- ➢ Order : Cyperales

- Family : Poaceae
- Genus : Vetiveria
- Species : zizanioides

The plant description

✧ **Description:**

Perennial grass that can grow upto 1 or 2 meters high with wide clumps. These tillers are known as stem produced by grass plants. The clumps or tillers arise from rhizomes. The root system of the plant is somewhat massive but compact, structured and very strong. The leaves are narrow, keel and erect with rough margins. The inflorescence panicles are long whorl branches. These are purple brown in colour. The spikelet are of grey, green or purple in colour.

✧ **Distribution:**

This plant grows in the tropical areas of Asia, Africa, Australia, India, China, Brazil, Haiti, Indonesia and Guatemala. In India it grows in the regions of Punjab, Utter pradesh, Rajasthan, Kerala, Karnataka, Madhya predesh and Assam.

✧ **Parts used:** Root, oil.

✧ **Chemical constituents:**

Allokhusiol, benzoic acid, cyclocapacamphene, epikhusinol, epizizanal, 2-epizizanone, B-eduesmol, eugenol, iskhusimol, isokhusinoloxide, isovalencenol, isovalenic, khusimylacetate, khusinodiol, khusinol, khusitoneol, laevojujenol, levojuneol, vanillin, vertiselinenol, B- & J vetivene, vetivenic acid, vetiverol, zizaene, zizanol[17].

✧ **Properties and uses:**

1) The plant is used as anthelmintic for children. It is also used for boils, burns, epilepsy, fever, scorpion sting, snakebite and sores in the mouth. The paste of fresh roots used for burn, snakebite and scorpion sting.

2) Decoction of the roots as a tonic for weakness; the Lodhas of West Bengal use the root paste for headache, rheumatism and sprain, and a stem decoction for urinary tract infection; the Mandla and Bastar tribes of Madhya Pradesh use the leaf juice as anthelmintic; the tribes of the Varanasi district inhale the root vapour for malarial fever. The root ash is given for acidity by the Oraon tribe[18].

2. Plectranthus vettiveroides

Common name: *Vilamicham ver*

Scientific classification:

- Kingdom : Plantae
- Division : Eudicots
- Class : Magnoliosida
- Order : Lamiales
- Family : Lamiaceae
- Genus : Plectranthus
- Species : vettiveroides

The plant description

✧ **Description:**

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

✧ **Distribution:**

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

✧ **Parts used:** Arial part of root

✧ **Chemical constituents:**

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

✧ **Properties and uses:**

1) Aril: Spasmolytic, root: hypertensive, spasmolytic and given to children in constipation: decoction as tonic and in the treatment of worms, parts to allay. Burning festering boils: mixed with mustard oil, grounded root externally applied to eczema and skin disease.

2) Forskolin, isolated from roots, is a bronchodilatator, cardiac tonic in the treatment of congestive heart failure, glaucomotherapy, anti-hypertensive, remedy for metastatic condition and thrombosis[19].

3. Gymnema sylvestre

Common name: *Chiru-kurinjan*

Scientific classification:

- Kingdom : Plantae
- Division : Magnoliophyta
- Class : Magnoliopsida
- Order : Gentianales
- Family : Apocynaceae
- Genus : Gymnema
- Species : sylvestre

The plant description

✧ **Description:**

A large stout, woody, climber with demely appressed hairy branchlets. Leaves- elliptic or obovate, acute, rarely pubescent above. Flower small, crowded, umbelliform cymes. Fruits slender, follicles, glabrous. Seeds narrowly ovoid-oblong, flat with a broad, thing wing, pale brown.

✧ **Distribution:**

Gymnema sylvestre grows in tropical areas of India, Africa and Australia, Sri lanka, Southern China and Japan.

✧ **Parts used:** whole plant, leaves, seeds and root.

✧ **Chemical constituents:**

Alanine, gamma-aminobutyric acid, isoleucine, valin, adenine, alkaloid, cyclic alcohol, inositol, d-quercitol, alpha and beta chlorophylls, lupeol, stigmasterol, gymnamo saponins, gymanamagenin, gymnestrogenin[20].

✧ **Properties and uses:**

1) Sushrutha describes Gymnema sylvestre as a destroyer of madhumegha (glycosuria) and other urinary disorders. On account of its property of absorbing the taste of sugar it has been given the name of 'Gurmar' meaning sugar destroyer. Therefore that it might neutralized the excess of sugar present in the body in Diabetes melitus.

2) Root powder has long been reputed as a remedy for snake bite[16a].

4. Acalypha indica

Common name: *Kuppaimeni*

Scientific classification:

- Kingdom : Plantae
- Division : Tracheophyta

- Class : Equisetopsida
- Order : Malpighiales
- Family : Euphorbiaceae
- Genus : Acalypha
- Species : indica

The plant description

✧ **Description:**

Annual herb to 60cm tall. Stem striate, pubescent; Leaves broadly ovate, base rounded to shortly attenuate; spikes axillary, monoecious, rachis endind in a triradiate hood at the tip. Male flowers above, ebracteate, minuate clustered, anthers vermicuniform. Female flowers below subtended by foliaceous. Ovary hisbid, 3 lobed, styles 3. Capsules 3 valved, concealed by bract, hispid.

✧ **Distribution:**

Acalypha indica widely throughout tropical Africa and the Indian ocean islands. It also occurs in the south east Asia , Yemen and Oceania.

✧ **Parts used:**

Leaves, stalk (young shoots), flowers and root.

✧ **Chemical constituents:**

Alkaloids acalypus and acalyphene. The arial parts contain a cyanogenic glycoside called acalyphin (a 3-cyanopyridone derivative) as well as flavanoids. Such as kaempferol glycosides mauritianin, clitorin, nicotiflorin and biorobin. Tannins β-sitosterol, acalyptamide, aurantiamide, succinimide and flindersin (a pyranoquinollinone alkaloid) have also isolated. The chemicals that attracts cats are the iridoid compounds isodihydronepetalactone and isoiridomyrmecin.

✧ **Properties and uses:**

1) The leaves are used in the form of powder or decoction. While it mixing with garlic they are used as anthelmintic. Mixing it with common salt they are used to treat scabies.

2) By instilling the decoction in the ear it cures earache and also used fomentation around the aching ear[16b].

5. Tephrosia purpurea

Common name: *Kozhunji*

Scientific classification:

- Kingdom : Plantae
- Division : Magnoliophyta
- Class : Magnoliopsida
- Order : Fabales
- Family : Fabaceae
- Genus : Tephrosia
- Species : purpurea

The plant description

✧ **Description:**

A suberect, much branched, polymorphis, perennial herb, 30-60cm in height. Leaves imparripinnate, perennial herb, 5-15cm long. Leaflets 9-21, glabrous above, obscurely silky beneath, narrow, oblanceolate. Flowers borne in leaf opposed racemes, blue or purple in colour. Pods linear, slightly curved, glabrescent. Seeds 5-10, smooth, greenish grey colour.

✧ **Distribution:**

It is found throughout India and Srilanka in poor soils.

✧ **Parts used:** Whole plant, seeds, root-bark, root.

✧ **Chemical constituents:**

Betasitosterol, lupeol, retin, delphinidinchloride, cyaniding chloride, isolonchocarpin, lanceolatins A&B, pongamol, karangin, kangone, 5,7-dimethoxy-8, flavanone, 2-methoxy-3-9-dihydroxycoumestone, flevichapparins B and C, methyl karanjic acid and purpurin[21].

✧ **Properties and uses:**

1) Leaves are used in dyspepsia, pectrol disease, syphilitis, gonorrhoea and bruritis.

2) A decoction of herb when administered in Bright's diseases with dropsy, showed mild diuretic effect causing reversed oedema. The herb was also tested for ascites and was found to be responsible for improving the function of liver.

3) Seeds are used to treat skin diseases and rat poisoning. Seeds are used against worm infestations in children[22].

6. Aegle marmelos

Common name: Vilvam

Scientific classification:

- Kingdom : Plantae
- Division : Magnoliophyta
- Class : Magnoliopsida
- Order : Sapindales
- Family : Rutaceae
- Genus : Aegle
- Species : marmelos

The plant description

✧ **Description:**

A spinous, deciduous, aromatic tree, spines, straight, strong, axillary. It grows upto 18meters tail and bears long throns. Leaves usually 3or 5 foliate. Leaflets ovate, lanceolate, lateral sessile, terminal long petioled. Flowers borne in few flowered, axillary panicles, greenish white, sweet scented. Fruit large upto 15cm diameter, globose, ovoid or pyriform, grey or greenish yellow woody, pulp orange sweet. Seeds numerous, aromatic pulp and mucilaginous.

✧ **Distribution:**

Aegle marmelos is native across the Indian subcontinent and southern Asia and it is also present in Srilanka, Thailand and Malesia.

✧ **Parts used:** Leaves, flower, fruit, seed, bark, root.

✧ **Chemical constituents:**

Beta sitosterol, amino acids, dictamnine, marmesin, marmin, umbelliferone, skimmianine, carbohydrate, carotene, tannins an vitamins, imperatorin(marmelosin) and its isomers, alloimperatorin and marmelide, psorallen and tannic acids, alpha-d-phellandrene[23].

✧ **Properties and uses:**

1) Unripe or half ripe fruit pulp, owiry to the pressence of tannins or mucilagenous substances which act as a demulcent. These fruit pulp dried and made into powder is prescribed in chronic diarrhoea and dysentry.

2) Rind of the beal fruit 5, coculus corditobus 4, mix and make it as a decoction used to treat vomiting[16c].

7. Stereospermum colais

Common name: *Pathiri*

Scientific classification:

➢ Kingdom : Plantae

- Division : Eudicots
- Class : Asterids
- Order : Lamiales
- Family : Bignoniaceae
- Genus : Stereospermum
- Species : Colais

The plant description

✧ **Description:**

It is large deciduous tree, about 10-20m tall with velvet-hairy branches. Leaves are compound with 3-4 pairs of leaflets. Leaflets broadly elliptic, long pointed. Flower are borne in large lax panicles. Flowers brownish white in colour. Inflorescence terminal panicles. Sepal cup is bell shaped. Stamens are 4, remaining inside the flower tube. Seed pod is long, cylindric, ribbed, rough.

✧ **Distribution:**

It is distributed throughout the moist parts of India and widely distributed in the deciduous forests of kerala. It is also found in tropical Himalayas, Assam and Meghalaya.

✧ **Parts used:** Leaves, flower, bark and root.

✧ **Chemical constituents:**

n-Triacontanol, β-sitosterol, lapachol, dehydro-α-lapachone, dehydrotectol, 6-0glucosylscutellarein and stereolensin. Leaves contain a flavone named stereolensin and has been characterised as 5,7,3,4- tetrahydroxy 6-0-β-D glucopyranosyl flavones[24].

✧ **Properties and uses:**

1) Root bark is one of the constituents of “Dashmula” which is the preparation used as tonic and diuretic.

2) Root has anti inflammatory, anti asthmatic, anti emetic and febrifuge properties.

3) It has biliary stimulant and cardio tonic activity. The root is also used in piles and nervous disorders[16d].

8. Ocimum sanctum

Common name: *Thulasi*

Scientific classification:

- Kingdom : Plantae
- Division : Magnoliophyta
- Class : Magnoliopsida
- Order : Lamiales
- Family : Lamiaceae
- Genus : Ocimum
- Species : sanctum

The plant description

✧ **Description:**

Ocimum sanctum is an erect, many branched subshrub. 30-60cm tall with hairy stems. Leaves are green or purple in colour. They are simple, petioled with an ovate upto 5cm long blade which usually has a slightly toothed margin. They are strongly scented and have a decussate phyllotaxy. The purplish flowers are placed in close whorls on elongate racemes.

✧ **Distribution:**

Ocimum sanctum is considered indigenous to the Indian subcontinent including the Himalayas, Malesia and other tropical and subtropical parts of Asia and now widely cultivated and naturalized in places around the world.

✧ **Parts used:** Leaves, seeds.

✧ **Chemical constituents:**

Oleanolic acid, ursolic acid, rosmarinic acid, eugenol, carvacrol, linalool, β-caryophyllene. Tulasi essential oil consists mostly of eugenol(70%), β-elemene(11.0%), β-caryophyllene(8%) &germacrene(2%) with the balance being made up of various trace compounds mostly terpenes.

✧ **Properties and uses:**

1) Infusion of the leaves is given in malaria and used to treat gastric diseases of children and hepatic infections.

2) Dried plant decoction is a domestic remedy for cough, catarrh, bronchitis and diarrhoea[16e].

9. Mukia maderaspatana

Common name: *Musumuskkai*

Scientific classification:

- Kingdom : Planate
- Division : Tracheophyta
- Class : Magnoliopsida
- Order : Cucurbitales
- Family : Cucurbitaceae
- Genus : Mukia
- Species : maderaspatana

The plant description

✧ **Description:**

Climbing scabrid herbs, tendrils simple. Leaves ovate, deltoid, angular or shallowly 3-5 lobed, base cordate, margin denticulate, apex accuminate. Flowers in

axillary, sessile clusters. Calyx tube 2mm, villous, lobes subulate, erect. Petals 5, ovate-oblong, obtuse, yellow. Stamens 3 free, inserted at base of calyx tube, anthers oblong, ciliate. Female flowers solitary or cluster. Ovary villous. Berry-globose, red. Seeds lenticular, rugose.

✧ **Distribution:**

Mukia maderaspatana is globally distributed in the paleotropic. Within India, it is said to be found throughout Andaman and Nicobar Islands.

✧ **Parts used:** Leaves, root.

✧ **Chemical constituents:**

Leaves contains mainly dichloroacetic acid, 4-methylpentyl ester, 2-butyn-1-0-1, 4-methoxy and also showed the pressence of other constituents like flavanoids, saponins, carbohydrates, steroids, tannins and phenolic compounds.

✧ **Properties and uses:**

1) The leaves and tender shoots are useful as aperient, diuretic, stomachic, antipyretic, antiflatulent, anti asthmatic, anti histaminic and expectorant.

2) The leaf tea is administered for the alleviation of jaundice.

3) The leaf tea is also claimed to possess hypotensive properties in human subjects with concomitant beneficial effects on serum anti oxidant potential, plasma lipid profile, fibrinogen, serum bilirubin and body mass index[25].

10. Rivea ornata

Common name: *Musuttai*

Scientific classification:

- Kingdom : Plantae
- Division : Magnoliophyta
- Class : Magnoliatae
- Order : Polemoniales

- Family : Convolvulaceae
- Genus : Rivea
- Species : ornata

The plant description

✧ **Description:**

Rivea ornata is a woody climber, branchless stout, white tomentose. Leaves ovate, cordate, 3-5 in diameter often broader than long, glabrous above, white silky tomentose beneath while young. Petiole 1-2 in. Flowers large, white, short, mostly 3-fid. Peduncles bracts 1/2 inch. Fruit 2/3 in diameter, indeniscent, globose, shining, yellow brown nearly dries.

✧ **Distribution:**

It is distributed throughout southern part of India.

✧ **Parts used:** Whole plant

✧ **Chemical constituents:**

Rivea ornata seed oil was found to contain 12,13-epoxy-octadec-cis-9-enoic acid (vernolic acid 22.0%) along with the other normal fatty acids like palmitic acids (24.2%), stearic acid (8.9%), oleic acid (17.1%) and linoleic acid (27.8%).

✧ **Properties and uses:**

1) *Rivea ornata* is a potential medicinal plant used topically in haemorrhagic diseases and piles.
2) Leaf juice is mixed with butter and made into oinment used to treat pityeriasis[16f].

11. Limonia acidissima

Common name: *Vila*

Scientific classification:

- Kingdom : Plantae
- Division : Tracheophyta
- Class : Magnoliopsida
- Order : Sapindales
- Family : Rutaceae
- Genus : Limonia
- Species : Acidissima

The plant description

✧ **Description:**

Moderate sized desiduous tree. The leaves are alternate, dark greeen, leathery, 3-5inch long. It dotted with oil glands and slightly lemon scented when crushed. Flowers small, numerous, dull red greenish colour. Fruits is berry, rounded to oval, 2.5 inch wide, greyish white in colour.

✧ **Distribution:**

Limonia acidissima is native to India so it is distributed throughout India and also cultivated in Bangladesh, Pakistan and Srilanka.

✧ **Parts used:** Leaves, fruit, bark, resin and root.

✧ **Chemical constituents:**

Fruit of this plant contains stigmasterol. Pericarp contains umbelliferone, dictamine, xanthotoxol, scoparone, torin, xanthotoxin, isopimpinelin, isoimperatorin and marmerin. Leaves contains stigmasterol, psorallen, bergapten, orientin, vitedin, saponin, tannins and an essential oil. Marmesin, feronone have been isolated from bark. Seeds contains fixed oil, carbohydrates, proteins and amino acids. Root contain feronia lactone, geranylum, belliferone, bargaapten, osthol, isopimpinellin, marmesin and marmin.

✧ **Properties and uses:**

1) Limonia Fruits are refrigerant, stomachic, stimulant, astringent, aphrodisiac, diuretic, cardiotonic, tonic to liver and lungs.

2) It cures cough, hiccup and good for asthma, tumours, opthalmia and leucorrhoea.

3) It's seeds are used in heart diseases.

4) The fruits are used as a substitute for bael (Aegle marmelos) in diarrhea and dysentery[26].

12. Hemidesmus indicus

Common name: *Nannari*

Scientific classification:

- Kingdom : Plantae
- Division : Magnoliophyta
- Class : Magnoliopsida
- Order : Gentianales
- Family : Apocyanaceae
- Genus : Hemidesmus
- Species : indicus

The plant description

✧ **Description:**

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

to greenish purple on the outside and yellow to light purple on the inside. It has fused corolla that numbers twice that of the calyx. Pistil is bicarpellary with free ovaries many ovuled and having distrint styles.

✧ **Distribution:**

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

✧ **Parts used:** Roots.

✧ **Chemical constituents:**

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

✧ **Properties and uses:**

1) Root bark of this plant known as "Indian sarsaparilla" are prescribed in dyspepsia, loss of appetite, nutritional disorders, fever, skin diseases and ulceration especially those of syphilitic origin, constitutional syphilitis, chronic rheumatism and leucorrhoea.

2) Hot infusion of root bark with milk and sugar is used as tonic especially for children in chronic cough and diarrhoea[16g].

13. Sida rhombifolia

Common name: *Kurunthotti*

Scientific classification:

- Kingdom : Plantae
- Division : Angiosperms
- Class : Eudicots
- Order : Malvales
- Family : Malvaceae

- Genus : Sida
- Species : rhombifolia

The plant description

✧ **Description:**

A summer annual with yellow flowers and very small spines at the base of each leaf and branch. Leaves arranged alternately along the stem approximately 3/4 inches long with petioles that are less than 1/3 the length of the leaves. The upper 1/2 of the leaves have toothed or serrated margins while the remaining of the leaves are untoothed. Flowers occur singly on flower stalks that arise from the area between the stems and leaf petioles. Flowers consist of 5 yellow petals that are 4 to 8mm long. Seeds 2, heart shaped cotyledons. The small spines that occur at the base of each leaf petiole and the rhombic leaves are characteristic that help in the idetification of jelly leaf.

✧ **Distribution:**

This is a weed very common in India and Srilanka in the dry country. The species is usually confined and rocky areas.

✧ **Parts used:** Whole plant

✧ **Chemical constituents:**

Sitosterol Ia, stigmasterol Ib, sinosterol-3-0-β-D glucopyranoside 2a, phaeophysin A, 17^3-ethoxypheophorbide A, 13^2-hydroxyphaephyrin B, 17^3-ethoxypheophorbide B, 5,7- dihydroxy-4'-methoxyflavone, cryptolepinone, salt of cryptolepine[27].

✧ **Properties and uses:**

1) Root of these weeds is held in great repute in treatment of rheumatism.

2) Mucilage is used by chemists in oxidizing mercury. It also used to treat scorpion sting[16h].

14.Withania somnifera

Common name: *Amukkura-kizhangu*

Scientific classification:

- Kingdom : Plantae
- Division : Magnoliophyta
- Class : Magnoliopsida
- Order : Solanales
- Family : Solanaceae
- Genus : Withania
- Species : somnifera

The plant description

✧ **Description:**

An erect, evergreen, tentose shrub about 0.5 to 2 cm high. Leaves simple, ovate, glabrous. Flower inconspicuous, greenish or lurid yellow, inaxillary, umbellete cymes. Fruits- the berries are glabose, orange red when mature, enclosed in the persistent calyx. Seed- yellow, reniform. Flowers and fruits during november, february.

✧ **Distribution:**

Withania somnifera grows abundantly in India(especially Madhya pradesh), Pakistan, Bangla desh, Srilanka and parts of Northern Africa.

✧ **Parts used:** Leaves, fruit, seeds, root.

✧ **Chemical constituents:**

Withanolides- withaferin A, withanone, withanolides I, II, III, III A, C, D, E, F, G, H, I, J, K, L, M, WS-I, P and S, withasomidienone, cuscohygrine, anahygrine, tropine, pseudotropine, anaferine, isopellatierine, 3- tropyltigloate.

All arial parts contains proteins and amino acids. Leaves contains erggostane which include withanone, alkaloides, fatty acids, beta sitosterol, polyphenols, phytosterols, withaferia, withanolide C,D,E[28].

✧ **Properties and uses:**

1) Root and bitter leaves are used to treat alcoholism and emphysematous dyspnoea.

2) Leaves are used as anthelmintic and as an application to carbuncles.

3) Root is used to treat senile debility, rheumatism, in all cases of general debility, nervous exhaustion, brain fog, loss of memory, loss of muscular energy and spermatorrhoea[16i].

15. Smilax china

Common name: *Parangippattai*

Scientific classification:

- Kingdom : Planate
- Division : Tracheophyta
- Class : Magnoliopsida
- Order : Liliales
- Family : Liliaceae
- Genus : Smilax
- Species : china

The plant description

✧ **Description:**

It is a bushy spiny plant, grew about 3 feet high. The leaves are thin, membranous, ovate to ovate-oblong, five nerved, acute or obtuse at both ends. Root or rhizome are stipules distinct obtuse and hard, large, blackish outer, pale and whitish

internally. Flowers are small found in umbels greenish yellow. Fruits are red and small.

✧ **Distribution:**

It is found in tropical and subtropical regions. It is native to China, Korea, Taiwan, Japan, Philippines, Vietnam, Thailand, Myanmar and Assam.

✧ **Parts used:** Roots.

✧ **Chemical constituents:**

Kaemperol-7-0-beta-D-glucopyranoside, engeletin, isoengeletin, kaempferol, dihydrokaempferol, dihydrokaempferol-5-0-P-D glucopyranoside, rutin, kaempferol-5-0-beta-D-glucopyranoside, 3,5,4'-trihydroxystibene, vanillic acid, 3,5-dimthoxy 4-0-beta-D-glucopyranosylcinnamic acid, beta sitosterol, beta daucosterol[29].

✧ **Properties and uses:**

1) Root used to treat rheumatism, gout, chronic nervous diseases, cachexia and constitutional syphilis.

2) Inhalation of root used in asthma[16j].

16. Crataeva magna

Common name: *Maavilangapattai*

Scientific classification:

- Kingdom : Plantae
- Division : Angiosperms
- Class : Dicotyledons
- Order : Parietales
- Family : Capparaceae
- Genus : Crateva
- Species : magna

The plant description

✧ **Description:**

A small much branched deciduous tree. Leaves 3 foliate. Leaflets 5-15cm long, ovate, lanceolate or obovate. Flowers many, in dense terminal corymbs, petals nearly 2.5cm long, greenish white, stamens longer than the petals. Fruit ovoid, woody berry.

✧ **Distribution:**

The plant is distributed along the riparian vegetation of India, Burma, Malaysia, China, Nepal, Myanmar, Srilanka and Indonesia. In India it is found in peninsular, western gangetic plains and eastern India upto Tripura and Manipura. It is mostly found along the banks of rivers and streams and near to temple side.

✧ **Parts used:** Leaves, bark, root.

