PRECLINICAL STUDY OF SIDDHA DRUG VENTHAMARAIYATHI CHOORANAM FOR IT'S THROMBOLYTIC, VASODILATOR, HYPOLIPIDEMIC & CARDIO PROTECTIVE ACTIVITIES

Dissertation submitted to

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for the award of the degree of

DOCTOR OF MEDICINE (SIDDHA)

BRANCH-II-GUNAPADAM

POST GRADUATE DEPARTMENT OF GUNAPADAM

THE GOVERNMENT SIDDHA MEDICAL COLLEGE

TIRUNELVELI-627002

OCTOBER 2016

GOVT. SIDDHA MEDICAL COLLEGE,
PALAYAMKOTTAI

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"Pre-clinical study of herbal formulation Venthamaraiyathi chooranam for its Thrombolytic, Vasodilator ,Hypolipidemic, & Cardioprotective activities"** is a bonafide and genuine research work carried out by me under the guidance of **Dr.A.KINGSLY M.D(s), Reader, Head of the Department,** Post Graduate Department of Gunapadam, Govt. Siddha Medical College, Palayamkottai, Tirunelveli-02 and the dissertation has formed the basis for the award of any Degree, Diploma, Fellowship or other similar title.

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ABBREVATIONS

ALT - Alanine transaminase

ANOVA- - Analysis of variance

AST - Aspartate transaminase

CMC - Carboxyl Methyl Cellulose

CDC - Council of Disease control and prevention

CPCSEA - Committee for the purpose of control and supe

DC - Differential Count

EDTA - Ethylene Diamine Tetra Aceticacid

ESR - Erythrocyte Sedimentation Rate

FTIR - Fourier transform infrared spectroscopy

Hb - Haemoglobin

DC - Differential Count

EDTA - Ethylene Diamine Tetra Aceticacid

ESR - Erythrocyte Sedimentation Rate

FTIR - Fourier transform infrared spectroscopy

IAEC - Institutional Animal Ethical Committee.

ICP-OES - Inductively coupled plasma optical emission spectrometry

Ig E - Immunoglobulin E

LDH - Lactate Dehydrogenase

MCV - Mean Corpuscular Volume

VTC	-	Venthamaraiyathi Chooranam
OECD	-	Organisation for Economic Co-operation and Development
PCV	-	Packed Cell Volume.
PGE	-	Prostaglandin E
RBC	-	Red Blood Corpuscles
SEM	-	Scanning electron microscope
TLC	-	Thin Layer Chromatography
HPTLC	-	High performance thin layer chromatography

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PALAYAMKOTTAI

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CERTIFICATE OF APPROVAL

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Dissertation Topic	PRE CLINICAL EVALUATION OF SIDDHA HERBAL FORMULATION " **VEN THAMARAIYATHI CHOORANAM"** FOR ITS THROMBOLYTIC, VASODILATOR, HYPOLIPIDEMIC &CARDIO PROTECTIVE ACTIVITIES
Documents Filed	1) Protocol
Clinical / Non Clinical Trial Protocol	Non Clinical Trial Protocol
Informed Consent Document	NA
Any other Documents	NA
Date of IEC Approval & its Number	GSMC-II-IEC/2015-Br.-II/01/16.07.2015

We approve the trial to be conducted in its presented form.

The Institutional Ethical Committee expects to be informed about the process report to be submitted to the IEC atleast annually of the study, any changes in the protocol and submission of final report.

16/7/2015 16/7/15

Chairman (Prof. Dr. M. Logamanian)

Member Secretary (Prof. Dr. S. Soundararajan)

Certificate of Botanical Authenticity

Certified the following plant drugs used in Siddha formulation ***"Venthamariyathi Chooranam"*** taken up for Post Graduation Dissertation Studies by **Dr. J. Indrakumar (Reg. No:321312001)**, PG Dept. of Gunapadam, are correctly identified and authenticated through Visual inspection / Organoleptic Characters / Experience, Education & Training Morphology / Microscopical and Taxonomical methods.

Drug : VENTHAMARAYATHI CHOORANAM

INGREDIENTS:

S.N	Name	Botanical Name	Parts used
1.	Elarisi	Elettaria cardomum	Seed
2.	Chukku	Zingiber officinalis	Dried Rhizome
3.	Thippili	Piper longum	Dry Fruit
4.	Cheeragam	Cuminum cyminum	Seeds
5.	Athimathuram	Glycyrrhiza glabra	Root
6.	Chatha kuppai	Anethum Sowa	Seed
7.	Venthamarai Ithal	Nelumbo nucifera	Flower

08/07/15

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Date : 08.07.2015

Authorized Signature.

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for AYUSH Post Graduates & Researchers

Organised by the Department of Siddha

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1. INTRODUCTION

Siddha system is considered to be one of the ancient systems of medicine in the world. Siddha is a complete holistic medical system that has been practiced in India for more than thousands of years.

"உடம்பார் அழியில் உயிரார் அழிவர்
திடம்பட மெய்ஞானம் சேரவும் மாட்டார்
உடம்பை வளர்க்கும் உபாயம் அறிந்தே
உடம்பை வளர்த்தேன் உயிர்வளர்த் தேனே".

- திருமந்திரம்

So Siddhars devised healing techniques and drugs to alletiate diseases, live long and have a healthy soul that is healthy uyir.

Siddha means "Perfection" or "Eternal bliss". Siddhi refers to the eight supernatural powers that are attainable by man. Those who attained these supernatural power or perfection or siddhi were called "Siddhars". Siddhars realised that if the body is made strong and perfect, they could get rid of death and diseases.

Siddha System of Medicine is one of the classical system of medicine consisting of Herbal , Mineral and Animal origin. This system approaches the diseases both mentally and physically.

Its origin strictly belongs to southern part of India. This system has its own basic principle i.e mukkutram (*Vatha, Pitta and Khapa*) and theories of Five Elements *(Aimpootham). "Health sustainment or decline is defined by the Normal or abnormal state of the Humors"* .

" மருந்தென வேண்டாவாம் யாக்கைக்கு அருந்தியது
அற்றது போற்றி யுணின்"

- திருக்குறள்

The doshas within any person keep changing constantly due to lifestyle, foods and environment.The loss of balance among the humors causes energy disharmony and physical and mental disequilibrium which may appear at any time and become the cause of diseases. According to the *Siddhar ',Aagasthir'* the diseases are widely classified into 4448 types. Kuruthi Azhal Noi is one the disease in the above classification.

Symptoms of *Kurthi Azhal Noi* are Weight gain, loss of energy, vomiting, cough, indigestion , burning sensation of the body, bleeding in vomitus and phlegm, Heaviness of Head, dryness of body, hesitation of food , excess secretion of saliva, irritation of throat and shortness of breath

"பித்தந் தானே குருதியிற் றோன்றும்"

So the disequilibrium of pitha reflects as a vascular diseases. The symptoms quoted under " *Kuruthi Azhal Noi*" in *Noinadal and Noi muthal Nadal Thiratu* more or less correlate with Hypertension .

Hypertension

Hypertension is a chronic condition of concern due to its role in the causation of coronary heart disease, stroke and other vascular complications. It is the commonest cardiovascular disorder, posing a major public health challenge to population in all socio-economic and epidemiological transition.

It is very common disease with annul incidence of more than 10 million cases for per year in India .

The symptoms of Hypertension are pulsating headache, insomnia, dizziness, lack of concentration, loss of memory, shortness of breath and occasional palpitation.

Normal Blood pressure	-	120/80 mmHg
Pre Hypertension	-	140/90 mmHg
Hypertension stage I	-	140-159/90-99 mmHg
Stage II	-	Above 160 /100mmHg

With the increasing of side effects and dependance of Modern drugs. It has become imperative to find out an Appropriate Siddha treatment for pithathikam that is way a preclinical study has been conducted to assess the effect of the drug ***Venthamaraiyathi Chooranam*** in essentional Hypertension.

2. AIM AND OBJECTIVES

AIM:

The aim of this study is to validate the **Thrombolytic,Vasodilator, Hypolipidemic** and **Cardioprotective** activity of ***Venthamaraiyathi Chooranam*** and thus ensuring a holistic approach by controlling the blood pressure levels, significantly decreasing the development and progression of complication of ***Kuruthi Azhal Noi*** (**HT**).

OBJECTIVES:

The main objective of the present study is to highlight the safety and efficacy of ***Venthamaraiyathi Chooranam*** in the treatment of ***Kuruthi Azhal Noi***, the following methodology was adopted to evaluate the drug and its standardization studies.

- Collection of literature evidence regarding the trial medicine.
- Identification of the drugs in the ***Venthamaraiyathi Chooranam*** .
- Preparation of the trial medicine as per the text.
- Physico-chemical analysis of Test drug.
- Evaluation of the toxicity of Test drug.
- Evaluation of Thrombolytic activity of the Test drug by in vivo Clot lysis in rat models.
- Evaluation of Hypolipidemic activity of Test drug by models of wistar albino rats.
- Evaluation of vasodilator activity of Test drug Student organ bath method.
- Evaluation of Cardioprotective activity of Test drug by against Doxorubicin induced myocardial toxicity in albino rats.

3. REVIEW OF LITERATURES

3.1. GUNAPADAM ASPECT

3.1.1. ஏலரிசி

(*Elettaria cardamomum*, Maton)

Other Name :

Anji, Thudi, Korangam

Types:

- Peria Elam,
- Chirttialam,
- Kattuyelak-kay,
- Malayelam

Habitat :

Cultivated for its fruits in many parts of Western of Southern India,

Part Used:

Dried ripe seeds, oil from fruits

Taste - Spicy
Character - Veppam (Hot potency)
Class - Spicy

Actions:

- Stimulant
- Carminative
- Stomachic

General Characters: (Pothu gunam)

'தொண்டை வாய்கவுள் தாலுகு தங்களில்
தோன்றும் நோயதி சாரம்பன் மேகத்தால்
உண்டை போல் எழுங் கட்டி கிரிச்சரம்
உழலை வாந்தி சிலந்தி விஷஞ்சுரம்

பண்டை வெக்கை வசிதாக நோய் காசமும்
பாழுஞ் சோம்ப் பிணிவிந்து நட்டமும்
அண்டை யீளைவன் பித்தம் இவைக்கெல்லாம்
ஆல மாங்கமழ் ஏல மருந்ததே"

(தேரையர் குணவாகடம்)

It cures Throat diseases, oral diseases, cough , dysuria, expectoration, and animal bites and Pitha diseases

Therapeutic Uses:

Cardamom seeds which have a fragrant taste and aromatic odour . They are generally used as a masticatory (as a spice and a flavor agent). Cardamoms are employed to a small extent in Europe for flavoring sweet meats. They are valuable in many stomach complaints. An oil extract from the fruits is used in both Pharmacy and Perfumery.

Cardamom may be safely used us a carminative in convalescence after diarrhea. In the form of tincture are powder , cardamoms are used , both in eastern and western systems of medicine , as a frequent adjunct to other stimulants, bitter and purgatives .

A decoction of cardamom together with their pericarp and jiggery added is a popular home remedy to relieve giddiness caused by biliousness. A compound powder containing equal parts of cardamom seeds, ginger, clove and caraway is a good stomachic in half drachms doses in a tonic dyspepsia . Powder made of equal parts of parched cardamom seeds, aniseeds and caraway seeds given in one teaspoonful doses good digestive.

Other preparation of Elarasi

1**. Elathi Kirutham**

Dosage : 1-2 teaspoon (bd)

Indications : Cough , cold, Peptic Ulcer, Indigestion

(Kannusamy , Parambarai Vaithiyam, 2012, 9th edition , p 237.)

2. Vallarai Kirutham

Dosage : 2-3 teaspoon

Duration : 40 days (bd)

Indicatons : Scabies , Venereal disease, Primary complex.

(Kannusamy , Parambarai Vaithiyam, 2012, 9th edition , p 237.)

3. Maha Elathi chooranam

Dosage : Thirikadi piramanam

Indications : Fever, Vomiting ,Headache, Bone Tuberculosis, Chest burning

(Kannusamy , Parambarai Vaithiyam, 2012, 9th edition , p 105.)

4**. Thalisapathiri Chooranam**

Dosage : Thirikadi piramanam

Indication : Tuberculosis, Body Burning, Peptic Ulcer, Jaundice .

5. Seethobalathi Chooranam

Indiacation : Venereal disease, Diarrhoea, Tuberculosis

6.Asuvaganthi Legium

Dosage : 5 -10 gm Bd

Indication : Health Tonic for Tuberculosis Patients. Strengthening. Infertility and fertility.

7. Ingi Legium

Dosage : 5-10 gm Bd

Indication : Cure for Pitha diseases, Control for Diarrhoea, Indigestion

3.1.2. BOTANICAL ASPECT

Elettaria cardamomum

Scientific classification

Kingdom	:	Plantae
(unranked)	:	Angiosperms
(unranked)	:	Monocots
(unranked)	:	Commelinids
Order	:	Zingiberales
Family	:	*Zingiberaceae*
Genus	:	Elettaria
Species	:	E. cardamomum
Binomial name	**:**	*Elettaria cardamomum*(L.) Maton
Common name	**:**	**Cardamom, Malabar cardamom , Ceylon cardamom**

VERNACULAR NAMES

Hindi	:	Elaichi
Marathi	:	Elachi
Tamil	:	Elam Ancha
Malayalam	:	Elatarri
Telugu	:	Elaki
Kannada	:	Elakki
Urdu	:	Elaichi
Sanskrit	:	Trutih

Description

Overview:

A herbaceous perennial growing up to 6 m tall. Leaf-shoots arising from a stout rhizome. Growing in a thick clump of up to 20 leafy shoots.

Leaves:

Dark green, long and sword-shaped. The underside is paler and may have a covering of tiny hairs.

Flowers:

Borne on a flowering stalk that can reach over 1 m in length. The pale green flowers contain both male and female parts. One of the petals is white and streaked with violet.

Fruits:

Pale green to yellow and elongate oval-shape. Each fruit has three chambers filled with small aromatic seeds, each about 3 mm long. The fruits and seeds dry to a straw-brown colour and are widely used as flavouring.

Uses

Food and drink

The dried fruits and seeds of cardamom are used to add a unique taste to rice, meat (including hamburgers, pizza sausages and Swedish meatballs), vegetables and other savoury dishes. Whole and ground cardamom seeds are added to flavor coffee, tea, liqueurs, ice cream, confectionery and baked goods (including Danish pastries).

Cardamom is highly valued in Kashmir as an essential ingredient of the fragrant drink *kahwa* and sweet Kashmiri black tea.

Cardamom essential oil is extracted from the seeds. It is mainly used in the flavouring of processed foods and drinks such as cordials, bitters and liqueurs and occasionally in perfumery.

Cardamom oleoresin has similar applications to the essential oil. It is mainly used to flavour meat products with a short shelf life, such as sausages. Because the oil has antibacterial activity it has been added to foods as a preservative at low levels. It is used in low quantities so it does not affect the flavour of the food.

Traditional medicine

In Ayurvedic medicine, cardamom is used to treat disorders of the stomach and urinary system, asthma, bronchitis and heart problems. When mixed with neem and camphor, cardamom is used as a nasal preparation to treat colds. An infusion of cardamom can be used as a gargle to relieve sore throats, which has led to its use in cough sweets.

Cardamom seeds have been used in a range of preparations. Roasted seeds were boiled with betel nuts (fruits of the palm *Areca catechu*) to make a drink that is used to treat indigestion and nausea. They are also added to tea to make a tonic to relieve the symptoms of stress due to overwork or depression. Cardamom seeds are given to patients with bad breath and a capsule of cardamom taken with honey is reputed to improve eyesight.

The traditional uses of cardamom to treat skin conditions have attracted the attention of those developing plant-based cosmetics, especially as it has been used traditionally to treat areas of the body that have red-pigmentation. It is often incorporated into soaps and hand creams.

Blood Pressure

As a diuretic and fiber rich spice, cardamom significantly lowers blood pressure.

Blood Clots

Cardamom prevents dangerous blood clots by preventing platelet aggregation and the sticking to the artery walls.

Antioxidant

Many of the vitamins, phytonutrients, and essential oils in cardamom act as antioxidants, cleaning up free radicals and resisting cellular aging.

Pathogens

The volatile essential oils in cardamom inhibit the growth of viruses, bacteria, fungus, and mold.

Anti-inflammatory

Like ginger and turmeric, its relatives, cardamom has some anti-inflammatory properties that limit pain and swelling, especially in mucus membranes, the mouth, and throat.

Hiccups

Cardamom is an anti-spasmodic that can help get rid of hiccups. This also applies to other involuntary muscle spasms, like stomach and intestinal cramps

3.1.3. LATERAL RESEARCH

Blood pressure lowering, fibrinolysis enhancing and antioxidant activities of cardamom (Elettaria cardamomum).

Abstract

Elettaria cardamomum (L.) Maton. (Small cardamom) fruit powder was evaluated for its antihypertensive potential and its effect on some of the cardiovascular risk factors in individuals with stage 1 hypertension. Twenty, newly diagnosed individuals with primary hypertension of stage 1 were administered 3 g of cardamom powder in two divided doses for 12 weeks. Blood pressure was recorded initially and at 4 weeks interval for 3 months. Blood samples were also collected initially and at 4 weeks interval for estimation of lipid profile, fibrinogen and fibrinolysis. Total antioxidant status, however, was assessed initially and at the end of the study. Administration of 3 g cardamom powder significantly ($p<0.001$) decreased systolic, diastolic and mean blood pressure and significantly ($p<0.05$) increased fibrinolytic activity at the end of 12th week. Total antioxidant status was also significantly ($p<0.05$) increased by 90% at the end of 3 months. However, fibrinogen and lipid levels were not significantly altered. All study subjects experienced a feeling of well being without any side-effects. Thus, the present study demonstrates that small cardamom effectively reduces blood pressure, enhances fibrinolysis and improves antioxidant status, without significantly altering blood lipids and fibrinogen levels in stage 1 hypertensive individuals.

3.2. GUNAPADAM ASPECT

3.2.1. சுக்கு

(***Zingiber officinale***)

Other Name :

Artharagam, Arukkan, Athagam, Upakullam, Ularntha Inji, Sudupathiram, Chukku, Chundi, Chondi, Choupannam, Chouvarnam, Navasuru, Naakaram, Manoushatham, Vichvapechajam, Vidamoodiya amirtham, Verkompu. Dried Zingier is called Chukku

Habitat :

Ginger is cultivated in many part of India. On large scale in the warm, moist regions, chiefly in Madras , Cochin and Thavancore, and to a some what less extent in Bengal and Punjab.

Part Used:

Tuber (Dry)

Taste - Spicy
Character - Veppam(Hot potency)
Class - Spicy

Actions

- Stimulant
- Carminative
- Stomachic

General Characters: (Pothu gunam)

"சூலைமந்தம் நெஞ்செரிப்பு தோடமேப் பம்மழலை
மூலம் இரைப்பிருமல் மூக்குநீர் வாலகப
தோடமதி சாரந் தொடர்வாத குன்மநீர்த்
தோடம்ஆ மம் போக்குஞ் சுக்கு."

It cures Indigestion, Chest Burning, Sour Belching, Oral diseases , Respiratory diseases, Diarrhea, Ulcer, Sinusitis, Abdominal unfortable, Ear pain, Face disease, Head ache , Anemia, Cold fever.

Therapeutic Uses:

Ginger is prepared from the dried rhizomes. Ginger being aromatic and pleasantly pungent, is commonly used us a spice and the preparation of condiments curries ginger bread , and conserve and syrup are made from the fresh younger rhizomes. Rhizomes are also pickled.

Dried ginger is of two types , peeled and unpeeled , the latter being mearly the cleaned rhizomes dried in the sun in the cases of dry specimen the out layer should be scraped off . when the fresh drug is used for extracting in the juice , the supernatant fluid alone should be used and the sediment discarded (Chunnam). “Ginger was at one time much employed for spicing beer and the modern equivalent, ginger beer, is highly esteemed today as a beneficial cordial in a cold weather” (chopra).

Dry ginger is much used as a carminative adjunct along with black pepper and long pepper under the name of trikatu. Ginger is extremely valuable in dyspepsia, flatulence, colic, vomiting, spasms and other painful affection of the stomach and the bowels an attended by fever; for cold , cough, asthma, dyspepsia and the indigestion is highly recommended a preparation called “allaepauk” or ginger Jam or conserve; it consist of ginger juice, water and sugar in sufficient quantites .

Other Preparation of Chukku

1. Mullangi Legium

Dosage	:	5- 10 gm Bd
Indication	:	Loss of appetite , vomiting, Abdominal Pain, Peptic Ulcer pain cure for the best this medicine . (Milk must taken after medicine)

2. Kandakathiri Legium

Dosage : 5 -10 gm bd

Indication : Peptic Ulcer, Indigestion, Constipation, vomiting, Throat irritation etc.

3. Sangu Mathirai

Dosage : 1 bd Hot water

Indication : Constipation, fever, cough, Tabe warm ,

4. Mantha Mathirai

Dosage : 65 gm tablet od for 1 week

Indication : Respiratory disease, Fever and Rhinitis, Body Pain.

5. Jeevaracha amirtha Chooranam

Dosage : Thirikadi Piramanam

Indication : Indigestion

6. Vaivilangathi Chooranam

Dosage : Thirikadi Piramanam

Indication : Gastritis , Hiccup , Vomiting, Asthma and Pitha disease

7. Thair Chunndi Chukku Chooranam

Dosage : Thirikadi Piramanam

Indication : Indigestion, loss of appetite, Flatulence, Abdominal uncomfortable

3.2.2. BOTANICAL ASPECT

Zingiber Officinale

Classification

According to Bentham and Hooker's Classification (1862-83) Zingiber Officinale Rose. in Trans. Linn. SOC is classified as follows.

Kingdom	-	Vegetable Kindom
Division	-	Spermatophyta
Sub-Division	-	Angiospermae
Class	-	Monocotyle donae
Series	-	Epigynae
Family	-	*Scitominae*
Sub –Family	-	*Zingiberaceae*
Genas	-	Zingiber
Species	-	Officinale

Vernacular Names:

Tamil	-	Dried – Shukku, Chukku –
English	-	Ginger
Sanskrit	-	Dried –Sunta- Nagara , Nagaram,
Hindi	-	Adrak, ada
Ger	-	Ingwer
Kannada	-	(Dried) – Vona – Shunti
Malayalam	-	Chukka
Telugu	-	Dried – Sonti
Bengali and	-	Dried – Sonth
Panjabi	-	Fresh – Adrak, Ada, Adi
Gujarathi	-	Adu

Distribution:

Widely cultivated in tropical Asia, Ginger is cultivated in many parts of India, and on large scale in the warm, moist regions, chiefly in madras, cochin and Travancore, and to a some what less extent in Bengal and the Punjab.

Botanical Description

It is an underground rhizome.

Habit:

A herbaceous, rhizomatous perennial, reaching up to 90cm in height under cultivation.

Root :

Adventitious

Leaves:

Narrow, distichous, Sub-sessile, linear – lanceolate, 17.0 cm x 1.8cm, dark green, evenly narrowed to form a slender tip with stem –clasping sheaths.

Inflorescence:

Spikes terminating Elongate Peduncles, sheathed by scarious bracks. Spikes Oblong-cylindric, 1.5-3 inch long 4-1 inch wide, glabrous. Pedeincle slender, Sheathing scales glabrous, about inch long. Lip 3 – 10bed, mid lobe oblong – ovate, lateral short, ovate, obtuse

Floral Characters:

Flowers : Zygromarphic, asymmetrical, bisexual, epigynous. Flowers in spikes , Scape radical or terminating the leafty stem. bracts persistent, usually 1- flowered. Greenish with a small dark purple or Purplish black tip in radical spikes 3.8 – 7.5 cm long and 2.5cm diameter . on peduncles 15-30 cm. long stamen dark puple, as long as the lip, rather shorter than the corolla.

Perianth	:	6 in two whorls (3+3) distinguishable in to calyx and corolla.
Calyx	:	Cylindric, Shortly 3- lobes
Corolla	:	Tubular, Unequally three lobed. Lobes calceolate, the upper concave.

Androecium

Stamens 6, in two whorls, only median posterior stamen of the inner whorl is functional and epipetalous, the other two stamens of the innter whole are united to form a 2-lipped staminodium (or) labellum, the anterior stamen of the outer whorl is absent and the remaining two modified into petaloid staminodes (or) absent.

Gynoecium:

Corples 3; syncarpous, ovary inferior, Axile Placentae, Ovules many, style and stigma 1, the slender style held in a groove in the fertile stamen.

Fruit	:	An oblong, tardily dehiscing capsule.
Seeds	:	Large, globose, arillate.
Part used	:	Rhizone
Rhizome	:	Thick and Fleshy
Propagation	:	By rhizomes

Adulteration

Adulteration of ginger by exhausted or not well prepared on limed drug can be determined by the above standards.

Sometimes in exhausted ginger, capsicum (or) Paradise grains are added to increased pungency, Paradise grains are the seeds of aframomum melgueta (Zingiberaceae) pungency of capsicum and paradise grains is not destroyed by boiling with alkali while it is destroyed in case of ginger. Adulteration of ginger by capsicum and paradise grains can be determined by this way.

Phytochemical Aspects:

Active Constituents:

Ginger Contains 1 to 2 % volatile oil, camphene, phellandrene, zingiberene, Zingiberol, eucalyptol, Citral, bomecol, Linalol, Methyl heptenone, Monyl – Alcohol esters of acetic, caprylic acid, yellow oil gingerol, Phenols, resin, Shogaol

5 to 8.7% pungent principle, resinous mass and starch. Volatile Oil is responsible for the aromatic smell and consists of Zingiberene 6% sesquiterpene hydrocarbon Zingiberol a sesquiterpene alcohol and bisabolene. Gingerol is a yellow pungent oily Liquid and yields gingerone a ketone and aliphatic aldehyde shogaol is formed by loss of water from gingerol. Shogaol and gingerone are less pungent. The pungency of gingerol and ginger is destroyed when boiled with 5% potassium hydroxide or other alkalies.

Chemical Compositions:

The composition of ginger varies according to the type and the agroclimatic conditions under it is grown.

Vitamins

Thiamine	-	0.06mg / 100gm
Riboflavin	-	0.03 mg/100gm
Niacin	-	0.60 mg/ 100gm
Vitamin-c	-	6.0 mg/ 100gm
Carotene	-	40 mg/ 100gm

Ginger contains small quantities of glucose fructose and sucrose, rattinose is probably present in traces.

The principal carbohydrate of the rhizome is starch. Besides starch the rhizomes are reported to contain Pent sans. (7.6% on dry basis in one sample). Ginger contains 1.60-2.44% Nitrogen on dry basis.

Extraction of the freeze – dried and powdered rhizome by the conventional Protein – solvents showed that albumin globuline, prolamine and glutelin formed respectively – 35.6, 16.9, 11.0 and 17.9% of the total proteins. 18.6% of the total proteins remained un extracted.

The free amino acids present in ginger include glutamic acid, aspartic acid, serine, glycine threonine, alanine, glutamine arginine,aminobutyric acid, valine, leucines, proline, phenylalanine, asparagines, lysine, cystine, histidine and pipecolic acid.

(***Connell Mclachlan. J.Chromat)***

Essential Oil

The Characteristic Pleasant and aromatic odour of ginger is due to an essential oil which can be separated from the rhizome by stream distillation. The oil is generally obtained from unscripted ginger. The oil containing cells are particularly numerous in the epidermal tissue and scraped ginger is therefore poor in oil content. The essential oil derived from the dried ginger known in trades as oil of ginger.

Ginger

Ginger was known in china as early as 400 B.C. It was also used as a spice by the Greeks and Romans who considered it an Arabian product because it was received from India by the way of the Red Sea. It was introduced in to Jamaica and other islands of the west indies by the Spaniards and ginger was exported from the west indies to spain in considerable quantity even during the year 1547 A.D.

Ginger has been under cultivation in India from times immemorial the plant does not occur in a truly wild state, and rarely flowers, though the cultivated plants on the west coast of India are said to bear flowers quite frequently in October.

Cultivation

For cultivation the rhizome is cut into pieces and each piece containing a bud is planted into trenches in well-drained and loamy soil in March or April Sandy loan rich in humens and warm humid conditions are ideal for its cultivation.

The plant requires about 80 inches rainfall per year and if rainfall is in adequate water may be supplied by irrigation. Collection is done in December or January when the plants wither after flowering period. Rhizomes are carefully dug out, aerial stems, fibrous roots and buds are removed. They are washed to remove mould and clay attached to them. Rhizome is peeled on that surface as well as between the fingers and thoroughly washed in running water. Drug is then dried completely by keeping in the sun on mats

which are covered over. nights and in rainy and cloudy seasons. If moisture is present, drug may become moudly. After drying it loses about 70% of its weight 1500 kg rhizomes are required per hectare Around 12.500 kg of rhizome per hectare is obtained.

Curing of Ginger

After the flowers have disappeared and the stems have withered. ginger is ripe for collection. The rhizome are dug up and prepared for the market in different ways.

Ginger is marketed both in the peeled and unpeeled condition, the forms as scraped ginger and later us unscraped ginger. In Scraped ginger the epidermal layer of the fresh rhizome is scraped off with the help of a sharpened bamboo- Splinter and then washed in water and dried in the sun for F-10 days as the essential oil is contained in the epidermal cells, excessive or careless scraping results in the loss of this oil and depreciate the quality of the spice. the quality of dry ginger, cured at Dodahoo, Himachal Pradesh, in the bright sun has been found to be better than that cured in a closed over with artificial heat. Iron – Knives are not used for scraping as they leave stains on the product.

In the middle – East countries which buy a very large part of the Indian produce there is a higher demand for white, polished rhizomes free from specks or spots. For this purpose the raw rhizomes are soaked in water for a day and later in thick milk of lime (1kg. slaked time/ 12 liters of water) This material is dried in the sun and then rubbed with gunny bag pieces to remove the last remnants of he skin. the treatment imparts a smooth finish to the final product.

