PRECLINICAL VALIDATION OF ANTI-ANXIETY, ANTI-DEPRESSANT AND ANTI- CATALEPTIC ACTIVITY OF CLASSICAL SIDDHA DRUG "*SADHAKUPPAI CHOORANAM*" IN ANIMAL MODEL

The dissertation Submitted by

Dr. A.ADAIKKALADEVI

Reg. No: 321512102

Under the Guidance of

Dr.M.D. SARAVANA DEVI, M.D.(S).,

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CHENNAI -106

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Dr. A.ADAIKKALADEVI

Dr. A.ADAIKKALADEVI

Dr. A.ADAIKKALADEVI

Dr. A.ADAIKKALADEVI

GOVT. SIDDHA MEDICAL COLLEGE, CHENNAI-106

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **Preclinical Validation of Anti-Anxiety, Anti-Depressant and Anti-Cataleptic activity of classical Siddha drug "*SADHAKUPPAI CHOORANAM*" in Animal Model** is a bonafide and genuine research work carried out by me under the guidance of **Dr.M.D.SARAVANADEVI M.D(S).,** Post Graduate Department of *Gunapadam*, Govt. Siddha Medical College, Arumbakkam, Chennai-600 106 and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or other similar title.

Date :

Place :

Signature of the candidate

Dr. A. Adaikkaladevi

GOVT.SIDDHA MEDICAL COLLEGE, CHENNAI-106

CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled **Preclinical Validation of Anti-Anxiety, Antidepressant and Anti-Cataleptic activity of classical Siddha drug "*SADHAKUPPAI CHOORANAM*" in animal model** is submitted to the Tamilnadu Dr.M.G.R.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 A.Adaikkaladevi 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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Place :

Signature of the Guide

ENDORSEMENT BY THE HOD, PRINCIPAL OF THE INSTITUTION

This is to certify that the dissertation entitled **Preclinical Validation of Anti-Anxiety, Antidepressant and Anti-Cataleptic activity of classical Siddha drug "*SADHAKUPPAI CHOORANAM*" in animal model** is a bonafide work carried out by **A.Adaikkaladevi** under the guidance of **Dr.M.D.SARAVANADEVI M.D(S),** Post Graduate Department of Gunapadam, Govt. Siddha Medical College, Chennai – 106.

Signature of the HOD **Signature of the Principal**

Date : **Date :**

Place : Chennai **Place : Chennai**

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FIGURE CONTENTS

TABLE CONTENTS

20.	**Food intake (gm/day) of Wistar albino rats group exposed to *SKC***	
21.	**Body weight of wistar albino rats group exposed to *SKC* in Repeated dose of oral toxicity**	
22.	**Water intake (ml/day) of Wistar albino rats group exposed to *SKC***	
23.	**Food intake (gm/day) of Wistar albino rats group exposed to *SKC***	
24.	**Haematological parameters of Wistar albino rats group exposed to *SKC***	
25.	**Biochemical Parameters of Wistar albino rats group exposed to *SKC***	
26.	**Renal function test of Wistar albino rats group exposed to *SKC***	
27.	**Liver Function Test of Wistar albino rats group exposed to *SKC***	
28.	**Effect of *SKC* in Elevated Plus Maze**	
29.	**Elevated plus maze number of entries & Average of time spent in open arms**	
30.	**Effect of *SKC* on locomotion of mice during forced swim and tail suspension test**	
31.	**Effect of *SKC* on Haloperidol induced catalepsy in rats.**	

FIGURE CONTENTS

CHART CONTENTS

ABBREVIATIONS

ADIS	**:**	**Anxiety Disorders Interview Schedule**
ANOVA	**:**	**Analysis Of Variance**
ASSOC-HAM	**:**	**Associated Chambers Of Commerce and Industry of India.**
AYUSH	**:**	**Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homoeopathy**
BDNF	**:**	**Brain Derived Neurotropic Factor**
BUN	**:**	**Blood Urea Nitrogen**
BQL	**:**	**Below Quantificial Level**
CNS	**:**	**Central nervous system**
CPCSEA	**:**	**Committee for the Purpose of Control Supervision and Experiments on Animals**
CSF	**:**	**Cerebro Spinal Fluid**
DPPH	**:**	**1,1-Diphenyl-2-picrylhydrazyl**
ED_{50}	**:**	**Effective Dose**
EFA	**:**	**Ethyl Acetate Fraction**
EPM	**:**	**Elevated Plus Maze**
FST	**:**	**Forced Swim Test**
FTIR	**:**	**Fourier Transform Infrared Spectroscopy**

GAD	**:**	**Generalized Anxiety Disorder**
GABA	**:**	**Gamma-Aminobutyric acid**
HPLC	**:**	**High Performance Liquid Chromatography**
IAEC	**:**	**Institutional Animal Ethical Committee**
ICPMS	**:**	**Inductively Coupled Plasma Mass Spectrometry**
ISM	**:**	**Indian System of Medicine**
SKC	**:**	***Sadhakuppai Chooranam***
MAO	**:**	**Monoamine Oxidase Inhibitors**
mCPP	**:**	**1-(3-Chlorphenyl) Piperazine**
OECD	**:**	**Organization For Economic Co-operation and Developement**
PTSD	**:**	**Post Traumatic Stress Disorder**
PCV	**:**	**Packed cell volume**
PRF	**:**	**Polyphenol Rich Fraction**
RBC	**:**	**Red blood corpuscles**
SEM	**:**	**Scanning Electron Microscope**
SSNRIs	**:**	**Selective Serotonin Norepinephrine Reuptake Inhibitors**
SSRIs	**:**	**Selective Serotonin Reuptake Inhibitors**
TCA	**:**	**Tricyclic Antidepressants**

TST	**:**	**Tail Suspension Test**
UV	**:**	**Ultra violet**
WHO	**:**	**World health organization**
XRD	**:**	**X-ray Power Diffraction**
5HT	**:**	**5 Hydroxytryptymine**

1. INTRODUCTION

Human race is gratified inventiveness of God. They are born with the power of thinking, reasoning and act independently. From ancient time to till now humans finds more and more evolution in all walks of life. Today more Technologies occupy the world. The bitter part behind this modernization is that though the man made Technologies seems to be an advancement, at times he could not come out of it and atlast gets addicted to that it. This makes him get separated from his harmonious life with nature.

The modern lifestyle which puts people under constant stress could severely damage major organs and lead to heart attack, kidney disease and dementia. Most researchers agree that to some extent modern lifestyle indirectly impact psychological life of the individual. Modern technologies has been breaking people's connection with the natural world. Different modern lifestyle patterns affects our health physically, psychologically and socially[1].

Mind and body are very closely interrelated and all our activities and behaviour depend in an intimate manner on the nervous system[2].

It has long been established in traditional forms of medicine and in anecdotal knowledge that the health of the body and the mind are inextricably linked. Strong and continually developing evidence now suggest a link between disorders which involve Hypothalamic-Pituitary-Adrenal axis (HPA) dysregulation and developing psychiatric disease. For instance adverse or excessive responses to stressful experiences are built into the diagnostic criteria for several psychiatric disorders including depression and anxiety disorders[3].

A neurological disorder is any disorder of the nervous system. Structural, biochemical and electrical abnormalities in the brain, spinal cord or other nerves can result in seizures, confusion, altered level of consciousness, muscle weakness, pain, loss of sensation, paralysis, poor coordination[4]. Adrenaline, noradrenaline, serotonin and dopamine are neurotransmitters. Noradrenaline and serotonin normally provide drive to the limbic system to increase a person's sense of well-being to create

happiness, contentment, good appetite, appropriate sex drive and psychomotor balance[5].

A Neurotic patient is fundamentally mistrustful of his own general level of competence and basic merit. Anxiety, depression, obsession and various physical symptoms are experienced by many people in response to stress and strains of everyday life. There is no internal homeostasis because the normal defence mechanism are inadequate to deal with anxiety[6].

Recurrent stressful situation increase the activity of cerebral cortex, especially of the psychic centre. It can be confirmed by EEG changes and also biochemically by observing increased acetylcholine content of the blood with a decrease in the acetylcholine content of brain. As a result of these changes the patient becomes more irritable and readily becomes a victim of sleeplessness, nervousness, worry, palpitation and also develops fine tremors in the hand[7].

According to a survey conducted by the Associated Chamber of Commerce and Industry (ASSOC-HAM), 68% of working women in the age bracket of 21-52 years were found to be afflicted with lifestyle ailments such as obesity, depression, chronic backache, diabetes and hypertension. The study 'Preventive Healthcare and Corporate Female Workforce' also said that long hours and working under strict deadlines cause up to 75% of working women to suffer from depression or general anxiety disorder, compared to women with lesser levels of psychological demand at work[8].

Depression is caused by diminished activity of noradrenaline and serotonin also[5a]. Anxiety is a feeling of worry, nervousness or unease about something with an uncertain outcome. Anxiety patients always anticipates problem and dangers. Anxiety and depression refers to an array of abnormal variations in the mood of a person. These conditions originate as a result of chemical reactions in the brain. 90% of patients with anxiety disorders develope depression, they generally exhibit feeling of guilt, loss of pleasure or interest, low self esteem and loss of appetite[9].

Catalepsy is a condition characterized by inactivity, decreased responsiveness to stimuli and a tendency to maintain an immobile posture. It may be associated with

the nervous system drug toxicity, psychotic disorders and other conditions[10].

Epidemiological studies consisting of 33572 persons in 6550 families yeilded an estimate prevalence rate of 58.2per thousand population. Organic psychosis 0.4, schizophrenia 2.7, neurotic disorder 20.7, epilepsy 4.4, alcohol drug addiction 6.9. There are 1.5 crore people suffering severe mental disorders in India. According to WHO, depression is the most common illness worldwide and the leading cause of disability. They estimate that 350 million people are affected by depression, globally[11].

In modern medicine many drugs are used for depression and anxiety. Prolonged use of anti anxiety and anti depressant drugs results in many side effects like nausea, increased appetite, weight gain, loss of sexual desire and other sexual problems, such as erectile dysfunction and decreased orgasm, fatigue, drowsiness, insomnia, dry mouth, blurred vision, constipation, dizziness, agitation, irritability[12].

Siddha system is a most indigenous and foremost system in south India. Siddha system of medicine have direct impact on person's physical and mental health. Siddhars laid foundation for this system. Traditional Siddha medicines are formulated by Siddhars. Siddhars have a concept that only an healthy body can develop an healthy soul, so they developed Aasanas, yoga practices, periodic fasting, meditations and medicines. Through this, they attained the supernatural powers and gained the supreme wisdom and overall immortality.

In sage Agathiyar's Gnanakaviyam,[13]

மனமதுசெம்மை யானால் மந்திரஞ் செபிக்க வேண்டா
மனமதுசெம்மை யானால் வாயுவை வுயர்த்த வேண்டா
மனமதுசெம்மை யானால் வாசியை நிறுத்த வேண்டா
மனமதுசெம்மை யானால் மந்திரஞ் செம்மையாமே

In Siddha system, concept of health and diseases were based on the concept of Panchabootha and the Tridhosha theory. The Panchabootha was the five elements

theory constituting the world of nature as well as the human body, namely, Earth (prithivi), Fire (agni), Water (neer), Air (vayu) and Ether (akasha). The thridhosha theory stated that there are three humors within the body (known as mukkutrangal)which comprise Vali (air), Anal (heat) and Eeram (moisture). The three humor represents Creation, Protection, Destructions which can be correlated as Anabolism, Metabolism, and Catabolism. Harmonious equilibrium of this three meant health and disruption of this balance leads to diseases.

Physical illnesses are accompanied by psychological symptoms. The comprehensive division of psychotic disorders referred to as Unmadam into 18 Kirikas. The "KirikaiNool64" of Agasthiar is the noteworthy book of Siddha system. It describes about 18Kirikas. Siddhars believed that the diseases are occurred due to sleeplessness and breaking of varma points [14].

Mental illness is old as in mankind. In olden days, people's belived that the mental illness is caused by evil, ghost or spiritual powers and there is no medicines to treat them. But the great scientist Siddhars mentioned in their literature the psychiatric illness are curable. They can be treated by both internal as well as external therapies.

Siddhars approach is more scientific. The etiological description of mental illness as given by sage Agasthiyar in his work *Agathiya Kanagamani Nooru*, encompasses hereditary enviroinment, socio-cultural, physiological, toxic and psychological spheres.In Siddha system of medicine many treatises are available in clinical varieties for mental illness. Among them the valuable literature are *Agasthiyar Maanidar Kirigai Nool-64* and *Yoogi Chinthamani-800*[15].

A few ailments has been emphasized in Siddha literature that are already used in the treatment of mental illness are as follows:

- Abraga Parpam
- Peranda Parpam
- Ekkhu Chendhuram
- Kaandha Chendhuram
- Brahmi Nei
- Vallarai nei
- Thurusu Parpam
- Velli Parpam
- Naaga Parpam
- Kandhaga Parpam.

In Siddha system of medicine,

"வேர்பாரு தழைபாரு மிஞ்சினக்கால்
மெல்ல மெல்லபற்பம் செந்தூரம் பாரே"[16]

From all the above facts I find a better way to reduce the neurotic disorders. Siddhars has mentioned several medicines in their literary works for mental illness. One of such medicine is *SadhakuppaiChooranam* indicated as a potent drug in treating mental illness in Siddha literature "*Agathiyar Attavanai Vagadam*"[17]. It will be very effective, less expensive and aimed to reveal the desired effect. Still now no scientific research works has been carried out on this herbal preparation, the author is interested in validating anti-anxiety, anti-deprassant and anti- cataleptic activities of *Sadhakuppai chooranam* through standardization, physico chemical, bio-chemical analysis, instrumental analysis, toxicity and pharmacological studies.

2. AIM AND OBJECTIVES

Aim :

The aim of this dissertation is to do a scientific review, to validate the safety and efficacy of the *Sadhakuppai Chooranam* for *Paithiyam* by preclinical studies.

Objectives:

Besides the scientific study, the basic concepts of Siddha science are also our aim. Hence, the following methodology was adopted to evaluate the safety and efficacy of the test drug.

- Collection of various Siddha and modern literature relevant to the study.
- Preparation of the drug according to the classical Siddha literature.
- Identification of the drugs in the ***Sadhakuppai Chooranam.***
- Physicochemical and phytochemical investigation of the test drug.
- Evaluate bio-chemical analysis of the test drug to derive acidic and basic radicals.
- To estimate the present of elements, functional groups and particle size through instrumental analysis of the trial drug.
- Evaluation of the Acute and 28 days repeated dose oral Toxicity of test drug according to OECD guidelines.
- Evaluation of pharmacological study of the drug through the following activities
  - Evaluation of Anti-Anxiety activity
  - Evaluation of Anti-depressant activity
  - Evaluation of Anti-Cataleptic activity of ***Sadhakuppai Chooranam***.

3.REVIEW OF LITERATURE

3.1 DRUG REVIEW

3.1.1.GUNAPADAM ASPECT

SADHAKUPPAI CHOORANAM

The following are the ingredients of *Sadhakuppai Chooranam*,

Table.No. 1

S.No.	TAMIL NAME	BOTANICAL NAME
1.	Sadhakuppai	*Anethum graveolans*
2.	Seeragam	*Cuminum cyminum*
3.	Perungeeragam	*Pimpinella anisum*
4.	Karungeeragam	*Nigella sativa*
5.	Elam	*Elettaria cardomomum*
6.	Adhimadhuram	*Glycyrrhiza glabra*
7.	Kothamalli	*Coriandrum sativum*
8.	Lavangam	*Syzygium aromaticum*
9.	Cinna lavangapattai	*Cinnamomum verum*
10.	Seena karkandu	*Saccharum officinarum*

Sadhakuppai

Scientific name	**:**	*Anethum graveolans*
Other names	**:**	Soyikkeerai vidhai, Madhurigai.
Vernacular names		
Tamil	**:**	Sadhakuppai
English	**:**	Dill seeds, Garden dill
Telugu	**:**	Soyikuravittulu
Malayalam	**:**	Shatakuppa
Kannadam	**:**	Sabbasagi
Taste	**:**	Sweet
Character	**:**	Heat
Division	**:**	Pungent
Parts used	**:**	Leaf, Flower, Seed.
Actions	**:**	Antispasmmodic Carminative Diuretic Emmenagogue Stimulant

General Properties:

வாதமொடு சூதிகா வாதம் சிரசுநோய்
மோதுசெவி நோய்கபநோய் மூடொசுரம் – ஓதுகின்ற
மூலக் கடுப்பு முதிர்பீனசம் போகும்
ஞாலச் சதகுப்பை நாடு.

- அகத்தியர் குணவாகடம்[18]

Uses:

- It cures sinusities, vadha diseases, anorexia.
- It strengthens liver, lungs and stomach.
- Seeds soaked water cures especially indigestion in children and adults.

Seeragam

Scientifiic name	**:**	*Cuminum cyminum*
Other names	**:**	Asai, Seeri, Ubakumbapesam, Narseeri, Thuthasaambalam, Prathiviga Pithanaasini, Bosanakudori, Metthiam.
Vernacular names		
Tamil	**:**	Seeragam
English	**:**	Cumin seeds
Telugu	**:**	Jilakarra
Malayalam	**:**	Jirakam
Kannadam	**:**	Jirlga
Taste	**:**	Pungent, Sweet
Character	**:**	Coolant
Division	**:**	Sweet
Parts used	**:**	seeds
Action	**:**	Carminative
		Stomachic
		Astringent
		Stimulant

General properties:

பித்தமெனு மந்திரியைப் பின்னப் படுத்தியவன்
சத்துருவை யுந்துறந்து சாதித்து – மத்தனெனும்
ராசனையு மீவென்று நண்பைப் பலப்படுத்தி
போசனகு டாரிசெயும் போர்.

தேரன் வெண்பா[18 a]

Uses:

- It cures abdominal pain, psychotic conditions, liver disease, renal calculi, dysentry, sinusites,.
- It strengthens the body.
- Seeragam powder with sugar powder cures cough.
- Seeragam powder with butter cures peptic ulcer.
- Seeragam powder merged in *Eclipta prostata* juice given with sugar and dried ginger powder twice a day cures jaundice and vadha diseases.

Perunjeeragam

Scientific name: : *Pimpinella anisum*

Other names : Sombu, venseeragam.

Vernacular names

Tamil : Perunjeeragam.

English : Anise seeds.

Telugu : Pedha-jilakara.

Malayalam : Perinchirakam.

Kanadam : Sombu, Sopu-gida.

Taste	**:**	Pungent, Sweet
Character	**:**	Heat
Division	**:**	Pungent
Parts used	**:**	Flowers, seeds, root.
Actions	**:**	Carminative Stomachic

General properties:

யோனிநோய் குன்மம் உரூட்சைமந் தம்பொருமல்
பேனமுறு காசம் பீலிகமிரைப் – பீனஉரை
சேர்க்கின்ற வாதமுபோஞ் சீர்பெரிய சீரகத்தால்
மூக்குநோ யில்லை மொழி.

அகத்தயர் குணவாகடம்[18 b]

Uses:

- It cures uterine disorders, abdominal pain, indigestion, abdominal distention, cough, liver disease.

Karunjeeragam

Scientific name	**:**	*Nigella sativa.*
Other names	**:**	Aranam, Ubakunjigai.
Vernacular names		
Tamil	**:**	Karunjeeragam.
English	**:**	Black cumin, Small fennel.
Telugu	**:**	Nalla-jilakarra
Malayalam	**:**	Karinchirakam

Kanadam	**:**	Kari-jirige.
Taste	**:**	Bitter
Character	**:**	Heat
Division	**:**	Pungent
Parts used	**:**	seeds.
Actions	**:**	Diuretic
		Emmenagogue
		Galactogogue
		Anthelmintic
		Parasiticide
		Emollient

General properties:

கருஞ்சீ ரகத்தான் கரப்பனொடு புண்ணும்
வருஞ்சிராய்ப் பீநசமு மாற்றூம் – அருந்தினால்
காய்ச்சல் தலைவலியுங் கண்வலியும் போமுலகில்
வாய்ச்ச மருந்தெனவே வை.

-அகத்தியர் குணவாகடம் [18 c]

Uses:

- It cures eczema, wounds, eye diseases, jaundice, scabies.
- Karunjeeragam powder with kaadineer act as anthelmintic.
- For rabies half to four grams twice day for 3-7 days.

