PRECLINICAL VALIDATION OF THE POSSIBLE MECHANISMS OF ANTI – HYPERTENSIVE, DIURETIC AND ANTI - OXIDANT ACTIVITY OF *"VENTHAMARAIYATHI CHOORANAM"* IN RODENTS

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CHENNAI-106

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"Preclinical validation of the possible mechanisms of Anti-Hypertensive, Diuretic and Anti-oxidant Activity of *Venthamaraiyathi chooranam* in Rodents"** is a bonafide and genuine research work carried out by me under the guidance of **Dr.R. Karolin Daisy Rani M.D(S),** Lecturer, Post Graduate Department of *Gunapadam*, Govt. Siddha Medical College, Arumbakkam, Chennai-106 and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or other similar title.

Date: **Signature of the Candidate**

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CHENNAI-106

CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled **"Preclinical validation of the possible mechanisms of Anti-Hypertensive, Diuretic and Anti-oxidant Activity of *"Venthamaraiyathi chooranam* in Rodents"** is submitted to the Tamilnadu, Dr.MGR Medical University in partial fulfillment of the requirements for the award of degree of M.D (*Siddha*) is the bonafide and genuine research work done by **B.Kiruthika.** Under my supervision and guidance and the dissertation has not formed the basis for the award of any Degree, Diploma, Associateship, Fellowship or other similar title.

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PRINCIPAL/HEAD OF THE INSTITUTION

This is to certify that the dissertation entitled **"Preclinical validation of the possible mechanisms of Anti-Hypertensive, Diuretic and Anti-oxidant Activity of *Venthamaraiyathi chooranam* in Rodents"**a bonafide work carried out by **Dr. B. Kiruthika** under the guidance of **Dr.R.Karolin Daisy Rani,M.D(S),** Lecturer**,** Post Graduate Department of *Gunapadam*, Govt. Siddha Medical College, Chennai - 106

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CONTENTS

CHART TABLE

ABBREVIATIONS

ACE inhibitors	Angiotensin converting enzyme
ALT	Alanine amino transferase
ANOVA	Analysis of variance
ARBs	Angiotensin receptor blockers
AST	Aspartate aminotransferase
AS	Arsenic
AOAC	Association of analytical communities
Bp	Blood pressure
BUN	Blood urea nitrogen
BQL	Below quantifiable limit
CCBs	Calcium channel blockers
CCF	Congestive cardiac failure
CMC	Carboxyl methyl cellulose
COX-2	Cyclooxygenase 2
CVD	Cardiovascular disease
CD	Cadmium
CPCSEA	Committee for the purpose of control and supervision of Experiments on animals
CO	Carbon monoxide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DOCA	Deoxycorticosterone acetate
DPPH	2,2-diphenyl-1-picrylhydrazyl
ECG	Electro cardio gram
ECF	Extracellular fluid
EDTA	Ethylenediaminetetraacetic acid

EDS	Energy dispersive X-ray spectroscopy
ETA	Endothelin a receptor
FTIR	Fourier Transform Infra-Red Spectroscopy
GFR	Glomerular filtration rate
GAE/g	Gallic acid equivalent
HT	Hypertension
HCL	Hydrochloric acid
Hb	Haemoglobin
HDL	High density lipoprotein
Hg	Mercury
HNO_3	Nitric oxide
IAEC	Institutional animal ethical committee
ICF	Intracellular fluid
ICPMS	Inductively coupled plasma Mass spectroscopy
IHD	Ischemic heart disease
JNC	Joint national committee on prevention, detection , evaluation and treatment of high Blood pressure
KEV	Kilo electron volte
LD	Lethal dose
LDL	Low density lipoprotein
LV	Left ventricle
LOX	Lipoxygenase
MI	Myocardial infarction
NO	Nitric oxide
NAOH	Sodium hydroxide
NSAIDs	Non-steroidal, anti-inflammatory drugs
NF-Kb	Nuclear factor kappa light chain enhancer of activated B cells
OECD	Organization for economic co-operative development

PCV	Packed cell volume
PCB'S	Polychlorinated Biphenyls
Pb	Lead
PVD	Peripheral vascular disease
RAAS	Renin angiotensin aldosterone system
RBC	Red blood corpuscles
SBP	Systolic blood pressure
SEM	Scanning electron microscope
SEM	Standard error meaning
SHR	Spontaneously hypertensive rats
SLE	Systemic lupus erythematous
STP	Standard test procedure
SGOT	Serum Glutamate oxaloacetate transaminase
SGPT	Serum Glutamate pyruvate transaminase
TGR(m Ren2)	Transgenic rats over expressing the mouse Ren2
TIA	Transient ischemic attack
TNTH	Tamilnadu test house
H-A	Hyaluronic acid
HPLC	High performance liquid chromatography
TSH	Thyroid stimulating hormone
UV	Ultra violet
VTC	*Venthamaraiyathi chooranam*
WBC	White blood corpuscles
WHD	World Hypertension Day
WHL	World Hypertension League
WHO	World health organization
2K1C	Two kidney one clip
XRD	X-ray powder diffraction

1.INTRODUCTION

He who has health, has hope and he who has hope, has everything. So for a healthy soul we need balanced diet, regular physical activities and life style management.

In this modern world our lifestyle has been changed a lot. Even in our food habits and physical activities. These changes lead to life style disorder. All the diseases that are linked with the way, people lead their lives are called life style diseases [1]. Over 61% of all deaths in India are due to life style or non-communicable diseases [2]. Lifestyle diseases include Atherosclerosis, Heart diseases, Hypertension, stroke, obesity, Diabetes, Colon cancer etc [1a].

Getting away from our nature cause life style diseases. So getting into our nature through herbal medicine is the one and only one best way to come out these problems. Among the system of medicine practiced all over the world for past many centuries, the *Siddha* system is the ancient and transcending system. *Siddha* system of medicine is a gift for our mankind by the *Siddha*r's the greatest scientists in ancient period [3]. The word *Siddha* means established truth. *Siddha* system is a treasure house of secret science, embodying the results of the ordent pursuit there of by the ancient *Siddha*rs [4].

Prevention and cure are the basic aims of all systems of medicine whereas the *Siddha* system has the transcendental motivation of what we called the immortality of the body. The basic emphasis of *Siddha* system is on positive health viz to prevent diseases by careful dieting and proper relaxation of mind to achieve a totality of health, that assures not only longevity but also immortality. The same is expressed by Thirumoolar in Thirumanthiram as,

உடம்பா ரழியி லுயிரா ரழிவர்

திடம்பட மெஞ்ஞானஞ் சேரவு மாட்டார்

உடம்பை வளர்க்கு முபாய மறிந்தே

உடம்பை வளர்த்தே னுயிர் வளர்த்தேனே.

-திருமந்திரம் சரீர சித்தியுபாய செய்யுள்(1) [5]

Hence the *Siddha* system of medicine have attained widespread acceptability and it is the best way of treating hypertension.

Hypertension is also known as high blood pressure or raised blood pressure. It is a condition in which the blood vessels have persistently raised pressure or putting them under increased stress.

Normal adult blood pressure is defined as a blood pressure of 120 mm Hg when the heart contracts (Systolic), and a blood pressure of 80 mm Hg when the heart relaxes (Diastolic). In the case of Hypertension systolic blood pressure is equal to or above 140 mmHg and diastolic blood pressure is equal to or above 90 mm Hg.

The majority of patients with hypertension, the cause will be unknown. Hypertension will come under the category of Non-communicable disease and it is also known as Silent Killer, the biggest menace of present generation . Headache, shortness of breath, dizziness, chest pain , palpitation of the heart and nose bleeds can be the most common symptoms of hypertension.

Hypertension is one of the most common cardiovascular problem and major public health problem in both developed and developing countries. This disease affects both sex even during the younger age which may increase the risk of cardiovascular disease (CVD), it may lead to death worldwide.

Hypertension is the most preventable cause of heart disease and stroke worldwide. It is identified that more than 1 in 5 adults have hypertension, which cause around half of all deaths from stroke and heart disease. In every year, it is considered that 9.5 million deaths were happened due to the complications of hypertension [6]

According to the WHO rates, it is estimated that 7.5 million deaths caused due to hypertension, that is 12.8% at the total of all deaths. By 2025, it is estimated that 1.56 billion adults will be living with hypertension [7]

In south india, it is showed in a survey that 26,000 of adults are having hypertension, that is a prevalence of 20% and 67% were unaware of their disease [8]. In Tamilnadu, it is about 24.5% of death in people aged between 45-59 years are happened due to the circulatory system problem [9].

Hypertension management deals not only in reducing the blood pressure but also minimise the cardiovascular risk by lifestyle measures, lipid managements, smoking cessation, dietary intervention, weight reduction and physical activity. Uncontrolled blood pressure can lead to stroke, aneurysm, heart failure, vision loss, metabolic syndrome and even memory loss [10].

In modern medicine, there are number of Anti-Hypertensives were used to reduce the high blood pressure or Hypertension. Medicines like diuretics, ACE Inhibitors, ARBs (Angiotensin Receptor Blockers), Calcium channel blockers, Central sympatholytics are commonly used as Anti-Hypertensives drugs. But these medicines have side effects like dry cough, headache, swollen ankles, dizziness, tiredness, urinary frequency, sleep disturbances, gastro intestinal disturbance [11].

In *Siddha*, there are many medicines for Anti-Hypertensive which are not having side effects. *Siddha* is one of the oldest system of medicine in the south india. According to the ancient *Siddha*, a human body is made up of several microscopic components of the universe that is "Panchaboothas". The elements of panchabootha are Earth (Munn), Fire(Thee), Water (Neer), Air (vayu) and Space (Akasam) from a human body.

In addition to this, there are three humors or Doshas called the Vatha, Pitta, Kapha. These three Doshas are considered as the pillars of health and support the structure and functions of the body. Tridoshas are involved in regulating the body functions and maintain physical, emotionals, and psychological balance. In *Siddha* system, any distubances or imbalance of these humors is considered as the diseased state [12]. If any disturbances happened to Pitta which leads to Kuruthi Azhal Noi. The symptoms of Kuruthi Azhal Noi such as headache, dizziness**,** giddiness, vomiting, sleeplessness, sweating and tachycardia which are correlated with the symptoms of hypertension. The above said was mentioned by Thiruvalluvar in Thirukural were as follows,

மிகினும் குறையினும் நோய் செய்யும் நூலோர்

வளிமுதலா எண்ணிய மூன்று

குறள்-941 (or)

Three things beginning with wind,say experts

In excess or lacking cause disease.

Kural -941 [13]

After a long period our traditional medical system has been a resurgence worldwide, based on the holistic natural approach of healing. In this treatment there are three distinct categories Deva maruthuvam (Divine method), Manida maruthuvam (Rational method) and Asura maruthuvam (Surgical method). In divine method, medicines like parpam, chenduram, Guru, kuligai are prepared from metals. In rational method, medicine like chooranam, kudineer, vadagam, are prepared from herbs. In surgical method, incision, excision, heat application, blood hitting, leech application etc [14].

Here to overcome this serious consequence of Hypertension we need proper medication and improved quality of life. Hence, in the treatment aspect of *Siddha* system the present investigations decided to choose the polyherbal formulation of ***Venthamaraiyathi chooranam***. I hope this formulation of polyherbal trail drug will be effective in the management of hypertension after preclinical validation of Anti-Hypertensive, Diuretic, Anti-Oxidant activity.

2. AIM AND OBJECTIVES

AIM:

The aim of this study is to validate the **Anti-hypertensive, Diuretic and Anti -oxidant** Activity of ***Venthamaraiyathi chooranam*** and thus ensuring a holistic approach by controlling the blood pressure level, 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 standardization studies.

The key objectives of the study are:

- Collection of various Siddha and modern literature relevant to the study.
- Preparing the drug according to Siddha classical text.
- Subjecting the drug into Physico-chemical standardization.
- Analyzing the drug chemically for detection of acid and basic radicals.
- Focusing the drug for analytical assessment through sophisticated analytical modern techniques like FTIR, ICP-MS, SEM, XRD, HPLC.
- Studying the toxicity profile of *Venthamaraiyathi chooranam* according to OECD guidelines.
- Evaluation of the pharmacological activity of the test drug *Venthamaraiyathi chooranam* through the following activities,
    - Anti-hypertensive, Diuretic Activities in wistar albino rats
    - Anti- Oxidant activity - Through DPPH assay
- Evaluation of Microbial load for this formulation.
- Analyzing all the above study results to evaluate the benefits of ***Venthamaraiyathi Chooranam***

3.REVIEW OF LITERATURE

DRUG REVIEW:

Ingredients of the trail drug:

- *Elarisi* ***(Elettaria cardamomum)***
- *Chukku* **(*Zingiber officinale*)**
- *Thippili* ***(Piper longum)***
- *Adhimadhuram* ***(Glycyrrhiza glabra)***
- *Cadhakuppai* ***(Anethum graveolens)***
- *Ceeragam* ***(Cuminum cyminum)***
- *Ven Thamarai poo ithalkal* ***(Nelumbo nucifera)***

3.1 GUNAPADAM ASPECT OF THE TRIAL DRUG:

Elarisi (Elettaria cardamomum)

Other names : *Anji, Korangam, Thudi.*

Vernacular names :

English **:** Cardamomum seeds

Telugu **:** Elakulu

Malayalam **:** Elattari

Hindi **:** Elachi

Sanskrit **:** Ela

Part used **:** Seeds

Properties :

Taste : Acrid

Character : Hot

Division : Acrid

Actions:

- Carminative
- Stimulant
- Stomachic

General character:

"தொண்டைவாய்கவுள் தாலுகு தங்களில்
தோன்றும் நோயதி சாரம்பன் மேகத்தால்
உண்டை போல்எழுங் கட்டி கிரிச்சரம்
உழலை வாந்தி சிலந்தி விஷஞ்சுரம்
பண்டை வெக்கை விதாகநோய் காசமும்
பாழுஞ் சோமப் பிணிவிந்து நட்டமும்
அண்டை யீளைவன் பித்தம் இவைக்கெல்லாம்
ஆல மாங்கமழ் ஏலமருந்ததே"[15].

-தேரன் குணவாகடம்

Uses:

- It cures Cough, Dysuria, Dysentery and it increases the sperm count.

***Chukku*: (*Zingiber officinale*)**

Other names:

Arukkan, Sundi, Sondi, Vidamoodiya Amirtham, Verkombu, Athakam, Navasuru, Nagaram.

Vernacular name:

English : Dried Ginger

Telugu : Sonti

Malayalam : Chukku

Sanskrit : Nagaram

Hindi : Sonth

Part used : Rhizome (Dry)

Properties:

Taste **:** Acrid

Character **:** Hot

Division **:** Acrid

Actions:

- ❖ Carminative
- ❖ Stimulant
- ❖ Stomachic

General character:

சூலைமந்தம் நெஞ்செரிப்பு தோடமேப் பம்மழலை
மூலம் இரைப்பிருமல் மூக்குநீர்-வாலகப
தோடமதி சாரந் தொடர்வாத குன்மந்
தோடம்ஆ மம்போக்குஞ் சுக்கு[15a].

-அகத்தியர் குணப்பாடம்.

Uses :

- ➢ It is used in the treatment of Indigestion, Ulcer, Cough, Cold and Anaemia.
- ➢ Decotion of chukku used for abdominal pain, Vomitting, Chronic fever.
- ➢ Powdered chukku with sugarcane juice used for gastritis.

Thippili (Piper longum)

Other names:

Aarkathi, Kaman, Saram, Aathi marunthu, Vaitheki, Thulavi, Kudari, Kozhaiyarukki, Ambu.

Vernacular name:

English **:** Long pepper

Telugu **:** Pippilu

Malayalam **:** Thipili

Part used : Seed, Fruit

Properties:

Taste **:** Sweet

Character **:** coolant

Division **:** Sweet

Actions:

- Carminative
- Stimulant
- Stomachic

General character:

கட்டி யெதிர்நின்று கடுநோயெல் லாம்பணியும்
திட்டி வினையகலும் தேகமெத்த – புட்டியாம்
மாமனுக்கு மாமனென மற்றவர்க்கு மற்றவனாங்
காமமெனுந் திப்பிலிக்கும் கை[15b].

-தேரன் வெண்பா

Uses:

- It is used in the treatment of Ulcer, Cough, Cold, Anaemia, ENT diseases and Headache.

Athimaduram (Glycyrrhiza glabra)

Other names: *Athingam, atti madhugam, Kundri ver.*

Vernacular names:

English **:** Jequitity, Indian or Jamaica liquorice

Telugu **:** Ati-Madhuramu, Yasti-Madhukam

Malayalam **:** Ati-Madhuram, Iratti-Madhurarr

Sanskrit **:** Yashti-Madhukam

Hindi **:** Jathi-Madh, Mulath

Part used : Root

Properties:

Taste : Sweet

Character : coolant

Division : Sweet

Actions:

- Emollient
- Demulcent
- Expectorant
- Laxative
- Tonic

General character:

கத்தியரி முப்பிணியால் வருபுண் தாகங்

கண்ணோய் உன்மாதம்விக்கல் வலிவெண் குட்டம்

பித்தமெலும் புருக்கி கிரிச்சரம் ஆவர்த்த

பித்தமத மூர்ச்சை விட பாகம் வெப்பந்

தத்திவரு வாதசோ ணிதங்கா மாலை

சருவவிடங் காமியநோய் தாத்ய் நட்டங்

குத்திருமல் ஆசியங்கம் இதழ்நோய் இந்து

குயப்புணும்போம் மதூகமெனக் கூறுங் காலே[15c].

-தேரன் குணவாகடம்.

Uses:

- The root of Indian liquorice is chewed for cough.
- It is also indicated for Jaundice, Arthritis, Eye diseases, Skin diseases, Leukoderma and Migraine.

Chadhakuppai (Anethum graveolens)

Other names:

Soyikeerai vithai, Madhurigai.

Vernacular name:

English **:** The Dill, Gardendill, Anet

Telugu **:** Soyikuravittulu

Malayalam **:** Shatakuppa

Sanskrit **:** Misi

Hindi **:** Suva

Part used : Leaves, Flower, Seed

Properties:

Taste **:** Sweet, Acrid

Character **:** Hot

Division **:** Acrid

Action :

- ❖ Carminative
- ❖ Emmenagogue
- ❖ Diuretic
- ❖ Stimulant

General characters :

வாதமோடு சூதிகா வாதம் சிரசு நோய்

மோதுசெவி நோய்கப நோய் மூடுசுரம்-ஓதுகின்ற

மூலக் கடுப்பு முதிர்பீனிசம் போகும்

ஞாலச் சதகுப்பை நாடு[15d]

அகத்தியர் குணவாகடம்

Uses :

It cures vadha disease, Headache, Haemorrhage, Rhinitis and Mental disorders.

Ceeragam (Cuminum cyminum)

Other names:

Asai, Seeri, Thuthasambalam, Posanakudori, Pithanasini, Methiyam, Ubakumbapesam.

Vernacular name:

English **:** Cumin Seeds

Telugu **:** Jilakarra

Malayalam **:** Jirakam

Sanskrit **:** Jirakams

Hindi **:** Zira

Part used : Seeds

Properties:

Taste **:** Acrid, Sweet

Character **:** Coolant

Division **:** Sweet

Action :

- ❖ Carminative
- ❖ Stimulant
- ❖ Stomachic
- ❖ Astringent

General Character :

வாயுவோடு நாசிநோய் வன்பித்தஞ் சேராது

காயம் நெகிழாது கண்குளிருந்- தூயமலர்க்

காரளகப் பெண்மயிலே! கைகண்ட தித்தனையுஞ்

சீரகத்தை நீதினமுந் தின்[15e].

-அகத்தியர் குணவாகடம்

Uses :

- It cures Abdominal pain, Liver diseases, Asthma and Renal calculi.
- Powdered Ceerakam with butter used for Peptic ulcer.
- Chiraka thylam used for Eye diseases, Giddiness, Vomiting and Headache.

Venthamarai poo ithalkal (Nelumbo nucifera)

Other names :

Erasivam, Erumbu, Maraipoo.

Vernacular names :

English : The sacred Lotus

Telugu : Tamara

Malayalam : Aravindam

Sanskrit : Pankaja

Part used : Flower, Petals, Seed, Rhizome

Properties :

Taste : Sweet, Astringent

Character: Coolant

Division : Sweet

Action:

- Coolant
- Astringent
- Expectorant
- Sedative

General character:

ஈரலைப் பற்றிமிக ஏறுகின்ற வெப்பமும்போங்
கோர மருந்தின் கொடுமையறும்- பாருலகில்
தண்டா மணத்தையுள்ள தாழ்குழலே! காந்தல்விடும்
வெண்டா மரைப்பூவால் விள்[15f].

– குணபாடம் மூலிகை வகுப்பு

Uses :

- Abdominal pain, itching, thirst, fever and Heart diseases.

3.2 BOTANICAL ASPECT:

Elettaria cardamomum

Taxonomical classification

Kingdom : Plantae

Division : Tracheophyta

Class : Magnoliopsida

Order : Zingiberales

Family : Zingiberaceae

Genus : *Elettaria*

Species : *cardamomum*

Elettaria cardamomum

Distribution:

Throughout in India.

Description:

Stem perennial, erect, joined, 6-9 feet, enveloped in the sheaths of leaves; leaves lanceolate, acuminate, sub-sessile, entire, 1-2 feet long; sheaths slightly villous; scapes several, flexuose, joined, branched, 1-2 feet long; flowers alternate, short stalked, solitary at each point of the receme; calyx funnel shaped, 3-toothed, inely striated, corolla tube as long as the calyx; limb doubled exterior portion of 3 oblong, concave, nearly equal division; inner lip obovate, longer then the exterior division, curled at the marings.

Apex 3- lobed, marked in the centre with purple white stripes; capsule oval, somewhat 3-sided, 3- celled, 3-valved; seeds numerous, angular; flowers pale-greenish white.

Part used: Seeds

Chemical constituents:

α-pinene, β-pinene, sabinene, myecene, a-phyllandrene, limonene; 1,8-cineole, y-terpinene, p-cymene, terpinolene, linalool, linalyl acetate, terpinen-4-oil, α-terpineol, α-terpineol acetate, citronellol, nerol, geraniol, methyl eugenol and trans-nerolidol.

Properties and Uses:

As cordial and stimulant the seeds are frequently used medicinally,but more frequently as corrective in conjunction with other medicines. A volatile is produced from them by distillation, which has a strong aromatic taste, soluble in alcohol. It loses its odour and taste by being kept too long. The natives chew the fruit with bettle, and use it in decoction for bowel-complaints and to check vomiting in infusion it is given in cough[16].