In certain localities the lime treated rhizomes are treated further with sulphur dioxide fumes at the rate 3.2 kg sulphur per tonne of rhizomes for 12 hours in specially constricted chambers to bleach the product.

In Jamaica, a small quantity of lime-Juice is sometimes added to the water used for washing the pealed rhizomes to produce whiter ginger.

Gradual drying is said to give a better product and so the rhizomes are dried in the morning and then air – dried in shade during the rest of the day this is continued for 8-12 days. The dry rhizomes are graded on the basis of colour and size before packing the product for market or shipment.

Medicinal Uses in Siddha

Uses

Used to treat Indigestion hyperacidity, cough, Asthma, dyspepsia, Polyuria, ascites, diarrhea, oedema, stomach disorders, anemia, disease of kapham, cardiac diseases.

In painful affections of the bowels stomach, etc., infusion of dry ginger is given with the addition of a tablespoonful or two of castor oil the dose of the infusion. In chronic rheumatism, infusion of south (1 in 24) taken warm just before going to bed, the body being covered with blankets so as to produce copious perspiration, is often attended with the best results. The same treatment has also been found beneficial in cold or cat arrhol attacks and during the cold stage of intermittent fever.

3.2.3. LATERAL RESEARCHES

Zingiber officinale Mitigates Brain Damage and Improves Memory Impairment in Focal Cerebral Ischemic Rat

Abstract

Cerebral ischemia is known to produce brain damage and related behavioral deficits including memory. Recently, accumulating lines of evidence showed that dietary enrichment with nutritional antioxidants could reduce brain damage and improve cognitive function. In this study, possible protective effect of Zingiber officinale, a medicinal plant reputed for neuroprotective effect against oxidative stress-related brain damage, on brain damage and memory deficit induced by focal cerebral ischemia was elucidated. Male adult Wistar rats were administrated an alcoholic extract of ginger rhizome orally 14 days before and 21 days after the permanent occlusion of right middle cerebral artery (MCAO). Cognitive function assessment was performed at 7, 14, and 21 days after MCAO using the Morris water maze test. The brain infarct volume and density of neurons in hippocampus were also determined. Furthermore, the level of malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) in cerebral cortex, striatum, and hippocampus was also quantified at the end of experiment. The results showed that cognitive function and neurons density in hippocampus of rats receiving ginger rhizome extract were improved while the brain infarct volume was decreased. The cognitive enhancing effect and neuroprotective effect occurred partly via the antioxidant activity of the extract. In conclusion, our study demonstrated the beneficial effect of ginger rhizome to protect against focal cerebral ischemia.

3.3. GUNAPADAM ASPECT

3.3.1. திப்பிலி

(Piper longum)

Other Name :

Aargathi, Unsaram, Ulavainasi, Kaaman, Kudaari, Kolakam, Koli, Kolaiyarukki, Saram, Saadi, Thulavi, Mahathi, Sunai, Chounndi, Thanduli, Kanam, Kalini, Paanam, Pippili, Vaitheki, Ampu, Aathi Marunthu

Habitat :

This plant is indigenous to northeastern and southern India and Ceylon and cultivated in Eastern Bengal.

Types:

- Arichi Thippili
- Aanai Thippili

Part Used:

Vegetable and Rice

Taste	-	Sweet
Character	-	Thatpam (Coolant)
Class	-	Sweet

Actions:

- Stimulant
- Carminative

General Characters: (Pothu gunam)

"ஆசனநோய் தொண்டைநோய் ஆவரண பித்தமுதல்
நூாசிவிழி காதிவைநோய் நாட்புழுநோய் - வீசிடுவி
யங்கலாஞ்ச னஞ்சிதையும் அம்பாய் அழிவிந்தும்
பொங்கலாஞ்ச நங்கையர்கோட்போல்.
திட்டி வினையகலும் தேகமெத்த – புட்டியாம்
மாமனுக்கு மாமனென மற்றவர்க்கு மற்றவனாங்
காமமெனுந் திப்பிலிக்கும் கை." *(தேரன் வெண்பா)*

It cures Kapha disease and General Tonic.

Therapeutic Uses:

Old long piper is more efficacious in medicine than fresh article (U.C.Dutt). powdered long piper administrated with honey will relieve cough, cold, asthma , hoarseness and hiccup. For catarrh and hoarseness a mixture of long piper root, long piper, black piper and ginger in equal part is a useful combination . A compound powder consisting of the same ingredient equal parts and called chuturushana chooranam is useful in colic and flatulence besides cough and coryza . it was tested and found successful. Dose is 10 to 60grm twice a day. A compound powder consisting of long piper , ginger , black piper , cinnamon and caraway in equal part is a good expectorant . Cow milk 350 ml was boiled and then 8 gm of Thippili powder added , mixed well and taken in night time will cure cough, gastritis, dyspnea, and Thiridhosa.

திப்பிலி கற்பம்

….. ….மேற்கணை தனைக்கிளர் பொடியாக்கியே தேனிற்
குழைத்துணத் தேமல்போ மதியினில். (தேரையர் காப்பியம்)

Thippili powder taken with honey will cure Tinea.

Other preparation Thipili

1. Thippili Chooranam

Dosage : Thirikadi Piramanam

Indication : Indigestion, cough , Asthma, Tuberculosis.

2.Dharunasura Kiyalam

Dosage : 60 ml

Indication : Fever and associated disease

3. Kasakulanthaga Mathirai

Dosage : 1 bd

Indication : Respiratory disease

4. Balasanjeevi Mathiri

Dosage	:	1 (30mg) tablet bd
Indication	:	Cough, Fever and associated disease

5. Thippili Legium

Dosage	:	5-10 gm
Indication	:	Abdominal Pain, Tuberculosis, cough, Asthma , Wheezing

3.3.2. BOTANICAL ASPECT

Piper Longum, Linn

Scientific Classification:

Kingdom	:	Plant
Division	:	Angiosperms
Order	:	Piperales
Family	:	Piperaceae
Genus	:	Piper
Species	:	Longum

Vernacular Names:

English	:	Long pepper
Telugu	:	Pippilu
Malayamlam	:	Hippili
Sanskrit	:	Pippali
Duk	:	Pipliyan
Persian	:	Daraife-fil
Bengali	:	Pipul
Gujarati	:	Lindi pippal
Oriya	:	Pippalimula
Kanada	:	Hippali, modi
Punjabi	:	Maghan
Urdu	:	Filfil Daraz

Habit:

It is a creeping aromatic herb, slender climber.

Stems:

Creeping jointed

Leaves:

Lower leaves are broadly ovate, deeply cordate with big lobes at base, upto 10 cm long, upper leaves are dark green, oblong-ovate, cordate.

Flowers:

Flowers are in spikes, bracts or male spikes narrow and female spikes circular.

Inflorescence:

Inflorescence is spike

Fruits:

Fruits are small, ovoid, shining, blackish green in colour, 2.5 to 4 cm long, light green when immature.

Chemical Constituents:

The fruit of long pepper contains piperine (4-5%) and piplartine alkaloid, starch, resin, gumand fat.

The dried fruits on steam distillation yield 0.7% of essential oil with spicy odour. Besides that it contains sesamin and piplasterol Major chemical constituents are piperine, piperlongumine (piplartine), piperlonguminine and also methyl – 3,4,5 – trimethoxycinnamate.

Others:

Sesamin, a lignan, Dihydrostigmasterol and two low melting unstable compounds, one of which appeared to be isobutylamide of an unsaturated acid, n-isobutyl-deca-trans-2-trans-4 dien eamide, essential oil consisting of n-hexadecane n-heptadecane, n-octadecane etc.

Therapeutic actions:

- Carminative, stimulant, stomachic, flatulence
- The dried unripe fruits are useful in cold, cough, chronic bronchitis and Diarrhoe
- It is also used in liniments for rheumatic pains and paralysis. Fruits and roots are used in Ayurvedic and Unani systems of drugs.
- It is also used to treat insomnia, epilepsy, obstruction of bile duct and gall bladder, dysentery and leprosy
- Used for the diseases of respiratory track, cough and bronchitis as counter-irritant
- Analgesic when applied locally for muscular pains and inflammation general tonic and Haematinic.

3.3.3. LATERAL RESEARCH

Piperine from the Fruits of *Piper longum* with Inhibitory Effect on Monoamine Oxidase and Antidepressant-Like Activity

Abstract :

A bioassay-guided isolation of the ethanol extract from the fruits of *Piper longum* yielded a known piperidine alkaloid, piperine, as a monoamine oxidase (MAO) inhibitor. Piperine showed an inhibitory effect against MAO-A (IC_{50} value: 20.9 μM) and MAO-B (IC_{50} value: 7.0 μM). Kinetic analyses by a Lineweaver–Burk plot clearly indicated that piperine competitively inhibited MAO-A and MAO-B with K_i values of 19.0±0.9 μM and 3.19±0.5 μM, respectively. The inhibition by piperine was found to be reversible by dialysis of the incubation mixture. In addition, the immobility times in the tail suspension test were significantly reduced by piperine, similar to that of the reference antidepressant fluoxetine, without accompanying changes in ambulation when assessed in an open-field. These results suggest that piperine possesses potent antidepressant-like properties that are mediated in part through the inhibition of MAO activity, and therefore represent a promising pharmacotherapeutic candidate as an antidepressant agent.

3.4. GUNAPADAM ASPECT

3.4.1. சீரகம்

Cuminum cyminum

Other Name :

Asai, Seeri, Upakumpapeesam, Narseeri, Thutthasampalam, Pirathi-viga, Pithanasini, Posanakudori, Methiyam

Habitat :

Extensively cultivated as a cold season crop on the plains and as a summer crop in the hills in northern India Himalayas and the Punjab, Baluchisthan, Kashmir, Kumaon, Garhwal etc. also imported from Persia and Asia Minor.

Part Used:

Seeds

Taste	-	Spicy and Sweet
Character	-	Thatpam (Coolant)
Class	-	Sweet

Actions:

- Stimulant
- Carminative
- Stomachic
- Astringent

General Characters: (Pothu gunam)

பித்தமெனு மந்திரியைப் பின்னப் படுத்தியவன்
சுத்துருவை யுந்துறந்து சாதித்து – மத்தனெனும்
ராசனையு மீவென்று நண்பைப் பலப்படுத்தி
போசனகுடாரிசெய்யும் போர்

(தேரன் வெண்பா)

It cures the Abdominal pain , Oral diseases, Hepatic, Tuberculosis, Calculi, Bleeding dysentery and asthma.

சீரக கற்பம்

போசன குடாரியைப் புசிக்கில்நோ யெலாமறுங்

காசமி ராதக் காரத்தி லுண்டிட

(தேரன் - வெண்பா)

Cuminum seeds separately taken directly all diseases will cure. Just add to Palm sugar then taken will cure the cough disease.

Therapeutic Uses:

Cumin seeds are largely used as a condiment or space in curries, pickles, etc., i.e, in cookery they are medically useful in hoarseness of voice, dyspepsia and chronic diarrhea, the dose is from 10 to 30 grains.

Seeds are also cooling in effect and therefore form an ingredient of most prescriptions for gonorrhea chronic diarrhea and dyspepsia, externally they are applied in the form of poultice to allay pain and irritation of worms in the abdomen; seeds reduced to powder, mix with honey salt and clarified butter are applied to scorpion bite. Seeds mixed with lime juice are administered in cases of bilious nausea in pregnant female and cumin seeds taken internally shortly after child birth increases secretion of milk.

A quantity of seeds lightly smeared with ghee, put in to pipe and smoke relief the hiccup. a confection called Jirakady modaka is prepared thus : - take of the three myrobalans tubers of cyperus rotundus , watery extract of gulancha , prepared talc, flowers of mesua ferrea , leaf called Thejapatra.

Other preparation of Seerakam

1. Keelanelli Karkam

Dosage : 10-30 gm

Indication : Jaundice ,Liver diseases

2. Mullangi Chooranam

Dosage : Thirikadi Piramanam

Indication : Diarrhoea, peptic ulcer, Abdominal pain and gastritis, indigestion

3. Bhaskaralavana chooranam

Dosage : ¼ rubai wt

Indication : Pitha disease, indigestion, flatulence , loss of appetite

4.Sharasvatha chooranam

Dosage : Arai kal Rubai wt.

Indication : Increase memory power

5. Nannari Mathirai

Dosage : 1 (130 mg) tablet bd

Indication : gastritis , burning micturation, insominia

6. Seeraga Legium

Dosage : 5-10 gm Piramanam

Indication : Indigestion,loss of appetite, pitha and associated diseases

7. Ingi Legium

Dosage : 5-10 gm Bd

Indication : Cure for Pitha diseases, Control for Diarrhoea, Indigestion

3.4.2. BOTANICAL ASPECTS

Cuminum cyminum, Linn

Scientific classification:

Kingdom	:	Plant
Class	:	Dicotyledonae
Subclass	:	Polypetalae
Family	:	Umbelliferae
Genus	:	Cuminum
Species	:	Cyminum
Botanical Name:		Cuminum cyminum, Linn

Vernacular Names:

Tamil	:	Seeragam	German	:	Kreuzkummel
Bengali	:	Jira	Burma	:	ziya
English	:	Cumin	Arabic	:	Kamuna
Hindi	:	Zira	Gujarathi	:	Jiru
Marathi	:	Jiraghi	Syria	:	Kemun
Telugu	:	Jiraka			
Urdu	:	Jirah			

Identification of the Family:

Leaves have sheathing bases. Stems hollow. Inflorescence umbels. The involucres of bracts stand out prominently, G (2). Ovary inferior, two chambered with one pendulous anatropous ovule in each loculus. Fruit cremocarp and odoriferous.

(Genera about 270: species about 2700)

Identification of the plant:

The slender annular herb about 1 feet height with a much branched angular or striated stems bearing 2 or 3 particle linear leaves twice or thrice 3 partite, ultimate segments filiform. Umbels compound, rays few, bracts and bracteoles, several, linear, rigid, calyx-teeth small, subulate, unequal. Petals oblong or obovate, emarginate, white often unequal fruits cylindrical.

Description:

Habitat:

It is cultivated throughout the temperate, sub-tropical regions like in India, Persia and Afghanistan.

Habit:

A small slender,erect and annual herb about with the much branched angular and striated stem.

Leaves:

2 or 3 ,bipinnate,dissected,ultimate segments linear,filiform,sheaths white margined.

Inflorescence:

In compound umbels.

Flowers:

Bisexual, regular, actinomorphic epigynous, white in terminal leaf opposed, few rayed.

Calyx:

Calyx teeth small on the inferior ovary

Corolla:

5 petals at various sizes, free, yellow in color

Androecium:

5 stamens, free

Gynoecium:

Bicarpellary, syncarpous pistil, inferior ovary, 2 chambers, one ovule in each chamber.

Fruit:

The fruits are greyish about ¼ inch long, tapering towards both base and apex and compressed laterally with ridges covered by pupillose hairs. The hairs may be absent in some forms.

Chemical Constituents:

The chief constituent is cumaldenyde C_{10} H_{12} O (P-iso-propyl benzaldehycte) which forms nearly 20 to 40% of the oil. Besides the aldehyde, the oil contains P-cymene, Pinene, dipentene, cumene, cuminic alchohol, B-phellandrene and –terpenol.

Seeds analysis carbohydrates 36.3%, moisture 11.9, protein 18.7 fibre 12, calcium 1.08, phosphorous 0.49%, Iron 31mg/100gms, Vitamin A-870 I.u/100g and vitamin C 3mg/100g.

Therapeutic uses:

Cumin seeds are aromatic and spicy, extensively used as condiment. In digenous medicine, cumin seeds hare long been considered stimulant and carminative, stomachic, astringent and useful in diarrhoea and dyspepsia

3.4.3. LATERAL RESEARCH

Effects of the fruit essential oil of *Cuminum cyminum* Linn. (Apiaceae) on pentylenetetrazol-induced epileptiform activity in F1 neurones of *Helix aspersa*

Abstract

The effect of the fruit essential oil of *Cuminum cyminum* Linn. (Apiaceae) (*syn. Cuminum odorum* Salisb) on the epileptiform activity induced by pentylenetetrazol (PTZ) was evaluated, using intracellular technique. The results demonstrated that extracellular application of the essential oil of *Cuminum cyminum* (1% and 3%) dramatically decreased the frequency of spontaneous activity induced by PTZ in a time and concentration dependent manner. In addition it showed protection against pentylenetetrazol-induced epileptic activity by increasing the duration, decreasing the amplitude of after hyperpolarization potential (AHP) following the action potential, the peak of action potential, and inhibition of the firing rate. These membrane effects suggest cellular mechanisms by which the essential oil of *Cuminum cyminum* can inhibit the PTZ-induced epileptic activity.

Keywords

- Cuminum cyminum*;*
- Anticonvulsant effect;
- Pentylenetetrazol;
- Current clamp

3.5.GUNAPADAM ASPECT

3.5.1. அதிமதுரம்

(*Glycyrrhiya glabra*)

Other Name :

Athingam, Atti, Mathookam, Kunriver

Habitat :

Arabia, Persian gulf , Afghanistan, Turkestan, Asia minor , Siberia etc., but the root is cultivated in the Punjab, Sub Himalayan tracts from the Chenab east wards, Sinthu, Peshawar valley , Burma and Andaman Island

Part Used:

Peeled Root

Taste	:	Sweet
Character	:	Thatpam (Coolant)
Class	:	Sweet

Actions:

- Emollient
- Demulcent
- Mild Expectorent
- Laxative
- Tonic

General Characters: (Pothu gunam)

கத்தியரி முப்பிணியால் வருபுண் தாகங்
கண்ணோய்உடன் மாதம்விக்கல் வலிவெண்குட்டம்
பித்தமெலும் புருக்கி கிரிச்சரம் ஆவர்த்த
பித்தமத மூர்ச்சை விட பாகம் வெப்பந்
தத்திவரு வாதசோணிதங்காமாலை
சருவவிடங் காமியநோய் தாது நட்டங்
குத்திருமல் ஆசியங்கம் இதழ்நோய் இந்து
குயப்புணும்போம் மதுாகமெனக் கூறுங் காலே. *(தேரன் குணவாகடம்)*

It cures diseases like hiccup, vitiligo, eye disease, bone, thristiness, Burning Micturation, toxicity and etc.

Therapeutic Uses:

Roots also used in scorpion sting . root in infusion decoction , extract or lozenge is useful as a demultent in inflammatory affection or irritable condition of the bronchial tube bowels and catarrh of the Genito Urinary passages as cough hoarseness sore throat, asthma, dysuria etc., also used as a tonic and as slight laxative.

It is much used as adjunct in pharmaceutical preparation as compound decoction and tincture of aloes , compound mixture and confection of Senna etc., also used for flavouring infusion , lozenges , oil and ghritas. Liquid extract is especially in the disguising the bitter or acid taste of many nauseous drugs. Particularly senna (leaf), aloes, Chloride of Ammonium, Senega, Hyocyamus, turpentime etc and sweeten tobacco.

Other preparation of Athimathuram

1. Thapa Sura Kiyalam

Dosage : 10 ml Children, 30ml adult

Indication : Thrist, vomiting

2. **Drashathi chooranam**

Dosage : Thirikadi piramanam

Indication : Urinary Track Infection, Burning Micturation,

3**. Athimathura Chooranam**

Dosage : Thirikadi piramanam

Indication : Vomiting, Nausea, giddiness, bitterness in mouth, pitha disease.

4.**Irumal Mathirai**

Dosage : 1 (500mg) tablet 4 hours once

Indication : Cough

5**. Nellikai Legium**

Dosage : 5- 10 gm

Indication : Renal disease, Immune power, general tonic, vomiting, jaundice, Anemia, Palpitation. Giddiness

3.5.2. BOTANICAL ASPECT

Glycyrrhiya glabra

Scietific Classification

Kingdom	:	Plantae
(unranked)	:	Angiosperms
(unranked)	:	Eudicots
(unranked)	:	Rosids
Order	:	Fabales
Family	:	Fabaceae
Subfamily	:	Faboideae
Tribe	:	Galegeae
Genus	:	Glycyrrhiza
Species	:	G. glabra
Binomial name	:	Glycyrrhiza glabra

Vernacular Name

Common name	:	Licorice, Liquorice, Sweetwood
Hindi	:	Jethi-madh, kubas-susa, mithilakdi
Kannada	:	Atimadhura, jestamaddu
Malayalam	:	Atimadhuram, erattimadhuram
Marathi	:	Jashtimadh
Sanskrit	:	Jalayashti, klitaka, madhu, madhu-yashtikam
Tamil	:	Adimaduram
Telugu	:	Athimathuram
Urdu	:	Mulhatti, mulathi

Habitat:

Dry, open scrubland, damp ditches or near streams; often in soils with high nitrogen content.

Description

It is a herbaceous perennial, growing to 1 m in height, with pinnate leaves about 7–15 cm (2.8–5.9 in) long, with 9–17 leaflets. The flowersare 0.8–1.2 cm (1⁄3–1⁄2 in) long, purple to pale whitish blue, produced in a loose inflorescence. The fruit is an oblong pod, 2–3 cm (3⁄4–1 1⁄6 in) long, containing several seeds. The roots are stoloniferous.

Chemistry

The scent of liquorice root comes from a complex and variable combination of compounds, of which anethole is up to 3% of total volatiles. Much of the sweetness in liquorice comes from glycyrrhizin, which has a sweet taste, 30–50 times the sweetness of sugar. The sweetness is very different from sugar, being less instant, tart, and lasting longer.

The isoflavene glabrene and the isoflavane glabridin, found in the roots of liquorice, are phytoestrogens.

Cultivation and uses

Liquorice, which grows best in well-drained soils in deep valleys with full sun, is harvested in the autumn two to three years after planting.Countries producing liquorice include India, Iran, Afghanistan, the People's Republic of China, Pakistan, Iraq, Azerbaijan, Uzbekistan, Turkmenistan, and Turkey.

The world's leading manufacturer of liquorice products is M&F Worldwide, which manufactures more than 70% of the worldwide liquorice flavours sold to end users.

Medicine

Glycyrrhizin has also demonstrated antiviral, antimicrobial, anti-inflammatory, hepatoprotective, and blood pressure-increasing effects in vitro and in vivo, as is supported by the finding that intravenous glycyrrhizin (as if it is given orally very little of the original drug makes it into circulation) slows the progression of viral and autoimmune hepatitis. In one clinical trial liquorice demonstrated promising activity, when applied topically, against atopic dermatitis. Additionally, liquorice may be effective in treating hyperlipidaemia (a high amount of fats in the blood).] Liquorice has also demonstrated

efficacy in treating inflammation-induced skin hyperpigmentation. Liquorice may also be useful in preventing neurodegenerative disorders and dental caries.

The antiulcer, laxative, antidiabetic, anti-inflammatory, immunomodulatory, antitumour and expectorant properties of liquorice have been investigated.

The compound glycyrrhizin (or glycyrrhizic acid), found in liquorice, has been proposed as being useful for liver protection in tuberculosis therapy, but evidence does not support this use, which may in fact be harmful.

Known hazards:

It has been reported that excessive liquorice consumption can lead to cardiac dysfunction and severe hypertension.

3.5.3. LATERAL RESEARCH

In vitro and in vivo neuroprotective effect and mechanisms of glabridin, a major active isoflavan from *Glycyrrhiza glabra* (licorice)

Abstract

Stroke is a life-threatening disease characterized by rapidly developing clinical signs of focal or global disturbance of cerebral function due to cerebral ischemia. A number of flavonoids have been shown to attenuate the cerebral injuries in stroked animal models. Glabridin, a major flavonoid of ***Glycyrrhiza glabra*** (licorice), possesses multiple pharmacological activities. This study aimed to investigate whether glabridin modulated the cerebral injuries induced by middle cerebral artery occlusion (MCAO) in rats and staurosporine-induced damage in cultured rat cortical neurons and the possible mechanisms involved. Our study showed that glabridin at 25mg/kg by intraperitoneal injection, but not at 5mg/kg, significantly decreased the focal infarct volume, cerebral histological damage and apoptosis in MCAO rats compared to sham-operated rats. Glabridin significantly attenuated the level of brain malonyldialdehyde (MDA) in MCAO rats, while it elevated the level of two endogenous antioxidants in the brain, i.e. superoxide dismutase (SOD) and reduced glutathione (GSH). Co-treatment with glabridin significantly inhibited the staurosporine-induced cytotoxicity and apoptosis of cultured rat cortical neurons in a concentration-dependent manner. Consistently, glabridin significantly reduced the DNA laddering caused by staurosporine in a concentration-dependent manner. Glabridin also suppressed the elevated Bax protein and caspase–3 proenzyme and decreased bcl–2 induced by staurosporine in cultured rat cortical neurons, facilitating cell survival. Glabridin also inhibited superoxide production in cultured cortical neurons exposed to staurosporine. These findings indicated that glabridin had a neuroprotective effect via modulation of multiple pathways associated with apoptosis. Further studies are warranted to further investigate the biochemical mechanisms for the protective effect of glabridin on neurons and the evidence for clinical use of licorice

Keywords

- Glabridin
- Stroke

3.6.GUNAPADAM ASPECT

3.6.1. சதகுப்பை

(***Anethum graveolens***)

Other Name :

Shoyikkeerai vithai, Mathurikai

Habitat :

Cultivated in Indian Gardens for Culinary Purpose " As the fruit of Indian Variety is much more narrowly winged then the variety met with in Europe, Its is considered by same to belong to a distinct specie anitham sowa or peudecanum sowa

Part Used:

Leaf, Flower and Seed

Taste	:	Sweet and Spicy
Character	:	Veppam (Hot potency)
Class	:	Spicy

Actions:

- Stimulant
- Carminative
- Deobstruent
- Diuretic
- Emmenagogue
- Stomachic
- Antispasmodic

General Characters: (Pothu gunam)

வாதமொடு சூதிகா வாதம் சிரசுநோய்
மோதுசெவி நோய்கப நோய் மூடுசுரம் - ஓதுகின்ற
மூலக்கடுப்பு முதிர்பினசம் போகும்
ஞாலச் சதகுப்பை நாடு. *(அகத்தியர் குணவாகடம்)*

It cures bleeding disorder, headache, Earpain , Rhinitis, and etc., Strengthens the liver, lung and stomach

Therapeutic Uses:

Essential oil contain in the fruit and distilled water of the fruit are much used in flatulence , hicc up and colic and abdomen pain in children and adult. It may combined with Na_2co_3 or little of lime water in hiccup and flatulence.

It is used to diminish the griping of purgatives , and the tormina of dysentery . and infusion of the bruised fruits or seeds (1 in 30) is also very useful. Seeds bruied and boiled in water and mixed with the roots are applied externally in rheumatic and others swelling of the joints. Seeds are used as a worm remedy especially in horses.

Leaves are moistened with little oil and warmed and applied to the boils and abscesses to hasten suppuration .

Other preparation of Sathakuppai

1. Panjakavviya Legium

Dosage : 5-10 gm bd

Indication : Menorrhoghea, Gonococcal arthritis ,
Peptic ulcer, gastritis,

2. Masha thylam

Indication : Tremor in Head and hands

3. Panjakavviya kirutham

Dosage : 5 -10 ml bd

Indication : Menorrhoghea, Gonococcal arthritis , Peptic ulcer,
gastritis, Anemia, Jaundice, Ascities

4.Karpurathi Chooranam

5. Poosanikai Nei

6. Soothaga vayu pattai legiyam

3.6.2. BOTANICAL ASPECT

Anethum graveolens

Scientific classification

Kingdom	:	Plantae
(unranked)	:	Angiosperms
(unranked)	:	Eudicots
(unranked)	:	Asterids
Order	:	Apiales
Family	:	Apiaceae
Genus	:	Anethum L.
Species	:	A. graveolens
Binomial name	:	Anethum graveolens L.

Vernacular Names

Sans	:	misroya
Eng	:	Dill
Hind	:	sowa
Punj	:	soya
Maha	:	shepu
Guj	:	surva-nu-bi
Telu	:	shataupivittulu
Tamil	:	satakuppi
Mal	:	chatukuppa
Can	:	sabbasige
Singh	:	sadakuppa
Malay	:	adaspudus

BOTANICAL DESCRIPTION

Anethum graveolens L. is the sole species of the genus Anethum, though classified by some botanists in the related genus Peucedanum as Peucedanum graveolens (L.) A variant called east Indian dill or Sowa(Anethum graveoeloens var sowa Roxb. ex, Flem.) occurs in India and is cultivated for its foliage as a cold weather crop throughout the Indian sub-continent, Malaysian archipelago and Japan.

Plant description

Anethum grows up to 90 cm tall, with slender stems and alternate leaves finally divided three or four times into pinnate sections slightly broader than similar leaves of fennel. The yellow flower develops into umbels.The seeds are not true seeds. They are the halves of very small, dry fruits called schizocarps. Dill fruits are oval, compressed, winged about one-tenth inch wide, with three longitudinal ridges on the back and three dark lines or oil cells (vittae) between them and two on the flat surface. The taste of the fruits somewhat resembles caraway. The seeds are smaller, flatter and lighter than caraway and have a pleasant aromatic odor.

Cultivation

Dill prefers rich well-drained, loose soil and full sun. It tolerates a pH in the range 5.3 to 7.8. It requires warm to hot summers with huge sunshine levels; even partial shade will reduce the yield substantially. The plant quickly runs into seeds in dry weather. It often self sows when growing in a suitable position. Propagation is through seeds. Seeds are viable for 3–10 years. The seed is harvested by cutting the flower heads off the stalks when the seed is beginning to ripe.