Elam

Scientific name	**:**	*Elattaria cardamomum*
Other names	**:**	Aangi, Korangam, Thudi.
Vernacular names		
Tamil	**:**	Elam
English	**:**	Cardomom seeds
Telugu	**:**	Elakulu
Malayalam	**:**	Elattari
Kanadam	**:**	Elakki
Taste	**:**	Pungent
Character	**:**	Heat
Division	**:**	Pungent
Parts used	**:**	Seeds
Actions	**:**	Stimulant Carminative Stomachic

General properties:

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

-அகத்தியர் குணவாகடம்[18 d]

Uses:

- It cures pitha related diseases.
- It cures throat problems, cough, dysuria, dysentry.
- It increases sperm count.

Adhimadhuram

Scientific name	**:**	*Glycyrrhiza glabra*
Other names	**:**	Adhingam, Atti (Ashti), Madhugam, kundriver.
Vernacular names		
Tamil	**:**	Adhimadhuram
English	**:**	Jequitity, Indian or jamaica liquorice
Telugu	**:**	Ati- madhuramu,yashti-madhukam
Malayalam	**:**	Iratti-madhuram
Hindi	**:**	Jathi-madh, Mulath.
Taste	**:**	Sweet
Character	**:**	Coolant
Division	**:**	Sweet
Parts used	**:**	Root
Actions	**:**	Emollient
		Demulcent
		Mild expectorant
		Laxative

Tonic

General properties :

கத்தியரி முப்பிணியால் வருபுண் தாகங்
கண்ணோயுன் மாதம்விக்கல் வலிவெண்குட்டம்
பித்தமெலும் புருக்கி கிரிச்சரம் ஆவர்த்த
பித்தமத மூர்ச்சைவிட பாகம் வெப்பந்
தத்திவரு வாதசோ ணிதங்கா மாலை
சருவவிடங் காமியநோய் தாது நட்டங்
குத்திருமல் ஆசியங்கம் இதழ்நோய் இந்து
குயப்புணும்போம் மதூகமெனலக் கூறுங்காலை.

தேரன் குணவாகடம்[18 e].

Uses:

- It reduces the virulence of eye disease, psychotic conditions, hiccup, leucoderma, burning micturation and jaundice.
- Adhimadhuram powder with breast milk for external application in eye.

Kothumalli

Scientific name	**:**	*Coriandrum sativum*
Other name	**:**	Urul arusi, Dhania.
Vernacular name		
Tamil	**:**	Kothumalli
English	**:**	Coriander seeds
Telugu	**:**	Kotimiri
Malayalam	**:**	Kotta-malli

Kanadam	**:**	Kottamari-bija
Taste	**:**	Pungent
Character	**:**	Heat
Division	**:**	Pungent
Partsused	**:**	Leaf, seeds
Actions	**:**	Stomachic
		Carminative
		Stimulant
		Diuretic

General properties :

கொத்துமல்லி வெப்பம் குளிர்காய்ச்சல் பித்தமந்தஞ்
சர்த்திவிக்கல் தாகமொடு தாதுநட்டம் – கத்தியெழும்
வாத விகார்மடர் வங்கர்த்த பிவிரணம்
பூதலத்தில் லாதகற்றும் போற்றும்.

-அகத்தியர் குணவாகடம்[18 ரி]

Uses:

- It cures psychotic condition, indigestion, vomitting, anorexia, fever.
- It strengthens the body and increase the sperm count.

Lavangam

Scientific name	**:**	*Syzygium aromaticum*
Othernames	**:**	Anjugam, urkadam, Karuvaai kirambu, Sosam, thirali, varaangam.

Vernacularnames

Tamil	**:**	Lavangam
English	**:**	**C**loves, clove tree.
Telugu	**:**	Lavangalu,Lavanga pu
Malayalam	**:**	Karambu
Kannadam	**:**	Lavangam
Taste	**:**	Pungent
Character	**:**	Heat
Division	**:**	Pungent
Partsused	**:**	Flower bud
Actions	**:**	Antispasmodic Carminative

General properties:

பித்த மயக்கம் பேதியொடு வாந்தியும்போம்
சுத்தவிரத் தக்கடுப்புந் தோன்றுமோ – மெத்த
இலவங்கங் கொண்டவருக் கேற் சுகமாகும்
மலமங்கே கட்டிமென வாழ்த்து.

சுக்கிலநட் டங்கர்ண சூர்வியங்க லாஞ்சனந்தாட்
சிக்கல்விடாச் சர்வா சியப்பிணியு – மக்கிக்குட்
டங்கப் பூவோடு தரிபடருந் தோன்றிலில்ல்
வங்கப்பூ வோடுரைத்து வா.

-அகத்தியர் குணவாகடம்[18 g].

Uses:

- It is one of the ingredient of drugs which is used for pitha diseases, Prepare this lavangam, like a paste with water and apply in the forehead and nose region will relives the sinusities pain.
- It cures giddiness, vomitting, dysentry, chronic diarrhoea.
- Roasted in mild heat and chewing the lavangam will strengthens the gums and cures the throat ulcer.

Ilavangpattai

Scientific name	**:**	*Cinnamomum verum*
Other names	**:**	Karuvappattai
Vernacular name		
Tamil	**:**	Ilavangapattai
English	**:**	Bark of cinnamon
Telugu	**:**	Lavanga- patta, Sanna-lavangapatta.
Malayalam	**:**	Cheria-ela, Vanna-tolif.
Kanadam	**:**	Dala-chinni, lavanga-patta.
Taste	**:**	Pungent, Sweet
Character	**:**	Coolant
Division	**:**	Sweet
Parts used	**:**	Bark.
Actions	**:**	Stimulant

carminative

Aphrodisiac

General properties:

தாதுநட்டம் பேதி சருவவிஷம் ஆகியநோய்
பூதகிர கஞ்சிலந்திப் பூச்சிவிடஞ் – சாதிவிடம்
ஆட்டுமிரைப் போடிருமல் ஆகியநோய்க் கூட்டமற
ஒட்டுமில வங்கத் துரி.
சன்னலவங்க கப்பட்டை தாங்குளிர்ச்சி
இன்னுமிரத் தக்கடுப்பை யீர்க்குங்காண் – முன்னமுறும்
உந்திக் கடுப்பகற்றும் உண்மூலப் புண்போக்கும்
கந்தமிகு பூங்குழலே! காண்.

-அகத்தியர் குண்வாகடம்.[18h]

Uses:

- It cures asthma, cough, internal haemorrhoids.
- It act as refrigerant.
- It has uterine contraction activity, so it is used in menorrhagia conditions.
- Toxins from snake bite and spider bite can be removed by cinnamomum verum.
- Oil extracted from leaf is used to cure body pain, head ache and tooth ache in 2-5 drops.

Seenakarkandu

Scientific name : *Saccharum officinarum*

Other names : Punarpoosam, Ikku, vei.

Vernacular names

Tamil	**:**	Karumbu
English	**:**	Sugarcane, Noblecane
Telugu	**:**	cheruku
Malayalam	**:**	Karinpa
Kannadam	**:**	Khappu
Taste	**:**	Sweet
Character	**:**	Coolant
Division	**:**	Sweet
Partsused	**:**	Sugarcane juice, Sugar, Root.
Actions	**:**	demulcent
		antiseptic
		stimulant
		diuretic
		nutrient

General properties :

ஈறின் தடிப்பு மிருமலும்பல் வாந்திகளுஞ்
சீறுகப முட்டினமுஞ் சேராதே – தேறியநற்
சொற்கண் டிளங்குயில்கள் சூழ மடவனமே!
கற்கண் டெனவுரைக்குங் கால்.

-அகத்தியர் குணவாகடம்[18i]

Uses:

- It cures fever, vomitting and hiccough.
- It cures vadha fever, common cold and sinusities.
- Paste of sugar with bee wax used to treat acne.
- It cures eye diseases.

3.1.2. BOTANICAL ASPECT

Anethium graveolans

Scientific classification

Botanical name **:** **Anethum graveolan**

Kingdom **:** plantae

Class **:** Dicotyledons

Order **:** Umbellales

Family **:** Umbelliferae

Genus **:** *Anethum*

Species **:** *graveolans*

Description:

A glabrous herb, about 1.5m high. Leaves 2-3 pinnate, ultimate segments of the leaves linear, toothed, or entire. Flowers yellow borne in compound umbells. Fruits narrowly winged, plano convex, 2-3times broad as thick. Flowers and fruits during January and march.

Distribution:

Cultivated throughout India including Assam, West bengal, Uttar pradesh, Punjab, Gujarat and Maharashtra.

Parts used **:** Fruits, seeds and leaves

Chemical constituents:

Carvone, dihydrocarvone, limonene,apiol, dill-apiol, α-bergamotene, trans-dihydrocarvone, β-caryophyllene, eugenol, myrcene, myristicin, d-limonene, p-menth-2,4(8)-diene, cis-ocimene, α-phelladrene, alanine, leucine, isoleucin, threonine, tyrosine, arachidic acid, linoleic acid and protein, nicotinic acid, riboflavin,

thiamin, vitamins A,C,K and P (rutin) isolated from the green herb.

Therapeutic uses:

Oil from the seeds is well-known remedy for flatulencein children. Also used as anthelmintic, antipyretic, aromatic, diuretic, emmenogogue, galactogogue, stimulant and specially as a stomachic, colic[19].

Cuminum cymiinum

Scientific classification:

Botanical name **:** *Cuminum cyminum*

Kingdom **:** plantae

Class : Dicotyledoneae

Order **:** Apiales

Family **:** Apiaceae

Genus **:** *Cuminum*

Species **:** *cyminum*

Distribution **:** Cultivated in Punjab and South India

Description :

A slender annual, glabrous herb. leaves twice or thrice 3-partite, ultimate segments filiform. Flowers in compound umbels, bracteate, bracts linear regid. calyx teeth small, Sabulate unequal. Petals oblong are obovate emarginate, white often unequal. Fruit cylindrical tip narrowed. Seed somewhat dorsally compressed.

Parts used : Fruits

Chemical constituents:

Amino acids, carotene, essential oils, cuminaldehyde, cuminyl alcohol, p-

cymene, dipentine, perilla aldehyde, alpha- and beta- pinene, alpha and beta phellandrene, alpha- terpenol, alpha- terpinene, cuminin, linoleic acid, ethanolamine, glycerol and inositol, luteolin and its glycosides, oxalic acid, acetyl choline, choline and antraquinones have been isolated from the plant.

Therapeutic uses:

Fruits antidiarrheal anti dysentery aromatic astringent carminative cooling galactagogue diuretic stimulant stomach beneficial in hoarseness of voice based externally applied to a leg pain and irritation due to worms in the abdomen oil useful in Eczema[19a].

Foeniculum vulgare

Scientific classification:

Botanical name	:	*Foeniculum vulgare*
Kingdom	:	Plantae
Class	**:**	Dicotyledonaea
Order	**:**	Apiales
Family	**:**	Apiaceae
Genus	**:**	*Foeniculum*
Species	**:**	*vulgare*

Distribution:

Commonly cultivated throat India ascending up to 2000m, often grows wild.

Description:

A glabrous, biennial or perennial tall herb. Leaves 2-3-4 pinnate, ultimate segments long, linear. Bracts and bracteoles absent or few small linear. Petals yellow emarginate. Carpels 1/2 Terete, ridges prominent Sub equal furrows 1-vittate, carpophore. Fruit oblong or ellipsoid. Seed somewhat dorsally compressed, inner face slightly concave. Flowers and fruits in January-may.

Parts used: Fruits, leaves and root.

Chemical constituents:

Ascorbic acid, niacin, riboflavin, alpha-, beta- and gamma tocopherol, alpha- and beta-tocotrienol, choline, trigonelline, p-cymene, anethole, anisaldehyde, camphene, estragole, foeniculin, methylchaviol, anisic, caffeic, chlorogenic, hydroxybenzoic and hydroxycinnamic acids, psoralen, scoparone, seselin, xanthotoxin, linoleic acids, 3-arabinosides, quercetin-3-glucuronoide.

Therapeutic uses:

Fruits: anthelmintic, aromantic, carminative, emmenagogue, stimulant and stomachic, beneficial in diseases related to chest and kidney, juice useful to improve eyesight, hot infusion given in amenorrhea, Fennal water prescribed in colic and flatulence in children. Oil is anodyne, diuretic, stimulant and vermicide, checks the griping pain due to excessive use of purgative leaves 0diuretic and increases the secretion of perspiration, root: diuretic and purgative[19b].

Nigella sativa

Scientific classification

Botanical name	**:**	*Nigella sativa*
Kingdom	:	Plantae
Class	**:**	Magnoliophyta
Order	**:**	Ranaunculales
Family	**:**	Ranunculaceae
Genus	**:**	*Nigella*
Species	**:**	*sativa*

Distribution:

A native of syria and lebanon, cultivated in Assam, Bihar, Himachal pradesh and Punjab.

Description:

An erect herb, 45 cm tall, leaves alternate,2-pinnately dissected, stipules small. Flowers white, blue or yellowish, terminal, peduncled, sometimes eithin an involucre or bracts. Sepals 5, regular, decidious, petaloid, imbricate. Petals5, with small 2-fid limb and long claw. Stamens numerous. Carpels3-10. Styles usually long. Fruits spherical capsules, seeds triangular, black. 3.2m long. Flowers in the autumn and fruits in the winter.

Parts used: Seeds.

Chemical constituents:

Seeds have been reported to yeild esters of unsaturated fatty acids with C15 and higher terpenoids, carvone, d-limonene, cymene, nigellone, aliphatic alcohols and alpha, beta-unsaturated hydroxy ketone, alkaloids, steroids and hederagenin glycoside.

Therapeutic uses:

Seeds are diuretic, lactiferous, stimulate uterine contraction, recommended in menstrual troubles and in puerperal fever. Powdered seeds mixed with sesamum oil are externally applied in boils and for scorpion-sting. Essential oils from seeds is used in common cold and cough[20].

Elatteria cardomomum

Scientific classification:

Botanical name	**:**	*Elatteria cardomomum*
Kingdom	**:**	Plantae
Class	**:**	Monocotyledone
Order	**:**	Zingiberales
Family	**:**	Zingiberaceae

Genus : *Elatteria*

Species : *cardomomum*

Distribution:

Moist evergreen forests of south India,growing wild in the western ghats, between 800-1600m. Commonly cultivated in Kerala, Karnataka and Tamilnadu.

Description:

A tall herbaceous perennial with branching subterranean root stock and several erect stems up to 3m high. Leaves 30-90 cm long, subsessile elliptic or lanceolate with sheathing base. Flowers borne in panicles, 60-120cm long, arising from the base of vegetative shoots; white or pale green. Fruits trilobular, subglobose or fusiform to ovoid capsule. Seeds 15-20 per pod, brownish black, angled, rugoose, covered with a thin mucilaginous membrane.

Parts used: Seeds

Chemical constituents:

Alpha-pinene, myrcene, limonene, cineole, cymene,methyl heptenone, linalool, linalyl acetate, alpha and beta terpineol, alpha-terpinyl aacetate, borneol, geraniol, neolidol, heptacosane, camphene,.

Therapeutic uses:

Seeds are aromatic, cooling, stimulant, carminative, digestive, stomachic, diuretic, caardiotonic and abortifacient. They are useful in anorexia, vomitting, giddiness, dyspepsia, haemorroids, strangury, renal and vesical calculi, halitosis, debility and defects of vision[21].

Glycirrhiza glabra

Scientific classification

Botanical name : *Glycyrrhiza glabra*

Kingdom : Plantae

Class	**:**	Dicotyledone
Order	**:**	Fabales
Family	**:**	Fabaceae
Genus	**:**	*Glycyrrhiza*
Species	**:**	*glabra*

Distribution:

Cultivated in Punjab and sub-Himalayan tracts

Description:

A tall perennial under shrub about 1m high; leaves compound, leaflets4-7 pairs; flowers violet in raceme; pods oblong to linear, flattened seeds reniform. The liquorice of commerce is thedried underground stems and roots.its outer surface is pale chocolate brown in colour. It has a characteristic pleasant sweet taste.

Parts used: Roots[22]

Chemical constituents:

Root is attributed to the flavonoid content, especially liquiritin and isoliquiritin. Plant gums, resin and essential oils have been extracted; however the root is cultivated for the principal active glycoside glycyrrhizin. The amount of glycyrrhizin varies from 7 % to 10 % or more depending on growing conditions. Glycyrrhizin, glycyrrhizic acid and glycyrrhizinate amount to10% to 25% of the root extract[23].

Therapeutic uses:

They are useful in hyperdipsia, cough, bronchitis,vitiated conditions of vata, gastralgia, cephalgia, fever, skin diseases. An extract of the root is good for treating gastric ulcers. A decoction of the root is good wash for falling and greying of hair. Externally the root is applied for cuts and wounds[22]

Coriandrum sativum

Scientific classification

Botanical name	**:**	*Coriandrum sativum*
Kingdom	:	Plantae
Class	:	Dicotyledone
Order	**:**	Apiales
Family	**:**	Apiaceae
Genus	**:**	*Coriandrum*
Species	**:**	*Sativum*

Distribution:

Cultivated throughout India

Description:

An annual herb, glabrous. leaves decompound, ultimate segments of the lower leaves ovate or lanceolate. Flowers in compound umbels. Calyx teeth acute, often unequal, petals obovate, emarginate, white or purplish. Fruits subglobose. Seeds about thrice as broad as thick.

Parts used: whole plant, fruits,seeds and leaves.

Chemical constituents:

Beta-carotene (also in leaves), eugenol, glucose, fructose, sucrose, bergapten,

5- and 8-methoxypsoralen, scopoletin, umbelliferone, umbelliprenin, beta-sitosterol

(also in seeds) and its glucosides(coriandrinol) in fruits, borneol, p-cymene, p-cymol, 1,8-cineole, camphene, camphor, beta-caryophyllene and its oxide, citronellol, dipentene, n- decyclic aldehyde, esters of acetic and decyclic acids elemol, geraniol and its acetate, limonene, d-linalool and its acetate.

Therapeutic uses:

- Whole plant: juice (fresh juice) beneficial in erythema;
- Strong decoction in milk with little sugar useful in dyspnea, flatulence, indigestion and bleeding piles;
- Fruits : antibilous, anthelmintic, aphrodisiac, aromatic, carminative, diuretic, refrigerant, stimulant, stomachic and tonic.
- Leaves : antibilious and carminative.
- Coriander water: useful in indigestion and other bowel complaints,
- commom catarrh, allays internal heat and thirst; decoction of dried fruits
- beneficial in bilious complaints[19c].

Syzygium aromaticum

Scientific classification

Botanical name **:** *Syzygium aromaticum*

Kingdom : Plantae

Class **:** Magnoliopsida

Order **:** Myrtales

Family **:** Myrtaceae

Genus **:** *Syzygium*

Species **:** *aromaticum*

Distribution:

They are native to Maluku islands (or Molouccas) in Indonesia and are commonly used as a spice. Commercially harvested primarily in Bangladesh, Indonesia, India, Madagascar, Pakistan, Srilanka. Cloves are available throughout the year[24].

Description:

It is a small- medium sized evergreen tree 8-30m tall. Canopy medium sized, crown base low, branches semi-erect and numerous, leaves glabrous, with numerous oil glands on lower surface flowers small in terminal cymose clusters, fruit olive shaped one seeded, popularly referred to as mother of clove[25].

Parts used: Flower buds

Chemical constituents:

Eugenol composes 72-90% of the essential oil extracted from cloves and is the compound most responsible for clove aroma, acetyl eugenol, beta-caryophyllene and vanilin, crategolic acid, tannin such as bicornen, gallotannic acid, methyl salicylate(pain killer), the flavonoids eugenin, kaempferol, rhamnetin and eugenitin, triterpenoids such as oleanolic acid, stigmasterol and campesterol and several sesquiterpenes. Eugenol is toxic in relatively small quantities for example, a dose of 5-10 ml has been reported as a near fatal dose for a 2 year old child[24].

Therapeutic uses:

The Essential oil extracted from the dried flowers buds of clove, Eugenia caryophylla used as a topical application to relieve pain and to promote healing and also finds use in the fragrance and flavouring industries, it has antimicrobial, antioxidant, antifungal, and antiviral activity. Clove essential oil has antiinflammatory, cytotoxic, insect repellent and anaesthetic properties[26].

Cinnamomum verum

Scientific classification

Botanical name	**:**	*Cinnamomum verum*
Kingdom	:	plantae
Class	**:**	Magnoliopsida
Order	:	Laurels
Family	**:**	Lauraceae
Genus	**:**	*Cinnamomum*
Species	**:**	*verum*

Distribution:

It is native to Sri Lanka and found wild in western and Southern India. It is cultivated in Malay Islands

Description:

An evergreen tree, 8-16 m, with reddish brown bark having numerous small warts. Leaves ovate or elliptic ovate, thick, leathery, subacute or shortly acuminate, shining green on upper surface, main nerves 3-5 from petioles. Flowers minute, in axillary or sub-terminal cymes or panicles. Fruits dark purple single seeded berry.