Zingiber officinale

Taxonomical classification:

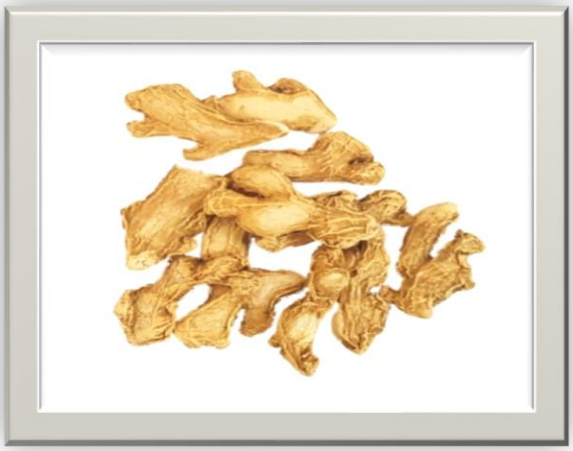
Kingdom : Plantae

Order : Zingiberales

Family : Zingiberaceae

Genus : *Zingiber*

Species : *officinale*



Zingiber officinale

Distribution:

Cultivated throughout India, run wild in some places in the Westernghats.

Description:

A slender, perennial rhizomatous herb; leaves linear, sessile, glabrous; flowers yellowish green in oblong, fruits oblong capsules. The rhizomes are white to yellowish brown in colour, irregularly branched.

Part used: Rhizomes (raw as well as dry)

Chemical constituents:

Ginger contains gingerols, a-curumene, citral, D-camphene, geraniol, zingiberenes, zingerone, phellandrene etc.

Properties and uses:

- The raw ginger is acrid, thermogenic, carminative, laxative and digestive. It is useful in anorexia, vitiated conditions of vata and kapha, dyspepsia and inflammations.

- Dry ginger is acrid, thermogenic, appetizer, laxative, stomachic, stimulant, and rubefacient, anodyne, aphrodisiac and carminative. It is useful in dropsy, otalgia, asthma, cough, diarrhoea, anorexia, nausea, vomiting and dyspepsia[17].

Piper longum

Taxonomical classification

Kingdom : Plantae

Order : Piperales

Family : Piperaceae

Genus : *Piper*

Species : *longum*

Piper longum

Distribution:

Throughout India, in evergreen forests, often cultivated.

Description:

A slender aromatic climber, rooting at the nodes; leaves alternate; flowers in solitary spikes; fruits berries, small, red when ripe. The mature spikes collected and dried form the commercial form of pippali. Roots are known as pippalimulam.

Part used: Roots, Dried spikes.

Chemical constituents:

It contains volatile oil, Resin, Piperin, Brachyamide A & B, Brachystine, sterols, Glycosides.

Proberties and Uses:

- The roots are bitter, thermogenic, tonic, diuretic, purgative, expectorant, anthelmintic, stomachic, digestive and emmenagogue. They are useful in vitiated conditions of vata, gout, dyspepsia and splenomegaly.
- The dried spikes are acrid, stomachic, aphrodisiac, carminative, expectorant, tonic, laxative and antiseptic. They are useful in anorexia, dyspepsia, asthma, flatulent colic, epilepsy and fever [18].

Glycirrhiza glabra

Taxonomical classification

Kingdom : Plantae

Division : Tracheophyta

Class : Magnoliopsida

Order : Fabales

Family : Fabaceae

Genus : *Glycyrrhiza*

Species : *glabra*

Glycirrhiza glabra

Distribution:

Cultivated in Punjab and the subhimalayas tract.

Description:

A tall perennial under shrub about 1m high, leaves compound, leaflet 4-7 pairs; flowers violet in recemes; pods, oblong to linear, flattened, seeds reniform. The liquorice of commerce in the dried underground stems and roots.

Its outer surface is pale, chocolate brown in colour, flexible, fibrous and internally has a light yellow colour. It has a characteristic pleasant sweet taste.

Part used: Roots.

Chemical constituents:

Glycyrrhizin, glycyrrhetic acid, glycyrrhetinic acid, 24-hydroxy glycyrrhetic acid, mixture of potassium and calcium salts of glycyrrhizinic (glycyrrhizic) acid, glabrin A and B, glycyrrhetol, glabrolide, isoglabrolide, formononetin, glabrone, neoliquiritin, hispaglabridin A and B; heriniarin, umbelliferone; licoagrodin, glabrol, onocerin, β-amyrin, stigmasterol, β-sitosterol, glabroisoflavanone A and B, glabrocoumarin, glychionide A and B and flavanoides.

Properties and uses:

- The roots are sweet; refrigerant, emetic, tonic, diuretic, demulcent, mild laxative, aphrodisiac, expectorant, emmanagogue, alexipharmic, and intellect promoting.
- They are useful in hyperdipsia, cough, bronchitis, vitiated conditions of vatha, cephalalgia, fever, skin diseases and opthalmopathy.
- An extract of the root is good for treating gastric ulcers. A decoction of the root is a good wash for falling and graying of hair. Externally the root is applied for cuts and wounds[19].

Chadhakuppai (Anethum graveolens)

Taxonomical classification

Kingdom: Plantae

Class: Dicotyledonae

Order: Apiales

Family: Apiaceae

Genus: *Anethum*

Species: *graveolens*

Anethum graveolens

Distribution:

It is cultivated widely throughout the world.

Description:

It is an annual herb, 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, width about one tenth.

Parts used: Fruit, Leaf

Chemical constituents:

Anethum graveolens contained essential oils, Fatty oils, Moisture (8.39%), Proteins (15.68%), Carbohydrates (36%), Fiber (14.80%), Furanocoumarin, Polyphenols and Mineral, Vitamin- A

Properties and Uses:

- Anti-microbial, Anti-inflammatory, Analgesic, Anti-convulsion, Anti-emetic, Hyperlipidaemic effect, and Anti fungal activity.
- Anethum is used as an ingredient in gripe water,given to relieve colic pain in babies and flatulence in young children.
- The essential oil in the seed relieves intestinal spasms, and griping.
- Chewing the seeds improves bad breath.
- It also cures urinary complaints, piles and mental disorders[20]

Cuminum cyminum

Taxonomical classification

Kingdom: Plantae

Class: Dicotyledonae

Order: Apiales

Family: Apiaceae

Genus: *Cuminum*

Species: *cyminum*

Cuminum cyminum

Distribution:

Cultivated throughout India.

Description:

A small slender glabrous annual herb about 30 cm in height with much branched angular or striated stem, leaves bluish green, two or three partite, ultimate segments filiform, leafbase sheathing, flowers small, white or rose coloured in compound umbels, fruits greyish, tapering towards both ends and compressed laterally with ridges covered over by papilose hairs.

Parts used: Fruits

Chemical constituents:

Cuminum cyminum contained cuminaldehyde (39.48%), gamma terpinene (15.21%), O-cymene (11.82%), beta-pinene(11.13%), 2-caren-10-al (7.93%), trans carveol (4.49%) and myrtenal (3.5%) as a major components.

Properties and Uses:

- The fruits are Acrid, Sweet, Cooling, Aphrodisiac, Astringent, Digestive, Carminative, Anthelmintic, Anti-inflammatory, Anodyne, Stomachic, Stimulant, Depurative, Galactagogue, Uterine, and Nervine stimulant.
- The fruits are useful in Dyspepsia, Colic, Helminthiasis, Inflammations, Flatulence, Anorexia, Vomiting, Haemorrhoids, Renal and Vesical calculi, Leucorrhoea, Chronic diarrhea, Skin diseases, Leprosy, Leucoderma, Fever, Cough, Asthma and Ulcers[21].

Nelumbo nucifera

Taxonomical classification

Kingdom: Plantae

Division: Tracheophyta

Class: Magnoliopsida

Order: Proteales

Family: Nelumbonaceae

Genus: *Nelumbo*

Species*:* *nucifera*

Nelumbo nucifera

Distribution:

Throughout in India, in marshes and ponds upto an elevation of 1,800m.

Description:

A large handsome aquatic herb with slender elongate, branched, creeping, rhizomes sending out roots at the nodes; leaves pelate, 60-90 cm or more in diameter; petioles very long, smoother or with small prickles, much raised out of water; flower solitary large, fragrant, white or rosy with a centrally located yellow obconical spongy tours in which capsules are shrunken; fruits ovoid, nut like achenes.

Part used: Whole plant

Chemical constituents:

Linalool, nonadecane, phytol, raffinose, neferine, nelumbine, liensinine, isoliensinine, nuciferine.

Properties and uses:

- The plant is astringent, bitter, sweet, cooling, emollient, diuretic, anti-fungal, anti-pyretic, cardiactonic. It is useful in hyperdipsia, vitiated conditions of pitta,

cholera, diarrhoea, helminthiasis, vomiting, burning sensation, haemorrhoids, nervous exhaustion, ringworm, dermopathy, intermittent fever, strangury and cardiac debility.

- The stem is astringent, cooling, fragrant, diuretic, anthelmintic and useful in vomiting, leprosy and skin diseases.
- The roots are bitter, cooling, emollient, diuretic, and useful in pharyngopathy, pectoralgia, spermatorrhea, smallpox, diarrhoea, dysentery, cough, vitiated conditions of pitta.
- The leaves are bitter, cooling, diuretic, and are useful in burning sensation, hyperdipsia, fever, strangury, haemorrhoids and leprosy.
- The flowers are sweet, astringent, refrigerant and cardiac tonic. They are useful in diarrhoea, cholera, fever, hepatopathy, hyperdipsia, internal injuries, bronchitis, cough, skin eruptions and vitiated conditions of pitta.
- The stamens are cooling, astringent, diuretic, aphrodisiac and are useful in diarrhoea, hyperdipsia, haemorrhoids, inflammations, stomatitis and menorrhagia.
- The fruits and seeds are bitter, sweet, cooling, diuretic, tonic, depurative and aphrodisiac. They are useful in hyperdipsia dermopathy, halitosis, burning sensation, vomiting, mennorrhegia, leucorrhoea, fever, pectoral diseases, leprosy and pruritis[22]

3.3 LITERATURE REVIEW OF DISEASE

3.3.1 SIDDHA ASPECT OF THE DISEASE

KURUTHI AZHAL NOI:

Definition:

It refers to the functional divergence in seneer thathu (blood), produced as a results of increased azhal (bio energy fire) eventully leading to consequent functional dearrangements in other udal thathus.

Vernacular names:

"இரத்த வமுக்கத் தியர்பெய ருரைத்திடிற்

இரத்தாதிக்கம் யழுத்தம் இரத்தக் கொதிப்பு

நந்திடும் நாடிஇறுக்கமும் நவில்வர்

பத்திடுங் காரணம் பகரக்கேண்மோ"

-நோய்நாடல் நோய்முதல் நாடல்திரட்டு[23]

Raktha soodu, Raktha azhutham, Raktha miguthi, Raktha perukkam, Rakthathikkam, Narambirukkam and Naadi irukkam.

Types of Disease:

The disease may be classified into eight types as follows:

1.Vali kuruthiazhal noi

2.Thee kuruthiazhal noi

3.Iya kuruthiazhal noi

4.Vali thee kuruthiazhal noi

5.Vali iya kuruthiazhal noi

6.Iya vali kuruthiazhal noi

7.Iya azhal kuruthiazhal noi

8.Mukkutra kuruthiazhal noi

Some other group of ancient physicians have classified this disease into four types depending upon the direction of flow of blood as follows:

1.Maelnokku kuruthiazhal noi

2.Keelnokku kuruthiazhal noi

3.Irunokku kuruthiazhal noi

4.Alavilla nokku kuruthiazhal noi[24]

Clinical features:

- Head ache most commonly seen in occipital region, dizziness, palpitation, easy fatigability, weakness.
- Dysponea, chest pain, pallor, perspiration, vertigo, syncope.
- Epistaxis, heamaturia, blurring of vision.

Epidemiology:

"கேட்டிடு பன்னாட்கெடு மலச்சிக்கல்
கூட்டிடும்மதுக் கள்கொள்ளல் புகையிலை
நாட்டிடும் மாவகை நற்கொழுப்பிறைச்சி
ஊட்டுதல் மிகுதல் ஊழைப்புமிகுதி
மிகுமனக்களிப்பு மிகுநாடித்துடிப்பு
தரு மனவோட்டம் நாடுதல்காமம்
தருமேகநோயிகள் தக்க பல்தொண்டை
பகு பித்தப்பை பலஉறுப்பினிலும்"

-நோய்நாடல் நோய்முதல் நாடல்திரட்டு

Kuruthi azhal noi is high in urban population and more common in men than women. In females the prevalence is closely related to age. This is increased presumeably related to the menopause.

- The elavenil (early summer), muthuvenil (late summer) aggressive the disease.
- Paalai (arid-tract) and neithal (coastal-tract) reported compared to other areas[23a]

3.3.2 MODERN ASPECT OF THE DISEASE

HYPERTENSION

The history of hypertension starts at 1628 when the book De Motu Cadrdis was published, and the book was written by an English physician Willium Harvey, who described the circulation of blood. Then the elergyman Stephen hales made the first measurement of blood pressure in 1733. Hypertension was described as a disease by thomus young and Richard in 1836. In 1884 the first report of elevated blood pressure in a person without evidence of kidney disease was recorded. Eventually in 1896 the invention cuff-based sphygmomanometer done by Scipione Riva-Rocci. Then the blood pressure was measured in the clinics, Nikolai korotkoff improved this technique by describing the korkoff sounds. These sounds are heard when the artery was ascultated with a stethoscope while the sphygmomanometer cuff was deflated.

Now in modern medicine, hypertension is classified into two types essential and secondary hypertension. The concept of essential hypertension was introduced in 1925 by the physiologist Otto frank, which has idiopathic origin. In 1928 the term malignant hypertension was coined by physicians from the mayo clinic. The essential hypertension may be the result of a combination of poor life style choices and genetics. Secondary hypertension that starts as a result of another disease especially kidney disease or disease associated with endocrine system[25

Definition:

Systemic hypertension is the persistent rise of basal blood pressure above the level of 140/90mmHg recorded on three or more successive occasions[26].

Classification of hypertension

- Essential or idiopathic hypertension
- Secondary or symptomatic hypertension

Table 1: Classification of blood pressure for adult (JNC7)[27]

Category	Systolic mmHg	Diastolic mmHg
Normal	90-119	60-79
High normal (pre-hypertension)	120-139	80-89
Stage I Hypertension	140-159	90-99
Stage II (Hypertension)	160-179	100-109
Stage III (Hypertensive emergency)	≥ 180	≥ 110
Isolated Hypertension	≥ 140	< 90

White coat Hypertension

It is commonly known as white coat syndrome or masked hypertension, is a phenomenon in which patient exhibit a blood pressure level above normal range, In a clinical setting, though they don't having exhibit other it in other settings. It is believed that the phenomenon is due to anxiety that those afflicted experience during a clinical visit [28].

Pathophysiology of Hypertension

Blood pressure is determined by the balance between cardiac output and vascular resistance rise in either of these variables, in the absence of a compensatory decrease in the other, increases mean Bp, which is the driving pressure.

Factors that affect cardiac output include the following:

- Baroreceptors
- Extracellular volume
- Effective circulating volume, Arterial natriuretic hormones, Mineralocorticoids, Angiotensin
- Sympathetic nervous syndrome

Factors that affect vascular resistance include the following:

- Depressors-angiotensin II, calcium (intracellular), catecholamines, vasopressin.
- Depressors-Artial natri uretic hormones, endothelial relaxing factors, kinins, prostaglandin, prostaglandin E_2, prostaglandin I_2

Changes in the electrolyte homeostasis particularly in sodium, calcium and potassium concentrations, affect some of these factors.

Under normal conditions, the amount of sodium excreted in the urine matches the amount of ingested, resulting in near constancy of extracellular volume. Retention of sodium results in increased extracellular volume, which associated with elevation of Bp. By means of various physical and hormonal mechanisms, elevation triggers changes in both the glomerular filtration rate(GFR) and the tubular resorption of sodium, resulting in excretion of excess sodium and restoration of sodium balance.

A rise in the intracellular calcium concentration, due to changes in plasma calcium concentration, increases vascular contractility. In addition, calcium stimulates release of renin, synthesis of epinephrine and sympathetic nervous system activity. Increased potassium intake suppresses the production and release of renin and induces natriuresis, decreasing the Bp.

Table 2:Common causes of hypertension by age[29]

Infants	**Children 1-6 yrs**	**Children 7-12**
Thrombosis of renal artery or vein	Renal artery stenosis	Renal parenchymal disease
Congenital renal anomalies	Renal parenchymal disease	Renovascular abnormalities
Coarctation of aorta	Wilms tumor Neuroblastoma	Endocrine causes
Bronchopulmonary dysplasia	Coarctation of aorta	Essential hypertension

Causes of primary hypertension:

Also commonly known essential hypertension, is a disorder which is associated with elevated blood pressure whose causes are not readily identifiable. Its prevalence tend to raise with age in most populations. Essential hypertension is common in adults.

The following risk factors for primary hypertension:

- Obesity or being overweight
- Excessive salt consumption
- Family history and genetics
- Sedentary life style [30]

Causes for secondary hypertension

- Renal causes- acute nephritic syndrome, chronic nephritis, Poly cystic kidney, Hydronephrosis, chronic pyelonephritis, Renal artery stenosis, Renin secreting tumour, Renal embolism.
- Endocrine causes- thyrotoxicosis and myxoedema, acromegaly, cushing's syndrome, cohn's syndrome, pheochromocytoma.
- Metabolic causes- diabetes mellitus, chronic gout, toxemias of pregnancy, atherosclerosis.
- Drugs- contraceptive pills, steroids, liquorice.
- Collagenosis and miscellaneous disease- SLE, polyarteritis nodosa, scleroderma, dermatomyositis, pseudoxanthoma elasticum.
- Congenital- coarctation of aorta.
- Psychogenic
- Neurological- encephalitis, brain tumour, cerebro vascular accident, diencephalic Syndrome.
- Blood disease- Polycythemia
- Renovascular hypertension- particularly in renal artery stenosis
- Miscellaneous- Pregnancy, Cyclosporine, NSAIDs.[29a]

Clinical features:

- Pulsating head ache often in occipital and occurs particularly in the morning.
- Easy fatiguability

- Insomnia
- Dizziness
- Lack of concentration
- Loss of memory
- Occational palpitation

Symptoms of associated disease may also present e.g. Cerebral Arteriosclerosis, Retinal Arteriosclerosis, Coronary Arteriosclerosis, Renal arteriosclerosis and Arteriosclerosis of limb of vessels [31].

Complication:

Cardiac hypertensive heart disease

Left Ventricular Hypertrophy develops in 10-30% of chronic cases. It may produce myocardial ischemia, ventricular arrhythmia, CCF and sudden death, LV diastolic dysfunction may also develop with CCF.

Cerebral

Cerebro vascular complications are more closely to systolic rather than diastolic blood pressure.

- Cerebral hemorrhages
- Cerebral thrombosis
- Lacunars infarct
- Hypertensive encephalopathy- this condition is acute cerebral ischemia from Hypertensive spasm, Cerebral edema and minor degree of hemorrhages or thrombosis.
- TIA- transient ischemic attack
- Subarachnoid hemorrhages.
- Dementia- both vascular and Alzheimer's type

Retinal

- Dimness of vision
- Thickening of arteries with Narrowing of lumen, Hemorrhage.
- Papilloedema.
- Detachment of Retina, Vitreous Hemorrhages

Renal

Patients with hypertensive nephropathy should have BP at 130/85mmHg or less if protenuria is present. Hypertension accelerates all forms of renal disease mostly diabetic nephropathy.

- Nephrosclerosis
- Uraemia
- Renal infarct

Aortic dissection

- The major cause of hypertension.

Atherosclerotic complications

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

- Cerebral arteriosclerosis
- Retinal arteriosclerosis
- Coronary arteriosclerosis
- Renal arteriosclerosis
- Arteriosclerosis of limb vessels.

Pregnancy induced hypertension

Gestational hypertension also referred to pregnancy induced hypertension (PIH). It is a condition of high blood pressure more than 140/90mmHg on two separate occations, more than 6 hours apart, without the presence of protein in the urine during pregnancy. It is diagnosed after 20 weeks of gestation. Gestational hypertension can lead to serious condition called pre-eclampsia[32].

Hypertensive crisis

In some situations in an hypertensive patients rapid reduction of blood pressure is required. These situations are include under the category of hypertensive crisis. These situations divided into two.

1. Hypertensive urgencies

Where blood pressure reduction is required comparatively slowly, Diastolic pressure is more than 130mmHg.

2.Hypertensive emergencies (Malignant hypertension)

Where immediate (within one hour) reduction of blood pressure is required. Systolic blood pressure is greater than 210mmHg of diastolic blood pressure 130mmHg.

Clinical features include

- Headache
- Confusion
- Visual loss
- Focal Neuralogic features
- Somnolence
- Coma
- Fundus shows Hemorrhages, Exudates and Papilloedema.

Mode of termination

- Acute left ventricular failure(60%)
- Cerebral hemorrhages (35%)
- Uraemia rare(5%)[33]

Differential diagnosis:

Aortic coarctation:

Differential blood pressure in upper and lower extremities. Absent of femoral pulses.

Obstructive sleep apnea:

Typically obese patients with day time somnolence, snoaring, or choking during sleep[34].

Investigations:

- Urine analysis, Protein and Glucose
- Blood, Urea, Electrolytes and creatinine
- Blood glucose

- Serum total and high-density lipoprotein(HDL) Cholesterol
- 12-lead ECG (left ventricular Hypertrophy, Coronary artery disease)
- Endocrine: Serum Sodium, Potassium, Calcium and TSH[33a]

3.4 TREATMENT FOR HYPERTENSION

3.4.1 *SIDDHA* ASPECT

In siddha system the treatment for *Kuruthi azhal noi* is based on the normalizing the altered hypertension.

Vanthi* and *kazhichal maruthuvam

(Bowel cleansing method for *azhal* and *vayu thathu*)

- *Meganatha thylam*-8-30ml at early morning
- *Vellai ennai*-15-30 ml at early morning
- *Karuda kizhangu thylam*-15 ml at early morning
- *Sanjeevi mathirai*-1-2 at early morning with goat's milk(50ml) kudineer
- *Kowshikar kuzhambu*-125-500 mg with ghee at early morning
- *Thiratchai kudineer*-40-80ml twice a day

The choices of medicine, doses and duration may be altered according to the condition of the patients and severity of the disease.