✧ **Chemical constituents:**

Cadabacine, cadabacine diacetate, (-) -catechin, (-) - epicatechin-5- glucoside, (-)epiafzelechin, isothicyanate glucoside, glucocapparin, taraxasterol, lupeol, 3-epilupeol, lupeol acetate, diosgenin, friedelin, betulinic acid, ceryl alcohol and spinasterol acetate.

Stem bark contains ceryl alcohol, friedelin,cadabicine, diacetate, lupeol, betulinic acid and diosgenin. Fruits contains glycocaparin, β-sitosterol, triacontane, triacontanol. Cetyl and ceryl alcohols. Leaves contain I-stachydrine. Root bark contains rutin, quercetin, lupeol, varunol and β-sitosterol.

✧ **Properties and uses:**

1) The leaf paste of Crataeva magna is applied externally on piles and the juice is drunk to get relief from bleeding piles.

2) Decoction along with the plant of Boerhaavia diffusa is given orally during inflammation.

3) Bark and roots are used for urinary disorder, fever, vomiting and gastric problems.

4) Stem bark paste mixed with powdered black pepper is taken twice daily with honey to cure infantile diarrhoea[30].

Saccharum officinarum

Common name: *Seeni-sarkkarai*

Scientific classification:

- Kingdom : Plantae
- Division : Angiosperms
- Class : Monocotyledons
- Order : Poales
- Family : Poaceae
- Genus : Saccharum
- Species : officinarum

The plant description

✧ **Description:**

It is a tropical, perennial grass that forms lateral shoots at the base to produce multiple stems, typically three or four meters high and about 5 cm diameter. The leaves grows from the nodes of the stem, arranged in two rows on either side of the stem. The leaves are tubular and blades like, thicker in the centres than at the margins and encircle than the stem. The inflorescence of sugar cane is a terminal panicle which possesses two spikelet and seeds protected by husks covered in silky hair.

✧ **Distribution:**

Sugar cane is indigenous to tropical south and Southeast Asia.

✧ **Parts used:** Juice, sugar, root.

✧ **Properties and uses:**

1) Sugar syrup can be used as preservative and also it cures Sinusitis and Rhinitis[31].

DISEASE REVIEW

3.3.SIDDHA ASPECT DISEASE REVIEW:

JAUNDICE (KAMALAI)

Other names: In *Siddha* literature *Kamalai* also known as *Manjal kamalai, Piththa kamalai, Kamala, Kamila.*

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

Aetiology:

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

Premonitory symptoms:

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

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

-யூகிமுனி.

Again, in this disease, palm, sole, face, eyes and the body will become pale; there would be severe fatigue in the extremities, shivering of the body, frequent dyspnoea, constipated and hardened feces, and yellowish discolouration of face, oedema, lassitude, deafness and heaviness of head[32].

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. *Mukkutra 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 of jaundice, seven types are curable and eight types are incurable. Curable types include *1.Udhu kamalai, 2.Varal kamalai, 3.Manjal kamalai, 4.Pitha kamalai, 5.Kabha kamalai, 6.Vatha kabha kamalai, 7.Pitha kabha kamalai*. The other varieties are not easily curable.

நாடி நடை:

"பண்பான பித்தத்தில் சேத்து மநாடி.....

கண்காது நயனமலம் நீரு மஞ்சள்"

(சதக நாடி)[33]

Treatment:

Intake of variable food and other deeds stimulates *pittha thathu, pitha* increases in its strength, joins *kaba* and becomes *pitha kaba* factor. This factor spoils

the spreading *vayu (paravu kal- Vyanan)* and prevents it from doing its normal work and thus it spoils the strength of blood. Because of this, liver gets affected and also the bile unable to flow in its normal route as there is an obstruction.

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

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

To induce vomiting:

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

To induce diarrhoea:

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

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

A decoction of the above substances may be made and given for jaundice for laxative purpose[32a].

Other medicines for vomiting and diarrhoea:

1.Malakudara oil

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

2.Malakudara mezhugu

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

3.Thithippu bedi mezhugu or legiyam

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

4.Sanjivi tablets

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

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

5.Marukkarai kaai

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

This can be ground and made into powder. ½ pinches can be given in the morning alone. Diarrhoea and vomiting will occur[34].

Medicines for Jaundice (Siddha Aspect):

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

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

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

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

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

Take each ingredient ¼ *palam*(8.5 gm) , Ground them all in the mortar and make tablets in the size of *Solanum torvum* (*Sundai*), dry them in shade and put them in a wide-mouthed bottle; pour good quality gingily oil and close it with a lid and put it in sunlight. Take each tablet twice a day. Jaundice along with oedema will get cured. Tamarind and salt should be avoided [35].

3.Jaundice powder (another process):

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

Mix all the above five ingredients and make it as a powder. Jaundice will be cured when this powder is taken in doses of 10 to 15 *Kundri* two to three times a day when with cow's milk or goat's milk or honey or in orange juice. It can also used as adjuvant to any other calyx or *Chenduram* or any other medicines prescribed for jaundice. By this, anaemia, oedema and liver diseases will get cured [36].

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

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

Each 17.5 gm (1/2 *palam*) is taken. All of them may be ground in a mortar with milk. Then this may be heated and processed for oil (*Thylam*) and filtered. 16 ml (the standard volume of a small spoon (*Uchikarandi*) may be consumed in the morning and evening. Jaundice will be cured. Salt-free diet is essential[36a].

5.*Karisalai ney* (Ghee of *Eclipta alba*):

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

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

It can be given with honey also or it can be taken separately. It can be used as an adjuvant for any other Calx or *Chenduram*. It will give an excellent cure for spleen and liver enlargement also[36b].

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

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

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

Phyllanthus distiches in the size of the fruit of *Alexandrian laurel* (*punnai kay*) may be taken and macerated on a stone slab. This may be given along with ¼ of a measure of sour buttermilk for 3 days in the morning. Jaundice will be cured. Rice with goat's milk can be taken. Salt should be avoided.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Put the iron pot on a stove. 8 gm of pepper may be put into the pot and fried. Make a powder of it keeping it in the pot itself. Then pour ½ measures of tender

coconut water into the pot over the pepper. Let it be boiled in the pot. One handful of tender leaves of *Thespesia populnea* may be taken and squeezed to get juice.

The juice may be poured into the pot when it starts boiling and the squeezed leaves may also be put in to the pot itself. Within a few minutes, the water portion in the pot will be reduced to 1/8 of a measure.

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

Saltiest and pungent diet should not be taken at the time of drinking this decoction for the whole day. Sweet substances are also to be reduced. Before consuming this medicine, the required food can be eaten[37].

The procedure for consuming this medicine

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

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

Then the needed food can be taken. Those who suffer from this disease for a longer duration can consume 3 doses of this medicine with a break of one day. For children, dosage should be adjusted suitably according to their age, body type and strength [38].

15.Decoction for jaundice:

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

2) 70 gm of *Sivanatha* powder along with cold water or honey or with the three fruit (three myrobalans-*kadu, tanri* and *nelli*) decoction may be consumed. Jaundice will get cured.

Diet for jaundice:

i. Salt should be restricted according to the strength of the patient. Porridge without salt and tamarind is good. Twice boiled rice can be given.

ii. As stated in above, when the bile flow is obstructed in the bile duct, fat will not be digested as bile is not available for digestion. Ghee, butter, oil and all other fatty substances should be avoided until the disease is cured completely.

iii. 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.

iv. Smoking, tobacco chewing, and alcohol–like substances should be fully avoided. Rest is essential until the disease is completely cured[39].

Liver diseases and medicinal plants:

- **Foeniculum vulgare:**

Hepatoprotective activity of Foeniculum vulgare (Family of Umbelliferae) essential oil was studied using a carbon tetrachloride induced liver fibrosis model in rats. The hepatotoxicity produced by chronic carbon tetrachloride administration was found to be inhibited by Foeniculum vulgare essential oil with evidence of decreased levels of serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and bilirubin.

- **Indigofera tinctoria:**

A bioactive fraction, indigtone (12.5100mg/kg p.o) characterized as trans-tetracos-15enoic acid (TCA), obtained by fractionation of a petroleum ether extract of the aerial parts of Indigofera tinctoria (Family of Fabaceae), showed significant dose dependent hepatoprotective activity against paracetamol (200mg/kg i.p) and CCl_4 (0.5ml/kg p.o mixed with liquid paraffin 1:1) induced liver injury in rats and mice. Pretreatment reduced Hexobarbitone induced sleep time, and zoxazolamine induced paralysis time. Pre and post treatment reduced levels of transaminases, bilirubin, TG, LPO and restored the depleted GSH in serum.

- **Rubia cordifolia:**

Rubiadin isolated from Rubia cordifolia Linn, (Family of Rubiaceae) at a dose of 50, 100 and 200 mg/kg was administered orally once daily for 14 days in rats. The substantially elevated serum enzymatic activities of serum GOT, GPT, ALP and GGT; decreased activities of glutathione Stransferase and glutathione reductase were restored towards normalization in dose dependent manner which were induce by CCl_4 treatment in rats. It also significantly prevents the elevation of hepatic MDA formation and depletion of reduced GSH content in the liver.

- **Solanum nigrum:**

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

- **Terminalia catappa:**

Punicalagin and Punicalin isolated from the leaves of Terminalia catappa L. (Family of Combretaceae) reduced hepatitis by reducing levels of AST and ALT which increased by APAP administration in rats[40].

3.4.MODERN ASPECT OF DISEASE REVIEW:

Liver Diseases:

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

Liver diseases also refered to as hepatic disease. 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[41].

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

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

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

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

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

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

10) Glycogen storage disease type II, the build-up of glycogen causes liberal muscle weakness (Myopathy) throughout the body and touches various body tissues, particularly the heart, skeletal muscles, liver and nervous system[42].

Causes for Liver diseases:

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

i. Congenital birth defects, or abnormalities of the liver present at birth

ii. Metabolic disorders, or defects in basic body processes

iii. Viral or bacterial infections

iv. Alcohol or poisoning by toxins

v. Certain medications that is toxic to the liver

vi. Nutritional deficiencies

vii. Trauma, or injury[7e]

Symptom of liver diseases includes:

Symptoms may begin slowly and slowly get worse. They may also begin suddenly and be severe from the start.

Early symptoms may be mild and include:

Breath with a musty or sweet odor, Change in sleep patterns, Changes in thinking, Confusion that is mild, Forgetfulness, Mental fogginess, Personality or mood changes, Poor concentration, Poor judgment, Worsening of handwriting or loss of other small hand movements.

More severe symptoms may include:

Abnormal movements or shaking of hands or arms, Agitation, excitement, or seizures (occur rarely), Disorientation, Drowsiness or confusion, Strange behavior or severe personality changes, Slurred speech, Slowed or sluggish movement, People with hepatic encephalopathy can become unconscious, unresponsive, and possibly enter a coma.

A rare but severe form of the liver infection called acute fulminant hepatitis causes liver failure. Symptoms of liver failure include:

An enlarged and tender liver, Enlarged spleen, Susceptibility to bleeding, Encephalopathy which is a disorder that affects how the brain functions, Changes in mental status or level of consciousness, Ascites which is an accumulation of fluid inside the abdomen, Edema or swelling under the skin, Aplastic anemia a condition in which the bone marrow cannot make blood cells[7f].

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[42a].

Viral Hepatitis:

Viral hepatitis is the cause of most cases of acute hepatitis. Types include Hepatitis A, Hepatitis B, Hepatitis C, Hepatitis B with D, Hepatitis E, Hepatitis F virus (existence unknown), and Hepatitis G or GBV-C.

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

this virus causes hepatitis, as it does not appear to replicate primarily in the liver. It is now classified as GB virus C.

- ✓ In addition to the hepatitis viruses, other viruses can also cause hepatitis, including cytomegalovirus, Epstein-Barr virus, yellow fever, etc. Non viral infection like Toxoplasma, Leptospira and Q fever also causes hepatitis[44].

Fatty Liver:

Fatty liver, also known as fatty liver disease (FLD), Steatorrhoeic hepatosis or Steatosis hepatitis, is a reversible condition where outsized vacuoles of triglyceride collect in liver cells via the process of Steatosis. Normal liver may cover as much as 5% of its weight as fat. Lipiotic liver may contain as much as 50% of its weight as fat, most of being triglycerides.

Severe fatty liver is sometimes accompanied by inflammation, a situation that is mentioned to as Steatohepatitis. The progression to cirrhosis may be influenced by the amount of fat and degree of Steatohepatitis and by a variety of other informing factors.

Cirrhosis:

Cirrhosis can be defined as a chronic disease condition giving morphological alteration of the lobular structure characterized by destruction and regeneration of the parenchyma cells and increased connective tissue. Major morphological changes induce granular or nodular appearance and are characterized by the presence of septate or collagen throughout the liver[45].

Infective Agents:

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

Toxic Agents:

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

dopa, Chlorpropamide, Phenylbu-tazone, Indomethacin, Paracetamol, Thiouracil, Acetaminophen, Halothen, Alcohol, Carbon tetrachloride, etc[46].

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	Pravastation	
Cimetidine	Quinidine	
Dantrolene	Rifampicin	
Erythromycin	Salicylates	
Galactosamine	Simvastatin	
Halothane	Sodium valproate	
Iproniazid	Sulphonamides	
Isoniazid	Tetracyclines	
Ketoconazole	Ethanol	

Liver Cancer:

The liver is inclined to cancer induction by a variety of human made and naturally occurring chemicals. Chemical substances include, aflatoxin B, cycasin, and safrole etc among human made substance are DDT, carbon tetrachloride, chloroform, thioacetamide. Studies in experimental animals designate quite clearly that development of cancer of the liver is associated with the number of obvious non-malignant lesions appearing prior to the occurrence of neoplastic malignancy.

Diagnosis:

Many further tests may also be used to support the diagnosis. These include blood test such as,

1) Liver function tests, which are blood tests that check a wide variety of liver enzymes and byproducts.

2) A complete blood count (CBC), which looks at the type and number of blood cells in the body.

3) Abdominal X-rays.

4) Ultrasounds, to show size of abdominal organs and the presence of masses.

5) An upper GI study, which can detect abnormalities in the esophagus caused by liver disease.

6) Liver scans with radio tagged substances to show changes in the liver structure.

7) ERCP, or endoscopic retrograde cholangiopancreatography. A thin tube called an endoscope is used to view various structures in and around the liver.

8) Abdominal CT scan or abdominal MRI, which provide more information about the liver structure and function.

Diagnosis of Drug-Related Hepatotoxicity:

There is no single test, including liver biopsy that can be used to diagnose drug-related Hepatotoxicity. Other causes of liver injury must first be considered with the use of a combination of serologic tests, imaging studies, and clues from the patient‟s history. CT denotes computed tomography, MRI magnetic resonance imaging, MRCP magnetic resonance cholangiopancreatography, ERCP endoscopic retro grade cholangiopancreatography, AST aspartate aminotransferase, ALT alanine aminotransferase, TIBC totaliron-binding capacity, and A1AT alpha1-antitrypsin[7g].

Hepatotoxicity:

Hepatotoxicity implies chemical-driven liver damage. The liver plays a Central role in transforming and clearing chemicals and is disposed to the toxicity from these agents. Certain medicinal agents when taken in overdoses and sometimes even when introduced within therapeutic ranges may injure the organ. Other chemical agents such as those used in laboratories and industries, and natural chemicals (e.g. microcystins) can also induce hepatotoxicity.

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

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

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

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

Some of the direct hepatotoxins comprise carbon 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[47]. 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[48].

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[49].

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[50].

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[51]. 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[52].

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

CCl_4 induced toxicity:

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

Paracetamol induced liver toxicity:

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

Alcohol induced toxicity:

Alcohol is metabolized by liver. This process produces a number of potentially dangerous byproduct. Alcohol is converted to acetaldehyde by the enzyme Alcohol dehydrogenase (ADH).The formed acetaldehyde is highly toxic. Normally the enzyme Aldehyde dehydrogenase (ALDH) rapidly oxidizes acetaldehyde to acetate. Both these enzymes ADH and ALDH are also involved in metabolism of vitamin A. Apart from ADH, the enzyme CYP2E1 (microsomal ethanol oxidizing system; MEOS) is also involved in metabolizing alcohol. MEOS plays a major role when blood ethanol levels are high. CYP2E1 produces a toxic byproduct *N*-acetyl-*p*-

benzo-quinone imine (NAPQI) which is responsible for damaging the hepatic protein[7h].

3.5.PHARMACOLOGICAL REVIEW

Models of Liver Fibrosis:

Several approaches to induce fibrosis in animals are designated and these models can be divided according to their stimulus from inciting injury. Liver fibrosis models are connected with

(1) Toxic damage (hepatocytes: CCl_4 dimethylnitrosamine (DMN), galactosamine; bile duct epithehal cells: thioacetamide (TAA),

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

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

(4) Alcohol-induced damage (baboon ethanol diet or Tsukamoto / French model in rats). Nowadays, fibrosis-related models are established that have their origin in fatty liver disease ,

(5) Fatty liver disease, in particular the 'malignant' inflammatory form non-alcoholic steatohepatitis (NASH), can increase to liver fibrosis and cirrhosis.

It is strongly associated with obesity and diabetes, two modern health problems in Western countries. Of the existing animal models for fatty liver disease, as reviewed by the genetic lepton-deficient (ob/ob) or lepton- resistant (db/db) mice[53].

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

BDL and CCl_4 are the most widely used rodent models[54] in liver fibrosis research to assess the effectively of experimental drugs on the pathogenesis, since these models represent features of human pathogenesis. Therefore, these models are the best categorized with respect to histological, biochemical, cell and molecular changes connected with the development of fibrosis.

In the past years, there is a tendency in fibrosis - related research to shift from rat to mice models, and most of the models originally described ferrates are now applied in mice. Moreover, new testing models arise due to the development of

transgenic or knock-out mice models, which were developed to elucidate the pathogenesis and common pathways in liver fibrosis. Examples of knockouts with spontaneous formation of liver fibrosis are mdr2-/- mice 1hx2-/- mice, and the mice models for NASH mentioned above[55].

Acute and Chronic Models with Carbon Tetrachloride (CCl_4)

CCl_4 intoxication results in hepatocyte necrosis and apoptosis with damage predominantly in zone III (around central vein) of the liver. The mechanism behind this hepatocyte damage is the activation of CCl_4 by cytochrome P450, which results in the formation of trichioromethyl radical in these cells and this free radical initiates lipid peroxidation[56] .

The damage to hepatocytes by CCl_4 is replicated by high plasma alanine transaminase (ALT) and aspartate transminase (AST) levels after CCl_4 administration, CCl_4causes also fatty changes in the hepatocytes. This initial damage is followed by hepatic stellate cell activation and tissue fibrosis.

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

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

The models for CCI_4 that are used in liver fibrosis research, are

(1) Acute damage (72 hours after a single injection of CCI_4) with HSC activation

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

(3) Early cirrhosis (8 week of twice weekly CCl_4 dosing)

(4) Advanced micronodular cirrhosis (12 week of twice weekly CCl_4 dosing). In addition for each of these models,

(5) Spontaneous recovery from fibrosis can be studied after cessation of dosing of CCl_4.This latter model is a valuable model to determine drug induced acceleration of recovery from established fibrosis after removal of the inciting stimulus.

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

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

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

Bile Duct Ligation (BDL) :

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

This latter is envisaged by elevated ALT and AST levels in plasma, in particular proximately after ligation (first week). Characteristics of obstruction of the bile are the appearance of bile products, such as bilirubin into the blood circulation, which causes jaundice in these animals[57].

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 sub-sequent this will lead to lipid peroxidation. In difference to the hepatotoxin $CC1_4$, DMN administration does not cause fatty changes, steatosis in the hepatocytes[53]. To induce the fibrosis, DMN (10 microliter/kg body wt., i.p) is given 3 days a week for 3 weeks to rats.

After administration of DMN, hemorrhagic necrosis is evident in centrolobular part (zone III) of the liver. Incomplete septa appear after 7 days and micronodular cirrhosis is developed after 3 weeks of treatment with DMN. Increased numbers of HSC and my fibroblasts are found in the formed septa. Influx of inflammatory cells, mainly lymphocytes, is noted early in DMN - induced liver injury.

Advantages of this model are that the disease induction is quite reproducible in the animals, and this model is associated with a prominent inflammatory reaction. Furthermore, this model can be used to study the transition from cirrhosis to hepatocellular carcinoma, and the effect of drugs on this process.

HSC in Culture (In Vitro System):

HSC are key players in fibrosis and these cells predominantly orchestrate the development of the disease. To evaluate the ant fibrotic efficacy of experimental drugs, these primary cultured cells are useful in assessing specific effects on HSC activities. In particular, the primary isolated HSC are valuable in drug research, because in vitro they spontaneously transform into my fibroblasts, and this transformation process is related with cellular activation proliferation and matrix production resembling cellular activities that also happen in vivo.

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

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

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

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

The yield of HSC after collagenase / pronase digestion and Nycodenz separation is about 20-40 x 10E6 cells per rat liver.

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

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

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

Roughly, two methods are used to isolate human stellate cells.
(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, CCl_4 intoxicated rats treated with TCW retained almost normal levels of these constituents. Decreased activities of antioxidant enzymes in CCl_4 intoxicated rats and their reversal of antioxidant enzyme activities in TCW treated rats, shows the effectiveness of TCW in combating CCl_4 induced oxidative stress.

Hepatoprotective outcome of TCW is also evidenced from the histopathological studies of liver, which did not show any fatty infiltration or necrosis, as observed in CCl_4 intoxicated rats[58].

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
- Inflammed 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):

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

Serum Albumin:

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

Prothrombin Time (PT):

The PT test measures in seconds the time it takes for blood clots to form (the longer it takes the greater the risk for bleeding[59].

3.6.LATERAL RESEARCH

Vetiveria zizanioides:

- **Anti tuberculosis activity:**

Vetiveria zizanioides (Family: poaceae) root extracts and fractions were evaluated for antimycobacterial activity against *Mycobacterium tuberculosis* $H_{37}Rv$ and $H_{37}Ra$ strains using radiometric BACTEC 460 TB system.

The ethanolic extrat of intact as well as spent root were showed potent antituberculosis activity at a minium concentration of 500µg/mL. The hexane fraction also showed antibacterial action by recording continuous decline in growth index (GI) of M.tuberculosis at 50µg/mL[60].

***Plectranthus vettiveroides*:**

- **In vitro Anti cancer and Anti oxidant activity:**

The efficacy of *Plectranthus vetiveroides* against various cancer cell lines showed that the incubation of cancer cells reduced the viability of all cancer cell lines and dead cells were significantly increased with high extract concentration. Hence hydro alcoholic extract of *Plectrathus vettiveroides* exhibited high cytotoxicity. Also the extract showed potent antioxidant activity against all the three tested methods.

Even at very low concentration *Plectranthus vettiveroides* showed high efficacy. In conclusion *Plectranthus vettiveroides* posses significant anti oxidant and anticancer activity[61].

Gymnema sylvestre:

❖ **Antidiabetic and Hypolipiemic activity:**

The aqueous leaf extract of *gymnema sylvestre* at the dose of 400, 600 and 800mg kg^{-1} body weight was administered orally once a day to the groups for 30days. Teh fasting blood glucose, cholesterol, HDL-cholesterol and serum triglyceride content were estimated in both normal and alloxan induced diabetic rats. All the content were found to be significantly reduced ($P<0.05$) (except HDL) in treated rats whereas the extract also showed the potent elevation in the level of serum HDL-cholesterol. The study reveals that *Gymnema sylvestre* has significant antidiabetic activity and a hypolipidemic activity in alloxan induced and normal fasting rats[62].

Acalypha indica:

❖ **Anelgesic activity and Anti inflammatory activity:**

The methanolic extract of *Acalypha indica* showed statistically significant (p,0.001) analgesic activity in mice in a dose dependent manner. A sustained and significant (p,0.001) inhibition of carrageenan induced inflammation of rat paw was observed with 125mg/kg and 250mg/kg body weight.