Parts used: Bark

Chemical constituents:

Linalool, benzyl acetate, cinnamic aldehyde, eugenyl acetate, cinnmyl acetate, benzyl benzoate, cinncassiol C1 glucoside, cinncassiol C2 and cinncassiol C3, cinncassiol D1, its glucoside, cinncassiol D2 and D3 and their glucoside dieterpenes (1-4), cinnamaldehyde, eugenol, benzaldehyde, methyl amyl ketone, phellandrene, pinene, cymene, cumic aldehyde, caryophyllene, esters of isobutyric acid, dipentinoids, safrole, methyl eugenol, cinnamyl alcohol.

Therapeutic uses:

The bark is aromatic, astringent, aphrodisiac, deodorant, stimulant, expectorant, febrifuge, diuretic and carminative. It is useful in bronchitis, asthma, cephalalgia, toothache, cardiac diseases, diarrhoea, nausea and vomiting, flatulence, urinary disorders, fever, halitosis and restoring normal skin colour on the face. Cinnamon oil is stomachic, carminative, emmenagogue, styptic and useful in anorexia, inflammations, abdominal pains, headache, vomiting, tubercular ulcers[21a].

Saccharum officinarum

Scientific classification

Botanical name : *Saccharum officinarum*

Kingdom : Plantae

Class :Monocotyledons

Order : poales

Family : poaceae

Genus : *Saccharum*

Species : *officinarum*

Distribution:

Sugarcane is indigenous to tropical south and Southeast Asia

Description:

It is a topical, perennial grass that forms lateral Shoots at the base to produce multiple stems, typically 3 or 4 m high and about 5 cm diameter and once harvested the stalk will regrow allowing the plant to live for between 8 to 12 years. The stem grows into cane stalk, which when mature stalk, which when mature constituents approximately 75% of entire plant.

A mature stalk is typically composed of 11 to 16 % fibre, 12 to 16 % soluble sugars 2 to 3 % non sugar and 63 to 73 % water. The leaves are grow from the nodes of the stem, arranged in two rows on either side of the stem. The leaves are tubular and blades like, thicker in the centres then at the margins and encircle than the stem. The inflorescence of sugarcane is a terminal panicle which possesses two spikelet and seeds protected by husks covered in silky hair

Parts used: Roots, stem[27].

Chemical constituent :

Sucrose is the product of sugarcane. The juice yeided flavones diosmetin-8-C-glucoside, vitrexin, schaftoside, isoschaftoside and 4',5'-dimethyl-luteolin-8-C glucoside[28].

Therapeutic uses:

The roots are useful in neuropathy. They are useful in fatigue, leprosy, gastropathy, cardiac debility, Hematemesis, cough, Bronchitis, anaemia, ulcers of the skin and mucous membrane, seminal weakness, emaciation and general debility[27].

3.2. DISEASE REVIEW

3.2.1. SIDDHA ASPECT – VERI NOI

Other names:

- Paiyethiya noi
- Piththu noi
- Piththa noi
- Unmadham

NATURE:

In this disease, the mental ability of the person is totally changed and the person becomes mad. The affected person will dance, talk, Sing, beating others, abusing etc. Are some of the action which he performs without any commands from others.

INDICATIONS:

In this the affected persons mental strength is reduced either the person will be too cheerful or to adamant, would like to have sex with any type of female, depression, totally looking like a different personality from a normal person[29].

CAUSES:

In Siddha, psychiatry is the importance laid on the imbalance of 3 humours especially or predominance of azhal humour over the other two (vadha and pitha).The siddhars approved of the possible predisposition to mental illness due to constitutional makeup, seasonal variation and other variables like dietary habits age and sex of individuals. Siddhars approach is more scientific and could be seen from the etiology enumerated by them, just to highlight one of them, we enumerate the etiological description of mental illness as given by *Agasthiya Kanagamani Nooru*. The description encompasses heriditary, environmental, socio-cultural, physiological, toxic and psychological Spheres.

Excessive anger, sexual perversion, offensive smell, sleeplessness,, conflict agitation and worry, sudden loss of wealth due to robbery, fear of enemy, exhaution due to wandering, toxic substances, excessive desire, improper practice of rajayoga, drug dependence like Ganja and abini, fear of higher authorit,y cruel, malicious and cunning activitie,s force full retention of urine and motion are some of the causes of mental illness[30]. Sometimes the disease may be hereditary[29].

TYPES:

In this disease, four types are present. They are

- *Vali varinoi*
- *Azhal verinoi*
- *Iyam verinoi*
- *Mukkutram verinoi*

***Vali Verinoi (Vadha Unmadham)*:**

Before this disease affects a person, he will not have desire to food. Moreover he will be eager to eat the food which is hard, cheap quality, stale etc. Due to this fact both the physical and mental health get spoiled. Stammering, singing, dancing, crying etc. are some of the indications.

***Azhal Verinoi* (*Pittha Unmadham*) :**

Consumption of very hot food made up of chillies, soar, food which generate lot of heat in the body etc. will lead a man to lose his physical health and mental health. In this type the affected person will always be agitated, impatient, removing clothes and run naked etc. are some of the indications.

***Iya Verinoi* (*Kabha Unmadham*):**

In work excess strain to the body beyond one's capacity, always thinking about some worried things etc. are some of the indications. The affected person will be desirous to have the company of women, frequent sleep, tastelessness, secretion of water in the mouth, paleness in the body etc. are some of the symptoms of this disease.

***Mukkutra Verinoi* (*Janni Unmadham)*:**

In this type, the symptoms described above three types are combined together and create a lot of complications to the affected person.

Soga Veri:

Losing the wealth accumulated or the family life, sudden fear are some of the causes of this disease. The affected person will reveal his mind views without any hidden facts, involuntarily talk, sing and dance are some of the indications.

***Nacchu Veri*:**

In this type the disease is caused due to consumption of poisonous items or medicines etc. The indications of the above mentioned types will also appear. The body appearance, redness in the eyes and the actions of five organs will deteriorate.

General Indications:

In this disease the mental balance will be lost, inconsistency, losing self-control capacity, always thinking, talk in loud voice, sleeplessness etc. are some of the symptoms.

Treatment:

Though the disease can be cured with different types of medicines available in Siddha but when this disease affect a person with protrusion of eyeball, dribbling of saliva in mouth, walking or running very fastly, shivering of extremities etc. cannot be cured easily[29].

In Siddha system treatment for neurosis are,

- Peranda Parpam
- Abraga Parpam.
- Vallarai nei
- Eggu Chenduram
- Gaandha Chenduram[29a].
- Pirandai Chooranam[31]
- Verpendhi ennai[31a]
- Kaariya Parpam[31b]
- Panjasootha Mezhugu [31c]
- Sithaadhi ennai[31d]
- Sangu Parpam[31e]

3.2.2. MODERN ASPECT – NEUROSIS

NEUROSIS :

Neurosis is a term generally used to describe a non-psychotic mental illness which triggers feelings of distress and anxiety and impairs functioning[32].

The term neurosis was coined by the Scottish doctor William Cullen in 1769 to refer to "disorders of sense and motion" caused by "general affection of the nervous system"[33].

Neurosis, plural neuroses also called psychoneuroses, mental disorder that causes a sense of distress and deficit in functioning. Neurosis are characterized by anxiety, depression or other feelings of unhappiness or distress that are out of proportion to the circumstances of a person's life. They may impair a person's functioning in virtually any area of his life, relationships or external affairs, but they are not severe enough to incapitate the person. Affected patients generally do not suffer from the loss of sense of reality seen in person with psychosis[34].

Aetiology

There is more than one reason why patients develop Anxiety disorders. Researchers and scientists are trying to find out more about the biological, psychological, and social factors which influence the development of anxiety disorders as there is still a lot more to learn about the role of these.

Genetics and Heredity :

There is clear evidence that anxiety disorders tend to run in families. If a parent or a sibling of a person suffers from an anxiety disorder, there are higher chances of that person developing this disorder. These findings suggest that a genetic factor combined with certain social factors predisposes certain people to develop anxiety disorders[35].

ANXIETY:

Anxiety disorders are a group of syndrome in which a heightened state of unease, worry or fear is the basis for the symptom. These syndromes are set apart from each other by kinds and degrees of anxious symptoms, along with the ways the individual has learned to try to prevent the symptoms. People with anxiety disorders often have some symptoms of depression and vice versa and sometimes a person has one or more anxiety disorders along with a depressive disorder. It is an emotional disturbances that may encompass many different feelings and symptoms[36].

Causes of anxiety disorders

Modern studies indicate that as a result of past, present or perceived circumstances that occurred to an individual, a chemical imbalance may have occurred in the brain. The emotions we feel are based on the release and reuptake of neurotransmitters in the brain. Feelings of anxiety are triggered by an imbalance of specific neurochemicals in the brain. The specific neurotransmitters that may be affects include seratonin, norepinephrine, GABA and dopamine. When we feel stressed, anxious or depressed, our brain may be releasing or absorbing (reuptake) chemicals either too rapidly or too slowly. If left untreated a chemical imbalance disorder may increase in severity as time passes

Chemical imbalance in the brain

Some theories suggest that chemical imbalances are a normal part of life. Everyone feels stressed or anxious at times, even depressed. This is a normal response by our body to events occurring around us. It is important to note that the physical or mental feelings or being caused by the release of chemicals and hormones in our brains.

Common chemical imbalances related to anxiety and depression related disorders that have been observed in clinical practice include:

- Reduced availability of neurotransmitters like Serotonin, Dopamine, Norepinephrine, GABA and acetylcholine.

- Increased levels of toxic neurochemicals such as Homocystine.
- Lower levels of serum magnesium, zinc or potassium
- Unhealthy or deficient levels of essential vitamins like B6 B9 B12 and Vitamin C
- Undersupply of key cofactors like amino acids that are used to help transport neurotransmitter precursors into the blood-brain barrier.
- Increased cortisol stress hormone levels.

Everything we do and every thought that goes through our mind happens as a result of the production, release and absorption of naturally occurring chemicals in our brain like hormones, neurotransmitters and amino acids. The complex set of chemicals in the brain are designed to process incoming Input and then return response. All of this process happened extremely quick in the brain. And, though the effects of chemical imbalance may lead to undue stress, nervousness and worry they are an important part of being human. Chemical imbalances or fluctuations, cannot be avoided because we are supposed to interpret and react to situations, whether they are stressful or joyful; this is simply human nature[36 a].

Types of anxiety

Anxiety attacks

Anxiety attack are a very common form of anxiety and are often experienced as a result of worrying about everyday things in the life such as family, finances or job. Initially the worry may seem completely normal, but overtime the worry more and more about specific issues in life. If the excessive worry continuous, it may begin to cause numerous symptoms that may include

- ✓ Increased Heartbeat
- ✓ Tremors
- ✓ Shaking

- ✓ Fear of dying
- ✓ Dizziness
- ✓ Lightheadedness
- ✓ Nausea
- ✓ Difficulty in breathing
- ✓ Chills
- ✓ Fear of losing control
- ✓ Feelings of being detached from reality

Social anxiety disorder

Social anxiety disorder is defined as a constant fear of being looked at or criticized by others. This is a very common anxiety disorder that is generally experience in a work place or during get-together with others. This anxiety disorder is usually accompained by symptoms such as distress, Panic, fear of the actual meetings or attempts to completely avoid being put in the situation.

Panic anxiety disorder

A panic anxiety disorder is an anxiety related effect that is commonly characterized by repeated panic attacks. Individuals have a panic disorder may experience stress and worry on a daily basis which may lead to severe symptoms including tremor, shaking, fear of dying, feelings of going crazy and detachment from reality.

Phobic anxiety disorders

A phobia is an abnormal fear and avoidance of an everyday object or situation. Many phobias of Medical stimuli exist (e.g.Doctors, Dentist, hospital, vomit, blood and injections) which affect the patients ability to receive adequate healthcare. individuals with specific phobia usually display symptoms of anxiety when faced with

their specific situation or thought common types of phobias include driving phobia, flying phobia, fear of heights and fear of dogs.

Social phobia

This is there fear and avoidance of social situations crowds, strangers, parties and meetings. Public speaking would be the sufferer's worst nightmare.

Generalized anxiety disorder (GAD)

It is probably the most common form of anxiety it is characterized by excessive worrying about daily life that may include more than one specific circumstances, that is worrying about the same things over and over like "what if I lose my job today?" Or "what if my child gets kidnapped if I let him play with his friends?". Although these seem like normal issues that most people deal with on a regular basis, Someone with GAD will have a constant worry and will not be able to get the thoughts out of their mind. It may cause symptoms such as stress, Insomnia, muscle tension, lump in the throat, nausea, clammy hand and even anxiety attack and panic attacks. In fact most people with GAD have experienced and anxiety or panic attack in the past.

Post traumatic stress syndrome (PTSD)

Someone who has been through a traumatic life experience may suffer future anxiety and panic over it. Severe wartime experiences, emotional abuse, devastating earthquake or hurricane, rape or sexual abuse are some of the post traumatic conditions. This form of anxiety disorder can be severe and more than likely require some type of behavioural therapy with a professional.

Obsessive-compulsive disorders

Obsessive- compulsive disorders are characterized by the irresistible entry of unwanted ideas, thoughts or feelings into consciousness or by the need to repeatedly perform ritualistic actions that the sufferer perceives as unnecessary or unwarranted. Obsessive ideas may include recurrent violent or obscene thoughts; compulsive behavior includes rituals such as, repetitive hand washing or door locking[36b]..

Somatoform disorders

Somatic disorders which include the so-called hysterical, or conversion, neuroses, manifest themselves in physical symptoms, such as blindness, paralysis, or deafness that are not caused by organic disease. Hysteria was among the earliest syndromes to be understood and treated by psychoanalysts, who believe that such symptoms result from fixations or arrested stages in an individual‟s early psychosexual development[37].

DEPRESSION:

A depressive disorder is an illness that involves the body, mood and thoughts. It affect the way a person eats and sleeps, the way one feel about oneself, and the way one thinks about things, it is not a sign of personal weakness or a condition that can be willed or wished away. People with a depressive illness cannot merely "pull themselves together" and get better [36c].

CATALEPSY:

Catalepsy is a nervous condition characterized by muscular rigidity and fixity of posture regardless of external stimuli, as well as decreased sensitivity to pain. Symptoms include rigid body, rigid limbs, limbs staying in same position when moved, no response, loss of muscle control and slowing down of bodily function such as breathing.

Catalepsy is a symptom of certain nervous disorders or condition such as Parkinson's disease and epilepsy.It is also a characteristic symptom of cocaine withdrawal, as well as one of the features of catatonia. It can be caused by treatment with antipsychotic drug such as haloperidol and by the anesthetic ketamine. protein kinase A has been suggested as a mediator of cataleptic behaviour[38].

Diagnosis

Patients with symptoms of mental illness should undergo a thorough physical examination and detailed patient history to rule out organic causes (such as brain tumor or head injury). If neurotic disorder is suspected, a psychologist or psychiatrist

will usually conduct an interview with the patient and administer clinical assessments (also called scales, inventories, or tests), to evaluate mental status. Tests which may be administered for the diagnosis and assessment of neurosis include the Neuroticism Extraversion and Openness (NEOR) scale, the Sixteen Personality Factor Questionnaire (16PF), and the Social Maladjustment Schedule. [39]

Laboratory tests for blood sugar (for diabetes) and thyroid function (for Hyperthyroid or Hypothyroid) are also commonly done. There are no laboratory teststhat can diagnose anxiety, although the doctor may order some specific tests to rule out disease conditions.

Although there is no psychiatric test that can provide definite diagnoses of anxiety disorders, there are several short answer interviews or symptom inventories that doctors can use to evaluate the intensity of a patient's anxiety and some of its associated features. These measures include the Hamilton Anxiety Scale and the Anxiety Disorders Interview Schedule (ADIS). [40]

In modern aspect treatment for neurosis

Anti-anxiety, Anti-depressant and Anti- cataleptic drugs:

Antianxiety drugs (Anxiolytics)

- **Benzodiazepines**

  Diazepam

  chlordiazepoxide

  lorazepam

  Alprazolam.

- **Beta- blockers**

  propranolol

- **5-HT agonist-antagonist**

  Buspirone

  Gepirone

  Ipsapirone

- **Sedative antihistamine**

  Hydroxyzine[41].

Antidepressants

Selective seratonin reuptake inhibitors (SSRIs)

Fluoxetin

Fluvoxamine

Paroxetin

Citalopram

Escitalopram

Sertraline

Tricyclic antidepressants

Imipramine,

Desipramine

Clomipramine

Amitryptyline

Nortryptilin

Doxepin

Serotonin norepinephrine reuptake inhibitors (**SNRIs**)

Venlafaxine

Desvenlafaxine

Duloxetin

Milnacipran

Atypical antidepressant

Mianserine

Amineptin

Tianeptin

Bupropion

Amoxapine

Mirtazapine

Monoamine oxidase (MAO) inhibitors

Phenelzine

Tranylcypromine

Moclobemide[41a].

AntiCataleptic drug:

L-DOPA and amantadine[42]

Mechanism of antianxiety drugs (Anxiolytics)

Benzodiazepines have good anti-anxiety actions and are the most commonly used to drugs for anxiety. They are CNS depressants. Alprazolam in addition has antidepressant properties.

Buspirone is an azapirone with good anxiolytic properties. It is a selective 5-HT_{1A} partial agonist. 5-HT_{1A} receptors inhibitory autoreceptors and binding of buspirone inhibits the release of 5HT. Buspirone is also a week D2 antagonist. It is useful in mild to moderate anxiety. Anti-anxiety effect develops slowly over 2 weeks. Unlike diazepam, it is not a muscle relaxant, not an anticonvulsant, does not produce significant solution, tolerance or dependence and is not much useful in panic attacks.

Buspirone is rapidly absorbed and metabolised in the liver, undergoes extensive first pass metabolism.

β-blockers are also useful in anxiety inducing states like public speaking and stage performance. They can be used as adjuvant to benzodiazepines.[41].

Mechanism of Antidepressants

SSRIs block the reuptake of serotonin from the synapse into the serotonergic nerve endings by inhibiting the serotonin transporter (SERT). About 80 % reuptake is inhibited and the more serotonin is available at the synapse which in turn results in transcription of certain proteins leading to the production of related proteins like BDNF responsible for the effects of SSRIs. Hence they enhance the serotonin levels in these synapses.[41a].

3.3. PHARMACEUTICAL REVIEW

Chooranam

Definition

Chooranam is a fine powders 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[43].

Method of preparation

Equipment required

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

Process of preparation

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

should be positively rejected.

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

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

The chooranam should be so 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*

தூான்னென்ற சூரணத்தின் சுத்திக்கேளு
துப்பாதே சரக்கெல்லாஞ் சூரணித்து
நானென்ற வாவின் பாலாற் பிசைந்து
நலமான சட்டியிலே பாலைவிட்டு
வானென்ற சுத்தசலம் பாதிவிட்டு
வளமாக மேற்சீலை கோடு கட்டிப்
பானென்ற சூரணத்தைப் பிட்டுபோல் வைத்து
பதறாதே வெந்தெடுக்கச் சித்தியமே!

- அகஸ்தியர் வைத்திய இரத்தினச் சுருக்கம்
-

The prepared *chooranam* is mixed with the milk in a pot half 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 ரவியுலர்த்திப் பொடிதான் செய்து
ஆப்பனே சமனாய்ச் சர்க்கரையைச் சேர்த்து

நாமப்பா கொண்டு வர தோசம் போச்சு

நான்றாகச் சுத்தி செய்யாச் சூரணந்தான்

தாமப்பத ரோகத்தை வெல்லா தப்பா

தளமான வியதி யெல்லாம் பாரிக்கும் பார்

வேமப்பா சுத்தி செய்து கொண்டாயானால்

வெகுசுறுக்காய் தீருமா வியாதி கேளு

- அகஸ்தியர் வைத்திய இரத்தினச் சுருக்கம்

Then the *chooranam* is placed in the sunlight and powdered. Equal the amount of sugar is added and taken internally. All type of diseases gets 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 cardboard boxes.