Chemical content:

Volatile oil, dillanoside, xanthone glucoside, coumarins, scopoletin, esculetin, bergapten, umbelliferone, umbelliprenine, daempferol, 3-gluucuronide, vicenin 6, 8-di-C-glucosyl-5.7.3′-trihydroxyflavone, flavonoids, petroselinic acid triglyceride, B-sitosterol glucoside, phenolic acids,caffeic, ferulic, and chlorogenic, carvone, d-limonene, ax-phellandrene, dihydrocarvone, eugenol, B-phellandrene, ax-pinene, anethole, dillapiole, myristicin, carveol, B-caryophellene, ax-phellandrene, limonene, terpinene, ax-pinene, dillapiole, myristicin, coumarans.

Medicinal uses

Anethum is used as an ingredient in gripe water, given to relieve colic pain in babies and flatulence in young children. The seed is aromatic, carminative, mildly diuretic, galactogogue, stimulant and stomachic. The essential oil in the seed relieves intestinal spasms and griping, helping to settle colic. The carminative volatile oil

improves appetite, relieves gas and aids digestion. Chewing the seeds improves bad breath. *Anethum* stimulates milk flow in lactating mothers, and is often given to cattles for this reason. It also cures urinary complaints, piles and mental disorders.

Sathakuppai use for		**% Daily Value***	
Total Fat 15 g			23%
Saturated fat 0.7 g			3%
Polyunsaturated fat 1 g			
Monounsaturated fat 9 g			
Cholesterol 0 mg			0%
Sodium 20 mg			0%
Potassium 1,186 mg			33%
Total Carbohydrate 55 g			18%
Dietary fiber 21 g			84%
Protein 16 g			32%
Vitamin A	1%	Vitamin C	35%
Calcium	151%	Iron	90%
Vitamin D	0%	Vitamin B-6	15%
Vitamin B-12	0%	Magnesium	64%

*Per cent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs.

3.6.3. LATERAL RESEARCH

Evaluation of Safety and Protective Effect of Combined Extract of Cissampelos pareira and Anethum graveolens (PM52) against Age-Related Cognitive Impairment

Abstract

The present study aimed to determine acute toxicity, the protective effect, and underlying mechanism of PM52, a combined extract of Cissampelos pareira and Anethum graveolens, against age-related cognitive impairment in animal model of age-related cognitive impairment. PM52 was determined as acute toxicity according to OECD guideline. Male Wistar rats, weighing 180–220 g, were orally given PM52 at doses of 2, 10, and 50 mg/kg at a period of 14 days before and 7 days after the bilateral administration of AF64A via intracerebroventricular route. All animals were assessed according to spatial memory, neuron density, MDA level, the activities of SOD, CAT, GSH-Px, and AChEI effect in hippocampus. It was found that all doses of PM52 could attenuate memory impairment and neurodegeneration in hippocampus. The possible mechanisms might occur via the suppression of AChE and the decreased oxidative stress in hippocampus. Therefore, our data suggest that PM52 may serve as food supplement to protect against age-related cognitive impairment such as mild cognitive impairment (MCI) and early phase of Alzheimer's disease. However, further researches are still essential.

3.7. GUNAPADAM ASPECT

3.7.1. தாமரை

(***Nelumbo nucifera***)

Other Name :

Aravintham, Ellimanai, Sooriynatpu, Ponmanai, Vintham, Pundareegam, Pathumam, Kamalam, Nalinam, Mulari, Mundagam, Malunthi, Sarogam, Koganagam, Indai, Kanjam, Appusam, Amporagam, Chalasam, Vanasam, Varisam, Saraseerugam, Pankerugam, Sarorugam, Pankasam

Habitat :

This large aquatic herbs with its elegant sweet scented flowers is generally met with in tanks and ponds throughout India.

Part Used:

Flower, Seed, Tuber

Taste	:	Sweet and Astringent
Character	:	Thatpam (Coolant)
Class	:	Sweet

Actions:

- Cooling
- Astringent
- Expectorant
- Sedative
- Demulcent
- Tonic
- Nutrient

General Characters: (Pothu gunam)

ஈரலைப் பற்றிமிக ஏறுகின்ற வெப்பமும்போம்
கோர மருந்தின் கொடுமையறும் - பாருலகில்
தண்டா மணத்தையுள்ள தாழ்குழலே காந்தல்விடும்
வெண்டா மரைப்பூவால் விள்.

Therapeutic Uses:

Flower and filaments and juice of flower stalk are useful in diarrhea , cholera and in liver complaints and also in fevers; it is recommended also as cardiac tonic . compound decoction it is useful in bilious fevers . “the root, flower, stalk and leaves in the form of infusion are used in fever as a refrigerant and diuretic” (Chopra). Honey formed in the flowers by the bees feeding upon the padma is called padma mathu or maharantha. This is very useful in eye disease. Syrup of flower is used in cough to check of Hemorrhoid from bleeding piles and Menorhogia and dysentery . Tubes of white lotus boiled in gingili oil are rubbed on the head to cool head and eyes. Express juice is also employed instead of speice of tuber. Root is mucilaginous and even in piles.

Seeds are used as an application in leprosy other skin affection. Lotus flower and fresh leaves ground with sandal wood and or embolic myrobalans also form a cooling application to the forehead in the cephalalgia , to the skin in erysipelas and cure other external inflammation.

Other Preparation of Venthamarai

1. Venthamarai Kudineer

Dosage : 15- 30 ml

Indication : Cardiac Tonic

2. Neithal Nei

3. Soodamani Mathirai

4. Aruganver Kizhangu

3.7.2. BOTANICAL ASPECTS

Nelumbo nucifera

Scientific classification:

Botanical Name(s)	:	Nymphaea Lotus, Nymphaea Nouchali
Family Name	:	Nymphaeaceae
Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Order	:	Nymphaeales
Family	:	Nymphaeaceae
Genus	:	Nymphaea
Species	:	N. lotus

Vernacular Names:

English	:	Sacred lotus
Hindi	:	Kanwal, Kamal
Sanskrit	:	Ambuja
Tamil	:	Ambal, Thamarai, Padma, Pankaja, Kamala;
Bengal	:	Padma
Gujarat	:	Suriyakamal
Malayalam	:	Tamara
French	:	Nelumbo
German	:	Indische lotosblume
Persian	:	Nilufer.

Habitat:

Throughout warmer parts of India, in tanks, ponds and ditches

Description:

A species of water lily, white lotus is a perennial plant growing to a height of 45 cm. Also known as tiger lotus, it grows in clear, warm, still and slightly acidic waters. The lily pads can be seen floating on water, while the blossoms rise above the water. The

flowers are white in color sometimes, with a pink tinge. The leaves vary from green to red-brown, with a number of purple spots. Tiger lotus is native to the Nile and is grown in various parts of East Africa and Southeast Asia. It is often used as an aquarium plant.

Plant Chemicals

Apomorphine, nuciferine, phytosterols, bioflavonoids, phosphoiesterase, glucose, fructose, sucrose, mannitol, galacturonic acid, raffinose, amoni acids.

Uses & Benefits of White Lotus

- White lotus was used in ancient Egypt, as a key to good health, sex and re-birth.
- The plant is an aphrodisiac for both men and women and a general remedy for all kind of illnesses.
- Continued use of tiger lotus enhances sexual vigor and general good health.
- It is a tonic richer than ginseng, pain reliever richer than arnica, circulation stimulant richer than ginkgo biloba and sexual stimulant richer than Viagra.
- The flowers of white lotus are used for preparing tea that creates a warm, euphoric glow.
- The dried flowers are smoked by themselves or mixed with other herbs to add flavor to smoking mixtures.
- The effects of tiger lotus are enhanced when soaked in wine or other alcohol.
- The plant is effectively used to increase memory and create a feeling of euphoria and ecstasy, without the use of narcotics.
- Its rhizomes are cooling, sweet, bitter and tonic and used in diarrhea, dysentery, dyspepsia and general debility.
- White lotus is used internally in treatment of gastrointestinal disorders and jaundice.
- The leaves are used in cutaneous, subcutaneous parasitic infection, eye treatments and pregnancy.
- The seeds are used in sauces, condiments, spices and flavorings.

(ref: Read more at http://www.iloveindia.com/indian-herbs/nymphaea-lotus.html#BWgzZFJgGWzLfS2k.99)

Chemical constituents

Flower

Several flavonoids have been identified in the stamens of *N.nucifera* . These include kaempferol and seven of its glycosides: kaempferol 3-O- β -D-galactopyranoside, kaempferol 3-O-β-D-glucopyranoside , kaempferol 7-O-β -D- glucopyranoside , kaempferol 3-O-a-L-rhamnopyranosyl-(1-6)-β -D-glucopyranoside , kaempferol 3-O-a-L-rhamnopyranosyl-(1-2)- β -D-glucopyranoside, kaempferol 3-Oa-L-rhamnopyranosyl -(1-2)- β- D-glucurono-pyranoside , kaempferol-3- O- β- D-glucurono-pyranoside , kaempferol 3-O-β-D-glucuronopyranosyl methylester , myricetin 3 0 ,5 0 -dimethylether 3-O- β -D-glucopyranoside , quercetin 3-O- β -D-glucopyranoside , nelumboroside A and nelumboroside B.

It also contains two isorhamnetin glycosides: isorhamnetin 3-O-β-D-glucopyranoside and isorhamnetin 3-O-a-L-rhamnopyranosyl- (1→6) -β -D-gluco-pyranoside . Some non-flavonoid compounds, including adenine, myo-inositol, arbutin and β-sitosterol glucopyranoside , have also been identified in stamen extract

PHARMACOLOGICAL ACTIVITIES

Flower

I. *Hypoglycaemic activity*

Sun-dried flower powder of *N.nucifera*, as well as the aqueous and alcoholic extract of the flower, produced significant hypoglycaemia in fasting normal albino rabbits. There was no significant difference in the activities of 1000 mg/kg of the test drug (sun-dried powder of the flower) and equivalent amounts of the extracts. Glucose tolerance studies with normal rabbits showed that oral doses of both extracts, equivalent to 1000 mg/kg of the test drug, produced significant depression of the peak rise in fasting blood sugar after glucose load; the effects of both extracts were 50% to that produced by 250 mg/kg of tolbutamide. A study of glucose tolerance curves shows that the duration of hyperglycaemia was also notably reduced compared with the controls. In normal rabbits, the extract at a dose of 1000 mg/kg significantly lowered hyperglycaemia induced by subcutaneous injection of 0.5 mg/kg adrenaline hydrochloride.

II. ***Antioxidant activity***

The potential of *N. nucifera* stamens to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals and peroxynitrites (ONOO–), and the inhibition of total ROS generation by kidney homogenates using 2v,7'-dichlorodihydrofluorescein diacetate (DCHF-DA) was examined.The methanol extract showed strong antioxidant activity in the ONOO– system and marginal activity in the DPPH and total ROS systems. Similarly, seven known flavonoids were isolated from lotus stamens, most of which also showed potent antioxidant activity. The glycosides nelumboroside A, nelumboroside B, isorhamnetin glycoside and isorhamnetin rutinoside isolated from N. nucifera stamens showed potent antioxidant activity in DPPH and ONOO– assays.[45] 'Yunyupju', a liquor made from the blossoms and leaves of lotus, has been reported to have antioxidant activity. The maximum scavenging activity on hydroxyl radicals (40%) could be achieved when lotus liquor was more than 500 μg. Lotus liquor also has a potent superoxide radical scavenging activity with value of 0.93 unit mg^{-1} as superoxide dismutase equivalents with an IC50 value of 1.07 ± 0.04 mg.

III. ***Antipyretic activity***

The ethanol extract of stalks of *N. nucifera* was evaluated for its antipyretic potential on normal body temperature and yeast induced pyrexia in rats. In the model of yeast provoked elevation of body temperature, the stalk extract showed significant activity in both models at oral doses of 200 and 400 mg/kg. The stalk extracts showed dose-dependent lowering of body temperature up to 4 h; the results were comparable to those with paracetamol.

IV. ***Aldose reductase inhibitory activity***

Two glycosides, namely kaempferol 3-O-a-L-rhamnopyranosyl-(1,6)-_-D-glucopyranoside and isorhamnetin 3-O-a-Lrhamnopyranosyl-(1!6)-_-D-glucopyranoside, isolated from the methanol extract of stamens of *N. nucifera* exhibited a high degree of inhibitory activity against rat lens aldose reductase *in vitro*, with IC50 values of 5.6 and 9.0 mM, respectively.

V. *Antibacterial Activity*

The hydroethanolic extract of flowers of *N.nucifera* Gaertn *in vitro* was reported to possess antibacterial activity. The antibacterial activity was screened against different bacterial strains like Escherichia coli Klebsiella pneumonia Pseudomonas aeruginosa Bacillus Subtilis Staphylococcus aureus by detecting zone of inhibition and minimum inhibitory concentration (MIC). The maximum zone of inhibition was exhibited by *N.nucifera* flowers against Escherichia coli (14mm), Bacillus Subtilis (13mm) and Staphylococcus aureus (11mm). The moderate zone of inhibition was found against Klebsiella pneumonia (10mm) and Pseudomonas aeruginosa (8mm). Gram-negative bacteria were more susceptible to the *N.nucifera* flower extracts than gram-positive bacteria which contradict the previous reports that plant extracts are more active against gram-positive bacteria than gram-negative bacteria. These results were compared with the standard antibiotic chloramphenicol (30µg/ml)

VI. *Antiplatellet activity*

The antiplatelet activity of hydroethanolic extract of N.Nucifera flowers using platelet-rich plasma in different concentrations (100 500µg/ ml) was reported. N.nucifera flower extracts showed dose-dependent effective antiplatelet activity with maximum activity at 500µg/ml concentration; prevention of platelet aggregation was 50% of that achieved with standard aspirin.

(ref: Nishkrati R . Mehta , Nelumbo nucifera (Lotus) A review on Ethanobotany Phytochemistry and Pharmacology (IJPBR) 2013,1(4):152-167)

3.7.3. LATERAL RESEARCH

Effects of *Nelumbo nucifera* Rhizome Extract on Cell Proliferation and Neuroblast Differentiation in the Hippocampal Dentate Gyrus in a Scopolamine-induced Amnesia Animal Model

Abstract

A large aquatic herb, *Nelumbo nucifera* Gaertn, has psychopharmacological effects similar to minor tranquillizers and antistress agents. This study examined the effects of *Nelumbo nucifera* rhizome extracts (NRE) on cell proliferation and neuroblast differentiation in the hippocampal dentate gyrus (DG) of a rat model of scopolamine-induced amnesia. Immunohistochemical markers included Ki67, an endogenous marker for active cell cycle, and doublecortin (DCX), a marker for immature neurons and migratory neuroblasts. Scopolamine was administered for 28 days via an ALzet minipump (44 mg/mL delivered at 2.5 µL/h). NRE was administered by gavage, 1 g/kg per day for 28 days. The administration of scopolamine significantly reduced the number of Ki67- and DCX-immunoreactive cells in the DG, whereas scopolamine did not induce any significant changes in mature neurons in the DG. The administration of NRE significantly ameliorated the scopolamine-induced reduction of Ki67- and DCX-immunoreactive cells in the DG. In addition, the administration of NRE significantly restored the scopolamine-induced reduction of brain-derived neurotrophic factor in DG homogenates. These results suggest that NRE can ameliorate the scopolamine-induced reductions of cell proliferation, neuroblast differentiation and BDNF levels. Copyright © 2010 John Wiley & Sons, Ltd.

3.8. PHARMACEUTICAL REVIEW

CHOORANAM

Definition:

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

Equipment Required:

1. A mortar and pestle
2. A fine sieve or fine cloth of close mesh (sieve no:100 mesh)

Note:

In large scale manufacture, in factories comminutors, pulverisers and ball mills are employed for powdering. Sieving is performed by mechanical sifters which handle large quantities of drugs in a short time.

Process of preparation:

The drugs enumerated in the recipe are clean and in well dried state.

The drugs which are to be used in the preparation should be taken from recently collected material.

However, drugs like embelia fruits, senna, long pepper, jiggery and cow's ghee are prepared from fairy 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 so find as to be called amorphous and should be never damp. The fineness of the sieve should be 100 mesh or still finer.

Storage:

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

The powder (*Chooranam*) is said to retain its potency for two months and then gradually deteriorate. However if properly packed, and stored they keep good for a year. The *chooranam* to facilitate easy handling and to assure exact dosage of administration could be pressed into tablets with the addiction of a suitable binder. These tablets could be packed in bottles or tubes made either of glass or plastics or packed in strips of metal foil or plastic sheets. In industry, the tablets are made, counted and packed by electronic devices.

3.9. DISEASE VIEW

3.9.1. SIDDHA ASPECT

KURUTHI AZHAL NOI (HYPERTENSION)

Ratha Pitham

As the name suggests the disease is due to excessive production of blood and heat in the body. In this disease, due to internal and external variations the heat is aggravated; this causes blood to become impure; this impure blood does not flow in its usually pathway but oozes out from the eye, nose, mouth , anus, vagina, urinary tract and skin and makes it as dangerous disease.

Usually, doshas stinulate the diseases. However , while naming this disease the dosha was not named first but blood has been named first. This is because it is only the blood which ecposed the mailfunctioned heat and hence blood was named first and the dosha (Heat) was named next.

Aetiopathogenesis

"வேர்வை யதனாலே மேலாகுந் தாதுக்களில்
ஆர்வுறப் பித்தமதன் வீரன் - சார்வாகிப்
பின்னர்ப் பெருகி பொருந்துன்ப மீந்துடலில்
மண்ணுவதே யாமென்னல் மாண்பு" - (*கையெழுத்துப்பிரதி)*

"அன்றியுளம் வாட்டம் யருவருப்புத் தொண்டை
துன்னுள் புண்போறல் தன் கண்முதல் - மனனுமுறுப்
பெருகுஞ்செம்மை மஞ்சளேற்ற பச்சையொன்றிவற்றில்
தங்குதலு மாமென்று சாற்று" - (*கையெழுத்துப்பிரதி)*

The following factors may be responsible for producing the disease; Excessive wandering in the sun rays , eating of food item in excess which generate heat, such as salt, bitter and sour substances, excessive indulgence insex; when yoga for controlling five senses and for protecting the body is not properly practiced; excessive heat dosha may dilute or concentrate the blood due to stimulation of vayu and may produce the disease. Further , due to bad effect of heat, belly, left and right portion of the liver, lungs, urinary bladder, large intestine, small intestine and heart will be damaged; the

impure blood come out from these organs and may produce the disease.

Types of disease:

The disease may be classified into eight types as follows:

1. Vali kuruthiazhal Noi (Vatha blood heat disease)
2. Thee kuruthiazhal Noi (Pitha blood heat disease)
3. Iya kuruthiazhal Noi (Kapha blood heat disease)
4. Vali thee kuruthiazhal Noi (Vathapitha blood heat disease)
5. Vali Iya kuruthiazhal Noi (Vathakapha blood heat disease)
6. Iyavali kuruthiazhal Noi (Kaphavatha blood heat disease)
7. Iyaazhal kuruthiazhal Noi (Kaphapitha blood heat disease)
8. Mukkutra kuruthiazhal Noi (Tri-dosha blood heat disease)
9. Some other group of ancient physicians have classified this disease into four types depending upon the direction of flow of blood as follows:
10. 1.Maelnoakku Kuruthi azhal Noi (Upward directional blood flow)
11. Keelnoakku kuruthiazhal Noi (Downward directional blood flow)
12. Irunoakku kuruthiazhal Noi (Bi directional blood flow)
13. Allavilla noakku kuruthiazhal Noi (Unlimited directional blood flow)
14. It is considered that the above mentioned four types of classification was made to identity the vayu which is responsible for the blood direction and for controlling the same.

Prodromal sysmptom:

Before the disease is clinically apparent the following signs and symptoms may occur. Heaviness of head, anorexia, severe cough, constriction of the skin due to body dryness , excessive salivation , non vegetarian smell from the mouth or ointment smell, severe nausea with vomiting, blood or bile strained vomiting, liking for sour substance and development of vomiting after eating them, burning sensation of throat and ulcers of the mouth. Later the following signs and symptoms may appear; Hoarseness of voice, pinching cough, mild wheezing , ageusia, emaciation; eyes cheek and throat may become yellow, red or green in colour . Finally the patient may lie down on the mat.

1. Vali kuruthiazhal Noi

In the disease the clinical features are similar to that of blood heat disease. In addition the following effects of vatha dosha may also appear; Body pain; the blood may attain black colour and and ooze out in diluted form, sometimes the blood may appear with froath; the stool will be hardened and passed like the stool of goat; painful defecation with blood in the stool.

2. Thee kuruthiazhal Noi

In this disease, the pitha dosha gets aggravated and hence the fire produces various calamities. There will be excessive discharge of blood . As a consequence of excessive activity of pitha the blood will be discoloured , become diluted and the colour will appear like water in which dried leaves is soaked. Sometimes , bile will also be, found mixed with the blood. Due to these the body will be pale and appear yellow in colour. Eyes, tongue and skin etc. will appear green. Sometimes the blood may also appear bright red in colour. Because of mixing of bile with the blood , the body becomes greenish in colour and the patient will appear like jaundiced.

3. Iya kuruthiazhal Noi

In this disease the heart disease is formed because of Iyam . The blood which is discharged will appear slightly pale mixed with excessive mucus . It will smell like meat and becomes hardened . In addition , there will be uncontrolled cough, slight fever and running nose.

4. Vali thee kuruthiazhal Noi

The signs and symptoms of this disease are similar to that of vali kuruthi azhal noi mentioned above. In addition certain signs and symptoms caused by thee dosha may also be formed.

5. Vali Iya kuruthiazhal Noi

The signs and symptoms of this disease are similar to that of vali kuruth azhal disease. In addition , blood will be discharged with some or many of the clinical features of Iya kuruthi azhal disease.

6. Iyavali kuruthiazhal Noi

In this disease, the signs and symptoms will be similar to that of excessive kapha and kuruthi azhal disease. In addition, signs and symptoms of the vatha dosha may also appear.

7. Iyaazhal kuruthiazhal Noi

In this disease, the signs and symptoms will be similar to that of kuruthiazhal and excessive kapha diseases. In additional , some of the signs and symptoms of the zhal dosha may be also found.

8. Mukkutra kuruthiazhal Noi

In this disease, all the three doshas become heperactive at the same time causing serious harm to the body. The signs and symptoms caused because of these, will appear similar to that of sanni disease. In addition, all the signs and symptoms of kuruthiazhal will be also found. This is not an easily curable disease.

General signs and symptoms of the disease:

Because of the activity of the tridosha, there will be cough, vomiting and wheezing. The cough and vomiting will be associated with excessive blood. Sometimes bleeding may be found from the nose, eye, ear, mouth, urinary tract and skin. The body strength will be reduced day by day. The skin may also shrink; it may become dry and may bleed. In view of the blood loss, the body will become pale, breathlessness, tiredness, polydipsia and hiccup may also appear. In additional , there will be swelling of face , upper and lower limbs.

1.Maelnoakku Kuruthi azhal Noi (Upward directional blood heat disease)

In this diseases, due to excessive heat, udhaana vavy is stimulated. This adversely affects the blood resulting in vomiting , cough, sneezing, hiccup and flatulence. There will be vigorour cough followed by vomiting. The vomiting may be stained with fresh frothy blood. If the udhaana vayu also affects abdomen, in association with kapha present there,bit makes the blood dry and causes pain in the abdomen; there may be

vomiting with drak coloured blood clots. If the disease attacks bronchi, left and right portion of the liver, there will be pain in these sites and oozing of blood; as a results there will be fatigue ,stickly sweating dryness of eye and paleness of body.

2. Keelnoakku kuruthiazhal Noi (Downward directional blood heat disease)

In this disease, abaaba vayu becomes hyperactive due to excessive heat. As a result the large intestine, urinary bladder, and ureter which are found below the level of umbilicus are affected. The samaana vayu also becomes hyperactive and produces inflammation of small intestine and kidney. In view of these , there will be excessive diarrhea, frequent urination and heamorrhage. There will be burning pain micturation and itching in the anus with burning pain .

3. Irunoakku kuruthiazhal Noi (Bi directional blood heat disease)

In the disease, the abaana as well as udhaana vayu are stimulated at the same times by the pitha . Hence, the clinical features of both upward and downward directional blood heat diseases will be found. Due to excessive activity of the downward directional blood heat disease , there will be excessive diarrhea, excessive urination with heamorrhage. Due to excessive activity of upward directional blood heat disease, there will be vomiting with blood. In view of the haemorrhage from upper and lower orifices, the body health will be severely affected.

4. Allavilla noakku kuruthiazhal Noi (Unlimited directional blood heat disease)

In this disease, the following four directional factors are stimulated at a time by the fire:

1) Abaanan (downward directional)
2) Udhaanan (upward directional)
3) Samaanan (mid directional)
4) Viyaanan (diffuse directional)

There will be haemorrhage from all the body orifices. Of all the four factors mentioned above, the viyaana vayu is much affected and produces uncontrollable burning sensation in the eye, ear and skin; the blood will ooze from the body and eye will appear yellow in colour.

3.9.2. MODERN ASPECT

HYPERTENSION

Hypertension (**HTN** or **HT**), also known as **high blood pressure** (**HBP**), is a long term medical condition in which the blood pressure in the arteries is persistently elevated. High blood pressure usually does not cause symptoms. Long term high blood pressure, however, is a major risk factor for coronary artery disease, stroke, heart failure, peripheral vascular disease, vision loss, and chronic kidney disease.

High blood pressure is classified as either primary (essential) high blood pressure or secondary high blood pressure. About 90–95% of cases are primary, defined as high blood pressure due to nonspecific lifestyle and genetic factors. Lifestyle factors that increase the risk include excess salt, excess body weight, smoking, and alcohol. The remaining 5–10% of cases are categorized as secondary high blood pressure, defined as high blood pressure due to an identifiable cause, such as chronic kidney disease, narrowing of the kidney arteries, an endocrine disorder, or the use of birth control pills.
Blood pressure is expressed by two measurements, the systolic and diastolic pressures, which are the maximum and minimum pressures, respectively. Normal blood pressure at rest is within the range of 100–140 millimeters mercury (mmHg) systolic and 60–90 mmHg diastolic. High blood pressure is present if the resting blood pressure is persistently at or above 140/90 mmHg for most adults. Different numbers apply to children. Ambulatory blood pressure monitoring over a 24-hour period appears more accurate than office best blood pressure measurement.

High blood pressure affects between 16 and 37% of the population globally. In 2010 hypertension was believed to have been a factor in 18% (9.4 million) deaths.

Signs and symptoms

Hypertension is rarely accompanied by any symptoms, and its identification is usually through screening, or when seeking healthcare for an unrelated problem. Some with high blood pressure report headaches (particularly at the back of the headand in the morning), as well as lightheadedness, vertigo, tinnitus (buzzing or hissing in the ears), altered vision or fainting episodes. These symptoms, however, might be related to

associated anxiety rather than the high blood pressure itself On physical examination, hypertension may be associated with the presence of changes in the optic fundus seen byophthalmoscopy The severity of the changes typical of hypertensive retinopathy is graded from I–IV; grades I and II may be difficult to differentiate. The severity of the retinopathy correlates roughly with the duration and/or the severity of the hypertension

Hypertensive crisis

Severely elevated blood pressure (equal to or greater than a systolic 180 or diastolic of 110) is referred to as a hypertensive crisis. Hypertensive crisis is categorized as either hypertensive urgency or hypertensive emergency, according to the absence or presence of end organ damage, respectively.

In hypertensive urgency, there is no evidence of end organ damage resulting from the elevated blood pressure. In these cases, oral medications are used to lower the BP gradually over 24 to 48 hours

Pregnancy

Hypertension occurs in approximately 8–10% of pregnancies Two blood pressure measurements six hours apart of greater than 140/90 mm Hg is considered diagnostic of hypertension in pregnancy High blood pressure in pregnancy can be classified as pre-existing hypertension, gestational hypertension or pre-eclampsia.

Children

Failure to thrive, seizures, irritability, lack of energy, and difficulty breathing can be associated with hypertension in neonates and young infants. In older infants and children, hypertension can cause headache, unexplained irritability, fatigue, failure to thrive, blurred vision, nosebleeds, and facial paralysis.

Causes

Primary hypertension

Hypertension results from a complex interaction of genes and environmental factors. Numerous common genetic variants with small effects on blood pressure have been identified as well as some rare genetic variants with large effects on blood pressure.

Blood pressure rises with aging and the risk of becoming hypertensive in later life is considerable. Several environmental factors influence blood pressure. High salt intake raises the blood pressure in salt sensitive individuals; lack of exercise, obesity, stress, and depression can play a role in individual cases. The possible role of other factors such as caffeine consumption, and vitamin D deficiency are less clear. Insulin resistance, which is common in obesity and is a component of syndrome X (or the metabolic syndrome), is also thought to contribute to hypertension. Events in early life, such as low birth weight, maternal smoking, and lack of breast feeding may be risk factors for adult essential hypertension, although the mechanisms linking these exposures to adult hypertension remain unclear. An increased rate of high blood urea has been found in untreated people with hypertensive in comparison with people with normal blood pressure, although it is uncertain whether the former plays a causal role or is subsidiary to poor kidney function.

Secondary hypertension

Secondary hypertension results from an identifiable cause. Kidney disease is the most common secondary cause of hypertension. Hypertension can also be caused by endocrine conditions, such as Cushing's syndrome, hyperthyroidism, hypothyroidism, acromegaly, Conn'syndrome or hyperaldosteronism, hyperparathyroidism andpheochrom ocytoma Other causes of secondary hypertension include obesity, sleep apnea, pregnancy, coarctation of the aorta, excessive liquorice consumption and certain prescription medicines, herbal remedies and illegal drugs. Arsenic exposure through drinking water has been shown to correlate with elevated blood pressure.