The methanolic extract of *A.indica* also demonstrated anti inflammatory effect in a dose dependent manner. Maximum inhibition by the extract was observed at 250mg/kg body weight after three hours of injestion, which was comparable to that of the standard drug phenylbutazone at a dose of 100mg/kg body weight[63].

Tephrosia purpurea:

❖ **Anti ulcer activity:**

Antiulcer activity of aqueous extract of *Tephrosia purpurea*(AETP) was studied in rats in which gastric ulcers were induced by oral administration of ethanol or 0.6M HCL or by pyloric ligation or indomethacin and duodenal ulcers were

induced by oral administration of cysteamine HCL. AETP administered in the dose of 1 to 20mg/kg orally 30min peror of to ulcer induction. The result suggest that AETP possesses significant antiulcer property which could be either due to cytoprotecting action of the drug or by strenthening of gastric and duodenal mucosa and thus enhancing mucosal defence[64].

Aegle marmelos:

❖ **Antidiarrhoeal activity:**

The decoction of the unripe fruit of *Aegle marmelos* showed cidal activity against *Giardia* and rotavirus whereas viability of none of the six bacterial strains tested was affected. It significally reduced bacterial adherence to and invasion of HEp-2 cells. The extract affected production of cholera toxin and binding of both E.coli heat labile toxin cholera toxin and their binding to gangliosside monosialic receptor. The decoction of the unripe fruit of *Aegle marmelos* has limited antimicrobial activity, affected th bacterial colonisation to gut epithelium and production of certain enterotoxins. These obsevation suggest the mode of action of *A.marmelos* in infectious of diarrhoea[65].

Stereospermum colais:

❖ ***Anticancer activity:***

The anticancer activity was evaluated using standard methods like MTT and No assay methods. *S. colais* ethyl acetate and methanol extracts and *B. acutangula* ethyl acetate extract showed free radical scavenging and anti-cancer effect 54 against Colon cancer cell lines Colo320. Also plant extracts cause apoptosis analyzed by DNA fragmentation assay. Plant extracts when treated with cells causes apoptosis when assayed with CASPASE.

❖ **In-vitro antioxidant activity:**

Methanol extracts of *Stereospermum colais* showed concentration dependent antibacterial activity ranging from 62.5 µg /ml to 2000 µg /ml when compared with standard curcumin in different in vitro models[66].

Ocimum sanctum:

❖ **Antibacterial activity:**

Ocimum sanctum fixed oil showed good antibacterial activity against *Staphylococcus aureus, Bacillus pumilus and psedomonas aereginosa.* Antibacterial activity was evaluated by paper disc deffusion method[67].

Mukia maderaspatana:

❖ **Antidiabetic activity:**

Methanol extract of the dried whole plant (0.25 and 0.5mg/ml) were studies for teh inhibition of gluconeogenesis in rat liver slices. Mukia inhibited gluconeogenesis by 45% and with with insulin inhibition increased by 50%[68].

Rivea ornata:

❖ **anti inflammatory activity:**

The anti inflammatory activity of the methanolic extract of *R.ornata* at the doseof 400mg/kg has a significant reduction in the Carageenan induced paw oedema in rats. So 400mg/kg dose of methanolic extract exhibiting anti inflammatory effect against acute inflammation[69].

Limonia acidissima:

❖ ***Wound healing and anti oxidant activity:***

In incision wound healing model, the methanolic extract of *L.acidissima* has significant wound healing activity and antioxidant activity in animals treated with 400mg/kg of the extract[70].

Hemidesmus indicus:

❖ **in vitro antioxidant and antithrombotic activity:**

The methanolic extract of *H.indicus* roots was found to inhibit lipid peroxidation and and scavenge hydroxyl and superoxide radicals in vitro. The amount

rquired for 50% inhibition of lipid peroxide formation was 217.5µg/ml. The extract also inhibited ADP-induced platelet aggregation in vitro[71].

Sida rhombifolia:

❖ **Anti inflammatory, anticholinergic and cytotoxic activity:**

The ethyl acetate of plant extract exhibited the most significant antioxidant activities by scavening DPPH radicals and ferrous ions with EC50 of 380.5 and 263.4µg/mL, respectively. In contrast the n-hexane extract showed the strongest anti inflammatory activity with IC50 of 52.16 and 146.03µg/mL for no and protein denaturation inhibition assays. The same extract also revealed the strongest effects in anti cholesterase and cytotoxic tests at the concentration of 100µg/mL. AChE enzyme inhibition was 58.55% and human cancer cells. SNU-1 and Hep G2 inhibition was 68.52% and 47.82% respectively[72].

Withania somnifera:

❖ **Immunomodulatory activity:**

Administration of an extract from the powdered root of the plant W.somnifera was found to stimulate immunological activity in Bab1/c mice. The extract along with the antigen produced an enhancement in the circulatory antibody titre and the number of plaque forming cells(PFC) in the spleen. It also showed the enhancement in phagocytic activity of peritonial macrophages (76.5pigmented cells/200) when compared to control(31.5/200 cells) in mice[73].

Smilax china:

❖ **Anti inflammatory activity and anti nociceptive activity:**

The aqueous exract of the tuber *Smilax china* tested for its anti inflammatory activity in rats by egg-albumin induced oedema and nociceptive effects in mice using hot plate test and acetic acid induced abdominal consrticton test, at the dose of 1000mg/kg had a significant anti inflammatory and nociceptive effect compared to physilogical saline[74].

Crataeva magna:

❖ **Antiurolithiatic avtivity:**

The ethanol extract(400mg/kg) had significant urolithiasis activity in lactose(30%)+ethylene glycol(1%) and ammonium chloride(2%)+ethylene glycol(0.75%) methods induced urolithiasis in rats respectively. Both models resulted in serum creatinine and calcium, urine oxalate and kidney weight significantly increased in final body weight and urine volume output when compared to toxic group[75].

3.7.PHARMACEUTICAL REVIEW

CHOORANAM:

Definition:

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

Method of preparation:

Equipment required:

1. The drug enumerated in the recipe in clean and 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 storage or changed in colour, taste and scent and those that are insects infected or attacked by fungi should be positively rejected.

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

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

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

Purification of the prepared Chooranam:

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

-அகத்தியர் வைத்திய இரத்தினச்சுருக்கம்.

The prepared Chooranam is mixed with the milk,in 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 Chooranam 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"

-அகத்தியர் வைத்திய இரத்தினச்சுருக்கம்[89]

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[77].

According to AYUSH guidelines shelf life of Chooranam is one year[78].

Table: 2. ANALYTICAL SPECIFICATIONS OF CHURNA/CHOORANAM

Sl. No	TESTS
1.	Description Macroscopic, Microscopic
2.	Loss on drying at 105^0 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

DRUG SELECTION:

For this present study, the Herbal formulation ***"Maavilingapattai Chooranam"*** was taken as the compound drug preparation for Hepatoprotective activity mentioned in the classical Siddha literature ***"Sirorathina Vaidhiya Booshanam"*** written by Angamuthu Mudhaliyar, published by Thamarai Noolagam, Chennai-26, pg.no:148-149.

Table 3: Ingredients of *Maavilingapattai Chooranam:*

INGREDIENTS:

S.no	NAME OF DRUGS	BOTANICAL NAME	QUANTITY
1.	*Iruveli*	*Vetiveria zizanioides*	35gms (1 palam)
2.	*Vilamichu*	*Plectranthus vettiveroides*	35gms (1 palam)
3.	*Chiru kurinchan*	*Gymnema sylvestre*	35gms (1 palam)
4.	*Poonai vanangi*	*Acalypha indica*	35gms (1 palam)
5.	*Kozhunji*	*Tephrosia purpurea*	35 gms (1 palam)
6.	*Koovilam*	*Aegle marmelos*	35gms (1 palam)
7.	*Pathiri*	*Stereospermum colais*	35gms (1 palam)
8.	*Thulasi*	*Ocimum sanctum*	35gms (1 palam)
9.	*Musumusukkai*	*Mukia maderaspatana*	35gms (1 palam)
10.	*Musuttai*	*Rivea ornata*	35gms (1 palam)
11.	*Vila*	*Limonia acidissima*	35gms (1 palam)
12.	*Nannari*	*Hemidesmus indicus*	35gms (1 palam)
13.	*Kurundhotti*	*Sida rhombifolia*	35gms (1 palam)
14.	*Ashwagandhi*	*Withania somnifera*	35gms (1 palam)
15.	*Parangichakkai*	*Smilax china*	70gms (2 palam)
16.	*Maavilingapattai*	*Crataeva magna*	280gms (8 palam)
17.	*Seenisarkarai*	*Saccharum officinarum*	-

Collection of the Plant materials:

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

Identification and Authentication of the drug:

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

Purification of the drugs:

Purification process was done as per classical *Siddha* literature[79].

Table 4: Purification of drugs:

S.no	Raw Drug	Method of Purification
1.	*Iruveli*	The root was cleaned with a white cloth. Finally washed and dried.
2.	*Vilamichu*	The root was cleaned with a white cloth. Finally washed and dried.
3.	*Chiru kurinchan*	The root was cleaned with a white cloth. Finally washed and dried.
4.	*Poonai vanangi*	The root was cleaned with a white cloth. Finally washed and dried.
5.	*Kozhunji*	The root was cleaned with a white cloth. Finally washed and dried.
6.	*Koovilam*	The root was cleaned with a white cloth. Finally washed and dried.
7.	*Pathiri*	The root was cleaned with a white cloth. Finally washed and dried.
8.	*Thulasi*	The root was cleaned with a white cloth. Finally washed and dried.
9.	*Musumusukkai*	The root was cleaned with a white cloth. Finally washed and dried.
10.	*Musuttai*	The root was cleaned with a white cloth. Finally washed and dried.
11.	*Vila*	The root was cleaned with a white cloth. Finally washed and dried.

S.no	Raw Drug	Method of Purification
12.	*Nannari*	The root was cleaned with a white cloth. Finally washed and dried.
13.	*Kurundhotti*	The root was cleaned with a white cloth. Finally washed and dried.
14.	*Ashwagandhi*	Dried and powdered then it is steamed in milk.
15.	*Parangichakkai*	Dried and powdered then it is steamed in milk.
16.	*Maavilingapattai*	Outer skin of bark was peeled off.

4.1. PREPARATION OF THE DRUG:

❖ **Procedure:**

35grams of purified *Iruveli*, 35grams of purified *Vilamichu*, 35grams of purified *Chirukurinchan*, 35grams of *Poonaivanangi*, 35grams of *Kozhunji*, 35grams of *Koovilam*, 35grams of *Pathiri*, 35grams of *Thulasi*, 35grams of *Musumusukkai*, 35grams of *Musutai*, 35grams of *Vila*, 35grams of *Nannari*, 35grams of *Kurundhotti*, 35grams of *Ashwagandhi,* 70grams of *Parangichakkai*, 280grams of *Maavilingapattai* were taken and powered separately. Then all the powder were mixed together.

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

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

❖ **Purification of the Chooranam- Steaming process (*Pittaviyal murai):***

The ***"Maavilinga Pattai Chooranam"*** was purified by *pittaviyal* method (steam cooking in milk) as per *Siddha* classical literature. A mud pot was taken and it was half filled by milk and mixed with equal quantity of pure water. The mouth of the pot was sealed by a cloth. This chooranam was placed over a clean dry cloth and tied

firmly around the mouth of mud pot. The gap between mud pots was tied with a wet cloth to avoid evaporation. 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[80].

❖ **Storage of the drug:**

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

❖ **Administration of the drug:**

- Form of the medicine : *Chooranam*
- Route of Administration : Interal
- Dose : 2 gm twice a day depending on the severity
- Duration : 12- 24 Days.

❖ **Indication:**

Kamalai, *Pun, Purai, Megavettai, Megapadai, Themal.*

Fig 2: INGREDIENTS OF *MAAVILINGAPATTAI CHOORANAM:*

2.1.Vetiveria zizanioides

2.2Plectranthus vettiveroides

2.3.Gymnema sylvestre

2.4.Acalypha indica

2.5.Tephrosia purpurea

2.6.Aegle marmelos

2.7.Stereospermum colais

2.8.Ocimum sanctum

2.9.Mukia maderaspatana

2.10.Rivea ornata

2.11.Limonia acidissima

2.12.Hemidesmus indicus

2.13.Sida rhombifolia

2.14.Withania somnifera

2.15.Smilax china

2.16.Crataeva magna

2.17.Saccharum officinarum

2.18.Final Product (*Maavilingapattai Chooranam)*

4.2. STANDARDIZATION OF THE DRUG:

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

Method of standardization:

Techniques Involved In Standardization of Compound Drugs:

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

4.2.1. ORGANOLEPTIC CHARACTER:[81]

The organoleptic characters of the sample were evaluated which include evaluation of the formulation by its colour, odor, taste, texture etc.

Colour:

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

Odour:

Chooranam were smelled, the time intermission between two smelling was kept 2 minutes to nullify the effect of previous smelling.

Taste:

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

Size:

The *chooranam* was completely sieved through mesh size 88.

4.2.2 PHYSICOCHEMICAL ANALYSIS:[82]

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

1. Solubility Test:

A pinch of sample (*MPC*) was taken in a dry test tube and to it 2 ml of the solvent was added and shaken well for about a minute and the results are observed. The test was done for solvents like distilled water, Ethanol, Petroleum ether, Propylene glycol, Toluene, Benzene, Chloroform, Ethyl alcohol, Xylene, Carbon tetra chloride and the results are observed individually.

2. pH value:

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

3. Loss on Drying:

An accurately weighed 2gm of ***Maavilingapattai Chooranam*** formulation was taken in a tarred glass bottle. The crude drug was heated 105^0 c for 6 hours in an oven till a constant weight. The percentage moisture content of the sample was calculated with reference to the shade dried material.

4. Determination of total Ash:

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

5. Determination of acid insoluble ash:

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

6. Determination of water soluble ash:

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

7. Determination of water soluble extractive:

5gm of air dried drug. Coarsely powered ***Maavilingapattai Chooranam*** was macerated with 100ml of distilled water in a closed flask for twenty-four hours, shaking frequently. The solution was filtered and 25 ml of filtered was evaporated in a tarred flat bottom shallow dish, further dried at 1000^0c and weighted. The percentage of water soluble extractive was calculated with reference to the air dried drugs.

8. Determination of alcohol soluble extractive:

2.5gm of air dried drugs coarsely powdered ***Maavilingapattai Chooranam*** was macerated with 50ml. alcohol in closed flask for 24 hours. With frequent shaking.it was filtered rapidly talking precaution against loss of alcohol. 10ml of

filtrate was the evaporated in a tarred flat bottom shallow dish, dried at 1000^0c and weighed. The percentage of alchol soluble extractive was calculated with reference to air dried drug.

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[83].

❖ **Test for Alkaloids:**

Extracts were dissolved in dilute hydrochloric acid and filtered.

1) **Mayers's test**: Filtrates were treated with Mayer's reagent (Potassium, Mercuric iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.
2) **Wagnor's test**: Filtrates were treated with Wagner's reagent (Iodine in potassium iodide). Formation of brown/ reddish precipitate indicates the presence of the alkaloids.
3) **Dragendroff' test**: Filtrates were treated with Dragendroff's reagent (solution of Potassium with Bismuth iodide). Formation of red precipitate indicates the presence of alkaloids.
4) **Hager's test**: Filtrates were treated with Hager's reagent (saturated picric acid solution) presence of alkaloids conformed by the formation of yellow precipitate.

❖ **Test for Carbohydrates and Reducing Sugars:**

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

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

❖ **Test for Glycosides:**

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

1) **Modified borntrager' s test**: Extracts were treated with ferric chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose pink colour in ammonical layer indicates the presence of anthranol glycosides.
2) **Cardiac glycoside** (keller-killiani test): Extracts was shaken with distilled water (5ml). to this, glacial acetic acid (2ml) containing few drops of ferric chloride was added followed by sulphuric acid (1ml) along the side of the test tube. The formation of the brown ring at the interface gives positive indication for cardiac glycoside and a violet ring may appear below the brown ring.

❖ **Test for Saponins:**

1) **Froth test**: Extracts were diluted with distilled water to 20ml and this was shaken graduated cylinder for 15 minutes. Formation of 1cm layer of foam indicates the presence of saponins.
2) **Foam test**: 0.5gm of extract was shaken with 2ml of water if foam produced persists for ten minutes. It indicates the presences of saponins.

❖ **Detection of phytosterols:**

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

❖ **Detection of phenol ferric chloride test**:

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

❖ **Detection of tannins Gelatin test:**

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

❖ **Detection of flavonoids:**

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

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

❖ **Detection of Proteins and Amino acids**:

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

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

❖ **Detection of Diterpenes:**

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

❖ **Gum and Mucilage**:

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

❖ **Test for Fixed oils and Fats:**

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

❖ **Test for Quinones**:

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

HPLC - High Performance Liquid Chromatography (HPLC)[84]:

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

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

- Column : Symmetry C18, 5 µm, 4.6x250 mm
- Run Time : 30 minutes
- Injection Volume : 20 µl
- Wavelength (Dual) : 272 nm & 360 nm
- Solvent A : Acetonitrile
- Solvent B : 0.1% Phosphoric acid in water
- Flow rate : 1.0 ml/min.
- Pump Mode : Gradient

4.2.4.BIO-CHEMICAL ANALYSIS[85]:

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

Preparation of extract:

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

Preliminary Basic and Acidic radical studies:

Test for basic radicals:

1. **Test for Potassium:**

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

2. **Test for Calcium:**

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

3. **Test for Magnesium:**

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

4. **Test for Ammonium:**

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

5. **Test for Sodium:**

Hydrochloric acid was added with a pinch of the *MPC* sample and a paste was made and introduced into the blue flame of Bunsen burner and observed for the appearance of intense yellow color.

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

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

7. **Test for Zinc:**

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

8. **Test for Aluminium:**

To the 2m1 of the *MPC* extract sodium hydroxide was added in drops and noted for any characteristic changes.

9. **Test for Lead:**

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

10. **Test for Copper:**

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

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

11. **Test for Mercury:**

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

12. **Test for Arsenic:**

To 2 ml of the *MPC* extract 2ml of Sodium hydroxide solution was added and brown wash red precipitate if appeared was noted.

Test for acid radicals:

1. **Test for Sulphate:**

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

2. **Test for Chloride:**

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

3. **Test for Phosphate:**

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

4. **Test for Carbonate**:

The *MPC* extract was treated with concentrated HCL and observed for the appearance of effervescence.

5. **Test for Fluoride & Oxalate:**

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

6. **Test for Nitrate:**

To 1 gm of the *MPC*, copper turnings was added and again concentrated H_2SO_4 was added, heated and the test tube was tilted vertically down and viewed for any characteristic changes.

4.2.5.AVAILABILITY OF MICROBIAL LOAD:

Enumeration of bacteria by plate count – Agar plating technique[86]:

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

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 are 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:

1. Label the dilution blanks as 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}.
2. Prepare the initial dilution by adding 1 ml of the *MPC* into a 9 ml dilution blank labeled 10^{-1} thus diluting the original sample 10 times.
3. Mix the contents by rolling the tube back and forth between hands to obtain uniform distribution of organisms.
4. From the first dilution transfer 1 ml of the suspension while in motion, to the dilution blank 10^{-2} with a sterile and fresh 1 ml pipette diluting the original specimen to 100 times.
5. From the 10^{-2} suspension, transfer 1 ml of suspension to 10^{-3} dilution blank with a fresh sterile pipette, thus diluting the original sample to 1000 times.
6. Repeat this procedure till the original sample has been diluted 10,000,000 times using every time a fresh sterile pipette.
7. From the appropriate dilutions transfer 1ml of suspension while in motion, with the respective pipettes, to sterile petri dishes. Three petri dishes are used for each dilution.

8. Add approximately 15 ml of the nutrient medium, melted and cooled to 45^0c, to each petri dish containing the diluted sample. Mix the contents of each dish by rotating gently to distribute the cells throughout the medium.
9. Allow the plates to solidity.
10. Incubate these plates in an inverted position for 24-48 hours at 37^0c.

Observation:

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

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

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

4.2.6.SOPHISTICATED INSTRUMENTAL ANALYSIS:

❖ **FTIR - Fourier Transform Infra-red Spectroscopy[87]:**

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

APPLICATIONS:

- Quatitative scans and Qualitative scan
- Solids, liquids, gases
- Organic samples, inorganic samples
- Unknown identification and Impurities
- Screening formulation
- Pharmaceuticals.

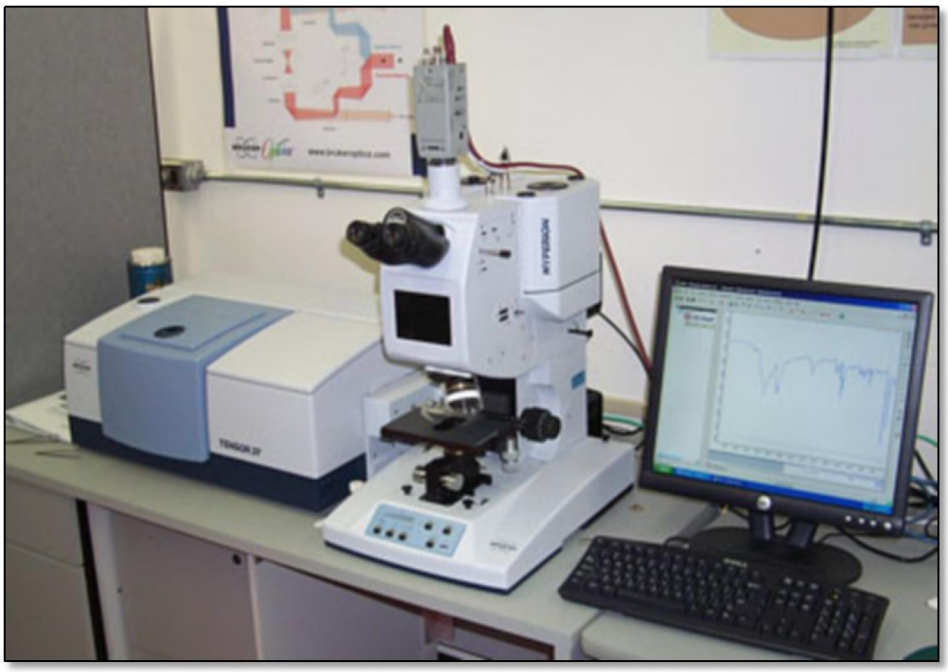


Fig no.3.1: FTIR INSTRUMENT

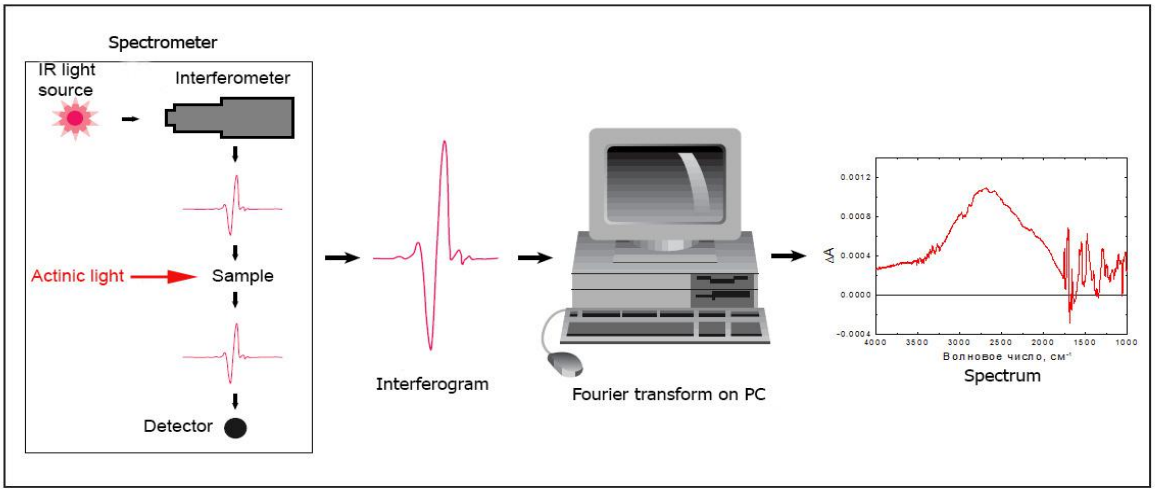


Fig no.3.2: FTIR MECHANISM

Principle:

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

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

- Triturate about 1 to 2 mg of the substance to be examined with 300 to 400 mg, unless otherwise specified, of finely powdered and dried potassium bromide. If the substance is a hydrochloride it is preferable to use potassium chloride.
- Carefully grind the mixture and spread it uniformly in a suitable die.
- Submit it to the pressure of about 800 mPa (8 tons/cm2).
- Examine the disc visually and if any lack of uniform transparency is observed, reject the disc and prepare again.
- Record the spectrum between 4000 to 650 cm-1 unless otherwise specified in individual standard test procedure.
- When sample and standard are measured for concordance, the transmittance obtained at the start of the scan range, should not deviate by more than 10% between them (For eg. If the standard shows a transmittance of 75%, the sample transmittance can be between 65% and 85%).