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

In industry the tablets are made, counted & packed by electronic devices. Then *chooranam* is said to retain its potency for 3 months and then gradually deteriorate.However if properly packed & stored they keep good for a year. (Formulary of Siddha Medicines, 1993)

According to AYUSH guidelines shelf life of *chooranam* is one year.[45]

Table no: 2. ANALYTICAL SPECIFICATIONS OF CURNA/ *CHOORNAM*

S.no	TESTS
1	Description Macroscopic, Microscopic
2.	Loss on drying at 1050 C
3.	Total – ash
4.	Acid – insoluble ash
5.	Water-soluble extractive
6.	Alcohol – soluble extractive
7.	Particle size (80-100 mesh for Churna; 40-60 mesh for churna)
8.	Identifications, TLC/HPTLC-with marker (wherever possible)
9.	Test for heavy/Toxic metals Lead Cadmium Mercury Arsenic
10.	Microbial contamination Total bacterial count Total fungal count Test for specific Pathogen E. coli
11.	Salmonella spp. S.aureus Pseudomonas aeruginosa Pesticide residue Organochlorine pesticides
12.	Organophosphorus pesticides Pyrethroids
13.	Test for Aflatoxins (B1,B2,G1,G2)

3.4. PHARMACOLOGICAL STUDY IN ANIMAL MODELS

ANTI-ANXIETY ACTIVITY:

Anti-anxiety test (Light-Dark Model) in mice and rats

Mice and rats tend to explore a novel environment, but they retreat from the observe sight of a brightly light opened field. Animals are placed in a two chambered system, where they can freely move between a brightly- light open field and a dark corner. After the treatment with anxiolytic they show more crossings between the two chambers and more locomotor activity. The number of crossings between the light and dark sites is recorded.

Animals required : Native mice or rats

Equipment‟s required : Dark and light chamber

Procedure :

The apparatus consists of a dark and a light chamber which are divided by a photocell equipped zone. A polypropylene animal cage of 44×21×21 cm dimensions is darkened with black spray over one-third of its surface. A partition containing a 13 cm long × 5 cm high opening is used for separating the dark one-third from the bright twothirds of the cage. This cage shows an activity monitor which counts total locomotor activity. Another electronic system consisting of four sets of photocells across the partition and records the time spent in the light and dark compartments.

Experiments are conducted on native mice or rats. They are treated 30 min before the experiments with test drugs or vehicle given i.p. placed in the cage and observed for 10 min. Groups of 6-8 animals should be used for each dose. Finally, the dose response curves are plotted and number of crossings through the partition between the light and the dark chamber are compared with total activity counts during the 10 min. It has been reported that anxiolytics like diazepam and meprobamate produce a dose dependent facilitatory effect whereas the non- anxiolytics are not effective in this model. The relative potency of anxiolytics in increasing the exploratory behaviour agrees well with their potency observed in lineal trails.

mCPP induced anxiety in rats

mCPP is a metabolite (1-(3-chlorphenyl) piperazine) of antidepressant drug trazodone, which has been classified as 5 HT 2c against. It has been shown to anxiogenic in man and in rats. mCCP includes induces hypophagia and hypolocomotion, inhibits social interaction in rats diminished exploratory activity of rats in the open field test and in the light-dark box test, induces hyperthermia and reduces ultrasound induced defensive behaviour in rats. Antagonism of these symptoms has been used for the screening of anxiolytic drugs.

Animals required : Male Sprague Dawley rats (200-250gm)

Chemicals required : mCCP 7 mg /kg (i.p)

Equipment‟s required : Locomotion activity cages

Procedure

Male Sprague Dawley rats (200-250gm) are housed in groups of six exposed to 12 hr light/dark cycle with free access to food and water.Locomotion study – Test compound or vehicle are administered orally 1 hr or i.p. 30 min before the locomotion test. mCCP is injected i.p. in a dose of 7 mg/kg 20 min before the test.Thereafter the animals are placed individually in an automated locomotors activity cages and locomotion is recorded for 10min. Hypophagia study-Rats are individually placed in cages on day 1. After getting acclimatized to their home cages, they are deprived of food on day 3 for 24 hr.They are then treated with the test drug or vehicle orally and returned with 5mg/kg mCCP or saline i.p. After a further 20 min weighed quantity of their normal food pellets are placed in their food hampers and the amount remaining after 1hr is measured. The quantity of food consumed by each animal during this period is calculated.

The effects of test compound of mCPP induced hypolocomotion is determined by oneway ANOVA and Newman-Keuls test and the effect on hypophagia is determined by one-way ANOVA and Dunnet‟s test. The dose producing 50% disinhibition of locomotion is also calculated for comparison with a standard drug. [46]

Behavioral tests

Elevated plus maze is an animal model of anxiety disorder following unconditioned reflex. It is a well-validated animal model to anxiogenic and anxiolytic effects of drugs to assess which was developed and modified by Kulkarni et al [47]. The test apparatus is a plus-shaped cross of two open (16 x 5 cm) and two enclosed arms (16 x 5 x 12 cm) opposite each at an angle of 900 connected with a central area called neutral zone elevated with 25 cm from the floor [48] .

Method

The experimental animals were divided into four groups of six animals each. The first group considered as a control group and was treated with vehicle (Normal saline) only. The second group considered as standard group which was treated with reference drug (Diazepam 2 mg/kg body weight). The third and fourth groups were considered as test groups and were treated with test drug at the dose levels of 100mg and 200mg/kg body weight respectively. Animals were fasted 18 h prior to the experiment. An adaptation period of about forty five minutes after the drug treatment, the experimental animals were placed individually in the centre of a platform facing one of the arms was closed, because animals naturally prefer the enclosed arms, as the aversion against the open arms predominates. Then the animal was observed for 5 minutes, recording the number of times that entered into the open or closed arms, and the average time spent by the animal was recorded.

ANTI-DEPRESSANT ACTIVITY

In vivo methods

Reserpine induces hypothermia

In this method depression is produced by inducing reserpine.

Animals required : Swiss albino mice (25-30 grams)

Chemicals required : Reserpine 2.5 mg/kg s.c

Equipment required : Temperature detector

Procedure

The test measures ability of compounds to inhibit Reserpine induced hypothermia in mice.Used to screen potential antidepressants mice (male albino Swiss 25-30 gm) 12 hr day-night cycle and free access to food and water. Reserpine in dose 2.5-5.0 mg/kg, s.c. induces ptosis, hyperthermia and catalepsy.

Reserpine given 2 hr before to test drug. Rectal body temperature is measured every 30 min for 3 hr after drug injection.Measure initial temperature. By measuring of temperature, according to intensity of temperature antidepressant activity of test drug is estimated.

Isolation induced hyperactivity

It is observed that rats when socially deprived for period of 15 days, exhibit depressive behaviour. There is a reduction in spontaneous locomotors activity, exploratory behaviour rearing and stereotypy.Adult Wistar rats of either sex (200-250gm) housed singly in cages (30cm×26cm×20cm) any visual or auditory their normally housed counter parts for 10-15 days.Animals are subjected to behaviour testing on an arbitrary scale for sleep, reduced response to external stimuli, ambulatory behaviour, and stereotype posture.Both classical and newer antidepressants reduce isolation induced depressive behaviour.[46a]

Forced swimming test (**FST**)

The forced swimming test adopted here is a modification of the method described by Porsolt et al[49] . In this model, rodents forced to swim in a position from which they cannot escape rapidly and become motionless, floating in an upright position and making only small movements to keep their heads above water.

Mice were individually forced to swim for 15 min in glass cylinders (height: 20 cm, diameter: 14 cm), containing 10 cm of water at 25 "C- which is a pre-test, and then mice were removed and dried before being returned to cages. Twenty-four hours later, mice were placed in the cylinders again for a 6-min test in the same system depicted above. The duration of immobility was recorded during the last 4 min of the 6-min testing period. The effect of pre-treatment with test drug was compared and analysed statistically with the tricyclic antidepressant imipramine (15 mg / kg) and recorded.

Tail suspension test (TST)

The tail suspension test (TST) was performed according to the method described by Steru et al with slight modifications [50]. Briefly, the mice were individually suspended by the tail from a metal rod using adhesive tape. The rod was fixed 50cm above the surface of a table. The total duration of immobility was measured for 6 minutes. Immobility' was defined as when they hung passively and were completely motionless. Control group of 6 mice were treated with vehicle (Distilled water 2 ml p.o.), test drug-treated and standard group imipramine (15 mg/kg, p.o.). After the administration, the mice were submitted to TST. The immobility of animals were observed and analysed with standard and control groups.

Catalepsy in Rodents

Catalepsy in rats is defined as a failure to correct an externally imposed, unusual posture over prolonged period of time. This is a typical effect of all agents, which inhibit dopaminergic system in the nigrostriatum.

Adult wistar rats of either sex, weighing between 180 to 220g each are randomly divided into two groups. One group is dosed with test drug and the other withstandard drug (haloperidol 0.5 mg /kg, ip). Catalepsy is evaluated according to theslighlity modified method of Delini-Stula and Morpurgo. After an appopriate pretreatment time of the drug, each at is tested with respect to its right and left front paws, which are first put on columns 3 cm and then 9cm high. The cataleptic state is scored as 1 and 2, respectively (maximum 6 points fo the right and left paws) if a rat maintains an abnomal body posture for mre than10seconds. Catalepsy is scored for 2 or 3h at 30 min intevals. Three trials are conducted for each animal[51].

3.5. LATERAL RESEARCH

Anethum graveolans **[52]**

Anti-inflammatory and analgesic effects

The hydro alcoholic extract of the *Anethum graveolans* seeds caused significant decrease in the inflammation and pain in rats.

Antimicrobial effects

Aqueous and organic extracts of seeds have exhibited potent antibacterial activity. The essential oil and different extracts of *Anethum graveolens* seeds exerted antimicrobial activity of against wide range of microorganisms. The essential oils and acetone extracts shown antimicrobial activity against staphylococcus aureus, Bacillus cereus, Enterococcus fecalis, Escherichia coli, Salmonella typhii.

Anethum graveolens seeds extract have also been reported to possess anti-ulcer activity, and have shown moderate activity against Helicobacter pylori.

Cuminum cyminum[53]

Antibacterial and antifungal activity

essential oil extracted by hydrodistillation from Tunisian variety of Cuminum cyminum was characteized by means of GC and GC-MS. Twenty one components were identified and C.cyminum contained cuminaldehyde, gamma-tepinene, cymene, beta-pinene, 2-caren-10-al, trans carveol and myrtenal as a major components and exhibited higher antibacterial and antifungal activities with a high effectiveness against Vibro spp.

Foeniculum vulgare[54]

Antioxidant activity

The water and ethanol extracts of Foeniculum vulgare seeds showed strong antioxidant activity. 100 microgram of water and ethanol extracts exhibited 99.1% and 77.5% inhibition of peroxidation in linoleic acid system, respectively, and grreater than the same dose of α-tocopherol (36.1%). The both extracts of *Foeniculum vulgare* have effecting reducing power, freer radical scavenging, hydrogen peoxide

scavenging and metal chelating activities.

***Nigella sativa*[55]**

Cytotoxic and immunopotentiating effects

In-vitro cytotoxic screening of extracts of Nigella sativa seeds indicated cytotoxicity in the ethyl-acetate fraction (EFA) against different classes of cancer cell lines, P388,Molt4, Wehi 164, LL/2, HepG2, as measurred by 3-(4,5-dimethylthiazole-2-yl)-2,5- diphenyltetrrazolium bromide(MTT)assay.

Antidiabetic activity [56]

Oral administration of ethanolic extract of *Nigella sativa* seeds (300mg/kg body weight/day) to streptozotocin induced diabetic rats for 30 days significntly reduced the elevated levels of blood glucose, lipids, plasma insulin and improved altered levels of lipid peroxidation products (TBARS and hydroperoxides) and antioxidant enzymes like catalase, superoxide dismutase, reduced glutathione and glutathione peroxidase in lver and kidney.

***Elatteria cardomomum*[57]**

Gastropotective effect

The crude methanolic extract (TM), essential oil(EO), petroleum ether soluble (PS) and insoluble (PI) fractions of methanolic extract, were studied in rats at doses of 100-500, 12.5-50,12.5-150 and 450mg/kg, respectively for their ability to inhibit the gastric lesions induced by aspirin, ethanol and pylorous ligature. In addition their effects on wall mucus and gastric acid output were recorded. All fractions (TM, EO, PS, PI) significantly inhibited gatric lesions induced by ethanol and aspirin but not those induced by pylorus ligation.

Glycyrrhiza glabra

Memory enhancing activity[58]

Elevated plus maze and passive avoidance paradigm were employed to test learning and memory. Three doses (75,150,and 300mg/kg p.o) of aqueous extract of *Glycyrrhiza glabra* were administered for 7 successive days in separate groups of animals. The dose of150 mg/kg of the aqueous extract of liquorice significantly

improved learning and memory of mice.

Pharmacological effects of *Glycyrrhiza glabra*[59]

Liquorice root is a traditional medicine used mainly for the treatment of peptic ulcer, hepatitis c and pulmonary and skin diseases, several other useful pharmacological properties such as antiinflammatory, antiviral, antimicrobial, antioxidant, anticancer activities, immunomodulatory, hepatoprotective and cardioprotective effects.

Coriandrum sativum

Anthelmintic activity[60]

In vitro anthelmintic activities of crude aqueous and hydro-alcoholic extracts of the seeds of *coriandrum sativum* were investigated on the egg and adult nematode parasite *Haemonchus contortus*. Both extracts of coriandrum sativum inhibited hatching of eggs completely at a concentrration less than 0.5mg/ml. ED_{50} of aqueous extract of coriandrum sativum was 0.12 mg/ml while that of hydro-alcoholic extract was 0.18 mg/ml. The hydro-alcoholic extract showed better in vitro activity against adult parasites the aqueous one.

Essential oil of *Coriandum sativum*[61]

The Coriandrum sativum essential oil and extracts possess prromising antibacterial, antifungal and anti-oxidative activities as various chemical components in different parts of the plant, which thus play a great role in maintaining the shelf life of foods by preventing their spoilage.

Syzygium aromaticum

Immunomodulatory activity[62]

Clove essential oil increased the total White blood cell (WBC) count and enhanced the delayed type hypersensitivity (DTH) response in mice. Moreover it

restored cellular and humoral immune responses in cyclophosphamide-immunosuppressed mice in a dose dependent manner.

Cinnamomum verum

Antioxidant activity[63]

The antioxidant activity of the methanolic extract of cinnamomum Verum barks were evaluated with reference to antioxidant compounds like butylated hydroxyl anisole, trolox and ascorbic acid. By virtue of their hydrogen donating ability, all of the tested compounds and CBE exhibited reducing power. They were found to be potent in free radical scavenging activity especially against DPPH radical and ABTS radical cations.

Saccharum officinarum

Immunostimulating effect [64]

The phagocytic activity of peripheral blood leukocyte (PBL) in chickens orally administered sugar cane extract (SCE) or polyphenols rich fraction (PRF) of SCE (500mg/kg/day) for 3 consecutive days increased significantly, when compared with that of saline-administered control chickens. It showed significantly higher antibody response against sheep red blood cells and *Brucella abortus* then control chicken.

4. MATERIALS AND METHODS

Drug selection

In this dissertation *Sadhakuppai chooranam* was taken as a trial drug for anti-anxiety, antidepressant and anti-cataleptic activities from the Siddha literature[65].

Collection of the raw materials

All the raw materials were brought from the Ramaswamysetty country drug store in Parrys Corner, Chennai.

Identification and authentication of the drug

All the raw materials are identified and authenticated by the Botanist *Gunapadam* experts in Government Siddha Medical College, Arumbakkam, Chennai 106.

Specimen sample of all the herbs have been preserved in PG Gunapadam department individually for future reference.

4.1. Preparation of the trial drug

1. Sadhakuppai	-	Dill seeds (Anethum graveolens)	35gms
2. Seeragam	-	Cumin seeds (Cuminum cyminum)	35gms
3. Perunjeeragam	-	Anise seeds (Foeniculum vulgare)	35gms
4. Karunjeeragam	-	Black cumin (Nigella sativa)	35gms
5. Lavangam	-	Cloves (Syzygium aromaticum)	35gms
6. Kothammalli	-	Coriander seeds (Coriandrum sativum)	35gms
7. Elam	-	Cardamom seeds (Elatteria cardamomum)	35gms
8. Adhimadhuram	-	Indian liquorice (Glycyrrhiza glabra)	35gms
9. Lavangapatttai	-	Bark of cinnamomum (Cinnamomum verum)	35gms
10. Seenakarkandu	-	Rock sugar (Saccharum officinarum)	70gms

Purification of the drugs

All the drugs mentioned here were purified as per the Siddha literature [66].

- Sadhakuppai, Seeragam, peunjeeragam, kaunjeeragam, were cle.aned well without any dust and impurities and dried in sunlight.
- Flower buds of lavangam was removed.
- Kothamalli seeds were tied in white cloth and hanged in hot water pot heated for some time and was dried in sunlight.
- Elam was dried in sunlight.
- Roots of Adhimadhuram was cleaned with water and cut in to small pieces and then dried.
- Lavangapattai was dried in sunlight.

Preparation of the drug

Procedure

All the purified ingredients were grounded seperately and powdered well and mixed with fine rock sugar. The powder was sieved through a white cloth and kept in a air tight container. Finally it was labelled as "*Sadhakuppai chooranam" (SKC).*

Purification of the Chooranam

Pittaviyalmurrai (steaming process):

The *Sadhakuppai chooranam* was purified by *Pittaviyal* method (steam cooking in milk) as per Siddha classical literature.A mud pot was taken and it was quarter filled by milk and quarter filled by pure water. The mouth of the pot was sealed by a cloth. This *choornam* then placed over the cloth and the pot was heated. The same drug was later dried and powdered then sieved again. It was used for the further study[67].

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	:	2-4 grams twice a day.
Adjuvant	**:**	Hotwater.
Indication	:	*Paithiyam*

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

Organoleptic character

The organoleptic characters of the sample drug where evaluated. One gram of the test drug was taken and the colour, texture, particle size and other morphology reviewed by naked eye under sunlight. Then the result is noted. Results were noted and tabulated in Table.No.7.

4.2.1. Physicochemical analysis

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

Solubility: **A.** A little of the*Sadhakuppai Chooranam*was shaken well with Distilledwater.

B. A little of the *SKC* was shaken well with con Hcl and Con $H2SO_4$.

Sparingly soluble character indicates the presence of Silicate.

pH value: Potentio-metrically pH value was determined by a glass electrode and a suitable pH meter.

1. **Loss on drying**

An accurately weighed 2gm of ***Sadhakuppai Chooranam*** formulation was taken in a tarred glass bottle. The crude drug was heated at 105^0 Celsius 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.

2.**Determination of total Ash**

Weighed accurately 2gm of ***Sadhakuppai chooranam*** formulation was added in crucible at a temperature 600^0 celsius in a muffle furnace still carbon free as was obtained. It was calculated with the reference to the air dried drug.

3. **Determination of acid insoluble Ash**

Ash above obtained, was boiled for 5 min with 25 ml of 1 M hydrochloric acid and filtered using and 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 muffler furnace. The percentage of acid insoluble ash was calculated with the reference to the air dried drug.

4. **Determination of water soluble Ash**

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

5**. Determination of water soluble extractive**

5 gram of air dried drug, coarsely powdered ***Sadhakuppai chooranam*** was macerated with 100 ml of distilled water in a closed flask for twenty-four hours, shaking frequently. Solution was filtered and 25 ml of filtrated was evaporated in tarred flat bottom shallow dish, further dried at 100^0 Celsius and weighted. The percentage of water soluble extractive was calculated with reference to the air dried drugs.

6. **Determination of alcohol soluble extractive**

2.5 gram of air dried drugs, coarsely powdered ***Sadhakuppai chooranam*** was macerated with 50 ml alcohol in closed flask for 24 hours. With frequent shaking, it was filtered rapidly taking precaution against loss of alcohol. 10 ml of filtrate was then evaporated in a tarred flat bottom Shallow dish, dried at 100^0 C and weighted. The percentage of alcohol soluble extractive was calculated with reference to air dried

drug.

Results were noted in the Table No. 8

4.2.2. Phytochemical analysis

The preliminary phytochemical screening test was carried out for each extracts of ***Sadhakuppaichooranam*** as per the standard procedure.

1. Detection of alkaloids:

Extracts were and dissolved individually in dilute Hydrochloric acid and filtered.

a) Mayer's Test: Filtrate where treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of yellow coloured precipitate indicates the presence of alkaloids

b) Wagner's test: Filtrate were treated with the Wagner's reagent (Iodine in Potassium Iodide). Formation of brown / reddish precipitate indicates the presence of alkaloids

c) Dragendroff's test: Filtrates were treated with the Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

d) Hager'sTest: Filtrate were treated with the Hager's reagent (saturated picric acid solution).

Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

2. Detection of carbohydrates

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrate where used to test for the presence of Carbohydrates.

a) Molisch's test

To 2 ml of plant sample extract, two drops of alcoholic solution of □-naphthol are added. The mixture is shaken well and few drops of conc. sulphuric acid

is added slowly along the sides of the test tube. A violet ring indicates the presence of carbohydrates.

b)Benedict test

Filtrates were treated with the Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

3.Detection of glycosides

Extracts were hydrolysed with dil. Hcl, and then subjected to test for 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 the ammonical layer indicates the presence of anthranol glycosides.

b) Cardiac glycoside (Keller killiani test):

Extract was shaken with the distilled water 5 ml to this glacial Acetic Acid to ML containing a few drops of ferric chloride was added, followed by H2 S o4 1 ml along the sides of the test tube. The formation of brown ring and the inference interface gives positive indication for cardiac glycoside and a wild thing may appear below the brown ring.

4. Detection of saponins:

a)Froth Test:

Extracts where diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

b)Foam test:

0.5 gram of extract was shaken with 2 ml of water. If phone produced persist

for 10 minutes it indicates the presence of saponins.

5.Detection of phytosterols:

a) Salkowski's test:

Extracts were treated with the chloroform and filtered. The filtrate were treated with a few drops of conc. sulphuric acid shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

6. Detection of phenols Ferric Chloride Test:

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

7. Detection of tannins Gelatin test:

The extact is dissolved in5ml of 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 a 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:

Extract were treated with few drops of lead acetate solution. Formation of yellow colour indicates the presence of flavonoids.

9. Detection of proteins and amino acids:

a) Xanthoproteic Test:

The extracts were treated with a few drops of conc. 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 copper Acetate test:

Extract 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 paper. Oil stain on the paper indicates the presence of fixed oils.

13. Test for quinones:

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

Results were noted in the Table No. 9.

4.2.3. HPLC - High Performance Liquid Chromatography (HPLC) [68].

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

of Waters Company while the software used was Empower.

Two methods using different mobile phases were used for chromatographic separation of the research drugs – Method I (binary gradient method of Acetonitrile & 0.1% Phosphoric acid in Water) and Method II (binary gradient method of Methanol & 1:25 Acetic acid in Water).

Results obtained during Method I have been discussed since better separation of compounds was observed during this analysis. The chromatographic conditions for Method I are as given below:

- Column : Symmetry C18, 5 µm, 4.6x250 mm
- Run Time : 30 minutes
- Injection Volume : 20 µl
- Wavelength (Dual) : 272 nm & 360 nm
- Solvent A : Acetonitr6ile
- Solvent B : 0.1% Phosphoric acid in water
- Flow rate : 1.0 ml/min.
- Pump Mode : Gradient

Processing Method

Table.no.3

Time (min.)	% A	% B
0	15	85
12	25	75
20	25	75
22	15	85
30	15	85

A- Acetonitrile

B- 0.1%Phosphoric acid in water.

Results were noted and tabulated in Table No: 10.

4.2.4. Bio-chemical analysis

Methodology for chemical analysis

Preparation of extract :

5gm of *SKC* was weighed accurately and placed in a 250ml clean beaker and added with 50ml of distilled water. Then it was boiled well for about 20 minutes.

Then it was cooled and filtered in a 1000ml volumetric flask and made up to 100ml distilled water [69].

Table no: 4 Test for basic radicals

PROCEDURE	OBSERVATION	INFERENCE
Test for Potassium: A pinch of sample is treated with 2ml of sodium nitrate solution and then treated with 2ml of cobalt nitrate in 30% of glacial acetic acid.	Formation of Yellow colour precipitate	Presence of Potassium
Test for Calcium: Taken2 ml of *SKC* extract in a clean test tube. Then acetic acid and potassium chromate solution were added	No Yellow precipitate	Presence of Calcium
Test For Magnesium: 2ml of *SKC* extract was taken in a clean test tube, few drops of Magnason reagentwas added in drops.	Formation of Blue colour precipitate	Presence of Magnesium

Test For Sodium: 2 pinches of *SKC* was mixed with HCl and made it into paste. And introduced into the blue flame of Bunsen burner.	Appearance of intense Yellow colour	Presence of Sodium
Test for Iron (Ferrous): 2ml of *SKC* extract was taken in a clean dried test tube and conc. HNO3 and ammonium thiocyanate were added.	Appearance of Blood red colour	Presence of Ferrous iron
Test For Zinc: 2 ml of the *SKC* extract was taken in a test tube and Potassium ferro cyanide solution was added	Formation of White colour precipitate	Presence of Zinc
Test For Aluminium: To the 2m1 *SKC* of the extract was taken in a test tube sodium hydroxide drops were added to it.	White precipitate obtained	Presence of Aluminium
Test For Lead: 2 ml of *SKC* extract was taken in a test tube and added with 2ml of potassium iodide solution	Formation of yellow colour precipitate	Presence of Lead
Test for Copper: To a small portion of *SKC* extract dilute hydrochloric acid was added and then hydrogen sulphide gas is passed through the solution.	Black precipitate	Presence of Copper
Test For Mercury: 2m1 of the *SKC* extract was taken in a test tube and treated With 2ml of sodium hydroxide solution	Formation of Yellow precipitate	Presence of Mercury

PROCEDURE	OBSERVATION	INFERENCE
Test for Arsenic: 2m1 of the *SKC* extract was taken in a test tube and treated with 2ml of sodium hydroxide solution	Formation of brownish red precipitate	Presence of Arsenic

Results were noted and tabulated in Table No:11

Table no.5 Test for acidic radical

PROCEDURE	OBSERVATION	INFERENCE
Test for Sulphate: 2 ml of the *SKC* extract was taken in clean, dry test tube and 5 % barium chloride solution was added to it	Formation of white precipitate	Presence of Sulphate
Test for Chloride: The *SKC* extract was taken in a test tube and then treated with Silver nitrate solution.	Formation of White precipitate	Presence of Chloride
Test for Phosphate: The *SKC* extract was taken in a test tube and treated with ammonium molybdate and conc. HNO3.	Formation of Yellow precipitate	Presence of Phosphate
Test for Carbonate : The substance was taken in a clean dry test tube and then treated with Conc. HCl.	Formation of Effervescence	Presence of Carbonate
Test for fluoride &oxalate: 2ml of extract was taken in a test tube and added with 2ml of dil.acetic acid,	Formation of cloudy appearance	Presence of Fluoride & Oxalate

2ml calcium chloride solution and then heated.		
Test For Nitrate: 1gm of the *SKC* was heated with copper turnings and concentrated H2SO4 and observed the test tube vertically down.	Characteristic changes	Presence of Nitrate

Results were noted and tabulated in Table no :12

4.2.5 ANTIMICROBIAL LOAD

Availability of microbial load:

Enumeration of bacteria by plate count – agar plating technique[70]

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 are 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 sample 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 used for each dilution.
- Add approximately 15 ml of the nutrient medium, melted and cooled to 450 c, to each petri dish containing the diluted sample. Mix the contents of each dish by rotating gently to distribute the cells throughout the medium.

- Allow the plates to solidity.
- Incubate these plates in an inverted position for 24-48 hours at 370 c.

Observation:

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

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

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

Results were noted and tabulated in Table No.13

4.2.6 Following instrumental analysis is carried out to study quantitative analysis of *Sadhakuppai chooranam.*

SOPHISTICATED INSTRUMENTAL ANALYSIS

FT IR - Fourier Transform Infra-red Spectroscopy[71].

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. 16 FT IR Instrument

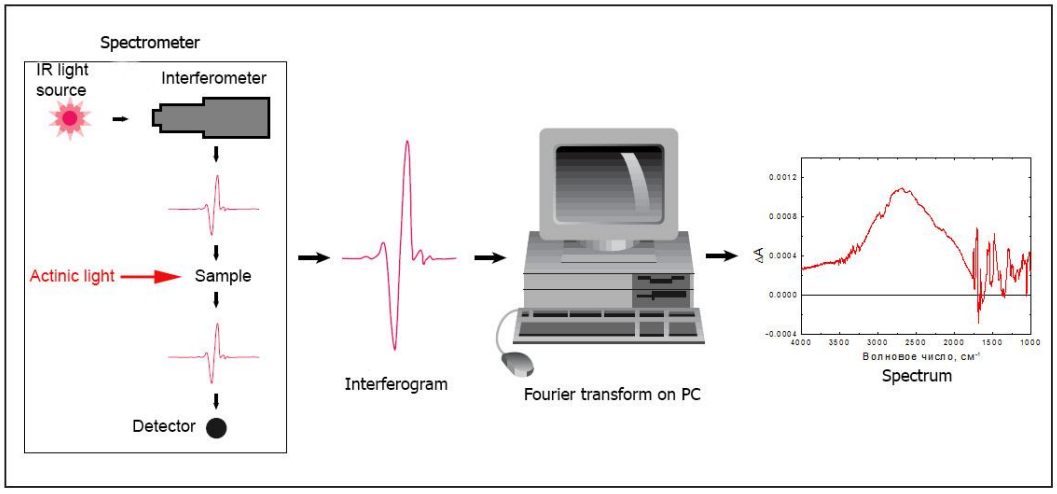


Fig. No.17 FT IR Mechanism

APPLICATIONS:

- Quantative scans
- Qualitative scan solids, liquids, gasess
- 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 die.
- Submit it to the pressure of about 800 mPa (8 tons/cm2).
- Examine the disc visually and if any lack of uniform transparency is observed, reject the disc and prepare again.
- Record the spectrum between 4000 to 650 cm-1 unless otherwise specified in individual standard test procedure.
- When sample and standard are measured for concordance, the transmittance

obtained at the start of the scan range, should not deviate by more than 10% between them (For eg. If the standard shows a transmittance of 75%, the sample transmittance can be between 65% and 85%).

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

- Speed
- Sensitivity
- Mechanical Simplicity
- Internally Calibrated

Results were noted and tabulated in Table No:14

ICP-MS -Inductively Coupled Plasma Mass Spectrometry

Analysis of Trace Metal and Inorganic Materials:[72]

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:18 ICP-MS INSTRUMENT

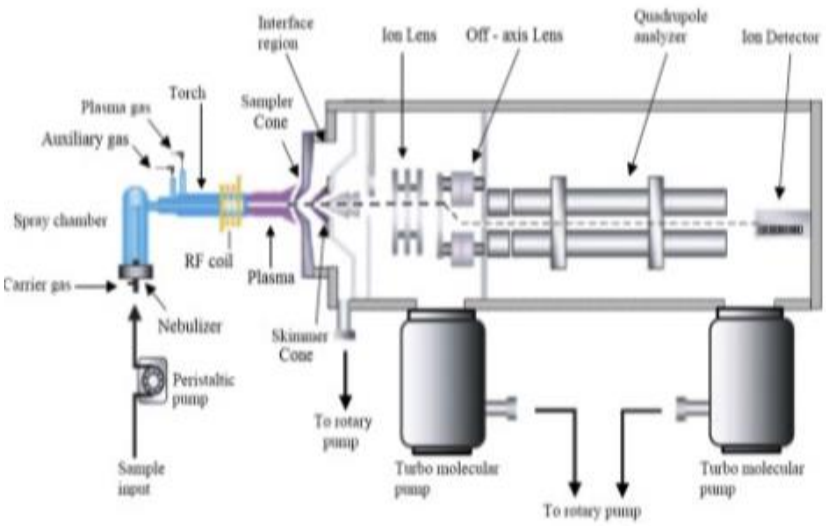


Fig. No:19 ICP- MS MECHANISM

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

Results were noted and tabulated in Table No:15

SEM (SCANNING ELECTRON MICROSCOPE)[73]

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 [83].

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: 20 SEM INSTRUMENT

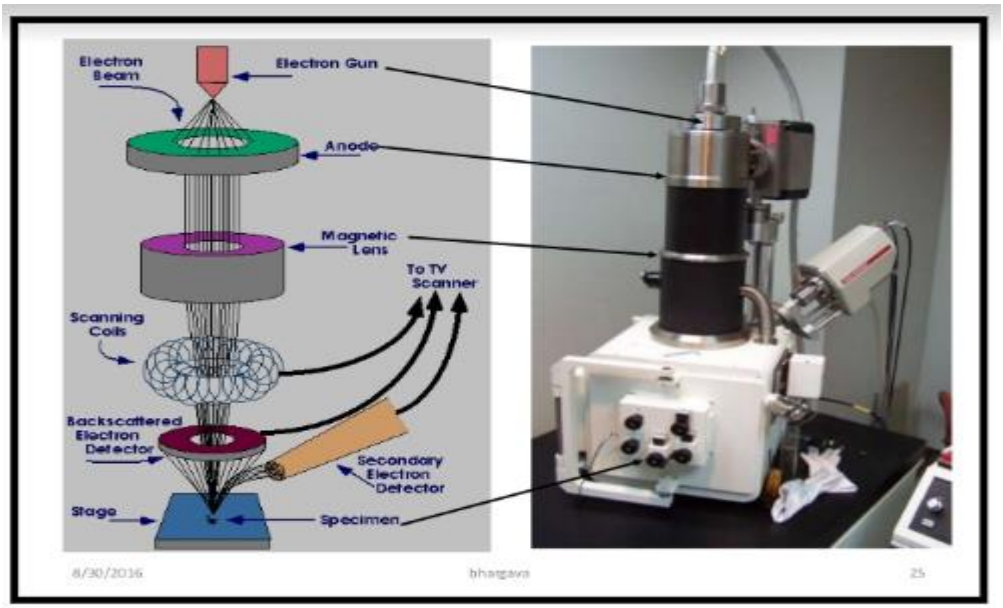


Fig.No: 21 SEM MECHANISM

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)[74]

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

DEFINITION

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

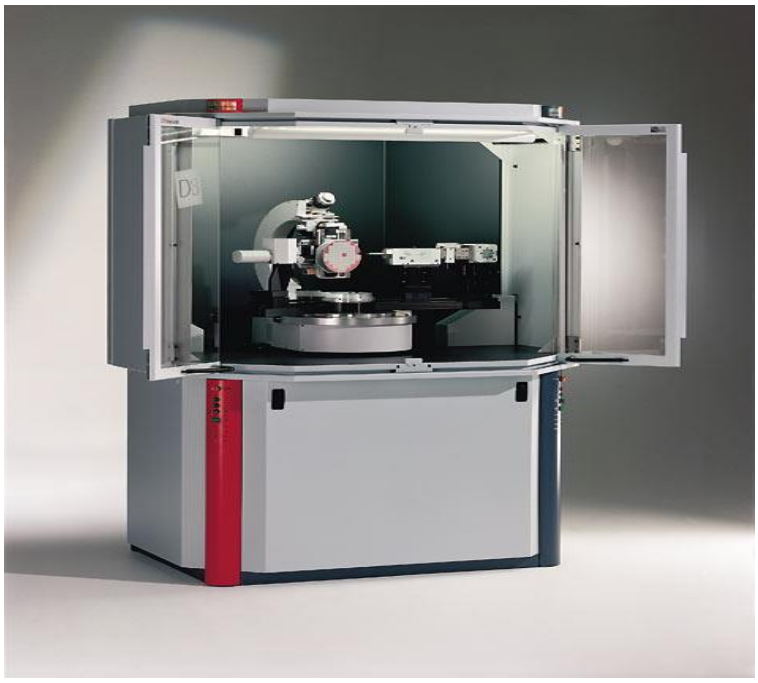


Fig.No.22 XRD Instrument

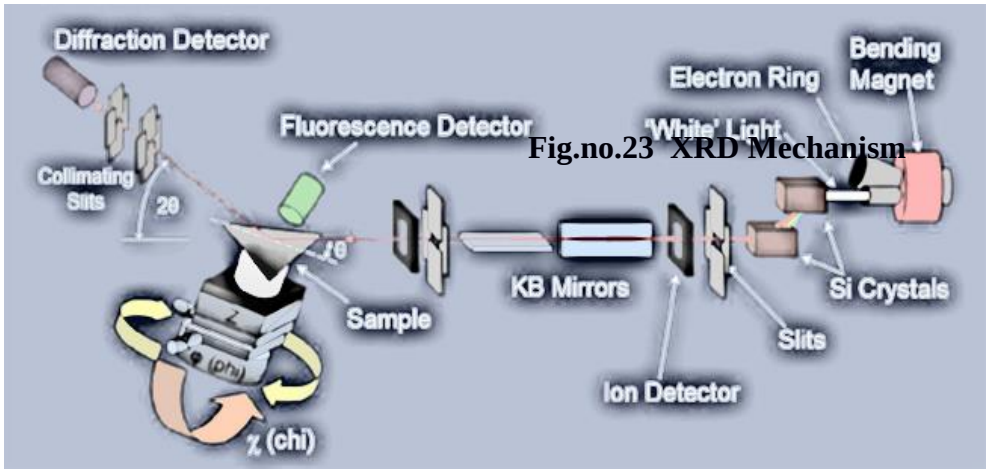


Fig.no.23 XRD Mechanism

Fig.No.23 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 μm(or 200-mesh) in size is preferred

Place into a sample holder or onto the sample surface.

4.3 TOXICOLOGICAL STUDY

4.3.1 ACUTE ORAL TOXICITY STUDY OF *SADHAKUPPAI CHOORANAM (SCM)* (OECD GUIDELINE – 423) [75]

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 of the Test:

It was the principle of the test that based on a stepwise procedure with the use of a minimum number of animals per step, sufficient information was obtained on the acute toxicity of the test substance to enable its classification. The substance was administered orally to a group of experimental animals at one of the defined doses. The substance was 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 was needed
- dosing of three additional animals, with the same dose
- dosing of three additional animals at the next higher or the next lower dose level. The method will enable a judgment with respect to classifying the test substance to one of a series of toxicity classes.

Methodology:

Selection of Animal Species

The preferred rodent species was the wistar albino rat, although other rodent species may be used. Healthy young adult animals are commonly used laboratory strains should be employed. Females should be nulliparous and non-pregnant. Each animal, at the commencement of its dosing, should be between 6 to 8 weeks old and the weight (150-200gm) 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 for Acute Toxicity Studies

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

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

IAEC approved Number: IAEC/XLVIII/14/CLBMCP/2016

Test Substance	: **SADHAKUPPAI CHOORANAM (SKC)**
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.

Numberofanimals : 3 Female/group,

Routeofadministration : 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 ± 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:

SADHAKUPPAI CHOORANAM (SKC) 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 5, 50, 300 and 2000 mg/kg body weight was administered stepwise. After the substance has been administered, food was withheld for a further 3-4 hours. The principle of laboratory animal care was followed. Observations were made and recorded systematically and continuously as per the guideline after substance administration. The visual observations included skin changes, mobility, aggressively, sensitivity to sound and pain, as well as respiratory movements. Finally, the number of survivors was noted after 24 hrs and these animals were then monitered for a further 14 days and observations made daily. The

toxicological effect was assessed on the basis of mortality.

Observations:

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

a. Mortality

Animalswillbeobservedintensivelyat0.5,2.0,4.0,6.0,12.0,24.0and48.0hoursfollowing drugadministrationonday1oftheexperimentanddailytwicethereafterfor14days.

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 behavioral patterns. Attention should be directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma.

d.Grossnecropsy

All animals (including those which die during the test period are removed from the study) was subject edto gross necropsy. Gross necropsy includes examination of the external surface of the body, allorifices, 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 was carried out in organs to show the evidence of any toxicity in gross pathology.

Data and reporting

All data were summarized in tabular form, (Table-1-4) showing for each test group the number of animals used, the number of animals displaying signs of toxicity, the number of animals found dead during the test, description of toxic symptoms, weight changes, food and water intake.

Test substance and Vehicle

In order to ensure the uniformity in drug distribution in the medium the suspension was made by mixing *SKC* with water and it was found suitable for do sea ccuracy.

Justification for choice of vehicle [76]

The vehicles elected as per the standard guideline was pharmacologically inert and easy to employ for new drug development and evaluation technique

4.3.2 28-DAYS REPEATED ORAL TOXICITY (407) STUDY OF *SADHAKUPPAI CHOORANAM (SKC)* [77]

Test Substance	: ***SADHAKUPPAI CHOORANAM* (*SKC*)**
Animal Source	: TANUVAS, Madhavaram, Chennai.
Animals	: Wister Albino Rats (Male -24, and Female-24)
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±3ºC.

Relative humidity : between 30% and 70%,

Air changes : 10 to 15 per hour

Dark and light cycle : 12:12 hours.

Duration of the study : 28 Days.

Justification for Dose Selection:

As per OECD guideline three dose levels were selected for the study. They are low dose (X), mid 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), X dose is (30mg/kg), 5X dose is (150mg/kg), 10X dose is (300mg/kg).

Preparation and Administration of Dose:

SADHAKUPPAICHOORANAM **(SCM)** suspended in with water, 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.