Chooranam

- *Marutham pattai chooranam*-1-2 g twice a day with hotwater (50ml)
- *Ceeraga chooranam*-1-2 g twice a day with hotwater 50ml)
- *Thirachathy chooranam*-1-2g twice a day with honey (5ml)
- *Seenthil chooranam*-1-2g twice a day with ghee (5ml)
- *Thirippala chooranam*-1-2g twice a day with hotwater (50ml)
- *Thalisathy chooranam*-1-2g twice a day with honey (5ml)
- *Elathy chooranam* 1-2g twice a day with warm water (50ml)
- *Amukkara chooranam*1-2g twice a day with milk (50ml)

Nei

- *Brahmi nei* 10-15ml twice a day with milk (50ml)

Manappagu

- *Thurunchi manappagu* 15ml twice a day with luke warm water (50ml)
- *Nannari manappagu* 15ml twice a day with luke warm water (50ml)
- *Madhulai manappagu* 10-15 ml twice a day with luke warm water (50ml)

Ilagam

- *Thetran ilagam* 5-10g twice a day
- *Vilvathy ilagam* 5-10g twice a day
- *Madhulai manappagu* 3g twice a day

Karpam

- *Bavana kadukkai* (500mg) 1 before and after food twice day

Parpam

- *Silasathu parpam* 300-600mgtwice a day with hotwater (50ml)
- *Kungiliya parpam* 100-300mg twice a day tendercoconut water (50ml)
- *Sangu parpam* 100-300mg twice a day with ghee (5ml)
- *Nathai parpam* 65-130mg twice a day with ghee (5ml)
- *Siringi parpam* 65-130mgtwice a day with brahmi nei (10ml)

Chenduram

- *Vediannabethi chenduram* 100-200mg twice a day with honey (5ml)
- *Aya chenduram* 65-130mg twice a day with honey (5ml)
- *Ayakandha chenduram* 65-130mg twice a day with honey (5ml)

Karpa marunthugal

Pothu karpam

- *Kattrazhai karpam* for 4 days
- *Ponnangani karpam* for 4 days
- *Kaiyan karpam* 1-2gms for 2 months

Sirappu karpam

- *Kadukkai karpam* *1-2* gms with hotwater at evening for 48 days
- *Panai ver kudinner* 60ml twice a day for 48 days
- *Vilva karpam* for 48 days

- *Elumitchai pazha karpam* for 48 days
- *Orilai thamarai karpam* with ghee for 48 days.

External medicines

Oil bath may be adviced twice a week with any of the following medicated oil.

- *Kaiyan thylam*
- *Ceeraga thylam*
- *Keezhaneli thylam*
- *Lahusandhanathy thylam*
- *Arakku thylam*
- *Thirippala thylam*

Pathiyam (diet)

Diet to be added

- Rice kanji-double boiled rice, *savvarisi kanji, pori kanji, barley kanji, manakkathai, kuruvai rice.*
- Vegetables- *athi, avarai, kathari, vazhai, vendai, murungai, sundai, mullangi, pagal, sambal poosani, thoothuvalai, pirandai.*
- Greens- *puliyarai, manathakkali, ponnangani, kaiyan, sukkan, vasalai keerai, pasalai keerai.*
- Pulses- *ulunthu, pasipayaru*
- Dairy products- cow's butter milk
- Non-vegetarian diet- *ayrai meen* (loach), *velladu* (capea hircus)

Diet to be avoided

- Intake of excessive salt, oil, fried food.
- Excessive hot, sour and salt tastes.
- *Sarkkarai valli kizhangu* (Ipomoea batatas), *Seppankizhangu* (Colacasiaesculanta), *Kothavarai* (Cyamopsis tetragonoloba), *Kollu* (Macrotylorum uvifiorum), *Verkadalai* (Arachis hypogea), *Kaaramani* (Vigna ungiculata), *Pattani* (Pisum sativum), *Motchai* (lablab purpureus).

Other instructions

- Avoid smoking and alcohol intake
- Dietary management
- Regular physical activity (brisk walking) at least 30 min/day
- Weight reduction, maintain normal body weight
- Relief of stress.
- Rejuvenation therapy

Karpayogam

- *Pranayamam*
- *Singasanam*
- *Sarvangasanam*
- *Puyangasanam*

3.4.2TREATMENT FOR HYPERTENSION IN MODERN ASPECT

Table 3: Classification of Anti-Hypertensive Drugs

S.NO	TYPES OF DRUG	NAME OF THE DRUG
1.	ACE inhibitors	Captopril Enalapril Lisinopril Ramipril
2.	Angiotensin antagonist	Losartan
3.	Calcium channel blockers	Nifidepine Felodipine Amlodipine Verapamil Diltiazem
4.	Diuretics	Hydrochlorothiazide Fruosemide Indapamide Spironolactone Triamterene Amiloride

5.	β-adrenergic blockers	Propranolol Atenolol Metaprolol
6.	α-adrenergic blockers	Prazocin Terazocin Phentolamine
7.	Central sympatholytics	Clonidine Methyldopa
8.	Vasodilators (i)Arteriolar (ii)Arteriolar and venular	Hydralazine Minoxidil Diazoxide Sodium nitroprusside Pinacidil

Anti-hypertensive drugs act by influencing the Blood pressure regulatory systems viz, the autonomic nervous system, Renin Angiotensin System. Calcium channels or sodium and water balance in plasma volume[35].

Classification of anti-hypertensive drugs

Diuretics

Diuretics lowers the blood pressure by increasing urination. The anti hypertensive action of diuretics is mild BP falls by 15-20mmHg over 2-4 weeks.

Diuretics enhance the excretion of sodium and water resulting in decreased plasma volume of CO and reduce BP.

- Thiazides (Hydrochlorothiazide, Chlorothiazide, Indapamide)
- Loop diuretics (Fruosemide, Bumetanide, Torsemide)
- K^+ (Spiranolactone, Amiloride, Trimterene)

Drugs acts on Rennin Angiotensin System

- Angiotensin converting enzyme inhibitors (ACE inhibitors) (Captopril, Enalapril, Lisinopril, Ramipril, Perindopril)

- Angiotensin II receptor antagonists (Losartan, Candesartan, Valsartan, Eprosartan, Irbesartan, Olmesartan)
- Renin inhibitor(Aliskien)
- ACE inhibitors are presently the first line anti hypertensives.ACE inhibitors are useful in the treatment of hypertension of all grades due to all causes. They are specially indicated as Anti-hypertensives in hypertension with left ventricular hypertrophy.
- Angiotensin II is powerful vasoconstrictor. ARBs are used in the treatment of hypertension in similar indications as that of ACE inhibitors as alternatives of ACE inhibitors, they can also be considered as the first line drug in hypertension.
- Renin inhibitors blocks the effects of renin there by reducing blood pressure. Use of several drugs like ACE inhibitors, ARBs and diuretics tend to bring about a compensatory rise in the plasma renin levels. Because aliskiren blocks the effects of renin, its action is synergistic with these drugs.

Sympatholytics

- Centrally acting drugs (Clodine, Methyldopa, Guanfacine)
- Ganglion blockers (Trimethaphan)
- Adrenergic receptor blockers (Guanethidine, Reserpine)
- Adrenergic receptor blockers
- α-blockers (Prazosin, Terazosin, Doxazosin, Phenoxybenzamine, Phentolamine)
- β-blockers (Propranolol, Atenolol, metoprolol)
- α and β blockers (Labetalol, Carvedilol).

Sympatholytics drugs may be used to interfere with sympathetic activity at different levels including centrally, at the ganglia, neurons and receptors.

Ganglion blockers block both sympathetic and parasympathetic ganglia resulting in decreased sympathetic tone and a fall in BP.

Adrenergic receptor blockers depletes the stores of nor adrenaline in the adrenergic neurons blockers its release.

α-blockers are used in the treatment of hypertension due to Pheochromacytoma. They block the α1 receptors in the arterioles and venules and thereby dilate both arteriole and venules. Peripheral vascular resistance is decreased leading to fall in BP with only mild tachycardia. β-blockers are mild anti-hypertensives. Blockage of β1 receptors results in decreased myocardial contractility and cardiac output. Thus they reduce BP due to a fall in the cardiac output [36].

Ca+ channel blockers

CCBs are another important group of anti-hypertensives. They are particularly used in elderly patients. CCBs may be used as mono therapy or in moderate to severe Hypertension along with other hypertensives (Nifedipine, Nicardipine, Nimodipine, Amlodipine, Felodipine, Verpamil).

Vasodilators

- Arteriolar dilators (Hydralazine, Minoxidil, Diazoxide)
- Arteriolar and venular dilators (sodium nitroprusside)

Vasodilators relax the vascular smooth muscle thus reducing BP due to decreased peripheral vascular resistance. Nitroprusside is the drug of choice in hypertensive emergencies. It is used in situations where short-term reduction of myocardial work load is required as serve heart failure and myocardial infarction.

Adverse effects of anti-hypertensive drugs

- Angiotensin receptor blockers cause Hypotension and Hyperkalaemia.
- Sympatholytics like Methyldopa cause dryness of mouth and nose, depression, vertigo, extra-pyramidal signs, raised prolactin levels, postural hypertension.
- Vasodilators like Hydralazine cause headache, dizziness, flushing, palpitation, nausea, anorexia, hypotension and salt and water retension.
- Sodium nitroprusside cause palpitation, sweating, weakness, nausea, vomiting and hypotension[37].

Future vaccine:

DNA vaccine

DNA vaccine that targets angiotensin II- a hormone that raises blood pressure by causing blood vessels to constrict. The narrowing can increases blood pressure and force heart to work harder.

In the study, researchers immunized hypertensive rats three times at two-week intervals with needless injections. The vaccine not only lowered blood pressure for up to six months, but also reduced tissue damage to the heart and blood vessels associated with hypertension. There were no signs of damage to other organs such as the kidney or liver.

The DNA vaccine works similar to common ACE inhibitor blood pressure medications which help blood vessels relax and open up, which in turn lowers blood pressure.

Other type of vaccine: Peptide vaccine[38].

3.5 PHARMACOLOGICAL REVIEW

PHARMACOLOGY STUDY OF ANTIHYPERTENSIVE ACTIVITY IN ANIMAL MODELS

HYPERTENSION MODELS

IN-VITRO MODEL

- Endothelin receptor antagonism in porcine isolated hearts
- Monocrotaline induced pulmonary hypertension

Endothelin Receptor model

Endothelins (ET) have been implicated in the pathophysiology of cardiovascular diseases. In this model isolated porcine coronary artery is used since the smooth musculature of artery is considered to contain the ETA receptors. ET results in potent long lasting contractions of isolated blood vessel strips. An increase of blood pressure in vitro studies has been elicited by Endothelin peptides.

Monocrotaline-induced pulmonary hypertension

Monocrotaline is a hepatotoxic pneumotoxic agent used in Rats for pulmonary hypertension. It is a pyrolizidine alkaloid derived from Crotalaria spectabilis and single injection leads to progressive pulmonary hypertension followed by right ventricular hypertrophy and cardiac failure. Ultrastructural changes such as degeneration and fragmentation of endothelial cells and mascularization of pulmonary arteries, arterioles are also observed. Monocrotaline administration of rats can results in severe right ventricular hypertrophy accompanied by rats and pleural effusion.

IN-VIVO MODELS

RAT MODELS

I. Reno-vascular induced

- Two-kidney one clip method (Goldblatt hypertension, 2K1C)
- Chronic renal hypertension in rats (1-kidney-1-clip method)
- Chronic renal hypertension in rats (Two kidney two clip method)

II. Neurogenic induced

Blood pressure in pithed rats

III. Diet induced

- Fructose induced
- Increased salt induced

IV. Endocrine induced

DOCA-salt rats

V. Psychogenic

Air-jet stimulation induced hypertension

VI. Genetically induced

- Salt-sensitive Dahl rats
- Spontaneously hypertensive rats

DOG MODEL OF HYPERTENSION

- Chronic renal hypertension
- Neurogenic hypertension

MONKEY MODEL OF HYPERTENSION

- Renin inhibition in monkeys

TRANSGENIC MODEL OF HYPERTENSION

- Transgenic rats over expressing the mouse Ren-2 gene [TGR(mRen 2)27]

Two kidney one clip (Goldblatt hypertension2k1c)

Sprague dawley rats used for this model

Ischemia of the kidney causes elevation of blood pressure by activation of renin angiotensin system. In rats clamping the renal artery for 4 hours can activates peripheral RAAS and sympathetic nervous system and induce renal hypertension. After reopening the vessel, accumulated renin is released into circulation leading to acute hypertension. Renin is secreted by the kidneys when sympathetic activity is increased. Renin converts Angiotensin to Angiotensin I, angiotensin II is a potent vasoconstrictor and increase blood pressure. Angiotensin II also causes release of aldosterone leading to salt and water retention result in increased blood volume and hypertension. This model is used to evaluate anti-hypertensive activities of drugs.

Chronic renal hypertension (one kidney one clip method)

The one kidney one clip method is the technique has been described for several animal species. The most effective modifications in rats in which one kidney is removed. Constriction of one kidney is done on one side and the contralateral kidney is removed. There is an increase in blood pressure within few hours. Since there is no other kidney, there is no blood pressure, diuresis, natriuresis, so there is rapid salt and water retention. Plasma renin activity is usually normal hypertension soon becames volume dependent.

Blood pressure in pithed rats

Male wistar rats (250-350 gms) are used in this model

The pithed rat model is divided for neurogenic reflex control that may modulate the primary drug effect. It is frequently used to evaluate drug action on the cardiovascular system.

Salt-sensitive Dahl rats

The kidneys have the ability to excrete easily the daily salt load without allowing a marked rise in extracellular volume. Chronic ingestion of excess salt produces hypertension in rats, which mimics human hypertension. The salt-sensitive dahl develop severe and fatal hypertension when feed high salt diets. This is the model of genetic hypertension, with the extra feature of Salt sensitive.

Fructose induced hypertension in rats

Feeding a high fructose diet induces hypertension and insulin resistance in Sprague dawley rats. Fructose feeding also causes insulin resistance, hyperinsulinemia and hypertriglyceridemia in normal rats. Fructose feeding induces hypertension in normal or high salt feed animals and it is associated with an increased expression of the Angiotensin II type I receptor in adipose tissue. AT 1 receptor play a role in the pathophysiology of metabolic and hemodynamic abnormalities induced fructose feeding.

DOCA-salt rats

Mineralocorticoids induces hypertension by causing in increased plasma and extracellular volume. The administration of deoxycorticosterone acetate (DOCA) , a mineralocorticoid in combination with high salt diet and unilateral nephrectomy induces hypertension. There is increased DOCA induced reabsorption of salt and water leading to increased blood volume and hence increased blood pressure[39].

Spontaneously hypertensive rats

By breeding strain of spontaneous hypertensive wistar rats with a female having slightly raised blood pressure, Okamoto and Aoki obtained a strain of rats spontaneous hypertension, Spontaneous Hypertension was devopled by meticulous genetic in breeding that uniformly resulted in 100% of progeny having naturally occurring hypertension. Several researchers reported that the spontaneous hypertension is an excellent model of experimental hypertension as well as model for complication of hypertension[40].

Transgenic rats overexpressing the mouse Ren2 gene [TGR(mRen2)27]

The ability to specifically introduce genetic constructs and thereby breed transgenic animal has opened new possibilities for hypertension research.Recently a transgenic rat has been obtained after introduction of the entire mouse Ren 2d gene. The introduction and over expression of mouse Ren2 in this rat leads to severe, lethal in the homozygous rat.

Method for the measurement of blood pressure in rats

- Tail-cuff method in rats
- Indwelling catheter for measurement of blood pressure in conscious rats.

Tail cuff method for measuring Bp

The indirect tail cuff method allows the measurement of Bp without any surgical procedure. The principle used in this method is that the pulse obliterate when the cuff is inflated will above suspected systolic blood pressure. The pulse reappears as the pressure in the cuff is slowly released and it falls below the systolic blood pressure. The method is analogous to sphygmomanometry in humans.

The indirect tail cuff method is widely used to evaluate the influence of anti hypertensive drugs in spontaneously and experimentally induced hypertensive rats.

Indwelling catheter method

This method allows directly measurement of blood pressure in conscious rat. The influence of anesthesia on the cardiovascular regulation is eliminated by this method.

7 cm long cannulae are prepared by cutting pe 10 and pe 20 tubing respectively. A style wire is inserted into the pe10 tubing is also slipped over the style wire. The tube ends are heated in a current of hot air and fused together. Using ridges the style wire is left inside the cannula and the cannula is heated in a jet of hot air. When the polyethelene at the point of heating becomes soft, the cannula is pressed slightly and the ridges are formed.

Dog models of hypertension

- Chronic renal hypertension
- Neurogenic hypertension

Chronic renal hypertension

Partial constriction of renal arteries in dogs produces hypertension. This method is modified and is now known as the wrapping technique, a sheet of cellophane is placed around the kidney and held in place by silk sutures tied loosely around the renal hilus. Both kidneys are wrapped or one kidney is wrapped and other is removed. A fibro collagenous shell is formed around the kidney in 3-5 days because of reaction of the tissue to the foreign material. This shell compresses renal vascular pressure. This expands the extracellular volume leading to increased peripheral resistance and hence increased blood pressure.

Neurogenic hypertension

Baroreceptor situated in the carotid sinus and aortic arch play an important part in the regulation of blood pressure. Stimulation of baroreceptor causes inhibition of vasomotor center leading to vasodilatation, bradycardia and decrease in blood pressure. Sectioning of the baroreceptor nerves leads to persistant raise in blood pressure. Thus this produce acute neurogenic hypertension in dogs.

Renin inhibition method in monkey

Blood pressure is mainly regulated by the renin Angiotensin system and can be influenced by the inhibition of renin. Renin inhibitors developed for have a high specifically for primate renin and cause only weak inhibition of renin sub primate species. It suggests that most commonly laboratory animals such as dog are not suitable for in-vivo evaluvation of rennin inhibitor. Marmosets (callithrix jacchus) of 300-400 gare feed pellet diet supplemented with fruits. These animals were used for renin inhibition method[41].

ANIMAL MODEL FOR THE DISSERTATION

SPONTANEOUSLY HYPERTENSIVE RATS:

Systolic blood pressure (SBP) and heart rate measurement of SH rats was carried out using tail-cuff method plethysmography (LE 5001 Pressure Meter). A mean of six measurements was obtained for each animal. For blood pressure measurement, the animals were warmed up to 42°C for 5 min in a confinement cage. The animals were first submitted to a period of adaptation for 15 days before the experiments and only SHR with an SBP > 170mmHg were selected for this study.

During the final week of the treatment, the rats were allowed to acclimatize to the experimental conditions of non invasive SBP measurements by allowing them to stand in rat restrainers for 30 min every day. SBP measurements were recorded 24 hours after the last treatment dose. At least 8-10 recordings were taken for each rat and the mean of the lowest 4 values within less than 10 mmHg difference was taken as the mean SBP.

3.6 PHARMACEUTICAL REVIEW

CHOORANAM

Definition:

Chooranam is a fine powder of drugs. The "Chooranam" may be applied to the powders of a single drug or a mixture of two or more drugs, which are powdered separately prior to their being mixed to homogeneity[42].

Method of preparation:

Equipment required

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

Process of preparation:

The drugs which are to be used in the preparations should be taken from recently collected material. Drugs which are aged by prolonged storages or changed in colour,

taste and scent, and those that are insects infested or attacked by fungi should be positively rejected.

However drugs like Embelia fruits, Senna, Long Pepper, Jaggery and cows ghee are preferred from fairly aged stock, provided they are not infested with pests, deteriorated or spoiled or developed racidity.

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

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

Purification of the prepared chooranam:

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

-அகஸ்தியர் வைத்திய இரத்தினச்சுருக்கம்[43]

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

"ஆமப்பா ரவியுலர்த்திப்பொடி தான்செய்து
அப்பனே சமனாய்ச் சர்க்கரையைச்சேர்த்து
நாமப்பா கொண்டுவர தோஷம்போச்சு
நன்றாக்ச் சுத்திசெய்யாச் சூரணந்தான்
தாமப்பா ரோகத்தை வெல்லாதப்பா
தளமான வியதியெல்லாம் பாரிக்கும்பார்
வேமப்பா சுத்திசெய்து கொண்டாயனால்
வெகுசுறுக்காய் தீருமா வியாதிகேளு"

-அகஸ்தியர் வைத்திய இரத்தினச்சுருக்கம்

Then the chooranam is placed in the sunlight and powdered. All type of diseases get cured. If the drug is taken without purification, the disease does not cure. If taken after purification the disease cures easily.

Storage:

The prepared chooranam should be allowed to cool by spreading and mixing, prior to packing. They should be stored in tightly stoppered glass, polythene or tin containers, or in polythene or cellophane bags and sealed. These bags should in turn be enclosed in cupboard boxes.

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

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

Then chooranam is said to retain its potency for 3 months and then gradually deteriorate. However if properly packed and stored, they keep good for a year. (Formulary of Siddha Medicines, 1993)[42a]

According to AYUSH guidelines shelf life of chooranam is one year.[44]

Table 4: ANALYTICAL SPECIFICATIONS OF CURNA/CHOORNAM

Sl.No	TESTS
1.	Description
	Macroscopic, Microscopic
2.	Loss on drying at 105˚ C
3.	Total – ash
4.	Acid – insoluble ash
5.	Water-soluble extractive
6.	Alcohol – soluble extractive
7.	Particle size (80-100 mesh for Churna; 40-60
	mesh for churna)
8.	Identifications,HPLC-withmarker(wherever possible)
	Test for heavy/Toxic metals
9.	Lead
	Cadmium
	Mercury
	Arsenic
	Microbial contamination
10.	Total bacterial count
	Total fungal count
	Test for specific Pathogen
11.	E. coli
	Salmonella spp.
	S.aureus
	Pseudomonas aeruginosa
	Pesticide residue
12.	Organochlorine pesticides
	Organophosphorus pesticides
	Pyrethroids
	Test for Aflatoxins (B1,B2,G1,G2)

3.7 LATERAL RESEARCH

1. Elettaria cardamomum:

Anti-ulcerogenic activity of *Elettaria cardamomum*

The gastro protective action of petroleum ether soluble fractions and essential oils of *Elettaria cardamomum* is due to increase in gastric motility and it has inhibitory effect in over production of some products of 5-lipoxygenase pathway[45].

Anti-convulsant activity:

The methanolic extract of *Elettaria cardamomum* against chemically (pentylentetrazole) and electrically (maximal electric shock) induced seizures in mice.Various pharmacological activities Anti -inflammatory, Analgesic, Anti -oxidant, Anti- microbial effects[46] .

2. *Zingiber officinale:*

Anti hypertensive activity:

Zingiber officinale showed antihypertensive activity while experimentally induced hypertension in rats.

Anti Oxidant activity:

Zingeberene possesses anti oxidant activity as well as significant anti -inflammatory and anti nociceptive property.

Anti Tumour activity:

Zingiberene inhibits the viability of breast cancer cells caspase mediated pathway

Zinger and its active ingredients reduce the chance of various diseases by suppression of NF-Kb , COX-2, LOX (inflammation mediators) and induction of apoptosis and tumour suppressor genes[47].

3. Piper longum:

Immunomodulatory and Anti tumor activity:

Alcoholic extract of the *Piper longum* fruits was 100% toxic at a concentration of 500 microg/ml Dalton's lymphoma ascities (DLA) cells and 250 microg/ml to Ehrlich ascities carcinoma (EAC) cells. Piperine was found to be cytotoxic towards DLA and EAC cells at a concentration of 250 microg/ml. Alcoholic extract and piperine was also found to produce cytotoxicity towards L929 cells in culture at a concentration of 100 and 50 microg/ml, respectively[48].