Pathophysiology

In most people with established essential hypertension, increased resistance to blood flow (total peripheral resistance) accounts for the high pressure while cardiac output remains normal. There is evidence that some younger people with prehypertension or 'borderline hypertension' have high cardiac output, an elevated heart rate and normal peripheral resistance, termed hyperkinetic borderline hypertension. These individuals develop the typical features of established essential hypertension in later life as their cardiac output falls and peripheral resistance rises with

age. Whether this pattern is typical of all people who ultimately develop hypertension is disputed. The increased peripheral resistance in established hypertension is mainly attributable to structural narrowing of small arteries and arterioles, although a reduction in the number or density of capillaries may also contribute. Whether increased active arteriolarvasoconstriction plays a role in established essential hypertension is unclear. Hypertension is also associated with decreased peripheralvenous compliance which may increase venous return, increase cardiac preload and, ultimately, cause diastolic dysfunction.

Pulse pressure (the difference between systolic and diastolic blood pressure) is frequently increased in older people with hypertension. This can mean that systolic pressure is abnormally high, but diastolic pressure may be normal or low — a condition termed isolated systolic hypertension. The high pulse pressure in elderly people with hypertension or isolated systolic hypertension is explained by increased arterial stiffness, which typically accompanies aging and may be exacerbated by high blood pressure.

EPIDEMIOLOGY

Hypertension in India

- High blood pressure (BP) is a major public health problem in India and its prevalence is rapidly increasing among both urban and rural populations. In fact, hypertension is the most prevalent chronic disease in India.
- The prevalence of hypertension ranges from 20-40% in urban adults and 12-17% among rural adults. The number of people with hypertension is projected to increase from 118 million in 2000 to 214 million in 2025, with nearly equal numbers of men and women.
- A survey of 26,000 adults in South India showed a hypertension prevalence of 20% (men 23% and women 17%) but 67% of those with hypertension were unaware of their diagnosis. Majority of hypertensive subjects still remain undetected and the control of hypertension is also inadequate. This calls for urgent prevention and control measures for hypertension.
- Recent (2012) studies show that for every known person with hypertension there are two persons with either undiagnosed hypertension or prehypertension.

- Reducing blood pressure can decrease cardiovascular risk and this can be achieved by lifestyle measures in mild cases and should be the initial approach to hypertension management in all cases. This includes dietary interventions weight reduction, tobacco cessation, and physical activity.
- A large-scale study undertaken in Tamil Nadu recently has established the high prevalence of hypertension in rural India. The study reported 21.4 per cent hypertension prevalence in about 10,500 people aged between 25 to 64 in 11 villages in the State with both sexes being affected equally. It was published in the International Journal of Public Health. Urban areas had a prevalence of hypertension of 22-30 per cent.

Investigation

- ECG
- X-Ray of the Chest
- Echo Cardiography
- Urine shows usually no change except RBC in Malignant hypertension
- Blood Urea may be high in case of malignant hypertension
- Serum Electrolytes
- Ultra Sonogram is also helpful to find out renal disease
- In other tests include IV urogram, Isotope renogram, CT Scan MRI etc.,

Complications:

Cardiac hypertensive Heart Disease

Left ventricular hypertophy develps in 10% to 30% of chronic cases.

Cerebral

Cerebrovascular complicationare more closely related to systeolic rather than diastolic BP

1. Cerebral haemorrhate
2. Cerebral thrombosis
3. Lacunar infarcts
4. Hypertensive encephalopathy
5. TIA
6. Subarachnoid haemorrhage.
7. Dementia - both vascular and Alzheimer's types

Retinal:

1. Dimness of vision
2. Thickening of arteries with narrowing of lumen, haemorrhage and exudates, papiloedea, as metnioned before.
3. Detachment of retina, vitreous haemorrhage

Renal:

1. Renal arteriosclerosis (nephrosclerosis)
2. Uraemia
3. Renal infarct.

Aortic dissection

The marory cause of its is hypertension

Atherosclerotic complications

Many paitents of hypertension die out of these complications but the relationship is much less close than other complications

Mode of Termination

1. Acute letft ventricular failure (60%)
2. Cerebral haemorrhage and allied episodes (35%)
3. Uraemia rare (5%)

Management

According to one review published in 2003, reduction of the blood pressure by 5 mmHg can decrease the risk of stroke by 34%, of ischaemic heart disease by 21%, and reduce the likelihood of dementia, heart failure, and mortality from cardiovascular disease.

Prevention

Much of the disease burden of high blood pressure is experienced by people who are not labeled as hypertensive . Consequently, population strategies are required to reduce the consequences of high blood pressure and reduce the need for antihypertensive drug therapy. Lifestyle changes are recommended to lower blood pressure, before starting drug therapy. The 2004 British Hypertension Society guidelines proposed the following lifestyle changes consistent with those outlined by the US National High BP Education

Program in 2002 for the primary prevention of hypertension:

- Maintain normal body weight for adults (e.g. Body mass index 20–25 kg/m^2)
- Reduce dietary sodium intake to <100 mmol/ day (<6 g of sodium chloride or <2.4 g of sodium per day)
- Engage in regular aerobic physical activity such as brisk walking (≥30 min per day, most days of the week)
- Limit alcohol consumption to no more than 3 units/day in men and no more than 2 units/day in women
- Consume a diet rich in fruit and vegetables (e.g. At least five portions per day);

Effective lifestyle modification may lower blood pressure as much as an individual antihypertensive drug. Combinations of two or more lifestyle modifications can achieve even better results.

Lifestyle modifications

The first line of treatment for hypertension is lifestyle changes, including dietary changes, physical exercise, and weight loss. Though these have all been recommended in scientific advisories, a review by Cochrane found no evidence for effects of weight loss diets on death or long-term complications and adverse events in persons with hypertension. The review did find a decrease in blood pressure. Their potential effectiveness is similar to and at times exceeds a single medication. If hypertension is high enough to justify immediate use of medications, lifestyle changes are still recommended in conjunction with medication.

Dietary changes shown to reduce blood pressure include diets with low sodium, the DASH diet, vegetarian diets and high potassium diets.

Physical exercise regimens which are shown to reduce blood pressure include isometric resistance exercise, aerobic exercise, resistance exercise, and device-guided breathing. Stress reduction techniques such as biofeedback or transcendental meditation may be considered as an add-on to other treatments to reduce hypertension, but do not have evidence for preventing cardiovascular disease on their own.

Medications

Several classes of medications, collectively referred to as antihypertensive medications, are available for treating hypertension.

First line medications for hypertension include thiazide-diuretics, calcium channel blockers, angiotensin converting enzyme inhibitors and angiotensin receptor blockers. These drugs may be used alone or in combination; the latter option may serve to minimize counter-regulatory mechanisms that act to revert blood pressure values to pre-treatment levels. The majority of people require more than one medication to control their hypertension.

4. MATERIALS AND METHODS

4.1 Drug selection:

In this pre clinical study of Herbal Drug ***Venthamaraiyathi Chooranam*** is taken as a trial drug for **Thrombolytic,vasodilator, Hypolipidemic** and **Cardioprodective** activity from the Siddha literature "**Pharmacopoeia of hospital of Indian medicine"** authored by **Dr.v.Narayanaswami.**

Ingredients

*Elarasi(***Elettaria cardamomum,.**)	-	2.5g(3%)
Chukku(**Zingiber officinale, linn.**)	-	5g(6%)
Thippili(**Piper longum, linn.**)	-	7.5g(9%)
Adhimadhuram(**Glycyrrhiza glabra, linn.**)	-	10g(12%)
*Sadhakuppai(***Anethum sowa, linn.**)	-	12.5g(15%)
Seeragam(**Cuminum cyminum, linn.**)	-	15g(18%)
Venthamarai Poo Ithalkal(**Nelumbo nucifera, linn.**)	-	30g(36%)

Purification of Drugs

Elarasi

Fried at low flame and taken

Chukku

Cleaned with clean cloth and the outer skin is scrubbed off.

Sadhakuppai

Impurities are removed and dried in sunlight.

Thippili

Soaking it with lemon juice and. Fried at low flame and taken.

Adhimadhuram

Fried at low flame and taken

Seeragam

Impurities are removed and dried in sunlight.

Venthamarai Poo Ithalkal

Impuritis are removed and dried cool dark places

Process of preparation:

All the above mentioned ingredients are taken in the specified quantity and roasted and powdered and filtered individually (fine process).Then all are thoroughly mixed to make Ven thamaraiyathi chooranam.

Shelf life :

3 months

Dosage:

500mg to 1g two times a day

Vehicle:

Milk

Indication:

Kuruthi Alal Noi

Fig. 1 (a) : INGREDIENTS OF VENTHAMARAIYATHI CHOORANAM

Elettaria cardamomum - Seed

Zingiber officinale, linn – Dried Rhizome

Piper longum, linn - Dry Fruit

Cuminum cyminum, linn - Seeds

Glycyrrhiza glabra, linn - Root

Anethum sowa, linn - Seed

Fig. 1 (b) : VENTHAMARAIYATHI CHOORANAM

NELUMBO NUCIFERA, LINN.- PETALS

VENTHAMARAIYATHI CHOORANAM

4.2 STANDARDIZATION OF THE DRUG:

The identification of herbal drugs & mineral drugs an authenticated by P.G.Gunapadam Department & Herbal Botany Dept. GSMC, Palayamkottai. Standardization of drugs helps in confirming its identify and determination of its quality and effectiveness. Standardization of herbomineral drug is based on qualitative and quantitative analysis through physico-chemical properties and instrumental studies. Physico-chemical analysis and elemental analysis of this herbomineral formulation have been done in Govt. Siddha medical college, Palayamkottai. IITM (FTIR in Dept of Chemistry) and SEM in Dept of mechanics, Chennai.

Organoleptic character

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

4.2.1 PHYSICO CHEMICAL ANALYSIS

Physicochemical studies of the trial drug have been done according to the WHO guidelines.

Determination of Ash Values:

Total Ash:

3g is accurately weighed and incinerated in a crucible dish at a temperature not exceed 450°C until free from carbon. It is then cooled and weighed. The % w/w of ash with reference to the air-dried powder is calculated.

Water Soluble Ash:

The total ash is obtained as the above method for preparation of total ash. The ash is boiled for 5minutes with 25ml water. The insoluble ashes is collected using filter paper and washed with hot water and then transferred to the silica crucible then ignite for 15minutes at temperature not exceeding 450°C. The silica crucible and residue are weighed until constant weight is attained for determination of weight of insoluble ash. The weight of the water soluble ash is determined by substracting the weight of insoluble ash from the weight of total ash.

Acid insoluble Ash:

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

Determination of Extractive Value:

Alcohol Soluble Extractive Value:

3g of test drug powder is weighed and macerated with 100ml of ethanol in a closed container for 24 hours. The resulting solution is shaken continuously for 6 hours and allowed to stand and soak for 18 hours. The solution is filtered and evaporated of the filtrate in a flat bottomed shallow dish and dried at 105°C then cooled and weighed.

Water soluble Extractive value:

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

Loss on Drying:

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

Thin layer chromatography (TLC)

Thin-layer chromatography(TLC) is a chromatographic technique that is useful for separating organic compounds. Because of the simplicity and rapidity of TLC, it is often used to monitor the progress of organic reactions and to check the purity of products.TLC is a simple, quick and inexpensive procedure that gives how many components are in a mixture.TLC is also used to support the identity of a compound in a

mixture when the R_f of a compound is compared with the R_f of a known compound(preferably both run on the same TLC plate).Chromatography works on the principle that different compounds will have different solubilities and adsorbtion to the two phases between which they are to be partitioned. As the solvent rises by capillary action up through the adsorbent, differential partitioning occurs between the components of the mixture dissolved in the solvent stationary adsorbent phase. The more strongly a given component of a mixture is adsorbed on to the stationary phase, the less time it will spend in the mobile phase and the more slowly it will migrate up the plate.

4.2.2 CHEMICAL ANALYSIS:

Preliminary Basic and Acidic radical studies:

Preparation of the extract:

5gms of the test drug is weighed accurately and placed in a 250ml clean beaker. Then 50ml of distilled water is added and dissolved well. Then it is boiled well for about 10 minutes. It is cooled and filtered in a 100ml volumetric flask and then it is made up to 100ml with distilled water. This preparation is used for the qualitative analysis of acidic/ basic radicals and biochemical constituents in it.

QUALITATIVE ANALYSIS FOR BASIC RADICALS:

Test for Calcium:

2ml of the above prepared extract is taken in a clean test tube. To this add 2ml of 4% Ammonium oxalate solution. Formation of white precipitate indicates the presence of calcium.

Test for Iron (Ferric):

The extract is acidified with glacial acetic acid and potassium ferro cyanide. Formation of blue colour indicates the presence of ferric iron.

Test for Iron (Ferrous):

The extract is treated with concentrated Nitric acid and ammonium thio-cyanate solution. Formation of blood red colour indicates the presence of ferrous iron.

Test for Zinc:

The extract is treated with potassium ferro-cyanide. Formation of white precipitate indicates the presence of zinc.

QUALITATIVE ANALYSIS FOR ACIDIC RADICALS:

Test for Sulphate:

2ml of extract is added to 5% barium chloride solution. Formation of white precipitate indicates the presence of sulphate.

Test for Chloride:

The extract is treated with silver nitrate solution. Formation of white precipitate indicates the presence of chloride.

Test for Phosphate:

The extract is treated with ammonium molybdate and concentrated nitric acid. Formation of yellow precipitate indicates the presence of phosphate.

Test for Carbonate:

On treating the extract with concentrated hydrochloric acid giving brisk effervescence indicates the presence of carbonate.

Test for starch:

The extract is added with weak iodine solution. Formation of blue colour indicates the presence of starch.

Test for albumin:

The extract is treated with Esbach`s reagent. Formation of yellow precipitate indicates the presence of albumin.

Test for tannic acid:

The extract is treated with ferric chloride. Formation of bluish black precipitate indicates the presence of tannic acid.

Test for unsaturation:

The extract is treated with potassium permanganate solution. The dis-colourization of potassium permanganate indicates the presence of unsaturated compounds.

Test for the reducing sugar:

5ml of Benedict`s qualitative solution is taken in a test tube and allowed to boil for 2 minutes and added 8-10 drops of the extract and again boil it for 2 minutes. Any colour change indicates the presence of reducing sugar.

Test for amino acid:

One or two drops of the extract is placed on a filter paper and dried it well. After drying, 1% Ninhydrin is sprayed over the same and dried it well. Formation of violet colour indicates the presence of amino acid.

4.2.3. INSTRUMENTAL ANALYSIS

Fig. 2. Scanning Electron Microscope (SEM)

The microstructure of the powders was examined using a Hitachi S 3000H scanning electron microscope (Fig). The scanning Electron Microscope is one of the most versatile instruments available for the examination and analysis of the micro structural characteristics of solid objects. The primary reason for the SEM's usefulness is the high resolution which can be obtained when bulk objects are examined; values of the order of 5nm (50degreeA) are usually quoted for commercial instruments. Advanced research instruments have been described which have achieved resolutions of about 2.5nm (25 degree A). Any solid material can be studied. Sample size is limited to specimens less than about 10μm in diameter. An electron beam passing through an evacuated column is focused by electromagnetic lenses onto the specimen surface. The beam is then rastered 0ver the specimen in synchronism with the beam of a cathode ray tube display screen. In eleastically scattered secondary electrons are emitted from the sample surface and collected by a scintillator, the signal from which is used to modulate the brightness of the cathode ray tube. In this way the secondary electron emission from the sample is used to form an image on the CRT display screen. (Goldstein, et. al., 1992)

Differences in secondary emission result from changes in surface topography. If (elastically) back-scattered electrons are collected to form the image, contrast results from compositional differences. Cameras are provided to record the images on the display screen. Since an electron is a charged particle, it has a strong interaction with the specimen (due to coulomb interaction). When an electron beam images on a specimen, it is scattered by atomic layers near the surface of the specimen. As a result, the direction of electron motion changes and its energy is partially lost. Once an incident electron (primary electron) enters a substance, its direction of motion is influenced by various obstructions (multiple scattering), and follows a complicated trajectory which is far from a straight line. Also, when electrons with the same energy are incident on the specimen surface, a portion of electrons is reflected in the opposite direction (back scattered) and the remainder is absorbed by the specimen (exciting X- rays or other quanta in the process). If the specimen issufficiently thin, the electron can pass all the way through the specimen (transmitted electrons, scattered or non-scattered).

The depth at which various signals are generated due to electron beam – specimen interaction indicates the diffusion area of the signals in the specimen in addition to the local chemistry of the specimen. Secondary electrons mainly indicate information about the surface of a specimen. Since secondary electrons do not diffuse much inside the specimen, they are most suitable for observing the fine-structures of the specimen surface. That is to say, sharp scanning images with high resolution can be expected from secondary electrons, because of the smaller influence on resolution by their diffusion.

As the incident electron energy increases, the probability of incident electrons colliding with elemental components of the specimen and releasing secondary electrons also increases. In other words, as the incident energy increases, the emission of electrons from the specimen also increases. However, as the energy increases beyond a certain level, the incident electrons penetrate deeper into the specimen with the result that the specimen derived electrons use up most of their energy to reach the specimen surface. Consequently, the electron emission yield decreases. Therefore, the peak secondary electron emission yield occurs at a specific entry level of the incident electrons.

In order to verify the existence of a substance and recognize its shape, the image contrast must be well defined. In other words, even if a system boasts extremely high

resolution, if image contrast is poor, it would be extremely difficult to determine the existence of a substance, let alone recognize its shape. Another important feature of the SEM is the three-dimensional appearance of the specimen image, which is a direct result of the large depth of field. The SEM is also capable of examining objects at very low magnification. This feature is useful in viewing particle size and shape of any composition at various stages of preparation in siddha system as well as other fields.

The large depth of field available in the SEM makes it possible to observe 3-dimensional objects in stereo. Today, a majority of SEM facilities are equipped with X-ray analytical capabilities. Thus topographic crystallographic and compositional information can be obtained rapidly, efficiently and simultaneously from the same area.

The author was chosen this analysis for detecting Particle size of the classical siddha herbal drug ***Venthamaraiyathi Chooranam*** . SEM results of ***Venthamaraiyathi Chooranam*** was represented in results section.

FOURIER TRANSFORM - INFRA RED SPECTROSCOPY (FT-IR)

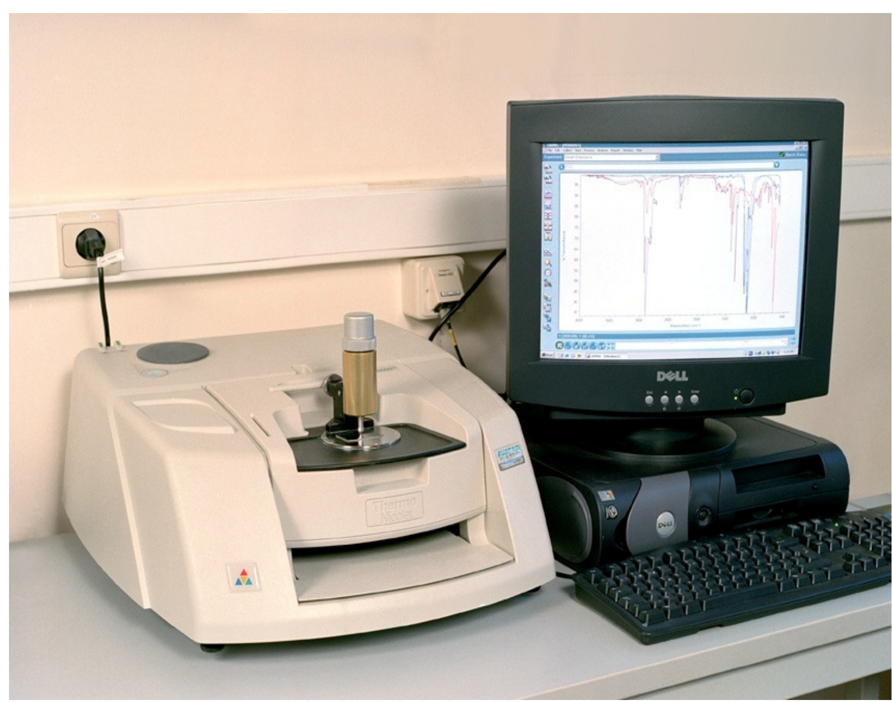


Fig. 3: FTIR Apparatus

FTIR-Mechanism

Fixed Mirror
Moving Mirror
Sample Cell
IR Sensor
Half Silvered Mirror
IR Source

Introduction

Vibrational spectroscopy is an extremely useful tool in the elucidations of molecular structure. The spectral bands can be assigned to different vibrational modes of the molecule. The various functional groups present in the molecule can be assigned by a comparison of the spectra with characteristic functional group frequencies. As the positions of the bands are directly related to the strength of the chemical bond, a large number of investigations including intermolecular interactions, phase transitions and chemical kinetics can be carried out using this branch of spectroscopy. In IR spectroscopy, the resonance absorption is made possible by the change in dipole moment accompanying the vibrational transition. The Infrared spectrum originates from the vibrational motion of the molecule. The vibrational frequencies are a kind of fingerprint of the compounds. This property is used for characterization of organic, inorganic and biological compounds. The band intensities are proportional to the concentration of the compound and hence qualitative estimations are possible. The IR spectroscopy is carried out by using Fourier transform technique.

Principle

Infra red spectroscopy involves study of the interaction of electromagnetic radiation with matter. Due to this interaction, electromagnetic radiation characteristic of the interacting system may be absorbed (or emitted). The experimental data consist of the nature (frequency of wave length) and the amount (intensity) of the characteristic radiation absorbed or emitted. These data are correlated with the molecular and electronic structure of the substance and with intra- and inter molecular interactions.

Source : Nernst Glower

Beam splitter	:	It is made up of a transparent material. Thin films of Silicondeposited on Potassium bromide (KBr)
Bromide (KBr) Detectors	:	Deutrated TriGlycine Sulphate (DTGS).
MIR Range	:	4000 to 450 cm-1
Resolution	:	4.0 cm-1

Sampling Techniques

There are a variety of techniques for sample preparation depending on the physical form of the sample to be analyzed.

Solid	:	KBr or Nujol mull method.
Liquid	:	Csl / TIBr Cells
Gas	:	Gas cells

KBr Method

- The sample is grounded using an agate mortar and pestle to give a very fine powder.
- The finely powder sample is then mixed with about 100mg dried KBr salt.
- The mixture is then pressed under hydraulic press using a die to yield a transparent disc and measure about 13mm diameter and 0.3mm in thickness.

Nujol Mull Method

- The sample is ground using an agate mortar and pestle to give a very fine powder.
- A small amount is then mixed with nujol oil to give a paste and this paste is then
- applied between two sodium chloride plates.
- The plates are then placed in the instrument sample holder ready for scanning.

Liquids

- Viscous liquids can be smeared in the cell and directly measured.
- For dilute solutions, liquid cells and variable path length cells are employed.

Measurements Techniques

The procedure for recording the %T or %A is as follows:

- Air is first scanned for the reference and stored. The sample is then recorded and
- finally the ratio of the sample and reference data is computed to give required %T or %A at various frequencies.
- Study of substances with strong absorbance bands and weak absorbance bands aswell as possible.
- Small amount of samples are sufficient
- High resolution is obtained.

Procedure

- Preparation of samples for infrared measurements and infrared spectra Typically,1.5 mg of protein, dissolved in the buffer used for its purification, were centrifuged in a 30 K Centric on micro concentrator (Amicon) at 3000_g at 4oC until a volume of approximately 40 Al.
- Then, 300 Al of 20 mM This buffer, prepared in H20 or 2H20, pH or p2H 7.2, were added and the sample concentrated again. The p2H value corresponds to the pH meter reading + 0.4. The concentration and dilution procedure was repeated several times in order to completely replace the original buffer with the Tris buffer.
- The washings took 24 h, which is the time of contact of the protein with the 2H20 medium prior FT-IR analysis. In the last washing, the protein was concentrated to fine a volume of approximately 40 Al and used for the infrared measurements.
- The concentrated protein sample was placed in CaF2 windows and a 6 Am tin spaceror a 25 Am Teflon spacer for the experiments in H20 or 2H20, respectively. FT-IR spectra were recorded by means of a Perkin-Elmer -Spectrum-1 FT-IR spectrometer using a deuterated triglycine sulfate detector.
- At least 24 h before, and during data acquisition, the spectrometer was continuously purged with dry air at a dew point of 40oC. Spectra of buffers and samples were acquired at 2 cm_1 resolution under the same scanning and temperature conditions. In the thermal denaturation experiments, the temperature was raised in 5oC steps from 20 to 95oC.
- Before spectrum acquisition, samples were maintained at the desired temperature for the time necessary for the stabilization of temperature inside the cell (6 min).
- Spectra were collected and processed using the SPECTRUM software from Perkin- Elmer. Correct subtraction of H20 was judged to yield an approximately flat baseline at 1900-1400 cm_ 1, and subtraction of 2H20 was adjusted to the removal of the2H20 bending absorption close to 1220 cm_ 1

INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROMETRY (ICP-OES),

Fig. 5: ICPOES Apparatus

Inductively coupled plasma optical emission spectrometry (ICP-OES)is an analyticaltechnique used for the detection of trace metals. It is a type of emission spectroscopy thatuses the inductively coupled plasma to produce excited atoms and ions thatemit electromagnetic radiation at wavelengths characteristic of a particular element. Theintensity of this emission is indicative of the concentration of the element within the sample.

Mechanism

The ICP-OES is composed of two parts: the ICP and the optical spectrometer. TheICP torch consists of 3 concentric quartz glass tubes. The output or "work" coil of the radiofrequency (RF) generator surrounds part of this quartz torch. Argon gas is typically usedto create the plasma.

When the torch is turned on, an intense electromagnetic field is created within thecoil by the high power radio frequency signal flowing in the coil. This RF signal is createdby the RF generator which is, effectively, a high power radio transmitter driving the "workcoil" the same way a typical radio transmitter drives a transmitting antenna.

The argon gasflowing through the torch is ignited with a Tesla unit that creates a brief discharge arcthrough the argon flow to initiate the ionization process. Once the plasma is “ignited”, theTesla unit is turned off.

The argon gas is ionized in the intense electromagnetic field and flows in a particularrotationally symmetrical pattern towards the magnetic field of the RF coil. A stable, hightemperature plasma of about 7000 K is then generated as the result of the inelastic collisionscreated between the neutral argon atoms and the charged particles. A peristalticpump delivers an aqueous or organic sample into a nebulizer where it is changed into mistand introduced directly inside the plasma flame. The sample immediately collides with theelectrons and charged ions in the plasma and is itself broken down into charged ions. Thevarious molecules break up into their respective atoms which then lose electrons andrecombine repeatedly in the plasma, giving off radiation at the characteristic wavelengths ofthe elements involved.

Within the optical chamber(s), after the light is separated into its differentwavelengths (colors), the light intensity is measured with a photomultiplier tube or tubesphysically positioned to “view” the specific wavelength(s) for each element line involved,or, in more modern units, the separated colors fall upon an array of semiconductor photodetectors such as charge coupled devices (CCDs). In units using these detector arrays, theintensities of all wavelengths (within the system’s range) can be measured simultaneously,allowing the instrument to analyze for every element to which the unit is sensitive all atonce. Thus, samples can be analyzed very quickly.

The intensity of each line is then compared to previously measured intensities of known concentrations of the elements, and their concentrations are then computed by interpolation along the calibration lines. In addition, special software generally corrects for interferences caused by the presence of different elements within a given sample matrix.Examples of the application of ICP-OES include the determination of metals, arsenicpresent in Traditional medicines, and trace elements bound to proteins. ICP-OES is widelyused in minerals processing to provide the data on grades of various streams, for theconstruction of mass balances.

The author used for elemental identification and quantitative compositional information of the ***VENTHAMARAIYATHI CHOORANAM*** .

4.3. TOXICITY STUDY

A) ACUTE TOXICITY STUDY

FEMALE WISTER RATS TO EVALUATE TOXICITY PROFILE OF *VENTHAMARAIYATI CHOORANAM*

OBJECTIVES

The aim of this Study is to evaluate the toxicity of the test substance ***VENTHAMARAIYATI CHOORANAM***, when administered orally to Female Wister Rats with different doses, so as to provide a rational base for the evaluation of the toxicological risk to man and indicate potential target organs.

Guidelines followed:

(a) OECD Guidelines No**. 423**,

Study Design and Controls:

- Female Wister Rats in controlled age and body weight were selected.
- ***Venthamaraiyathi Chooranam*** was administered at **5 mg/kg, 50 mg/kg, 300 mg/kg, 1000 mg/kg, and mg/kg** body weight as (Water) as suspension along with blank.
- The results were recorded on day 0, with single oral dosing period of 14 days.

EXPERIMENTAL PROCEDURE

ANIMALS

Supply

A total of 15 Female Wister Rats with an approximate age of 6 weeks and purchased from M/s.Venkateshwara Enterprises Pvt. Ltd, Bangalore. On their arrival a sample of animals was chosen at random and weighed to ensure compliance with the age requested. The mean weights of Female Wister Rats were 100-150 g respectively. The animals were housed in metabolic cages (55 x 32.7 x 19 cm), with sawdust litter, in such a way that

each cage contained a maximum of 3 animals of the same sex.

All animals underwent a period of 20 days of observation and acclimatization between the date of arrival and the start of treatment. During the course of this period, the animals were inspected by a veterinary surgeon to ensure that they fulfilled the health requirements necessary for initiation of the Study.

Housing

The Female Wister Rats were housed in metabolic cages (55 x 32.7 x 19 cm), placed on racks. From the week before initiation of the treatment, each cage contained a maximum of 6 mice of the same sex and treatment group.

Each cage was identified by a card, color coded according to the dose level. This card stated the cage number, number and sex of the animals it contained, Study number, test substance code, administration route, dose level and Study Director's name, date of the arrival of the animals and initiation of treatment.

The temperature and relative humidity were continuously monitored. Lighting was controlled to supply 12 hours of light (7:00 to 19:00 hours) and 12 hours of dark for each 24-hour period.

The cages corresponding to each experimental group were distributed on racks in such a manner that external factors, such as environmental conditions, were balanced as far as possible.

DIET

All the rats had free access to a pelleted rat diet. The diet was analyzed by the manufacturer to check its composition and to detect possible contaminants.