Table.No.6

Groups	No of Rats
Group I Vehicle control (water)	12(6male, 6female)
Group II SKC- Low dose X(30mg)	12(6male, 6female)
Group III SKC-Mid dose 5X(150mg)	12(6male, 6female)
Group IV SKC-High dose 10X(300mg)	12(6male, 6female)

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.

Necropsy:

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

Laboratory Investigations:

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

Haematological Investigations

Blood samples of control and experimental rats was analyzed for haemoglobin 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 glutamateoxaloacetate transaminase/ Aspartateaminotransferase (GOT/AST), glutamatepyruvate transaminase/ Alanine amino transferase (GPT/ALT) and alkaline phosphatase were estimated as perthe colorimetric procedure.

Histopathology

Histopathological investigation of the vital organs was done. The organ pieces(5-6µmthick)of the high estdose level of 300mg/kg were preserved and were fixed in 10% formal in for 24 hours and washed in running water for 24 hours.

Samples were dehydrated in anauto technique and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and the ninacubical block of paraffin made by the "L" moulds. It was followed by micro to meandtheslides were stained with Haematoxylin-eosin. The organs included heart, kidneys, liver, ovary, pancreas, brain, spleen and stomach, of the animals were preserved they were subjected to Histopathological examination.

Statistical analysis:

Findings such as body weight changes, water and food consumption, hematology and blood chemistry were subjected to One-way ANOVA followed by dunnet t test using a computer software programme – Graph pad version 7. All data were summarized in tabular form, (Table-6 to 12)

4.4 PHARMACOLOGICAL STUDY

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 Female

4.4.1 Anti-anxiety activity of *Sadhakuppaichooranam*

Experimental Protocols

Wister rats were randomly selected to form groups of 6 each. The animals were acclimatized one hour before for behavioral tests.Animal were treated with tests and standard drugs for 7 consecutive days (once a day). The test was performed on the seventh day after 60 min administration of test drugs per oral by bulb tipped gastric gavage needle (18 gauge) and 30 min standard drug diazepam 2 mg/kg IP.

Group I: Test animals received no drugs and kept as control.

Group II: These animals received 2mg/kg of the diazepam drug as standard.

Group-III: These animals received 100mg/kg of SKC.

Group-IV: These animals received 200mg/kg of SKC.

The anxiety inducing process

Prior to testing the anti anxiety effect of each drug, rats were undergone the restraint-induced anxiety. Restraint-anxiety and immobilization procedure are most common to induce stress-related behavior Take a cylindrical or semi-cylindrical tube with ventilation holes, then the animal is kept for 120-180 min for restraint stress. After this, the animals show the signs of elevated levels of anxiety in the Elevated Plus Maze (EPM)

Method

The Elevated Plus Maze (EPM) is made up of 4 arms forming a plus sign with 2 open arms (35×5cm) and 2 closed arms (35×5×20 cm). The arms were connected together with a central square of 5×5cm. It was positioned 25 cm above the floor. The animal was placed at the center of the EPM, head facing towards the closed arms. Then the animal was observed for 5 minutes, recording the number of times that entered into the open or closed arms, and the average time spent by the animal was recorded. The time spent in open arm and closed arm was recorded during 5 min periods. Arm entry was counted when the animal had placed all of its four paws on it. The procedure was conducted in a sound attenuated room[78]

Average time was calculated by the following formula.

Average time = total duration in the arms / number of entries.

During the experiment, all the animals were allowed to socialize to avoid unnecessary anxiety. After each animal, the test apparatus was carefully cleaned.

4.4.2 Anti- depressant activity of *Sadhakkuppai chooranam*

In the present study, the antidepressant-like activity was assessed in mice model of depression derived, namely, the forced swimming test (FST) and Tail suspension test (TST).

Experimental Design for anti-depressant activity:

The rats were divided four groups (n=6). Drugs/ vehicle were administered to the animals 60min prior to study.

Group I - Control, administered saline 1 ml/kg orally.

Group II - Receive standard drug Imipramine (10 mg/kg orally)

Group III - Received *Sadhakuppai Chooranam* (SKC) 100 mg/kg orally

Group IV - Received *SadhakuppaiChooranam* (SKC) 200 mg/kg orally

Forced Swim Test (FST)

The forced swimming test adopted here is a modification of the method described by Porsolt et al.

Rats of either sex were individually forced to swim in an open cylindrical container (diameter 10 cm, height 25 cm) containing 19 cm of water at 25±1°c which is a pre-test, and then mice were removed and dried before being returned to cages. Twenty-four hours later, rats were placed in the cylinders again for a 6-min test in the same system depicted above. Treatment was given 60min prior to study as described by study design.

All animals were forced to swim for 6 min and the duration of immobility was observed and measured during the final 4 min interval of the test. Each rats was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements to keep its head above water. A decrease in the duration of immobility is indicative of an antidepressant like effect. The duration of immobility was recorded during the last 4 min of the 6-min testing period. The effect of pretreatment with *Sadhakuppai Chooranam* was compared and analysed statistically with the tricyclic antidepressant imipramine (10 mg / kg) and recorded. [79]

Tail suspension test (TST)

The tail suspension test (TST) was performed according to the method described by Steru et al[50]with slight modifications. Briefly, the rats were

individually suspended by the tail from a metal rod using adhesive tape. The rod was fixed 50cm above the surface of a table. The total duration of immobility was measured for 6 minutes.

Immobility' was defined as when they hung passively and were completely motionless. Control group of 6 mice were treated with vehicle (Distilled water 2 ml p.o.), test drug-treated group (Sadhakuppai Chooranam 200mg/kg, p.o.), and standard group imipramine (15 mg/kg, p.o.). After the administration, the rats were submitted to TST. The immobility of animals were observed and analysed with standard and control groups.

4.4.3 Anti-cataleptic Activity

Bar test was used to study the effect of *Sadhakuppai Chooranam* on various doses on haloperidol induced catalepsy. Haloperidol (1 mg/kg, IP) was injected to rats (n = 6) pretreated 30 min before with vehicle (1 ml/kg, i.p.), Standard drug Levodopa 30 mg/kg i.p. and two dose levels of *Sadhakuppai Chooranam*

Group I: Control, administered saline 1 ml/kg orally.

Group II: Received Haloperidol 1mg/kg IP

Group III: Receive standard drug levodopa (30 mg/kg orally)

Group IV: Received *SADHAKUPPAI CHOORANAM* (*SKC*) 100 mg/kg orally

Group V: Received *SADHAKUPPAI CHOORANAM* (*SKC*) 200 mg/kg orally

The forepaws of rats were placed on horizontal bar (1 cm in diameter, 3 cm above the table) and the time required to remove the paws from bar was noted for each animal and the durations of catalepsy was measured at 0, 30, 60, 90 min. The standard bar test was used to determine the intensity of catalepsy. Both forelegs of a mouse were placed on a horizontal bar. The latency from paw placement until the rest complete removal of one paw from the support was measured (maximal test duration 180 s) and termed here as descent latency. If the mice did not assume the position on the bar after three attempts, it received a descent latency of 0s. [80].

Preparation of the *Sadhakuppai chooranam*:

Fig.No.1 *Anethum graveolans*

Fig.No. 4 *Nigella Sativa*

Fig.No. 2 *Cuminum cyminum*

Fig.No.5*Elatteria cardomomum*

Fig.No.3 *Foeniculum vulgare*

Fig.No : 6 *Glycyrrhiza glabra*

Fig.No.7 ***Coriandrum sativum***

Fig.No.9 ***Syzygium aromaticum***

Fig.No.8 ***Cinnamomum verum***

Fig.No.10 ***Saccharum officinarum***

Fig.No.11 Pounding

Fig.No.12 Sieveing

Fig.No.13 ***Vasthirakayam***

Fig.No.14 ***Sadhakuppai chooranam***

Sadhakuppai chooranam **in a air tight glass container**

Fig.No.15

5. RESULTS AND DISCUSSION

There are several studies have been done to bring the efficacy and potency of the drug "***Sadhakuppai Chooranam".*** The study includes

- Literary collections,
- Organoleptic characters,
- Physicochemical analysis,
- Phytochemical analysis,
- Acid-Base radical test,
- Bacterial load,
- Instrumental analysis,
- Toxicological study and Pharmacological study.

The drug "***Sadhakuppai Chooranam"*** has been selected for **Anti-Anxiety, Anti-depressant and Anti-cataleptic** activity in reference with the text book ***"Agasthyar Attavanai Vagadam".pg no:82-83.***

- 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 neurosis.
- Gunapadam review brings the general properties, uses and effectiveness of the drug in treating neurosis.
- Botanical aspect explains the identification, description, active principle and medicinal uses of the plants.
- Pharmaceutical review describes about the chooranam and its properties.
- The pharmacological review explains about the methodology of Anti-Anxiety, Anti-depressant and Anti- cataleptic Activity and the drugs used in neurosis in both Siddha snd Modern system.
- Siddha and Modern aspect of the disease was reviewed.

Standardization of the test drug

Standardization of the drug is more essential to derive the efficacy, potency of the drug by analyzing it by various studies. The following results of physicochemical, phytochemical analysis, physical characterization and estimation of basic and acidic radicals have been done and tabulated. Toxicological results of the drug and pharmacological activity of the drug are derived. Its result has been tabulated and interpretation is made below. Thus, the results give a complete justification, to bring the effectiveness of the trial drug *Sadhakuppai Chooranam*.

Siddha parameters of testing for *Chooranam,*

Chooranam tends to be amorphous.

It should be never damp.

The fineness of the sieve should be100 mesh or still finer and

the *Chooranam* gave the inference of amorphous, it doesn't damp.

ORGANOLEPTIC CHARACTERS

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

Table No. 7 Organoleptic character

S.No	Parameters	Results
1	Colour	Brown
2	Odour	Aromatic
3	Taste	Bitter
4	Texture	Fine powder
5	Particle size	Completely pass through sieve no 88

Table. No.8 Physicochemical analysis:

S.no	Parameters	Percentage
1	pH	5.65
2	Loss on drying	3.35%
3	Total ash value	7.23%
4	Acid insoluble ash	Less than 1%
5	Water soluble ash	2.74%
6	Water soluble extraction	20.2%
7	Alcohol soluble extraction	26.38%
8	Solubility	
	a) Distilled water	Soluble
	b) Benzene	Soluble
	c) chloroform	Soluble

The physicochemical analysis of the drug result reveals the pH, moisture, total ash value, acid insoluble ash, water soluble ash and Solubility

pH: It is a measure of hydrogen ion concentration; it is the measure of the acidic or alkaline nature. 7.0 is a neutral, above 7.0 is an alkaline and below are acidic.

Interpretation:

- **pH :** pHof SKC is 5.65 is acidic in nature. The acidic medium is higher oral availability and was likely to be the result of better solubility and lower clearance. So, the result concludes that the oral bioavailability of the drug is very high
- **Loss on drying:** Loss on drying of *SKC* is 3.35. The moisture present in the drug was established in loss on drying. The moisture content of the drug reveals the stability and its shelf-life. High moisture content can adversely affect the active ingredient of the drug. Thus low moisture content could get maximum stability and better shelf life.

- **Ash**: Ash constitutes the inorganic residues obtained after complete combustion of a drug. Thus Ash value is a validity parameter describe and to assess the degree of purity of a given drug.

- **Total ash value:** The total ash value of *SKC* is 7.23%, it determines the purity of the drug.The amount of minerals and earthy materials present in the drug are represented by Total ash value.

- **Acid insoluble ash**: The acid insoluble ash value of the drug denotes the amount of siliceous matter present in the plant. The acid insoluble ash value of *SKC* is less than 1%, which determines the superior quality of the *Sadhakuppai chooranam*.

- **Water soluble ash:** Water soluble ash represents easy facilitation of diffusion and osmosis mechanism. Here the value of *Sadhakuppai chooranam* is 2.74% will denote its diffusion capacity.

- **Water soluble extractive:** Solubility is the basic requirements for the absorption of the drug from GIT. Here the water soluble nature of Sadhakuppai chooranam is 20.2%. so, there is a chance for better absorption. [81].

PHYTOCHEMICALS ANALYSIS

Table No. 9 Phytochemicals screening test for *SKC*

S.No	Phytochemicals	Test name	H2O Extract
1.	Alkaloids	Mayer's Test	Positive
2.	Carbohydrates	Benedict's Test	Positive
		Molisch's Test	Positive
3.	Glycoside	Modified Borntrager's Test	Positive
4.	Saponin	Froth Test	Positive
5.	Tannins	Gelatin Test	Positive
6.	Flavanoids	Alkaline Reagent test	Positive
		Lead acetate test	Positive
7.	Diterpines	Copper Acetate Test	Positive

The phytochemical analysis reveals the presence of alkaloids, carbohydrates, glycosides, saponins, diterpenes, flavonoids and tannins.

Interpretation:

- Phytochemicals are natural bioactive compound, found in plants and fibers, which act as a defense system against diseases and more accurately to protect against diseases[82].
- Alkaloids for known modulators of different biochemical and the molecular markers regulating a number of signal transduction Pathways, which are attributed to their potential as disease modifying agent against the complex nature of neurological disorders[83].
- Flavanoids that possesses an effective anti-depressant and anxiolytic activity. It modulates GABAergic neurotransmission, enhances the inhibitory tone of the nervous system, improves the symptoms like restlessness, disturbed

sleep and calms depression and anxiety.[84]

- Flavonoids are known by their antioxidannt activities thus preventing oxidative stress, which is belive to be one of the cause of CNS disorders.[85]
- Flavonoids and glycosides which reach the brain tissues through the metabolizing process, protecting brain function from CNS disturbance and consequently, exerting an antidepressant effect[86].
- Glycosides are naturally occuring plant secondary metabolite with significant medicinal potential.
- Phytoglycosides have been evaluvated for their depression alleviation. The efficacy is mediated by the modulation of brain - derived neurotrophic factor (BDNF) in the hippocampus, that is known for promoting synaptic efficacy, neuronal connectivity and neuroplasticity.[87]
- Tannins act as potential therapeutic agent, which may be beneficial in patients with neurological disease[88].
- Diterpenes to have neurobiological activities like neuro-protection, anti-epileptic, anxiolytic, anti-alzheimer's disease, anti-parkinsons disease, anti-neuropathic pain and anti-neuro inflammatory[89].
- Chemical constituents like flavanoids, alkaloids, saponins, carbohydrates show a wide range of therapeutic potencies like anticataleptic, antimicrobial, immunomodulator.[90].

A synergetic effect of all these alkaloids, carbohydrates, glycosides, saponins, diterpenes, flavonoids, tannins increases the potency of the trial drug *SKC* against Neurosis.

HPLC - High performance liquid chromatography

HPLC has been used to find out the retention time(RT) which depends upon the separation of compounds in the C18 column under high pressure and different solvents in gradient pattern of Acetonitrile and 0.1% phosphoric acid in water for 30 minutes.

Table.No.10 Results of HPLC for *SKC*

S. NO	PARAMETERS	UNITS	RESULTS
1	Total Polyphenol as gallic acid Equivalent	mg/100 g	0.017
2	Total Flavonoids as Quercetin Equivalent	mg/100 g	3.39
3	Total Alkaloids	mg/100 g	2.16
4	Total Tannin as Tannic Acid Equivalent	mg/100 g	0.4

Interpretation:

- Gallic acid is a well-known antioxidant compounds which has neuroprotective actions in different models of neurodegeneration, neurotoxicity and oxidative stress. [91]
- Flavanoids and tannins having highly effective role in the treatment of depression. The mechanism of flavanoids and tannins in anti-depressant action is by inhibit the non selective re-uptake of neurochemicals (Dopamine, Norepinephrine, Seratonin, GABA and Glutamate) that decreases neurochemical degradation, enhances binding to many receptors and supresses depression[84].
- Quercetin in invitro studues in experimental animals and humans have provided supporting evidence for neuroprotective effects against neurotoxic chemicals or in various models of neuronal injury and neurodegenerative diseases. [92]

- Quercetin, health benefits includes preventing allergies, preventing free radicals, controlling stress level, increases stamina, supporting cardiovascular health.[93]

- The neuroprotective ability of alkaloids has been exerted against Alzheimer's disease, anxiety, depression, cognitive impairment and dementia, neurotoxicity, multiple sclerosis, psychological disorders, epilepsy and so on. [83]

From these facts, all the above compounds exhibit the neuroprotective effect and antioxidant properties. Due to the antioxidant properties the oxidative stress will be reduced ,which one is the major cause for stress. This will enhance potency of the drug against anxiety, depression, catalepsy.

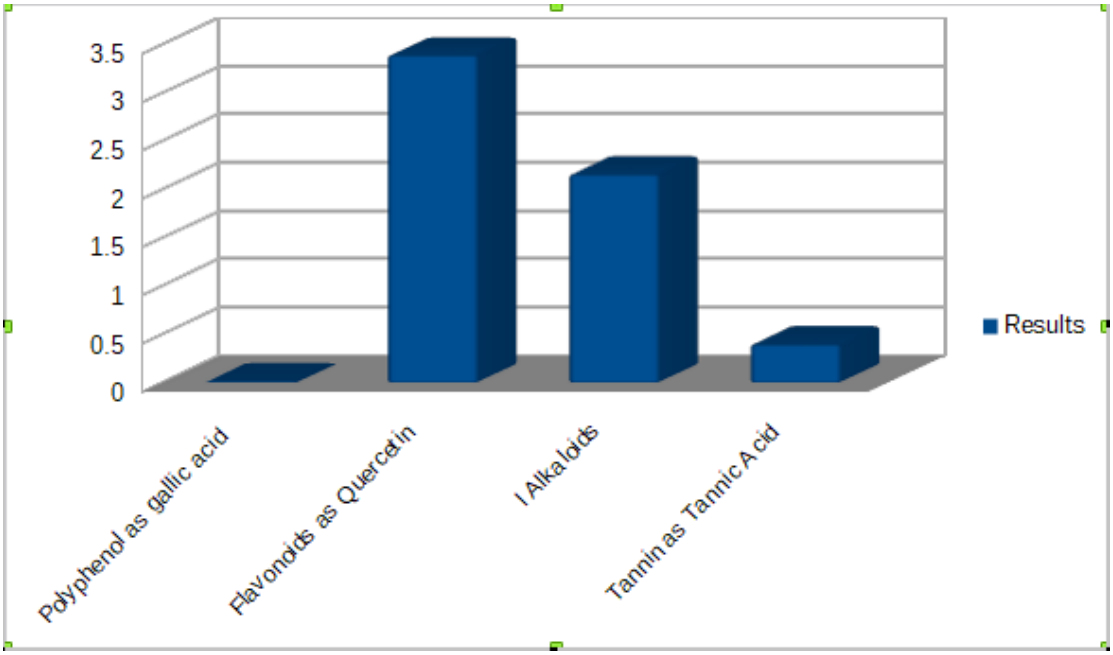


Chart No 1: Results of HPLC for SKC

Table.No.11 Results of basic radicals studies

S.NO	Parameter	Result
1	Test for Potassium	Negative
2	Test for Calcium	Positive
3	Test For Magnesium	Positive
4	Test For Ammonium	Negative
5	Test For Sodium	Negative
6	Test for Iron (Ferrous)	Negative
7	Test For Zinc	Negative
8	Test For Aluminium	Negative
9	Test For Lead	Negative
10	Test for Copper	Positive
11	Test For Mercury	Negative
12	Test for Arsenic	Negative

Interpretation

The presence of **Calcium, Magnesium and Copper** in the basic radical test and absence of heavy metals such as lead, arsenic and mercury.

- Presence of Calcium in the drug improves neurotransmission and cell metabolism. [94]
- Calcium is a vital element in the process of neurotransmitter release, when calcium channels are blocked, neurotransmitter release is inhibited. [95]
- Calcium then triggers neurotransmitters release within a few hundred microseconds by activating synaptotagmins calcium ions.[96]
- Magnesium, the body's fourth most abundant cation. The derangement in the metabolism of magnesium, profoundly affects the nervous system. So,

presence of magnesium in the drug improves the function of nervous system.[97]

- Magnesium plays a vital modulatory role in brain biochemistry, influencing several neurotransmission pathways associated with the development of depression. [98]
- Magnesium is well-known for curing some of the common psychiatric disorders like stress, panic attacks.[99]
- Copper plays most important roles in the human body its promoting neurological function by playing a role in antioxidant defense and neurotransmitter synthesis.[100].

Table .No.12.Results of acid radical studies

S.NO	Parameter	Result
1.	Test for Sulphate	- ve
2.	Test for Chloride	+ve
3	Test for Phosphate	+ve
4	Test for Carbonate	- ve
5	Test for fluoride &oxalate	-ve
6	Test For Nitrate	- ve

Interpretation:

The acid radicals test shows the presence of **Chloride and Phosphate.**

Table .No.13. Availability of Microbial load in *Sadhakuppai Chooranam*

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

Interpretation:

The availability of bacterial load in the *SKC* has been performed by Plate count- Agar plate technique.