Anti hyperglycemic activity:

The Aqueous and Methanolic extracts of *Piper longum* root produced significant Anti hyperglycemic activity at a dosage of 200 mg/kg b.w in diabetic treated rats[49].

Anti anaphylatic activity:

The fruit effectively reduce the passive cutaneous anaphylaxis in rats and protect guinea pigs against antigen induced bronchospasm[50]

4. Glycyrrhiza glabra:

Glycyrrhiza glabra possesses Anti bacterial, Anti -oxidant, Anti -malarial, Anti spasmodic, Anti -inflammatory, Anti hyperglycemic properties. Various other effects like ulcer, Anti-viral, Hepatotoxic, and Anti- fungal have also been studied[51].

Memory enhancing activity:

The dose of 150 mg/kg of the aqueous extract of liquorice significantly improved learning and memory of mice[52]

5. Anethum graveolens:

Anti ulcer activity:

Anethum graveolens seed extracts have significant mucosal protective and anti secretory effects of gastric mucosa in mice[53]

Anti fungal activity:

The essential oil of dill could be used to control food spoilage as a potential source of food preservative[54]

Hypolipidemic activity:

Anethum graveolens powder and essential oil significant lipid lowering effect and cardioprotective agent[55].

6. Cuminum cyminum:

Anti Microbial activity:

In the present study *Cuminum cyminum* essential oil exhibited a strong anti microbial activity against the microbial flora of the teeth with failed endodontic treatments and it was biocompatible for L929 mouse fibroblasts[56].

Anti Oxidant activity :

Anti oxidant properties were assayed using DPPH free radical scavenging, inhibition of metal induced oxidation of proteins & lipids and protection of DNA against H_2O_2 induced oxidative stress. IC 50 value of cumin was estimated by these mechanisms. Cumin extract had polyphenols (7.45+.10 mg GAE/g dryseeds) as major anti oxidant principle[57].

7. Nelumbo nucifera:

Cardioprotective effect of Nelumbo nucifera flower extract Against isoproterinol induced oxidative stress in male albino Swiss rats

The present study investigate the cardioprotective effect of *Nelumbo nucifera* flower in isoproterinol induced rats. The positive hypertrophy response of isoproterinol caused a severe oxidative stress in the myocardium through increased lipid peroxidation. *Nelumbo nucifera* was administered intraperitonealy at a dose of 200mg/kg for a period of 30days.[58]

Psycopharmacological activity:

Methanolic extract of rhizomes of *Nelumbo nucifera* was investigated for different psychopharmacological action in rats and mice. The extract was found to cause reduction in spontaneous activity, decrease in exploratory behavioral pattern by the head dip and Y-maze test, reduction in the muscle relaxant activity by rotarod 30^0 inclined screen and traction test and potentiated the pentobarbitone induced sleeping time in mice significantly[59].

4. MATERIALS AND METHODS

4.1 Drug selections

In this dissertation purified and prepared "***Venthamaraiyathi chooranam"*** was taken as a trial drug for Anti-Hypertensive activity from the Siddha Literature **"Pharmacopoeia of Hospital of Indian Medicine"**authored by Dr.Narayanaswami page no:17

Table 5: Ingredients of *Venthamaraiyathi Chooranam:*

S.NO	TAMIL NAME	BOTANICAL NAME	PART USED
1.	*Elarisi*	*Elettaria cardamomum*	Seed
2.	*Chukku*	*Zingiber officinale*	Dried Rhizome
3.	*Thippili*	*Piper longum*	Dry Fruit
4.	*Adhimathuram*	*Glycyrrhiza glabra*	Root
5.	*Chadhakuppai*	*Anethum graveolens*	Seed
6.	*Ceeragam*	*Cuminum cyminum*	Seed
7.	*Venthamarai Poo Ithalkal*	*Nelumbo nucifera*	Flower

Quantity of Ingredients:

Elarisi (Elettaria cardamomum)	250gm
Chukku (Zingiber officinale)	500 gm
Thippili (Piper longum)	750gm
Adhimadhuram (Glycyrrhiza glabra)	500 gm
Cadhakuppai (Anethum graveolens)	500gm
Ceeragam (Cuminum cyminum)	500gm
Ven Thamarai poo ithalkal (Nelumbo nucifera)	3kg

Collection of the Plant materials

- The raw materials of Cardomum, Dry fruit Piper longum, Dry Zinger, Glycyrrhiza glabra root, Cumium cyminum and Anethum graveolens seeds were collected from the raw drug country shop at Parrys corner, Chennai, Tamilnadu.
- The Petals of White Lotus were collected at Kumbakonam.

Identification and Authentication of the drug

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

Purification of the drugs

All the drugs mentioned here were purified as per the Siddha literature. [61]

Elarisi:

Fried at low flame.

Chukku:

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

Thippili:

Soaking it with lemon juice and fried at low flame.

Adhimdhuram:

The root of Indian liquorice was cleaned with water and cut into small pieces and then dried.

Cadhakuppai:

Impurities are removed and dried in sunlight.

Ceeragam:

Impurities are removed and dried in sunlight.

Ven Thamarai Poo Ithalkal:

Impurities are removed and dried in cool dark place.

Preparation of the Drug

Procedure

All the above-mentioned ingredients were purified and dried in the shade until complete evaporation of the moisture content. It was roasted and powdered and filtered individually. (fine process). Then all are thoroughly mixed to make *Venthamaraiyathi Chooranam* and kept in an air tight container. It was labelled as "***Venthamaraiyathi Chooranam" (VTC).***

Purification of the Chooranam:

Steaming process (*Pittaviyalmurai*)

The *Venthamaraiyathi Chooranam* was purified by *pittaviyal* method (steam cooking in milk) as per Siddha classical literature. A mud pot was taken and it was half filled by mixture of milk with equal quantity of water. The mouth of the pot was sealed by a cloth. This *chooranam* was placed over the cloth and tied firmly around the mouth of mud pot by another pot. The gap between mud pots was tied with a wet cloth to avoid evaporation. The mud pot was kept on fire and boiled until the cow's milk 3/4 reduced in the lower pot. The same drug was later dried and powdered then sieved again. It was used for the further study [62].

Storage of the drug

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

Administration of the drug

Form of the medicine	:	*Chooranam*
Route of Administration:		Enteral
Dose	:	500 mg twice a day
Vehicle	:	Milk
Indications	:	Kuruthi Azhal Noi (Hypertension)

Ingredients of Venthamaraiyathi *Chooranam:*

Elettaria cardamomum (Elarisi)

Fig no:1

Zingiber officinale (Chukku)

Fig no:2

Piper longum (Thippili)

Fig no:3

Glycyrrhiza glabra (Adhimathuram)

Fig no:4

Anethum graveolens (Cadhakuppai)

Fig no:5

Cuminum cyminum(Ceeragam)

Fig no:6

Nelumbo nucifera (Venthamarai poo ithal)

Fig no:7

Preparation of Chooranam:

Fig no:8 Pounding

Fig no:9 Sieving

Fig no:10 Final product of ***Venthamaraiyathi chooranam***

4.2 STANDARDIZATION OF THE DRUG

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

4.2.1 ORGANOLEPTIC CHARACTER

The organoleptic characters of the sample drug were evaluated.1gm of the test drug was taken and the colour, texture, particle size and other morphology were viewed by naked eye under sunlight. Then the result was noted [63].

4.2.2 PHYSICOCHEMICAL ANALYSIS

Physico - chemical investigations like Solubility, pH value, Loss on drying at 105°C, and Ash test have been done at The Tamilnadu Dr M.G.R Medical University, Anna salai, Guindy, as per the guide lines of WHO [64].

Solubility:

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

pH value:

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

Loss on Drying:

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

Determination of total Ash:

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

Determination of acid insoluble ash:

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

Determination of water soluble ash:

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

Determination of water soluble extractive:

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

Determination of alcohol soluble extractive:

2.5gm of air dried drugs coarsely powdered *VTC* was macerated with 50ml. alcohol in closed flask for 24 hours. With frequent shaking.it was filtered rapidly talking precaution against loss of alcohol .10ml of filtrate was the evaporated in a tarred flat bottom shallow dish, dried at 100^0 c and weighed. The percentage of alcohol soluble extractive was calculated with reference to air dried drug.

4.2.3 PHYTOCHEMICAL ANALYSIS

The Phytochemical screening of the extract gives general idea regarding the nature of chemical constituents present in the crude drug. The phytochemical tests were done as the method illustrated in [65].

1.Detection of alkaloids:

Extracts were dissolved in dilute hydrochloric acid and filtered.

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

2.Detection of carbohydrates:

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

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

3.Detection of glyoscides:

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

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

4.Detection of saponins:

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

5.Detection of phytosterols:

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

6.Detection of phenol ferric chloride test:

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

7.Detection of tannins Gelatin test:

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

8.Detection of flavonoids:

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

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

9.Detection of Proteins and Amino acids:

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

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

10. Detection of Diterpenes:

a) copper acetate test:

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

11. Gum and Mucilage:

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

12.Test for Fixed oils and Fats:

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

13.Test for Quinones:

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

HPLC - High Performance Liquid Chromatography (HPLC) [66]

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

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

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

4.2.4 BIO-CHEMICAL ANALYSIS

Preliminary Basic and Acidic radical studies [67]

Preparation of extract

5gm of *VTC* was taken in a 250 ml of clean beaker and 50 ml of distilled water was added to it. Then it was boiled well for about 10 minutes. Then it was allowed to cool and filtered in a 100 ml volumetric flask and made up to 100 ml with distilled water. This preparation was used for the qualitative analysis of acidic/ basic radicals and biochemical constituents in it.

Test for Basic radicals

1. Test for Potassium

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

2. Test for Calcium

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

3. Test for Magnesium:

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

4. Test for Ammonium:

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

5. Test for Sodium

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

6. Test for Iron (Ferrous)

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

7. Test for Zinc

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

8. Test for Aluminium

To the 2m1 of the *VTC* extract sodium hydroxide was added in drops and changes are noted.

9. Test for Lead

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

10. Test for Copper

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

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

11. Test for Mercury

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

12. Test for Arsenic

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

Test for acid radicals

1. Test for Sulphate

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

2. Test for Chloride

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

3. **Test for Phosphate**

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

4. **Test for Carbonate**

The *VTC* extract was treated with conc.Hcl and observed froth appearance of effervescence indicate the presence of carbonate.

5. Test for Fluoride & Oxalate:

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

6. Test for Nitrate:

To 1 gm of the *VTC*, copper turnings was added and again conc.H_2SO_4 was added, heated and the test tube was tilted vertically down and observed for any changes.

4.2.5 AVAILABILITY OF MICROBIAL LOAD:

Enumeration of bacteria by plate count – agar plating technique [68]

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

Principle:

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

Dilution:

A small measured volume is mixed with a large volume of sterile water or saline called the diluent or dilution blank. Dilution are usually made in multiples of ten.

A single dilution was calculated as follows:

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

Requirements:

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

Procedure:

- Label the dilution blanks as 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}.
- Prepare the initial dilution by adding 1 ml of the *VTC* into a 9 ml dilution blank labelled 10^{-1} thus diluting the original sample 10 times.
- Mix the contents by rolling the tube back and forth between hands to obtain uniform distribution of organisms.
- From the first dilution transfer 1 ml of the suspension while in motion, to the dilution blank 10^{-2} with a sterile and fresh 1 ml pipette diluting the original specimen to 100 times.
- From the 10^{-2} suspension, transfer 1 ml of suspension to 10^{-3} dilution blank with a fresh sterile pipette, thus diluting the original sample to 1000 times.
- Repeat this procedure till the original sample have been diluted 10,000,000 times using every time a fresh sterile pipette.
- From the appropriate dilutions transfer 1ml of suspension while in motion, with the respective pipettes, to sterile petri dishes. Three petri dishes are to use for each dilution.
- Add approximately 15 ml of the nutrient medium, melted and cooled to 45^0c, to each petri dish containing the diluted sample. Mix the contents of each dish by rotating gently to distribute the cells throughout the medium.
- Allow the plates to solidity.

- Incubate these plates in an inverted position for 24-48 hours at 37^0c.

Observation:

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

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

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

4.2.6 INSTRUMENTAL ANALYSIS

SOPHISTICATED INSTRUMENTAL ANALYSIS

FT IR - Fourier Transform Infra-red Spectroscopy [69]

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

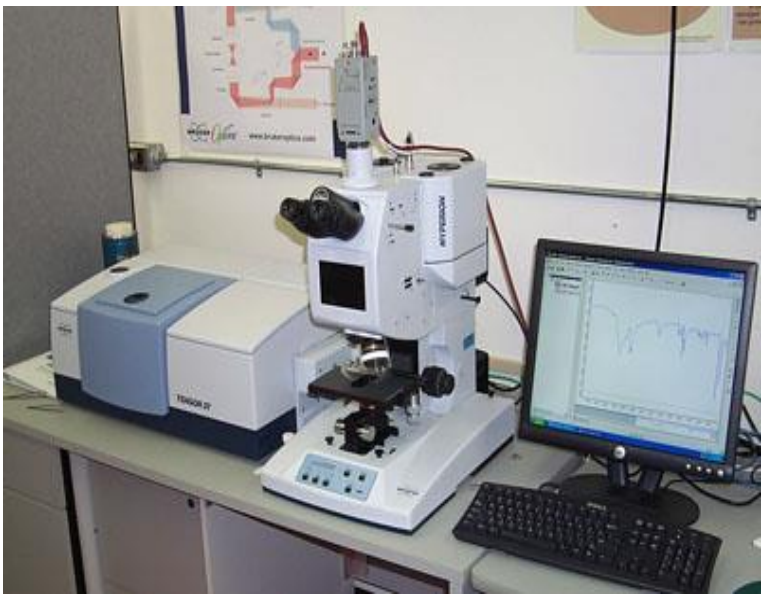


Fig no:11 FTIR INSTRUMENT

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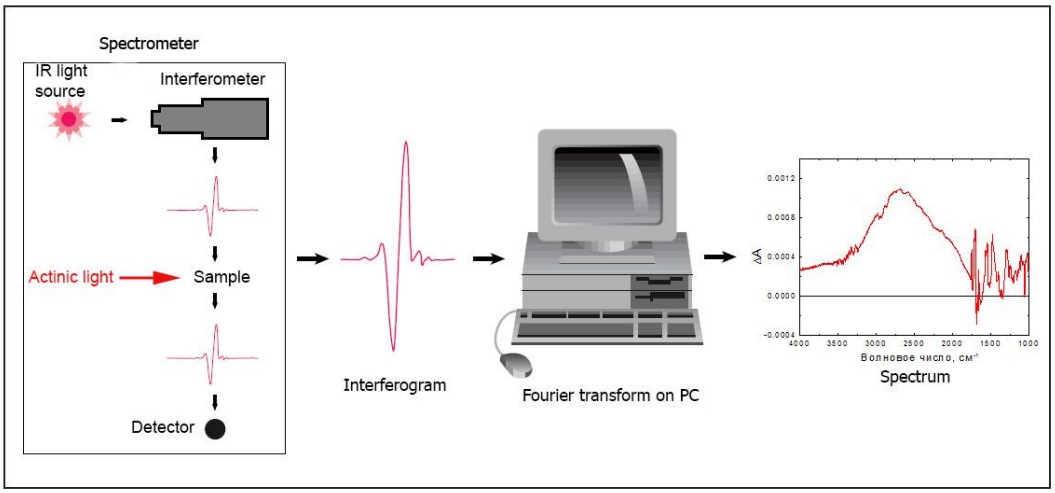


Fig no:12 FTIR MECHANISM

APPLICATIONS:

Quantitative scans

Qualitative scan solids, liquids, gases

Organic samples, inorganic samples

Unknown identification

Impurities screening

Formulation

Pharmaceuticals

Principle:

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

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

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

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

- Speed
- Sensitivity
- Mechanical Simplicity
- Internally Calibrated

ICP-MS - INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY [70]

Analysis of Trace Metal and Inorganic Materials

Inductively Coupled Plasma Mass Spectrometry is a technique routinely used to analyse trace levels of a wide range of inorganic elements. The ICP-MS allows for the detection and quantification of elements with atomic mass ranges 7 to 250. This covers Lithium to Uranium.

The typical detection limits are in the parts per billion (ppb) range and even parts per trillion (ppt) in some cases. The ICP-MS analysis methods available at LPD Lab Services allow the detection, identification and quantification of a wide array of elements using a Perkin Elmer ELAN 6000 ICP-MS

Fig no: 13 ICP-MS INSTRUMENT

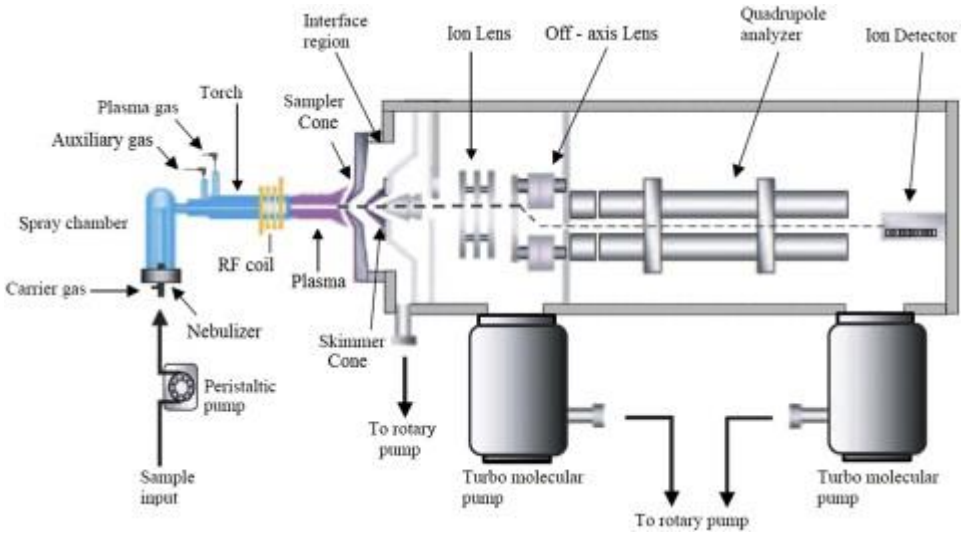


Fig no: 14 ICP MS MECHANISM

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

Applications of ICP-MS

- Monitoring of trace metals in drinking water, ground water, rainwater, wastewater or industrial effluent streams.
- Trace elements in product / raw materials or from washed or rinsed surfaces.
- Analysis of additives and purity in metal alloys.
- Analysis of low level contaminants in chemical products, beverages, foods, cosmetics, pharmaceuticals.
- Analysis of soluble / leachable material from solid samples such as medical devices, polymers, PCB`s.
- Analysis can be performed on a diverse range of sample

SEM - Scanning Electron Microscope [71]

DEFINITION

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

SEM ANALYSIS APPLICATIONS

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

External morphology (texture)

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

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

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

SEM Analysis with EDS – qualitative and semi-quantitative results

Magnification – from 5x to 300,000x

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

Materials analysed – solid inorganic materials including metals and minerals.

Fig no: 15 SEM INSTRUMENT

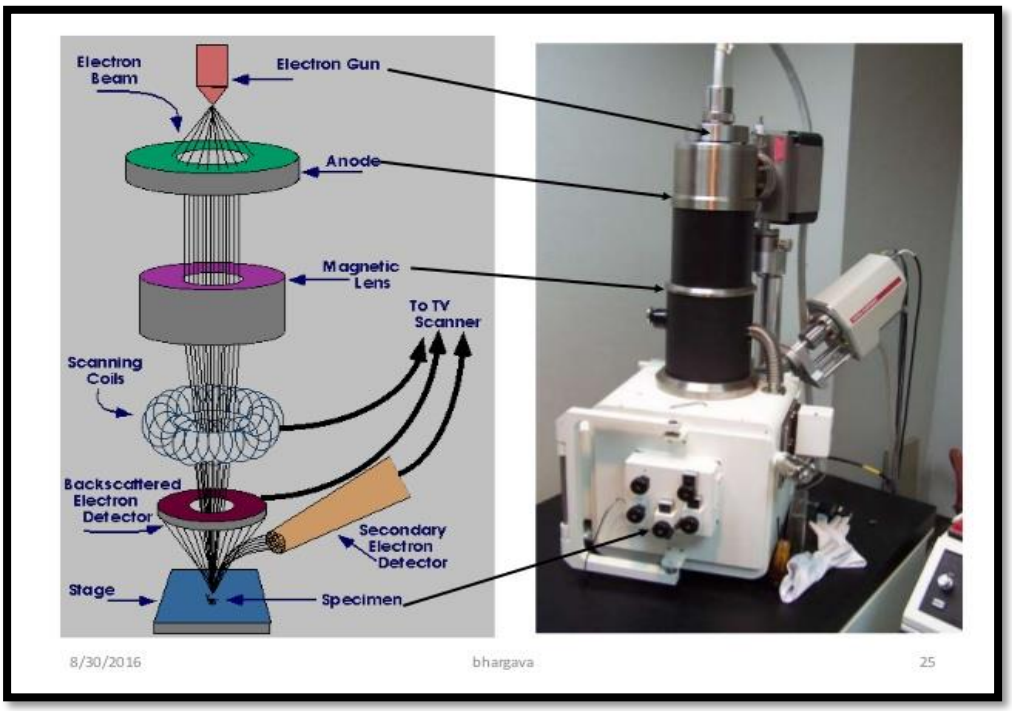


Fig no: 16 SEM MECHANISM

THE SEM ANALYSIS PROCESS

Scanning Electron Microscopy uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens. In most SEM microscopy applications, data is collected over a selected area of the surface of the sample and a two-dimensional image is generated that displays spatial variations in properties including chemical characterization, texture and orientation of materials. The SEM is also capable of performing analyses of selected point locations on the sample. This approach is especially useful in qualitatively or semi-quantitatively determining chemical compositions, crystalline structure and crystal orientations.

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

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

- Secondary electrons
- Back scattered electrons
- Characteristic x-rays light
- Specimen current
- Transmitted electrons.

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

XRD - X-ray Powder Diffraction (XRD) [72]

X-ray powder diffraction (XRD) is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions. The analysed material is finely ground, homogenized, and average bulk composition is determined.

