# PRECLINICAL SAFETY EVALUATION OF

# "AAGASAGARUDAN KIZHANGU CHOORANAM"


The dissertation Submitted by

**Dr. V.M. KARTHIC M.D(S)**

Under the Guidance of

**Dr. S. MURUGESAN M.D(S)**

Lecturer and Guide, Department of Nanju Noolum Maruthuva Neethi Noolum,

National Institute of Siddha, Ch-47.


Dissertation Submitted to

**THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY, CHENNAI – 32**

*For the partial fulfillment of the requirements to the Degree of*

**DOCTOR OF MEDICINE (SIDDHA)**

**BRANCH-VI - DEPARTMENT OF NANJU NOOLUM**

**MARUTHUVA NEETHI NOOLUM**

**2015 – 2018**


**NATIONAL INSTITUTE OF SIDDHA,**

**Chennai–47**

## DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"Preclinical safety evaluation of Aagasagarudan Kizhangu Chooranam**" is a bonafide and genuine research work carried out by me under the guidance of **Dr.S.Murugesan, M.D(S)**, Guide, **Department of Nanju Noolum Maruthuva Neethi Noolum**, National Institute of Siddha, Chennai -47 and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or another similar title.

Date: Signature of the Candidate

Place: Chennai-47 Dr. V.M.KARTHIC

## BONAFIDE CERTIFICATE

Certified that I have gone through the dissertation submitted by **Dr.V.M.Karthic (Reg No: 321516204)** a student of final year MD(S) Branch VI, **Department of Nanju Noolum Maruthuva Neethi Noolum**, National Institute of Siddha, Tambaram Sanatorium, Chennai – 47, and the dissertation work has been carried out by the individual only. The dissertation does not represent or reproduce the dissertation submitted or approved earlier.

Place: Chennai-47
Date:

**Dr. S. MURUGESAN M.D(S)**
Dept. of Nanju Noolum Maruthuva Neethi Noolum,
National Institute of Siddha,
Tambaram Sanatorium,
Chennai -47.

**Dr. R. MADHAVAN M.D(S)**
Dept. of Nanju Noolum Maruthuva Neethi Noolum,
National Institute of Siddha,
Tambaram Sanatorium,
Chennai-47.

Forwarded by the Head of Institution

**Prof. Dr.V.BANUMATHI M.D(S)**
Director,
National Institute of Siddha,
Tambaram Sanatorium,
Chennai-47.

**ACKNOWLEDGEMENT**

- I thank **God** for giving me this occasion, as long as strength and power to accomplish this obligation.
- I acknowledge my sincere thanks to the **Secretary, Ministry of AYUSH**, Health & Family welfare, New Delhi for giving great opportunity to carry out P.G and this dissertation work in National Institute of Siddha, Chennai-47
- I express my sincere thanks to the **Vice-chancellor,** The Tamil Nadu Dr. M.G.R. Medical University, Chennai-32
- I express my profound sense of gratitude to **Prof. Dr. V. Banumathi, M.D(S),** Director, National Institute of Siddha, Chennai- 47 for granting permission to undertake a study in this dissertation topic and also for providing all the basic facilities in order to carry out this work.
- I cordinally register my sincere thanks to my guide **Dr. S. Murugesan M.D(S)** Lecturer, Department of Nanju Noolum Maruthuva Neethi Noolum, National Institute of Siddha for his valuable guidance and encouragement in carrying out this work.
- I express my sincere thanks to **Dr. R. RengaSundari, M.D(S)**, Assosiate professor, Department of Nanju Noolum Maruthuva Neethi Noolum, National Institute of Siddha, Chennai-47 for the ceaseless care and support throughout my academic period and in my work.
- I would like to express my sincere thanks to **Dr. R. Madhavan M.D(S),** Lecturer & Head of the Department.i/c, Department of Nanju Noolum Maruthuva Neethi Noolum, National Institute of Siddha, Chennai-47 for his encouragement and support to my study.
- I would like to express my heartfelt thanks to my Lecturer **Dr.P.Shanmugapriya M.D(S),** Department of Nanju Noolum Maruthuva Neethi Noolum, National Institute of Siddha, Chennai-47 for her presence and valuable guidance in my study
- I express my grateful to my Lecturer, **Dr. V. Manjari, M.D(S),** Department of Nanju Noolum Maruthuva Neethi Noolum, National Institute of Siddha, Chennai-47 for the guidance, memorable support and continuous encouragement for giving valuable suggestions to do this study.

- I am thankful to **Dr. D. Aravind M.D(S),** Associate professor, Department of Botany, National Institute of Siddha, chennai-47 for his guidance in drug authentication
- I extend my thanks to **Dr. A. Muthuvel M.Sc, Ph.D**(Biochemistry) Associate professor, National Institute of Siddha, Chennai-47 for his guidance in doing chemical studies.
- I am thankful to the team of **Noble Research Solutions**, Chennai for providing the support and genuine reports in Toxicity studies.
- My special acknowledgements to **Mr. M. Subramanian M.Sc (Statistics),** Senior Research Officer, National Institute of Siddha, Chennai- 47, for his valuable help in statistical analysis.
- I would like to thank **Dr. V. Suba M.Pharm, Ph.D,** Associate professor of Pharmacology, National Institute of Siddha, Chennai-47 for her interesting teaching of pharmacology and valuable guidance to do this study
- I am grateful to the assistance provided by **Dr. Gayatri Natarajan M.V.Sc,** Veterinarian, National Institute of Siddha, Chennai-47 in this study.
- I wish to dedicate this work to my wife, **Dr. B. Poongodi M.D(S)** for her dedicated support in every stage of this work and sacrifice for me.
- I thank **my family members** for their support in this work.
- I thank all my department juniors for providing their cooperation throughout the study, without whom this work will be impossible.
- I remind thankfully all the animals that lost their lives for the sake of my study and without which I would not have been successful in my study.

# CONTENTS

# INTRODUCTION

The Siddha system (Tamizh Maruthuvam) of medicine is the traditional medical systems in the world. The uniqueness of Siddha system is evident by its constant service to the humankind by the great Siddhar's for more than 5000 years in combating diseases with various herbals, metals/minerals and animal products having several therapeutic indications.

The Siddhar's believes there is a divine power beyond them in achieving their goal and it is not been attained by searching outside. The following stanzas from *"Kagapusundar Gnanam 80"* texts written by Siddhar Kagapusundar clearly states that "the divine power is inside you, without knowing it why this mankind is wasting their time in searching for several forms of divine power.

"தானென்ற பிரமத்தை யடுத்திடாமல்
தரணியில் தெய்வமடா அனந்த மென்றும்"
- காகபுசுண்டர்

The Siddha System contains unlike blend of remedy for similar ailment. Medicine will be handled by the Siddha physician based on Ayim bootham, Aaru aatharam, suvai, pragiruthi, Paalinam, three humors like Vali, Azhal, Iyam etc. This indicates individualism of siddha medicine purely depends on Siddha physiology to validate the root cause of disease by Envagai thervugal, especially Naadi-pulse reading method. It is good for a physician to diagnose the disease, trace its cause, how it may be moderated and then to use his expert skill.

Siddha system of medicine provides the healthiness and care through Prophylaxis (*Noyillaneri*), Treatment (*Maruthuvam*) and Rejuvenation (*Kayakarpam*). For a physician, it nourishes knowledge about the art of diagnosis (*Noi Nadal*), pharmacology (*Gunapadam*) and toxicology (*Nanjumurivu nool*).

Siddhars are excellent in the documentation work and they taught the above said sciences to their disciples too. They wrote their works in manuscripts which is the spinal column for generations of Traditional healers and Siddha physicians.

In Siddha system of medicine, Chooranam based on pure herbals is one of the first line of medicine for the several diseases. The shelf-life of chooranam is three months[1]

Siddha system of medicine is self sufficient to meet the needs of public health. It is high time to work on the feature assertion, safety and efficiency of Siddha formulations to make our system advantageous for inhabitants at large.

The branch of Toxicology is developed by our forefathers to save the mankind. They discussed about the toxic effect of drugs and noxious bites with treatment to prevent the mortality circumstances. Siddhar's and traditional healers have well knowledge about the safety aspects in therapeutic practice.

The *Aagasagarudan kizhangu* is commonly called as "*Kollankovai kizhangu", "Garudan", "Aagayagarudan", "Peicheenthil"*, etc. According to Thiru.T.V.Sambasivam pillai dictionary, the word *'Aagasagarudan'* refers to 'root living in air'. It is a climbing shrub with yellow flowers[2]. The therapeutic uses of *Aagasagarudan kizhangu* are indicated for Virulent poison (Kodiya nanju), Anemia (Paandu), Pruritus (Namaichal), Thyroidism (Kazhuththu Kazhalai), Leprosy (Perunoi), Herpes zooster (Akkipun)[3].