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

- Speed
- Sensitivity
- Mechanical Simplicity
- Internally Calibrated

❖ **ICP-MS - Inductively Coupled Plasma Mass Spectrometry[88]:**

Analysis of Trace Metal and Inorganic materials.

Inductively Coupled Plasma Mass Spectrometry is a technique routinely used to analyse trace levels of a wide range of inorganic elements. The ICP-MS allows for the detection and quantification of elements with atomic mass ranges 7 to 250. This covers Lithium to Uranium. The typical detection limits are in the parts per billion (ppb) range and even parts per trillion (ppt) in some cases. The ICP-MS analysis methods available at LPD Lab Services allow the detection, identification and quantification of a wide array of elements using a Perkin Elmer ELAN 6000 ICP-MS.

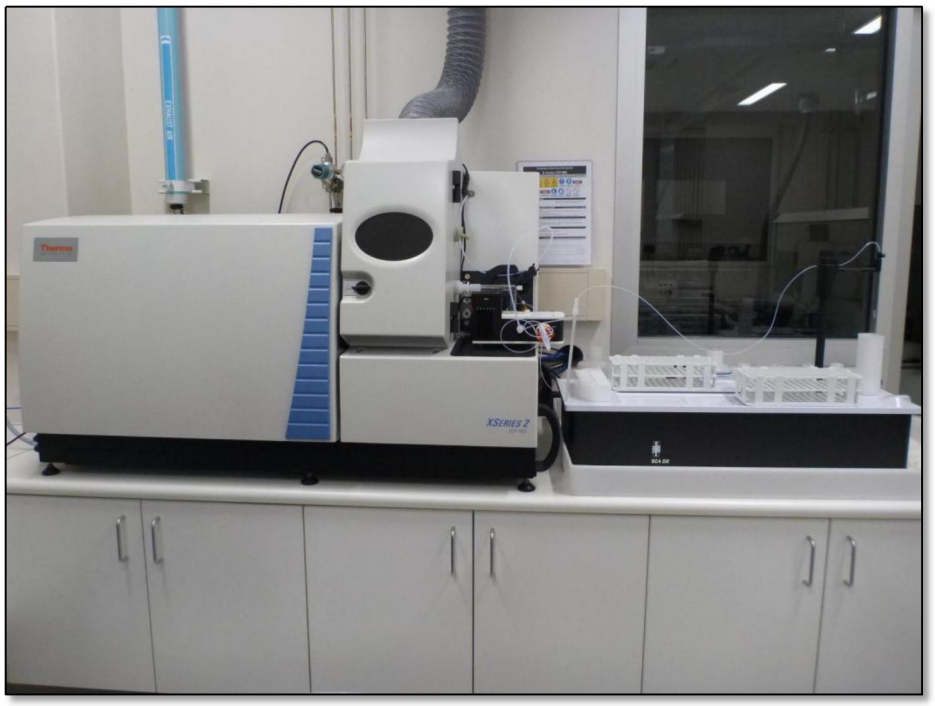


Fig no:4.1.: ICPMS- INSTRUMENT

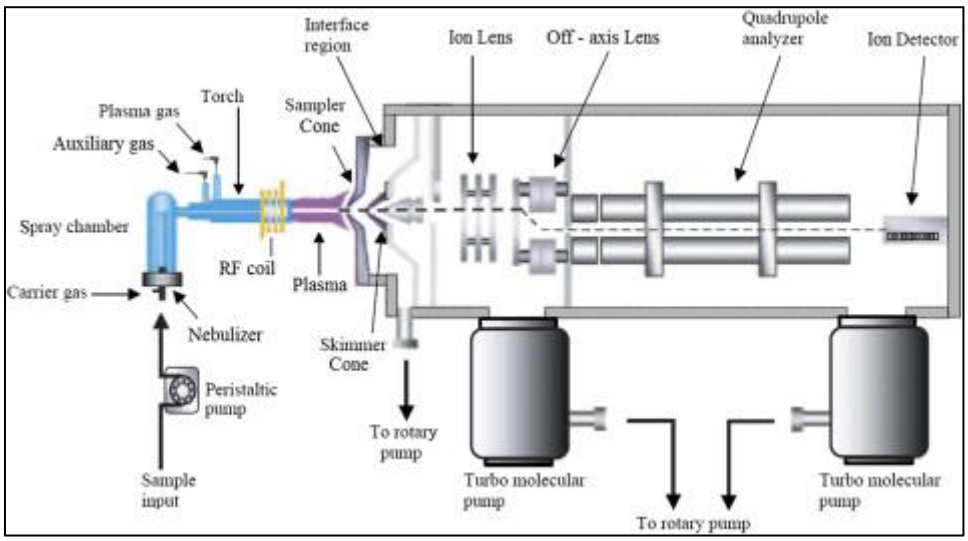


Fig no:4.2. ICP MS MECHANISM

Analysis: Analyze according to the manufacturer suggestions for program and m/z. Calculate and report results based on the original sample size.

Applications of ICP-MS:

- Monitoring of trace metals in drinking water, ground water, rainwater, wastewater or industrial effluent streams.
- Trace elements in product / raw materials or from washed or rinsed surfaces.

- Analysis of additives and purity in metal alloys.
- Analysis of low level contaminants in chemical products, beverages, foods, cosmetics, pharmaceuticals.
- Analysis of soluble / leachable material from solid samples such as medical devices, polymers, PCB`s.
- Analysis can be performed on a diverse range of sample.

❖ **SEM - Scanning Electron Microscope[89]:**

DEFINITION:

Scanning Electron Microscopy (SEM), also known as SEM analysis or SEM microscopy, is used very effectively in microanalysis and failure analysis of solid inorganic materials. Scanning electron microscopy is performed at high magnifications, generates high-resolution images and precisely measures very small features and objects.

SEM ANALYSIS APPLICATIONS:

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

External morphology (texture)

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

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

- Determine elements in or on the surface of the sample for qualitative information
- Measure elemental composition for semi-quantitative results
- Identify foreign substances that are not organic in nature and coatings on metal

SEM Analysis with EDS – qualitative and semi-quantitative results

Magnification – from 5x to 300,000x

Sample Size – up to 200 mm (7.87 in.) in diameter and 80 mm (3.14 in.) in height

Materials analysed – solid inorganic materials including metals and minerals.

Fig no:5.1: SEM INSTRUMENT

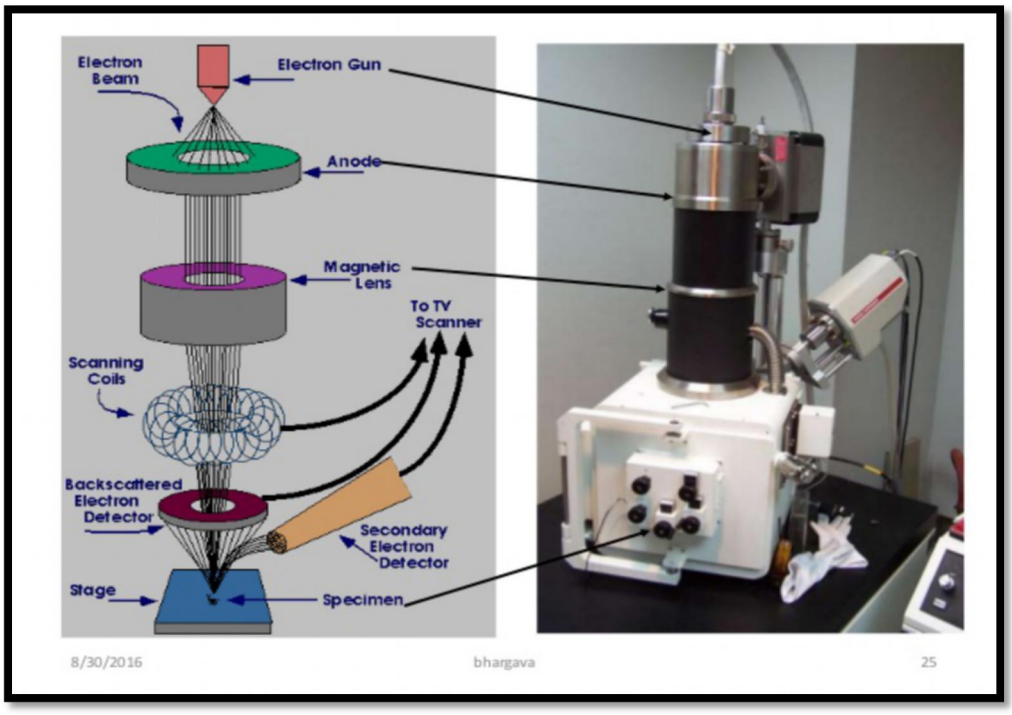


Fig no:5.2: SEM MECHANISM

THE SEM ANALYSIS PROCESS:

Scanning Electron Microscopy uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens. In most SEM

microscopy applications, data is collected over a selected area of the surface of the sample and a two-dimensional image is generated that displays spatial variations in properties including chemical characterization, texture and orientation of materials. The SEM is also capable of performing analyses of selected point locations on the sample. This approach is especially useful in qualitatively or semi-quantitatively determining chemical compositions, crystalline structure and crystal orientations.

The EDS detector separates the characteristic X-rays of different elements into an energy spectrum and EDS system software is used to analyse the energy spectrum in order to determine the abundance of specific elements. A typical EDS spectrum is portrayed as a plot of X-ray counts vs. energy (in keV). Energy peaks correspond to the various elements in the sample. Energy Dispersive X-ray Spectroscopy can be used to find the chemical composition of materials down to a spot size of a few microns and to create element composition maps over a much broader raster area. Together, these capabilities provide fundamental compositional information for a wide variety of materials, including polymers. In scanning electron microscope high-energy electron beam was focused through a probe towards *MPC*. Variety of signals was produced on interaction with the surface of the sample. This results in the emission of electrons or photons and it was collected by an appropriate detector.

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

- Secondary electrons
- back scattered electrons
- characteristic x-rays light
- specimen current
- Transmitted electrons.

This gives the information about the sample and it includes external morphology, texture, its crystalline structure, chemical composition and it displays the shape of the sample.

❖ **XRD - X-ray Powder Diffraction (XRD)[90]:**

X-ray powder diffraction (XRD) is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit

cell dimensions. The analyzed material is finely ground, homogenized, and average bulk composition is determined.

DEFINITION:

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

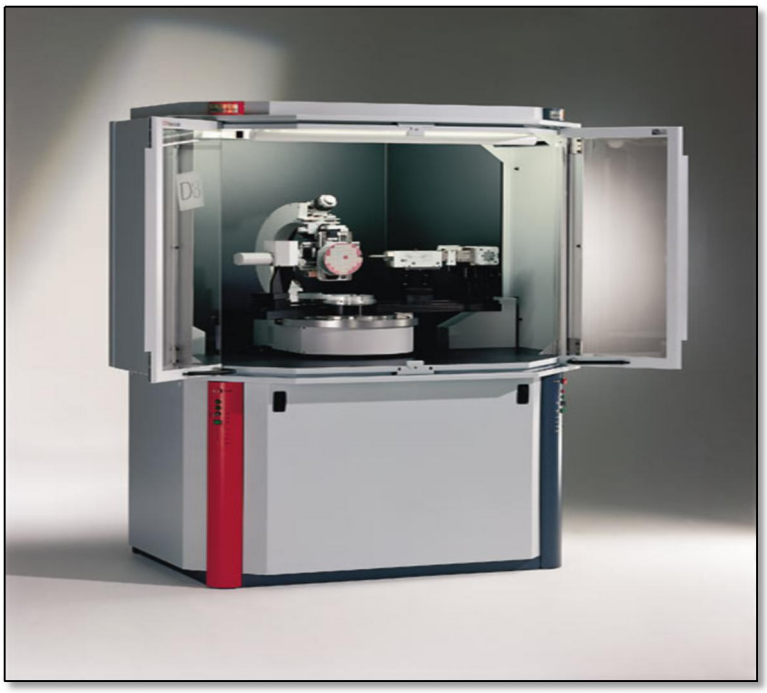


Fig no:6.1: XRD - X-ray Powder Diffraction

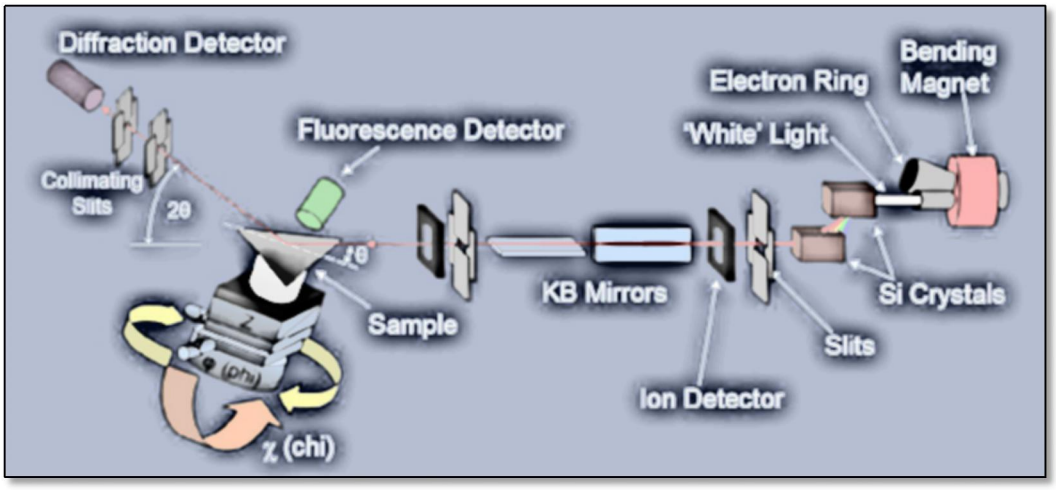


Fig no:6.2: XRD Mechanism

APPLICATIONS:

- ✧ Characterization of crystalline materials[88].
- ✧ Identification of fine-grained minerals such as clays and mixed layer clays that are difficult to determine optically
- ✧ Determination of unit cell dimensions.
- ✧ With specialized techniques, XRD can be used to:
- ✧ Determine crystal structures using Rietveld refinement
- ✧ Determine of modal amounts of minerals (quantitative analysis)
- ✧ Characterize thin films samples by:
  - ➢ determining lattice mismatch between film and substrate and to inferring stress and strain.
  - ➢ determining dislocation density and quality of the film by rocking curve measurements
  - ➢ measuring super lattices in multilayered epitaxial structures
  - ➢ determining the thickness, roughness and density of the film using glancing incidence X-ray reflectivity measurements
- ✧ Make textural measurements, such as the orientation of grains, in a polycrystalline sample.

Strengths and Limitations of X-ray Powder Diffraction:

Strengths:

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

Limitations:

- ➢ Homogeneous and single phase material is best for identification of unknown
- ➢ Must have access to a standard reference file of inorganic compounds
- ➢ Requires tenths of a gram of material which must be ground into a powder
- ➢ For mixed materials, detection limit is ~ 2% of sample

- For unit cell determinations, indexing of patterns for non-isometric crystal systems is complicated.

Sample Collection and Preparation:

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

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

4.3.TOXICOLOGICAL STUDIES:

Introduction:

The acute toxic class method is a stepwise procedure with the use of 3 animals of a single sex per step. Depending on the mortality and/or the moribund status of the animals, on average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance. Morbid animals or animals obviously in pain or showing signs of severe and enduring distress shall be humanely killed, and are considered in the interpretation of the test results in the same way as animals that died on test. The method allows for the determination of an LD_{50} value only when at least two doses result in mortality higher than 0% and lower than 100%.

4.3.1.ACUTE ORAL TOXICITY – OECD GUIDELINES - 423:

- Acute toxicity study was carried out as per OECD guideline (Organization for Economic Co - operation and Development, Guideline-423[91].
- The experimental protocol was approved by the institutional ethical committee (IAEC) under CPCSEA (IAEC approved Number: **IAEC/XLVIII/20/CLBMCP/2016**)
- These studies were conducted in C.L. Baid Metha College of Pharmacy, Dhuraipakkam, Chennai**.**

Introduction:

- The acute toxic class method was stepwise procedure with the use of 3 animals of a single sex per step.
- Depending on the mortality and/or the moribund status of the animals, on average 2-4 steps may be necessary to allow judgement on the acute toxicity of the test substance.
- This procedure is reproducible, uses very few animals and is able to rank substances in a similar manner to the other acute toxicity testing methods.
- The acute toxic class method is based on biometric evaluations with fixed doses, adequately separated to enable a substance to be ranked for classification purposes and hazard assessment.
- In principle, the method is not intended to allow the calculation of a precise LD50, but does allow for the determination of defined exposure ranges where lethality is expected since death of a proportion of the animals is still the major endpoint of this test.
- The method allows for the determination of an LD50 value only when at least two doses result in mortality higher than 0% and lower than 100%.
- The use of a selection of pre-defined doses, regardless of test substance, with classification explicitly tied to number of animals observed in different states improves the opportunity for laboratory to laboratory reporting consistency and repeatability.

Principle of the Test:

It is the principle of the test that based on a stepwise procedure with the use of a minimum number of animals per step, sufficient information is obtained on the acute toxicity of the test substance to enable its classification. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals of a single sex. Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.

– no further testing is needed

− dosing of three additional animals, with the same dose

− dosing of three additional animals at the next higher or the next lower dose level. The method will enable a judgment with respect to classifying the test substance to one of a series of toxicity classes.

Methodology:

Selection of Animal Species:

The preferred rodent species is the wistar albino rat, although other rodent species may be used. Healthy young adult animals are commonly used laboratory strains should be employed. Females should be nulliparous and non-pregnant. Each animal, at the commencement of its dosing, should be between 8 to 12 weeks old and the weight (200-220gm) should fall in an interval within ± 20 % of the mean weight of the animals.

Housing and Feeding Conditions

The temperature in the experimental animal room should be 22ºC ± 3ºC. Although the relative humidity should be at least 30% and preferably not exceed 70% other than during room cleaning the aim should be 50-60%. Lighting should be artificial, the sequence being 12 hours light, 12 hours dark. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water. Animals may be group-caged by dose, but the number of animals per cage must not interfere with clear observations of each animal.

Test Animals and Test Conditions:

Sexually mature Female Wistar albino rats (200-220gm) were obtained from TANUVAS, Madhavaram, Chennai. All the animals were kept under standard environmental condition (22 ± 3°C). The animals had free access to water and standard pellet diet (Sai meera foods, Bangalore).

Preparation of animals:

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

Preparation for Acute Toxicity Studies

Rats were deprived of food overnight (but not water 16-18 h) prior to administration of the, ***Maavilingapattai Chooranam***.

The principles of laboratory animal care were followed and the Institutional Animal Ethical Committee approved the use of the animals and the study design

Test Substance : ***MAAVILINGAPATTAI CHOORANAM***
Animal Source : TANUVAS,Madhavaram, Chennai.
Animals : Wister Albino Rats (Female-3+3)
Age **:** 8-12 weeks
Body Weight on Day 0 **:** 200-220gm.
Acclimatization **:** Seven days prior to dosing.
Veterinary examination **:** Prior and at the end of the acclimatization period.
Identification of animals **:** By cage number, animal number and individual marking by using Picric acid.
Number of animals : 3 Female/group,
Route of administration : Oral
Diet **:** Pellet feed supplied by Sai meera foods Pvt Ltd, Bangalore.
Water **:** Aqua guard portable water in polypropylene bottles.
Housing & Environment **:** The animals were housed in Polypropylene cages provided with bedding of husk.
Housing temperature **:** between 22ºC $\pm$ 3ºC.
Relative humidity **:** between 30% and 70%,
Air changes **:** 10 to 15 per hour and
Dark and light cycle **:** 12**:**12 hours.
Duration of the study **:** 14 Days
Administration of Doses:

Maavilingapattai Chooranam was suspended in water and administered to the groups of wister albino rats in a single oral dose by gavage using a feeding needle. The control group received an equal volume of the vehicle. Animals were fasted 12 hours prior to dosing. Following the period of fasting, the animals were weighed and then the test substance was administered. Three Female animals are used for each group. The dose level of 5,50,300 and 2000 mg/kg body weight was administered stepwise. After the substance has been administered, food was withheld for a further 3-4 hours. The principle of laboratory animal care was followed. Observations were made and recorded systematically and continuously as per the guideline after substance administration.

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

Limit test:

The limit test is primarily used in situations where the experimenter has information indicating that the test material is likely to be nontoxic, i.e., having toxicity only above regulatory limit doses. A limit test at one dose level of 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 to be carried out.

Observations:

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

It should be determined by the toxic reactions, time of onset and length of recovery period, and may thus be extended when considered necessary. The times at which signs of toxicity appear and disappear are important, especially if there is a

tendency for toxic signs to be delayed. All observations are systematically recorded with individual records being maintained for each animal.

a. Mortality:

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

b. Body weight

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

C. Cage-side observation

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

d. Gross necropsy:

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

Histopathology:

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

Data and reporting:

All data were summarized in tabular form, (Table) showing for each test group the number of animals used, the number of animals displaying signs of toxicity, the number of animals found dead during the test, description of toxic symptoms, weight

changes, food and water intake.

Test substance and Vehicle:

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

Justification for choice of vehicle:

The vehicle selected as per the standard guideline was pharmacologically inert and easy to employ for new drug development and evaluation technique[92].

4.3.2.REPEATED DOSE 28-DAY ORAL TOXICITY (407) STUDY OF *MAAVILINGAPATTAI CHOORANAM:*[93]

Test Substance	: ***Maavilingapattai Chooranam***
Animal Source	: TANUVAS,Madhavaram, Chennai.
Animals	: Wister Albino Rats (Male -40, and Female-40)
Age	**:** 6-8 weeks
Body Weight	**:** 150-220gm.
Acclimatization	**:** Seven days prior to dose.
Veterinary examination	**:** Prior and at the end of the acclimatization period.
Identification of animals	**:** By cage number, animal number and individual marking by using Picric acid
Diet	**:** Pellet feed supplied by Sai meera foods Pvt Ltd, Bangalore
Water	**:** Aqua guard portable water in polypropylene bottles.
Housing & Environment	**:** The animals were housed in Polypropylene cages provided with bedding of husk.
Housing temperature	**:** between 22ºC $\pm$ 3ºC.
Relative humidity	**:** between 30% and 70%,
Air changes	**:** 10 to 15 per hour
Dark and light cycle	**:** 12**:**12 hours.
Duration of the study	**: 28 Days.**

Justification for Dose Selection:

The results of acute toxicity studies in Wistar albino rats indicated that ***Maavilingapattai Chooranam (MPC)***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 as per OECD guideline three dose levels were selected for the study. They are low dose (X), mid dose (2X), high dose (4X). X is calculated by multiplying the therapeutic dose (195 mg) and the body surface area of the rat (0.018). i.e X dose is (100mg), 2X dose is 200mg/animal, 4X dose is 400mg/animal.