Water

The water was offered ad libitum in bottles.

ADMINISTRATION ROUTE AND PROCEDURE

The test substance was administered orally. The Female Wister Rats belonging to the control group were treated with the vehicle (Water) at the same administration volume as the rest of the treatment groups.

Numbering and Identification

The animals were marked on body with picric acid solution prepared in water. The marking within the cage was as below.

Table-1 Numbering and Identification

Group No	Animal Marking
1	Head
2	Body
3	Tail

The group no., cage no., sex of the animal and animal no. were identified as indicated below using cage label and body marking on the animals

Table-2 Numbering and Identification

Cage No	Group No	Animal Marking	Sex
1	I	H,B,T	Female
2	II	H,B,T	Female
3	III	H,B,T	Female
4	IV	H,B,T	Female
5	V	H,B,T	Female

Doses

The doses for the study were selected based on literature search and range finding study. Following the period of fasting, the animals were weighed and then drug was administered orally as single dose using a needle fitted onto a disposable syringe of approximate size at the following different doses.

Table-3 Doses

GROUP	DOSE
Group-I	**5 mg/kg**
Group-II	**50 mg/kg**
Group-III	**300 mg/kg**
Group-IV	**1000 mg/kg**
Group-V	**2000 mg/kg**

The test item was administered as single dose. After single dose administration period, all animals were observed for day 14.

Dose Preparation

Venthamaraiyathi Chooranam was added in distilled water and completely dissolved to form oral for administration. The dose was prepared of a required concentration before dosing by dissolving, in distilled water. It was mixed well. The preparation for different doses was vary in concentrations to allow a constant dosage volume.

Administration

The test item was administered orally to each Female Wister rats as single dose using a needle fitted onto a disposable syringe of appropriate size at the following different doses. The concentration was adjusted according to its body weight. The volume was not exceeding 10 ml/kg bodyweight. Variability in test volume was minimized by adjusting the concentration to ensure a constant volume at all dose levels.

Observation period

All animals were observed for any abnormal clinical signs and behavioral changes. The appearance, change and disappearance of these clinical signs, if any, were recorded for approximately 1.0, 3.0 and 4.0 hours post-dose on day of dosing and once daily thereafter for14 days. Animals in pain or showing severe signs of distress were humanely killed. The cageside observation was included changes in skin, fur, eyes and

mucous membranes, occurrence of secretions and excretions. Autonomic activity like lacrimation, piloerection, pupil size and unusual respiratory pattern, changes in gait, posture, response to handling, presence of clonic or tonic movements, stereotypes like excessive grooming and repetitive circling or bizarre behavior like self-mutilation, walking backwards etc were observed. At the 14th day, sensory reactivity to stimuli of different types (e.g. auditory, visual and proprioceptive stimuli) was conducted. Auditory stimuli responses were measured by clicker sound from approximately 30 cm to the rats; visual stimuli response were measured with the help of shining pen light in the eye of rats and placing a blunt object near to the eye of rats. Response to proprioceptive stimuli was measured by placing anterior/dorsal surface of animals paw to the table edge. The responses of reactions for these three exercises were normal in animals belonging to both the controls as well as drug treatment dose groups.

Mortality and Morbidity

All animals were observed daily once for mortality and morbidity at approximately 1.0, 3.0 and 4.0 hours post dose on day of dosing and twice daily (morning and afternoon) thereafter for 14 days.

B. SUB-ACUTE TOXICITY STUDY

WISTER RATS TO EVALUATE TOXICITY PROFILE OF VENTHAMARAIYATHICHOORANAM

1. Objective

The objective of this '**Sub-Acute Toxicity Study of *Venthamaraiyathi Chooranam* ON Wister Rats**' was to assess the toxicological profile of the test item when treated as a single dose. Animals should be observed for 28 days after the drug administration. This study provides information on the possible health hazards likely to arise from exposure over a relatively limited period of time.

2. Test Guideline Followed

OECD 407 Method - Sub-Acute Toxic Class Method (Repeated Dose 28-Day Oral Toxicity Study in Rodents)

3. Test Item Detail

Name: ***Venthamaraiyathi Chooranam***

4. Test System Detail

The study was conducted on 5 male 5 female Wister rats. These animals were selected because of the recommended rodent species for oral studies as per followed guideline and availability of Animals 8-12 weeks old male and female rats were selected after physical and behavioral examination. The body weight range was fallen within ± 20% of the mean body weight at the time of Randomization and grouping. The rats were housed in standard laboratory condition in Polypropylene cages, provided with food and water *adlibitum* in the Animal at M/s. Sree Venkateshwara Enterprises Pvt. Ltd, Bangalore. The experimental protocol was approved by Institutional Animal Ethical Committee as per the guidance of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, government of India.

5. Acclimatization

The animals were selected after veterinary examination by the veterinarian. All the selected animals were kept under acclimatization for a week.

6. Randomization & grouping

One day before the initiation of treatment (days 0- last day of acclimatization), the selected animals were randomly grouped into three different groups containing minimum 6 male animals per group.

7. Numbering and Identification

The animals were marked on body with picric acid solution prepared in water. The marking within the cage was as below.

TABLE-4 NUMBERING AND IDENTIFICATION

Group No	Animal Marking
1. CONTROL	H,B,T,HB,NM (MALE) H,B,T,HB,NM (FEMALE)
2. LOW DOSE OF VENTHAMARAIYATHICHOORANAM 300MG/KG	H,B,T,HB,NM (MALE) H,B,T,HB,NM (FEMALE)
3. MIDDLEDOSE OF VENTHAMARAIYATHICHOORANAM 600MG/KG	H,B,T,HB,NM (MALE) H,B,T,HB,NM (FEMALE)
4. HIGH DOSE OF VENTHAMARAIYATHICHOORANAM 900MG/KG	H,B,T,HB,NM (MALE) H,B,T,HB,NM (FEMALE)

The group no., cage no., sex of the animal and animal no. were identified as indicated below using cage label and body marking on the animals:

Table – 5 Husbandry

Cage No	Group No	Animal Marking	Sex
1.	1. CONTROL	H,B,T,HB,NM H,B,T,HB, NM	Male Female
2.	2. LOW DOSE OF VENTHAMARAIYATHICHOORANAM 300mg/kg	H,B,T,HB,NM H,B,T,HB, NM	Male Female
3.	3. MIDDLEDOSE OF VENTHAMARAIYATHICHOORANAM 600mg/kg	H,B,T,HB,NM H,B,T,HB, NM	Male Female
4.	4. HIGH DOSE OF VENTHAMARAIYATHICHOORANAM 900mg/kg	H,B,T,HB,NM H,B,T,HB ,NM	Male Female

Husbandry

Housing

The Wister rats were housed in standard polypropylene cages with stainless steel top grill. Paddy husk was used as bedding. The paddy husk was changed at least twice in a week. From the week before initiation of the treatment, each cage contained a maximum of 6 mice of the same sex and treatment group.

Environmental conditions

The animals were kept in a clean environment with 12 hour light and 12 hour dark cycles. The air was conditioned at 22±3^{0}C and the relative humidity was maintained between 30-70% with 100% exhaust facility. The cages corresponding to each experimental group were distributed on racks in such a manner that external factors, such as environmental conditions, were balanced as far as possible.

Feed & feeding schedule

'Sai Durga Animal Feed, Bangalore. Feed was provided *adlibitum throughout* the study period, except over night fasting (18-20 hours) prior to dose administration. After the substance has been administered, food was with held for a further 3-4 hours.

Water

The water was offered *adlibitum* in bottles. There was periodically analyzed to detect the presence of possible contaminants

Doses

The doses for the study were selected based on literature search and range finding study. Following the period of fasting, the animals were weighed and then extract was administered orally as single dose using a needle fitted on to a disposable syringe of approximate size at the following different doses.

Table-6 Dose level

TEST GROUP	DOSE TO ANIMALS (mg/kg body-weight/day)	NUMBER OFANIMALS
Group-1	**1. CONTROL**	10 (5MALE and5 FEMALE)
Group-II	**2. LOW DOSE OF VENTHAMARAIYATHICHOORANAM 300mg/kg**	10(5MALE and 5 FEMALE)
Group-III	**3. MIDDLEDOSE OF VENTHAMARAIYATHICHOORANAM 600mg/kg**	10(5MALE and 5 FEMALE)
Group-IV	**4. HIGH DOSE OF VENTHAMARAIYATHICHOORANAM 900mg/kg**	10(5MALE and 5 FEMALE)

The test item was administered as single dose. After single dose administration period, all animals were observed for 28 days.

Dose Preparation

VENTHAMARAIYATHICHOORANAM was added in distilled water and completely dissolved to for oral for administration. The dose was prepared of a required concentration before dosing by dissolving ***Venthamaraiyathi Chooranam*** in distilled water. It was mixed well. The preparation for different doses was vary in concentrations to allow a constant dosage volume.

Administration

The test item was administered orally to each rat as single dose using a needle fitted on to a disposable syringe of appropriate size at the following different doses. The concentration was adjusted according to its body weight. The volume was not exceeding 10 ml/kg body weight. Variability in test volume was minimized by adjusting the concentration to ensure a constant volume at all dose levels.

OBSERVATIONS

These observations were also performed on week-ends. The observations included but were not limited to changes in skin and fur, in the eyes and mucous membranes, in the respiratory, circulatory, central nervous and autonomous systems, somatomotor activity and behavior.

Clinical signs of toxicity

All the rats were observed at least twice daily with the purpose of recording any symptoms of ill- health or behavioral changes. Clinical signs of toxicity daily for 28 days.

Food intake

Prior to the beginning of treatment, and daily, the food intake of each cage was recorded for period of 28 days and the mean weekly intake per rats was calculated.

Water intake

Water intake was checked by visual observation during the Study. In addition, the water consumption in each cage was measured daily for a period of 28 days.

Bodyweight:

The body weight of each rat was recorded one week before the start of treatment, and during the course of the treatment on the day of initial, 3rd, 7th, 10th, 14th, 17th, 20th, 24th and 28th days (day of sacrifice). The mean weights for the different groups and sexes were calculated from the individual weights.

Blood Collection

Blood was collected through retro-orbital sinus from all the animals of different groups on 28th day. The blood was collected in tubes containing Heparin/EDTA as an anticoagulant. Animals were fasted over night prior to the blood collection.

LABORATORY STUDIES

During the 4th week of treatment, samples of blood were withdrawn from the orbital sinus of 6 males from each group, under light ether anesthesia after fasting for 16 hours. The blood samples are used to evaluate Hematological parameters like RBC, WBC, and PLATELETS etc….. The collected blood samples also centrifuged 10000 rpm in 10 minutes to separate the serum. The separated serum used to evaluate biochemical parameters like SGOT, SGPT, ALP and BILIRUBIN etc.,

Hematology

The following hematological parameters were analysed using Autoanalyser

Hb	:	Haemoglobin (g %)
PCV	:	Packed Cell Volume
WBC	:	White Blood Corpuscles (x103/cmm)
RBC	:	Red Blood Corpuscles (x106/cmm)
		Blood Platelet count (x103/cmm)

Differential WBC count:

N	:	Neutrophils (%)
L	:	Lymphocytes (%)
M	:	Monocytes (%)
E	:	Eosinophils (%)
RDW	:	Red Cell Distribution Width.
MPV	:	Mean Platelet Volume

Clinical Biochemistry:

The following clinical Bio parameters were analysed using Auto analyser

Total serum protein (g/dl)

ALT/SGPT	:	Alanine amino transferase (U/L)
AST/SGOT	:	Aspartate amino transferase (U/L)
ALP	:	Alkaline serum phosphatase (U/L)
CHL	:	Cholesterol (mg/dL)
HDL	:	High density lipoprotein
TG	:	Triglyceride

TERMINAL STUDIES

Sacrifice and macroscopic examination

On completion of the 4 weeks of treatment, 18 Wister rats were sacrificed by ether inhalation. A full autopsy was performed on all animals which included examination of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents both *in situ* and after evisceration. As the number of animals exceeded the number that could be sacrificed in one day, the autopsies were carried out over three consecutive days at the end of the treatment period.

Organ weights:

After the macroscopic examination the following organs were weighed after separating the superficial fat: Brain, Heart, Spleen Kidneys, Testes, Liver, Lungs, pancreas and stomach.

HISTOPATHOLOGICAL STUDIES

Anatomy of the liver was studied immediately after sacrificing the animals. A small portion was fixed in 10% neutral buffered formalin as described by Luna 14 . Thin sections of 4-5 µm were taken, stained with Haematoxylin and Eosin and histology was studied.

ESTIMATION OF HEMATOLOGICAL PARAMETERS:

Collection of blood for hematological studies

After the treatment period the animals were anaesthetized by ketamine hydrochloride and the blood was collected from Retro-orbital sinus by using capillary into a centrifugation tube which contains EDTA for haematological parameters The haematological parameters like RBC, WBC and Hb percentage, Differential cell count, MCV, MCHC, Hematocrit, MCH, platelet count were estimated by the following procedures.

ENUMERATION OF RED BLOOD CELLS: [1] Ramnic 2007)

Reagents : RBC diluting fluid

Procedure:

Using a red blood cell pipette of haemocytometer, well mixed blood was drawn up to 0.5 mark and RBC diluting fluid was taken up to mark II. The fluid blood mixture was shaken and transferred onto the counting chamber. The cells were allowed to settle to the bottom of the chamber for 2 min. See the fluid does not get dried. Using 45X or high power objective the RBC’s were counted uniformly in the larger corner squares.

The cells were expressed as number of cells x10^{12}/l

ENUMERATION OF WBC: [2] **John 1972)**

Reagents:

Turk's fluid: Turk's fluid was prepared by mixing 2ml of acetic acid with 100 ml of distilled water. To this 10 drop of aqueous methylene blue 3 % w/v) was added. This solution haemolysis the red cells due to acidity so that counting of white cells becomes easy.

Procedure:

Using a white blood cell pipette of haemocytometer, well mixed blood was drawn up to 0.5 mark and WBC diluting fluid was taken up to mark II. The fluid blood mixture was shaken and transferred onto the counting chamber. The cells were allowed to settle to the bottom of the chamber for 2 min. See the fluid does not get dried.

Using 10X or low power objective the WBC's were counted uniformly in the larger corner squares.

The cells were expressed as number of cells/10mm.

DIFFERENTIAL LEUCOCYTE COUNT: John 1972

Reagent:

Leishmann's stain: 150mg of powdered leishmann's stain was dissolved in 133ml of acetone free methanol.

Procedure:

A blood film stained with leishmann's stain was examined under oil immersion and the different types of WBCs were identified. The percentage distribution of these cells was then determined. Smears were made from anticoagulant blood specimens and stained with leishmann's stain. The slides were preserved for counting the number of lymphocytes and neutrophils, per 100 cells were noted.

From the different Leukocyte count and WBC count, absolute lymphocyte and neutrophil count were calculated.

$$\text{Absolute neutrophil count} = \frac{\text{Number of neutrophils}}{100} \times \text{TWBC}$$

$$\text{Absolute lymphocyte count} = \frac{\text{Number of lymphocytes}}{100} \times \text{TWBC}$$

J. C. Dacie and S. M. Lewis, Practical haematology, London: Churchill Livingstone, 1984, pp. 5.

Measurement of biochemical parameters estimation

Haemoglobin (Hb), was estimated using whole blood. Remaining parameters were measured in serum. All of the above biochemical parameters were estimated using semi-autoanalyzer (Photometer 5010 $_{V5+}$, Germany) with enzymatic kits procured from Piramal Healthcare limited, Lab Diagnostic Division, Mumbai, India.

Determination of aspartate aminotransferase (AST)

Aspartate aminotransferase, also known as Glutamate Oxaloacetate Transaminase (GOT) catalyses the transamination of L-aspartate and α keto glutarate to form oxaloacetate and L- glutamate. Oxaloacetate formed is coupled with 2,4- Dinitrophenyl hydrazine to form hydrazone, a brown coloured complex in alkaline medium which can be measured colorimetrically.

Reagents

Buffered aspartate (pH 7.4); 2,4- DNPH reagent; 4N sodium hydroxide; working pyruvate standard; solution I (prepared by diluting 1 ml of reagent 3 to 10 ml with purified water).

Procedure

Rietman and Frankle method was adopted for the estimation of SGOT. (Reitmann S, Frankel S, 1957. A colorimetric method for the determination of serum oxaloacetic and

glutamic pyruvate transminases. American Journal of Clinical Pathology.28: 56-63.The reaction systems used for this study included blank, standard, test (for each serum sample) and control (for each serum sample). 0.25 ml of buffered aspartate was added into all the test tubes. Then 0.05 ml of serum was added to the test group tubes and 0.05 ml of working pyruvate standard into the standard tubes. After proper mixing, all the tubes were kept for incubation at 37°C for 60 min, after which 0.25 ml each of 2,4-DNPH reagent was added into all the tubes. Then, 0.05 ml of distilled water and 0.05 ml of each serum sample was added to the blank and the serum control tubes respectively. The mixture was allowed to stand at room temperature for 20 min. After incubation, 2.5 ml of solution I was added to all test tubes. Mixed properly and optical density was measured in a spectrophotometer at 505 nm within 15 min.

The enzyme activity was calculated as:-

AST (GOT) activity in IU/L) = [(Absorbance of test **-** Absorbance of control)/ (Absorbance of standard - Absorbance of blank)] x concentration of the standard

Determination of alanine aminotransferase (ALT)

Alanine aminotransferase, also known as Glutathione Peroxidase (GPT) catalyses the transamination of L-alanine and α keto glutarate to form pyruvate and L- Glutamate. Pyruvate so formed is coupled with 2,4 – Dinitrophenyl hydrazine to form a corresponding hydrazone, a brown coloured complex in alkaline medium which can be measured colorimetrically.

Reagents

Buffered alanine (pH 7.4), 2,4–DNPH, 4N sodium hydroxide, working pyruvate standard, solution I (prepared by diluting 1 ml of reagent 3 to 10 ml with purified water).

Procedure

Rietman and Frankle method was dopted for the estimation of SGPT. The reaction systems used for this study included blank, standard, test (for each serum sample) and control (for each serum sample). 0.25 ml of buffered alanine was added into all the test tubes. This was followed by the addition of 0.05 ml of serum into the test group tubes and

0.05 ml of working pyruvate standard into the standard tubes. After proper mixing, all the tubes were kept for incubation at 37°C for 60 minutes, after which 0.25 ml each of 2,4-DNPH reagent was added into all the tubes. Then, 0.05 ml of distilled water and 0.05 ml of each serum sample was added to the blank and the serum control tubes respectively. The mixture was allowed to stand at room temperature for 20 min. After incubation, 2.5 ml of solution **I** was added to all test tubes. Mixed properly and optical density was read against purified water in a spectrophotometer at 505 nm within 15 min.

The enzyme activity was calculated as:- ALT (GPT) activity in IU/L) = [(Absorbance of test **-** Absorbance of control)/ (Absorbance of standard - Absorbance of blank)] x concentration of the standard.

Determination of alkaline phosphatase (ALP)

Alkaline phoshatase from serum converts phenyl phosphate to inorganic phosphate and phenol at pH 10.0. Phenol so formed reacts in alkaline medium with 4-aminoantipyrine in presence of the oxidising agent potassium ferricyanide and forms an orange-red coloured complex, which can be measured spectrometrically. The color intensity is proportional to the enzyme activity.

Reagents:

Buffered substrate

Chromogen Reagent

Phenol Standard, 10 mg%

Procedure:

ALP was determined using the method of Kind (Kind PRM, King EJ, 1972. *In-vitro* determination of serum alkaline phosphatase. Journal of Clinical Pathology 7: 321-22\). The working solution was prepared by reconstituting one vial of buffered substrate with 2.2 ml of water. 0.5 ml of working buffered substrate and 1.5 ml of purified water was dispensed to blank, standard, control and test. Mixed well and incubated at 37^{0}C for 3 min. 0.05 ml each of serum and phenol standard were added to test and standard test tubes respectively. Mixed well and incubated for 15 min at 37^{0}C. Thereafter, 1 ml of

chromogen reagent was added to all the test tubes. Then, added 0.05 ml of serum to control. Mixed well after addition of each reagent and the O.D of blank, standard, control and test were read against purified water at 510 nm.

Serum alkaline phosphatase activity in KA units was calculated as follows [(O.D. Test-O.D. Control) / (O.D. Standard- O.D. Blank)] x 10

Determination of bilirubin

In toxic liver, bilirubin levels are elevated. Hyperbilirubinemia can result from impaired hepatic uptake of unconjugated bilirubin, such a situation can occur in generalized liver cell injury, certain drugs (e.g Rifampin and probenecid) interfere with the rat uptake of bilirubin by the liver cell and may produce a mild unconjugated hyperbilirubinemia. Bilirubin level rises in diseases of hepatocytes, obstruction to bilirubin excretion into duodenum, in haemolysis and defects of hepatic uptake and conjugation of Bilirubin pigment such as Gilbert's disease.

Elevation of total serum bilirubin may occur due to:

- Excessive haemolysis or destruction of the red blood cells.Eg:Haemolytic disease of the new born.
- Liver diseases.Eg.Hepatitis and cirrhosis.
- Obstruction of the biliary tract.Eg.Gall stones.

The method is based on the reaction of Sulfonilic acid with sodium nitrite to form azobilirubin which has maximum absorbance at 546nm in the aqueous solution. The intensity of the color Produced is directly proportional to the amount of direct or total bilurubin concentration present in the sample.

Reagents

1. Diazo A-(Reagent-R1) :Ready to use
2. Diazo B-(Reagent-R2):Ready to use
3. Bilirubin Activater :Ready to use

Procedure

Kind & King's method was followed for the estimation of Bilirubin. Five hundred µl of working reagent was added to 50 µl of rat serum & incubated for 5 min at 37°C. Absorbance was measured AT 546 NM in semi auto analyzer against the standard.

The Bilirubin content was calculated using the following equation: Total bilirubin (mg/dt) = Abs of the sample blank x 15.

Estimation of Urea

Urea is the nitrogen-containing end product of protein catabolism. States associated with elevated levels of urea in blood are referred to as hyper uremia or azotemia.

Method

Estimation of urea was done by Urease-GLDH: enzymatic UV test.

Principle

$$Urea + 2H_2O \xrightarrow{Urease} 2NH_4 + 2HCO_3$$

$$2\text{- Oxoglutarate} + NH_4^+ + NADH \xrightarrow{GLDH} L\text{- Glutamate} + NAD^+ + H_2O$$

Table 7. Reagents

R 1	TRIS pH 7.8	120 mmol/l
	2-Oxoglutarate	7 mmol/l
	ADP	0.6 mmol/l
	Urease	≥ 6 KU/l
	GLDH	≥ 1 KU/l
R 2	NADH	0.25 mmol
R 3	Standard	40 mg/dl

Procedure

a. Take 1000 µl of reagent-1 and 250 µl of reagent-2 in 5 ml test tube.
b. To this, add 10 µl of serum.
c. Mix well and immediately read the test sample at 340 nm Hg 334 nm Hg 365 nm optical path 1 cm against reagent blank (2-point kinetic).
d. And note down the value.

Normal range: 10 – 50 mg/d

ESTIMATION OF URIC ACID

Uric acid and its salts are end products of the purine metabolism. In gout the most common complication of hyperuricemia, ie. Increased serum levels of uric acid lead to formation of monosodium urate crystal around the joints.

Method

Enzymatic photometric test using TOOS (N ethyl- N (hydroxyl -3- sulfopropyl)- m- toluidin)

Principle

$$\text{Uric acid} + H_2O + O_2 \xrightarrow{\text{uricase}} \text{Allantoin} + CO_2 + H_2O_2$$

$$\text{TOOS} + 4\ \text{aminoantipyrine} + 2H_2O_2 \xrightarrow{\text{POD}} \text{Indamine} + 3H_2O$$

Table 8.reagents

R1	Phosphate buffer pH 7.0	100mmol/l
	TOOS	1mmol/l
	Ascorbate oxidase	≥1 KU/l
R2	Phosphate buffer pH 7.0	100mmol/l
	4- amino antipyrine	0.3mmol/l
	K_4 (Fe(CN)$_6$)	10µmol/l
	Peroxidase	≥1KU/l
	Uricase	≥50U/l

Procedure

a. Take 800µl of reagents -1 in a2ml centrifuge tube.
b. To this add 20µl of serum.
c. Mix well and incubate at 30°c for 5 minutes.
d. Then add 200µl of reagent2
e. Mix well incubate for 5min at 37°c
f. Measure the not down the values.

Normal range: 1.9-8.2mg/dl

ESTIMATION OF CREATININE:

Estimation of Creatinine by Jaffe Method (modified)

Principle:

Creatinine forms a coloured complex with picrate in alkaline medium. The rate of formation of the complex is measured.

Reagents:

Reagent 1 Standard Creatinine (2mg/100ml)
Reagent 2 Picric acid solution
Reagent 3 Sodium hydroxide solution

Procedure:

Take 500 µl of reagent -2 and 500 µl of reagent -3 in a 5ml test tube. To this add 100 µl of serum. Mix well and immediately read the test sample at Hg 492 nm 1cm light path and note down the values.

Normal range is 0.6 -1.1 mg/dl.

4.4. PHARMACOLOGY STUDY

4.4.1.EVALUATION OF IN VIVO CLOT LYSIS IN RAT MODELS

In vivo studies were carried out by the oral administration of aril and rind of ***VENTHAMARAIYATHI CHOORANAM*** to examine the clot lysing ability, antioxidative and cytotoxic effect.

Thrombus induction

Experimental rats were anaesthetized with i.p injection of ketamine hydrochloride (100 mg/kg). To attain effective clot formation κ – carrageenan (1 mg/kg) dissolved in saline was injected into the rat tail vein at a site 12 cm from the tip of the tail with a ligation. After a period of 10 minutes, the ligature was removed. The length of the infarct was monitored for thrombus formation. Once thrombus was formed, the animals were treated with respective extracts and monitored for the reduction in the length of the thrombus in rat tail for 30 days.

Table – 9 Dose level

TEST GROUP	DOSE TO ANIMALS (mg/kg body-weight/day)
GROUP-1	CONTROL
GROUP-II	ONLY CARRAGEENAN
GROUP-III	CARRAGEENAN+STD
GROUP-IV	CARRAGEENAN+ LOW DOSE OF VENTHAMARAIYATHICHOORANAM 300MG/KG
GROUP-V	CARRAGEENAN+ MIDDLEDOSE OF VENTHAMARAIYATHICHOORANAM 600MG/KG
GROUP-VI	CARRAGEENAN+ HIGH DOSE OF VENTHAMARAIYATHICHOORANAM 900MG/KG

The test item was administered as single dose. After single dose administration period, all animals were observed for 30 days.

Estimation of D-dimer

(Marder and Francis, 1983)

Fibrin degradation products (FDP), which are highly heterogeneous soluble fragments, are a result of 2simultaneous phenomena:

- Fibrinogen is coagulated by thrombin and factor XIIIa toform stabilized fibrin,
- The fibrin clot is dissolved by plasmin into solublefragments released into the blood. The terminal productof fibrinolysis is D-dimer.

Principle

The assay principle combines a two-step enzymeimmunoassay sandwich method with a final fluorescentdetection (ELFA). The Solid Phase Receptacle (SPhR) serves as the solid phase with an anti-FDP monoclonal antibody adsorbed on its surface.

Procedure

The sample was taken and transferred into the wellcontaining an alkaline phosphatase – labeled anti-FDP monoclonal antibody. The sample mixture was cycled in and out of the SPhR several times to increase the reaction speed. The antigen binds to antibodies coated on the SPhR and to the conjugate forming a "sandwich". The remaining free antigen sites were saturated by cycling the conjugate in the fifth well of the strip in and out of the SPhR. Unbound components wereeliminated during the washing steps.

Two detection steps were then performed successively. Duringeach step, the substrate (4-Methyl-umbelliferyl phosphate) wascycled in and out of the SPhR. The conjugate enzymecatalyzes the hydrolysis of this substrate into a fluorescentproduct (4-Methyl-umbelliferone), the fluorescence of whichis measured at 450 nm. The intensity of the fluorescence isproportional to the concentration of antigen present in thesample.

Estimation of tissue plasminogen activator

(Kit method - Roche)

Tissue-type plasminogen activator (tPA) is one of two major physiologic activators of plasminogen in plasma. The activation of plasminogen by tPA is dependent on the presence of a fibrin cofactor. The binding of both tPA and plasminogen to fibrin is mediated in part through lysine binding sites within the kringle structures of both enzyme

and substrate, but also through the finger domain of tPA. Activation of plasminogen by tPA occurs by cleavage after residue Arg560 to produce the two-chain active serine protease plasmin.

Principle

Affinity-purified antibody to tPA is coated onto the wells of a microtitre plate. Any remaining binding sites on the plastic wells are blocked with an excess of bovine serum albumin. The plates are washed and plasma or other fluids containing tPA are applied. The coated antibody will capture the tPA in the sample. After washing the plate to remove unbound material, a peroxidase conjugated second antibody to tPA is added to the plate to bind to the captured tPA. After washing the plate to remove unbound conjugated antibody, the peroxidase activity is expressed by incubation with O-enylenediamine (OPD). After a fixed development time the reaction is quenched with the addition of H_2SO_4 and the colour produced is quantified using a microplate reader. The colour generated is proportional to the concentration of tPA present in the sample.