I wished for working in the field of Siddha toxicology and was permitted to do the safety evaluation of '*Aagasagarudan kizhangu Chooranam*'- a Siddha herbal preparation which is indicated for several illness and virulent noxious bite. Snake bite is a life threatening problem causing mortality from ancient period to till date. About 94,000 snake bite deaths are recorded globally and 15000 in India per year[4]. Even today treating the noxious cases is a dare to the medical profession, but treating such noxious cases through the Siddha system of medicine seems to be more abundant and cost effective, hence this meticulous formulation had been preferred in a broad vision.

# AIM AND OBJECTIVES

**AIM:**

To evaluate the safety profile of **"AAGASAGARUDAN KIZHANGU CHOORANAM"** – A herbal preparation

**OBJECTIVES:**

- ***Aagasagarudan Kizhangu*** authenticated by Botanist.
- To do the following studies on ***Aagasagarudan Kizhangu Chooranam***.
  - Physicochemical analysis
  - Biochemical analysis
  - Heavy metal analysis using ICP-OES
  - Phytocompound analysis using GC-MS
  - Organic Functional group analysis using FT-IR
  - Acute Oral Toxicity study as per WHO guideline
  - Long term Oral Toxicity study as per WHO guideline

# REVIEW OF LITERATURE

## 3.1. ஆகாசகருடன்

**வேறு பெயர்:**

- ஆகாசகெருடன்
- ஆகாயகருடன்
- கருடன்
- கொல்லன் கோவை
- பேய்ச்சீந்தில்

இது ஏறுகொடித் தாவரம் ஆகும். இந்தியாவில் எங்கும் பயிராகும். தென்னிந்தியாவில் தமிழ்நாடு, கர்நாடகம், மேற்குத் தொடர்ச்சி மலையின் அடிவாரக் காடுகளில் வளர்கின்றது.

வேர்க்கிழங்கில் கசப்பான **'ப்ரியோனின்'** என்னும் வேதிப்பொருள் உள்ளது[5].

**பயன்படும் உறுப்பு** - வேர் முதலியன, சிறப்பாகக் கிழங்கு

**சுவை** - கைப்பு

**தன்மை** - வெப்பம்

**பிரிவு** - கார்ப்பு

**செய்கை:**

- உடற்றேற்றி
- உரமாக்கி

**வேரின் பண்பு:**

சூலையாண் டூதைதிரி தோடமக்கி வெப்புகண்ட
மாலை குடலின்வலி மாகுட்டம் - ஆலவிடம்
உட்கரப்பான் மெய்யரிப்பும் உண்டோகொல் லங்கோவை
கைகு ளிருக்கவின்னு ங்காண் (அகத்தியர் குணவாகடம்)

கொல்லன் கோவை வேர் முதலியவைகளால்,

- ✓ சூலை
- ✓ பாண்டு
- ✓ முக்குற்றம்
- ✓ அக்கிப்புண்

- ✓ உட்சூடு
- ✓ கழுத்துக் கழலை
- ✓ குடல்வலி
- ✓ பெருநோய்
- ✓ நஞ்சுகள்
- ✓ கரப்பான்
- ✓ நமைச்சல் ஆகிய இவைகள் நீங்கும்.

கிழங்கின் குணம்:

துட்டவிடம் பாண்டுவெப்பு சூலைவா தங்கிரந்தி
குட்டம் அரிப்பக்கி கோண்குடல் நோய் - கெட்டகண்ட
மாலைபோம் கொல்லன்கோ வைக்கிழங்கால் முத்தோட
வேலைபோம் பாரில் விளம்பு. (அகத்தியர் குணவாகடம்)

ஆகாசகருடன் கிழங்கு

- ✓ கொடிய நஞ்சு
- ✓ பாண்டு
- ✓ உட்சூடு
- ✓ சூலை
- ✓ கிரந்தி
- ✓ பெருநோய்
- ✓ நமைச்சல்
- ✓ அக்கிப்புண்
- ✓ குடல் நோய்
- ✓ கழுத்துக்கழலை
- ✓ முக்குற்றக்கேடு ஆகிய இவைகளைப் போக்கும்[3]

**தூய்மையாக்கும் வழி (பொதுவானது):**

கிழங்கின் மேற்றோலை நீக்கி நிழலிலுலர்த்தி இடித்துச் சூரணம் செய்து ஒரு புதுப்பாண்டத்தில் பாலைவிட்டு சீலையால் ஏடுகட்டி அதில் இச்சூரணத்தை

வைத்து மேல்சட்டி மூடி, ஒரு சாமம் எரித்த பின்பு சூரணத்தை ரவிமுகத்தில் உலர்த்தி அரைத்தெடுத்துக் கொள்க[6].

**வழக்கு:**

- **சகல நாகங்களின் விஷம் தீர:**
  குப்பைமேனி, அவுரி, ஆவிரை, கொல்லன் கோவை, கீழ்காய் நெல்லி - இவைகளின் சாறு 1/16 படி உள்ளுக்கு கொடுத்து மேலுக்கும் பூசவும்.
- **பூரான் கடி நஞ்சுக்கு முறிவு:**
  ஆகாசகருடன் கிழங்கைச் சிறு சின்னியிலையின் சாறுவிட்டு அரைத்து வேளைக்குக் கழற்சிக்காயளவு காலை மாலை இரண்டு வேளையும் மூன்று நாள் உண்டு அம்மூன்று நாளும் வெளியில் வராமல் அறையினுள்ளேயே இருக்க வேண்டும். (குப்பைமேனி சாற்றைப் பூசுவதினாலும் தீரும்) இதற்கு ஏழு நாள் வரைக்கும் புளியாகாது.
- **கருந்தேள் கடித்த நஞ்சு தீர:**
  கொல்லன் கோவைக் கிழங்கை அரைத்துக் கொடுக்க வேண்டும்.
- **நட்டுவக்காலி நஞ்சிற்கு முறிவு:**
  கொல்லன் கோவை கிழங்கை வெந்நீரிலரைத்து உள்ளுக்குக் கொடுக்க நஞ்சு இறங்கும்.

**பாம்பு நெருங்காமற் தடுக்கும் மனை மூலிகைகள்:**

கண்டுகொள்வாய் சொல்லுகிறே னுலகோர்க் கெல்லாம்
காரமா மூலியடா பங்கம் பாளை
கொண்டுவந் துன்மனையில் வைத்தி ருந்தால்
கொடியவிட மணுகாது குடியோ டிப்போம்
நன்றான நாகதாளிக் கிழங்கு தானும்
நன்மனையி லிருக்கவிடம் நாடா தப்பா
அன்றான ஆகாசக் கருடன் மூலி
அம்மனையி லிருக்கவிட மற்றுப் போமே.

(சி.ஆ செய்யுள் 27)

ஒவ்வொரு வீட்டிலும் மூலிகைகளை வைத்து வளர்த்து வருதல் நலம். அவற்றில் சிறந்த இனங்கள் வீட்டில் வளர்க்கப்படுவதால் பாம்பு போன்ற உயிர்கள் வராது. அம்மூலிகைகள் பின்வருமாறு:

- பங்கம்பாளை
- ஆடுதின்னாப்பாளை
- நாகதாளிக் கிழங்கு
- ஆகாசகருடன் கிழங்கு
- சிறியாநங்கையும் இவ்வினத்தைச் சார்ந்த மூலிகையாகும்[7]

# CORALLOCARPUS EPIGAEUS

A genus of climbing and trailing herbs, distributed in the tropical Asia, Africa and Malagasy. About five species occur in India.

## HABITAT:

A perennial climber, found in dry, arid areas throughout India. Roots conical or napiform, yellowish white, marked externally with circular rings; stems slender, grooved with simple, glabrous tendrils; leaves variable in shape and size, usually 2 – 10 cm long, pubescent, 3 – 5 lobed, long petioled; male flowers 4 – 15, in clusters, yellowish green; female flowers solitary or fasciculated on glabrous peduncles; fruits scarlet except in beak and base, ellipsoid or ovoid, beaked, 12 – 16 cm x 7 – 8 mm; seeds 6 – 9, ellipsoid or pyriform, brown or yellow, embedded in orange pulp.