Preparation and Administration of Dose:

Maavilingapattai Chooranam suspended, It was administered to animals at the dose levels of X, 2X. The test substance suspensions were freshly prepared every two days once for 28 days. The control animals were administered vehicle only. The drug was administered orally by using oral gavage once daily for 28 consecutive days.

Methodology:

Randomization, Numbering and Grouping of Animals:

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

Table 5

Groups	**No of Rats**
Group I Vehicle control (Water)	20 (10 male,10 female)
Group II MPC- low dose X (100mg)	20 (10 male,10 female)
Group III MPC- Mid dose 2X(200mg)	20 (10 male,10 female)
Group IV MPC- High dose 4X (400mg)	20 (10 male,10female)

MPC- MaavilingaPattai Chooranam

Observations:

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

Body Weight:

Weight of each rat was recorded on day 1,15,28, at biweekly intervals throughout the course of study. From the data, group mean body weights and percent body weight gain were calculated.

Food and water Consumption:

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

Clinical signs:

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

Mortality:

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

Functional Observations:

At the end of the 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 animals fasted over-night. Blood samples were collected from orbital sinus using sodium heparin (200IU/ml) for Bio chemistry and potassium EDTA (1.5 mg/ml) for Hematology as anticoagulant. Blood samples were centrifuged at 3000 r.p.m. for 10 minutes.

Haematological Investigations:

- Haematological parameters were determined using Haematology analyzer.
- 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:

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

Necropsy:

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

Histopathology:

Histopathological investigation of the vital organs was done.Control and highest dose group animals will be initially subjected to histopathological investigations. If any abnormality found in the highest dose group than the low, then the mid dose group will also be examined. Organs will be collected from all animals and preserved in 10% buffered neutral formalin for 24 h and washed in running water for 24 h. The organ sliced 5 or 6µm sections and were dehydrated in an auto technicon and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the “L” moulds. It was followed by microtome and the slides were stained with Haematoxylin-eosin red.

Statistical analysis:

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

4.4.PHARMACOLOGICAL STUDIES:

4.4.1.HEPATO PROTECTIVE ACTIVITY OF "*MAAVILINGA PATTAI CHOORANAM" (MPC)* IN CCL_4 INDUCED HEPATOTOXICITY RATS MODEL:

Experimental design:

Animals were divided into 5 groups of 6 rats each.

Group I animals served as control and received liquid paraffin (LP) subcutaneously at the dose of 3 ml/kg body weight of each animal.

Group II animals received CCl_4+ LP (for 14 days) at the dose 1 ml CCl_4/kg body weight, in a suspension of double the volume of LP (which served as vehicle) subcutaneously at lower abdomen on every 14 days of the treatment.

Group III and IV animals received subcutaneous administration of CCl_4+ LP. They also received test drug *Maavilingapattai Chooranam* orally at the dose of 100, 200 mg/kg body weight respectively as a suspension of water.

Group V 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 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 CCL_4.

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[94].

4.4.2.HEPATOPROTECTIVE ACTIVITY OF *"MAAVILINGA PATTAI CHOORANAM" (MPC)* AGAINST PARACETAMOL INDUCED HEPATOTOXICITY IN RATS MODEL:

Experimental design:

The animals were randomly divided into five groups each consisting of six rats (n=6).

Group I animals were treated with vehicle for 8 days and served as normal control.

Group II animals were first treated with vehicle for 7 days and then, hepatotoxicity was tried to induce on 8th day by oral administration of Paracetamol 3 g/kg b. wt. in a single dose.

Group III, IV and V animals were first treated with MPC 100, MPC 200 and silymarin 100 mg/kg/day

respectively for 7 days and then, hepatotoxicity was tried to induce on 8th day by oral administration of Paracetamol 3 g/kg b. wt. in a single dose. 24 h after the last dosing by Paracetamol, the blood was obtained through the retro-orbital plexus under light anesthesia and the rats were sacrificed. Serum for the analysis of various biochemical parameters was separated by centrifugation at 3000 rpm at 4 C for 20 min. A small portion of tissue was fixed in formalin for histopathological examination.

Biochemical parameters studied:

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

Statistical analysis:

The data obtained from the study were subjected to statistical analysis by one way ANOVA followed by Dunnett t-test, and results were expressed in terms of Mean±SEM values. Statistical analysis was performed using INSTAT- V3 Software programme[95].

4.4.3.HEPATOPROTECTIVE ACTIVITY OF *"MAAVILINGA PATTAI CHOORANAM" (MPC)* AGAINST ETHANOL INDUCED HEPATOTOXICITY IN RATS MODEL:

Experimental design:

The animals were divided into five groups, six animals in each group.

Animals in Group 1 were treated with Vehicle only twice a day P.O for 25 days served as Normal Control Group.

Group 2 animals were treated with 40% ethanol 3.76 gm/kg twice a day P.O for 25 days which served as Positive Control Group.

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

Biochemical parameters studied:

The activities of serum glutamate pyruvate transaminase, and serum glutamate oxaloacetate transaminase 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 Ethanol.

Statistical analysis:

The data obtained from the study were subjected to statistical analysis by one way ANOVA followed by Dunnett t-test, and results were expressed in terms of Mean±SEM values. Statistical analysis was performed using INSTAT- V3 Software programme[96].

5. RESULTS AND DISCUSSION

The well known Traditional medicine *Maavilingapattai Chooranam* had been subjected to various studies and standardization to establish the works of *Siddhars* to be true. The study includes literary collections, organoleptic character, physicochemical and phytochemical analysis, instrumental analysis, toxicological study and pharmacological study. The drug ***Maavilingapattai Chooranam*** has been selected for Hepatoprotective activity in reference with the text ***"Sirorathina Vaidhiya Booshanam".***

Discussion on review of literature:

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

Standardization of the test drug:

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

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

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

- ***Iruveli*** is indicated for curing Jaundice (*Kaamaalai*).
- ***Vilamichu*** is used in the treatment of biliousness.
- ***Kozhunji*** is indicated for the treatment of Liver diseases. It also known to improving the fuctions of liver.
- ***Vilvam*** is indicated for curing Jaundice (*Kaamaalai*).
- ***Thulasi*** has an action of tonic to Liver.
- ***Musumusukkai*** is used in the treatment of jaundice (*Kaamaalai*).
- ***Vila*** is indicated for curing Jaundice (*Kaamaalai*).
- ***Ashuwagandhi*** has an action of tonic to Liver.
- ***Mavilingapattai*** is indicated for the treatment of Liver diseases.

Discussion on pharmacological aspect:

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

Discussion on Pharmaceutical review:

- This review explained the preparation of *Chooranam* in detail including the purification of raw drugs, methods of manufacturing of *Chooranam* and the *Siddha* parameters for the standardization of analyzing *Chooranam.*
- 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. *Chooranam* are fine, dry powders of drugs. The term *Chooranam* may be applied to the powder of single drug or a mixture of two or more drugs.
- On purification (pittaviyal), the weight of the *Chooranam* is different from the exact value but not from the mean value when calculated.
- The *Chooranam* were also subjected to *Siddha* parameters of the testing like,
    - *Chooranam* 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 characters:

Table: 6. Organoleptic characters of *Maavilingapattai Chooranam*:

Colour	Brown
Odour	Pleasant
Taste	Bitter
Texture	Fine powder
Particle size	Completely pass through sieve no 88

Physicochemical Analysis:

Table: 7. Physicochemical Analysis:

S.NO	Parameter	Result
1	pH	6.2
2	Loss on drying(at 105^0 C)	5.66
3	Total ash value (%)	7.21
3	Acid Insoluble ash (%)	1.37
4	Water soluble ash (%)	3.03
5	Solubility	
	1.Distilled water	Soluble
	2.Benzene	Soluble
	3.Chloroform	Soluble

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

Interpretation:

1. pH:

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

The pH of the drug *Maavilingapattai Chooranam* is 6.2 which is weak acidic in nature. Acidic drug is essential for its bioavailability and effectiveness. Acidic drugs are better absorbed in stomach.[97]

2. 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 *Maavilingapattai Chooranam* is 5.66.[98]

3. Total Ash:

Ash constitutes are the inorganic residues obtained after complete combustion of a drug. Thus Ash value is a validity parameter to describe and to assess the degree of purity of a given drug. Total ash value of plant material indicated the amount of minerals and earthy materials present in the drug. The total ash value of *Maavilingapattai Chooranam* is 7.21 which determine the absence of inorganic content.

5. Acid insoluble ash:

The acid insoluble ash value of the drug denotes the amount of siliceous matter present in the plant. The quality of the drug is better if the acid insoluble value is low. Acid insoluable ash value of *Maavilingapattai Chooranam* is 1.37.

6. Water soluble ash:

Water-soluble ash is the part of the total ash content, which is soluble in water. Decreased water soluble ash value indicates easy facilitation of diffusion and osmosis mechanism. Water soluble ash value of *Maavilingapattai Chooranam* is 3.03.

7. Solubility:

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

The most frequent causes of low oral bioavailability are attributed to poor solubility and low permeability .[99]

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

Phytochemical analysis:

The phytochemical analysis of *Maavilingapattai Chooranam* result were given below:

Table: 8. Phytochemicals screening test:

SNO	Phytochemicals	Test	Result
1.	**Carbohydrates**	Molisch's test	Present
		Benedict's test	Present
2.	**Glycosides**	Modified Borntrager's test	Present
3.	**Saponins**	Froth test	Present
4.	**Phenols**	Ferric chloride test	Present
5.	**Flavanoids**	Alkaline reagent test	Present
		Lead acetate test	Present
6.	**Diterpenes**	Copper acetate test	Present
7.	**Gum & Mucilage**	Extract + Alcohol	Present

The phytochemical analysis of the drug (*MPC*) result reveals Carbohydrats, Glycosides, Saponins, Phenols, Flavanoids, Diterpenes and Gum & Mucilage. The interpretation of the result were given below.

Interpretation:

❖ **Carbohydrate:**

- Carbohydrate contains plenty of antioxidants, vitamins and fiber that are necessary for our health.
- Carbohydrate diet can improve the liver function in people with fatty liver diseases.
- Carbohydrates plays an important role in storage of glucose. It regulates the blood glucose level and provides energy to the body.
- Carbohydrates helps in fat metabolism. It plays an important role in homeostasis.
- Carbohydrates help us to fight inflammation and cancer, improve our digestive system, heart and bone health.[100]

❖ **Glycosides:**

- In the liver, glycosides helps in the process of detoxification.

- Glycosides have antibacterial activity, so they protect our body from bacteria and infectious diseases.
- Glycosides increased the intestinal motility. So it produces laxation.[101]

❖ **Saponins:**

- Saponins include, supporting kuffer cells in the liver and encouraging normal detoxification.
- In the digestive tract, saponins produce an emulsification of fat soluble molecules. Saponins bind with bile acids and helps to eliminate them from the body, preventing cholesterol from being reabsorbed.
- Saponins can boost the immune system, have an antioxidant effect and may even support bone strength.[102]

❖ **Phenols:**

- Phenols possess rich anti-oxidant property and protect the body from oxidative stress.
- Phenols inhibit the LDL cholesterol levels.
- Phenols reduces cell death and it regulate glucose metabolism.
- Phenols increase the vasodilation of blood vessels to promote circulation. It is a Effective anti-hyperglycaemic agent. [103]

❖ **Flavanoides:**

- It is the most important group of polyphenolic compounds in plants.
- Flavonoids can exert their anti-oxidant activity by scavenging the free radicals, by chelating metal ions or by inhibiting enzymatic systems responsible for free radical generation.
- Flavanoids are immunomodulator. It also possesses anti-microbial activity which is confirmed by the various anti-microbial assays.[104]

❖ **Diterpenes:**

- Diterpene has an anti-oxidant effect.

- Diterpenes helps to cure hypertension. It also have tumour inhibitory properties as well as a stimulating effect on the immune system.
- It is used widely as a stomachic.[105]

❖ **Gum & Mucilage:**

- It is used as a bulk laxatives.
- Gum and mucilage are used for their demulcent properties for cough suppression.[106]

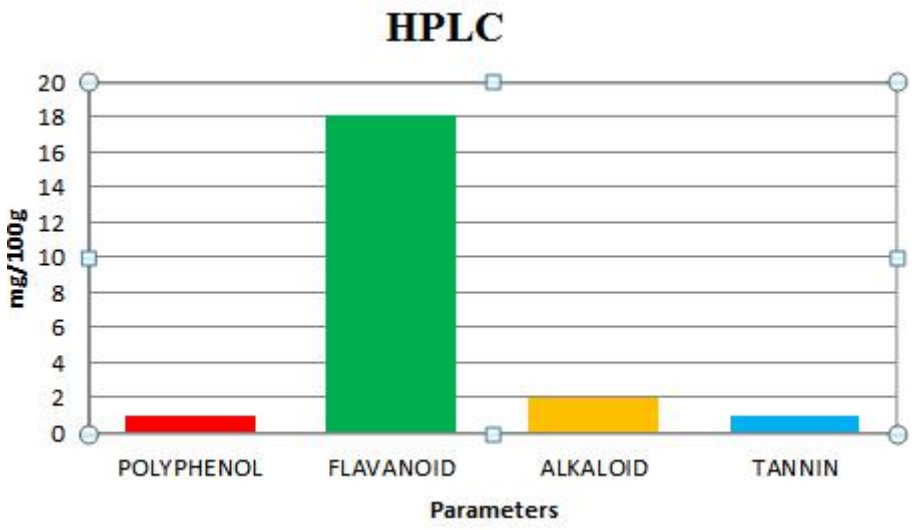
High Performance Liquid Chromatography (HPLC):

HPLC analysis were done. HPLC analysis performed with *Maavilingapattai Chooranam* revealed the pressence of following compounds:

Table:9.

S. NO	PARAMETERS	METHOD	UNITS	RESULTS
1.	Total Polyphenol as gallic acid Equivalent	Indian Pharmacopoeia 2014	mg/100g	0.09
2.	Total Flavonoids as Quercetin Equivalent	TNTH/STP/FOOD/110	mg/100g	18.15
3.	Total Alkaloids	TNTH/STP/FOOD /426	mg/100g	1.93
4.	Total Tannin as Tannic Acid Equivalent	AOAC 20th Edn.2012, 955.35	mg/100g	0.98

HPLC analysis reveals the pressence of polyphenols, Flavanoids, Alkaloids and Tannins.



Graph 1

Interpretation:

- Polyphenols are the member of very large family of plant derived compounds which had the anti lipidogenic effect. This is mainly due to reduced fatty acid and triglycerol synthesis, increased in fatty acid oxidation and reduction of oxidative stress and inflammation.
- Beneficial effects of polyphenols in the prevention and treatment of liver steatosis have been reported.
- Polyphenols are biomolecules which produce hepatoprotective effects which reduce the liver fat accumulation, mainly by reducing lipogenesis and by increasing fatty acid oxidation and decrease oxidative stress and inflammation are the main factors responsible for liver damage[107].
- Flavanoids a group of plant compounds which have the beneficial effects against Non Alcoholic Fatty Liver Disease.
- Flavanoids prevent Hepatosteatosis by increasing fatty acid oxidation in liver. They can also reduce caloric intake and decrease body weight and fat deposition in viseral tissues.
- Flavanoids are the unique antioxidant. It also correct dislipidemia and blood pressure[108].

- Tannins and alkaloids contains anti oxidant effect which produce many essential effects in protecting the body.

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

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

Interpretation:

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

Potassium:

- Potassium levels may be an indicator of impending liver problems.
- Potassium is absorbed through the small intestine. Severe lack of potassium can disturb the liver function and if potassium level falls below 30% to 40% causes Non Alcoholic Fatty Liver Diseases.
- Potassium is important for maintaining the integrity of cell membranes and functions as a vital electrolyte.[109]

Magnesium:

- Magnesium is essential for liver to prevent liver diseases. It enhances immune system.

- Depletion of magnesium levels leads to Cirrhosis and Fatty liver syndrome.
- It also helps to regulate blood glucose levels and aid in the production of energy and protein[110].

Iron:

- Iron is an essential micronutrient that is an critical component of oxygen transport proteins (Haemoglobin & Myoglobin).
- Chronic iron deficiency results in decreased haemoglobin production and anaemia which may result chronic liver diseases.
- Iron is essential for oxygen transport, energy production, other cellular growth and proliferation.
- Iron is an essential element for blood production and also needed for energy metabolism.[111]

Zinc:

- The liver plays a central role in zinc homeostasis. Zinc is a trace mineral that is essential to the normal functioning of the immune system.
- Zinc is essential for many metabolic and enzymatic functions. In Liver it act as a powerful antioxidant.
- Deficiency of zinc leads to malabsorption syndrome and Cirrhosis of liver[112].

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 reveals 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 *Maavilingapattai Chooranam:*

Table: 12. Bacterial and fungal dilutions:

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

Interpretation:

- The availability of bacterial load in the *Maavilingapattai Chooranam* has been performed by Agar plate techniques..
- As *Maavilingapattai Chooranam* 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 various disease instead of disease being cured.
- The contamination of *Maavilingapattai Chooranam* has been examined by bacterial and fungal load.
- Total bacterial load in 10^{-4} dilution is 5 and 10^{-6} dilution 4.
- 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 of *Maavilingapattai Chooranam* . They are within the normal limits.[113]

INSTRUMENTAL ANALYSIS:

FTIR Spectrum Analysis:

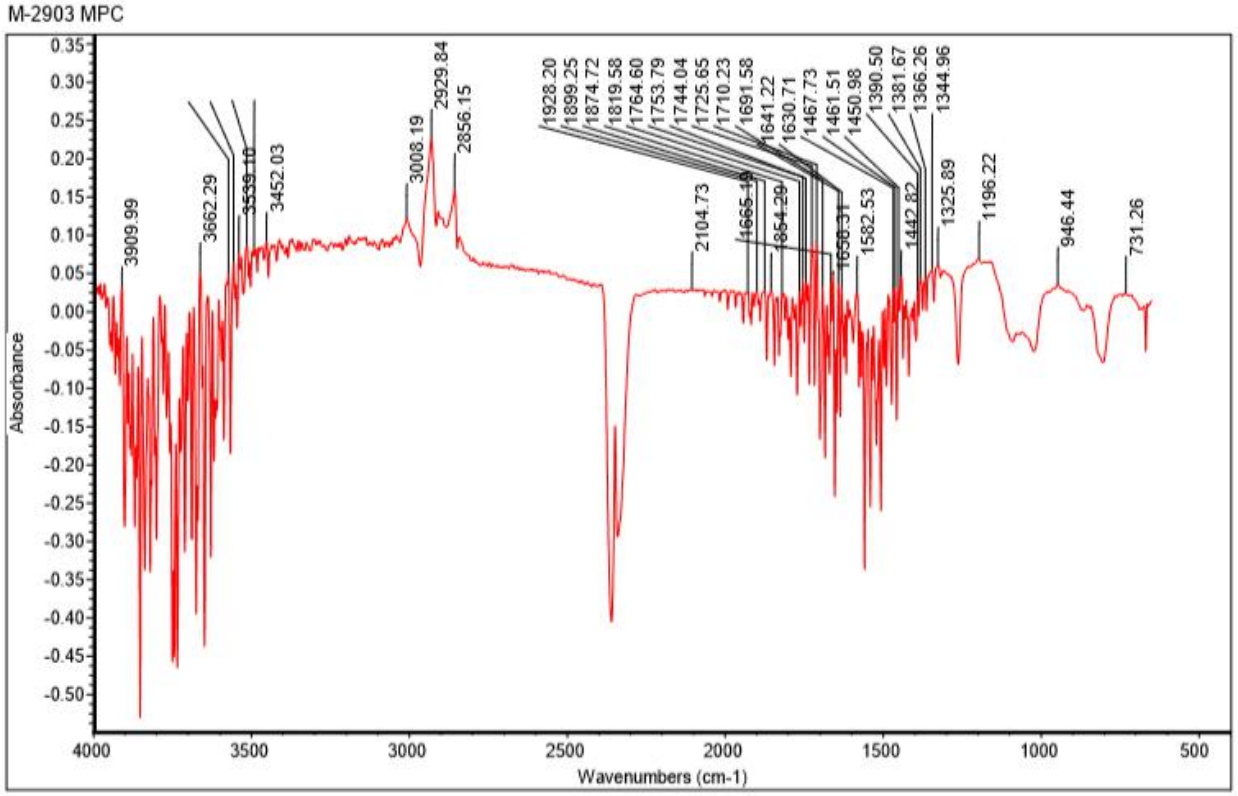


Fig 7: FTIR Spectrum analysis

Table 13: FT-IR INTERPRETATION:

Absorption peak cm^{-1}	Stretch	Functional group
3909	O-H stretch, Free hydroxyl	Alcohols Phenols
3452	N-H stretch	1,2 Amines Amides
3008	C-H stretch =C-H Stretch	Aromatics alkines

Absorption peak cm^{-1}	Stretch	Functional group
2104	-C=N Stretch	Alkynes
1665	C=O stretch	α,β unsaturated aldehydes, ketones
1325	C-N stretch	Aromatic amines
1196	C-H wag(-CH_2 x)	Alkylhalides
946	O-H bend	Carboxylic acids
1764	C=O stretch	Esters, saturated aliphatic
1710	C=O stretch	α,β unsaturated aldehydes, ketones
1641	-C=C- stretch	Alkenes
1630	N-H bend	1º amines
1390	C-H bend	Alkanes
1344	N-O symmetric stretch	Nitro compounds
1582	C-C stretching (ring)	Aromatics

The above table shows the presence of alcohol, phenols, alkane, acid, alkynes, amide, aromatic, alkylhalide, ether, alkene, nitro compounds, α,β unsaturated aldehydes, ketones which represents the peak value. The interpretation of the result were given below.

Interpretation:

FTIR instrumental analysis was done. The test drug (*MPC*) was identified to have 35 peaks. They are the functional groups present in the trial drug *Maavilingapattai Chooranam.*

- **Phenols** possess high anti-oxidant property which enhances the drug effect against the liver disease.
- **Phenolic** compounds are also present in a number of biological systems and natural products such as neurotransmitters, flavouring agents.[114]
- **Aromatic amines** has anti-oxidant property. In liver aromatic amines invovles metabolic activation and also involved in detoxification. [115]
- **OH group** has higher potential towards inhibitory activity against microorganisms.
- **Amines** enhance the drug effect against the hepatic disease. It also act on the neurotransmitters, it is involved in the protein synthesis.[116]
- **Alkanes** have little biological activity, it protect against Microorganism.
- **α, β unsaturated aldehydes** involved in free radical scavenging activity and also used in the treatment of cancer.
- **Phenols and flavanoids** possess diverse biological activities, for example, hepatoprotective activity, antioxidant and antidepressant activities[117].

SEM (SCANNING ELECTRON MICROSCOPE):

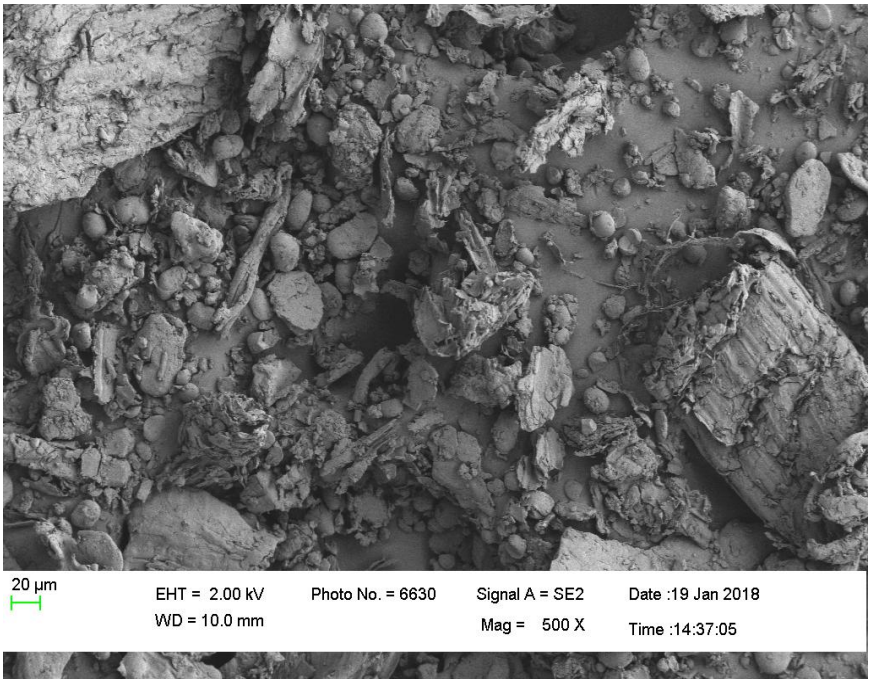


Fig: 8. SEM (SCANNING ELECTRON MICROSCOPE)

The SEM image is done by 500X magnification via 20µm aperture shows maximum depth focused.