Reagents

1. *Capture antibody* (TPA–EIA-C)
2. *Detecting antibody* (TPA-EIA-D)
3. ***Coating Buffer***: 50 mM Carbonate: 1.59g of Na_2CO_3 and 2.93g of $NaHCO_3$ up to 1 litre. Adjust pH to 9.6.
4. ***PBS*** (base for wash buffer and blocking buffer): 8.0g NaCl, 1.15g Na_2HPO_4, 0.2g KH_2PO_4 and 0.2g KCl, up to 1 litre. Adjust pH to 7.4, if necessary.
5. ***Blocking Buffer***: PBS-BSA (1%, w/v) Dissolve 2.5 g of Bovine Serum Albumin (Sigma-RIA grade) in 200 ml of PBS. Adjust pH to 7.4, if required, then make up to 250 ml with PBS.
6. ***Sample Diluent*** (HBS-BSA-T20): 5.95g HEPES (free acid), 1.46 g NaCl, 2.5 g Bovine Serum Albumin dissolved in 200 ml H_2O. Add 0.25 ml of Tween- 20, check and adjust pH to 7.2 with NaOH, then make up to a final volume of 250 ml with H_2O.

7. ***Substrate Buffer*** (Citrate-Phosphate buffer - pH 5.0) 2.6g Citric acid and 6.9g Na_2HPO_4 up to a final volume of 500 ml with purified H_2O.
8. ***OPD Substrate*** (o-Phenylenediamine.2HCl) TOXIC!: Make up immediately before use. Dissolve 5mg OPD in 12 ml substrate buffer then add 12 µl 30% H_2O_2.
9. Stopping Solution: 2.5 M H_2SO_4 (Where stock sulphuric acid is 18 M, add 13.9 ml to 86 ml H_2O).

Procedure

1. ***Coating of plates***: Dilute the capture antibody 1/100 in coating buffer (in a polypropylene tube) and immediately add 100 µl per well in the plate. Incubate for 2 hours at ambient temperature or overnight at 2-8^{0}C.
2. ***Blocking***: Empty contents of plate and add 150 µl of blocking buffer to every well and incubate for 60 minutes at 22^{0}C. Wash plate X 3 with wash buffer.
3. ***Preparation of tPA Reference Standards***: Reconstitute vials of tPA standard and tPA/PAI-1 deficient plasma according to manufacturer's instructions. After reconstitution, dilute the tPA standard into tPA/PAI-1 deficient plasma to achieve six reference standard plasmas with final tPA concentrations of 50, 25, 12.5, 6.25, 3.13 and 1.56 ng/ml respectively.
4. ***Samples***: Reference plasmas prepared in step 3 and test plasmas are diluted 1/4 and 1/8 in HBS-BSA-T20 sample diluent. Samples should be run in duplicate. Apply 100 µl/well and incubate plate at 22^{0}C for 90 minutes. Wash plate X 3 with wash buffer.
5. ***Detecting Antibody***: Dilute the detecting antibody 1/100 in HBS-BSA-T20 sample diluents and apply 100 µl to each well. Incubate plate at 22^{0}C for 90 minutes. Wash plate X 3 with wash buffer.
6. ***OPD Substrate***: Apply 100 µl of freshly prepared OPD substrate to every well. Allow colour to develop for 5-10 minutes then stop colour reaction with the addition of 50µl/well of 2.5 M H_2SO_4. The plate can be read at a wavelength of 490 nm.

Determination of Bleeding Time

(Brown, 1993)

Apparatus

Blood lancet, spirit, pieces of filter paper, stop watch

Procedure

With usual aseptic precautions, a puncture wound was inflicted. It is to be more deep than usual and should be done in a standard manner. The time of puncture and first appearance of the blood was noted. The blood was gently blotted with a filter paper. Care should be taken not to press or wipe the wound. It was repeated with a fresh piece of filter paper for every 10 seconds till no blood appears on the paper. The number of filter paper that shows the blot of blood on it was counted. Number of blot x 10 seconds will be bleeding time. Similar experiments were carried out on a healthy animal and the result obtained was considered as a control value. Normal bleeding time is 1 – 5 minutes.

Blood clotting time

The ability of N. laevis extract to inhibit in vitro coagulation of blood was quantitatively assayed following a standard procedure adopted by Koffuor and Amoateng (2011). Briefly, 1.0 ml of whole blood drawn from the marginal ear vein of rabbits was added separately to 0.2 ml of 5 % and 10 % w/v of NLMLE in test tubes placed in a water bath at 37 °C. The time taken for the blood to clot in the separate concentration of the extract was recorded. A repeat of each test was performed to obtain five determinants in coagulation times exerted with differences in concentration of the extract. Controls were coagulation time for blood in 0.2 ml distilled water or blood alone as baseline clotting times.

Determination of euglobulin clot lysis time

(Nordby*et al*., 1980)

The euglobulin clot lysis time is a test that reflects the overall fibrinolytic activity of plasma.

Principle and Method:

Venous blood is collected into chilled tubes containing trisodium citrate as an anticoagulant and placed on ice. The sample is then centrifuged at 4°C and the plasma sample is collected, diluted with acetic acid and incubated on ice for 15 minutes. A precipitate forms [the euglobulin fraction of plasma] which contains plasminogen, plasminogen activators [primarily t-PA] and fibrinogen. The supernatant is collected by centrifugation in refrigerated centrifuge at 4°C. The supernatant is discarded and the precipitate is dissolved in buffer. This is then clotted with thrombin and the time to clot lysis is determined by inspection every 15 minutes. A control plasma sample collected at the same time must be run in parallel.

4.4.2. VASODILATOR ACTIVITY OF STUDENT ORGAN BATH METHODS

Materials and Methods

Chemicals

Phenylephrine chloride (PE), Nω-nitro-L-arginine (L-NA), atropine sulfate, acetylcholine chloride (Ach), rutin hydrate, oleuropein, kaempferol disaccharides, and quercetin were purchased from Sigma-Aldrich Chemical, USA. All other chemicals used were of analytical grade.

Animals

Wistar rats (*Rattusnorvegicus*) and ICR mice (*Musmusculus*) used were obtained from the National Laboratory Animal Center,Baide Metha Pharmacy College, Thuraipakkam, Chennai. All animals were acclimatized for 1 week before starting of the experiments in controlled environmental conditions (25 ± 1°C) with a 12 h light/dark cycle and allowed access to standard food and tap water *ad libitum*. Animal welfare was under control by IACUC of the Baide Metha Pharmacy College.

Plant Material and Extraction

The flowers Venthamarai were collected from thovalai , kanniyakumari dist in October 2015. The specimen voucher was TISTR no. 160309, and the samples were deposited at TISTR. Approximately 3.1 grams of the flowers were dried at 50°C for 42 h, powdered, and macerated in 18 mL of 95% ethanol at room temperature overnight. After

elution, the flower residues were repeatedly macerated with equal volume of ethanol overnight and eluted again. The ethanol elutes were combined, filtered through Whatmann filter paper no. 42, and evaporated under reduced pressure at 50°C. The semisolid light yellow materials were stored in desiccators until used. Percentage yield of the extract was 17.68% yield (w/w). The extract was dissolved in 0.05% dimethxylsulfoxide (DMSO) for *in vitro* experiments and in 1% Tween or 1% gum tragacanth for *in vivo* studies.

Vasodilatation Effect Test

Each rat was killed by cervical dislocation. Its thorax was opened, and the aortic vessel was removed from fat and connective tissues and kept in a Petri dish containing Krebs' solution. Two adjacent aortic rings of 3-4 mm in length were cut. In only one ring, endothelium was removed mechanically by gently rubbing the intimal surface of the vessel using the method as previously described. The organ bath contained the Krebs-Henseleit solution (NaCl 119, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4{\cdot}7H_2O$ 1.0, KH_2PO_4 1.2, $NaHCO_3$ 25.0, and glucose 11.1 mM). This solution was maintained at 37°C and continuously bubbled with 95% O2 and 5% CO2. The two rings were individually suspended horizontally between two stainless steel hooks in a 20 mL organ bath containing Kreb's solution. One of the hooks was fixed to the bottom, and the other was connected to a force displacement transducer that connected to a MP100 (BIOPAC System, Inc., Model MP100 WSW) for the isometric tension record. The stabilization period was of 45 min under a resting tension of 1.0 g and the solution was changed every 15 min to prevent the accumulation of metabolites. After equilibration, the rings were preconstricted with 1 × 10−6 M phenylephrine (PE) until the responses curve reached plateaus (5–8 min), and dilator responses to 1 × 10−5 M acetylcholine (Ach) were detected. The absence of the relaxation to acetylcholine was taken as evidence that the vessel segment was functionally denuded of endothelium, and at least 70% vasodilatation to acetylcholine for theendothelium-intact thoracic aorta rings was observed.

Effect of the VenthamaraiChoornam Extract on PE-Induced Tonus in the Endothelium-Intact or Endothelium-Denuded Thoracic Aorta Rings

After 45 minutes re-equilibration, the rings with and without endothelium intact were pre-contracted with 1 × 10−6 M PE for 20 minutes. After several washings and re-

equilibration for 30 minute, five different concentrations of the VTC extracts (50, 100, 200, 300, and 400 μg/mL) were added 5 min and the rings were incubated with PE for another 20 min. The contractions were measured by comparing the developed tension before and after the addition of the extract and expressed as percentage of contraction from induced tonus.

Effect of the Extract on PE-Induced Tonus in the Endothelium-Intact Thoracic Aorta Rings with Atropine and Nω-Nitro-L-arginine

After 45 min re-equilibration, the endothelium-intact aortic rings were pre-constricted with 1 × 10−6 M PE for 20 min. After several washings and re-equilibration for 30 minutes, the rings were exposed to atropine (1 × 10−6 M) or Nω-nitro-L-arginine (L-NA), a nitric oxide synthase inhibitor (1 × 10−4 M), for 5 minutes. Then, the same serial concentrations of the extracts were added, and the percentage of the contractions before and after the addition of the extracts was determined as described

4.4.3. HYPOLIPIDEMIC ACTIVITY OF SIDDHA FORMULATION *VENTHAMARAIYATHI CHOORANAM* IN HYPERLIPIDEMIC MODELS OF WISTAR ALBINO RATS

INTRODUCTION:

Many people with diabetes have conditions called "risk factor" that contribute to atherosclerosis and its complications. These include high blood pressure, excess weight and high blood glucose levels. Dyslipidemia further raises risk of atherosclerosis in people with diabetes. Dyslipidemia affects people with type 2 diabetes more often than those with type 1 diabetes. The most common Dyslipidemia in diabetes is the combination of high triglycerides and low HDL levels. People with diabetes may also have elevated LDL cholesterol.

Among the drugs available to treat Dyslipidemia, statins are often the first choice for lowering total and LDL cholesterol levels, other drugs that lowers cholesterol include cholesterol-adsorption blockers, bile acids, sequestrants, and nicotinic acids. These may be used in combination, if a single drug is not effective in reaching target levels. Fibrates and extended release niacin may be used to lower triglycerides (or) raise HDL cholesterol levels.

Hyperglycemia and Dyslipidemia are significant and independent risk factors for the vascular complications and suggested to cause cardio vascular pathological changes in diabetic states through the following molecular mechanism, formation and accumulation of advanced glycation products, increased oxidative stress, activation of proteinkinase C pathway , increased activity of hexosamine pathway and vascular inflammation and the impairment of insulin action in the vascular tissues. As siddha formulation venthaa marayathy chooranam have been traditionally claimed for the treatment of HYPOLIPIDEMIC, hence in the present study an attempt has been made to screen the siddha formulation *Venthamaraiyathi Chooranam* for the Hypolipidemic activity..

The present investigation is undertaken to study the effect of siddha formulation *Venthamaraiyathi Chooranam* on changes in Total cholesterol, Triglycerides, HDL, LDL, VLDL, AI, and LDL/HDL.

Materials and methods

Animals

Wister albino rats were obtained from central animal house, K.M.College of pharmacy, Madurai. The animals were given standard rodent diet and water ad libitum throughout the study. The rats used in the present study were maintained in accordance with guidelines of the national institute of nutrition, Indian council for medical research, Hyderabad, India and study approved by Institutional animal ethical committee.

Materials:

- Siddha formulation venthaa marayathy chooranam
- Cholesterol extra pure for feeding purpose was obtained from S D fine-chem. limited, Mumbai, India. Coconut oil was used as a vehicle for cholesterol feeding.
- *Atorvastatin* was obtained from Micro labs, Bangalore, India.

Experimental procedure:

All the animals were weighed and divided into five groups each of six animals.

Group I	:	Normal control.
Group II	:	Cholesterol control. Fed ***cholesterol*** at a dose of 400mg/kg body weight for 30 days.
Group III	:	fed cholesterol as in group II and ***Atorvastatin*** 1mg/kg body weight from days 15 to day 30. (3)
Group IV	:	fed cholesterol as in group II and siddha formulation *Venthamaraiyathi Chooranam* at a dose of 200mg/kg body weight from days 15 to day 30.
Group V	:	Fed cholesterol as in group II and siddha formulation *Venthamaraiyathi Chooranam* at a dose of 400 mg/kg body weight from days 15 to day 30.

At the end of 30 days all the rats were sacrificed, blood was collected, allowed to clot and serum was obtained by centrifugation. The serum samples were used for various biochemical procedures.

Biochemical analysis:

The serum was analyzed for total cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL), by using standard protocol methods. (Auto Analyzer)

Atherogenic index (AI) and LDL-C/HDL-C ratio

- The AI was calculated by the following formula
- AI = (total cholesterol – HDL-C)/HDL-C
- LDL-C/HDL-C ratio was calculated as the ratio of plasma LDL-C to HDL-C Levels

Statistical analysis

- All the values were expressed as mean ± SEM.
- Data was analyzed by one way analysis of variance (ANOVA) followed by Newmankeuls multiple test.
- P values <0.05 were considered as statistically significant.

4.4.4. CARDIOPROTECTIVE EFFECT OF VENTHAA MARAYATHY CHOORANAM AGAINST DOXORUBICIN INDUCED MYOCARDIAL TOXICITY IN ALBINO RATS

INTRODUCTION

Doxorubicin (DOX), an anthracycline antibiotic, is an excellent drug for the treatment of a wide variety of human solid tumors and leukemias. However, its clinical uses are limited by seriously high incidence of cardiotoxicity. An initial acute effect includes hypotension and transient electrocardiographic abnormalities. Meanwhile, the chronic effects may occur several weeks or months after cumulative DOX administration. Cardioxmyophathy is dose dependent which accounts for high mortality . The possible mechanisms proposed for cardiotoxic effects of DOX include free radical induced myocardial injury, lipid peroxidation, mitochondrial damage, decreased activity of Na+–K+ adenosine triphosphate, vasoactive amine release and cellular toxicity . Increased oxidative stress and release of free radicals, including super oxide anion (O2-) and other reactive oxygen intermediates as well as endogenous antioxidant deficits have been suggested to play a major role in Dox-induced cardiomyopathy and heart failure . Additionally, DOX has a very high affinity by cardiolipin, a phospholipid that is present in mitochondrial membranes of heart, resulting in the accumulation of DOX inside cardiac cell Moreover, in recent years, it has been observed that there is a growing interest in the usage of natural antioxidants as a protective strategy against cardiovascular-related problems in experiments such as ischemia reperfusion [10] and Dox-induced cardiotoxicity .The aim of this study was to evaluate the carioprotective properties of *Venthamaraiyathi Chooranam* against Dox-induced cardiotoxicity in rats.

Chemicals and drugs

Dox was a gift from Get Well Pharmaceuticals, India. Other chemicals were obtained from Sigma and analyzing kits were obtained from ERBA. All the chemicals were of the analytical grade.

Animals

Albino wister rat (150-225 gm) were produced from animal experimental laboratory, and used throughout the study. They were housed in micro nylon boxes in a control environment (temp 25±2°C) and 12 hrs dark /light cycle with standard laboratory

diet and water *ad libitum.* The study was conducted after obtaining institutional animal ethical committee clearance. As per the standard practice, the rats were segregated based on their gender and quarantined for 14 days before the commencement of the experiment. They were fed on healthy diet and maintained in hygienic environment in our animal house.

Treatment protocol

After one week of acclimatization, the animals were randomly divided into 5 groups of 6 animals and treated as follows- Group 1 served as Normal control and received normal saline 5 ml/kg body weight (i.p.). Group 2 animals were treated with Dox (2.5 mg/kg body weight, i.p.) in 6 equal injections alternatively for two weeks to make a total cumulative dose of 15 mg/kg body weight. Group 3 animals were treated with Dox (2.5 mg/kg body weight, i.p.) and Vitmanin E. Group 4 animals received *Venthamaraiyathi Chooranam* with low dose and Dox (200 mg/kg body weight). Group 5 animals received *VENTHAMARAIYATHI chooranam* extract with high dose and Dox (400 mg/kg body weight).

Evaluation of myocardial infarction

Biochemical analysis

Dissection and Homogenization

After the experimental period the rats were sacrificed by euthanasia method. Blood was collected and serum supernatant was for the assay of marker enzymes Alkaline Phosphatase(ALP), lactate dehydrogenase (LDH), Aspartate Transaminase (AST), Alanine Transaminase (ALT) and Creatine Phosphokinase (CPK).

Statistical analysis

The results are expressed as mean ± S.E.M. The results were analyzed using one-way ANOVA followed by Newman Keul's multiple rang. Data was computed for statistical analysis by using GraphPad InStat version 3.00 of GraphPad Software, Inc. (San Diego, CA).

4.5. ANTI - MICROBIAL ACTIVITIES BY WELL DIFFUSION METHOD

Aim:

The antimicrobial activity of ***Venthamaraiyathi Chooranam*** was adapted through Well diffusion method(Agar diffusion testing).

PRINCIPLE

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

Components of Muller Hinton agar medium:

Beef extract	-	2gm/lit
Acid Hydrolysate of Casein	-	17.5 gm/lit
Starch	-	1.5 gm/lit
Agar	-	17gm/lit
Distilled water	-	1000 ml
PH	-	7.3 $\pm$ 0.1 at 25^0C

PROCEDURE (Murray *et al.,* 1995)

Petriplates containing 20ml Muller Hinton medium were seeded with 24hr culture of bacterial strains. Wells were cut and 20 µl of the plant extracts (aqueous) were added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993). Streptomycin was used as a positive control. standard for an In-vitro antimicrobial activity of ***Venthamaraiyathi Chooranam*** was screened against bacteria strains such as *Klebsiella pneumonia, Streptococcus mutans, Staphylococcus aureus, Escherichia coli, Pseudomonas.aerugina, Enterococcus faecalis.*

Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken HR Manual of Clinical Microbiology, 6th Ed. ASM Press, Washington DC, 1995; 15-18.

5. RESULTS AND DISCUSSION

STANDARDIZATION OF THE TEST DRUG

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

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

Table : 10 Organoleptic Characters

S.No.	Parameter	Results
1.	a. Colour	Yellowish L.Green
2.	b. Odour	Pleasant Odour
3.	c. Sense of Touch	Nice
4.	d. Appearance	Powder
5.	e. Taste	L.Bitter, L.Sweet

Interpretation:

The organoleptic characters of the drug ***Venthamaraiyathi Chooranam*** showed that the colour of the *chooranam* is Yellowish L. Green in colour since prepared from dry herbs and minerals, L. Bitter, L. Sweet in taste which might be responsible for the activity mentioned earlier and on sight they are fine powder.

- The fineness of the *chooranam* represents easy absorption and better availability of the drugs.
- The size of the particle is reduced through various stages like pounding, sieving, filtering through white cloth (*vasthirakaayam*).
- Only if the size of the particle is reduced to micro and nano particles, the drug is easily assimilable in the digestive system.
- The above processes reduced the size of the particle so that the *chooranam* passes through the sieve.

TABLE : 11 PHYSICO CHEMICAL ANALYSIS

S.NO	Parameter	Results
1	Loss on Drying	6.3%
2	Total Ash	6.1%
3	Acid Insoluble ash	2.3%
4	Water soluble Extractive	26.7%
5	Alcohol Soluble Extractive	21.7%
6	Total Viable aerobic count	1.3×10^4 col/g
7	Total Enterobacteriaceae	Nil
8	Total fungal count	
9	Test for specific pathogens	3×10^2 col/g
10	Salmonella sp	Nil
11	Staphylococcus aureus	Nil
12	E.coli	Nil
13	Pseudomonas aeruginosa	Nil

Interpretation of Physico chemical analysis

Ash Values

The ash remaining following ignition of medicinal plant materials is determined by three different methods which measure total ash, acid insoluble ash and water insoluble ash.

The total ash method is designed to measure the total amount of inorganic material remaining after ignition.

It measures the total inorganic content (ammonium, silica, potassium, calcium, chloride, iron, etc.) present in the drug.

This includes both “physiological ash”, which is derived from the plant tissue itself and “non-physiological ash” which is the residue of the extraneous matter like sand and soil adhering to the plant surface.

Total Ash

The ash value of the drug was determined as 23.5% which is high when compared to other herbo mineral drugs but the increased ash content may be due to the presence of inorganic materials. Thus Ash value is a validity parameter describe and to assess the degree of purity of a given drug

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. It is 2.3% for VTC

Water soluble ash

Water-soluble ash is the part of the total ash content, which is soluble in water. It is 26.7%for VTC Water-soluble extractive value plays an important role in drug delivary system.

Alcohol-soluble ash:

Alcohol-soluble ash is the part of the total ash content, which is soluble in alcohol. It is 21.7% for VTC

- These are indicating the approximate measure of chemical constituents of crude drug.
- The percentage of soluble matters present in the drug is determined by the values of water extractive and ethanol extractive.
- Based on the extractive value suitable solvent can be selected. It also gives the percentage of drug which will correlate with the metabolism reactions.
- Water-soluble extractive value plays an important role in evaluation of crude drugs
- The alcohol-soluble extractive value was also indicative for the same purpose as the water-soluble extractive value

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.
- Since the drug has low loss on drying, the moisture content is less which is suitable for medicine preparation

Microbial Limit Tests

The total bacterial count and the total fungal count of the drug were found to be within the WHO prescribed limits which indicate that the drug is free from microbial condamination. The other pathogens like Escherichia coli, Salmonella sps, Pseudomonoas aeruginosa and Staphylococcus aureus were found to be completely absent in the drugs.

Thin Layer Chromotography :

Under UV 254nm and 366nm

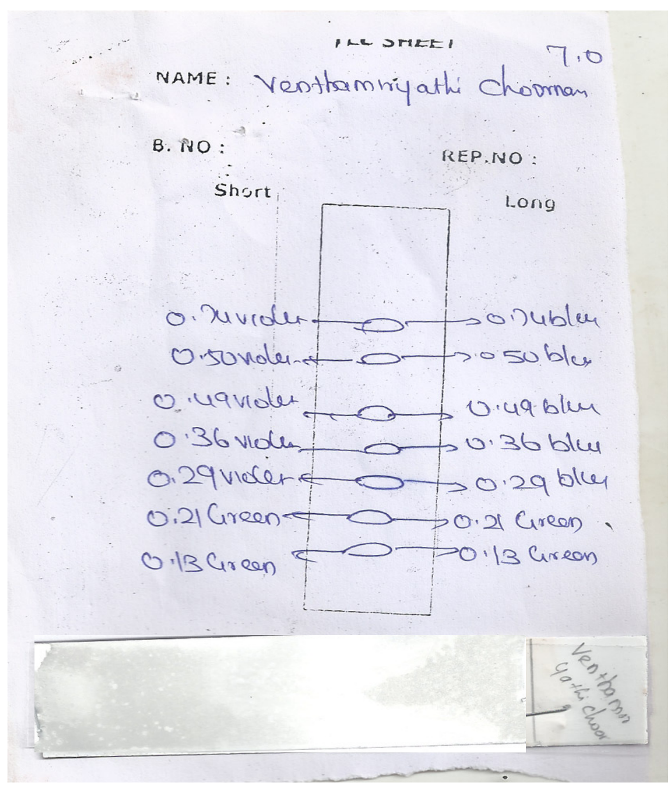


Fig : 5 TLC sheet

Interpretion:

Under UV 254 nm and 366 nm test related to alkaloids, it shows major spots short at Rf 0.74(violet), 0.50(violet), 0.49(violet), 0.36(violet), 0.29(violet), 021(green) and long at Rf 0.74(blue), 0.50(blue), 0.49(blue) ,0.36(blue) ,0.29(blue), 0.21(green) . show 2 compounds are found.

CHEMICAL ANALYSIS

Table:12 Result of Acidic and Basic Radicals

S.No	Test	Inference
1	Test for **calcium**	**+ve**
2	Test for **sulphate**	**+ve**
3	Test for **chloride**	**+ve**
4	Test for carbonate	-ve
5	Test for **starch**	**+ve**
6	Test of **iron ferrous**	**+ve**
7	Test for **phosphate**	**+ve**
8	Test for Albumin	-ve
9	Test for Tannic acid	-ve
10	Test for **unsaturation**	**+ve**
11	Test for **reducing sugar**	**+ve**
12	Test for **amino acid**	**+ve**
13	Test for zinc	-ve

Interpretation :

The chemical analysis of *venthamarayathi chooranam* contains the following chemical constituents ferrous Iron, calcium, Sulphate, chloride,Starch, iron ferrous, phosphate, Unsaturated, reducing sugar ,amino acid compounds.

Calcium

Presence of calcium improves the physical strength of skeletal tissue. Calcium ions are necessary for muscle contraction. Calcium ions are necessary for the normal transmission of nerve impulse

Unsaturated fats

Monounsaturated and polyunsaturated fats can replace saturated fat in the diet, trans unsaturated fats should not. Replacing saturated fats with unsaturated fats helps to lower levels of total cholesterol and LDL cholesterol in the blood.

Chloride

Chloride is an anion in the human body needed for metabolism (the process of turning food into energy) It also helps keep the body's acid-base balance. The amount of serum chloride is carefully controlled by the kidneys.

Chloride ions have important physiological roles. For instance, in the central nervous system, the inhibitory action of glycine and some of the action of GABA relies on the entry of Cl– into specific neurons

Starch

Digestive enzymes have problems digesting crystalline structures. Raw starch will digest poorly in the duodenum and small intestine, while bacterial degradation will take place mainly in the colon.

Iron

iron metabolism is the set of chemical reactions maintaining human homeostasis of iron at both the systemic and cellular level. The control of this necessary but potentially toxic metal is an important part of many aspects of human health and disease. Hematologists have been especially interested in systemic iron metabolism because iron is essential for red blood cells, where most of the human body's iron is contained.

Phosphate

Parathyroid hormone(PTH), and calcitriol also regulate phosphate in the body. PTH helps lower blood phosphate levels. It does this by reducing the reabsorption of phosphates dissolved in urine in the kidneys, causing more excretion of phosphates. Calcitriol raises the level of phosphate in the blood by promoting its absorption by the intestine.

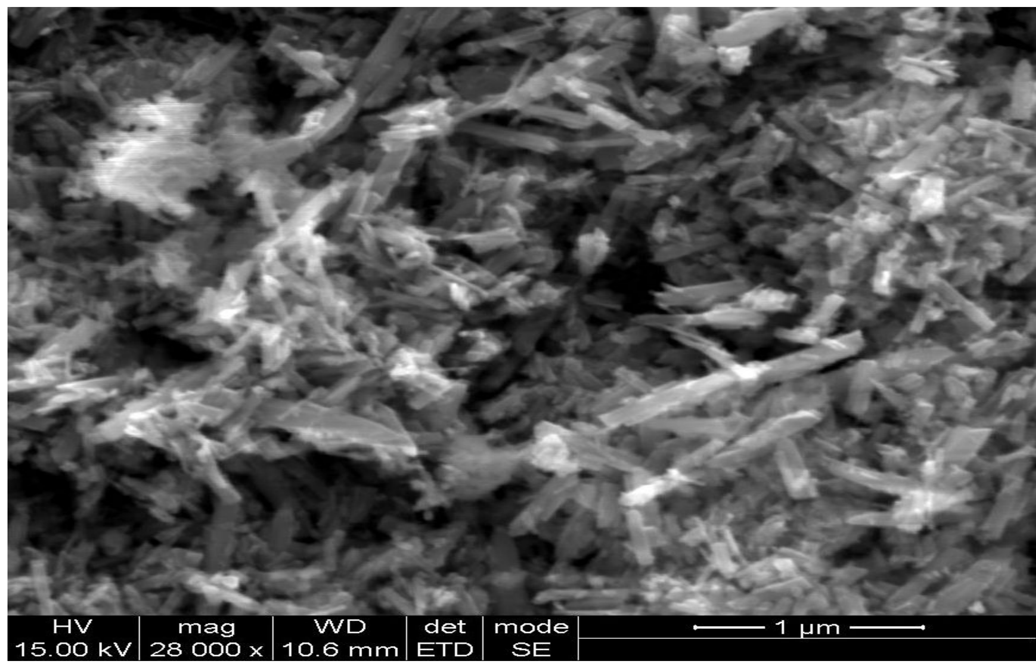
Amino acids

Amino acids derivatives include 5-HTP (5-hydroxytryptophan) used for experimental treatment of depression L-DOPA (L-dihydroxyphenylalanine) for Parkinson's treatment

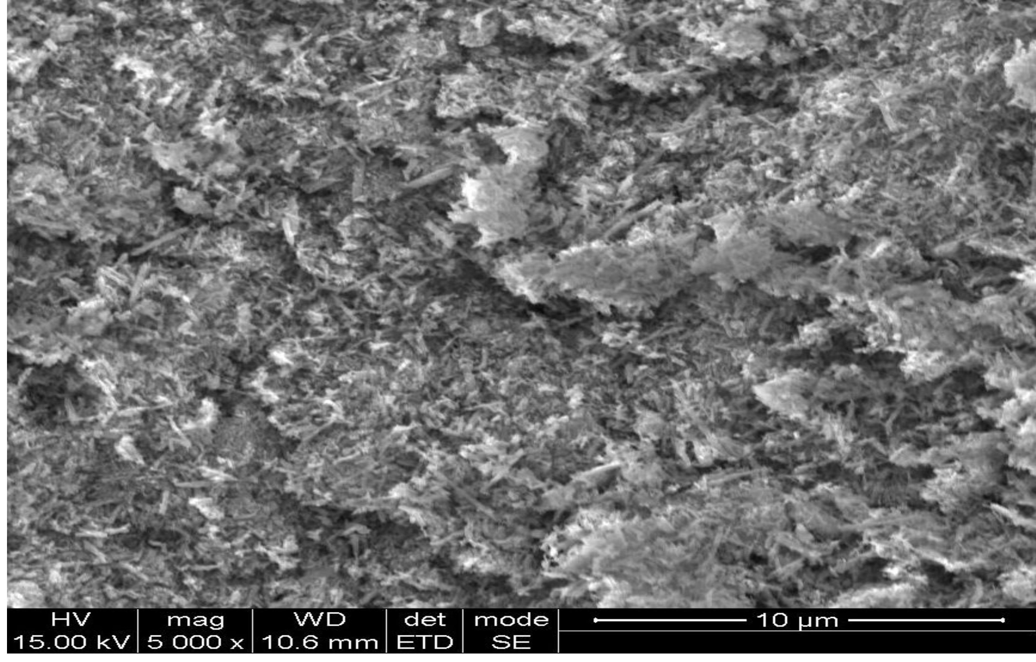
VTC drug that inhibits ornithine decarboxylase and used in the treatment of sleeping sicknes

SCANING ELECTRON MICROSCOPE (SEM) OFVENTHAMARAYATHI CHOORANAM

Fig. 6



SEM Picture 28, 000 Magnification of VT



SEM Picture 5,000 Magnification of VT

SEM images showing the shape and size of the particle size of the drug VTC

Interpretation

The morphology of the VT C sample can be determined by Environmental SEM (FEI Quanta). A representative portion of each sample must be sprinkled onto a double side carbon tape and mounted on aluminium stubs, in order to get a higher quality secondary electron image for SEM examination. We have observed from SEM photographs that particles are spherical in shapes and sizes are in the range from 0.1 micron to 0.5 micron. Although the particle sizes of different batches showed similarity, it seems that these particles are aggregates of much smaller particles. When dispersed in an aqueous medium, these preparations form a negatively charged hydrophobic particle suspension. This hydrophobicity gives these particles a tendency to aggregate together to form larger particles. Chooranam exhibited larger sizes and agglomeration of the particles. Therefore, the comparatively larger size may be due to the agglomeration of the particles by repeated purification process involved in preparation.