The root has a bitter and sub-acidic taste and is mucilaginous. When cut, it exudes a viscid juice which soon hardens into an opalescent gum. It contains bitter principles cucurbitacin B and another allied to bryonin. The root is credited with aperients, alternative and emetic properties; a paste of it is applied o swelling.[8] A liniment made from it along with cumin seeds, onion and castor oil is applied in chronic rheumatism. The fruit is a drastic purgative and emetic [9].

Flowers and fruiting on June to October [10]. The root contains phydroxybenzoyl ester, named epigaeusyl ester, a sesterpene lactone, viz. corallocarpscalaride, a pyridine carboxylic ester, designated as corallocarpeonyl ester [11]. Deccan and Mysore

the root has repute as a remedy for snakebite administered internally and applied to bitten part [12].

**Parts Used** : Whole plant and Rhizome.

**Odour** : Pungent

**Taste** : Bitter.

**Indications** :

It was used for many inflammatory diseases.

Effective in Asthma, Bronchitis. [13]

## SYNONYMS:

- **BRYONIA EPIGAEA**

**Common name** **:** Redfruit creeper[14]

**Tamil** **:** Akasagerudan kizangu, Kollankovai

**Malayalam** **:** Kollankova, Kollanhova-kizhauna, Nagadonda

**Telugu** **:** Akashagadda, Makkasagaddah, Nagadondagadda

**Kannada** **:** Akashagaruda-gaddah

**Bengali** **:** Rakasgaddah

**Gujarathi** **:** Kadavinai

**Hindi** **:** Akasgaddah, Rakasgaddah

**Marathi** **:** Akashgarudand, Kadavinai

**Madhya Pradesh** **:** Keerkand, Mirchakand

**Rajasthan** **:** Kadawi-nai, Mirchi-kand[8]

**Sanskrit** **:** Kadamba[14]


**Fig: 1. AAGASAGARUDA KIZHANGU PLANT**


**Fig: 2. AAGASAGARUDA KIZHANGU**

## TAXONOMICAL CLASSIFICATION[15]:

Kingdom : Plantae

Clade : Angiosperms

Clade : Eudicots

Clade : Rosids

Order : Cucurbitales

Family : Cucurbitaceae

Subfamily : Cucurbitoideae

Tribe : Coniandreae

Genus : Corallocarpus

Species : Epigaeus

**Table 1: Types of Species in Genus Corallocarpus[16]:**

| Types of Species | Native |
|---|---|
| Corallocarpus epigaeus | India, Oman, Africa |
| Corallocarpus bainesii | Africa |
| Corallocarpus boehmii | Africa |
| Corallocarpus Welwitschii | Africa |
| Corallocarpus triangularis | Africa |

In many developing countries, Traditional medicine plays an important role in meeting the primary health care needs of the population. Some studies have shown that individuals choose Traditional medicine for various reasons, including an increasing dissatisfaction with existing health-care services, and a rekindled interest in

'whole person care' and disease prevention which are more often advocated in Traditional medicine[17]

In Gunapadam (Mooligaivaguppu) text book, Aagasagarudan kizhangu (Corallocarpus Epigaeus) Chooranam is mentioned for Namaichal, which is the primary sign in the Kaanaakkadi. Several scientific researches on tuber of Aagasagarudan kizhangu reported to possess Anti-snake venom, anti-diabetic, anti-fungal, anti-steroidogenic, anthelmintic, anti-inflammatory, analgesic, spasmolytic activities[18] .

**NUTRITIONAL VALUES**: [19]

The Nutritive elements per 100 grams of methanolic extracts of tuber contain

Carbohydrates - 46.43 mg

Protein - 21.17mg

Fat - 2mg

Crude fibres - 5 mg,

Calcium - 0.225 mg,

Magnesium - 0.124 mg,

Phosphorus - 0.049 mg,

Iron - 0.006 mg

Vitamin C - 0.051 mg

Calories - 288.4mg

**CHOORANAM**:

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

**Shelf life of Medicines**:

The shelf life of medicines indicates the Potency of medicines. Siddha medicines can be classified into internal and external medicines. Chooranam comes

under internal medicines. As per Siddha literature Agamarunthu paadal in Gunapadam thathu jeevam text,

"உயர்சூர ணம்பிட்டு வடகம் வெண் ணெய்நான்கி
னுயிர்மூன்று திங்களெண்ணெய்....."

From the above quote the shelf life of chooranam (powder) is three months, but according to AYUSH guidelines the shelf life of chooranam is one year [20]

## RESEARCH ARTICLES PUBLISHED IN JOURNALS:

### Anti-allergic Activity

Kollankovai kizhangu is one of the ingredient in drug G7 (manufactured and marketed by Dr. JRK Siddha Research and Pharmaceuticals Pvt. Ltd., Chennai, India) which is effective in preventing histamine release from mast cells, reported to have anti allergic activity[21]

### Anti-diabetic Activity

The alpha amylase inhibition assay by DNS method revealed that the methanol extract showed inhibition of α-amylase enzyme activity at low concentration. The presence bioactive compound conferring the amylase inhibition in the methanol extract of tuber could possibly contribute to the anti-diabetic activity[22,23]

### Haemopoietic Activity

According to this clinical study, Corallocarpus epigaeus rhizome Choornam on Pandu Rogam with special reference to iron deficiency anemia showed moderately significant result in improving hemoglobin concentration and significant result in the relieving of symptoms like Exertional tiredness, Palpitation, Paleness and Tachycardia. Effectiveness of drug is discussed on the basis of suvai, Veeriya, Vipaka, action and properties of selected plant[24]

### Anti-cancer Activity

This current study is a preliminary effort to evaluate cytotoxic potential of whole plant ethanolic extract of Corallocarpus epigaeus against K562 cell lines

employing Trypan blue and MTT In-vitro cytotoxic assay. The study revealed a dose dependent reduction in number of K562 cell lines upon single exposure for 48hrs which indicates that the extract may be capable of altering the membrane permeability and functioning of mitochondrial dehydrogenase synthase. Further studies were implicated to identify individual phyto-constituents responsible for cytotoxicity and elucidation of probable mechanism of action may lead to the development of promising natural agents in the treatment of Chronic Myeloid leukaemia[25]

**Antimicrobial Potency**

This study revealed that the medicinal plant Corallocarpus epigaeus has the capability of Antifungal as well as antibacterial activity. The phytochemical analysis revealed that the phytochemicals present in the tuber contributes to antimicrobial activity[26]

**Antioxidant and anti-inflammatory Activities**

This study, the different extract of Corallocarpus epigaeus rhizomes was found in valuable levels total free phenol, tannins and flavonoid which is promising antioxidant activity. The extract of Corallocarpus epigaeus rhizomes exhibits potential anti-inflammatory effect. The extract is almost compared with the standard indomethacin. The present study supports the traditional medicine system, of the rhizome for their use in antioxidant and anti-inflammation[27]

**Invitro Cytotoxic Activity**

This study, the root extract of Corallocarpus epigaeus showed cytotoxic activity against HT-29 and MCF-7 cell lines with the percentage mortality increased with an increase in concentration[28]

**Antidote Activity**

Field survey undertaken in 12 mandal areas out of 62 mandals of Chittoor district revealed the occurence of 31 medicinal plants especially used as antidote ones against snake-bite. The possible number of tribal doctors that prefer the individual plant species for treating snake-bite. Of all the plants 3 plant species like

Aganosmacymosa, Corallocarpus epigaeus and Randiadumetorum were found to be preferred by more than 70% of tribal doctors to treat snake-bites[29]

**Chemical and Biological properties**

Bryonia epigaea (Rottler) is the most instinctive plant having wide range of activities. Wide ranges of compounds were analyzed in methanolic extract. The antimicrobial efficiency of Bryonia epigaea (Rottler) aerial part extracts showed good results against various pathogens Methanol extract showed good anti-oxidant activity. Bryonia epigaea (Rottler) aerial parts were analyzed for its biological activities and further isolation and charecterization of the compounds with biological activities will certainly add a valuable invention in the field of drug discovery[30]

**Anti-Snake Venom Activity**

Invivo antisnake venom studies of the methanolic extract of Corallocarpus epigaeus reveals significant antisnake venom activity and could have a promising role in the treatment of Russell's viper snake bite. The exact mechanism of antisnake venom and isolation of active constituents has to be evaluated[31]

**Antidiabetic Activity**

This study, it can be concluded that the ethanolic extract of Corallocarpus epigaeus rhizomes possesses the antidiabetic action which is comparable with that of the standard Glibenclamide drug employed. This work supports the traditional claim of the rhizomes for their use in diabetes[32]