Interpretation:

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

- Increased therapeutic effect of the drug
- Decreased toxicity/ side effects
- Protection from physical and chemical degradation
- Improves stability and Increased bioavailability[118]

The test drug *Maavilingapattai Chooranam* contains micro particles.

- Micro particles present in the drug results in a better bioavailability and facilitates absorption.
- The particles of micro size show that the drug may easily enter the cells at the molecular level to treat the disease rapidly and increase the therapeutic effect.

XRD (X-Ray Diffraction studies):

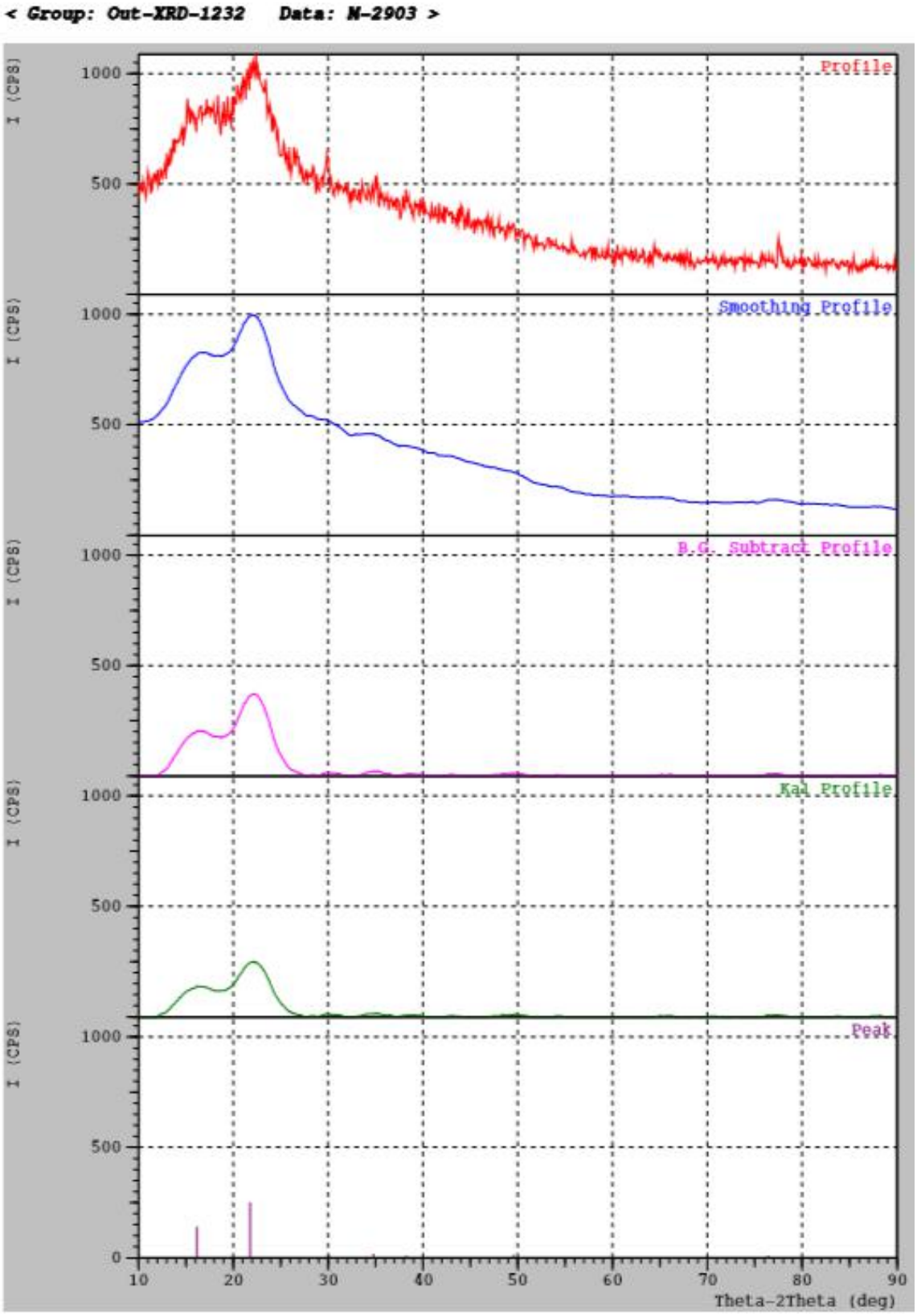


Fig 9: XRD

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 nano particles may enhance bio absorption of the drug.

The major diffraction peaks are identified after XRD analysis *MPC* concluded the range 16-34nm in association with organic molecules propably plays an important role in making it biocompatible and nontoxic at therapeutic doses. Other elements present in *MPC* act as additional supplement and possibly helps in increase the efficacy of the formulation.[119]

Table 14: ICP-MS results of *Maavilingapattai Choornam:*

S. no	Elements	units	Detected levels
1.	Arsenic	mg/kg	BQL (LOQ 0.1)
2.	Mercury	mg/kg	BQL (LOQ 0.1)
3.	Lead	mg/kg	BQL (LOQ 0.1)
4.	Cadmium	mg/kg	BQL (LOQ 0.1)

Discussion:

From the above results the heavy metals Cadmium, Lead, Mercury, Arsenic are observed with in permissible limits. Hence the safety of the drug is ensured.

TOXICITY STUDY RESULTS:

Acute oral toxicity study of *Maavilingapattai Chooranam* – OECD 423

Dose finding experiment and its behavioural Signs of acute oral Toxicity:

Table: 15. Observation done:

Group	**Day**
Body weight	Normal
Assessments of posture	Normal
Signs of Convulsion Limb paralysis	Absence (-)
Body tone	Normal
Lacrimation	Normal
Salivation	Normal
Change in skin color	No significant colour change
Piloerection	Normal
Defecation	Normal
Sensitivity response	Normal
Locomotion	Normal
Muscle gripness	Normal
Rearing	Mild
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. Piloerection 4. Grooming 5.Gripping 6. Touch Response 7. Decreased Motor Activity 8.Tremors 9.Convulsions 10.Muscle Spasm 11.Catatonia 12.Musclerelaxant 13.Hypnosis 14.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 *Maavilingapattai Chooranam:*

DOSE	DAYS		
	1	7	14
CONTROL	220.6±31.474	221.4 ± 34.324	224.2 ± 27.623
HIGH DOSE	220.5± 27.75	221.7 ± 3.64	223.4 ± 3.22
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 *Maavilingapattai Chooranam:*

DOSE	DAYS		
	1	7	14
CONTROL	58.5 ± 6.74	60±9.13	60.4±4.13
HIGH DOSE	66.4±2.12	67.6.2±1.10	67.9±6.12
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 *Maavilingapattai Chooranam:*

DOSE	DAYS		
	1	7	14
CONTROL	40.56±9.36	42.6±4.42	41.6±7.46
HIGH DOSE	43.4±1.14	44.3±1.12	46.2±2.20
P value (p)*	NS	NS	NS

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

Interpretation of Acute toxicity studies:

In the acute toxicity study, the rats were treated with different concentration of *Maavilingapattai Chooranam* from the range of 5mg/kg to 2000mg/kg.

- This dose level did not produce signs of toxicity, behavioral changes, and mortality in the test groups as compared to the controls when observed during 14 days of the acute toxicity experimental period.
- However the behavior changes, Body weight, Water intake, food intake does not produce much significant, Thus the results are non-significant.

- These results showed that a single oral dose of the extract showed no mortality of these rats even under higher dosage levels indicating the high margin of safety of this extract.
- In acute toxicity test the *Maavilingapattai Chooranam* was found to be nontoxic at the dose level of 2000mg/ kg body weight.

Repeated dose 28-day oral toxicity of *Maavilingapattai Chooranam* OECD – 407

Repeated dose 28 day oral toxicity study was done and the results were tabulated below.

Table: 20. Body weight of wistar albino rats group exposed to *Maavilingapattai Chooranam:*

DOSE	Days		
	1	15	28
CONTROL	302.1±1.14	302.2 ± 1.20	302.3± 0.22
LOW DOSE	297.1 ± 10.3	297.4 ± 1.11	298.4± 1.4
MID DOSE	298.6 ± 2.20	299.5 ± 2.1	299.4 ± 2.7
High DOSE	299.3± 1.20	299.5 ± 2.24	299.7 ± 2.20
P value (p)*	NS	NS	NS

*N.S- Not Significant,**(p < 0.01), *(p <0.05), n = 20 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 *Maavilingapattai Chooranam:*

DOSE	DAYS		
	1	15	28
CONTROL	84.2 ± 1.14	84±1.26	84.6±1.30
LOW DOSE	90.2±1.18	90.2±2.40	91.4±1.24
MID DOSE	90.8±2.20	92.1±1.60	93.1±1.20
HIGH DOSE	93.3±1.12	93.4±1.22	95.3±1.26
P value (p)*	NS	NS	NS

*N.S- Not Significant,**(p < 0.01), *(p <0.05), n = 20 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 *Maavilingapattai Chooranam:*

DOSE	DAYS		
	1	15	28
CONTROL	378.6± 1.20	378±1.32	378.8±2.30
LOW DOSE	382.1±3.24	381.2±3.20	381.4±3.40
MID DOSE	380.1±1.12	380.1±1.20	380.4±1.40
HIGH DOSE	382.1±1.10	382.2±2.20	383.6±1.20
P value (p)*	NS	NS	NS

*N.S- Not Significant,**(p < 0.01), *(p <0.05), n = 20 values are mean ± S.D (One-way ANOVA followed by Dunnett's test)*

Interpretation of weight, water and food intake:

There is no significant change in weight, water intake and food intake by the animals during the period of study.

Table: 23. Haematological parameters of Wistar albino rats group exposed to *Maavilingapattai Chooranam:*

Category	Control	Low dose	Mid dose	High dose	P value (p)*
Haemoglobin (g/dl)	13.4±0.06	15.2±0.23	15.6±0.5	16.6±0.60	N.S
Total WBC ($\times 10^3$ µL)	11.5±0.04	12.4±0.03	12.2±1.1	12.6±1.46	N.S
Neutrophils (%)	25.2±0.02	27.3±0.08	27.5±0.6	28.6±2.12	N.S
Lymphocyte (%)	70.1±1.21	69.2±1.10	69.7±0.8	70.3±1.28	N.S
Monocyte (%)	0.01±0.02	0.02±0.04	0.02±0.05	0.02±0.07	N.S
Eosinohil(%)	.04±0.23	.03±0.25	.03±0.35	.03±0.42	N.S

Category	Control	Low dose	Mid dose	High dose	P value (p)*
Platelets cells10^3/µl	233.13±2.16	244.14±4.30	245.3±3.5	246.4±3.14	N.S
Total RBC 10^6/ µl	6.64±0.01	6.68±0.40	6.68±1	6.69±1.04	N.S
PCV%	44.1±0.2	46.10±1.2	46.2±1.7	47.1±2.14	N.S
MCHC g/dL	35.8±1.10	38.8±0.12	38.5±0.6	38.3±1.10	N.S
MCV fL(µm^3)	56.1±1.01	57.1±3.10	57.2±1.6	57.9±1.22	N.S

*N.S- Not Significant,**(p < 0.01), *(p <0.05), n = 20 values are mean ± S.D (One-way ANOVA followed by Dunnett's test)*

Interpretation:

- The haematological parameters of animals were done. The results of repeated dose 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 *Maavilingapattai Chooranam* was good and safe drug for oral administration.

Table: 24. Biochemical Parameters of Wistar albino rats group exposed to *Maavilingapattai Chooranam:*

BIOCHEMICAL PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
GLUCOSE (R) (mg/dl)	189.02±01.15	187.10± 01.12	187±0.9	186.10±1.21
T.CHOLESTEROL (mg/dl)	74.98±1.04	73.08±1.28	73.7±1.2	72.18±1.84

BIOCHEMICAL PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
TRIGLYCERIDES (mg/dl)	48.21±2.32	46.22±1.18	44.14±1.7	43.12±2.22
LDL	78.6±2.13	78.7±2.05	77.04±0.22	77.40±01.32
VLDL	14.2±1.52	14.20±2.41	14.1±1.6	14.04±2.15
HDL	28.12±4.32	28.32±2.50	28.7±2.1	29.51±1.23
Ratio 1(T.CHO/HDL)	3.73±1.16	3.72±1.80	3.71±1.9	3.74±2.33
Ratio 2(LDL/HDL)	1.92±1.22	1.92±1.20	1.93±4.2	1.94±06.02
Albumin(g/dL)	4.84±0.04	4.24±0.42	4.55±1.2	4.74±0.60

*N.S- Not Significant,**(p < 0.01), *(p <0.05), n = 20 values are mean ± 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 *Maavilingapattai Chooranam* shows safe and non toxic effects on general body metabolism.

Table: 25. Liver Function Test of Wistar albino rats group exposed to *Maavilingapattai Chooranam:*

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
T BILIRUBIN (mg/dl).	0.20±0.04	0.17±0.08	0.17±0.03	0.14±0.03
SGOT/AST(U/L)	21.13±1.42	19.11±0.2	18.1±0.06	17.1±1.1
SGPT/ALT(U/L)	34.12±1.24	31.12±1.82	31.1±0.9	30.12±1.52
ALP(U/L)	162.02±2.18	144.24±1.18	140.15±1.4	132.24±2.24
T.PROTEIN(g/dL)	6.94±0.10	6.32±0.54	6.48±0.30	6.78±0.12

*N.S- Not Significant,**(p < 0.01), *(p <0.05), n = 20 values are mean ± S.D (One-way ANOVA followed by Dunnett's test)*

Interpretation

The above Liver function test showed that all the enzymes are normal in range. Thus the liver function test of *Maavilingapattai Chooranam* shows normal in this 28 day repeated oral toxicity study.

Table: 26. Renal function test of Wistar albino rats group exposed to *Maavilingapattai Chooranam:*

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
UREA (mg/dl)	14.50±0.29	14.50±0.29	14.45±1.1	14.40±1.22
CREATININE (mg/dl)	0.6±0.08	0.5±0.09	0.5±0.07	0.5±0.04
BUN(mg/dL)	23.06±0.82	21.13±2.20	22.31±0.03	22.10±1.14
URIC ACID(mg/dl)	4.02±0.04	4.02±0.21	4.01±0.9	4±0.10

*N.S- Not Significant,**(p < 0.01), *(p <0.05), n = 20 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 and not produce any nephro toxicity, thus it suggests that the trial drug *Maavilingapattai Chooranam* was safe for long term administration.

DISCUSSION:

Observations:

Overall observations were similar in both sex rats.

Clinical signs of toxicity

No clinical signs of toxicity were observed.

Mortality

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

Body weight

No significant alterations were observed in body weight.

Food and water consumption

No significant alterations were observed.

Physiological activities

No changes in the general behaviour.

Blood analysis

a. **Hematology**

No treatment related effects were observed.

b. **Biological parameters**

No treatment related effects were observed.

c. **Histological examination**

Histological examination of organs did not show any pathological changes.

Discussion

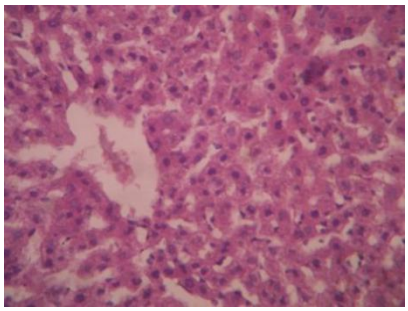
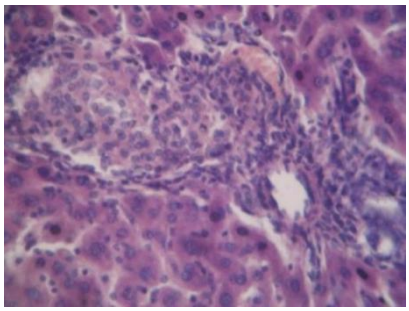
- The acute and repeated 28 days oral toxicity studies of *MPC* did not produce any toxicity signs in wistar albino rats. Daily administration of *MPC* at different doses 100mg/kg, 200mg/kg for 28 days was tolerated by the rats without any mortality and morbidity, indicates the drug tolerance.
- Hence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

HISTOPATHOLOGY STUDIES:

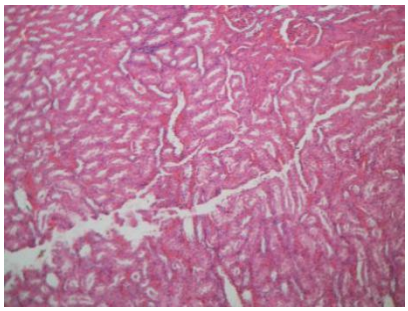
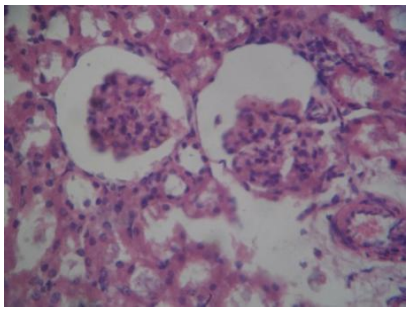
Fig: 10.

CONTROL: **HIGH DOSE:**

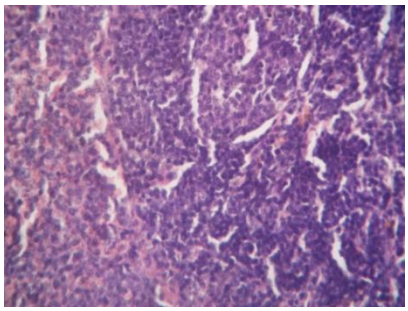
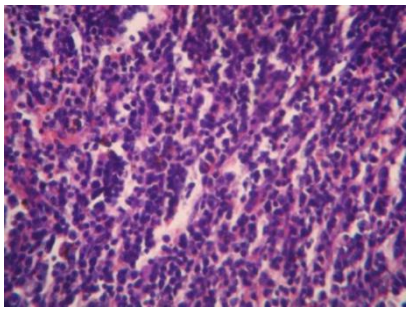
LIVER

KIDNEY

SPLEEN

Interpretation:

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

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

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

PHARMACOLOGY ACTIVITY:

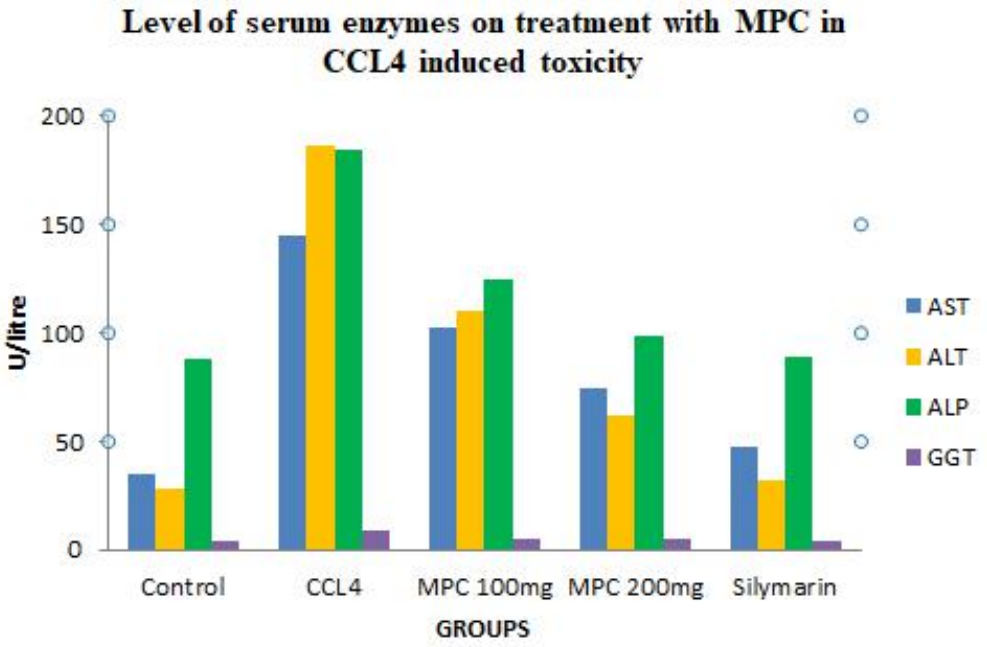
Pharmacological activity study result CCl_4 induced hepatotoxicity

Table: 27. Level of Serum Enzymes value (AST, ALT, ALP, GGT) with *MPC:*

Groups	Treatment	AST U/liter	ALT U/liter	ALP U/liter	GGT U/liter
Group 1	Normal	35.14±0.09	28.20±1.25	88.11±2.18	4.24±1.24
Group 2	CCL4+ LP	145.11 ± 1.54	186.56±1.00	184.72±1.8	9.22±1.20
Group 3	CCL4+Low dose (MPC) 100mg/kg	102.62±0.93	110.15±1.24	124.26±2.28	5.21±2.12*
Group 4	CCL4+High dose (MPC) 200mg/kg	74.24±0.62*	62.10±2.16*	98.64±2.14*	5.16±1.22*
Group 5	CCL4+ Silymarin 100mg/kg	47.28±1.14	32.16±0.24	89.42±1.42	4.04±1.21

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*



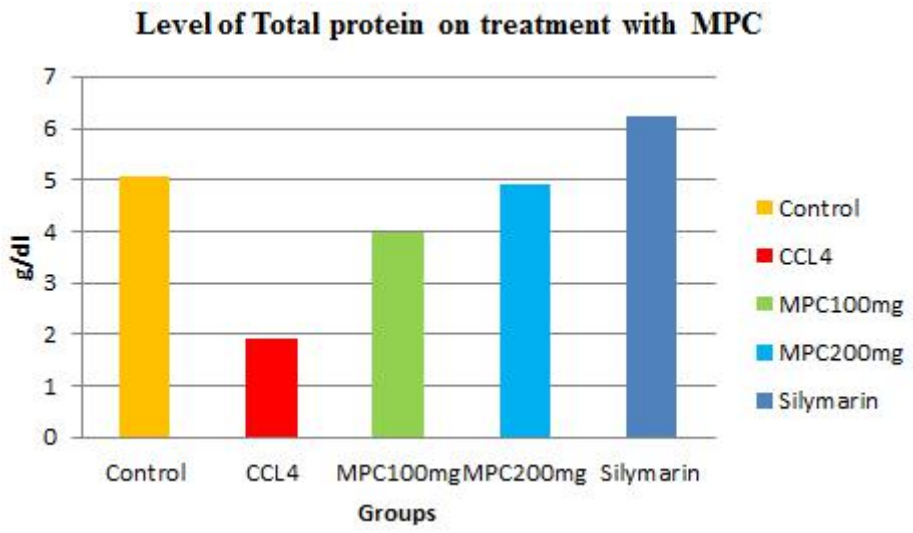
Graph 2

Table: 28. Effect of Total Protein and Bilirubin with *MPC:*

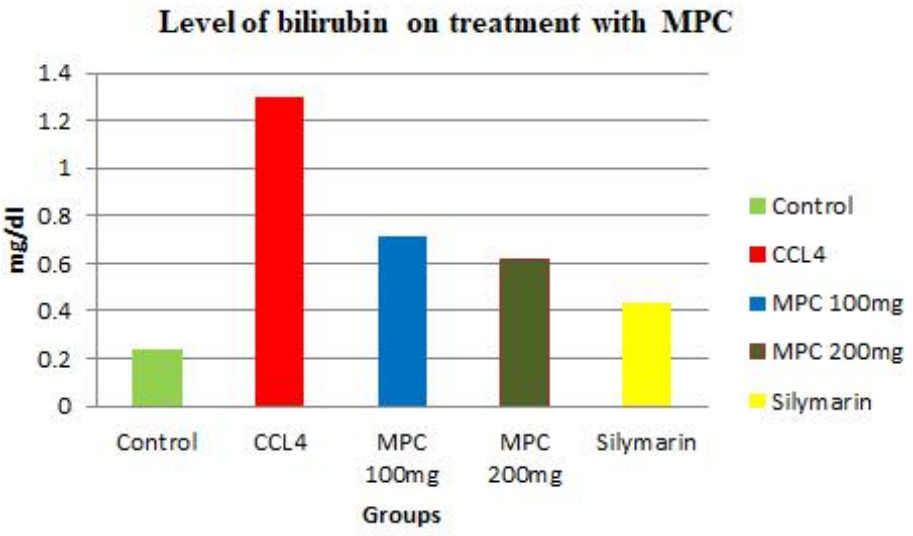
Groups	Treatment	Total Protein g/dl	Bilirubin mg/dl
Group 1	Normal	5.06±0.20	0.24±0.03
Group 2	CCL_4+ LP	1.90±0.46	1.3±0.09
Group 3	CCL_4+Low dose (MPC) 100mg/kg	3.97±0.48	0.71±0.07
Group 4	CCL_4+High dose (MPC) 200mg/kg	4.92±0.64*	0.62±0.03*
Group 5	CCL_4+Silymarin100mg/kg	6.24 ±0.44	0.43±0.04

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 3



Graph 4

HISTOPATHOLOGY SLIDES OF MPC ON CCL_4 INDUCED HEPATOTOXICITY:

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

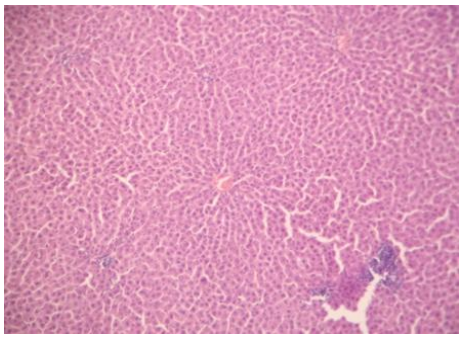
Group II: Showing dilated central vein and hepatocytes with degeneration.