DEFINITION

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

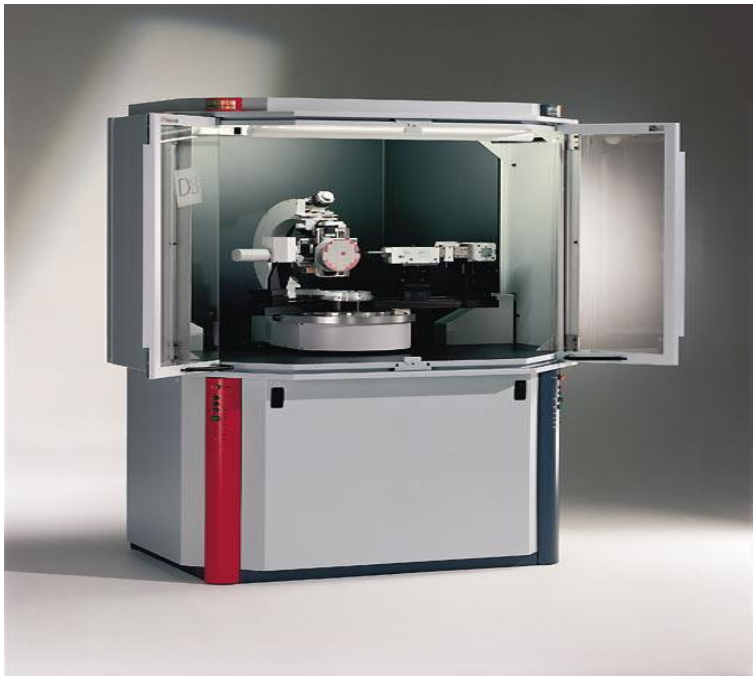


Fig no: 17 XRD - X-ray Powder Diffraction

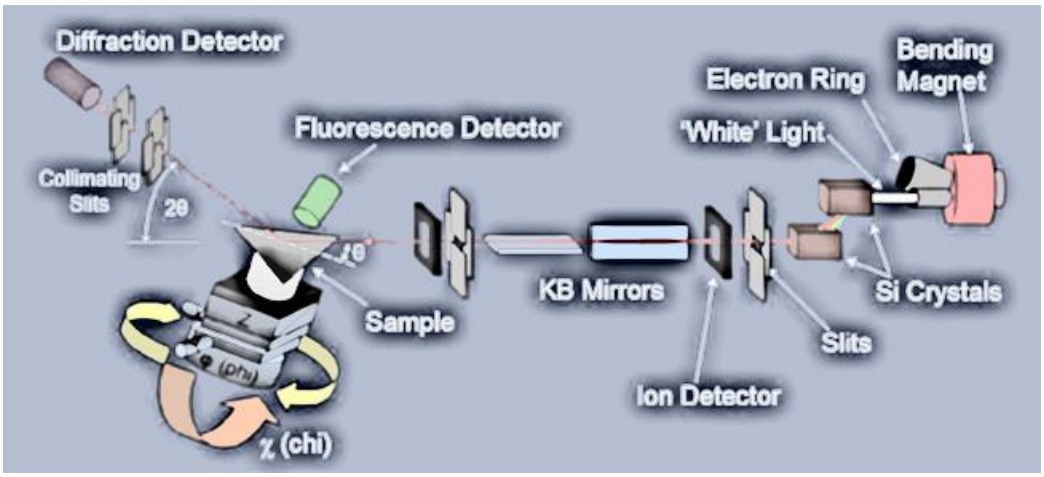


Fig no: 18 XRD Mechanism

Applications:

- Characterization of crystalline materials
- Identification of fine-grained minerals such as clays and mixed layer clays that are difficult to determine optically
- Determination of unit cell dimensions.

With specialized techniques, XRD can be used to:

- Determine crystal structures using Rietveld refinement
- Determine of modal amounts of minerals (quantitative analysis)
- Characterize thin films samples by:
- Determining lattice mismatch between film and substrate and to inferring stress and strain
- Determining dislocation density and quality of the film by rocking curve measurements
- Measuring super lattices in multilayered epitaxial structures
- Determining the thickness, roughness and density of the film using glancing incidence X-ray reflectivity measurements
- Make textural measurements, such as the orientation of grains, in a polycrystalline sample.

Strengths and Limitations of X-ray Powder Diffraction:

Strengths:

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

Limitations:

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

Sample Collection and Preparation:

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

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

4.3 TOXICOLOGICAL STUDIES

4.3.1 ACUTE ORAL TOXICITY STUDY

Acute toxicity study was carried out as per OECD guideline (Organization for Economic Co - operation and Development, Guideline-423 [73]. The experimental protocol was approved by the institutional ethical committee (IAEC) under CPCSEA **IAEC APPROVED NUMBER**: **IAEC/XL VIII/18/CLBMCP/2016.**

These studies were conducted in C.L.Baid Metha College of Pharmacy, Dhuraipakkam, Chennai.

INTRODUCTION:

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

The use of a selection of pre-defined doses, regardless of test substance, with classification explicitly tied to number of animals observed in different states improves the opportunity for laboratory to laboratory reporting consistency and repeatability.

PRINCIPLE:

It is the principle of the test that based on a stepwise procedure with the use of a minimum number of animals per step, sufficient information is obtained on the acute

toxicity of the test substance to enable its classification. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals of a single sex. Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.;

− No further testing is needed

− dosing of three additional animals with the same dose

− dosing of three additional animals at the next higher or the next lower dose level.

The method will enable a judgment with respect to classifying the test substance to one of a series of toxicity classes.

METHODOLOGY

Selection of Animal Species

The preferred rodent species is the wistar rat, although other rodent species may be used. Healthy young adult animals are commonly used laboratory strains should be employed. Females should be nulliparous and non-pregnant. Each animal, at the commencement of its dosing, should be between 6 to 8 weeks old and the weight (150-250gm) should fall in an interval within±20 % of the mean weight of any previously dosed animals.

Housing and Feeding Conditions

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

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

Test Animals and Test Conditions:

Sexually mature Female Wistar albino rats (150-200gm) were obtained from TANUVAS, Madhavaram, Chennai. All the animals were kept under standard environmental condition (22±3°C). The animals had free access to water and standard pellet diet (Sai Meera foods, Bangalore).

PREPARATION OF ACUTE TOXICITY STUDIES:

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

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

Test Substance	: ***VENTHAMARAIYATHI CHOORANAM***
Animal Source	: TANUVAS, Madhavaram, Chennai.
Animals	: Wister Albino Rats (Female-3+3)
Age	**:** 6-8 weeks
Body Weight on Day 0	**:** 150-200gm.
Acclimatization	**:** Seven days prior to dosing.
Veterinary examination	**:** Prior and at the end of the acclimatization period.
Identification of animals	**:** By cage number, animal number and individual marking by using Picric acid.
Number of animals	: 3 Female/group,
Route of administration	: Oral
Diet	**:** Pellet feed supplied by Sai meera foods Pvt Ltd, Bangalore
Water	**:** Aqua guard portable water in polypropylene bottles.
Housing & Environment	**:** The animals were housed in Polypropylene cages provided with bedding of husk.
Housing temperature	**:** between 22ºC $\pm$ 3ºC.
Relative humidity	**:** between 30% and 70%,
Air changes	**:** 10 to 15 per hour and
Dark and light cycle	**:** 12**:**12 hours.
Duration of the study	**:** 14 Days

Administration of Doses:

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

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

Limit test

The limit test was primarily used in situations where the experimenter has information indicating that the test material was likely to be nontoxic, i.e., having toxicity only above regulatory limit doses. A limit test at one dose level of 2000 mg/kg body weight was carried out with three animals per step.

The test substance-related mortality was not produced in animals, so further testing at the next lower level need not be carried out.

Observations

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

a. Mortality

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

b. Body weight

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

c. Cage-side observation

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

d. Gross necropsy

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

Histopathology

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

Data and reporting

All the data were summarised in tabular form, table showing the animals used, number of animals displaying signs of toxicity, the number animals found dead during the test or killed for humane reasons, a description and the time course of toxic effects and reversibility, and necroscopic findings.

Test substance and Vehicle

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

Justification for choice of vehicle

The vehicle selected as per the standard guideline is pharmacologically inert and easy to employ for new drug development and evaluation technique [74].

4.3.2 REPEATED DOSE 28 DAYS ORAL TOXICITY STUDY OF *VEN THAMARAIYATHI CHOORANAM* ON RATS – (OECD-407 guidelines) [75]

Test Substance : **VENTHAMARAIYATHI CHOORANAM**
Animal Source : TANUVAS, Madhavaram, Chennai.
Animals : Wister Albino Rats (Male -6, and Female-6)
Age **:** 6-8 weeks
Body Weight **:** 150-200gm.
Acclimatization **:** Seven days prior to dose.
Veterinary examination **:** Prior and at the end of the acclimatization period.
Identification of animals **:** By cage number, animal number and individual marking by using Picric acid
Diet **:** Pellet feed supplied by Sai meera foods Pvt Ltd, Bangalore
Water **:** Aqua guard portable water in polypropylene bottles.
Housing & Environment **:** The animals were housed in Polypropylene cages provided with bedding of husk.
Housing temperature **:** between 22ºC $\pm$ 3ºC.
Relative humidity **:** between 30% and 70%,
Air changes **:** 10 to 15 per hour
Dark and light cycle **:** 12**:**12 hours.
Duration of the study **: 28 Day**

Table no 6: Grouping of animals:

Groups	**No of Rats**
Group I Vehicle control (Water)	12(6male,6 female)
Group II VTC- low dose X (20mg)	12 (6male,6 female)
Group III VTC- Mid dose 5X (100mg)	12 (6male,6female)
Group IV VTC- High dose 10X (200 mg)	12(6male,6female)

Justification for Dose Selection:

The results of acute toxicity studies in Wistar albino rats indicated that *(VTC)* was non-toxic and no behavioural changes was observed up to the dose level of 2000 mg/kg body weight. On the basis of body surface area ratio between rat and human, the doses selected as per OECD guideline three dose levels were selected for the study. They are low dose (5X), high dose (10X). X is calculated by multiplying the acute toxicity dose 2000mg) and the body surface area of the rat (0.018), 5X dose is (100mg/kg), 10X dose is (200mg/kg) The oral route was selected for use because oral route is considered to be a proposed therapeutic route.

Preparation and Administration of Dose:

Venthamaraiyathi chooranam suspended in prescribed medium, it was administered to animals at the dose levels of X, 5X, 10X. The test substance suspensions were freshly prepared every two days once for 28 days. The control animals were administered vehicle only. The drug was administered orally by using oral gavage once daily for 28 consecutive days.

METHODOLOGY

Randomization, Numbering and Grouping of Animals

48 Wistar Albino Rats (24M + 24F) were selected and divided into 4 groups. Each group consist of 12 animals (Male -6, and Female-6). First group treated as a control and other three group were treated with test drug (low, mid, high) for 28 days. Animals were allowed acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. Each animal was marked with picric acid. The females were nulliparous and non-pregnant.

OBSERVATIONS

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

Body Weight:

Weight of each rat was recorded on day 0, at weekly intervals throughout the course of study.

Food and water Consumption:

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

Clinical signs:

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

Mortality:

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

Functional Observations:

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

Laboratory Investigations:

Following laboratory investigations were carried out on day 29 in animal's fasted over-night. Blood samples were collected from orbital sinus using sodium heparin (200IU/ml) for Bio chemistry and potassium EDTA (1.5 mg/ml) for Haematology as anticoagulant. Blood samples were centrifuged at 3000 r.p.m. for 10 minutes.

Haematological Investigations:

Blood samples of control and experimental rats was analyzed for hemoglobin content, total red blood corpuscles (RBC), white blood corpuscles (WBC) count and packed cell volume (PCV).

Biochemical Investigations:

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

Necropsy:

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

Histopathology:

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

Statistical analysis:

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

4.4 PHARMACOLOGICAL STUDIES

4.4.1 ANTI-HYPERTENSIVE ACTIVITY OF *VENTHAMARAIYATHI CHOORANAM* IN SPOTANEOUSLY HYPERTENSIVE RATS

Cardiovascular disease is a leading cause of death, and hypertension is a critical risk factor for cardiovascular events. The pathogenesis of hypertension is accompanied by decreased nitric oxide (NO) bioavailability in the vasculature and increased cardiovascular remodelling. Hypertensive patients frequently develop clinically evident cardiac hypertrophy 10 to 20 years after the onset of hypertension, as a result of adaptive and maladaptive responses to pressure overload. Cardiac hypertrophy has been linked to the development of a variety of cardiovascular diseases, including myocardial ischemia, arrhythmias, and sudden cardiac death. Therefore, treatment options that not only maintain stable pressure levels but also delay or even regress the structural and functional changes in resistance arteries and the heart are needed. Despite the current

availability of multiple anti-hypertensive medication types, a significant number of patients do not respond to treatment and remain hypertensive. As multiple mechanisms likely contribute to the development of hypertension, including angiotensin, oxidative stress and hemodynamic changes, multi-targeted therapeutic interventions will likely be required for effective management of hypertension.

Animals:

All animal experiments were performed in accordance with the Guidelines of OECD. All experiments were performed with the approval of IAEC of C.L. BAID METHA COLLEGE OF PHARMACY. SHR (9 weeks old) and age-matched Wistar rats male, weighing 250±20 g, were purchased from King Institute of Preventive Medicine and Research, Rats were kept in a room temperature-controlled room (25 °C), with 12 hours dark and 12 hours artificial illumination daily (7:00— 19:00). Food and water were available ad libitum.

GROUPING

The animals were divided into following groups:

- Group 1 control untreated group which received normal saline.
- Group 2 received Verapamil 12.5 mg/kg b.w
- Group 3 *VTC* 100 mg/kg b.w
- Group 4 *VTC* 200mg/kg b.w

The drug *VTC* was administered orally and once daily for 4 weeks.

In this study, the effect of a four weeks chronic administration of daily oral doses of 100 and 200 mg/kg body weight, *VTC* on blood pressure was measured.

The stock solution was prepared once every three days. Extract suspensions were stored at 4ºC and were allowed to reach room temperature before administration.

METHOD:

Systolic blood pressure (SBP) and heart rate measurement of SH rats was carried out using tail-cuff method plethysmography (LE 5001 Pressure Meter). A mean of six measurements was obtained for each animal. For blood pressure measurement, the animals were warmed up to 42°C for 5 min in a confinement cage. The animals

were first submitted to a period of adaptation for 15 days before the experiments and only SHR with an SBP > 170 mmHg were selected for this study.

During the final week of the treatment, the rats were allowed to acclimatize to the experimental conditions of non-invasive SBP measurements by allowing them to stand in rat restrainers for 30 min every day. SBP measurements were recorded 24 hours after the last treatment dose. At least 8-10 recordings were taken for each rat and the mean of the lowest 4 values within less than 10 mmHg difference was taken as the mean SBP [76].

4.4.2 DIURETIC ACTIVITY OF *VENTHAMARAIYATHI CHOORANAM* [77]

ANIMALS

Male Wistar rats (175-200g) were purchased from TANUVAS Chennai. They were maintained under standard conditions of temperature and humidity. The method of Lipschitz et al was employed for the assessment of diuretic activity. FOUR groups of six rats.

GROUPING

The animals were divided into following groups:

- Group 1----control untreated group which received normal saline.
- Group 2 ----received Furosemide12.5 mg/kg b.w
- Group 3-----100mg/kg b.w
- Group 4-----200mg/kg b.w

each were fasted and deprived of water for eighteen hours prior to the experiment. On the day of experiment, normal group of animals were given normal saline orally (25 ml/kg body weight.) And the treated groups were given 100,200 VTC mg/kg bodyweight of rats and water. The standard groups were given furosemide (20mg/kg) intraperitoneally. The rats were placed in metabolic cages specially designed to separate faecal matter and urine. The urine volume was collected at 24 hours post administration. During this period no food or water was given to the animals. The total urine volume was measured for both control and treated animals. The sodium, potassium and chloride ion concentration in the urine samples were determined.

The urine was collected for 5th hour and 24th hour after administration control, standard and test drug. The bladder was emptied by pull the base of tail of each rat[78]

OBSERVATION

- Animals are subjected to collect urine periodically by metabolic cages.
- Diuretic assay parameters were observed for each rat.
- The total volume of urine was measured.
- Urinary pH, urinary sodium excretion, urinary potassium excretion, urinary chloride excretions are determined. The concentration of sodium, potassium and chloride levels excreted in the urine were measured by flame photometry and the chloride concentration was estimated by titration with silver nitrate solution(N/50) using 3 drops of 5% potassium chromate as indicator [79]
- The data was analyzed using one-way analysis of variance (ANOVA).
- The statistical significance of the difference of the means was evaluated by Dunnett's multiple comparison test

4.4.3 ANTI-OXIDANT ACTIVITY OF *VENTHAMARAIYATHI CHOORANAM*

DPPH ASSAY (2, 2-diphenyl -1-picrylhydrazyl) IN-VITRO [80]

The radical scavenging activity of different extracts was determined by using DPPH assay according to [74] Chang et al [2001]. The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517 nm.

Ascorbic acid (10mg/ml DMSO) was used as reference.

PRINCIPLE

1,1-diphenyl-2-picrylhydrazyl is a stable free radical with red colour which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity. The scavenging reaction between (DPPH) and an antioxidant (H-A) can be written as,

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

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

REAGENT PREPARATION

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

PROCEDURE

Different volumes (1.25-20µg/µl) of *VTC* extracts were made up to 40µl with DMSO and 2.96ml DPPH (0.1mm) solution was added. There action mixture was incubated in dark condition at room temperature for 20min. After 20min, the absorbance of the mixture was read at 517 nm. 3ml of DPPH was taken as control. The % radical scavenging activity of the *VTC* extracts was calculated using the following formula,

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

5.RESULTS AND DISCUSSION

There are so many advanced studies have been carried out to bring the efficacy and potency of the drug "***Venthamaraiyathi chooranam".*** The study includes literary collections, organoleptic characters, physicochemical, phytochemical analysis, Acid-Base radical test, Microbial load, instrumental analysis, toxicological study and pharmacological study. The drug "***Venthamaraiyathi Chooranam"*** has been selected for **Anti-Hypertensive activity** in reference with the text **"Pharmacopoeia of Hospital of Indian Medicine"** authored by Dr.Narayanaswami. Literary collections about the drug from various text books were done. Siddha literatures related to the drug bring the evidence and importance of its utility in treating the hypertension.

- Botanical aspect explains the identification, description, active principle and medicinal uses of the plants.
- Gunapadam review brings the effectiveness of the drug in treating hypertension.
- The pharmacological review explains about the methodology of Anti-Hypertensive Activity and the drugs used.
- Pharmaceutical review describes about the *chooranam* and its properties.
- Lateral research gives the effectiveness of the drug in treating hypertension.
- Siddha and Modern aspect of the disease was also reviewed.

DISCUSSION ON STANDARDIZATION TECHNIQUES:

STANDARDIZATION OF THE TEST DRUG

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

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

ORGANOLEPTIC CHARACTERS

The following characters have been noted in *Venthamaraiyathi chooranam*.

Table no.7.Organoleptic Characters

Colour	Yellowish green
Odour	Pleasant
Taste	Bitter and sweet
Texture	Fine powder
Particle size	Completely pass through sieve no 88

Discussion:

The organoleptic characters of the drug *Venthamaraiyathi chooranam* showed yellowish green colour since prepared from dry herbs, Bitter and 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 drug.
- The size of the particle is reduced through various stages like pounding, sieving, filtering through white cloth *(vasthirakayam)*
- Only if the size of the particle is reduced to micro particles, the drug is easily absorbable in the digestive system.
- The above processes reduced the size of particle so that the *chooranam* passes through the sieve no 88.

PHYSICO CHEMICAL ANALYSIS

Table no.8. Results of physico chemical analysis

S.No	Parameter	Result
1	P^H	5.48
2	Solubility	Positive
	Distilled water	Soluble
	Benzene	Soluble
	Chloroform	Soluble
3	Loss on drying	8.21%
4	Total ash value	9.27%
5	Acid insoluble Ash (%)	1.27%
6	Water soluble ash (%)	4.8%

Discussion:

The physico chemical analysis of the drug result reveals the pH, Solubility, Loss of drying, Total ash value, Water soluble ash and Acid insoluble ash.

Solubility:

- Solubility is one of the important parameters to achieve desired concentration of drug in systemic circulation for desired (anticipated) pharmacological response.
- Oral ingestion is the most convenient and commonly employed route of drug administration; oral bio-availability depends on solubility**.**
- *VTC* is soluble in major solvents and sparingly soluble in some solvents, well soluble in Distilled water, Benzene and Chloroform. It improves that its efficiency of solubility and increases the bio- availability of the test drug [81].

pH:

- The p^H level plays a role in enzyme activity by maintaining the internal environment, thus it exhibits important role in regulating homeostasis.
- *Venthamaraiyathi chooranam* shows weak acidic p^H 5.48.

- Weak acid is more lipid soluble in an acidic solution, and more water soluble in alkaline solution.
- Whenever a weak acid drug is given, most of the drug in the stomach is in un-ionized form, which forms through the gastric mucosa. Weakly acidic drugs are more readily absorbed from an acid medium (stomach) than are weakly basic drugs.
- It is also important factor for drug absorption. which enhances the bio-availability [82]

Loss on drying:

- Loss on drying (LOD) gives the total amount of volatile content and moisture (water) present in the drug.
- The stability of a drug and its shelf-life are dependent on moisture content.
- Moisture increase can adversely affect the active ingredient.
- Low moisture content- drug could get maximum stability and better shelf life.
- Since the *VTC* has low loss on drying, the moisture content is 8.21% which is suitable for medicine preparation.

Ash:

- Ash values are helpful in determining the quality and purity of the drugs
- The ash content of a crude drug is generally taken to be the residue remaining after incineration. It usually represents the inorganic salts naturally occurring in the drug and adhering to it, but it may also include inorganic matter added for the purpose of adulteration [83].
- The total ash value of *Venthamaraiyathi chooranam* is 9.27%, which determines the presence of inorganic content.

Acid insoluble ash:

- The total ash is the residue remaining after incineration. The acid insoluble ash is the part of the total ash which is insoluble in diluted hydrochloric acid.
- Lower the acid insoluble value better will be the drug quality. The drug ensures a low value of acid insoluble ash indicating that the preparation did not contain any sand, dust and stones.
- The Acid insoluble ash of *Venthamaraiyathi chooranam* is 1.7% which ensure the trail drug does not contain any sand, dust and stones.

Water soluble ash:

- Decreased water soluble ash value indicates easy facilitation of diffusion and osmosis mechanisms.
- The Water-soluble ash of *Venthamaraiyathi chooranam* is 4.83% indication increase the facilitate of diffusion and osmosis.

PHYTOCHEMICAL ANALYSIS:

Table no 9: Results of phytochemical analysis

S.no	Phytochemicals	Test name	Result
1.	Alkaloids	Mayer's Test	Positive
		Wagner's Test	Positive
2.	Glycoside	Modified Borntrager's Test	Positive
3.	Saponin	Froth Test	Positive
4.	Phenols	Ferric chloride Test	Positive

5.	Tannins	Gelatin Test	Positive
6.	Flavanoids	Alkaline Reagent test	Positive
		Lead acetate test	Positive
7.	Diterpines	Copper Acetate Test	Positive
8.	Gum and Mucilage	Extract +Alcohol	Positive

Discussion

Phytochemicals are natural bioactive compounds, found in plants and fibers, which act as a defense system against diseases and more accurately protect against diseases. The phytochemical analysis reveals that the presence of Alkaloids, Glycosides, Saponin, Phenol, flavanoids and Diterpines, Tannin, Gum and Mucilage

Alkaloids:

- Alkaloids possess Vasodilators and anti-arrhythmic effects
- Alkaloids are the active principles producing many essential effects in protecting the body [84]**.**

Glycosides

Glycosides are Anti-oxidant activity in nature thus it plays major role in treating cardiac diseases [85].