**Anthelmintic Activity**

The anthelmintic activity was evaluated on adult Indian earthworms *Lampito marutii, Eudrillus eugine, Eisenla foetida* due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human being and also in intestinal roundworms. The method of Mathew et al was followed for anthelmintic screening[33,34,35,36]

**Antifungal Activity**

The antifungal activity of petroleum ether, hexane, chloroform, acetone and methanol extracts obtained from Corallocarpus epigaeus revealed that only the methanol extract showed a good antifungal activity against the fungal strains[37]

**Anti-inflammatory and analgesic Activities**

Kollankovai kizhangu is one of the ingredients in the sasthric Siddha herbo-mineral drug Gendhaga Vallaathi which is effective in rheumatoid arthritis. The ingredients of this drug possess various activities such as Anti-inflammatory, analgesic, antipyretic, antioxidant and immunomodulatory activity[38]

## IMPORTANCE OF SUDDHI IN SIDDHA SYSTEM OF MEDICINE

The word "*Suddhi*" means "to get rid of impurities"[39]. The concept of Suddhi (Purification) in Siddha text is not only a process of Purification/detoxification. It also enhances the potency and efficacy of the drug. Researches in various Herbal, Mineral and Metal purification process reveals the following changes occur,

1. Elimination of physical impurities
2. Organoleptic changes
3. Changes the hardness of a drug
4. Reduction in particle size
5. Reduction of toxic substance
6. Changes in chemical structure
7. Changes in Elements
8. Changes in the Pharmacological action

**Recent Researches in various purification process**

**1. Elimination of physical impurities**

- By purification process the physical impurities of the drug like, sand particle, mud, insect, foreign particles get removed.

**2. Organoleptic changes**

- Raw Croton was Blackish in colour, it was changed to Brown in purified one[40]
- The colour of sulphur before purification was bright yellow and shiny in nature, it was changed to yellow colour in after purification.
- The smell of sulphur in before purification is pungent in nature, after it was turned to odourless[41].

# 4. MATERIALS AND METHODS

## COLLECTION:

The Aagasagarudan kizhangu were collected from a reputed raw drug shop at Broadway Chennai.

## AUTHENTICATION:

The Herbal drug Aagasagarudan kizhangu is identified and authenticated by Assistant Professor, Department of Medicinal Botany, National Institute of Siddha, Chennai-47.

## PREPARATION OF TEST DRUG:

## SELECION OF TEST DRUG:

The test drug Aagasagarudan kizhangu was selected for the evaluation of toxicity studies in Wister albino rats.

## INGREDIENTS:

- Aagasagarudan kizhangu (Corallocarpus epigaeus) – 1 part
- Naattu sarkkarai – equal part

## METHOD OF PURIFICATION AND PREPARATION:

Decayed parts or the mud sticking to the Aagasagarudan kizhangu are removed and then the skin of tuber was peeled. The skinless tuber was sliced and dried in sunshade. Then it was grounded well to obtain fine powder and purified through *'pittavial* process. After this process, the dried powder was sieved through white cloth and it was mixed with equal quantity of Naattu sarkkarai and stored in an air tight container and it was labelled as *Aagasagarudan Kizhangu Chooranam***.**

## Pittaviyal Murai (Milk steaming process):

The *Aagasagarudan Kizhangu Chooranam* was purified by Pittaviyal process as per Siddha classical literature. A mud pot was taken and it was quarter filled by

water. The mouth of the pot was sealed by cloth. This Chooranam was then placed over the cloth and the pot was covered with lid and heated. After this process the powder was dried, sieved, mixed with sieved Naatu sarkkarai and stored in an air tight container [42].

**THERAPEUTIC DETAILS OF AAGASAGARUDAN KIZHANGU CHOORANAM:**

| | | |
|---|---|---|
| Form of the Medicine | : | Chooranam (Powder) |
| Route of Administration | : | Enteral |
| Clinical dose | : | Half to One Varaagan (2-4 grams) |
| Adjuvant | : | Naattu sarkkarai |
| Indication | : | Virulent poison (Kodiya nanju) |
| | | Anemia (Paandu) |
| | | Pruritus (Namaichal) |
| | | Thyroidism (Kazhuththu Kazhalai) |
| | | Leprosy (Perunoi) |
| | | Herpes zooster (Akkipun) |

Fig A

Fig B

Fig C

Fig D

Fig E

Fig A. Raw Aagasagarudan kizhangu

Fig B. Skinless Aagasagarudan kizhangu

Fig C. Unpurified Aagasagarudan kizhangu chooranam

Fig D. Aagasagarudan kizhangu chooranam under purification process

Fig E. Purified Aagasagarudan kizhangu chooranam

## 4.2. QUALITATIVE ANALYSIS

### 4.2.1 PHYSICO CHEMICAL ANALYSIS

The *Aagasagarudan kizhangu chooranam* was studied by physicochemical parameters. This study was done at The Tamil Nadu Dr.M.G.R. Medical University No.69, Annasalai, Guindy, Chennai-600032.

**1. Loss on drying of the sample at 105ºC**

4g of test drug was weighed in a previously weighed 100ml beaker and heated in an oven at 105ºC for 5hours. Cooled in a dessicator and weighed. Repeated the procedure till constant weight was obtained. The percentage loss in weight of the test drug was calculated by the following formula.

**Calculation:**

$$\text{Percentage of loss on drying at } 105^{o}C = \frac{\text{Loss in weight of test drug}}{\text{Weight of test drug taken}} \times 100$$

**2. Ash content**

**a. Total ash content**

4g of test drug was weighed accurately in a previously ignited and tarred silica dish. The material was evenly spread and ignited in a muffle furnace at $600^{0}$C until it became white indicating the absence of carbon. The dish was cooled in a dessicator and weighed. As carbon free ash cannot be obtained in this manner, the dish was cooled and the residue moistened with sufficient quantity of water. Dried on a water bath and then ignited in the electric furnace to get the constant weight. Cooled the dish in a dessicator and then weighed. The percentage of total ash of air-dried materials was calculated as per the formula given below.

**Calculation:**

$$\text{Percentage of total ash} = \frac{\text{Weight of the ash}}{\text{Weight of test drug taken}} \times 100$$

**b. Acid-insoluble ash**

The total ash of the test drug was found out as described above. To the dish containing the total ash was added 45 ml of 1: 5 hydrochloric acid in three portions of 13 ml each time.

Boiled gently for 5 minutes and filtered. Collected the insoluble matter on an ashless filter paper (Whatman No.41) and washed with distilled water until the residue was free from acid. Transferred the filter paper containing the insoluble mater to the original dish. Dried and ignited to the constant weight. Cooled the dish in a dessicator, and then weighed. Calculated the percentage of acid-insoluble of the air-dried material by the given following formula[43]

**Calculation:**

$$\text{Percentage of acid-insoluble ash} = \frac{\text{Weight of the acid-insoluble residue}}{\text{Weight of test drug taken}} \times 100$$

**i. Extractive of the test drug**

**a. Water-soluble extractive of the test drug**

4 g of the test drug was weighed accurately in a glass stoppered flask. Add 100 ml of distilled water and shakened occasionally for 6 hours and then allowed to stand for 18 hours. Filtered rapidly taking care not to lose any solvent and pipetted out 25 ml of the filtrate in a preweighed 100 ml beaker and evaporated to dryness on a water bath. Kept in an air oven at 105ºC for 6 hours. Cooled in a dessicator and weighed. Repeated the experiment twice, and taken the average value. The percentage of water soluble extractive was calculated by the formula given below.

**Calculation:**

$$\text{Percentage of water soluble extract} = \frac{\text{Weight of the extract}}{\text{Weight of sample taken}} \times \frac{100}{25} \times 100$$

### b. Alcohol-soluble extractive of the sample

4 g of the sample was weighed accurately in a glass stoppered flask. Added 100 ml of distilled alcohol (approximately 95%) and shaken occasionally for 6 hours and then allowed to stand for 18 hours. Filtered rapidly taking care not to lose any solvent and pipetted out 25 ml of the filtrate in a pre-weighed 100 ml beaker and evaporated to dryness on a water bath.

Kept in an air oven at 105ºC for 6 hours and cooled in a dessicator and weighed. Repeated the experiment twice, and taken the average value. The percentage of alcohol soluble extractive was calculated by the formula given below.

**Calculation:**

$$\text{Percentage of alcohol soluble extract} = \frac{\text{Weight of the extract}}{\text{Weight of sample taken}} \times \frac{100}{25} \times 100$$

### ii. Determination of pH

The pH of the Aagasagarudan kizhangu was estimated as per the method prescribed in the Indian standard (IS) - 6940(1982).