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

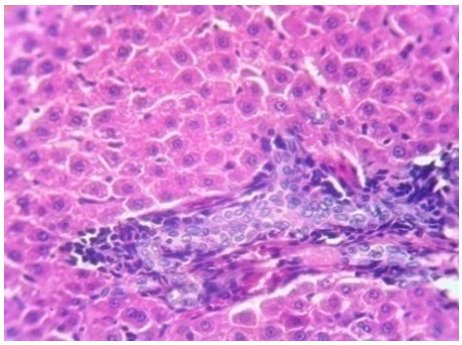
Group IV: Central vein showing normal hepatocytes with regenerating hepatocytes and mild inflammation in the portal area.

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

Fig:11.



Group 1



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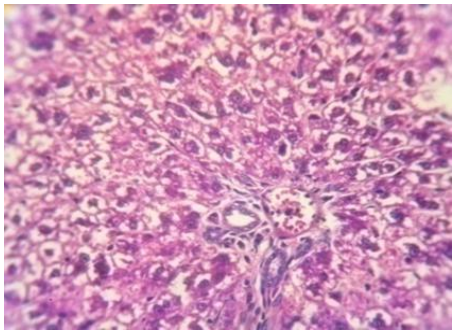

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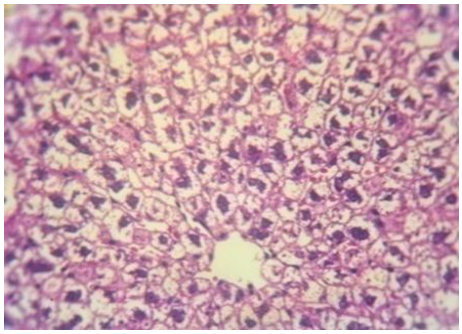

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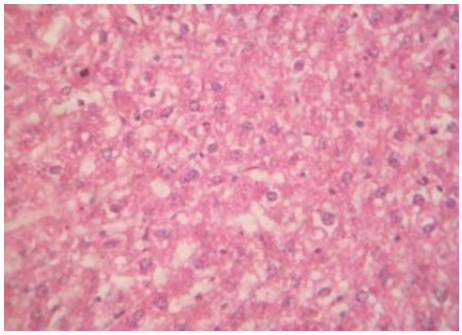


Group 5

Interpretation:

- The present studies were performed to assess the hepatoprotective activity in rats against Carbon tetrachloride as hepatotoxins.
- The serum marker enzymes, AST, ALT, ALP, GGT and Bilirubin 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.

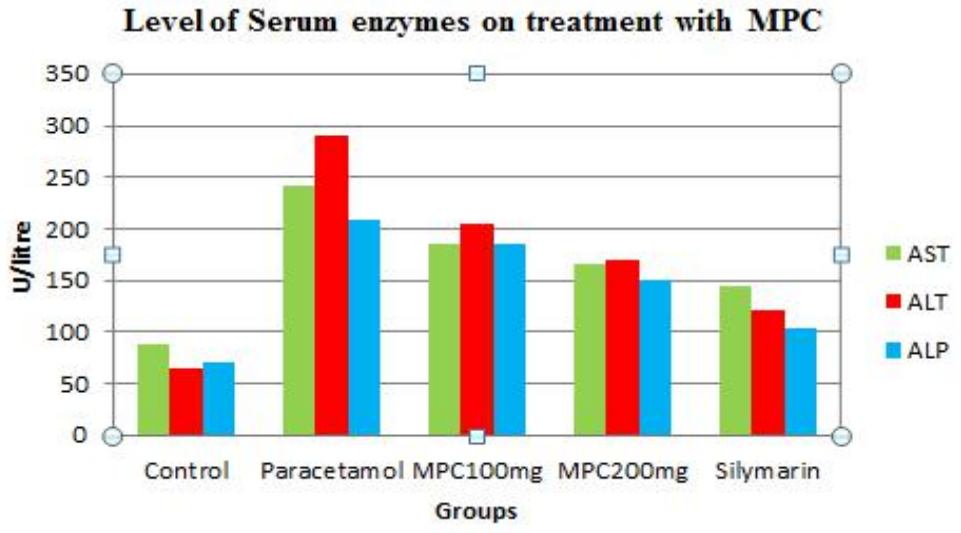
- The serum enzymes like AST, ALT, ALP, Bilirubin, GGT treated animals were significantly reduced by seven days pretreatment of MPC at two dose levels 100mg/kg and 200mg/kg, when compared with CCl_4 treated control. No significant clinical abnormalities in other groups.
- The changes associated with Carbon tetrachloride induced liver damage of the present study appeared similar to the acute viral hepatitis. In CCl_4 induced hepatotoxicity, the administration of the toxicant CCl_4 showed a distinct rise in the levels of serum marker enzymes namely AST, ALT, ALP, Bilirubin and as shown above Table no 27.
- A number of reports indicates that overdose of carbon tetrachloride can produce centrizonal hemorrhagic hepatic necrosis in humans and experimental animals.
- Carbon tetrachloride is biotransformed by the cytochrome P-450 system to produce the trichloromethyl free radical, which in turn covalently binds to cell membranes and organelles to elicit lipid peroxidation, disturb Ca 2+ homeostasis and finally result in cell death.
- The drug (*MPC*) treatment was carried out at two dose levels 100 and 200mg/kg, both of which along with the standard treated group showed a significant reduction in the elevated enzyme levels.
- The *MPC* treatments significantly reversed the levels of ALP. Reduction in ALP levels with concurrent depletion of raised bilirubin levels suggests the stability of the biliary function during injury with Carbon tetrachloride. Therefore the reduction in the activity of these enzymes may result in a number of deleterious effects.
- Administration of *MPC* increased the activities against CCL_4-induced liver damage in rats to prevent the accumulation of excessive fats and protected the liver[115].
- These data suggests a dose dependent hepatoprotective activity of *MPC*. Reduction in the levels of AST, ALT, and ALP towards the normal value is an indication of regeneration process. The protective effect exhibited by *MPC* at dose level of 200 mg/kg was comparable with the standard drug.
- These findings also suggested that the *MPC* administered has significantly neutralized the toxic effects of Carbon tetrachloride and helped in regeneration of hepatocytes.[120]
- This was further confirmed by histopathological slides in Fig 11.

Pharmacological activity study result Paracetamol induced hepatotoxicity

Table: 29. Level of Serum Enzymes value (AST, ALT, ALP) with *MPC:*

Groups	Treatment	AST U/liter	ALT U/liter	ALP U/liter
Group 1	Normal	88.67 ±1.085	64.83±0.600	70.50±0.763
Group 2	Toxicant Control(Paracetamol-1.25ml/kg)	242.5 ± 2.349	290.5±0.763	209.5±0.831
Group 3	Low dose (MPC) 100mg/kg	186 ± 0.577	204.8±0.600	184.50±0.563
Group 4	High dose (MPC) 200mg/kg	165.5 ± 0.763*	159±0.577*	130.5±0.436*
Group 5	Silymarin 100mg/kg	144.8 ± 0.600	120.8±0.945	104.5±0.672

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*



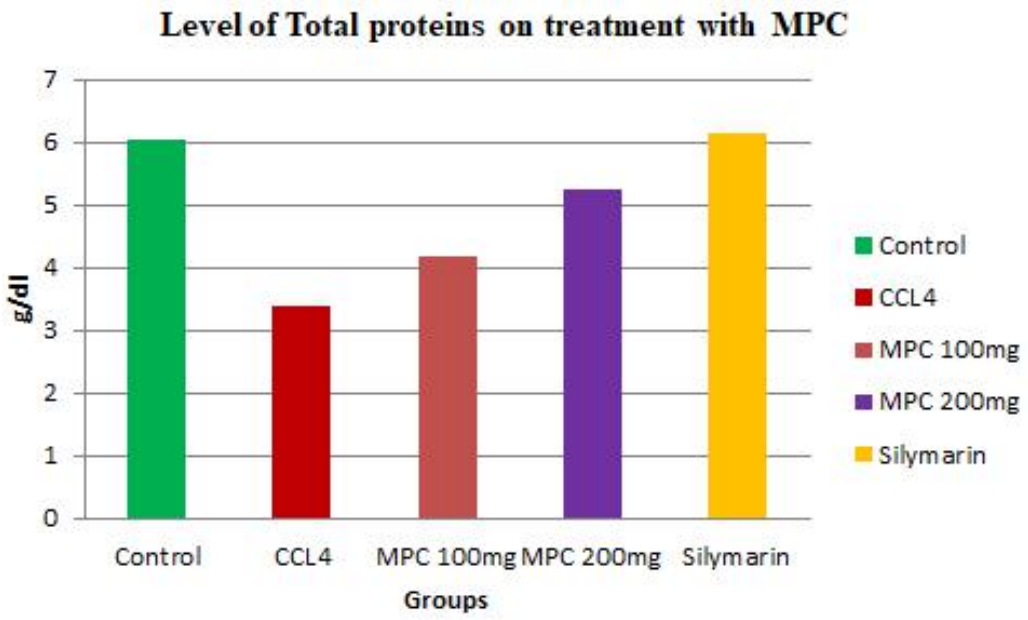
Graph 5

Table: 30. Effect of Total protein and Bilirubin with *MPC:*

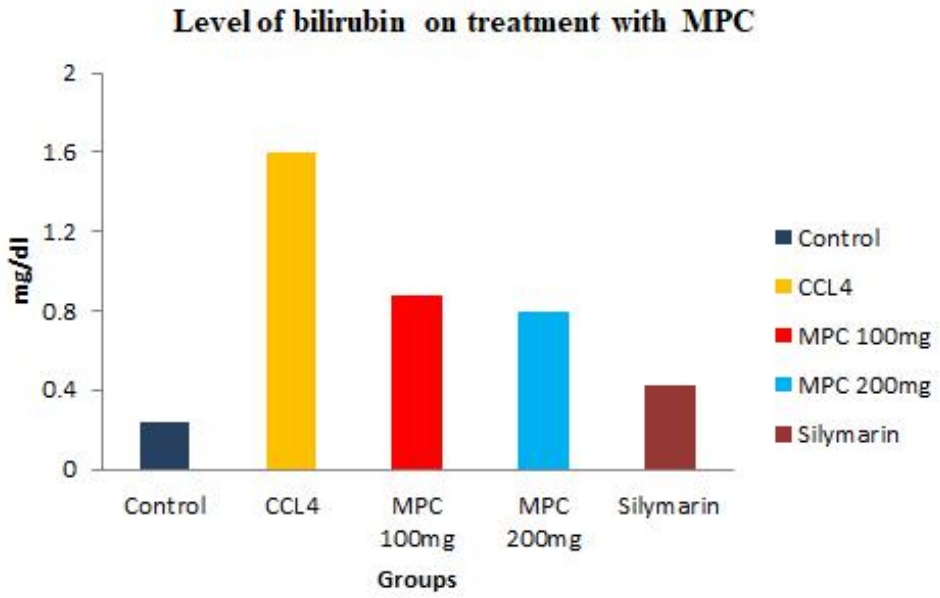
Groups	Treatment	Total protein g/dl	Bilirubin mg/dl
Group 1	Normal	6.04±0.31	0.24±0.6
Group 2	Toxicant Control(Paracetamol-1.25ml/kg)	3.39±0.39	1.6±0.09
Group 3	Low dose (MPC) 100mg/kg	4.18±0.21	0.88±0.03
Group 4	High dose (MPC) 200mg/kg	5.24±0.23*	0.79±0.07*
Group 5	Silymarin 100mg/kg	6.14±0.31	0.42±0.04

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



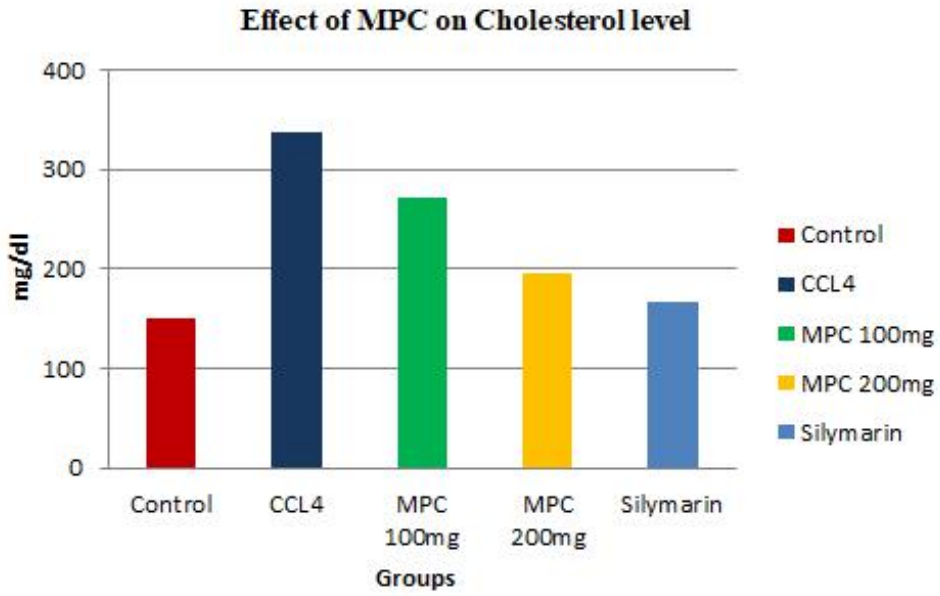
Graph 7

Table: 31. Effect of Cholesterol with *MPC:*

Groups	Treatment	Cholesterol (mg/dl)
Group 1	Normal	149.8±6.52
Group 2	Toxicant Control(Paracetamol-1.25ml/kg)	337.9±10.14
Group 3	Low dose (MPC) 100mg/kg	271±6.28
Group 4	High dose (MPC) 200mg/kg	194.6±6.38*
Group 5	Silymarin 100mg/kg	166±5.82

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*



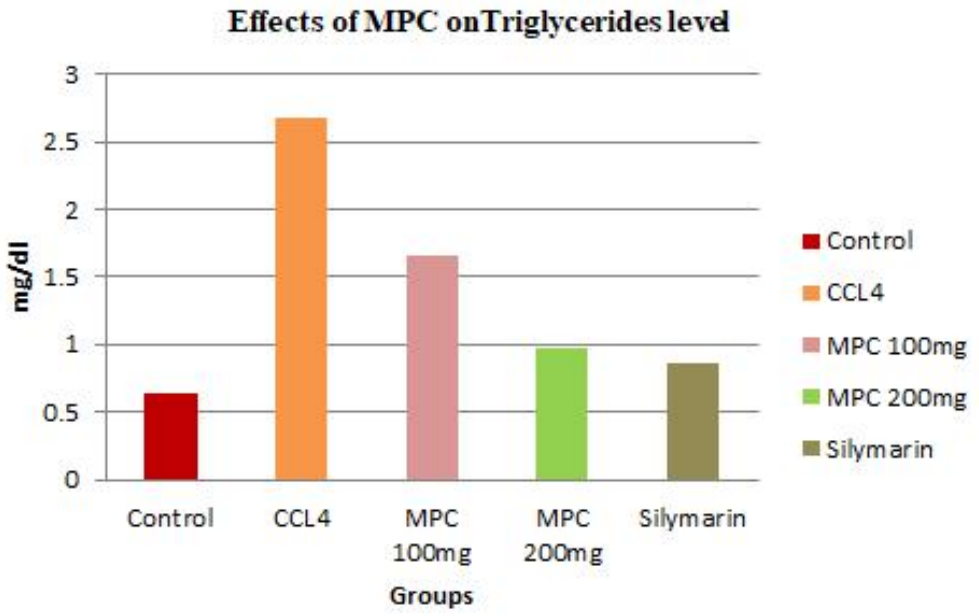
Graph 8

Table: 32. Effect of Triglycerides with *MPC:*

Groups	Treatment	Triglycerides (mg/dl)
Group 1	Normal	0.63±0.049
Group 2	Toxicant Control(Paracetamol-1.25ml/kg)	2.674±0.086
Group 3	Low dose (MPC) 100mg/kg	1.65±0.045
Group 4	High dose (MPC) 200mg/kg	0.974±0.261**
Group 5	Silymarin 100mg/kg	0.858±0.024

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*



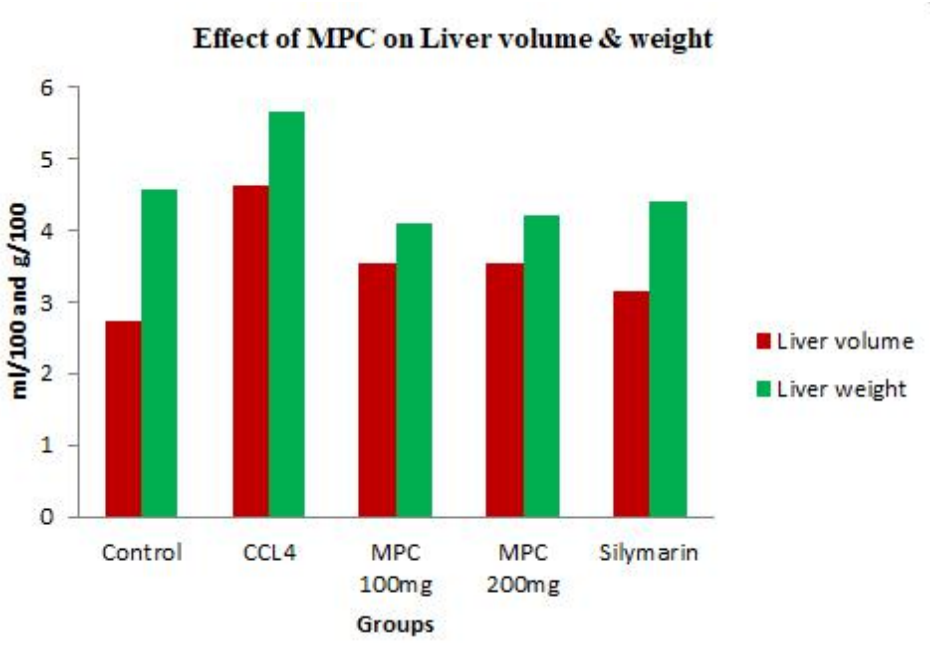
Graph 9

Table: 33. Effect of Liver Volume and Liver Weight with *MPC*

Groups	Treatment	Liver Volume(ml)	Liver Weight(g)
Group 1	Normal	2.721±0.054	4.56±0.71
Group 2	Toxicant Control(Paracetamol-1.25ml/kg)	4.624±0.086	5.66±0.61
Group 3	Low dose (MPC) 100mg/kg	3.546±0.95*	4.17±0.58*
Group 4	High dose (MPC) 200mg/kg	3.526±0.302*	4.29±0.63*
Group 5	Silymarin 100mg/kg	3.156±0.138	4.4±0.29

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*



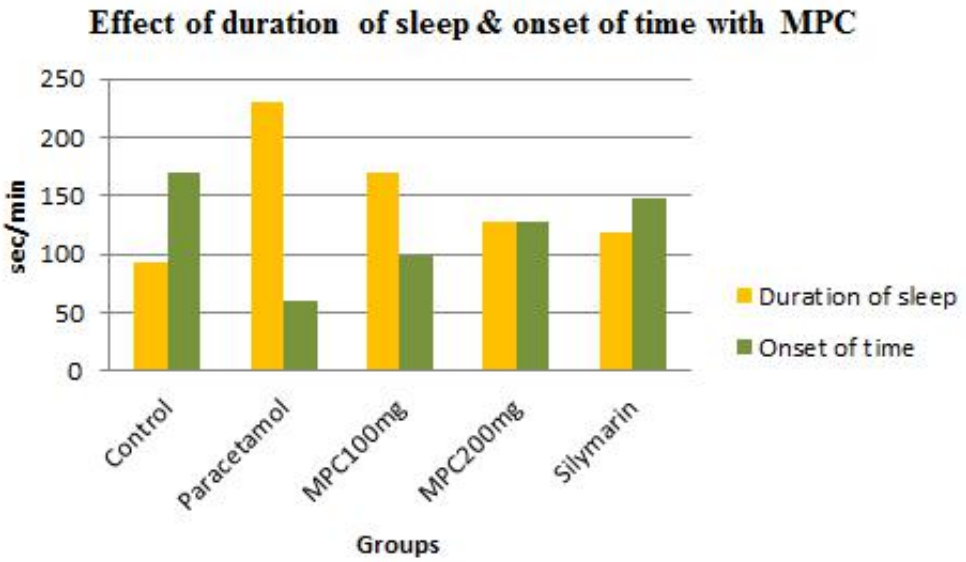
Graph 10

Table: 34. Effect of Direct Duration of Sleep and Onset of Time with *MPC:*

Groups	Treatment	Duration of Sleep (sec)	Onset of Time (min)
Group 1	Normal	94±2.711	169.9±1.70
Group 2	Toxicant Control(Paracetamol-1.25ml/kg)	230.2±1.79	59.9±2.96
Group 3	Low dose (MPC) 100mg/kg	169.9±2.11*	99±0.216*
Group 4	High dose (MPC) 200mg/kg	128.1±0.91*	127.70±2.29*
Group 5	Silymarin 100mg/kg	118±1.19	149±3.30

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 11

HISTOPATHOLOGY SLIDES OF MPC ON PARACETAMOL INDUCED HEPATOTOXICITY:

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

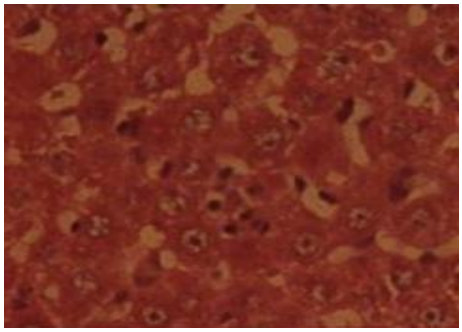
Group II: Showing extensive areas of confluent necrosis and also showing fatty changes and hydropic degeneration.