SKC is a herbal drug prepared by plants. It is easy to get contamination,If any contamiation present in drug, that decreases the potency and efficacy. The contamination of *SKC* has been examined by bacterial and fungal load.

- Total bacterial load in 10^{-4} dilution is 14 and 10^{-6} dilution is 8
- Total fungal load in 10^{-2} dilution is 8 and 10^{-3} dilution is 4

Here, the result shows presence of bacterial and fungal load in the trial drug(*SKC*).They present 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.

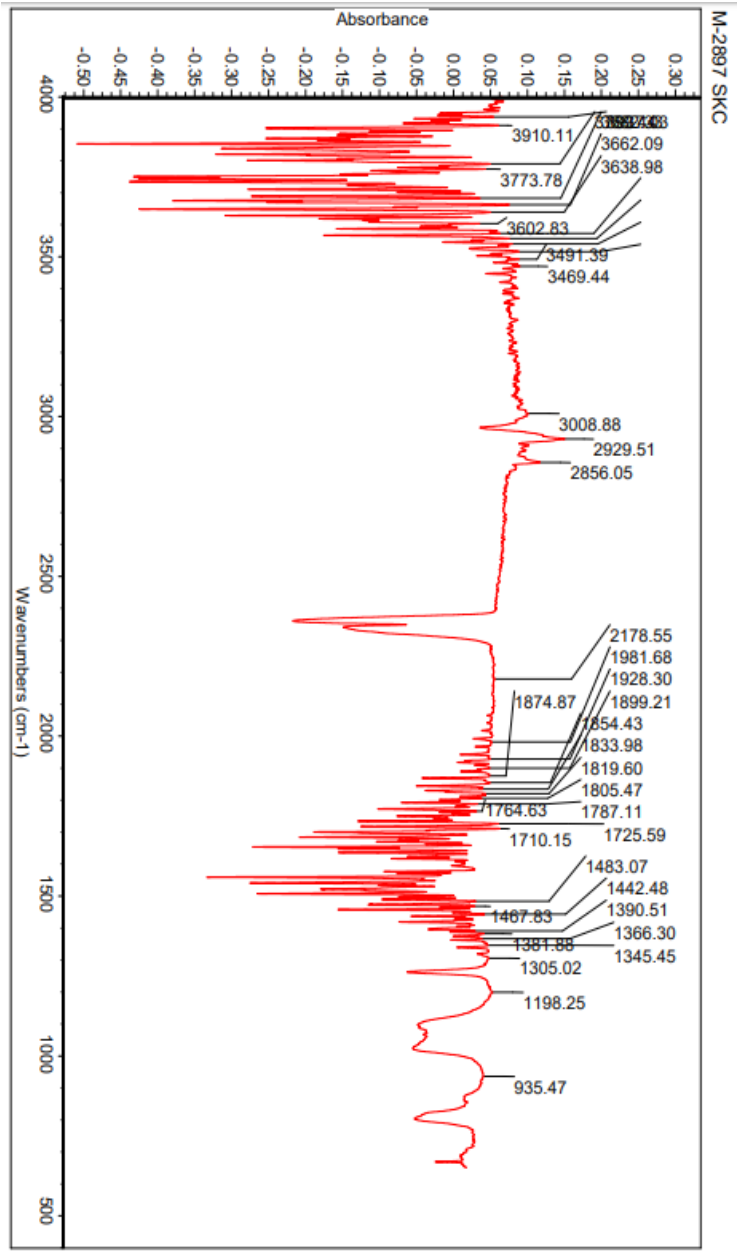


Fig No.24 FTIR results for *Sadhakuppai Chooranam*

Table .No.14 Results of FTIR

3638	O-H strech, free hydroxyl	Alcohols, phenols
3469	O-Hstrech, H-bonded	Alcohols, phenols
3008	O-H Strech	Carboxylic acids
2929	C-H strech	Alkanes
2856	C-H Strech	Alkanes
2178	-C≡C strech	Alkynes
1764	C=O Strech	Carboxylic acids
1710	C=O Strech	✔,✔ unsaturated aldehydes, ketones.
1483	N-O Asymmetric strech	Nitro compounds
1442	C-C Strech	Aromatics
1467	C-C Strech	Aromatics
1366	C-H Rock	Alkanes
1345	N-O Asymmetric strech	Nitro compounds
1305	C-O Strech	Alcohols, carboxylic acids, esters, ethers.
1198	C-O Strech	Alcohols, carboxylic acids, esters, ethers.
935	O-H Bend	Carboxylic acids

The above table shows the presence of alcohols, alkanes, aromatics, carboxylic acids, ✔,✔ unsaturated aldehydes, ketones, nitro compounds, esters, ethers which are represents the peak value.

Interpretation

FTIR instrumental analysis was done. The test drug was identified to have 16 peaks. They are the functional groups present in the trial drug *SKC.*

- Aromatics have played an important role in the management of anxiety,

depression, some cognitive disorders, insomnia and stress related disorders[101].

- phenolic compounds are pesent in a number of biological systems and natural products neurotransmitters, flavouring agents.
- Phenols improved the emotion and cognition related behaviours in depression effectively. Moreover it increased neurotransmitter concentration.
- The effect of phenols is currently of great awareness due to their anti-oxidative and it posses diverse biological activities like antioxidant and antidepressant activities[102]
- Nitro compounds have antidepressant activity.[103]

XRD (X-Ray Diffraction Analysis) results of *Sadhakuppai Chooranam:*

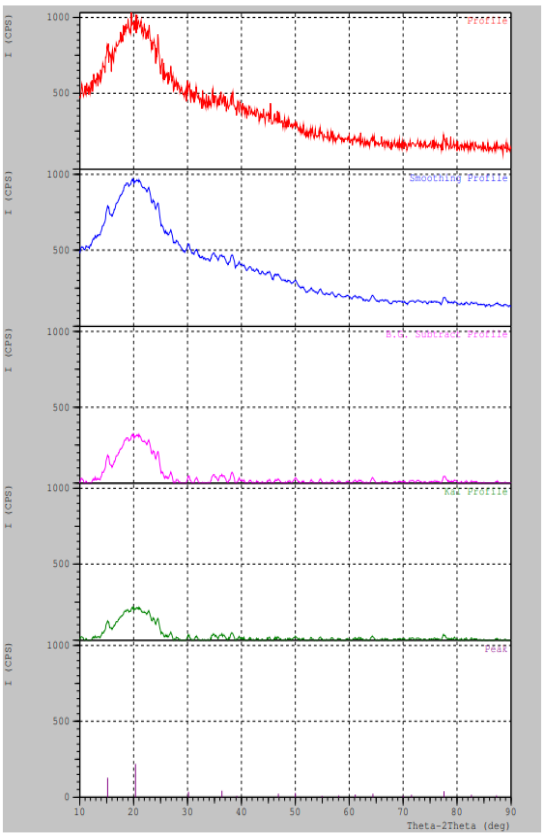


Fig.No.25 XRD results for *Sadhakuppai chooranam*

Interpretation:

- The structure and the size of the particles are highly dependent on the route of synthesis and high lights the efficacy of the drug. The micro particles may enhance bio-absorption of the drug.
- The major diffraction peaks are identified after XRD analysis *SKC* concluded that range 15-36nm is associated with organic molecules probably plays an important role in making it biocompatible and nontoxic at therapeutic doses.
- Other elements present in *SKC* act as an additional supplement and possibly helps in increase the efficacy of the formulation.

SEM: (SCANNING ELECTRON MICROSCOPE)

The particle size and the chemical elements were assesed by scanning electron microscope. SEM is one of the most widely used instruments in research side. The picture of SKC is shown below.

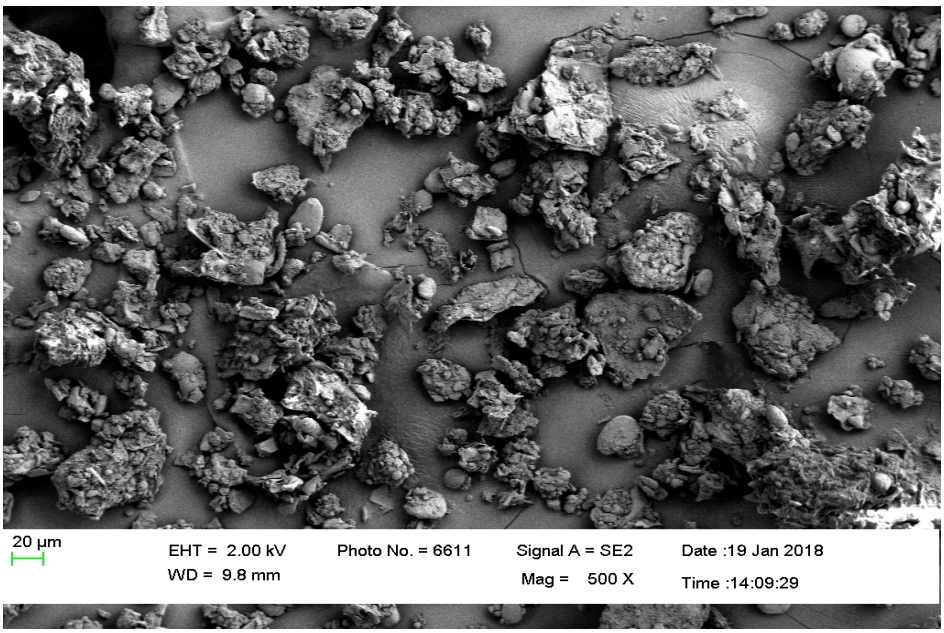


Fig.no.26 SEM picture of *SKC*

Interpretation for SEM

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

- Size and surface of micro particles can be easily manipulated to achieve both passive and active drug targeting.
- They control and sustain the release of drug during the transportation and at the site of localization, alters drug distribution in the body and subsequent clearance of the drug so as to achieve increased drug therapeutic efficacy thereby bio-availability and reduced side effects. [104]

ICP-MS (INDUCTIVELY COUPLED PLASMA MASS SPECTROSCOPY)

The drug SKC was analysed by the Inductively Coupled Plasma Mass Spectroscopy to detect the trace elements.

Table.No.15 Results of ICP-MS

Heavy metal analysis by ICP-MS			
1	Arsenic	mg/kg	BQL (LOQ 0.1)
2	Mercury	mg/kg	BQL (LOQ 0.1)
3	Cadmium	mg/kg	BQL (LOQ 0.1)
4	Lead	mg/kg	BQL (LOQ 0.1)

Interpretation:

- ICP-MS results of *SKC* showed the Arsenic, Cadmium, Mercury and Lead are in below detectable level.
- From the above results, the trial drug is safe as it contains heavy metals are observed within the normal limits.
- So this reveals the safety of the trial dug *SKC*.

TOXICITY STUDIES RESULTS

Acute oral toxicity study of *Sadhakuppai Chooranam:*

Dose finding experiment and its behavioral Signs of acute oral Toxicity

Table.No :16 Obsevation done

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

Table.No.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	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2.2.2.	SKC 2000mg	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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

(+ Present, - Absent)

Table .No.18 (Body weight observation of Wistar albino rats group exposed to *SKC)*

DOSE	DAYS		
	1	7	14
CONTROL	270.1±65.70	270.7± 09.71	270.6 ±2.10
HIGH DOSE	260.3± 4.44	261.4 ±7.12	263.2 ± 6.05
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 *(SKC)):*

DOSE	DAYS		
	1	7	14
CONTROL	60 ± 1.62	60±1.10	60.1±1.04
HIGH DOSE	59.5±1.04	59.4±2.07	59.8±2.04
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.No20: Food intake (gm/day) of Wistar albino rats group exposed to SKC

DOSE	DAYS		
	1	7	14
CONTROL	62.4±1.54	62.2±1.62	62.7±4.06
High DOSE	64.0±2.24	64.4±2.10	64.6±2.70

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

Interpretation

The acute toxicity shows no mortality up to 2000mg/kg. It showed changes in alertness, grooming, gripping and eye closure at touch response. The behavioural changes are normal. Hence the test drug *Sadhakuppai chooranam* is a safe herbal drug and can be used for long time administration.

REPEATED DOSE 28-DAY ORAL TOXICITY (407) STUDY OF *SKC*

Table.No21: Body weight of wistar albino rats group exposed to SKC

DOSE	DAYS				
	1	7	14	21	28
CONTROL	275.2±2.41	275.4±3.17	275.8 ± 2.64	276.2 ± 4.18	277 ± 3.30
LOW DOSE 30mg/kg	275.3 ±2.23	276.5 ± 1.67	276.3 ± 7.87	278.2± 1.40	279.6 ± 5.35
MID DOSE 150mg/kg	278.4 ± 5.75	278.9 ± 4.19	280.3± 5.21	281.2 ±1.40	282.6± 6.16
HIGH DOSE 300mg/kg	280.2±05.64	281.2 ± 10.04	281.9 ± 12.40	282.2±14.40	282.32 ± 12.10
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 SKC

DOSE	DAYS				
	1	7	14	21	28
CONTROL	56.5 ± 2.34	57.0±1.07	55.7±1.30	55.8±1.10	56.9±1.70
LOW DOSE 30mg/kg	54.1±1.81	54.6±2.43	55.6±1.72	57.7±2.36	58.7±1.30
Mid dose 150mg/kg	56 ±1.02	56.7 ±1.21	57.1 ±2.36	56.6 ±5.45	57.4 ±1.64
HIGH DOSE 300mg/kg	57.1±3.40	57.4±1.42	58.7±1.44	59.6±1.78	60.8±2.62

Pvalue (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 SKC

DOSE	**DAYS**				
	1	7	14	21	28
CONTROL	61±3.01	61.2±2.11	62.4±3.11	61.4±3.42	61.12±3.40
LOW DOSE 30mg/kg	57.5±7.12	58.5±1.44	58.9±1.50	59.4±1.20	59.8±3.92
MID DOSE 150mg/kg	58.2±2.14	58.9±3.41	59.1±1.21	61.2±2.18	59.9±2.43
HIGH DOSE 300mg/kg	60.3±1.55	60.6±1.54	61.6±2.16	63.1±2.50	64.16±1.72
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 SKC

Category	Control	Low dose 30 mg/kg	Mid dose 150mg/kg	High dose 300mg/kg	P value (p)*
Haemoglobin (g/dl)	34.3±0.43	34.6±0.30	33.2±0.12	34.6±0.23	N.S
Total WBC ($\times 10^3$ l)	9.1±0.40	10.12±0.01	12.13±1.3	12.96±3.30	N.S
Neutrophils (%)	15.1±0.20	17.12±0.23	17.94±1.67	16.14±1.07	N.S
lymphocyte (%)	80.10±1.36	80.10±1.20	81.48±1.02	81.20±1.34	N.S
Monocyte (%)	0.01±0.02	0.09±0.01	0.09±0.11	0.15±0.03	N.S
Eosinophil (%)	0.04±0.06	0.05±0.03	0.03±0.12	0.02±0.07	N.S
Platelets cells10^3/µl	1400.1±1.08	1417.3±4.84	1422.3±5.43	1429.4±6.32	N.S
Total RBC 10^6/ µl	9.32±0.64	9.32±0.652	9.43±0.98	9.66±0.05	N.S
PCV%	34.60±0.8	33.63±6.23	35.09±2.56	34.8±8.22	N.S
MCHC g/dL	35.2±1.42	36.4±1.22	37.18±2.76	36.8±1.23	N.S
MCV fL(µm^3)	54.8±1.21	53.8±1.20	54.3±0.11	54.7±1.10	N.S

*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.25 : Biochemical Parameters of of Wistar albino rats group exposed to *SKC*

BIOCHEMICAL PARAMETERS	CONTROL	LOW DOSE 30 mg/kg	MID DOSE 150mg/kg	HIGH DOSE 300mg/kg	P Value (p)*
GLUCOSE(R) (mg/dl)	85.10±1.22	85.13±1.31	87.14±2.11	86.7±6.20	N.S
T.CHOLESTEROL(mg /dl)	105.10±3.10	101.15±2.20	101.11±2.01	105.11±13	N.S
TRIGLY(mg/dl)	76.03±1.04	75.04±1.32	74.98±1.26	76.66±1.04	N.S
LDL	69.2±4.13	67.4±1.45	68.8±1.97	69.4±2.22	NS
VLDL	14.6±1.30	13.6±1.42	13.1±3.4	13.14±1.24	NS
HDL	24.12±2.30	25.12±2.30	25.14±5.31	27.65±1.34	NS
Ratio 1(T.CHO/HDL)	5.41±1.10	5.32±1.20	5.89±2.30	5.93±1.60	NS
Ratio 2(LDL/HDL)	2.85±2.13	3.05±1.20	2.96±3.56	2.89±4.02	NS
Albumin (g/dL)	3.23±0.10	3.12±0.64	3.10±1.55	2.93±3.24	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 SKC

PARAMETERS	CONTROL	LOW DOSE 30mg/kg	MID DOSE 150mg/kg	HIGH DOSE 300mg/kg	P Value (p)*
UREA (mg/dl)	22.11±0.10	21.10±0.15	22.09±1.63	22.12±3.45	N.S
CREATININE(mg /dl)	0.6±0.02	0.6±0.03	0.4±0.08	0.3±0.09	N.S
BUN(mg/dL)	27.5±0.03	26.5±0.14	26.02±0.11	27.8±1.40	NS
URIC ACID(mg/dl)	6.04±0.02	6.1±0.20	6.02±0.1	6.2±0.60	N.S

*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.27: Liver Function Test of of Wistar albino rats group exposed to (*SKC*)

PARAMETERS	CONTROL	LOW DOSE 30mg/kg	MID DOSE 150mg/kg	HIGH DOSE 300mg/kg	P Value (p)
T BILIRUBIN(mg/ dl).	0.07±0.07	0.05±0.02	0.06±0.03	0.07±0.01	N.S
SGOT/AST(U/L)	132.1±1.33	130.2±0.32	131±1.32	133.6±1.43	N.S
SGPT/ALT(U/L)	99.10±1.44	99.14±1.10	98.13±1.20	99.23±0.20	N.S
ALP(U/L)	182.40±1.12	180.2±1.14	181.1±1.1	183.3±2.51	N.S

T.PROTEIN(g/ dL)	6.5±0.13	6.3±0.21	6.4±0.22	6.7±0.34	N.S

*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 SKC. All animals survived to study termination period.

Body weight

Increased in body weights were compared to their initial 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 behaviour

Blood analysis

- **Hematology**

No treatment related effects were observed.

- **Biological parameters**

No treatment related effects were observed.

- **Histological examination**

Histological examination of organs did not show any pathological changes.

Discussion

- The acute and repeated 28 days oral toxicity studies of *SKC* showed did not produced any toxicity signs in wistar albino rats. Daily administration of *SKC* at different doses 30mg/kg, 150mg/kg, 300mg/kg for 28 days was tolerated by the rats without any mortality and morbidity, indicates the drug tolerance.

Hence the poly herbal formulation of *SKC* can be considered to be safe drug for prolonged duration use as revealed by toxicological studies.

REPEATED ORAL TOXICITY STUDY

HISTO PATHOLOGY

Interpretation

The above slides show the histopathology studies of sub-acute toxicity. There is no toxicological abnormality seen in the vital organs after administration of the test drug *sadhakuppai 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- ANXIETY ACTIVITY IN ELEVATED PLUS MAZE

The elevated plus maze (EPM) is a model of anxiety that is used as a screening test for putative anxiolytic compounds and as a general research tool in neurobiological anxiety research. The test setting consists of a plus-shaped apparatus with two open 30 x 5 and two enclosed arms 30 x 5 x 25, each with an open roof, elevated 40-70cm from the floor. The model is based on rodent's aversion of open spaces. This aversion leads to the behavior termed thigmotaxis, which avoidance of open areas by confining movement to enclose.

Table.No.28 Effect of *SKC* in Elevated Plus Maze.

Group	Dose	Time spent in open arms in 5 min period	Time spent in closed arm in 5min period
I	Untreated	27.52±2.20	220±1.20
II	Diazepam 2mg/kg	109.24±2.16***	131±2.20**
III	SKC 100mg/kg	79.22 ±6.24*	190±3.44*
IV	SKC 200mg/kg	94.62±4.20**	167±4.22**

The data is expressed as Mean ± SEM.; ANOVA followed by Dunnet's Multiple comparison test. *P<0.05vsControl.