One gram of the test drug was taken into a 100ml graduated cylinder containing about 50 ml of water. The cylinder was shaken vigorously for two minutes and the suspension was allowed to settle for hour at 25ºC to 27ºC, then 25 ml of the clear aqueous solution was transferred in to a 50 ml beaker and tested for pH using digital pH meter.

### 4.2.2 BIO-CHEMICAL ANALYSIS:

The bio-chemical analysis of **Aagasagarudan kizhangu** was done at Biochemistry lab, National Institute of Siddha, Chennai-47.

## Table 4.2.2 Experimental procedures of Chemical analysis

| S.No | EXPERIMENT | OBSERVATION | INFERENCE |
|---|---|---|---|
| 1. | Appearance of sample | Pale brown in colour | |
| 2. | **Test for Solubility:**<br>a. A little (500mg) of the sample is shaken well with distilled water.<br>b. A little (500mg) of the sample is shaken well with con. HCl/Con. $H_2So_4$ | Sparingly soluble<br>Completely soluble | Presence of Silicate<br>Absence of Silicate |
| 3. | **Action of Heat:**<br>A small amount (500mg) of the sample is taken in a dry test tube and heated gently at first and then strong. | White fumes evolved | Presence of Carbonate |
| 4. | **Flame Test:**<br>A small amount (500mg) of the sample is made into a paste with Con.HCl in a watch glass and introduced into non-luminous part of the Bunsen flame. | Bluish green flame not appeared. | Absence of Copper |
| 5. | **Ash Test:** A filter paper is soaked into a mixture of sample and dil. cobalt nitrate solution and introduced into the Bunsen flame and ignited | No yellow colour flame appeared | Absence of sodium |

**Preparation of Extract:**

5gm of ***Aagasagarudan kizhangu*** is weighed accurately and placed in a 250ml clean beaker and 50ml of distilled water was added with it. Then it was boiled well for about 10 minutes. Then it was allowed to cool and filtered in a 100ml volumetric flask and made up to 100ml with distilled water[44]

## Table4.2.3 Experimental procedures of Biochemical analysis

| S.No | EXPERIMENT | OBSERVATION | INFERENCE |
|---|---|---|---|
| | **I. Test For Acid Radicals** | | |
| 1. | **Test For Sulphate:**<br>2ml of the above prepared extract was taken in a test tube and 2ml of 4% dil. ammonium oxalate solution was added. | No Cloudy appearance | Absence of Sulphate |
| 2. | **Test For Chloride:**<br>2ml of the above prepared extracts was added with 2ml of dil-$HNO_3$ until the effervescence ceases off. Then 2 ml of silver nitrate solution was added. | No Cloudy appearance | Absence of Chloride |
| 3. | **Test For Phosphate:**<br>2ml of the extract was treated with 2ml of con.HNo3 and 2ml of dil.ammonium molybdate solution. | No Yellow precipitate | Absence of Phosphate |
| 4. | **Test For Carbonate:**<br>2ml of the extract was treated with 2ml dil. magnesium sulphate solution | Cloudy appearance present | Presence of Carbonate |
| 5. | **Test For Nitrate:**<br>1ml of the substance was heated with copper turning and concentrated $H_2SO_4$ and viewed the test tube vertically down. | Brown gas was not evolved | Absence of Nitrate |
| 6. | **Test For Sulphide:**<br>1ml of the substance was treated with 2ml of con. HCL | No Rotten Egg Smelling gas was evolved | Absence of Sulphide |

| | | | |
|---|---|---|---|
| 7. | **Test For Fluoride & Oxalate:**<br>2ml of extract was added with 2ml of dil. Acetic acid and 2ml dil.calcium chloride solution and heated**.** | No Cloudy appearance | Absence of fluoride and oxalate |
| 8. | **Test For Nitrite:**<br>3drops of the extract was placed on a filter paper, on that-2 drops of dil.acetic acid and 2 drops of dil.Benzidine solution were placed. | Characteristic changes not appeared | Nitrite absent |
| **II.** | **Test For Basic Radicals** | | |
| 1. | **Test For Lead:**<br>2ml of the extract was added with 2ml of dil.potassium iodine solution. | No Yellow Precipitate is obtained. | Absence of Lead |
| 2. | **Test For Copper:**<br>One pinch (50mg) of substance was made into paste with con. HCl in a watch glass and introduced into the non-luminuous part of the flame. | No Blue colour precipitate formed. | Absence of Copper |
| 3. | **Test For Aluminium:**<br>To the 2ml of extract dil.sodium hydroxide was added in 5 drops to excess. | No characteristic changes | Absence of Aluminium |
| 4. | **Test For Iron:**<br>a.To the 2ml of extract add 2ml of ammonium thiocyanate solution.<br>b.To the 2ml of extract 2ml ammonium thiocyanate solution and 2ml of con HN03 is added | Presence of mild red color appearance<br>Presence of mild Red colour was formed | Presence of Iron<br>Presence of Iron |

| | | | |
|---|---|---|---|
| 5. | **Test For Zinc:**<br>To the 2ml of the extract dil.sodium hydroxide solution was added in 5 drops to excess and dil.ammonium chloride was added. | No White precipitate formed | Absence of Zinc |
| 6. | **Test For Calcium:**<br>2ml of the extract was added with 2ml of 4% dil.ammonium oxalate solution | Cloudy appearance was formed | Presence of Calcium |
| 7. | **Test For Magnesium:**<br>2ml of the extract dil.sodium hydroxide solution was added in drops to excess. | white precipitate not formed | Magnesium absent |
| 8. | **Test For Ammonium:**<br>To 2ml of the extract 1 ml of Nessler's reagent and excess of dil.sodium hydroxide solution were added. | Brown colour formed | Presence of Ammonium |
| 9. | **Test For Potassium:**<br>A pinch (25mg) of substance was treated with 2ml of dil.sodium nitrite solution and then treated with 2ml of dil.cobalt nitrate in 30% dil.glacial acetic acid. | Mild Yellowish red precipitate formed | Presence of Potassium |
| 10. | **Test For Sodium**:<br>2 pinches (50mg) of the substance was made into paste by using HCl and introduced into the blue flame of Bunsen burner. | Yellow colour flame not appeared | Sodium absent |

| | | | |
|---|---|---|---|
| 11. | **Test For Mercury**:<br>2ml of the extract was treated with 2ml of dil.sodium hydroxide solution. | Yellow precipitate not formed | Mercury absent |
| 12. | **Test For Arsenic:**<br>2ml of the extract was treated with 2ml of dil.sodium hydroxide solution. | Brownish red precipitate not formed | Arsenic absent |

| **III. Other constituents** | | | |
|---|---|---|---|
| 1. | **Test For Starch :**<br>2ml of the extract was treated with weak dil.iodine solution | Blue colour developed | Presence of Starch |
| 2. | **Test For Reducing Sugar:**<br>5ml of Benedict's qualitative solution was taken in a test tube and allowed to boil for 2 minutes and added 8 to 10 drops of the extract and again boil it for 2 minutes. | The was no specific change in colour | Absence of Reducing sugar |
| 3. | **Test For The Alkaloids**:<br>a) 2ml of the extract is treated with 2ml of dil.potassium lodide solution.<br>b) 2ml of the extract is treated with 2ml of dil.picric acid. | Reddish brown precipitation formed<br>Yellow precipitation formed | Presence of Alkaloid<br>Presence of Alkaloid |

| | | | |
|---|---|---|---|
| 4. | **Test For Tannic Acid:**<br><br>2ml of extract was treated with 2ml of dil.ferric chloride solution | No Black precipitate obtained | Tannic acid absent |
| 5. | **Test For Unsaturated Compound:**<br><br>To the 2ml of extract 2ml of dil.Potassium permanganate solution was added. | Potassium permanganate was not decolourised | unsaturated compounds absent |
| 6. | **Test For Amino Acid:**<br><br>2 drops of the extract was placed on a filter paper and dried well, then 20ml of Biurette reagent was added in it. | Violet colour not developed | Amino acids absent |
| 7. | **Test For phenols:**<br><br>2ml of the extract was treated with 2 ml of dil.ferric chloride solution. | No specific colour formation | Phenols absent |

## 4.3. QUANTITATIVE ANALYSIS

### 4.3.1 HEAVY METAL ANALYSIS

The analysis of heavy metals and trace elements were estimated by using Inductively Coupled Plasma Optical Emission Spectrometry (ICP- OES). The Experimental Procedure was done at SAIF, IIT Madras, Chennai-32.