Group III: Liver tissue of rats treated with *MPC* at 100 mg /kg showing partial protection of hepatocytes and mild degree of necrosis.

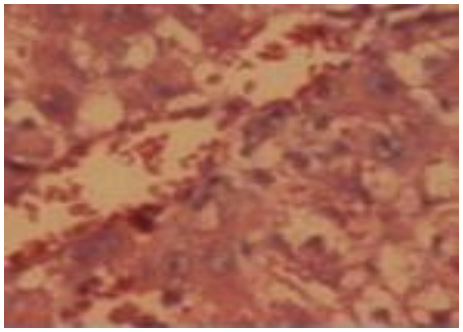
Group IV: Liver tissue of rats treated with *MPC* at 200 mg /kg showing centrizonal protection with regenerating hepatocytes and mild inflammation in the portal area.

Group V: Photo micrograph of liver tissue treated with silymarin showing normal histological architecture with central vein , portal vein and portal Artery.

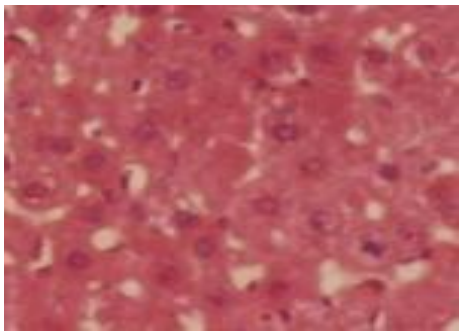
Fig: 12.



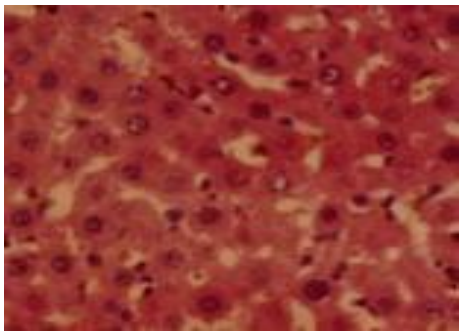
Group 1



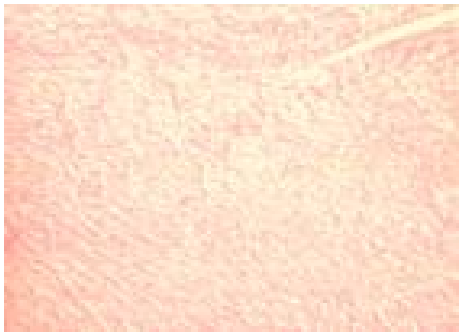
Group 2



Group 3



Group 4



Group 5

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 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 *Maavilingapattai Chooranam.*

- In paracetamol treated Wister albino rat the levels of serum marker enzymes (ALT, AST, and ALP) and triglyceride level elevated significantly. These increased 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.
- The liver volume and liver weight significantly increased. Duration of sleep significantly increased and onset of time siginificantly decreased by the treatment with *Maavilingapattai Chooranam* at 100 mg/kg p.o and 200 mg/kg p.o.
- Moreover, the increased levels of the serum enzymes were significantly decreased by the treatment with *Maavilingapattai Chooranam* at 100 mg/kg and 200 mg/kg, all the details implying that the drug prevent the liver damage[116].
- The *Maavilingapattai Chooranam* treatment confirmed dose dependent activity, *Maavilingapattai Chooranam* at 200 mg/kg revealed good result than 100mg/kg which is given in Table 29 for the determined levels of various serum enzymes.[121] This was further confirmed by histopathological slides in Fig 12.

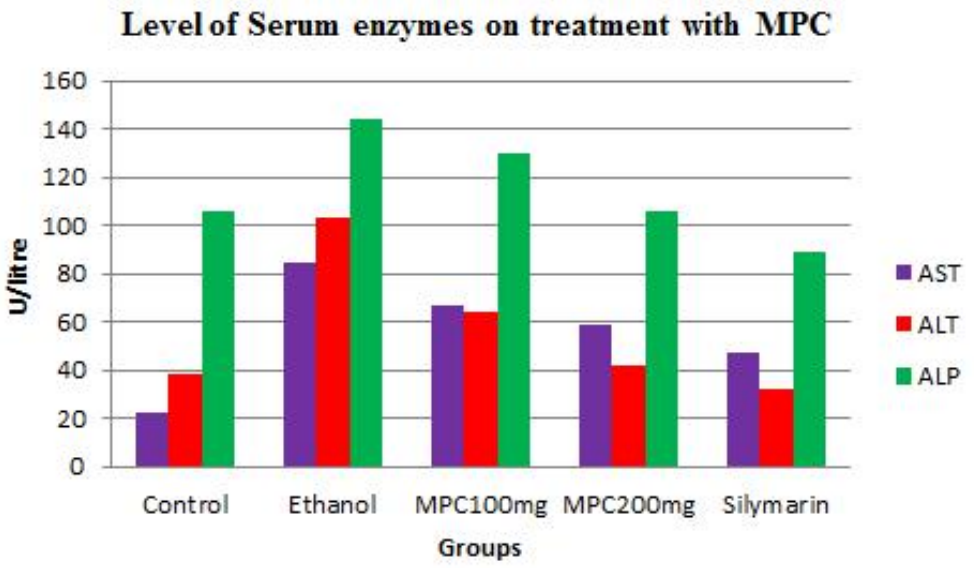
Pharmacological activity study result Ethanol induced hepatotoxicity

Table: 35. Level of Serum Enzymes value (AST, ALT, ALP) with *MPC:*

Groups	Treatment	AST U/liter	ALT U/liter	ALP KA units/100ml
Group 1	Normal	22.14±0.39	38.20±3.66	106.10±1.18
Group 2	Ethanol	85.11 ±1.44	103.24±1.00	144.32±1.43
Group 3	Ethanol+ (MCM) 100mg/kg	67.02±0.46*	64.38±1.04*	130.16±1.18
Group 4	Ethanol+ (MCM) 200mg/kg	59.14±0.42*	42.14±1.16*	106.44±1.14*
Group 5	Ethanol+ Silymarin 100mg/kg	47.28±1.14	32.16±0.24	89.42±1.42

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*



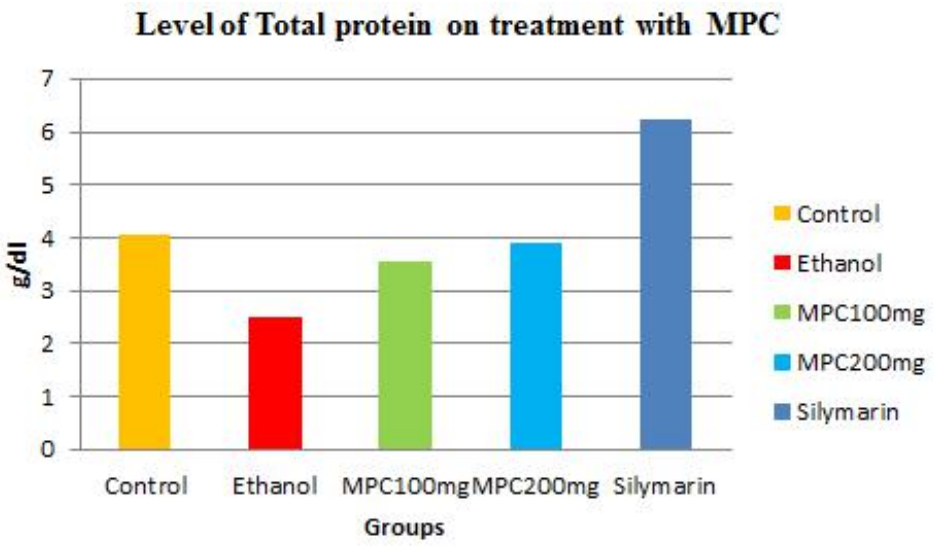
Graph 12

Table: 36. Effect of Total Protein and Bilirubin with *MPC:*

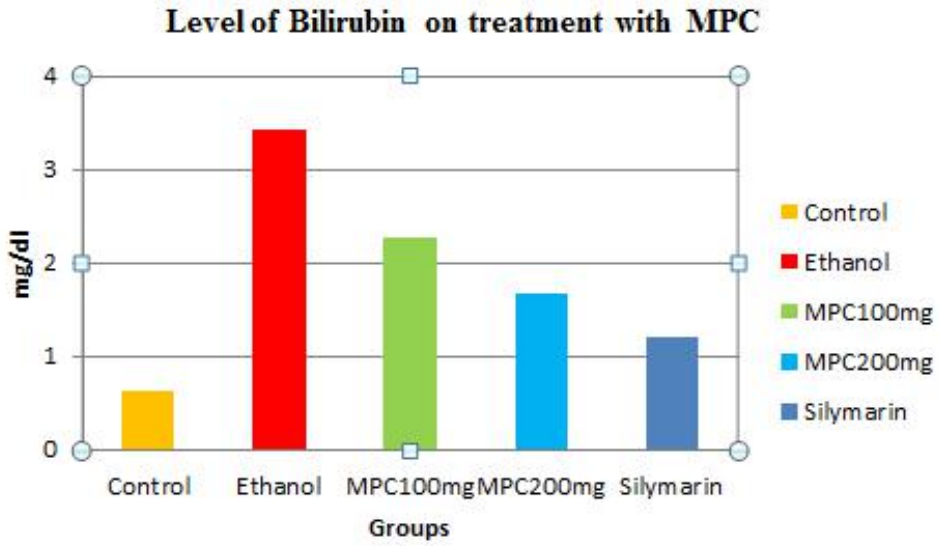
Groups	Treatment	Total Protein g/dl (TP)	Bilirubin mg/dl (BN)
Group 1	Normal	4.07±0.20	0.62 ± 0.32
Group 2	Ethanol	2.52±0.16	3.42±0.34
Group 3	Ethanol+ (MPC) 100mg/kg	3.56±0.18*	2.28±0.33
Group 4	Ethanol+ (MPC) 200mg/kg	3.92±0.64*	1.67±0.22*
Group 5	Ethanol+ Silymarin 100mg/kg	6.24 ±0.44 [a]	1.2±0.22

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 13



Graph 14

HISTOPATHOLOGY SLIDES OF MPC ON ETHANOL INDUCED HEPATOTOXICITY:

Group I: Control rat showing normal liver tissue.

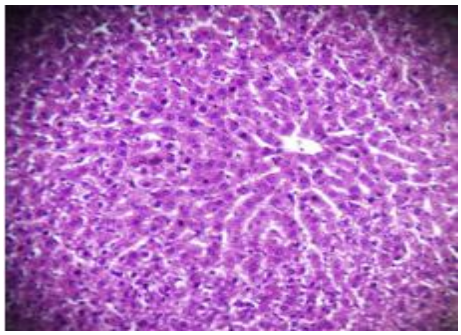
Group II: liver tissue of rat administered alcohol showing periportal necrosis with the presence of multinucleated hepatocytes and dilated central vein.

Group III: Liver tissue of rats treated with *MPC* at 100 mg/kg showing apparantly normal liver with few scattered hepatocytes.

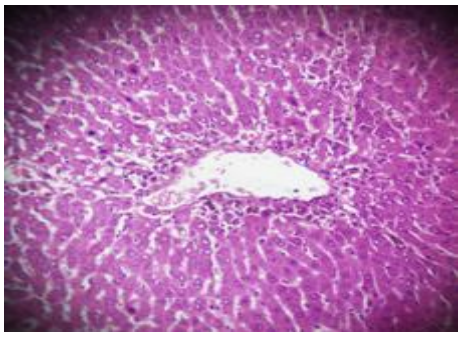
Group IV: Liver tissue of rats treated with *MPC* at 200 mg/kg showing normal hepatocytes with regenerating hepatocytes and mild inflammation.

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

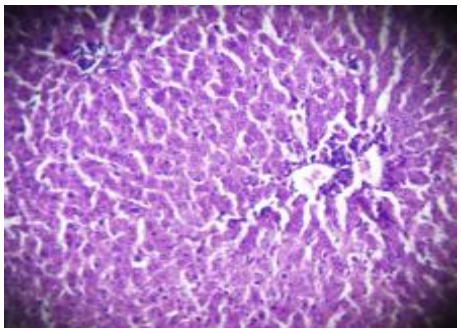
Fig: 13.



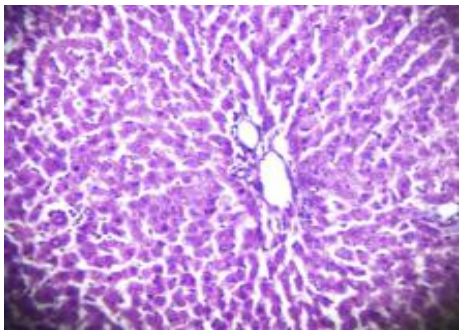
Group 1



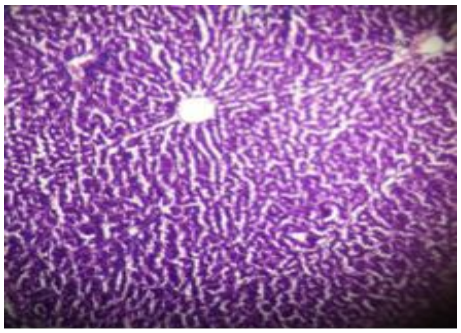
Group 2



Group 3



Group 4



Group 5

Interpretation:

- In the present study ethanol was used to induce Hepatotoxicity.
- Ethanol produces a constellation of dose related deleterious effects in the liver. Both acute and chronic ethanol administration cause enhanced formation of

cytokines, especially TNF-alpha by hepatic Kupffer cells, which have a significant role in liver injury.

- Elevated levels of AST and ALT are indications of hepatocellular injury.
- On the other hand suppression of elevated ALP activities with concurrent depletion of raised bilirubin level and an increase in the total plasma protein content suggests the stability of biliary dysfunction in rat liver during hepatic injuries with toxicants.
- In Ethanol treated rats the levels of serum marker enzymes (AST, ALT, ALP and Bilirubin) elevated significantly. Moreover, the increased levels of the serum enzymes were significantly decreased by the treatment with *Maavilingapattai chooranam* at 100 mg/kg p.o and 200 mg/kg p.o, implying that the drug prevent the liver damage[117].
- The *Maavilingapattai chooranam* treatment confirmed dose dependent activity, at dose level 200 mg/kg p.o revealed good result than 100mg/kg p.o.[122]
- This was further confirmed by histopathological slides in Fig 13.

6. CONCLUSION

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

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

The drug ***Maavilingapattai Chooranam*** was selected from the *Siddha* literature ***Sirorathina Vaidhiya Booshanam*** to validate the safety and its efficacy of CCL_4, Paracetamol and Ethanol induced hepatotoxicity.

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

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

Physico-chemical analysis:

- The pH of *Maavilingapattai Chooranam* was 6.2. This is weak acidic in nature. Acidic drug is essential for its bioavailability and effectiveness. Acidic drugs are easily absorbed in upper part of stomach. Hence, the drug should not produce any harmful effect to the mucus membrane of the GI tract.
- *Chooranam* 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.

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

Phytochemical analysis:

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

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

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

- HPLC analysis performed with *Maavilingapattai Chooranam* revealed the pressence of Polyphenols, Flavanoides, Alkaloides and Tannins.

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

Antimicrobial activity:

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

Instrumental analysis:

- Based on the results, *Maavilingapattai Chooranam* is preferably non-toxic to human in its therapeutic dose. The standardization of the drug was evaluated by chemical characterization with heavy metal analysis, functional group analysis,

elemental analysis and determination of particle size by ICP-MS, FTIR, and SEM respectively.

- ICP-MS reveals that in *Maavilingapattai Chooranam*, the heavy metals like Hg, As, Cd, Pb were below detectable level. This reveals the safety of the drug.
- The FTIR results showed the presence of O-H Stretching and bend, OH group has higher potential towards inhibitory activity against microorganisms. Phenols and flavanoides possess highly Anti-Oxidant property which enhances the drug effect against the disease.
- The major diffraction peaks are identified after XRD analysis. XRD pattern of the trail drug *Maavilingapattai Chooranam* shows some good crystanility.
- The SEM picture shown the presence of microparticle of size 100-1000 nm in the drug *Maavilingapattai Chooranam*. Further, the study shows that *Maavilingapattai Chooranam* is a kind of micromedicine which favours the advantages of bio availability, better absorption and non toxic with minimal dose level.

Pharmacokinetic aspect:

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

Toxicity studies:

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

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

In Conclusion, no toxic effect was observed up to 200mg/kg of *Maavilingapattai Chooranam* treated over a period of 28 days (OECD 407). So, it can be concluded that the *Maavilingapattai Chooranam* can be prescribed for therapeutic use in human with the dosage recommendations of up to 100mg/kg body weight p.o.

Pharmacological activities:

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

- CCL_4 induced Hepatotoxicity
- Paracetamol induced Hepatotoxicity
- Ethanol induced Hepatotoxicity

Hepatoprotective activity against CCL_4, Paracetamol and Ethanol induced Hepatotoxicity:

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

7. SUMMARY

The test drug ***Maavilingapattai Chooranam*** was selected from the *Siddha* literature ***Sirorathina Vaidhiya Booshanam*** written by Angamuthu Mudhaliyar for its hepatoprotective activity.

❖ The dissertation started with an introduction explaining about the *Siddha* concept, prevalence of jaundice and role of the test drug in treating hepatic diseases.

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

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

❖ The test drug *Maavilingapattai Chooranam* was prepared properly by the given procedure.

❖ All the ingredients were identified and authenticated by the experts.

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

❖ Pharmacological review was done to establish the methodologies.

❖ The drug was subjected to analysis such as physicochemical, phytochemical, biochemical and also instrumental analysis which provided the key ingredients present in the drug thus it accounts the efficacy of the drug.

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

❖ Pharmacological study was done. It revealed the Hepatoprotective activity of *Maavilingapattai Chooranam* in animal model Wistar albino rats.

❖ Results and discussion gives the necessary justifications to prove the potency of the drug.

❖ Conclusion gives a compiled form of the study and explains the synergistic effect of all the key ingredients and activities that supports the study.

❖ This current analysis authenticates that *Maavilingapattai Chooranam* has impressive Hepatoprotective activity, which exemplifies the intelligence of the *Siddha* literature to reach globally for the welfare of mankind. Thus the herbal formulation *Maavilingapattai Chooranam* is validated for its safety and efficacy for treating jaundice and it would be a great drug of choice.

8. FUTURE SCOPE

The Herbal formulation ***"Maavilingapattai Chooranam"*** was taken as the compound drug preparation for Hepatoprotective activity mentioned in the classical *Siddha* literature ***"Sirorathina Vaidhiya Booshanam"*** written by **Angamuthu Mudhaliyar.** The mechanism of action by which *Maavilingapattai Chooranam* produced its effect on hepatoprotective activity in experimental animals need to be validated in a scientific manner using specific experimental animal models and also multi-center clinical trials are required to understand the exact molecular mechanisms of action. So it could be used worldwide for hepatoprotective action.

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Government Siddha Medical College

Arumbakkam, Chennai – 600 106.

CERTIFICATE

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

Ingredients of *Maavilingapattai Chooranam*:

1. *Iruveli* - *Vetiveria zizanioides*
2. *Vilamichu* - *Plectranthus vetttiveroides*
3. *Chiru kurinchan* - *Gymnema sylvestre*
4. *Poonai vanangi* - *Acalypha indica*
5. *Kozhunji* - *Tephrosia purpurea*
6. *Koovilam* - *Aegle marmelos*
7. *Pathiri* - *Stereospermum colais*
8. *Thulasi* - *Ocimum sanctum*
9. *Musumuskkai* - *Mukia maderaspatana*
10. *Musuttai* - *Rivea ornata*
11. *Vila* - *Limonia acidissima*
12. *Nannari* - *Hemidesmus indicus*
13. *Kurundhotti* - *Sida rhombifolia*
14. *Ashwagandhi* - *Withania somnifera*
15. *Parangichakkai* - *Smilax china*
16. *Maavilingapattai* - *Crateva magna*
17. *Seeni sarkarai* - *Saccharam officinaram*

Date: 25.8.2017

Place: Chennai

PG Department of Gunapadam

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 MAAVILINGAPATTAI CHOORANAM & NILAKADAMBU*(Asarum europaeum)* CHOORANAM** in rats pigs 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/20/CLBMCP/2016

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

(Dr.P.Muralidharan)

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

IAEC Member Secretary

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

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

This Certificate is awarded to Dr/Mr/Mrs....I. SAMROOTHUL PARVEEN.....

For participating as ~~Resource~~ ~~Person~~ / Delegate in the Twentieth 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 07th to 11th March 2016.

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

Prof.**Dr.P.ARUMUGAM,** M.D.,
REGISTRAR i/c

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

SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

CHENNAI – 600 119

CENTRE FOR LABORATORY ANIMAL TECHNOLOGY AND RESEARCH

(CPCSEA Approved)

WORKSHOP ON

TOXICOLOGICAL PROFILING AND ASSESSMENT OF TOXICITY OF DRUGS ON LAB ANIMALS

CERTIFICATE

This is to certify that Dr./~~Mr.~~/~~Ms.~~ I. SAMROOTHUL PARVEEN

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

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

Convener
Dr. R. SELVARAJ
Scientist In-charge

NATIONAL SEMINAR ON

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

(RM & PHISSM – 2018)

6TH & 7TH APRIL 2018

प्रमाण पत्र
CERTIFICATE

सिद्ध क्षेत्रीय अनुसन्धान संस्थान
पूजप्पुरा, तिरुवनंतपुरम, केरल
SIDDHA REGIONAL RESEARCH INSTITUTE
Poojappura, Thiruvananthapuram, Kerala

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

This is to certify that Dr./Shri/Smt. Samsoothul Parveen I., GSMC, Chennai has participated/presented a paper entitled An Advantage of Siddha Medicine 'MAI' in Healing Eye Diseases. in the National Seminar on "Research Methodology and Public Health Initiative through Siddha System of Medicine" (RM & PHISSM – 2018) organized by Siddha Regional Research Institute, Thiruvananthapuram on 6th & 7th April 2018 at Dr. M R DAS Convention Centre, Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, Kerala.

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

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

CLINICAL RESEARCH & HOSPITALS (P) LTD

National Conference on

HERBAL MEDICINE AND ETHNOPHARMACOLOGY

Date: 06.04.2017; Venue: TICEL Biopark.

This is to certify that ~~Ms./Mr.~~/Dr. I. SAMROOTHUL PARVEEN *from* DEPT. OF PHARMACOLOGY, GOVT. SIDDHA MEDICAL COLLEGE, CHENNAI *attended the National Conference on "**Herbal Medicine and Ethnopharmacology**" conducted in V.S. Clinical Research & Hospitals (P) Ltd., Chennai, Tamil Nadu. He/She presented a paper/poster in the topic* MEDICINAL PLANTS USED FOR THE TREATMENT OF PARKINSON ....

T. Mathangi

Dr. T. Mathangi
Scientist & Coordinator

Dr. L. Lokoranjan
Chairman & Managing Director

GOVERNMENT SIDDHA MEDICAL COLLEGE

Arumbakkam, Chennai, 600106

This certificate is awarded to Dr./~~Mr.~~/~~Ms~~. I. SAMROOTHUL PARVEEN

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

"Orientation to research Methods"

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

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

Dr. K. Kanakavalli
Principal
Govt. Siddha Medical College