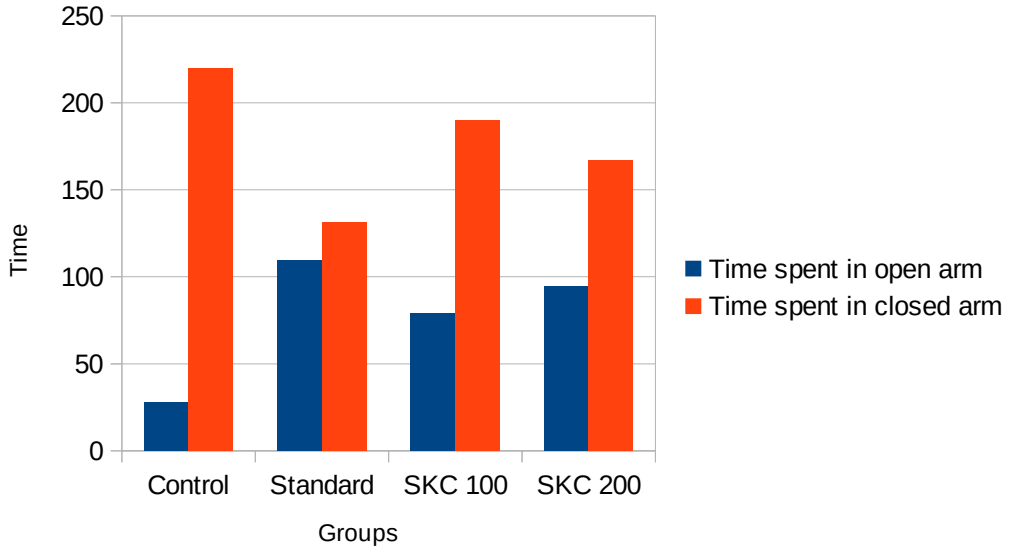


Chart.no.2 Effect of *SKC* in EPM

Table.No.29 Number of entries in open and closed arms

Groups	Time travelled	No of entries in open arm	Average Time Spent in open arm
Control	5 min	4.03±3.20	4.65±1.42
II Diazepam	5 min	8.32±2.22***	15.06±2.64**
III SKC 100	5 min	6.17±4.62*	11.2±3.45*
IV SKC 200	5 min	7.13±1.04**	13.01±3.12**

*The data is expressed as Mean ± SEM.; ANOVA followed by Dunnet's Multiple comparison test. *P<0.05vsControl.*

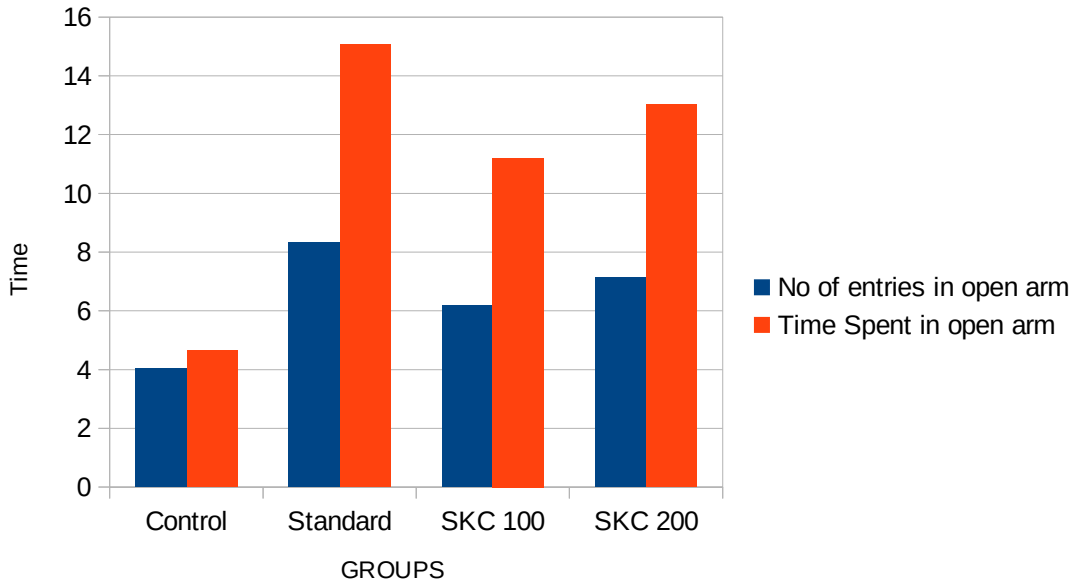


Chart.no.3 No of enteries in open and time spent in open arm.

Interpretation:

In EPM this translates into an estimation of movement to the enclosed arms. Anxiety reduction in the plus-maze is indicated by an increase in the proportion of time spent in the open arms and an increase in the proportion of entries and time spent in the arms. Total number of open arm entries and number of closed-arm entries are usually employed as measures of general activity. From the present study it was found that *SKC* significantly prolonged the time of animals spent in the maze compared to that of the control group. Normally, when rats are placed in a maze they prefer to hide rather than explore, because they are anxious. In this test, rats generally nervous and fearful in the maze were transformed by *SKC* and rats treated with SKC showed a quiet curiosity in exploring their environment.

Antianxiety activity was studied using EPM. In this study, time spent in open arm and closed arm was observed. Time spent in open arm was significantly increased in 200mg dose level than time spent in closed arm. To conclude, SKC 200mg/kg body weight shows the significant effect. Since time spent in open arm is more.

Table.No.30 Result of Antidepressant activity using FST and TST

Group and Treatment	Immobility period forced swim test (FST) (sec)	Immobility period in tail suspension test(TST) (sec)
I Control	114.44±5.42	81.11±2.21
II Imipramine10mg/kg	92..24±6.24**	69.01±2.24*
III SKC100mg/kg	102.22±4.14	73.5±1.48
IV SKC200mg/kg	95.18±3.20*	71.00±2.46*

Statistical analysis of data was carried by one-way ANOVA followed by Dunnet's multiple comparisons test. *p < 0.05 vs Control.

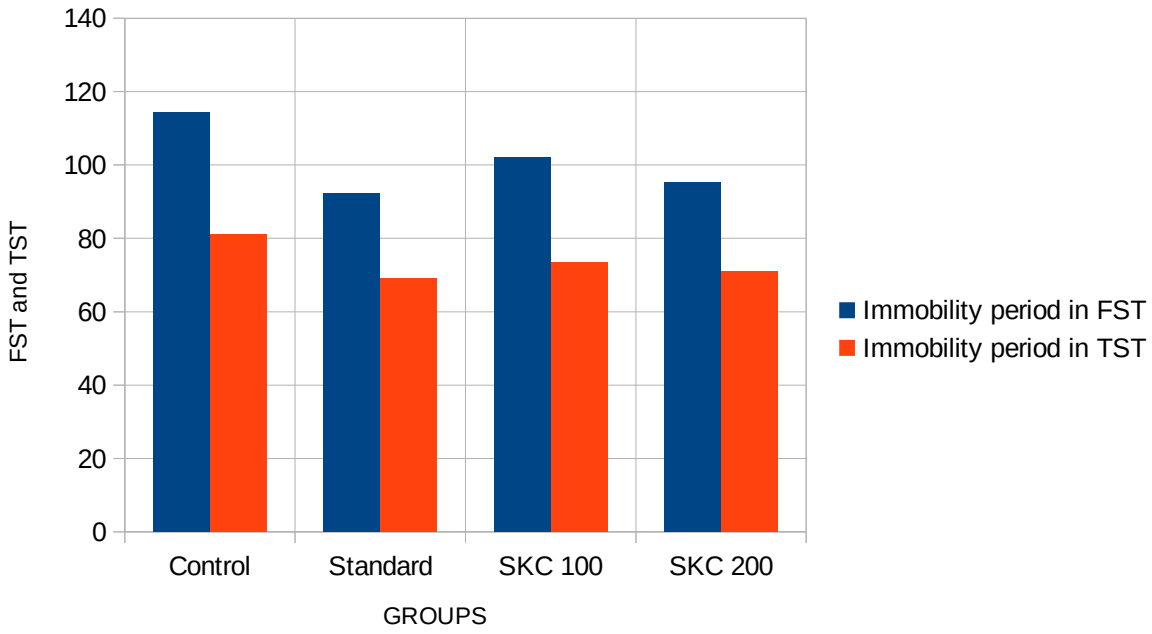


Chart.No.4 Results of anti depressant activity in FST and TST

Interpretation

In this present experiment, Forced swimming test and Tail suspension test were used to evaluate the antidepressant effects of *Sadhakuppai Chooranam* in rats and are tabulated in table. no.30. Both FST and TST are widely used to screen new antidepressant drugs. These tests are quite sensitive and relatively specific to all major classes of antidepressant drugs including tricyclics, 5-HT-specific reuptake inhibitors, MAO inhibitors, and atypical antidepressant drugs.

In FST, rats are forced to swim in a restricted space from which the cannot escape and are induced to a characteristic behavior of immobility.This behavior is reflecting a state of despair which is reduced by several agents which are therapeutically effective in human depression. The TST also induces a state of despair in animals like that in FST. This immobility referred to as behavioral despair in animals, is claimed to reproduce a condition similar to human depression.

The present study provides behavioral evidence for the antidepressant-like activities of *SKC*. From the result, it was observed that *SKC* administration showed a significant activity to reduce the immobility time at doses of 200 mg/kg in forced swimming test and tail suspension test in rats.

Though the antidepressant action of *SKC* was less potent than imipramine based on the given data, the effect of *SKC* as well as other herbal medicine, is slow, mild and prolonged effect without or with mild undesirable side-effects; these are advantages over the classical antidepressants.

Antidepressant activity was carried out using FST and TST in rats. Four groups were divided, each group has 6 rats. Imipramine was the standard drug used. Results show that SKC 200mg/kg body weight is having significant antidepressant activity. The duration of immobility is increased significantly.

ANTI-CATALEPTIC ACTIVITY

Table.No.31 Effect of *SKC* on haloperidol induced catalepsy in mice.

Group	Treatment	Duration of catalepsy (Sec)			
		0min	30 min	60 min	90 min
I	Control	0±0	1.24±1.26	1.10±1.22	1.2±1.10
II	Haloperidol	0±0	1.98± 2.38	4.96± 2.24	6.5±8.20
III	Levodopa	0±0	0±0***	0.24±0.15**	0.30±0.18**
IV	SKC 100mg	0±0	1.20±0.10	1.10±0.32	0.96±0.17
V	SKC 200mg	0±0	0.3±0.24	0.20±2.26**	0.29±0.12**

Statistical analysis of data was carried by one-way ANOVA followed by Dunnet's multiple comparisons test. *p < 0.05 vs Control.

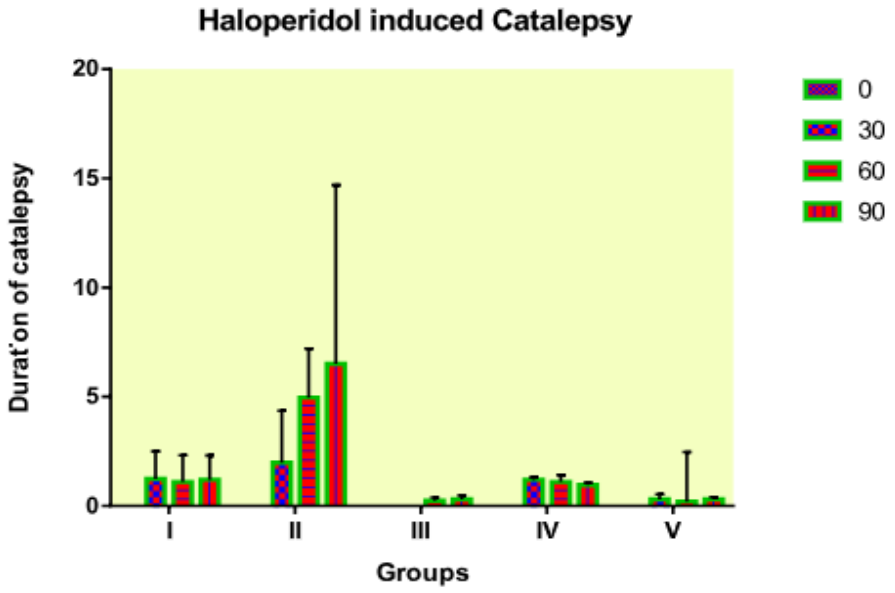


Chart.No.5 Effects of SKC in Haloperidol induced Catalepsy.

Interpretation:

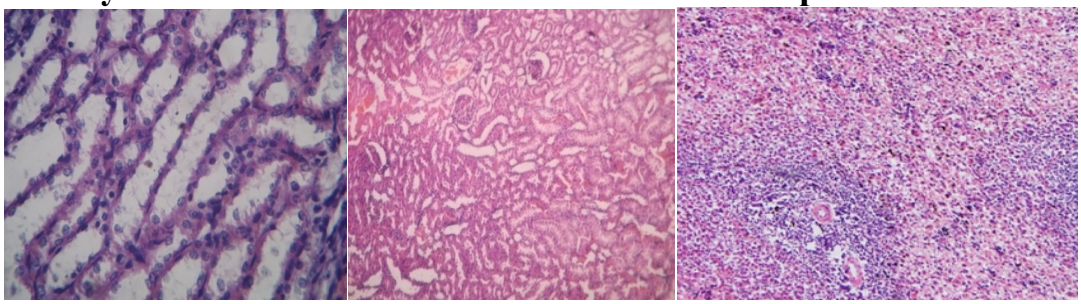
Anticataleptic study was done using Haloperidol induced method. Standard drug used was L-dopa(levodopa). In this study, *SKC* 100 and 200mg dose level was used for the study. *SKC* 200mg showed a significant decrease in the induration of catalepsy in the 60th minute of the study. It concluded that *SKC* 200mg showing potential anti cataleptic effect in Haloperidol induced method.

REPEATED ORAL TOXICITY STUDY

HISTO PATHOLOGICAL SLIDES

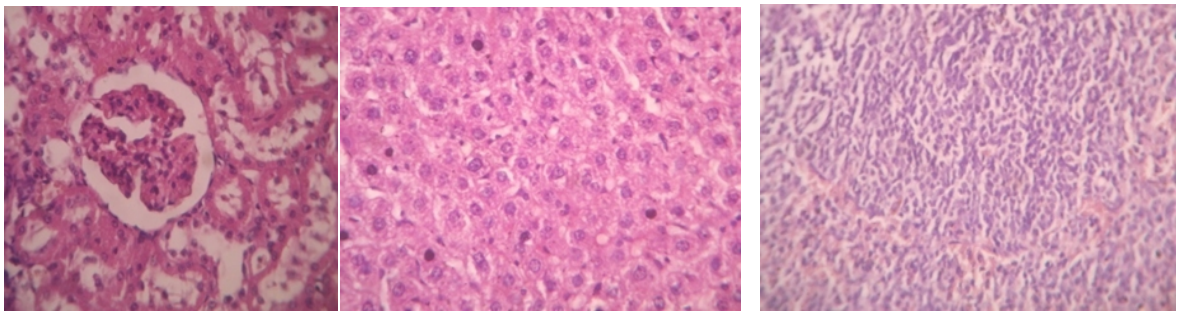
CONTROL GROUP

Kidney **Liver** **Spleen**



HIGH DOSE

Kidney **Liver** **Spleen**



6.CONCLUSION

- Siddha system is highly integrated system; *Maruthuvam* gives a detailed description of mental disorders. This system has a two-way interactive model of the mind-body relationship.

- Hence the author conducts the detailed scientific validation of *Sadhakuppai Chooranam* for Anti-anxiety activity, Anti-depressant activity and Anticataleptic activity.

- To collect the information about the drug in various classical Siddha and modern text books, literature were referred. From them, the author came to an idea about the drug and its efficacy on neurosis.

- The Phytochemical analysis of the drug evaluates that it contains, Alkaloids, carbohydrates, Glycosides, Diterpenes, Flavonoids and Tannins, which contributes much in relieving the symptoms of Neurosis.

- Chemical analysis of the drug contains Calcium, magnesium and copper which involves improving normal mental health in anxiety and depression.

- SEM analysis represents the drug contains Nano particles. And XRD analysis concluded that the range 15-36 nm of this drug.

- The Preclinical study showed that the drug has got safety and significant Anti-anxiety, Anti-depressant and Anticataleptic activities.

- An incredible action of this drug value against the disease of Neurosis has been revealed from this study of *Sadhakuppai Chooranam.* This must be implicated in the future clinical studies and preclinical chronic toxicity studies to prove its beneficial effect in the treatment of neurosis in future.

7. SUMMARY

The trial drug ***Sadhakuppai Chooranam*** was selected from the Classical Siddha literature, ***"Agasthiyar Attavanai Vagadam"*** for the evaluation of safety and efficacy of the drug in "*Paithiyam"* (Neurosis).

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

Various analysis such as physicochemical, phytochemical, biochemical analysis, availability of bacterial and fungal load, instrumental analysis were made. The physical character of *SKC* shows good solubility and the pH of the trial drug is 5.65. Phytochemical screening test showed the presence of Alkaloids, Carbohydrates Glycosides, Saponins, Diterpenes, Flavonoids and Tannins are responsible for the anti-anxiety, antidepressant and anticataleptic activity. The HPLC results were made and it shows the prescence of Gallic acid, Flavanoids as Quercetin , Alkaloids, Tannic Acid.

Biochemical analysis showed the presence of Calcium, Magnesium, Copper, Chloride and Phosphate. Calcium, Magnesium and Copper supports anti-anxiety and antidepressant activity of the trial drug *(SKC).*

The availability of bacterial load in the *(SKC)* 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 peak values present which are the functional groups responsible for its activity. SEM picture described its morphology and the particle size ranging from 10µm-20µm. The result of ICPMS shows the heavy metals like As, Hg, Cd and Pb were below detectable level. This reveals the safety of the drug.

Toxicological study of both acute and sub-acute toxicity study were carried out in animal model Wistar albino rat according to the OECD guidelines. The test

drug showed no acute toxicity as there was no mortality seen. The sub-acute toxicity after the repeated dose of 28 days was done

The mortality, functional observations, haematological and biochemical investigations were made. There was no significant change seen in the normal values. Thus the toxicological study of the test drug greatly establishes the safety and gives the justification for long time administration.

The pharmacological study was carried out in the animal model. Three activities were seen in the drug *Sadhakuppai Chooranam*. The activities were

- Anti-anxiety activity in Elevated plus maze
- Anti-depressant activity in Forced swimming test and Tail suspension test
- Anticataleptic activity in Haloperidol induced catalepsy in rats

Anti-anxiety activity was carried out in Elevated plus maze. The trial drug *Sadhakuppai Chooranam* - 200mg/kg b.w showed significant decrease in Anxiety condition. Thus this activity reveals the effect of the drug against Anxiety.

Antidepressant activity of *Sadhakuppai chooranam*-200mg/kg b.w showed significant activity to reduce depression. Thus, this activity therapeutically effective in depressed condition.

Anticataleptic activity of the test drug *Sadhakuppai Chooranam* -200mg/kg b.w was carried out in haloperidol induced catalepsy in rats. From the present study, it was concluded that the *SKC* extract has a marked Anti-cataleptict activity.

Thus by scrutinizing all the above mentioned factors it is concluded that the trial drug ***Sadhakuppai Chooranam*** is a safe and a potent Anti-anxiety, Antidepressant and Anticataleptict drug. Modern medicine has its own limit in

8. FUTURE SCOPE

The trial drug ***Sadhakuppai Chooranam*** has its own potency in treating Anxiety, Depression and Catalepsy in animal model which has been established in this study. An incredible action of this drug value against the disease of Neurosis has been revealed from this study of *Sadhakuppai Chooranam.* This must be implicated in the future clinical studies and preclinical chronic toxicity studies. So it could be used worldwide in treatment of Neurosis.

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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 SADHAKUPPAI CHOORANAM & PIDANGUNARI (*Premna tomentosa*) 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/14/CLBMCP/2016

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

IAEC Member Secretary

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

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Government Siddha Medical College

Arumbakkam, Chennai _ 600 106

CERTIFICATE

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

Ingredients:

1. Sadhakuppai (*Anethum graveolans*)
2. Seeragam (*Cuminum cyminum*)
3. Perungeergam (*Foeniculum vulgare*)
4. Karungeeragam (*Nigella sativa*)
5. Elam (*Elettaria cardomomum*)
6. Adhimadhuram (*Glycyrrhiza glabra*)
7. Kothamalli (*Coriandrum sativum*)
8. Lavangam *(Syzygium aromaticum*)
9. Cinna lavangapattai (*Cinnamomum verum*)
10. Seena karkandu (*Saccharum officinarum*)

Date: 25.8.2017

Place: Chennai

25/8/17

PG Department of Gunapadam

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

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

This Certificate is awarded to Dr/Mr/Mrs.....A. ADAIKKALADEVI.....

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

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