Saponin:

- Saponin reduce an emulsification of fat molecules. Saponin bind with bile salt and cholesterol in the intestinal tract. Bile salts form small micelles with cholesterol facilitating its absorption. Saponin cause a reduction of blood cholesterol by preventing its re-absorption.
- It has a property of Anti-oxidant and reduced the risk of heart diseases [86].

Phenols:

They possess rich Anti-Oxidant property and protect body from oxidative stress [87]

Diterpene:

- Diterpene has the property of exhibiting of vasorelaxant action and inhibiting of vasocontraction
- So, it is helpful in reduction of blood pressure [88].

Flavonoids

- It is the most important group of polyphenol compounds in plants.
- They improve the endothelial and capillary function
- Reduces the risk of atherosclerosis.
- They help in strengthening and protect the inner lining of the blood vessels
- Flavonoids are a group of plant metabolites which provide health benefits through cell signaling pathways and antioxidant effects.
- Flavonoids can exert their Anti-Oxidant activity by scavenging the free radicals, by chelating metal ions or by inhibiting enzymatic systems which are responsible for free radical generation [89].

Tannin:

- Tannins contains anti-oxidant effect which producing many essential effects in protecting the body.
- Tannin contains ACE inhibitory effect [90].

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) ANALYSIS OF *VENTHAMARAIYATHI CHOORANAM:*

Table no 10: HPLC analysis were done. HPLC analysis performed with *Venthamaraiyathi chooranam* revealed the presence of following compounds:

S. NO	PARAMETERS	METHOD	UNITS	RESULTS
1.	Total Polyphenol as gallic acid Equivalent	Indian Pharmacopoeia 2014	mg/100g	0.12
2.	Total Flavonoids as Quercetin Equivalent	TNTH/STP/FOOD/110	mg/100g	5.46
3.	Total Alkaloids	TNTH/STP/FOOD /426	mg/100g	2.47
4.	Total Tannin as Tannic Acid Equivalent	AOAC 20th Edn.2012, 955.35	mg/100g	0.96

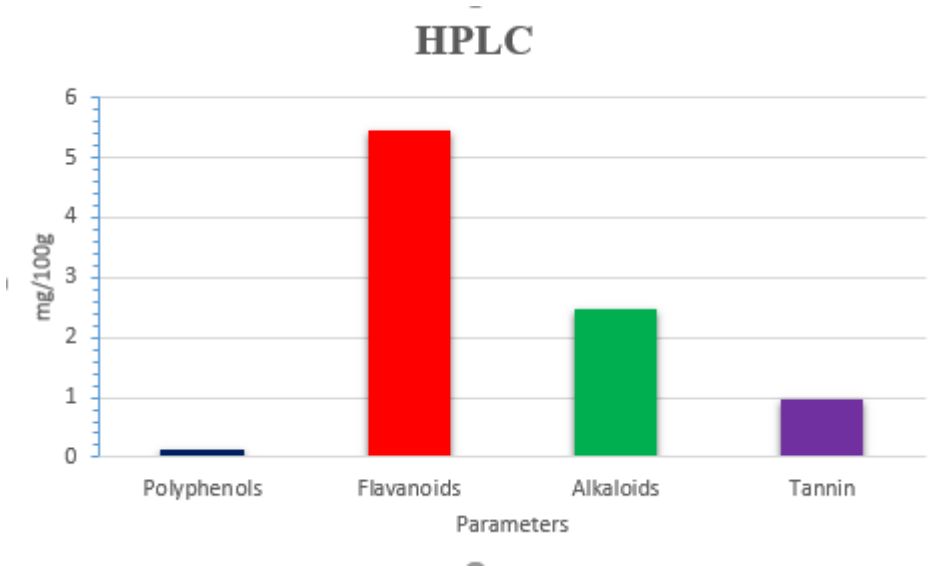


Chart no:1 Results of HPLC analysis

Discussion:

Polyphenols:

- Polyphenols are protection and improving endothelial function with vascular relaxation and improves vascular health, thereby significantly reducing the risk of hypertension and CVD.
- Polyphenols can stimulate the formation of vasoprotective factors such as nitric acid (NO) and endothelium derived hyperpolarizing factor to promote vasodilation.
- Polyphenols inhibits platelet aggregation in humans.
- Polyphenols also improve vascular smooth muscle function, by reducing the excessive vascular oxidative stress of pathological blood vessels associated with many cardiovascular risk factors.
- Polyphenols are the members of very large family of plant derived compound which had the anti-lipogenic effect. This is mainly due to the reduce fatty acid and triglycerol synthesis, increased in fatty acid oxidation and reduced oxidative stress and inflammation [91].

Flavanoids:

- Flavonoids are able to modulate blood pressure by restoring endothelial function, either directly or by affecting nitric acid levels.
- Flavanoids comprise large group of secondary metabolites occurring widely throughout the plant kingdom.
- Biological action of flavonoids, including anti-oxidant, anti aggregant and vasodilator affect.
- Flavanoids can also reduce caloric intake and decrease body weight and fat deposition in visceral tissues.
- Flavanoids are the unique antioxidant. It also corrects dislipidemia and blood pressure [92].

Tannin:

- Tannins contains anti-oxidant effect which producing many essential effects in protecting the body.
- Tannin contains ACE inhibitory effect [93].

BIOCHEMICAL ANALYSIS:

Table no.11.Results of basic radical studies of *Venthamaraiyathi chooranam*

S.No	Parameter	Observation	Result
1.	Test for Potassium	Formation of yellow colour precipitate	Positive
2.	Test for Calcium	Formation of white colour precipitate	Positive
3.	Test for Magnesium	Formation of white colour precipitate	Positive
4.	Test for Sodium	Appearance of intense yellow colour	Positive
5.	Test for Iron (Ferrous)	Appearance of blood red colour	Positive

Discussion

The results of basic radical test show that the presence of Potassium, Calcium, Magnesium, Sodium and Iron.

Potassium:

- Potassium is important for muscle function especially relaxing the wall of blood vessels.
- This lowers the blood pressure and protects against muscle cramping.
- This protects against an irregular heartbeat [94].

Calcium:

- Presence of calcium improves the physical strength of skeletal tissue. calcium ions are necessary for muscle contraction and transmission of nerve impulse.
- Calcium is important for healthy blood pressure because it helps in vasoconstriction and vasodilatation of blood vessels [95].

Magnesium:

- Magnesium also helps in regulation of blood pressure and relaxing the blood vessels.
- Magnesium act as a natural calcium channel blocker, increases nitric oxide, improves endothelial dysfunction and induces vasodilation [96].

Sodium:

- Sodium also important for regulation of blood pressure [97]

Iron:

- Iron helps in regulation of blood pressure.
- Iron is essential for proper functioning of immune system.
- It is essential for oxygen transport, energy production, other cellular growth and proliferation.
- Iron is an essential element for blood production and also needed for energy metabolism [98]

Table no.12.Results of acid radical studies

S.NO	Parameter	Observation	Result
1.	Test for Chloride	Formation of white precipitate	Positive
2.	Test for Nitrate	Formation of yellow precipitate	Positive

Discussion:

The acidic radicals test shows the presence of Chloride and Nitrate.

Chloride

- They help in maintenance of proper blood volume, blood pressure, pH of blood and also helps in balance between ECF and ICF of cells [99].

Nitrate

- Nitric oxide derived from nitrate, which helps in reduction of blood pressure by the vasodilatation of blood vessels [100].

MICROBIAL LOAD:

Table no.13.Availability Microbial load in *Venthamaraiyathi Chooranam*

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

Discussion:

- The availability of bacterial load in the *VTC* has been performed by Plate count- Agar plate technique.
- *VTC* is a Poly herbal drug prepared by plants. It is easy to get contamination, if any contamination present in drug, that decreases the potency and efficacy.
- The contamination of *VTC* has been examined by bacterial and fungal load.

Total bacterial load in 10^{-4} dilution is 5 and 10^{-6} dilution are 2

Total fungal load in 10^{-2} dilution is 4 and 10^{-3} dilution are Nil

Here, the contamination of *VTC* is within the WHO norms. Thus, the result shows presence of bacterial and fungal load in the trial drug (*VTC*) within the normal limits.

INSTRUMENTAL ANALYSIS

FT-IR (Fourier Transform Infra-Red spectroscopy)

Fourier Transform Infra-Red Spectroscopy (FTIR) analysis results in absorption spectra provide information about the functional group and molecular structure of a material. The results of Table no:14 and Fig no:7 shows the presence of functional group and inorganic compounds of *Venthamaraiyathi chooranam*

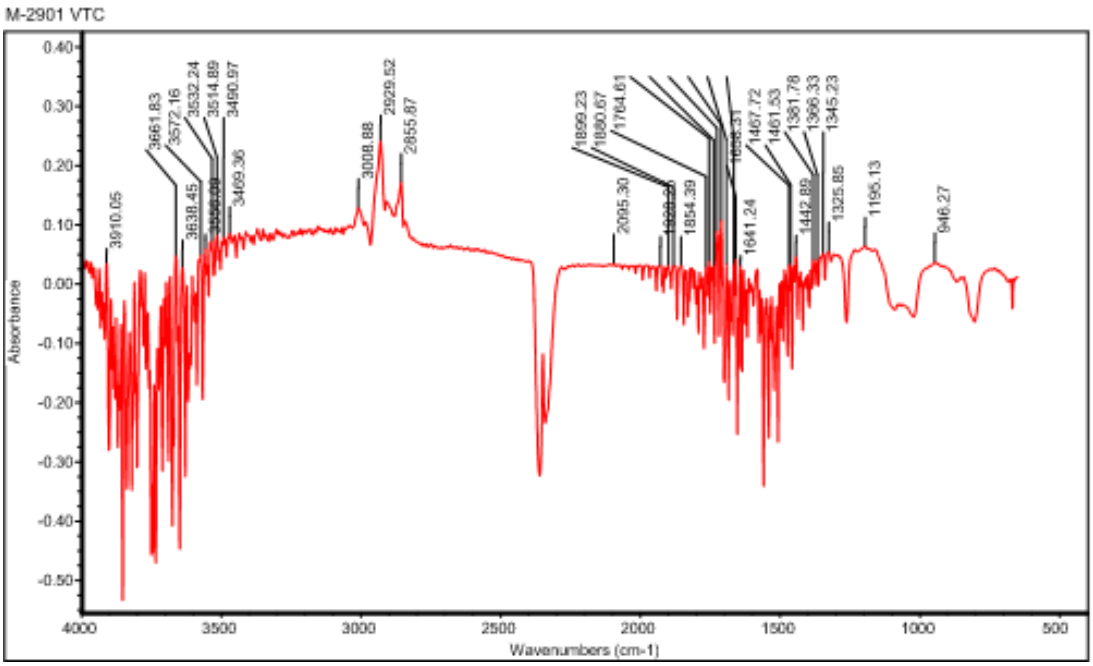


Fig no.19: FT-IR Spectrum analysis

Table no.14.FTIR-INTERPRETATION

Absorption peak cm^{-1}	**Stretch**	**Functional group**
3910	O-H stretch, free hydroxyl	Alcohols, Phenols
3469	O-H stretch, H bonded	Alcohols, Phenols
3008	=C-H Stretch	Alkene

2095	-C≡C- Stretch	Alkyne
1854	C=O Stretch	Carboxylic acid
1641	N-H bend	1^0Amines
1467	C-H Bend	Alkane
1345	N-O Symmetric stretch	Nitro compound
1325	C-H Wag-CH_2X	Alkylhalides
1195	C-N stretch	Aliphatic amines

Discussion:

FTIR instrumental analysis was done. The test drug was identified to have 10 peaks. They are the functional groups present in the trial drug "*Venthamaraiyathi Chooranam".* The above table shows the presence of alcohol, alkanes, alkenes, alkynes, amine, Alkyl halides, Aliphatic amines and nitro groups which represents the peak value.

Phenols:

It has possessed high Anti-Oxidant property which enhances the drug effect against the disease [101]**.**

Amines:

- It enhances the drug effect against the disease.
- Amines groups act as neurotransmitters. Amines are a class of compounds derived from ammonia by replacement of one or more effective antagonists of

SSTR5 (Somatostatin receptor 5) and are used for treatment, control and prevention of disorders such as lipid disorders and obesity[102].

Nitro groups:

Nitro groups containing drugs act directly on the vascular smooth muscle to cause relaxation and reduce the blood pressure [103]

Alkanes:

Alkanes protect the microorganisms.

Alkyl halides:

These 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 [104].

SEM: (SCANNING ELECTRON MICROSCOPE)

The particle size and the chemical elements were assessed by Scanning Electron Microscope. SEM is one of the most widely used instruments in research side. The SEM picture of *Venthamaraiyathi Chooranam* is shown below.

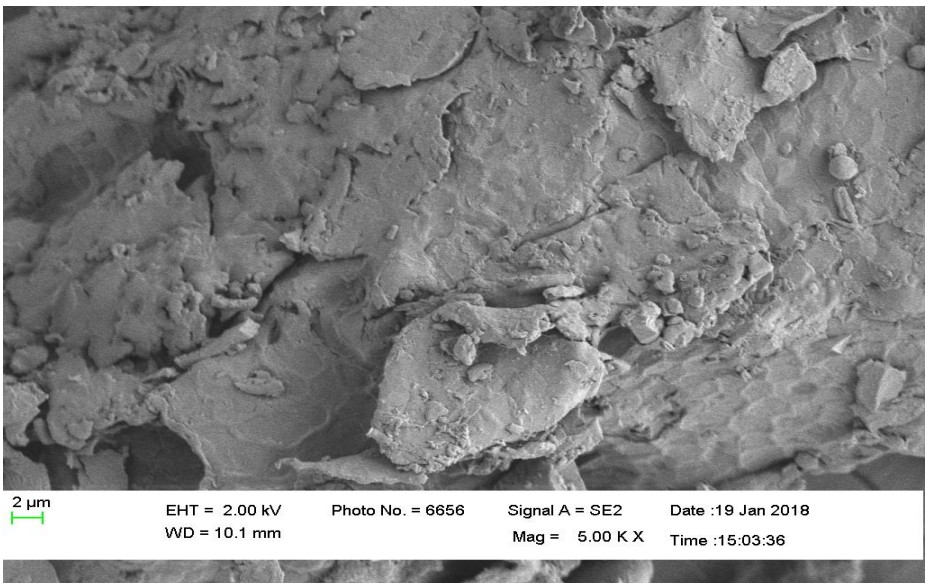


Fig no: 20 SEM analysis

The above SEM study shows of microscopic resolution of 5.00kx showed objects of sizes ranging from 2,5,10,20µm. The difference in morphology as evident from the micrograph is due to presence of various substances in the sample.

Discussion:

- Micro particles are defined as particulate dispersion or solid particles with a size in the range of 100-1000 nm in diameter.
- Size and surface of micro particles can be easily manipulated to achieve both passive and active drug targeting.
- They control and sustain the release of drug during the transportation and at the site of localization, alters the drug distribution in the body and subsequent clearance of the drug so as to achieve increased drug therapeutic efficacy there by it increases the bio-availability of the drug and reduced the side effects

Hence, *Venthamaraiyathi chooranam* which is prepared biologically contains micro particles to enhance the pharmacological action in the target site [105].

ICP-MS (Inductively Coupled Plasma Mass Spectrometry):

The drug sample *Venthamaraiyathi Chooranam* was analysed by the Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) to detect the trace elements and other elements quantitatively. The result of (ICP-MS) is given on the Table 15.

Table No.15 Results of ICP-MS

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

Discussion:

- The above results indicate that the trial drug is extremely safe as it contains heavy metals within specified limits.
- In *Venthamaraiyathi Chooranam*, the heavy metals like As, Cd, Hg, Pb and were below detectable level. This reveals the safety of the drug.

XRD (X-RAY DIFFRACTION METHOD):

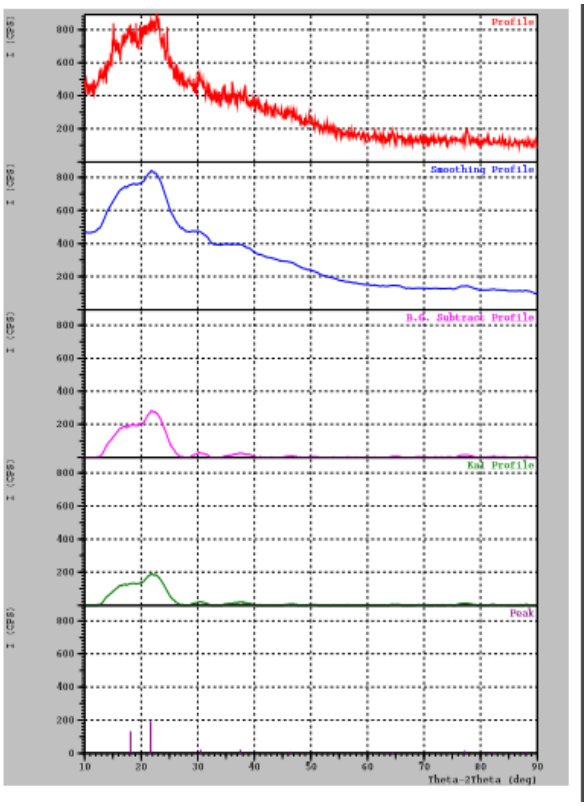


Fig no:21 XRD analysis

Discussion

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

XRD pattern of *Venthamaraiyathi chooranam* shows the good crystallinity. The major diffraction peaks are identified after XRD analysis *VTC* concluded that Nano crystalline range (18 -30nm) is association with organic molecules probably plays an important role in making it biocompatible and nontoxic at therapeutic doses. Other elements present in *VTC* act as additional supplement and possibly helps in increase the efficacy of the formulation [106].

TOXICITY STUDY RESULT

ACUTE ORAL TOXICITY IN RATS – OECD 423

Wistar albino rat was treated with the test drug *Venthamaraiyathi Chooranam* of single dose of 2000mg/kg in 2% CMC as suspension. This study was conducted as per the OECD guidelines. The result of acute toxicity of *Venthamaraiyathi Chooranam* has been tabulated below.

Dose finding experiment and its behavioural Signs of Toxicity for *Venthamaraiyathi Chooranam*

Table no.16. Observation done

SL	Group Control	Observation	SL	Group Test Group	Observation
1	Body weight	Normal	1	Body weight	Normal
2	Assessments of posture	Normal	2	Assessments of posture	Normal
3	Signs of Convulsion Limb paralysis	Normal	3	Signs of Convulsion Limb paralysis	Absence of sign (-)
4	Body tone	Normal	4	Body tone	Normal
5	Lacrimation	Normal	5	Lacrimation	Normal
6	Salivation	Normal	6	Salivation	Normal
7	Change in skin colour	No significant colour change	7	Change in skin colour	No significant colour change
8	Piloerection	Normal	8	Piloerection	Normal
9	Defecation	Normal	9	Defecation	Normal
10	Sensitivity response	Normal	10	Sensitivity response	Normal

11	Locomotion	Normal	11	Locomotion	Normal
12	Muscle gripness	Normal	12	Muscle gripness	Normal
13	Rearing	Mild	13	Rearing	Mild
14	Urination	Normal	14	Urination	Normal

Table 17: observational study results

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

1. Alertness 2. Aggressiveness 3. Piloerection 4. Grooming 5. Gripping 6. Touch Response 7. Decreased Motor Activity 8. Tremors 9. Convulsions 10. Muscle Spasm 11. Catatonia 12. Muscle relaxant 13. Hypnosis 14. Analgesia 15. Lacrimation 16. Exophthalmos 17. Diarrhoea 18. Writhing 19. Respiration 20. Mortality.

(+ Present, - Absent)

Table no18 : Body weight (g) changes of rats exposed to *Venthamaraiyathi Chooranam*

DOSE ¤	**DAYS**		
	1	7	14
CONTROL	210.6±31.474	211.2 ± 14.162	220.2 ± 24.22
HIGH DOSE(2000mg)	220.5± 27.75	221.4 ± 3.22	224.1 ± 12.72
P value (p)*	NS	NS	NS

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

Table no 19: Water intake (ml/day) of Wistar albino rats group exposed to *Venthamaraiyathi chooranam*

DOSE	DAYS		
	1	7	14
CONTROL	54 ± 2.22	53±7.42	58.4±2.54
HIGH DOSE (2000mg)	62.2±1.21	62.8±4.46	64.6±2.22
P value (p)*	NS	NS	NS

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

Table no 20: Food intake (gm/day) of Wistar albino rats group exposed to *Venthamaraiyathi chooranam*:

DOSE	DAYS		
	1	7	14
CONTROL	45.24±6.32	45.2±6.32	45.4±4.16
High DOSE (2000 mg)	42.2±1.44	44.8±2.32	43.1±4.14
P value(p*)	NS	NS	NS

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

Discussion:

- In the acute toxicity study, the rats were treated with different concentration of ***Venthamaraiyathi chooranam*** from the range of 5mg/kg to 2000mg/kg.
- The test groups compared to the controls when observed during14 days of the acute toxicity experimental period. This dose level of VTC did not produce signs of toxicity, behavioural changes, Body weight and mortality.

- No significant alterations were observed in food and water intake.
- These results showed that a single oral dose of the extract showed no mortality of these rats even under higher dosage levels indicating the high margin of safety of this extract.
- In acute toxicity test the *Venthamaraiyathi chooranam* was found to be nontoxic at the dose level of 2000mg/kg body weight.