ICP - OES RESULT:

Venthamarayathi Chooranam weight = 0.32515gm

Table No. 13

Elements symbol	Wave length (nk)	Concentration (Mg/ l)
Al	396.152	**BDL**
AS	188.979	**BDL**
Ca	315.807	12.170 mg/L
Cu	327.393	**BDL**
Fe	238.204	01.306 mg/L
Hg	253.652	**BDL**
K	766.491	33.801mg/L
Mg	285.213	01.304 mg/L
Na	589.592	24.310 mg/L
Ni	231.604	**BDL**
Pb	220.353	**BDL**
P	213.617	88.341 mg/L
S	180.731	01.324 mg/L
Zn	206.200	00.258 mg/L

Interpretation:

The result reveals the below detection limit (BDL) of Aluminimum (Al), Arsenic (As), Copper (Cu), Mercury (Hg), Nickel (Ni), Lead (Pb). This reveals the safety of the drug.

The result indicates the presence of Calcium (Ca), Iron, Pottasium (K), Magnesium (Mg), Sodium (Na), Phosphorus (P), Sulphur (S), Zinc (Zn).

Calcium:

Calicum was associated with the lower risk of Hypertension.

- By inhibiting calcium influx into cytosol, histamine release from mast cell, acetylcholine release from cholinergic nerve endings
- It also may increase the vasodilator effect of beta2-agonist by increasing the receptor affinity

Iron:

- The heme containing enzyme such as catalase and peroxidase protect cell against portentially damaging highly reactive species.
- Iron is essential for may numbers of biological functions such as growth, reproduction, healing and immune function.

- Iron is associated with effective immune competence of the body.
- Iron plays an important role within the cells that produce the energy.

Potassium

- Potassium dilates the arteries and relaxes the smooth muscles and increases the blood flow (F.J. Haddy, 2012).
- An adequate amount of potassium on consumption, reacts as vasodilator, brings blood flow freely in body ,
- Avoid the formation of clot and reduce the cause of stroke
- It controls electrical activity of heart

Magnesium

- Magnesium is a cofactor that regulates diverse biochemical reactions in the body, including protein synthesis, muscle, nerve function, blood glucose control and blood pressure regulation (Rude.R.K, 2012).
- It is an important mineral to several body functions
- It enhances the function of the endothelium, which is the inner most layer of blood vessels. & mucus membrane

Zinc

- Zinc is a micro nutrient and is required for wound healing.
- Zinc has effects against viruses. {Rhino virus}.
- Zing may be regarded as an antioxidant, protects the body against free radical damage and cell damage.
- Zinc is important for a healthy immune system
- It enhances absorbtion of iron.
- It can produce healthy veins and arteries that enhance the blood circulation

Sodium:

- Sodium regulates the body's acid base balance
- Sodium is required for the maintenance of osmotic pressure and fluid balance
- It is necessary for the normal muscle irritability and cell permeability

Phosphorus:

- It is requried for the formation of phospholipids & Nucleic acids .
- It palays a central role for the formation and utilization of high energy phosphate compounds
- Maintenance of PH in the blood as well as in the cells.

Sulphate

- Sulphate may prevent the occurrence of any infection
- Sulphate is potent anti oxidant activity in human body

Fourier Transform Infrared Spectroscopy Analysis

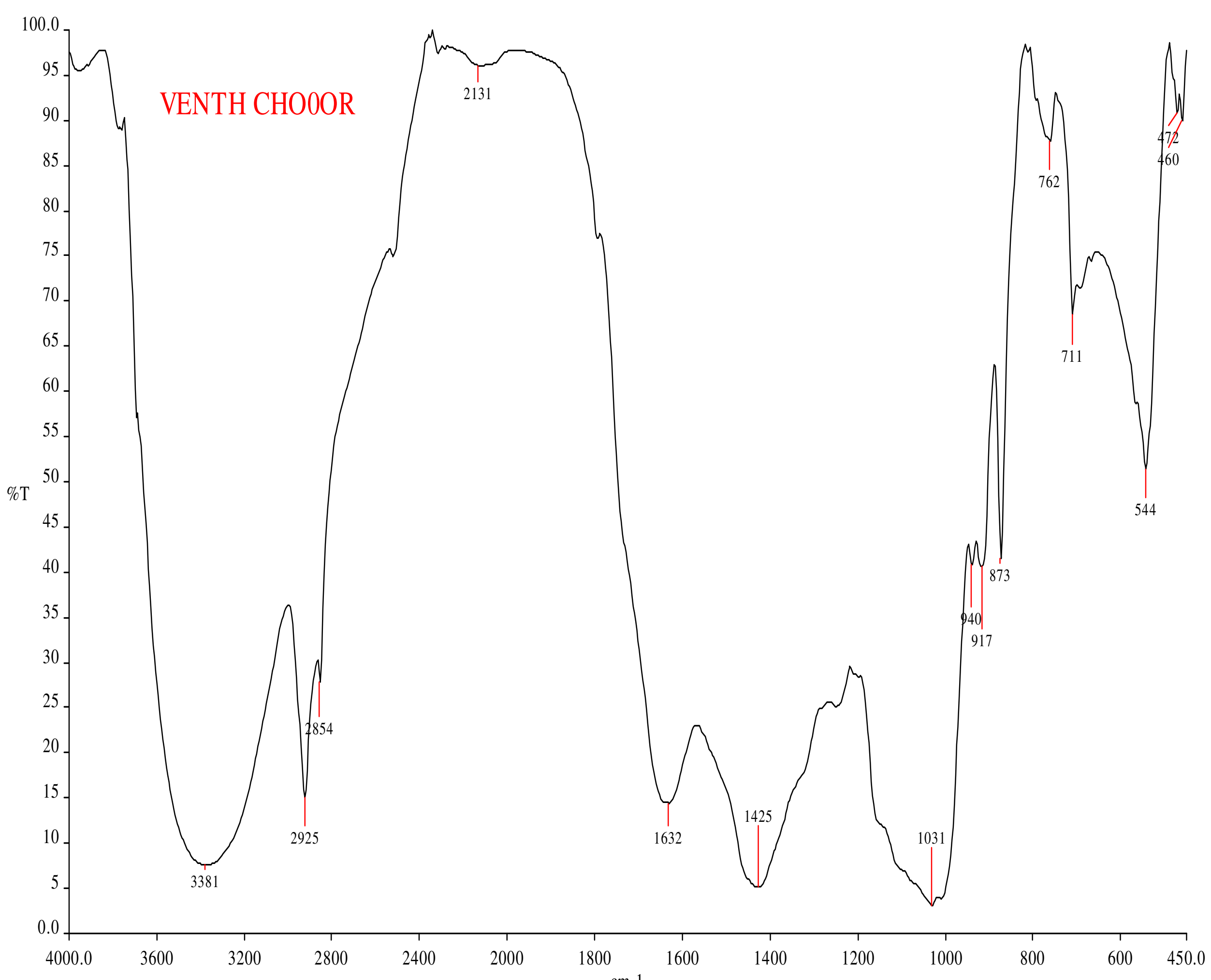


Fig : 7 Graph of FTIR analysis

Table : 14 Results of FTIR analysis of VTC

Frequency, cm-1	Bond	Functional group
3381 (m)	N – H stretch	Primary, secondary, amines, amides
2925 (m)	C – H stretch	Alkenes
2854 (m)	C – H stretch	Alkenes
2131 (w)	C (triple bond) C- stretch	Alkynes
1632 (m)	N – H bend	Primary amines
1425 (m)	C –C stretch (in – ring)	Aromatics
1031 (m)	C – N stretch	Aliphatic amines
900 (s)	C – H “oop”	Aromatics
762 (m)	O – H bend	Carboxylic acids
711 (m)	O – H bend	Carboxylic acids
675 (s)	C – H “oop”	Aromatics
544 (m)	C – CI stretch	Alkyl halides

M = medium, w = weak, s=strong,

Interpretation:

The FTIR has proven to be a valuable tool for the charecterisation and identification of compounds or functional groups present in the VTC

In FTIR the wavenumbers between $4000cm^{-1}$-$400cm^{-1}$ is known as functional group area. <$400cm^{-1}$ wave number is known as finger print area. The corresponding absorbtion frequency by FTIR shows the presence of, Alkanes, Amines,Aromatics,,Aliphatic amines , Alkyl halides, Carboxylic acids. The C-H stretch may be due to the presence of moisture content. Infrared bonds for inorganic materials appear in the lower wave numbers than those observed for organic materials.

In cofirms that Venthamarayathi chooranam contains Alkanes, Amines,Aromatics,,Aliphatic amines , Alkyl halides, Carboxylic acids

Amines groups:

- Amines groups act as neurotransmitters.
- It is involved in protein synthesis.
- This group of substances has antihistaminic and analgesic activity.
- Amines are a class of compounds derived from ammonia by replacement of one or more effective antagonists of SSTR5 (Stomatostatin receptor 5)and are used for treatment, control and prevention of disorders such as ,lipid disorders and obesity

Alkanes groups:

- Alkanes have little biological activity.
- It is predominate in plants. They protect against bacteria and fungi

Alkynes:

- Alkyne is an unsaturated hydrocarbon containing one carbon –carbon triple bond,Some alkyne compounds which are physiologically acceptable salts are used as MCH antagonist .Thus they influence eating behavior , reduce body weight and addressing obesity which often leads to diseases such as diabetes ,dyslipidemias, high BP and CHD.

Alkyl Halides:

- These are group of compounds derived from alkanes containing one or more halogens. Some are used as anesthetics and antiseptic agents. Some of them are used in medicine for the elimination of hook worms

TOXICITY STUDY RESULTS

ACUTE TOXICITY STUDY IN FEMALE WISTER RATS TO EVALUATE TOXICITY PROFILE OF *VENTHAMARAIYATI CHOORANAM*

Result:

From acute toxicity study it was observed that the administration of ***Venthamaraiyathi Chooranam*** at a dose of 2000mg/kg, to a rats. From acute toxicity study it was observed that the administration of ***Venthamaraiyathi Chooranam*** at a dose of 2000 mg/kg to the rats do not produce drug-related toxicity and mortality. So No-Observed-Adverse-Effect- Level (NOAEL) of ***Venthamaraiyathi Chooranam*** is 2000 mg/kg.

Discussion

Venthamaraiyathi Chooranam was administered single time at the dose of 5mg/kg, 50mg/kg , 300mg/kg, 1000mg/kg and 2000mg/kg to rats and observed for consecutive 14 days after administration. Doses were selected based on the pilot study and literature review. All animals were observed daily once for any abnormal clinical signs. Weekly body weight and food consumption were recorded. No mortality was observed during the entire period of the study. Data obtained in this study indicated no significance physical and behavioural signs of any toxicity due to administration of ***Venthamaraiyathi Chooranam*** at the doses of 5mg/kg, 50mg/kg , 300mg/kg, 1000mg/kg and 2000mg/kg to rats.

At the 14th day, all animals were observed for functional and behavioral examination. In functional and behavioral examination, home cage activity, hand held activity were observed. Home cage activities like Body position, Respiration, Clonic involuntary movement, Tonic involuntary movement, Palpebral closure, Approach response, Touch response, Pinna reflex, Sound responses, Tail pinch response were observed. Handheld activities like Reactivity, Handling, Palpebral closure, Lacrimation, Salivation, Piloercetion, Papillary reflex, abdominaltone, Limb tone were observed. Functional and behavioral examination was normal in all treated groups. Food consumption of all treated animals was found normal as compared to normal group.

Body weight at weekly interval was measured to find out the effect of ***Venthamaraiyathi Chooranam*** on the growth rate. Body weight change in drug treated animals was found normal.

SUB-ACUTE TOXICITY STUDY
WISTER RATS TO EVALUATE TOXICITY PROFILE OF VENTHAMARAIYATHICHOORANAM

RESULT:

Clinical Signs:

All animals in this study were free of toxic clinical signs throughout the dosing period of 28 days.

Mortality:

All animals in control and in all the treated dose groups survived throughout the dosing period of 28 days.

Body weight:

Results of body weight determination of animals Table-1 from control and different dose groups exhibited comparable body weight gain throughout the dosing period of 28 days.

Food consumption:

During dosing and the post-dosing recovery period, the quantity of food consumed by animals from different dose groups was found to be comparable with that by control animals.

Organ Weight:

Group Mean Relative Organ Weights (% of body weight) are recorded in Table No.4 Comparison of organ weights of treated animals with respective control animals on day 29 was found to be comparable similarly.

Hematological investigations:

The results of hematological investigations (Table 4) conducted on day 29 revealed following significant changes in the values of different parameters investigated when compared with those of respective controls; however, the increase or decrease in the values obtained was within normal biological and laboratory limits or the effect was not dose dependent.

Biochemical Investigations:

Results of Biochemical investigations conducted on days 29 and recorded in Table 2 revealed the following significant changes in the values of hepatic serum enzymes studied. When compared with those of respective control. However, the increase or decrease in the values obtained was within normal biological and laboratory limits.

Histopathology:

In histopathological examination, revealed normal architecture in comparison with control and treated animal.

Discussion:

- All the animals from control and all the treated dose groups up to 900 mg/kg survived throughout the dosing period of 28 days.
- No signs of toxicity were observed in animals from different dose groups during the dosing period of 28 days.
- Animals from all the treated dose groups exhibited comparable body weight gain with that of controls throughout the dosing period of 28 days.
- Food consumption of control and treated animals was found to be comparable throughout the dosing period of 28 days
- Haematological analysis conducted at the end of the dosing period on day 29, revealed no abnormalities attributable to the treatment.
- Biochemical analysis conducted at the end of the dosing period on day 29 no abnormalities attributable to the treatment.
- Organ weight data of animals sacrificed at the end of the dosing period was found to be comparable with that of respective controls.
- Histopathological examination revealed normal architecture in comparison with control and treated animal.

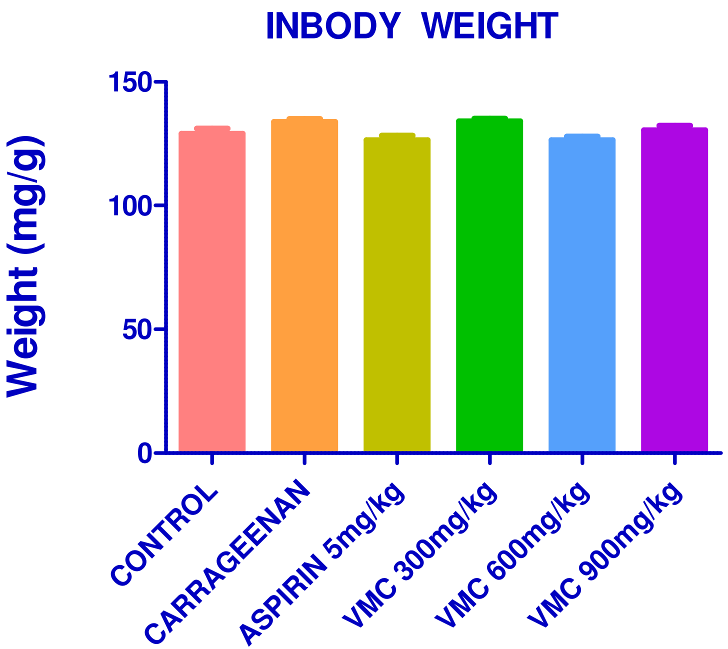
PHARMACOLOGICAL STUDIES RESULT

1. THROMBOLYTIC ACTIVITY RESULT
EFFECT OF *VENTHAMARAIYATHI CHOORANAM* ON BODY WEIGHT (PHYSICAL PARAMETER)

Table : 15 Results

GROUP	CONTROL	ONLY CARRAGEENAN	CARRAGEENAN +STD	CARRAGEENAN + VMC -300mg/kg	CARRAGEENAN + VMC -600mg/kg	CARRAGEENAN + VMC -900mg/kg
BODY WEIGHT	129±2.176	133.8±1.167	126.5±1.821	134.2±0.9804	126.5±1.408	130.5±1.765

Values are expressed as the mean ± S.D; Statistical significance (p)calculated by one way ANOVA followed by dunnett's $^{c}P < 0.001$, $^{b}P < 0.01$, $^{a}P < 0.05$ calculated by comparing treated group with CONTROL group



EFFECT OF *VENTHAMARAIYATHICHOORANAM* ON

Table : 16

GROUP	CONTROL	ONLY CARRAGEENAN	CARRAGEENAN +STD	CARRAGEENAN + VMC -300mg/kg	CARRAGEENAN + VMC -600mg/kg	CARRAGEENAN + VMC -900mg/kg
Total Tail Length	14.1±0.430116	13.24±0.421426	13.96±0.628172	13.7±0.463681	13.84±0.566216	13.34±0.366879
Total Clotting Tail Length	0±0	12.68±0.378682	7.98±0.235372	9±0.223607	6.76±0.2502	5.64±0.443396

Values are expressed as the mean ± S.D; Statistical significance (p)calculated by one way ANOVA followed by dunnett's $^{c}P < 0.001$, $^{b}P < 0.01$, $^{a}P < 0.05$ calculated by comparing treated group with CONTROL group

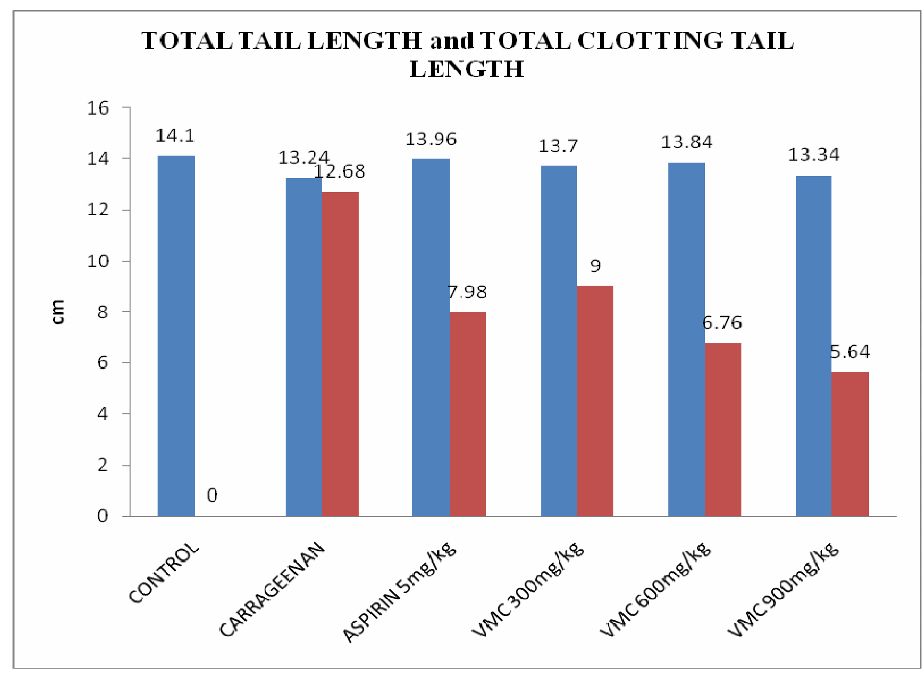


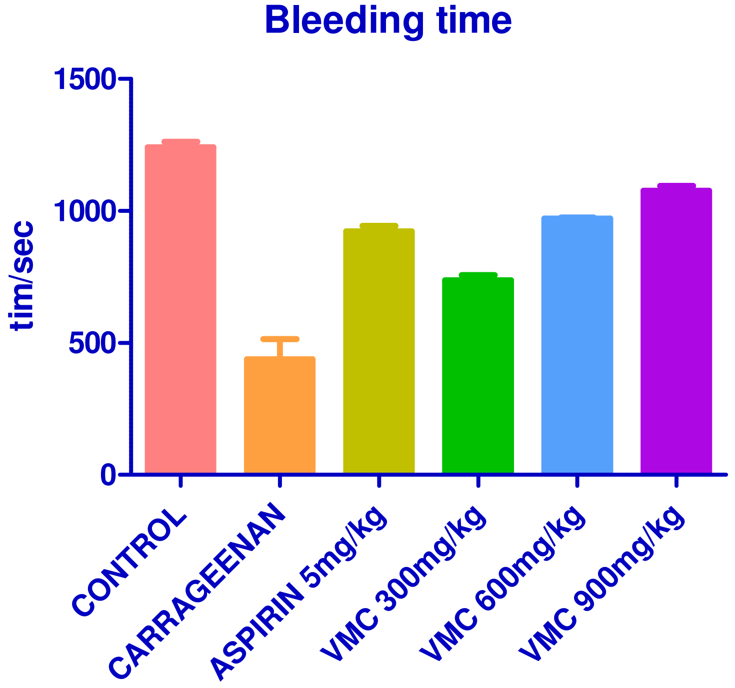
Fig. 8. Total Clotting Tail Length

EFFECT OF *VENTHAMARAIYATHICHOORANAM* ON BLEEDING TIME and BLOOD CLOTTING TIME.

Table : 17 Stastical significance

GROUP	CONTROL	ONLY CARRAGEENAN	CARRAGEENAN +STD	CARRAGEENAN + VMC -300mg/kg	CARRAGEENAN + VMC -600mg/kg	CARRAGEENAN + VMC -900mg/kg
Bleeding time	1241.33±20.0577	437.667±76.8117	923±20.4385	738.333±19.08	971.333±4.86256	1077.33±18.089
blood clotting time (sec.)	33.1667±0.909823	6±0.68313	24.1667±1.16667	14.5±0.885061	25.5±0.885061	31.1667±0.909823

Values are expressed as the mean ± S.D; Statistical significance (p)calculated by one way ANOVA followed by dunnett's $^{c}P< 0.001$, $^{b}P< 0.01$, $^{a}P < 0.05$ calculated by comparing treated group with CONTROL group



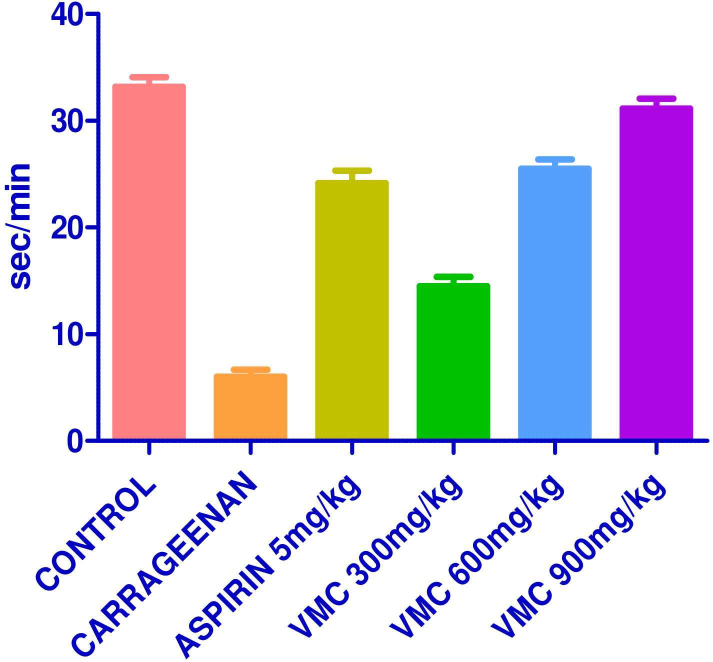


Fig. 9

Table : 18 Effect on Partial Thromboplastin Time (APTT), Prothrombin Time (PT), Fibrinogen Concentration AndEuglobulin Clot Lysis Time (ECLT) In Rats.

GROUP	APTT(S)	PT(S)	Fibrinogen Concentration	ECLT (S)
Control	18.26±0.475	34.93±1.536	238±1.93218	266±2.19089
Only Carrageenan	18.36±0.423	36±0.730	486±7.89937***	440±15.249***
Carrageenan +STD	20.46±0.735	25.33±1.115	218±0.730297***	206.67±2.764***
Carrageenan +Vmc 300mg/Kg	23.06±1.187***	31±0.632	307±6.46014***	246±5.11208***
Carrageenan +Vmc 600mg/Kg	17.73±0.830	45.33±3.451	292.667±19.25	270.667±3.67575
Carrageenan +Vmc 900mg/Kg	20.33±0.735	50±6.366**	278±5.26625*	224.667±5.18116**

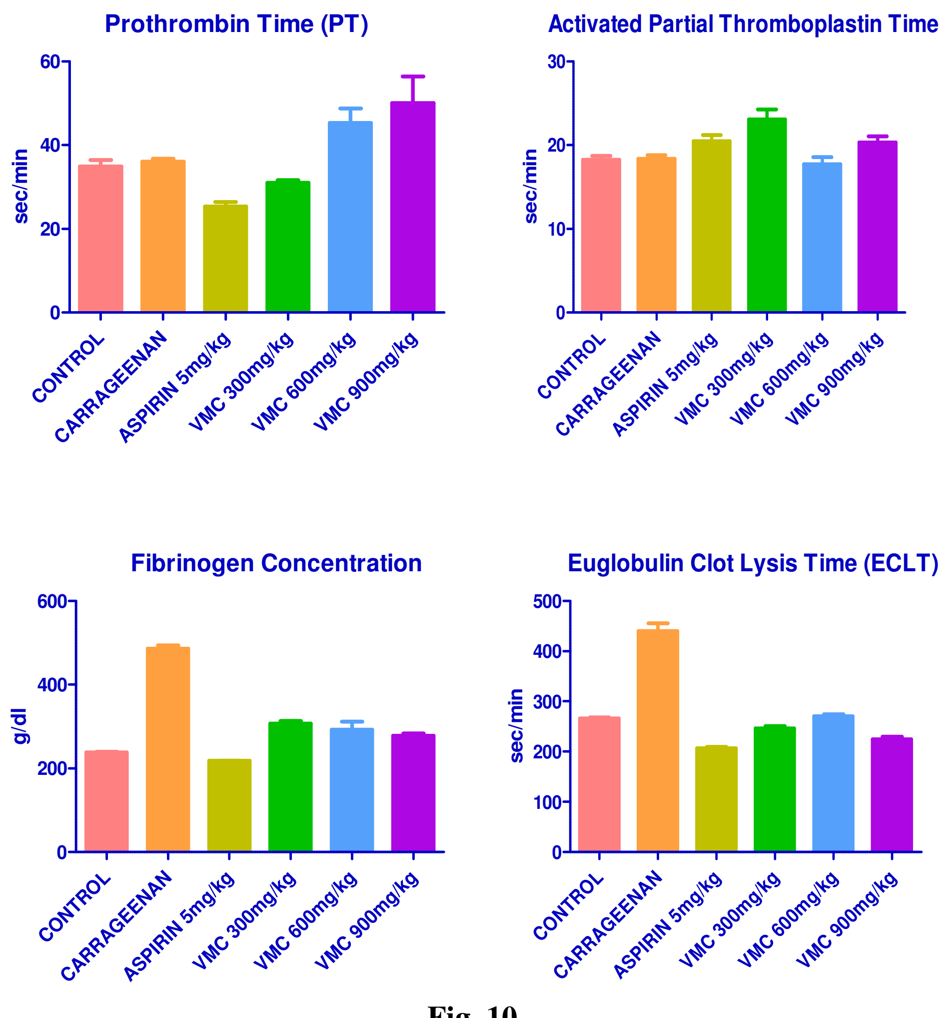


Fig. 10

Interpretation:

The test drug "***Venthamaraiyathi Chooranam*** " has got Significant thrombolytic activity.

2. VASODILATOR ACTIVITY RESULT

Vasodilatation Effect Test

PE at 1 μM produced a steady-state contraction in the aortic rings with or without endothelium. As shown in Figure 1, the extract (50–400 μg/mL) caused vasodilation of the endothelium-intact thoracic aorta ring pre-constricted with phenylephrine in a dose-response manner. However, this effect disappeared after preincubation of the aortic rings with atropine (1×10^{-6} M), L-NA (1×10^{-4} M) or by removal of the vascular endothelium.