**ICP-OES**:

Perkin Elmer Optima 5300DV was used for standard ICP-OES analysis. The optimized operating conditions are given in (table 5), the wave length of analytical lines is given in table (5) and the test drug *Aagasagarudan kizhangu* underwent microwave digestion for sample preparation. With respect to other kind of analysis where chemical speciation was relevant. Only total essential concentration was analyzed by ICP-OES. [45]

**Table 4.3.1: ICP- OES Operating Conditions**

| | |
|---|---|
| Rf frequency | 40 M Hz |
| Range | 165 – 782 nm |
| Detection limit | Up to ppm level using SCD detector |

### 4.3.2 ANALYSIS OF PHYTOCOMPOUNDS

The analysis of Phytocompounds is performed by using Gas chromatogram Mass spectroscopy (GC-MS). The Experimental Procedure was done at SAIF, IIT Madras, Chennai-32.

**GC-MS ANALYSIS:**

GC-MS analysis of the methanol extract of *Aagasagarudan kizhangu chooranam* was performed using a Agilant GC system comprising an AOC-20i auto-sampler and a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with a Elite-5MS (5% diphenyl/95% dimethyl poly siloxane) fused a capillary column (30 × 0.25 μm ID × 0.25 μm df). For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 2 μl was employed (a split ratio of 10:1).

The injector temperature was maintained at 250 °C, the ion-source temperature was 200 °C, the oven temperature was programmed from 110 °C (isothermal for 2 min), with an increase of 10 °C/min to 200°C, then 5 °C/min to 280°C, ending with a 9 min isothermal at 280 °C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 450 Da. The solvent delay was 0 to 2 min, and the total GC-MS running time was 36 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. . The mass-spectrometer used in this analysis was JEOL GC-MATE-II, and the software adopted to handle mass spectra and chromatograms was a JEOL Ver.2.0 and NIST library Ver.2.0 was used.

**4.3.3 ANALYSIS OF ORGANIC FUNCTIONAL GROUPS - FTIR**

The analysis of some organic functional groups was determined using FT-IR Spectra. The Experimental Procedure was done at SAIF, IIT Madras, Chennai-32.

The Perkine Elmer Spectrum One Fourier Transform Infrared (FTIR) Spectrometer was used to derive the FT IR Spectra of *Aagasagarudan kizhangu chooranam* in Potassium Bromide (KBr) matrix with scan rate of 5 scan per minute at the resolution 4cm-1 in the wave number region 450-4000cm-1. The samples were grounded to fine powder using agate motor and pestle and the mixed with KBr[46]

They were then Pelletized by applying pressure to prepare the specimen (the size of specimen about 13 mm diameter and 0.3 mm in thickness) to recorded the FT-IR Spectra under Standard conditions.

FT-IR is an important and advanced technique to identify the functional group to determine the quality and consistency of the sample material. And can determine the amount of compounds in the sample. It was an excellent for quantitative analysis.[47]

The spectrum that appears denotes the molecular absorption and transmission. The peaks seen in the spectrum indicates the amount of materials present in it.[48]

## 4.4 TOXICITY STUDIES OF AAGASAGARUDAN KIZHANGU CHOORANAM

To evaluate the safety profile of *Aagasagarudan kizhangu chooranam* with acute and sub chronic toxicity study carried out as followed

Principles of laboratory animal care were followed and the Institutional Animal Ethical Committee approved the use of animals and the study design. Institutional Animal Ethical Committee number: (IAEC). (NIS/IAEC-II/11/2016) dated 29.9.2016.

### 1. ACUTE TOXICITY STUDY OF AAGASAGARUDAN KIZHANGU CHOORANAM

| | | |
|---|---|---|
| Species | : | Wister albino Rats |
| Sex | : | Male and Female |
| Age/weight at start of test | : | 6 weeks/140-160g b.wt |
| Acclimatization Period | : | 7 days prior to dosing |
| Housing | : | Polypropylene cages |
| Husbandry | : | 12-h light/12-h dark artificial photoperiod/ Room temperature 22°C (±3°) and relative humidity 30–70% |
| Feed and Water | : | Rodent pelleted feed<br>RO purified water *ad libitum* |
| Identification | : | Animals will be kept in individual cages and numbered |

**Experimentation Details of Acute toxicity study:**

| | | |
|---|---|---|
| Groups/treatment regimen | : | Grouped by randomisation |
| Test guideline | : | WHO |
| Length of exposure to test substance | : | Single dose |
| No of animals | : | 5Male + 5 Female/group |
| Control group | : | Vehicle |
| Test groups | : | *Aagasagarudan kizhangu chooranam* (Low, Mid, High dose) |

Healthy Wister Albino rats of both sexes weighing 150-200g were obtained from authorized animal breeders of the animal laboratory in TANUVAS, Madhavaram, and Chennai and stocked in the animal house at National Institute of Siddha, Chennai. Animals were housed in a cage at 25℃ and have free access to standard rat pellet diet. The animals were administered with *Aagasagarudan kizhangu chooranam* by oral route for one day and monitored for behavioral parameters for the first 4 hours after drug administration. Body weight of the animal will be monitored at weekly intervals. The animals that die within this period will be subjected to necropsy. Remaining animals will be weighed and sacrificed under the injection of Pentothal sodium after 14 days of drug administration. The toxicological effect was assessed on the basis of mortality and behavioral parameters.

**Preparation of Test Drug Doses:**

| **Groups** | **No. of Rat** |
|---|---|
| Group I: Vehicle control (Water) | 10(5M+5 F) |
| GroupII: Test drug (Aagasagarudan kizhangu chooranam) – 2000mg/kg b.wt | 10(5M+5F) |

**Total 20(10M+10F)**

**Route of administration:**

Oral route was selected because it is the normal route of clinical administration.

**Administration of Dose:**

The animals were fasted (only food was with held) for 12hrs and weighed prior to dosing. Three animals were used for each step. A single dose of the test drug (2000mg/kg.b.wt) was consecutively administered by oral gavage using intubation cannula. The food was withheld for another 4hrs after dosing and administration of the drug.

**Observations**

Observation were made and recorded systematically and continuously observed after the substance administration as per the guidelines.

- Mortality, behavioural changes
- ½ hour , 1 hour, 2 hour ,4 hour and upto 24 hours observation
- All rats were observed twice daily for 14 days
- Body weight was observed once in a week.
- Feed intake was calculated daily.
- Water intake was calculated daily.

**a. Cage-side observation**

Clinical observation includes Mortality, convulsion, tremor, sedation, excitation, abnormal gait, motor coordination, piloerection, head movements, reactivity to touch, gripping, grooming, exophthalmos, diarrhoea, salivation, lacrimation, posture, dyspnoea and coma.

**b. Gross necropsy**

At the end of the 14$^{th}$day, all the animals were sacrificed by using the injection of Pentothal sodium Gross necropsy includes examination of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents like brain, lung, heart, spleen, liver, kidney, uterus, testes, ovary of all animals.

## 2. LONG TERM TOXICITY STUDY OF AAGASAGARUDANKIZHANGU CHOORANAM

**Experimental animals:**

| | | |
|---|---|---|
| Species | : | Wister albino Rats |
| Sex | : | Male and Female |
| Age/weight at start of test | : | 6 weeks/140-160g b.wt |
| Acclimatization Period | : | 7 days prior to dosing |
| Housing | : | Polypropylene cages with bedding with husk |
| Husbandry | : | 12-h light/12-h dark artificial photoperiod/ Room temperature 22°C(±3°) and relative humidity 30–70% |
| Feed and Water | : | Rodent pelleted feed<br>RO purified water *ad libitum* |
| Identification | : | Animals will be kept in Polypropylene cages and numbered |

**Experimentation details**

| | | |
|---|---|---|
| Groups/treatment regimen | : | Grouped by randomisation |
| Test guideline | : | WHO |
| Length of exposure to test substance | : | 90 days |
| No of animas | : | 10 Female +10 Male / group |
| Control group | : | Vehicle |
| Test groups | : | Aagasagarudan kizhangu chooranam (Low, Mid, High dose) |

Albino rats of both sexes are divided into four groups. The first group treated as vehicle control and second, third, fourth group were treated with Aagasagarudan kizhangu chooranam i.e., Low dose, mid dose and high dose respectively. The control animals were administered with Water as a vehicle. The other animals were

administered with Aagasagarudan kizhangu chooranam orally for 90 days and it was monitored for behavioral parameters for the first 4 hours after drug administration every day. Body weight of the animal was monitored at weekly intervals. The food and water intake were calculated daily. The animals that die within this period will be subjected to necropsy. Remaining animals were weighed and sacrificed at the end of the study (91 days) by using the injection of Pentothal sodium. Blood was collected from the anesthetized animals from abdominal aorta. And the following investigations like Haematology, Biochemical analysis and Histopathology are done.

| **Groups** | **No. of Rats** |
|---|---|
| Group I: Vehicle control (Water) | 20(10M + 10F) |
| Group II: Test drug - low dose (360mg/kg b.wt) | 20(10M + 10F) |
| Group III: Test drug - Mid dose (720mg/kg b.wt) | 20(10M + 10F) |
| GroupIV: Test drug - High dose (1.440mg/kg b.wt) | 20(10M + 10F) |

**Total: 80 (40 M + 40 F)**

## Observations

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

- Mortality, behavioural changes
- All rats were observed twice daily for 90 days
- Body weight was observed once in a week.
- Feed intake was calculated daily.
- Water intake was calculated daily.