RESULTS OF SUB-ACUTE ORAL TOXICITY 28 DAYS REPEATED DOSE STUDY IN RATS

Table no.21.Body weight (g) changes of rats exposed to *Venthamaraiyathi Chooranam*

DOSE	DAYS				
	1	7	14	21	28
CONTROL	260.4±12.42	261.4 ± 20.14	261.7 ± 19.60	262.6 ± 19.16	262.4 ± 12.12
LOW DOSE	240.2 ± 10.12	241.7 ± 38.24	243.4± 42.14	244 ± 52.16	245.42± 12.54
MID DOSE	243.4± 08.74	244.3 ± 12.14	246.2 ± 88.14	247.1 ± 13.66	249.4 ± 22.10
HIGH DOSE	255.6± 16.84	256.8 ± 12.42	258.4 ± 22.26	260 ± 24.18	261 ± 56.41
P value (p)*	NS	NS	NS	NS	NS

*NS- Not Significant, **(p < 0.01),*(p <0.05), n = 12 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Table no:22 Water intake (ml/day) of Wistar albino rats group exposed to *Venthamaraiyathi chooranam*

DOSE	DAYS				
	1	7	14	21	28
CONTROL	55.9 ± 9.72	56±8.22	57.2±2.20	59±2.16	59.4±2.16
LOW DOSE	58.2±1.21	58.8±3.22	58.9±1.62	60.2±1.28	60.8±1.23
MID DOSE	62.2±2.12	62.3±1.12	63.1±2.422	63.4±1.14	62.4±1.32
HIGH DOSE	63.1±1.21	63.2±1.24	63.4±1.14	63.6±1.42	63.8±2.52
P value (p)*	NS	NS	NS	NS	NS

*N.S- Not Significant, **(p < 0.01), *(p <0.05), n = 12 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Table no 23: Food intake (gm/day) of Wistar albino rats group exposed to *Venthamaraiyathi chooranam*

DOSE	DAYS				
	1	7	14	21	28
CONTROL	27±5.14	28.5±2.12	28.5±2.17	28.7±1.18	28.78±2.16
LOW DOSE	27.7±1.18	27.3±1.41	27.1±1.16	27.4±1.21	28.6±1.42
MID DOSE	28.2±2.44	28.3±3.60	28.4±4.25	28.5±2.18	28.7±1.44
HIGH DOSE	28.4±1.14	28.1±1.24	28.2±2.16	28.6±1.20	29.7±3.32
P value (p)*	NS	NS	NS	NS	NS

*N.S- Not Significant, **(p <0.01), *(p <0.05), n = 12 values are mean ± S.D (One way ANOVA followed by Dunnett's test*

Table no 24: Haematological parameters of Wistar albino rats group exposed to *Venthamaraiyathi chooranam*

Category	Control	Low dose	Mid dose	High dose	P value (p)*
Haemoglobin(g/dl)	14.8±0.58	14.80±0.64	15.4±0.66	15.18±0.44	N.S
Total WBC ($\times 10^3$ l)	7.91±0.52	7.25±0.16	7.48±0.17	7.20±1.32	N.S
Neutrophils (%)	30.25±0.04	31.22±0.12	32.10±1.32	33.06±1.20	N.S
lymphocyte (%)	61.14±1.42	60.12±2.10	60.10±2.22	60.40±2.26	N.S
Monocyte (%)	1.86±0.07	1.85±0.09	1.66±0.03	1.81±0.06	N.S

Eosinophil (%)	0.62±0.04	0.65±0.02	0.66±0.01	0.63±0.06	N.S
Platelets cells10^3/µl	786.14±4.42	788.41±4.16	783.13±7.0	787.16±6.74	N.S
Total RBC 10^6/ µl	6.88±0.12	6.86±0.46	6.62±0.44	6.15±0.22	N.S
PCV%	47.56±0.6	47.46±1.13	48±1.28	47.80±2.24	N.S
MCHC g/dL	34.4±1.32	34.6±1.28	34.28±1.20	34.33±1.12	N.S
MCV fL(µm^3)	52.07±3.24	52.20±1.21	53.10±1.34	54.24±1.42	N.S

*N.S- Not Significant, **(p < 0.01), *(p <0.05), n = 12values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Table no 25: Biochemical Parameters of Wistar albino rats group exposed to *Venthamaraiyathi chooranam*

BIOCHEMICAL PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE	P Value (p)*
GLUCOSE (R) (mg/dl)	80.24±10.6	80.16±6.14	81.22±14.10	81.62±10.2	NS
T. CHOLOSTEROL (mg/dl)	128.16±1.42	129.25±1.22	126.82±1.28	121.22±1.83	NS
TRIGLY (mg/dl)	58.36±1.42	57.32±1.28	56.56±1.32	51.66±1.23	NS
LDL	81.6±2.53	83.14±2.34	83±2.42	79.44±14.15	NS
VLDL	16.2±2.34	16.42±4.44	16.44±8.24	15.34±24.26	NS
HDL	30.16±6.18	30.26±2.25	32.28±4.26	34.48±20.12	NS
Ratio 1(T.CHO/HDL)	4.12±2.16	4.16±2.14	4.14±2.24	4.05±2.20	NS
Ratio 2(LDL/HDL)	2.70±1.18	2.72±2.12	2.70±3.10	2.10±18.02	NS
Albumin (g/dL)	4.93±0.26	4.63±0.42	4.62±12.42	4.60±15.68	NS

*NS- Not Significant, **(p <0.01), * (p <0.05), n = 12 values are mean ± S.D (One way ANOVA followed by Dunnett's test*

Table no 26: Renal function test of of Wistar albino rats group exposed to *Venthamaraiyathi chooranam*

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE	P Value (p)*
UREA (mg/dl)	23.32±0.99	23.28±0.46	23.16±1.28	23.18±1.22	NS
CREATININE (mg/dl)	0.62±0.08	0.61±0.04	0.61±0.06	0.61±0.02	NS
BUN (mg/dL)	17.1±0.13	17.10±0.80	17±0.42	17.01±1.12	NS
URIC ACID (mg/dl)	6.22±0.34	6.11±0.22	6.10±0.24	6.10±0.26	NS

*NS- Not Significant, **(p < 0.01), * (p <0.05) , n = 12 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Table 27: Liver Function Test of of Wistar albino rats group exposed to *Venthamaraiyathi chooranam*

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE	P Value (p)*
T. BILIRUBIN (mg/dl)	0.06±0.06	0.05±0.08	0.5±0.06	0.4±0.04	NS
SGOT/AST(U/L)	119.15±1.32	119.34±0.52	118.01±1.22	117.75±1.03	NS
SGPT/ALT(U/L)	70.13±2.18	70.21±1.44	70.14±1.28	70.12±0.48	NS
ALP(U/L)	133.52±4.26	134±12.14	132.12±4.04	132.23±12.25	NS
T. PROTEIN (g/dL)	7.79±0.36	7.78±0.32	7.76±0.24	7.53±0.48	NS

*NS- Not Significant, **(p < 0.01), * (p <0.05), n = 12 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Results:

Observations

Overall observations were similar in both sex rats.

Clinical signs of toxicity

No clinical signs of toxicity were observed.

Mortality

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

Body weight

No significant alterations were observed in body weight.

Food and water consumption

No effect of treatment was noted.

Physiological activities

No changes in the general behavior

Blood analysis

a. **Hematology**

   No treatment related effects were observed.

b. **Biological parameters**

   No treatment related effects were observed.

c. **Histological examination**

   Histological examination of organs did not show any pathological changes.

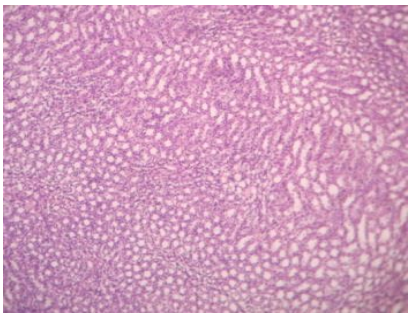
Sub-Acute Toxicity Discussion:

- The repeated 28 days' oral toxicity studies of *VTC* showed that the drug did not produce any toxicity signs in wistar albino rats. Daily administration of *VTC* at different doses 20mg/kg, 100mg/kg, 200mg/kg for 28 days were tolerated by the rats without any mortality and morbidity, indicates the drug tolerance.
- No physical changes were observed throughout the dosing period.
- No significant changes were observed in the values of different parameters studied when compared with controls and the values obtained were within normal biological and laboratory limits.
- No significant changes in Red blood cells (RBC) white blood cell (WBC), packed cell volume (PCV), Erythrocyte sedimentation rate (ESR) in all the treated groups as compared to respective control groups.
- Hence the Poly herbal formulation of *VTC* can be considered to be safe drug for prolonged duration use as revealed by toxicological studies.

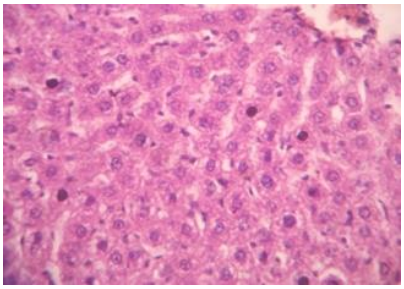
HISTOPATHOLOGY

Control group

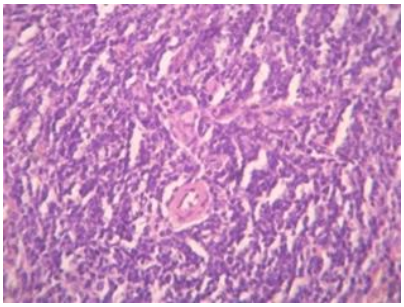
Kidney



Liver

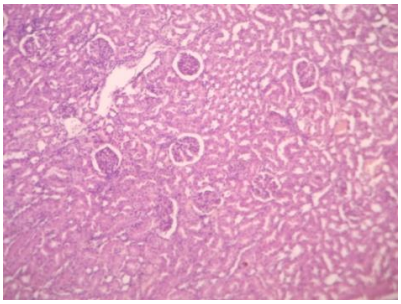


Spleen

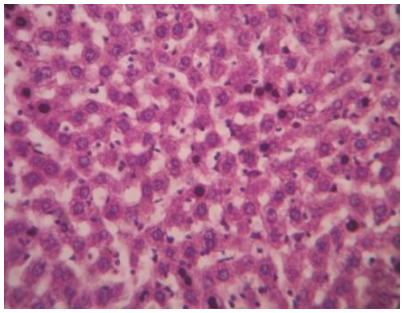


VTC 200 mg

Kidney



Liver



Spleen

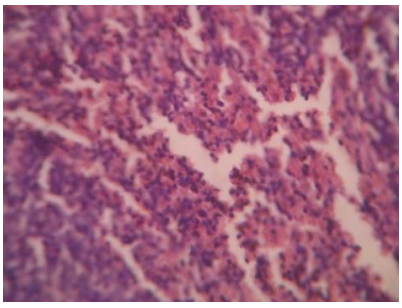


Fig no:22

Discussion

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

PHARMACOLOGICAL STUDY:

ANTI-HYPERTENSIVE ACTIVITY OF *VTC*:

SHR (9 weeks old) and age-matched Wistar rats male weighing 250±20g, Rats were kept in a room temperature-controlled room (25 ° C), with 12 h dark and 12 h artificial illumination daily (7:00— 19:00). Food and water were available ad libitum. Systolic blood pressure (SBP) and heart rate measurement of SH rats was carried out using tail-cuff method plethysmography (LE 5001 Pressure Meter).

GROUPING

The animals were divided into following groups:

Group 1----control untreated group which received normal saline.

Group 2 ----received Verapamil 12.5 mg/kg b.w

Group 3-----100mg/kg b.w

Group 4-----200mg/kg b.w

The drug *Venthaamaraiyathi chooranam* was administered orally and once daily for 4 weeks.

Table no.28.Effect on Systolic Blood Pressure (SBP) of *Venthamaraiyathi Chooranam* on various treatment groups on SH-rats

S:NO	Treatment group	**SBP** 1st day	7th day	14th day	21st day	28th day
1.	Control	196.2±8.62	190.4±6.46	186.2±2.86	184.6±2.64	180.3±8.65
2.	*VTC* 100 mg	194.4±6.86	186.8±6.82	176.2±4.62	170.8±8.42	168.4±4.64**
3.	*VTC*200 mg	190.8±6.24	180.2±6.24	168.2±4.82	150.8±8.26	142.2±2.62***
4.	Verapamil hydrochloride 12.5mg/kgb.w	182.6±4.28	170.2±2.46	158.6±4.86	142.4±6.40	130.2±6.68***

*Values represent mean ± SEM of 6 experiments. * P< 0.05; ** P< 0.01; ***P < 0.001, treatment versus control group*

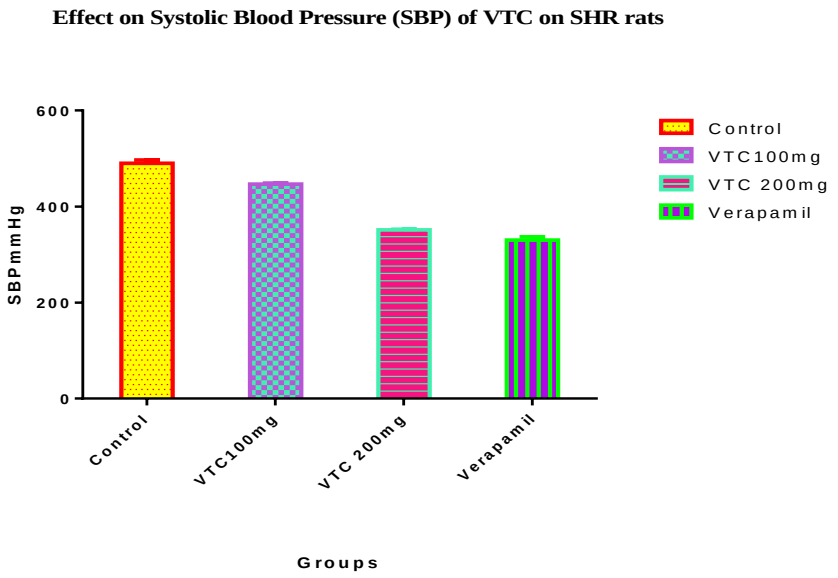


Chart no.2. Effect of Systolic Blood Pressure (SBP) of *VTC* on various treatment groups on SH-rats at 28th day

Table no.29Effect on Heart rate (HR) of *Venthamaraiyathi chooranam* various treatment groups on SH-rats

S:no	Treatment group	HR beats/min 1st day	7th day	14th day	21st day	28th day
1.	Control	499.2±5.81	497.6±2.86	494.7±4.01	492.8±8.10	490.2±6.43
2.	*VTC* 100	490.6±2.84	478.8±4.21	466.2±4.01	454.2±4.82	446.4±2.32**
3.	*VTC* 200	488.6±4.81	460.21±8.24	420.8±4.46	380.8±4.81	351.2±1.31***
4.	Verapamil hydrochloride 12.5mg/kg b.w	470.6±6.21	438.6±8.24	400.8±8.62	364.68±2.10	330.1±3.34***

*Values represent mean ± SEM of N= 6 experiments. * P< 0.05; ** P< 0.01; *** P < 0.001, treatment versus control group*

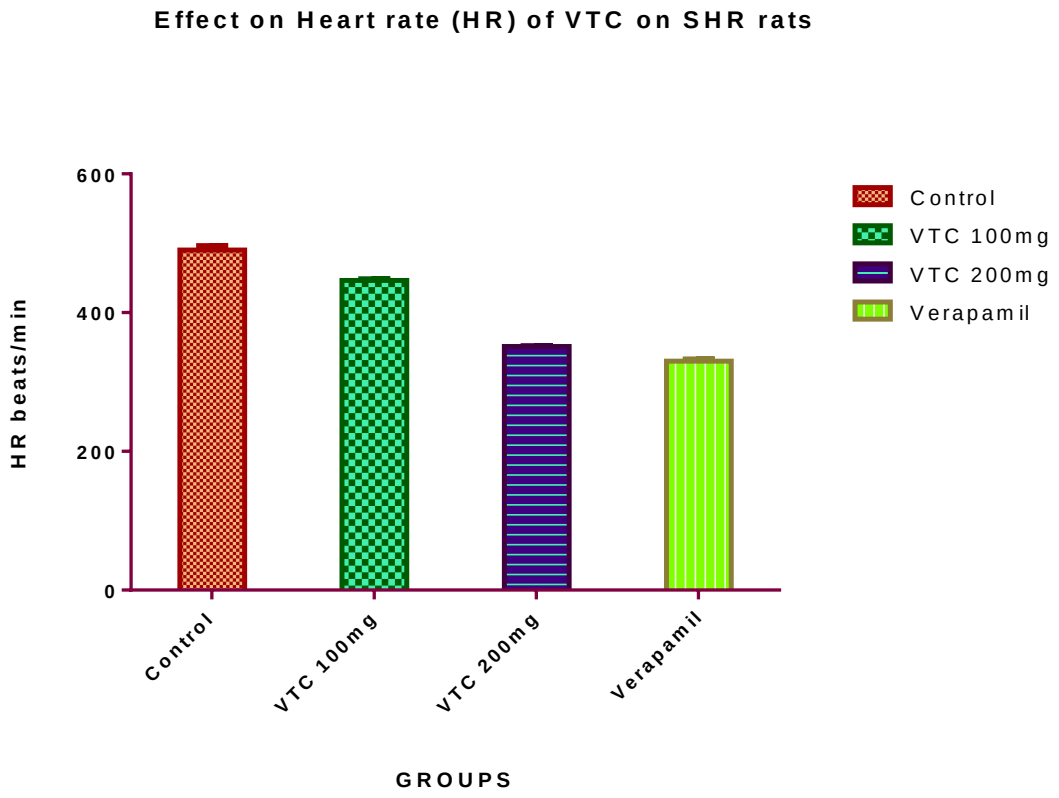


Chart no.3. Effect on Heart rate (HR) of *Venthamaraiyathi Chooranam on* various treatment groups on SH-rats at 28th day

Discussion:

- The systolic blood pressure and heart rate were recorded in the conscious animals in non-invasive tail cuff method.
- The results reveal that the *VTC* exhibits antihypertensive effect in the form of significant lower in systolic blood pressure and heart rate after continuous administration for 7 days.
- Heart rate was also decreased significantly in comparison to control
- The reduction in systolic blood pressure was measured and tabulated. The readings were compared with control group
- The systolic blood pressure on 7th day in group III treated with *VTC* 200m/kg body weight showed moderate reduction in Systolic blood pressure compared with 7th day of control
- But the reduction of Systolic blood pressure measured on 21st day of *VTC* 200mg /kg body weight treated group showed significant reduction of

Systolic blood pressure compared with 21st day of control group persistence highly significant antihypertensive effect was noticed even after cessation of dosing 7 days earlier. This suggests absence of rebound phenomenon after withdrawal of the test drug *VTC* which an advantage in the therapy of hypertension.

- The Siddha Polyherbal formulation *VTC*, according to their traditional uses and phytochemical constituents based on their therapeutic value leads to discovery of newer and safer alternative drug and herbal medicines having a protective role in cardiovascular diseases.

DIURETIC ACTIVITY OF *VTC*:

Lipschitz et al was employed this method for the assessment of diuretic activity. The animals were deprived of food and water for 16tn hours prior to the experiment. Before oral administration of test drug, the animals were dosed with 25ml/kg body weight of normal saline. The total volume of was measured. The urinary pH, sodium, potassium and chloride also determined. The diuretic activity result of *VTC* was derived and tabulated below.

Table no 30: Effect on urine volume of *Venthamaraiyathi Chooranam* on various treatment groups on SH-rats

S.no	Groups	Treatment	Urine volume/100gm/24hr	Diuretic index (24hr interval)
1	I	Control	4.33±1.26	-
2	II	Furosemide 12.5mg	10.12±3.42	2.74
3	III	VTC 100mg/kg	6.86±2.64	2.08
4	IV	VTC 200mg/kg	8.64±2.34	2.46

Diuretic index = volume of test group/volume of control group

Values are expressed in mean ± SD;n=6 . P< 0.05; ** P< 0.01; ***P<0.001, compared with control group (Kruskall Wallis and Mann Whitney test)*

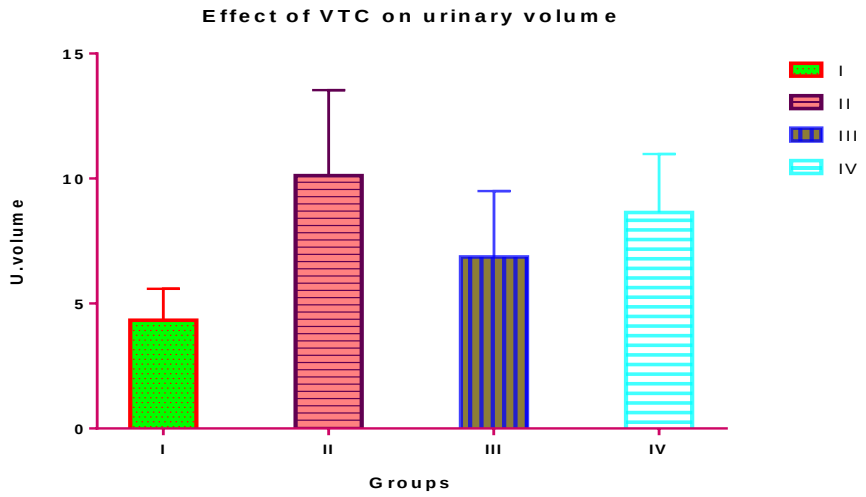


Chart no.4.Effect on urine volume of *Venthamaraiyathi Chooranam* on various treatment groups on SH-rats

Table no 31: Effect on urine electrolyte excretion of *Venthamaraiyathi Chooranam* on various treatment groups on SH-rats

Groups	Na^+ m.mol/L	K^+ m.mol/L	Cl^- m.mol/L	Na/K
Control	108±6.23	55±10.1	87.4±4.64	1.96
Frusemide 12.5mg/kg	132.2±1.42	85.4±4.24	98.6±1.54	1.40
VTC 100mg/kg	169±1.42	93.5±4.24	133.5±4.44	1.82
VTC 200mg/kg	154.24±9.23	82.32±6.22	100.4±2.46	0.96

Values are expressed in mean± SEM n=6. P< 0.05; ** P< 0.01; *** P < 0.001, compared with control group (Kruskall Wallis and Mann Whitney test)*

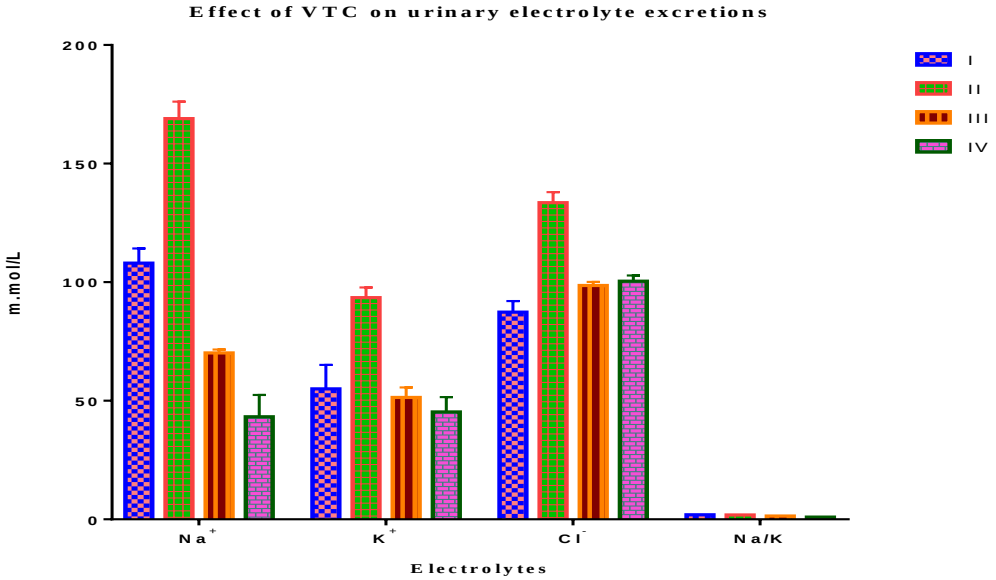


Chart no.5.Effect on urine electrolyte excretion of *Venthamaraiyathi Chooranam* on various treatment groups on SH-rats

Discussion:

- The results of diuretic activity of *VTC* showed marked increase in urine volume
- There was no evidence of dehydration of animals. Animals were observed normal at 5 hours and 24 hours interval.
- The standard diuretic Frusemide significantly increased in urine output when compared to normal
- The test drug *VTC* at 100mg/kg b.w, 200mg/kg b.w doses, showed statistically significant increase in the volume of urine with a dose dependent manner
- There is a significant change in the pH level of urine
- Excretion of Na^+, Cl^- followed by similar pattern. Chloride excretion highly significant with two doses.
- The diuretic activity of *VTC*, 5 hours after its administration was manifested in the form of an increase in urinary volume, which was highly significant with 100mg/kg b.w, 200mg/kg b.w doses at 5 hours urine analysis

- Analysis of 24 hours post dosing urine sample revealed similar results with regards to urinary volume, sodium, chloride and potassium are observed in 5th hour sample. That indicates a continuation of diuretic effect of *VTC* upto 24 hours
- An herbal preparation usually contains many active components (flavanoids, alkaloids, Phenols etc.)
- The Phyto chemical analysis of *VTC* shows significant presence of these compounds which either alone or in combination is responsible for the diuretic activity
- The diuretic study result of *VTC* clearly indicates, that possesses potential diuretic activity on SH-rats and diuretic agents plays an important role in decreasing high blood pressure by decreasing plasma volume and also reducing cardiac work load and the oxygen demand [107] [108]

ANTI-OXIDANT STUDY (In-Vitro) OF *VTC*:

- Antioxidants are substances that can prevent or slow down to the damage to cells caused by free radicals, unstable molecules that the body produces as a reaction to environmental and other pressures. They are sometimes called "free-radical scavengers."
- Antioxidants are said to help neutralize free radicals in our bodies, and this is thought to enhances the overall health.
- Antioxidants can protect against the cell damage that free radicals cause, known as oxidative stress.