Figure: 11 (a)

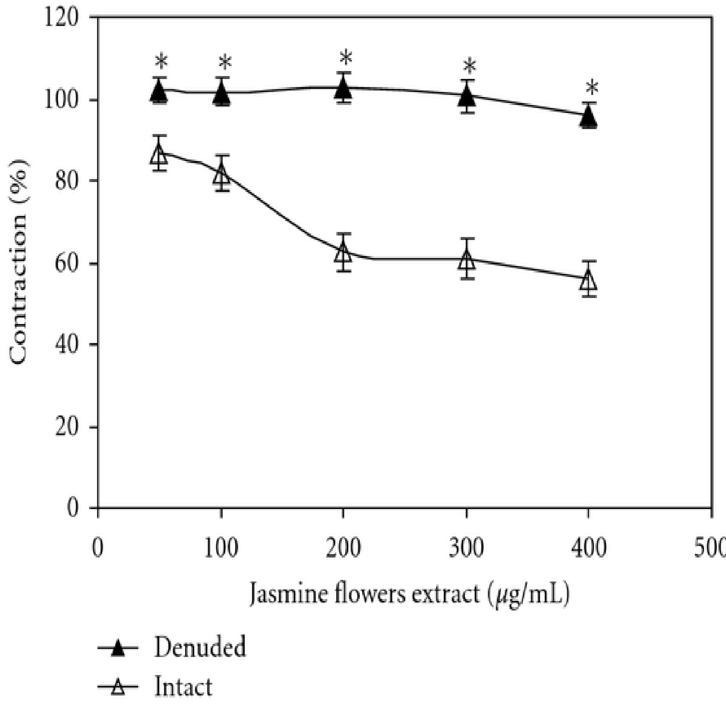


Figure: 11 (b)

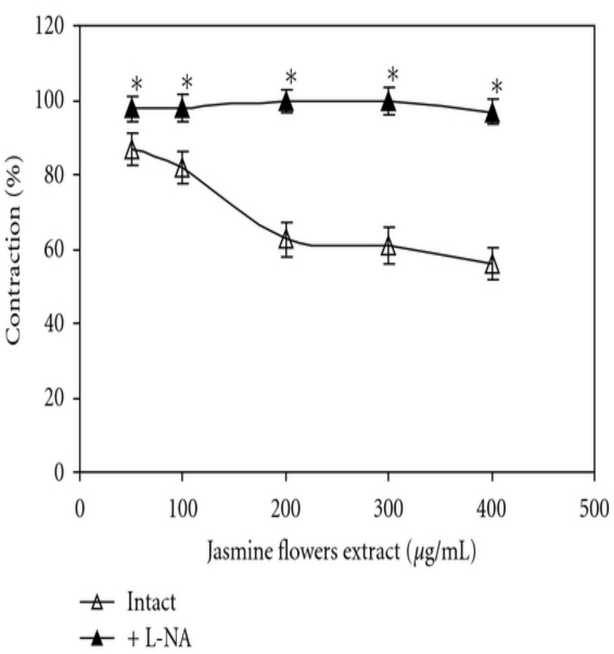
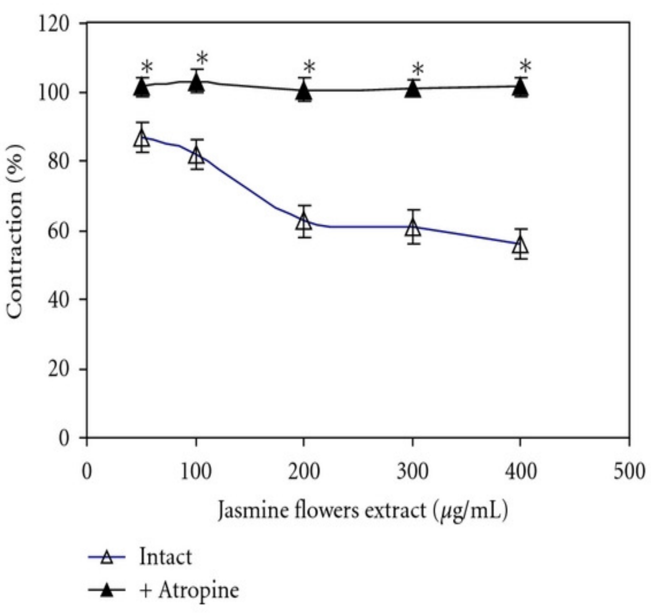


Figure: 11 (c)



Dose-response curves for the vasodilation effects of the ethanolicVenthamaraiyadhiChoornam extract on the preconstricted thoracic aorta rings. Results are presented as means ± SD ($n = 6$), (Student's t-test, $^{*}P < 0.05$). (a) Endothelium **..**

Discussion

Venthamarai Flower is one of the most well-famous fragrant plants worldwide and has been prescribed in folk medicines in many countries according to its multipurpose actions. Venthamaraiyathi Choornam possessed vasodilation activity. As shown in Figure 11(a), the vasodilation effect of the Venthamaraiyathi Choornam extract was mediated by endothelial cells in the aortic vessel. The Choornam extract at a dose of 400 μg/mL reduced the contraction to lower than 43% of the maximal contraction. This result suggests that the vasorelaxation effect of the ethanolic *Venthamaraiyathi Choornam* extract is endothelium dependent. It has been reported that the vasorelaxant property of most plant extracts was from flavonoids.

Thus, the vasodilation activity should be attributed to the high content of flavonoid mixtures found in the Venthamaraiyathi Choornam extract.Preincubating the thoracic aortic rings with atropine (10^{-6} M), a muscarinic receptor antagonist completely blocked the relaxant activity of the extract (Figure 11(b)). The pharmacologically relaxant effect of the Choornam extract was further determined. Pretreatment of endothelium-intact aorta with L-NA (1×10^{-4} M) also reduced the vasodilation effect of the extract to an extent equivalent to the effect in endothelium-denuded aorta (Figure 11(c)). Nowadays, the mechanism of nitric oxide (NO) and the function of endothelial cells in the relaxation of arteries are well described. NO is a potent vasodilator synthesized in the endothelium by NO synthase and causes vascular relaxation. The result from the present study suggests that the Venthamaraiyathi Choornam extract may exert its endothelium-dependent relaxation activity by stimulating the nitric oxide release from the vascular endothelium via muscarinic receptors.

3. HYPOLIPIDEMIC ACTIVITY RESULT

Table : 19 - Effect on siddha formulation ventha maraiyathy chooranam in Lipid Profile

GROUPS	Total cholesterol (Mg/dl)	Tri glycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	AI	LDL/HDL
Normal Control	50.70 ± 1.80	58.10± 0.90	27.40 ± 1.20	14.40 ± 0.75	31.22 ± 1.15	0.87 ± 0.50	0.54 ±
Cholesterol Control	115.40 ± 1.58$^{**(a)}$	162.65 ± 1.72$^{**(a)}$	11.92 ± 0.70$^{**(a)}$	30.95 ± 1.36$^{**(a)}$	13.10 ± 0.70$^{**(a)}$	8.51 ± 1.33$^{**(a)}$	2.60 $^{**(a)}$
Standard Control	76.60± 1.45$^{**(b)}$	83.15± 1.80$^{**(b)}$	21.70 ± 0.40$^{**(b)}$	20.15 ± 0.80$^{**(b)}$	24.90 ± 0.80$^{**(b)}$	2.34 ± 2.33$^{**(b)}$	0.92 $^{**(b)}$
Treatment control 200mg/kg	91.80 ± 1.30$^{**(b)}$	115.40 ± 1.92$^{**(b)}$	17.50 ± 0.50$^{**(b)}$	25.30 ± 0.60$^{**(b)}$	17.85 ± 0.42$^{**(b)}$	4.39 ± 1.48$^{**(b)}$	1.46$^{**(b)}$
Treatment control 400mg/kg	84.45 ± 0.95$^{**(b)}$	96.5 ± 1.10$^{**(b)}$	20.30 ± 1.32$^{**(b)}$	22.15 ± 0.70$^{**(b)}$	21.30 ± 0.52$^{**(b)}$	3.13 ± 0.24$^{**(b)}$	1.08 $^{**(b)}$

- Values are expressed as Mean ± SD.
- Values were find out by using ONE WAY ANOVA followed by Newman Keul's multiple range tests.
- ** (a) values were significantly different from normal control at P< 0.01.
- ** (b) Values were significantly different from hyperlidemic control at P< 0.01.

Table shows the levels of Cholesterol, Triglycerides, HDL, LDL and VLDL of control and experiment rats respectively. Serum of hyperlipidemic rats showed significantly increased levels of Cholesterol, Triglycerides, LDL - C and low HDL – C, when compared with normal rats. In rats treated with both doses of siddha formulation *Venthamaraiyathi Chooranam* and Atorvastatin there was significant decrease in the content cholesterol, TGs, LDL – C, and VLDL and increases HDL – C, when compared with cholesterol control rats.

Atherogenic index (AI) and LDL – C / HDL – C ratio:

Table shows the changes of Atherogenic Index and LDL – C / HDL – C ratio in control and treated rats. It appears clear form these results that the cholesterol induction significantly affects the cardio vascular risk markers.

Indeed, AI was statistically increased in cholesterol control group 90% compared with the values found in their normal control group.

Besides there were significant further increase of LDL – C / HDL – C ratio in cholesterol control group compared to normal control group.

Promising results in lowering of AI by siddha formulation venthaa marayathy chooranam was found in Table I. The siddha formulation *Venthamaraiyathi Chooranam* showed an improvement of the cardio vascular risk level by decrease of AI in the treated group by more than 73% and 63% ($p < 0.01$), when compared to the cholesterol control group.

The ratio of LDL – C to HDL – C is also a protective indicator of cardio vascular disease incidence. The cholesterol induction produced a significant increase of this marker. In contrast, elevated ratio in treated group and Atorvastatin group returned to basal value when the data were compared in the same period to the data found for cholesterol rats.

DISCUSSION

The reduction of plasma total cholesterol was associated with a decrease in its LDL fraction which is a major, potentially modifiable risk factor of cardio vascular disease and the target of drug. Many suggest that the cholesterol lowering activity of this product appears to be due to the enhancement of LDL – C catabolism through hepatic receptors .(4)In addition siddha formulation ventha maraiyathy chooranam showed protective action which is reported to have a preventive function against atherogenesis since an independent inverse relationship between blood HDL – C levels and cardio vascular risk incidence is reported.(5) The lipoprotein called "good cholesterol" facilitates the mobilization of triglycerides and cholesterol from plasma to liver where it is catabolised and eliminated in the form of bile acids. The possible mechanism of this activity may result from the enhancement of lecithin cholesteryl acyl transferase (LCAT)

and inhibition of Hepatic Triglyceride Lipase (HTL) on HDL which may lead to a rapid catabolism of blood lipids through enterohepatic tissues.(6)

It is also recently reported that triglycerides plays a key role in the regulation of lipoprotein interaction to maintain normal lipid metabolism. Indeed, the elevated plasma TG levels were associated with an increased incidence of coronary artery disease. Moreover these higher plasma TG levels have been attributed mainly to increase population of small, dense LDL deposits which are very atherogenic and enhanced cholesteryl ester mass transfer from apolipoprotein B containing lipoproteins (VLDL and LDL).(7) TG has also been proposed to be major determinant of cholesteryl esterification, its transfer and HDL remodeling in human plasma.(8)

Siddha formulation *Venthamaraiyathi Chooranam* significantly suppress the elevated blood concentration of TGs. This result suggests that the product is able to restore, at least partially, the catabolism of TG. The underlying the mechanism of this activity is not elucidated by the present study. However, as hypothesized by many works with other plants, the restoration of catabolic mechanism of TGs would be due to an increased stimulation of the lipolytic activity of Plasma Lipoprotein Lipase (LPL).(9)

Administration of siddha formulation *Venthamaraiyathi Chooranam* provides a beneficial action on rat lipid metabolism with regard to the reduction of AI. Infact, the AI was decreased in all treated groups. Similar results were reported by others when studying the hypolipidemic effects of natural products .(10) This ameliorative action was due to the plasma lipid lowering activity of different constituents of the plant.

It is also desirable to have higher plasma HDL and lower LDL – C to prevent atherogenesis, since there is a positive correlation between an increased LDL – C / HDL – C ratio and the development of atherosclerosis. Again, the administration of siddha formulation *Venthamaraiyathi Chooranam* significantly suppress the higher values of LDL – C / HDL – C ratio showing the beneficial effect of this preparation in preventing atherosclerosis incidence.

This result is considered as an important for the treatment of hyperlipidemia induced atherosclerosis and apparently validates the folk medicinal use of hyperlipidemic patients in India.

Interpretation:

Hyperlipidemia is considered to be major risk factor for the premature atherosclerosis and essentially the cholesterol in atherosclerotic plaque is derived from that of circulatory cholesterol. The antihyperlipidemic effect on siddha formulation ***Venthamaraiyathi Chooranam*** in particular could be considered as a possible therapeutic value.

3. CARDIOPROTECTIVE ACTIVITY RESULT:

EFFECT OF VENTHAA MARAYATHY CHOORANAM AGAINST DOXORUBICIN INDUCED MYOCARDIAL TOXICITY IN ALBINO RATS

Effect of *Venthamaraiyathi Chooranam* on Dox-induced cardiac toxicity was established by measuring cardiac biomarker enzymes and endogenous antioxidants and heart tissue histopathology.

General observation and mortality

The general appearance of all groups of animals was recorded throughout the study. In Dox-treated group, the animal fur became scruffy and developed a pink tinge. These rats also had red exudates around the eyes and nose, soft watery feces and enlargement of abdomen. These observations were significantly less in *Venthamaraiyathi Chooranam* treated group.

Serum enzyme biomarkers

Animals treated with Dox showed significant increase in the levels of CPK and LDH compared to normal Tab.no 1. *Venthamaraiyathi Chooranam* + Dox treated group shown significantly lower levels of CPK and LDH as compared to Dox treated group.The myocardial damage in the various treated groups was determined by estimating the activities of AST, ALT and ALP Tab no.1. These biochemical markers were significantly increased in the Dox group compared to control ($P<0.01$). *Venthamaraiyathi Chooranam* pretreatment group showed significant reduction in AST, ALT and ALP levels as compared to Dox-treated group.

Table 20: Effect of *Venthamaraiyathi Chooranam* on AST, ALT, ALP, CPK and LDH enzyme activities in doxorubicin-treated rats

Group	AST	ALT	ALP	CPK	LDH
Group 1 (Normal control)	66.60±1.40	30.60±1.10	122.82±3.12	153.4±3.60	220.20±8.52
Group 2 (Toxic control)	194.60±7.80*a	60.84±2.50*a	244.20±6.36*a	320.20±6.60*a	408.52±12.30*a
Group 3 (Standard control)	8.30±2.32*b	38.20±1.80*b	16.30±4.34*b	220.30±4.25*b	282.40±9.20*b
Group4 Treatment low dose	128.90±5.28*b	49.35±2.15*b	192.65±4.50*b	270.35±4.65*b	325.20±10.62*b
Group 5 Treatment high dose	108.40±4.92*b	42.64±1.98*b	175.32±3.60*b	243.25±3.60*b	298.40±9.45*b

Values are expressed as mean ± SD,

a* - values were significantly different from Normal control (GI) at P<0.01,

b* - values were significantly different from Toxic control (GI) at P<0.01.

DISCUSSION

The study reveals the cardioprotective effect of *Venthamaraiyathi Chooranam* against Dox-induced cardiotoxicity in rats. Following evidence can be emphasized from the present study. *Venthamaraiyathi Chooranam* has been traditionally used in medicine and culinary practices in India, possesses cardioprotective, hepatoprotective and lipid lowering properties. The present study is aimed to investigate the cardioprotective effects of oral administration of *Venthamaraiyathi chooranam* against Dox -induced cardiotoxicity.

In the Dox-treated group, the animal fur became scruffy and developed a pink tinge which in the later days of observation period was followed by red exudates around the eyes and nose. Necrosis was also observed at the site of Dox injection. These changes were less pronounced in case of *Venthamaraiyathi Chooranam* pretreated group animals,

which accounts for the effective cell protecting property of *Venthamaraiyathi Chooranam* with anti-inflammatory, antioxidant and antifibrotic effect.

The study revealed severe biochemical changes as well as oxidative damage in the cardiac tissue after the chronic treatment with Dox. Transaminases such as ALT and AST are liberated into the serum after extensive tissue injury. Because the heart muscle is rich in both (especiallyAST), it suggests that their increased level is an indicator of myocardial damage.

The Dox-treated group showed marked elevation in serum levels of AST and ALT as compared to vehicle-treated group. The mild elevations of AST have been associated with liver injury or myocardial infractions. Greater the injury size, higher the activity of AST. This result implies that the Dox when taken for long period of time could cause both liver and heart injury. A typical myocardial injury gives an AST/ALT ratio greater than 1. However, AST/ALT ratio less than 1 are found due to release of ALT from the affected liver [12]. Since the result showed AST / ALT ratio to be greater than 1 with higher doses over a long period of time, Dox is likely to lead myocardial damage.

In *Venthamaraiyathi Chooranam* +Dox treated group, AST and ALT levels significantly decreased as compared to Dox treated group; therefore, present results suggest that treatment of *Venthamaraiyathi Chooranam* may inhibit myocardial damage. These findings confirm that *Venthamaraiyathi Chooranam* is responsible for maintenance of normal structural and architectural integrity of cardiac myocytes, which can be accounted for membrane stabilizing property of *Venthamaraiyathi Chooranam* , as evident from the near normal serum enzymatic activities of AST and ALT.

The serum ALP, LDH and CPK enzyme activities are important measures of both early and late phases of cardiac injury. It is reported that serum LDH and CPK were increased after Dox administration in mouse. The present results are in good agreement with our earlier findings [13] ALP activity on endothelial cell surface is responsible, in part, for the conversion of adenosine nucleotides to adenosine, a potent vasodilator and anti-inflammatory mediator that can protect tissues from the ischemic damage that results from injury. This may account for the elevation of ALP in the Dox group, where tissue injury and inflammation are prominent. On the other hand, CPK and LDH are not specific for myocardial injury individually; however, evaluation of these enzymes

together may be an indication of myocardial injury. In the preventive group, i.e. *Venthamaraiyathi Chooranam* +Dox, the ALP, LDH and CPK enzyme levels were decreased to a level near to that of control group, suggesting that *Venthamaraiyathi Chooranam* may protect the myocardial tissue against Dox toxicity.

Interpretation

In conclusion, the present study shows that the cardiotoxicity induced by Dox is related with oxidative stress. Anti-proliferative, anti-initiation and free radical scavenging properties of *Venthamaraiyathi Chooranam* may boost myocardial integrity and attenuate the cardiac toxicity. *Venthamaraiyathi Chooranam* has shown to be cardioprotective, which may be attributed to its potent antioxidant properties.

ANTI –MICROBIAL ACTIVITY RESULT:

Table : 21 Anti - Microbial Activities By Well Diffusion Method

s.no	Organism (Culture)	Susceptibility	Zone inhibition	
			Streptomycin zone size	Medicine size
1.	E.coli	Sensitive	20mm	18mm
2.	Klebsiella pnemoniae	Sensitive	24mm	11mm
3.	Staphylococcus aureus	Resistant	24mm	–
4.	Streptococcus mutant	Sensitive	18mm	22mm
5.	Enterococcus faecalis	Moderate resistant	24mm	25mm
6.	Pseudomonas aeruginosa	Resistant	14mm	–

EFFECT OF VTC ON ANTI MICROBIAL ACTIVITY

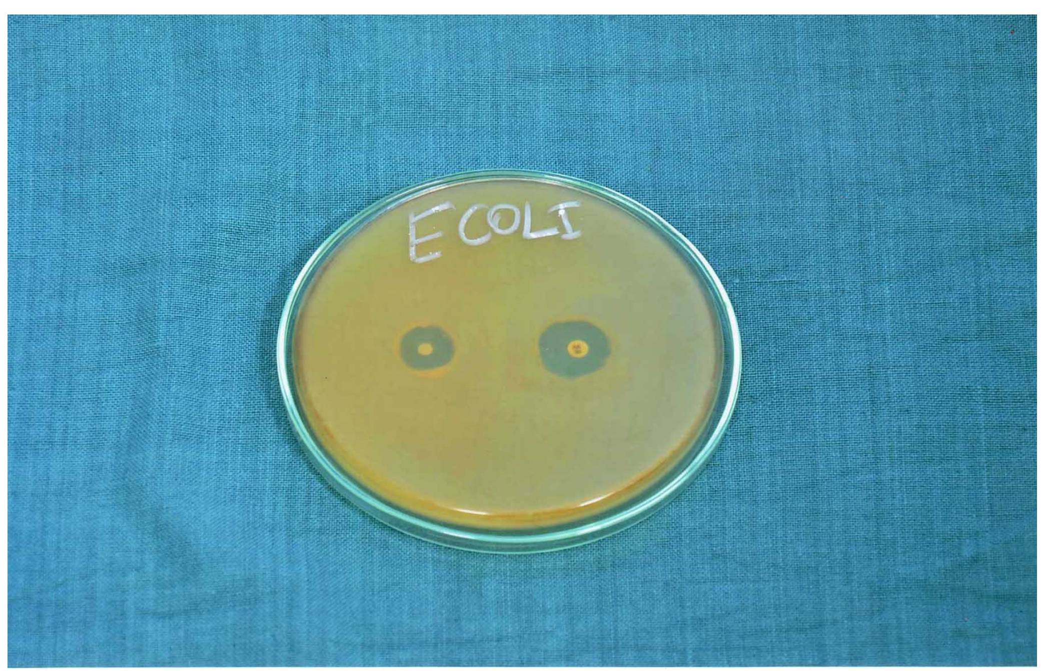


Fig. 12 (a)

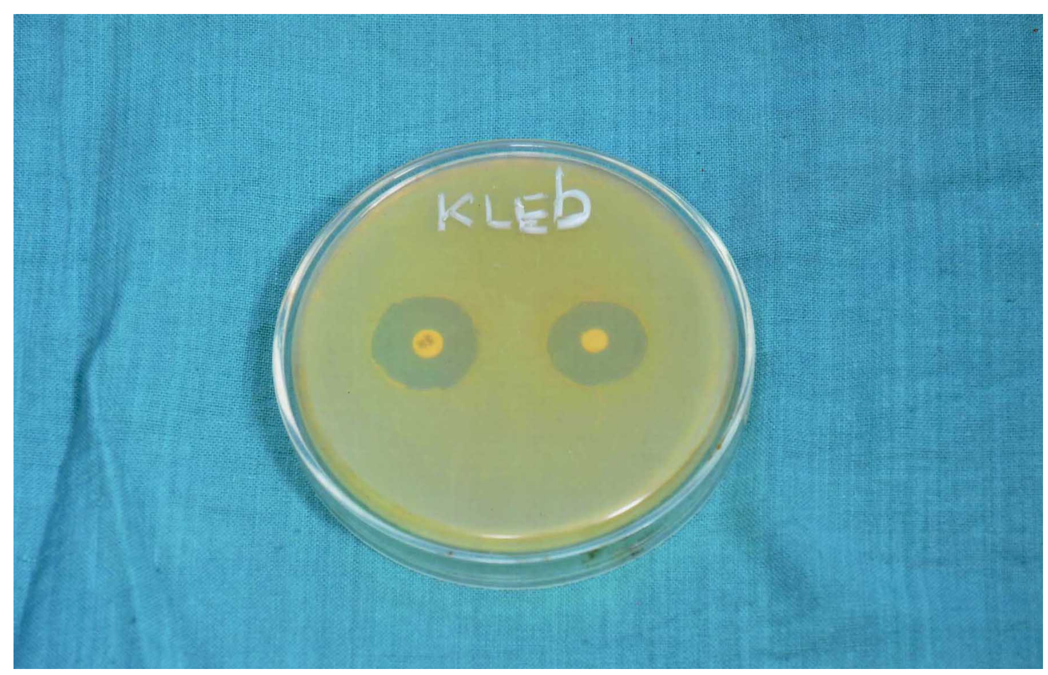


Fig. 12 (b)

Fig. 12 (c)

INTERPRETATION:

It was observed that anti microbial studies of ***Venthamaraiyathi Chooranam*** showed that it is sensitive against *Escherichia coli,* Klebsiella pnemoniae, Streptococcus mutant and moderately sensitive against Enterococcus faecalis when compared to the standard drug (Streptomycin) which was evident from the zone inhibition.The herbel drug VTC showed inhibition of the growth of the micro organism at 100mg/ml concentration for the organism.Our result confirmed the traditional use of VTC has Anti microbial activity.

6. SUMMARY

- The test drug "***Venthamaraiyathi Chooranam*** **"** was selected from the siddha literature "Pharmacopoeia of hospital of Indian medicine**"** authored by Dr.V.Narayanaswami for its Thrombolytic, vasodilator, Hypolipidemic and Cardioprotective activities**.** The dissertation started with an introduction explaining about the siddha concept, prevalence of hypertension and role of the test drug in treating it.

- The test drug was prepared properly by the given procedure. All the ingredients were identified and authenticated by the botanist and experts of *Gunapadam*,Govt. Siddha medical college, Palayamkottai.

- Review of literatue in various categories was carrid out siddha aspect botanical aspect abd pharmaceutical review disclosed about thae drug and disease pharmacological review was done to establish the methodologies

- According to siddha aspect hypertension (*Kruthi Azhal Noi*) is caused by derangement of vatha pitha humors. Spicy taste of test drug diminish the vatha humor the spicy taste where posses the seetha veriyam very much helpful to demines the aggrevated vatha pitha humors.

- Various analysis such as physicochemical , microbiological , phytochemical, biochemical analysis, instrumental analysis were made . From the above analysis we came who known the presence of active ingredients responsible for its activity. Phytochemical analysis showed the presence of potassium, calcium, chloride, iron,.

- Biochemical analyisis showed the the presence of calcium,sulphate, chloride,starch, iron ferrous, phosphate, unsaturation , reducing suger, amino acids .thus from these results we come to known the effectiveness of the drugs is due to the presence of these constituents.

- The instrumental analysis FTIR showed the peak values presence of Alkanes, Amines,Aromatics,,Aliphatic amines , Alkyl halides, Carboxylic acids

- ICP-OES analysis of these drug shows heavy metals Like Arsenic, cadmium, Nickel, copper and lead are found in below detecting level. The toxic metals are found in BDL. It reveals the drug in safer for long term use. Calicum was associated with the lower risk of Hypertension. Sodium regulates the body's acid base balance. Iron is essential for may numbers of biological functions such as growth, reproduction, healing and immune function. The phosphorus is involved in tissue repair Magnesium is regulates the blood pressure. Sulphate is potent anti oxidant activity in human body. Zinc has got potent anti-microbial activity.

- Acute and sub-acute toxicity were carried out in wistar albino rats according to OECD guidelines(423). This drug has no acute toxicity as there was no mortality seen sub-acute toxicity is carried by repeated dose of test drug for 28 days. Mortality, the functional observations, haematological and bio-chemical investigations were done. There were no significant changes in the bio-chemical and haematological profile. So the toxicological study of these test drug, *venthamaraiyathi chooranam* establish the safety of the drug for long time administration.

- In pharmacological studies, the thrambolytic activity is carried out in wistar albino rats by using enzymeimmunoassay sandwich method. The test drug *venthamaraiyathi chooranam* has significant thrambolytic activity.

- The vasodilator activity of test drug *venthamaraiyathi chooranam* carried out wistar albino rats by using students organ bath. The present study suggests that the Venthamaraiyathi Choornam extract may exert its endothelium-dependent relaxation activity by stimulating the nitric oxide release from the vascular endothelium via muscarinic receptors.

- The hypolipidemic activity of test drug *venthamaraiyathi chooranam* carried out wistar albino rats models. Hyperlipidemia is considered to be major risk factor for

the premature atherosclerosis and essentially the cholesterol in atherosclerotic plaque is derived from that of circulatory cholesterol. The antihyperlipidemic effect on siddha formulation *Venthamaraiyathi Chooranam* in particular could be considered as a possible therapeutic value.

- Cardioprotective activity present study shows that the cardiotoxicity induced by Dox is related with oxidative stress. Anti-proliferative, anti-initiation and free radical scavenging properties of *Venthamaraiyathi Chooranam* may boost myocardial integrity and attenuate the cardiac toxicity. *Venthamaraiyathi Chooranam* has shown to be cardioprotective, which may be attributed to its potent antioxidant properties

- Anti-microbial study of the test drug carried out by well diffusion method**.** It is observed that *Venthamaraiyathi Chooranam* is sensitive to Escherichia coli and Streptococcus mutant & Klebsiella pnemoniae. These *VTC* has significant anti-bacterial activity.
- Result and discussion gives the necessary and essential justification to prove the potency of test drug *Venthamaraiyathi Chooranam* with the scientific validation. Thus the herbel formulation is validated for its safety efficiency in the management of diarrhoea and it would be the way for a drug of choice.

7. CONCLUSION

It is concluded that the test drug *Venthamaraiyathi Chooranam* has significant activity to control blood clot possess thrombolytic activity and control to cholesterol level by possessing hypolipidemic action and also stimulating the nitric oxide release from the vascular endothelium via muscarinic receptors some significant vasodilator activity and boost myocardial integrity by possessing cardioprotective activity. Apart from this *Venthamaraiyathi Chooranam* has anti-bacterial sensitivity against various pathogens. Finally the drug *Venthamaraiyathi Chooranam* is scientifically validated by modern techniques.

8.FUTURE SCOPE

Pre clinical evaluation of the drug *Venthamaraiyathi Chooranam* has been done by physico chemical, bio chemical, Instrumental, pharmacological, toxicological, anti microbial procedures. In future, the drug has to be validated by clinical trials and should be used for patients.

The active principle which is responsible for the activity has to be find out, through modern scientific analysis. Having made up of nano particles, *Venthamaraiyathi Chooranam* holds extra promise for the treatment of hypertension.

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