## Cage-side observation

The animals were monitored for behavioural parameters like Alertness, Mortality, convulsion, tremor, sedation, excitation, abnormal gait, motor coordination, piloerection, head movements, reactivity to touch, diarrhoea, salivation, lacrimation, posture, dyspnoea, coma.

## Laboratory investigation:

On the 91th day, the animals were fasted overnight, then anesthetized to collect blood samples from the abdominal aorta in two tubes: one with EDTA for haematological parameters , another one without any anticoagulant and was centrifuged at 4000 rpm at $4^0$C for 10 minutes to obtain the serum for biochemical parameters.

## Clinical Biochemistry

At the end of the study, the animals were sacrificed, blood was collected in all the overnight fasted rats, through abdominal aorta .The blood sample was processed for the below mentioned investigations total cholesterol, triglycerides, Liver function test (SGOT, SGPT, alkaline phosphatase, total bilirubin, total protein, albumin, globulin), Renal function test (creatinine, urea).

## Haematological Investigation:

Blood samples of control and experimental rats were analysed for Haemoglobin (Hb), Total red blood corpuscles (RBC), Total white blood corpuscles (WBC), Platelet, Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) were calculated by auto analyser.

## Gross necropsy

All the animals were sacrificed on the 91th day. Gross necropsy includes examination of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents like Brain, eye, lung, heart, spleen, liver, kidney, testes and uterus of all animals were recorded.

## Histopathology

The organs included liver, kidneys, spleen, brain, heart, lungs, uterus, ovary, testes and stomach of the animals were preserved, and they were subjected to histopathological examination.

Control and highest dose groups animals were initially subjected to histopathological investigation. If any abnormality found in the highest dose group then the low and mid dose group were also examined. Various organs (brain, heart, lung, liver, kidney, spleen, stomach)will be collected from all the animals and preserved in 10% buffered neutral formalin, sliced , 5 or 6μm sections and was stained with Haematoxylin and Eosin. Examined for histopathological changes.

## Statistical analysis:

Findings such as body weight changes, food consumption, water intake, haematology and biochemical analysis were subjected to One-way ANOVA Dunnet's test using a computer software program followed by D *Graph Pad Instat-3.*

# 5. RESULTS

The prepared drug analyzed by various analytical procedures and studied by acute and long term toxicity studies through WHO guidelines. The results are given by the following tabulation, graphs and microscopical slides.

## 5.1 QUALITATIVE ANALYTICAL STUDIES ON AAGASAGARUDAN KIZHANGU CHOORANAM

### 1. Physico-chemical Analysis

**Table 1: Physico-chemical properties of Aagasagarudan kizhangu chooranam (AGC)**

| S.No | Parameters | Percentage |
|---|---|---|
| 1. | pH | 6.5 |
| 2. | Loss on drying | 6.19% |
| 3. | Total Ash value | 3.6% |
| 4. | Acid insoluble ash | 0.4% |
| 5. | Water soluble ash | 2.8% |
| 6. | Water soluble extraction | 14.09% |
| 7. | Alcohol soluble extraction | 11.69% |

**RESULTS AND INTERPRETATION:**

The pH level plays a role in enzyme activity by regulating the homeostasis. It is also an important factor for drug absorption. The pH of AGC is 6.5. Being weak acidic,the drug is more readily absorbed in an acid medium like stomach which enhances the bioavailability of the drug.

The moisture present in the drug was indicated in Loss on drying. The moisture content of the drug reveals the stability and its shelf-life. Low moisture content could get maximum stability and better shelf life. Being a chooranam without incineration process the moisture content of AGC is 6.19%. So the stability and shelf life was about one year.

The Total Ash value determines the amount of minerals and earthy materials present in the drug. The Ash value of AGC is 3.6% which denotes the purity of the drug as the ash value is low.

Acid insoluble ash value of the drug denotes the amount of siliceous matter (dust,sand etc.,)present in the drug.

The quality of the drug is better if the acid insoluble Ash value is low. Here, acid insoluble ash value is 0.4%.hence it represents the superior quality of the AGC.

Decreased Water soluble ash value(2.8 %) indicates easy facilitation of diffusion and osmosis mechanisms[49].

**Table 2: Colour and Nature of Aagasagarudan kizhangu chooranam (AGC)**

| S.No | Parameters | Results |
|---|---|---|
| 1. | Colour | Pale brown |
| 2. | Odour | Pungent |
| 3. | Taste | Bitter |
| 4. | Texture | Fine powder |
| 5. | Particle size | Completely passed through sieve no.88 |

**2. Biochemical Analysis**

**Table 3: Biochemical analysis of Aagasagarudan kizhangu chooranam (AGC)**

| **S.No** | **EXPERIMENT** | **INFERENCE** |
|---|---|---|
| 1. | Test for Silicate | + |
| 2. | Test for Carbonate | - |
| 3. | Test for Copper | - |
| 4. | Test for Sodium | - |
| 5. | Test for Sulphate | - |
| 6. | Test for Chloride | - |
| 7. | Test for Phosphate | + |
| 8. | Test for Nitrate | - |
| 9. | Test for Sulphide | - |
| 10. | Test for Fluoride &Oxalate | - |
| 11. | Test for Nitrite | - |

| | | |
|---|---|---|
| 12. | Test for Lead | - |
| 13. | Test for Aluminium | - |
| 14. | Test for Iron | + |
| 15. | Test for Zinc | + |
| 16. | Test for Calcium | + |
| 17. | Test for Magnesium | - |
| 18. | Test for Ammonium | + |
| 19. | Test for Potassium | + |
| 20. | Test for Mercury | - |
| 21. | Test for Arsenic | - |
| 22. | Test for Starch | + |
| 23. | Test for Reducing sugar | - |
| 24. | Test for Alkaloids | + |
| 25. | Test for Tannic acid | - |
| 26. | Test for Amino acid | - |
| 27. | Test for Phenol | - |

(+) Present (-) Absent

## RESULTS AND INTERPRETATION OF BIOCHEMICAL ANALYSIS

The chemical analysis shows the presence of Silicate, Phosphate, Zinc, Iron, Calcium, Magnesium, Ammonium, Potassium, Starch, Alkaloids in Aagasagarudan kizhangu Chooranam.

Phosphate is a charged particle that contains the mineral phosphorus.[50]. The mineral phosphorus is primarily used for growth and repair of body cells and tissues.[51]. It reduces the histamine release by activated mast cells[52]

Zinc is deeply involved in regulation of immune system. Deficiency of zinc leads to development of inflammatory and autoimmune disorders[53]. So the presence of zinc in AGC cures the inflammatory disorders thereby regulating the immune system.