Free radicals and their chemical reactions:

- A free radical can be defined as any molecular species capable of independent existence that contains an unpaired electron in an atomic orbital. The presence of an unpaired electron results in certain common properties that are shared by most radicals.
- Radicals are weakly attracted to a magnetic field and are said to be paramagnetic. Many radicals are highly reactive and can either donate an electron to or extract an electron from other molecules, therefore behaving as

oxidants. As a result of this high reactivity, most radicals have a very short half-life (10^{-6} seconds or less) in biological systems, although some species may survive for much longer.

- Oxidative stress has been linked to heart disease, cancer, arthritis, stroke, respiratory diseases, immune deficiency, emphysema, Parkinson's disease, and other inflammatory or ischemic conditions.

Antioxidant action in Hypertension:

During hypertension, our body went at the condition of oxidative stress. In this oxidative stress condition, oxygen radicals such as superoxide anion, hydroxyl radicals and peroxyl radicals are produced. These oxygen radicals are reactive oxygen species and they can lead to oxidative damage to cellular components such as proteins, lipids, and DNA. These free radicals are reactive molecules involved in many of the physiological process such as atherosclerosis, hypertension, ischemic heart disease. These oxygen free radicals formed during the oxidative stress are resulting in the disturbances of vasodilators system, particularly degrading of nitric oxide which are mainly due to the endothelial dysfunction are the effects of hypertension [109].

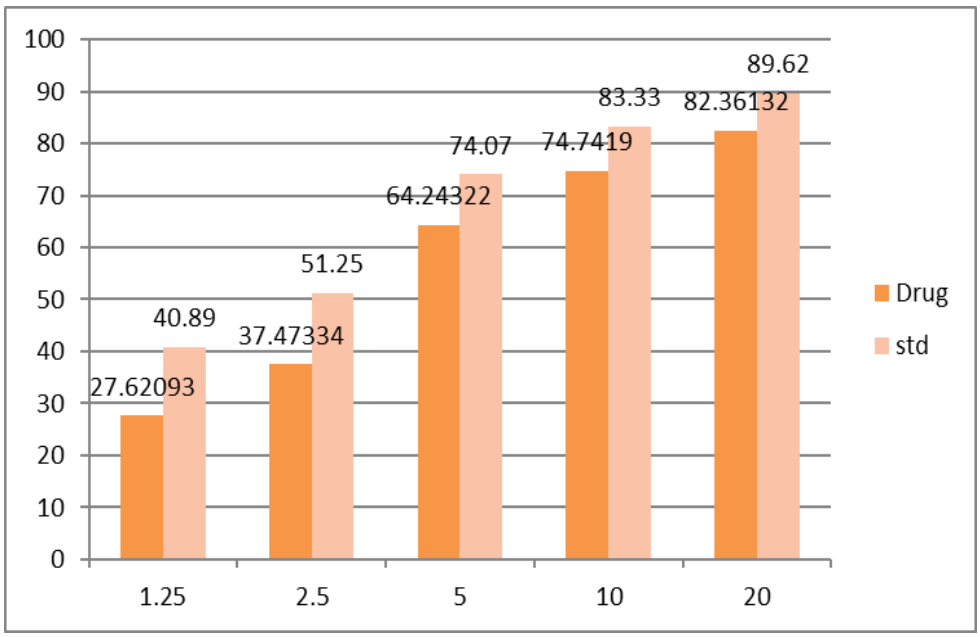
Result:

Table no-:32 DPPH assay on *Venthamaraiyathi chooranam*

Sample concentration (µg/ml)	Absorbance		Percentageof Inhibition	
	Drug	Standard	Drug	Standard
Control	0.5271	0.312	-	-
1.25	0.2403	0.278	27.62093	40.89
2.50	0.2347	0.202	37.47334	51.25
5	0.2201	0.084	64.24322	74.07
10	0.2122	0.052	74.7419	83.33
20	0.2063	0.034	82.36132	89.62

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

Chart no:6 DPPH assay on *Venthamaraiyathi chooranam*



IC50 Value –*VTC*- 51.258 µg/mL (Calculated using ED50 PLUS V1.0 Software)

Discussion on Antioxidant activity in DPPH assay:

DPPH assay were used for the determination of Anti-oxidant activity of the different extracts. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of VTC extract. The antioxidant molecules can quench DPPH free radicals by providing hydrogen atom or by electron donation and a colourless stable molecule 1, 1 diphenyl-2- picrylhydrazil is formed and as a result of which the absorbance at 517nm of the solution is decreased.

In the present study, the *VTC* extract was analysed able to decolorize DPPH and the free radical scavenging activity. Ascorbic acid (10 mg/ ml DMSO) was used as a reference and result was expressed as the percentage decrease in absorbance. In the present study, the extract of *VTC* was found to possess concentration dependent scavenging activity on DPPH radicals.

The values of DPPH free radical scavenging activity of the *VTC* extract was given in (Table 32) expressed in the percentage. The extract of *VTC* was found to possess concentration dependent scavenging activity on DPPH radicals. The extract of *VTC* showed the highest DPPH scavenging activity (82.36%) at 20µg/ml and the lowest percentage of inhibition (27.62%) at 1.25µg/ml. Ascorbic acid (Standard) showed highest percentage of inhibition (89.62%) at 20µg/ml and the lowest percentage of inhibition (40.89%) at 1.25µg/ml. This indicated that % of inhibition increased within increase in concentration of both the standard and *VTC* extract. The *VTC* extract has more or less equal DPPH scavenging activity when compared to the standard. From the present study, it was concluded that the *VTC* extract has a marked antioxidant activity at higher concentrations. Antioxidant compounds are highly present in plants and have protective effects against diseases without reducing their therapeutic efficacy [110]. So, using of natural antioxidant as a protective strategy against cardiovascular related problems [111].

6. CONCLUSION

The selection of a trial drug ***Venthamaraiyathi Chooranam*** was selected from the *Siddha* literature **"Pharmacopoeia of Hospital of Indian Medicine"** authored by Dr.Narayanaswami to validate the safety and its efficacy in treating Hypertension by Spontaneously Hypertensive Rat (SHR) model.

- The ingredients of the test drug were identified and authenticated by the Siddha experts. The drug was prepared as per the procedure and subjected to various studies to reveal its potency and effectiveness against the disease.
- Various analysis such as organoleptic character, physicochemical, phytochemical, biochemical analysis, availability of bacterial and fungal load, instrumental analysis was done.
- The organoleptic characters of the drug *Venthamaraiyathi chooranam* showed the yellowish green colour , Bitter and sweet in taste which might be responsible for the activity mentioned earlier. The fineness of the *chooranam* represents easy absorption and better availability of the drug.
- The physical character of *VTC* shows good solubility and the pH of the trial drug is 5.48 that indicates the better absorption and effectiveness.
- Phytochemical screening test showed the presence of alkaloids, glycosides, phenols, diterpenes, flavonoids, saponins and tannin may be responsible for the anti-hypertensive activity.
- The HPLC finger prints were made and it shows 13 peaks. In which the two major peaks denote presence of phytochemicals and Rf value of the trial drug supports the better standardization of the trial drug *VTC.*
- Biochemical analysis showed the presence of potassium, calcium, magnesium, sodium, iron, chloride and nitrate. The potassium, calcium, magnesium sodium, chloride, nitrate supports anti-hypertensive activity and it has a potent anti-oxidant activity of the trial drug *(VTC)).*
- The availability of bacterial load in the *VTC* has been performed and the result shows presence of bacterial and fungal load within the normal limits of trial drug.

- The instrumental analysis FTIR showed the 10 peak values present, in which the functional groups are alcohol, alkanes, alkenes, alkynes, amine, Alkyl halides, Aliphatic amines and nitro groups responsible for its activity.
- SEM picture described its morphology and the particle size. *VTC* which is prepared biologically contains micro particles to enhance the pharmacological action at the target site.
- The results of ICPMS shows that there is a mild presence of the heavy metals like As, Cd, Hg, Pb and were below detectable level. This reveals the safety of the drug in treating Hypertension.
- Toxicological study of both acute and sub-acute toxicity study was carried out in animal model Wistar albino rat according to the OECD guidelines. The test drug showed no acute toxicity as there was no mortality seen and then 28 days of repeated oral toxicity were done in 28days to show that the trail drug doesn't produce any toxic effect while it was given for long period.
- The mortality, functional observations, hematological and biochemical investigations were done. There was no significant change seen in the normal values. Thus, the toxicological study of the test drug greatly establishes the safety and gives the justification for long time administration.
- The pharmacological study was carried out in the animal model Spontaneously Hypertensive Rats. Three activities were seen in the drug *Venthamaraiyathi Chooranam*. The activities were

    - Anti-hypertensive activity
    - Diuretic activity
    - Anti-Oxidant activity [IN-VITRO]

- Anti-hypertensive activity was carried out in Spontaneously Hypertensive Rats. The trial drug *Venthamaraiyathi Chooranam*-200mg/kg b.w showed significant decrease in systolic blood pressure and heart rate. Thus, this activity reveals the effect of the drug against Hypertension.
- Diuretic activity of *Venthamaraiyathi chooranam*-200mg/kg b.w shows statistically increase in urine volume, excretion of urine electrolytes and there is no evidence of dehydration of animals were found, observed normal at 5 hours and 24 hours interval.

- Anti-oxidant activity of the test drug *Venthamaraiyathi Chooranam* was carried out in in-vitro model. The *VTC* extract has more or less equal DPPH scavenging activity when compared to the standard (ascorbic acid). From the present study, it was concluded that the *VTC* extract has a marked antioxidant activity at higher concentrations.

Thus, by scrutinizing all the above-mentioned factors it is concluded that the trial drug ***Venthamaraiyathi Chooranam*** is a safe and a potent anti-hypertensive drug. It also possesses diuretic and anti-oxidant activity which supports the effective treatment for managing Hypertension and its complications.

Modern medicine has its own limit in treating Hypertension and controls high blood pressure. Whereas in treating the disease with this trial drug it has a synergistic effect of controlling high blood pressure, diuretic effect and also has anti-oxidant property. Thus, it brings a complete treatment for Hypertension and its complications. From the study ***Venthamaraiyathi Chooranam*** has been proven as a safety and best drug for treating hypertension.

7. SUMMARY

The trial drug ***Venthamaraiyathi chooranam*** was selected from the siddha literature **"Pharmacopoeia of Hospital of Indian Medicine"** authored by Dr.Narayanaswami for Anti-hypertensive, Diuretic, Anti-oxidant activities**.** The dissertation started with an introduction explaining about the siddha concept, prevalence of hypertension and role of the test drug in treating hypertension,

- The test drug was prepared properly by the given procedure. All the ingredients were identified and authenticated by the experts.
- Review of literature in various categories was carried out. Siddha aspect, botanical aspect and pharmaceutical review disclosed about the drug and the disease. Pharmacological review was done to establish the methodologies.
- The drug was subjected to analysis such as organoleptic characters, physicochemical, phytochemical, biochemical and also instrumental analysis which provided the key ingredients present in the drug thus it accounts the efficacy of the drug
- Toxicological study was made according to OECD guidelines comprising both acute and sub-acute toxicity study. It showed the safety of the drug which attributes its utility in long time administration.
- Pharmacological study was done. It revealed the Anti-hypertensive, Diuretic and Anti-oxidant activity (in –vitro model) of *Venthamaraiyathi chooranam* in spontaneously hypertensive rat model.
- Results and discussion gives the necessary justifications to prove the potency of the drug.
- Conclusion gives a compiled form of the study and explains the synergistic effect of all the key ingredients and activities that supports the study.
- Thus, the Polyherbal formulation *venthamaraiyathi chooranam* is validated for its safety and efficacy for treating hypertension and it would be a one of the drugs of choice.

8. FUTURE SCOPE

The trial drug ***Venthamaraiyathi Chooranam*** has its own potency in treating Hypertension in Spontaneously Hypertensive rat model, which has been established in this study. However, the mechanism of action by which *Venthamaraiyathi Chooranam* produced its effect on decreasing the systolic blood pressure and heart rate in experimental animals need to be evaluated in a scientific manner using specific experimental animal models and also multi-center clinical trials are required to understand the exact molecular mechanisms of action. So, it could be used worldwide in treatment of Hypertension.

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C.L.BAID METHA COLLEGE OF PHARMACY

(An ISO 9001-2000 certified institute)

Jyothi Nagar, Old Mahabalipuram Road

Thoraipakkam, Chennai – 600 097

CERTIFICATE

This is to certify that the project entitled, **Toxicological and Pharmacological study on VENTHAMARAIYATHI CHOORANAM& PARUTHI*(Gossypium herbaceum)* CHOORANAM** in rats submitted in partial fulfilment for the degree of **M.D. (Siddha)** was carried out at C.L. Baid Metha college of Pharmacy, Chennai-97, in the Department of Pharmacology during the academic year of 2016-2017. It has been approved by the **IAEC**

No: IAEC/XLVIII/18/CLBMCP/2016

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

(Dr.P.Muralidharan)

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

IAEC Member Secretary

Government Siddha Medical College

Arumbakkam, Chennai – 600 106

CERTIFICATE

Certified that the samples submitted for identification by **DR.B.Kiruthika**, PG Scholar, Department of *Gunapadam*, Government Siddha Medical College, Arumbakkam, Chennai-600 106, were identified as:

Ingredients of *Venthamaraiyathi Chooranam:*

S.NO	TAMIL NAME	BOTANICAL NAME	PART USED
1.	*Elarisi*	*Elettaria cardamomum*	Seed
2.	*Chukku*	*Zingiber officinale*	Dried Rhizome
3.	*Thippili*	*Piper longum*	Dry Fruit
4.	*Adhimathuram*	*Glycyrrhiza glabra*	Root
5.	*Chadhakuppai*	*Anethum graveolens*	Seed
6.	*Ceeragam*	*Cuminum cyminum*	Seed
7.	*Venthamarai Poo Ithalkal*	*Nelumbo nucifera*	Flower

Date: 25.8.2017

Place: Chennai

25/8/2017

PG Department of Gunapadam

GOVERNMENT SIDDHA MEDICAL COLLEGE

Arumbakkam, Chennai, 600106

This certificate is awarded to Dr./~~Mr./Ms.~~ B. KIRUTHIKA

for participating as a ~~resource person~~ / delegate in the seminar on

"Orientation to research Methods"

Organised by Suzhumunai Scientific forum Government Siddha Medical College on 22 March 2018

Dr. P. Manickam
Scientist E
(ICMR) National Institute of Epidemiology

Dr. K. Kanakavalli
Principal
Govt. Siddha Medical College

Government of India
Ministry of AYUSH

Siddhar Agathiyar
Father of Siddha Medicine

Certificate of Achievement

This Certificate is proudly presented to

DR. KIRUTHIKA. B
GSMC, CHENNAI

for making oral presentation/poster presentation titled

DIABETIC VERSUS TRADITIONAL SIDDHA MEDICINE - A REVIEW

in the National Conference on "*Prevention and Management of Lifestyle Disorders through Siddha system of Medicine*" on **the first Siddha Day** held on **04.01.2018** – organised by Central Council for Research in Siddha (CCRS) jointly with Directorate of Indian Medicine and Homoeopathy, Govt. of Tamil Nadu, The Tamil Nadu Dr. M.G.R. Medical University and National Institute of Siddha.

மார்கழி
ஆயில்யம்
சித்த மருத்துவத்
திருநாள்

Prof. Dr. R. S. Ramaswamy
(Director General
Central Council for Research in Siddha)
Chairman

Prof. Dr. P. Parthiban
(Joint Director, DIM&H
Govt. of Tamil Nadu)
Organising Secretary

Certificate. No: FSD/Pres/035

Government of India
Ministry of AYUSH

Siddhar Agathiyar
Father of Siddha Medicine

Certificate of

Participation

This Certificate is proudly presented to

Dr. Km. Kiruthika. B
[GSMC, CHENNAI]

for participating

in the National Conference on "*Prevention and Management of Lifestyle Disorders through Siddha system of Medicine*" on **the first Siddha Day** held on **04.01.2018** – organised by Central Council for Research in Siddha (CCRS) jointly with Directorate of Indian Medicine and Homoeopathy, Govt. of Tamil Nadu, The Tamil Nadu Dr. M.G.R. Medical University and National Institute of Siddha.

மார்கழி
ஆயில்யம்
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Prof. Dr. R. S. Ramaswamy
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Central Council for Research in Siddha)
Chairman

Prof. Dr. P. Parthiban
(Joint Director, DIM&H
Govt. of Tamil Nadu)
Organising Secretary

Certificate. No: FSD/Part/ 063

IN COLLABORATION WITH

MINISTRY OF AYUSH Govt OF INDIA

सत्यमेव जयते

NORTHERN PROVINCIAL COUNCIL

1ST INTERNATIONAL CONFERENCE & EXHIBITION ON SIDDHA MEDICINE -2018

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

CERTIFICATE

THIS IS TO CERTIFY THAT Prof / Dr. / Mr. / Ms. .....B. KIRUTHIKA..... PARTICIPATED / PRESENTED A PAPER ON .AN INVITRO & IN-VIVO STUDIES OF ANTI-OXIDANT, PHYTOCHEMICALS PROPERTIES & ANTI-HYPERTENSIVE ACTIVITY OF GOSSIPIUM HERBACEUM. IN THE ABOVE CONFERENCE HELD ON 26TH & 27TH FEBRUARY 2018.

.....

Prof . R. VIGNESWARAN
VICE CHANCELLOR
UNIVERSITY OF JAFFNA

.....

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

One day National Seminar on

"ROLE OF NEURO INFLAMMATORY MEDIATORS IN NEURODEGENERATIVE CONDITIONS IN DRUG DISCOVERY"

Conducted by

Department of Pharmacology
K.K.COLLEGE OF PHARMACY

Gerugambakkam, Chennai-600128.

Co Sponsored by

The Tamil Nadu Dr. MGR Medical University, Chennai

CERTIFICATE

This is to Certify that Dr/ Mr/Ms..... B. KIRUTHIKA .....

has Participated as Resourse person / Chair person / Delegate ✓ in the Seminar in ***"Role of neuro inflammatory mediators in neurodegenerative conditions in drug discovery"*** on 24th January 2018

Dr.R.PRAKASH
Organizing Secretary

Dr.V.VAIDHYALINGAM
Director

Dr.A.MEENA
Convenor

SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY
CHENNAI – 600 119

CENTRE FOR LABORATORY ANIMAL TECHNOLOGY AND RESEARCH

(CPCSEA Approved)

WORKSHOP ON

TOXICOLOGICAL PROFILING AND ASSESSMENT OF TOXICITY OF DRUGS ON LAB ANIMALS

CERTIFICATE

This is to certify that Dr./~~Mr.~~/~~Ms.~~ B. KIRUTHIKA

of Govt. Siddha Medical college, Chennai has participated in the two-day workshop on "**TOXICOLOGICAL PROFILING AND ASSESSMENT OF TOXICITY OF DRUGS ON LAB ANIMALS**" organized by the Centre for Laboratory Animal Technology and Research, Sathyabama Institute of Science and Technology, Chennai during 31st January – 1st February 2018.

B. Sheela Rani

Chair Person & Coordinator
Dr. B. SHEELA RANI
Director (Research)

Convener
Dr. R. SELVARAJ
Scientist In-charge

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

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

This Certificate is awarded to Dr/Mr/Mrs.....B. KIRUTHIKA.....

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

"RESEARCH METHODOLOGY & BIOSTATISTICS"

*For **AYUSH Post Graduates & Researchers***

Organized by the Department of Siddha

The Tamil Nadu Dr. M.G.R. Medical University From 07th to 11th March 2016.

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

Prof.**Dr.P.ARUMUGAM,** M.D.,
REGISTRAR i/c

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

NATIONAL SEMINAR ON

"RESEARCH METHODOLOGY AND PUBLIC HEALTH INITIATIVE THROUGH SIDDHA SYSTEM OF MEDICINE"

(RM & PHISSM – 2018)

6TH & 7TH APRIL 2018

प्रमाण पत्र
CERTIFICATE

सिद्ध क्षेत्रीय अनुसन्धान संस्थान
पूजप्पुरा, तिरुवनंतपुरम, केरल
SIDDHA REGIONAL RESEARCH INSTITUTE
Poojappura, Thiruvananthapuram, Kerala

केन्द्रीय सिद्ध अनुसन्धान परिषद्
(आयुष मंत्रालय, भारत सरकार)
CENTRAL COUNCIL FOR RESEARCH IN SIDDHA
Ministry of AYUSH, Govt. of India

This is to certify that Dr./Shri/Smt. Kiruthika B., GSMC, Chennai has participated/presented a paper entitled A Role of Siddha Medicine "Kallikkam" in Healing Various Diseases in the National Seminar on "Research Methodology and Public Health Initiative through Siddha System of Medicine" (RM & PHISSM – 2018) organized by Siddha Regional Research Institute, Thiruvananthapuram on 6th & 7th April 2018 at Dr. M R DAS Convention Centre, Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, Kerala.

डॉ. ए. कनगराजन / Dr. A. Kanagarajan
Organizing Secretary and Convenor

RM & PHISSM 2018

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