## 5.2 QUANTITATIVE ANALYTICAL STUDIES ON AAGASAGARUDAN KIZHANGU CHOORANAM

### 3. ICP-OES Analysis

**Table 4: Inductively Coupled Plasma Optical Emission Spectrometry Analysis of Aagasagarudan kizhangu Chooranam (AGC)**

| Sl.No | Element name | Standard value | Obtained value |
|---|---|---|---|
| 1 | As | 188.979 | BDL |
| 2 | Ca | 315.807 | 24.150 mg/L |
| 3 | Cd | 228.802 | BDL |
| 4 | Cu | 327.393 | BDL |
| 5 | Fe | 238.204 | 2.340 mg/L |
| 6 | Hg | 253.652 | BDL |
| 7 | K | 766.491 | 120.821 mg/L |
| 8 | Mg | 285.213 | 01.020 mg/L |
| 9 | Na | 589.592 | 13.110 mg/L |
| 10 | Ni | 231.604 | BDL |
| 12 | Pb | 220.353 | BDL |
| 13 | P | 213.617 | 58.541 mg/L |
| 14 | Zn | 213.856 | 01.587 mg/L |

(BDL-Below Detection Limit)

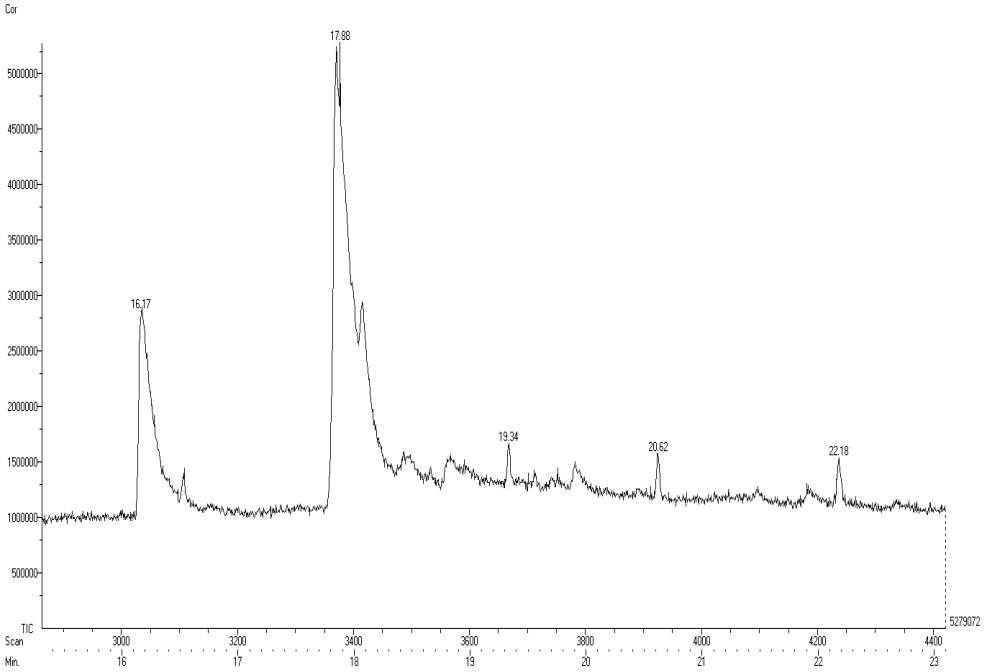
**RESULTS AND INTERPRETATION OF ICP – OES ANALYSIS:**

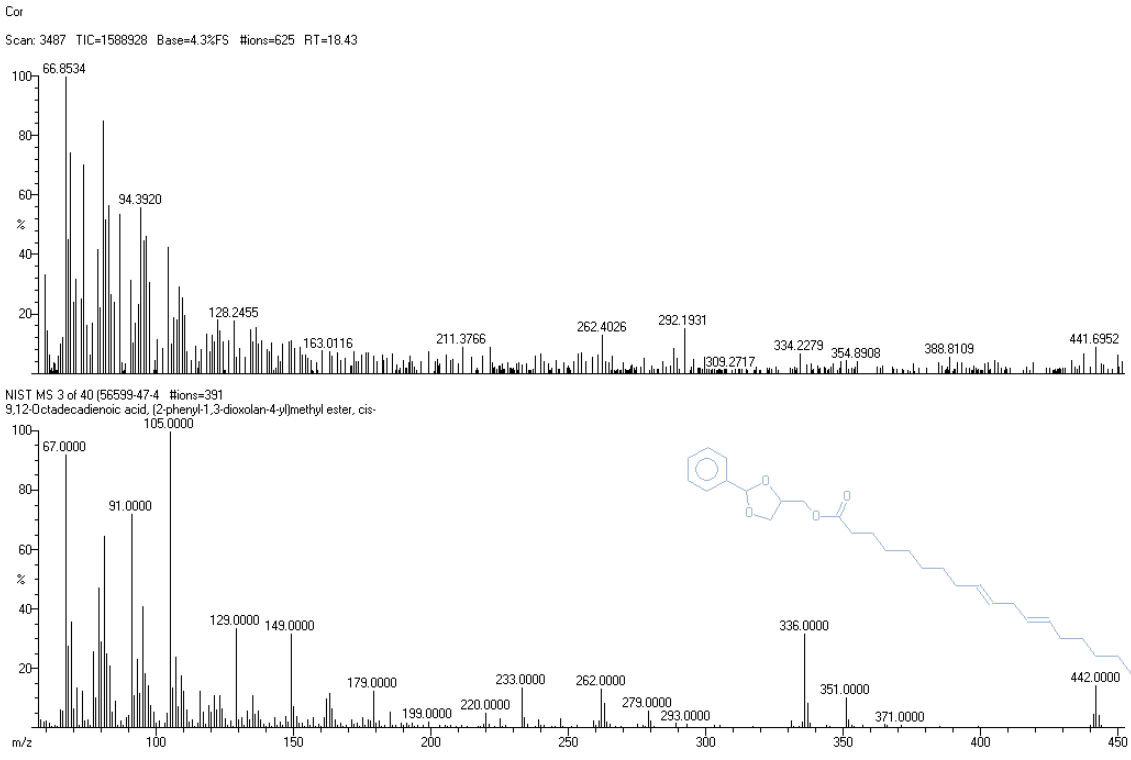
The presence of some metals such as Calcium, Iron, Mercury, Potassium, Manganese, Sodium, Phosphorus and Zinc were detected in the sample of *Aagasagarudan kizhangu chooranam*.

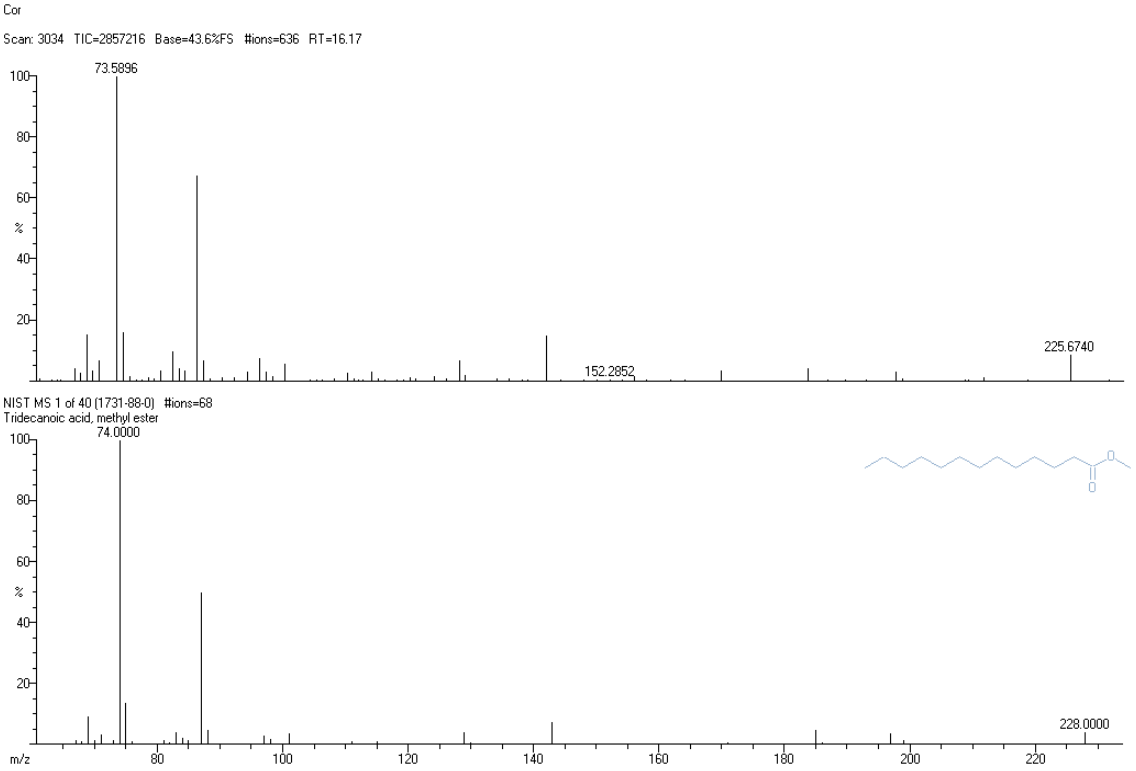
The most important heavy metals such as lead, mercury, nickel, copper, arsenic and cadmium are present in BDL as per the WHO permissible levels in this purified sample.

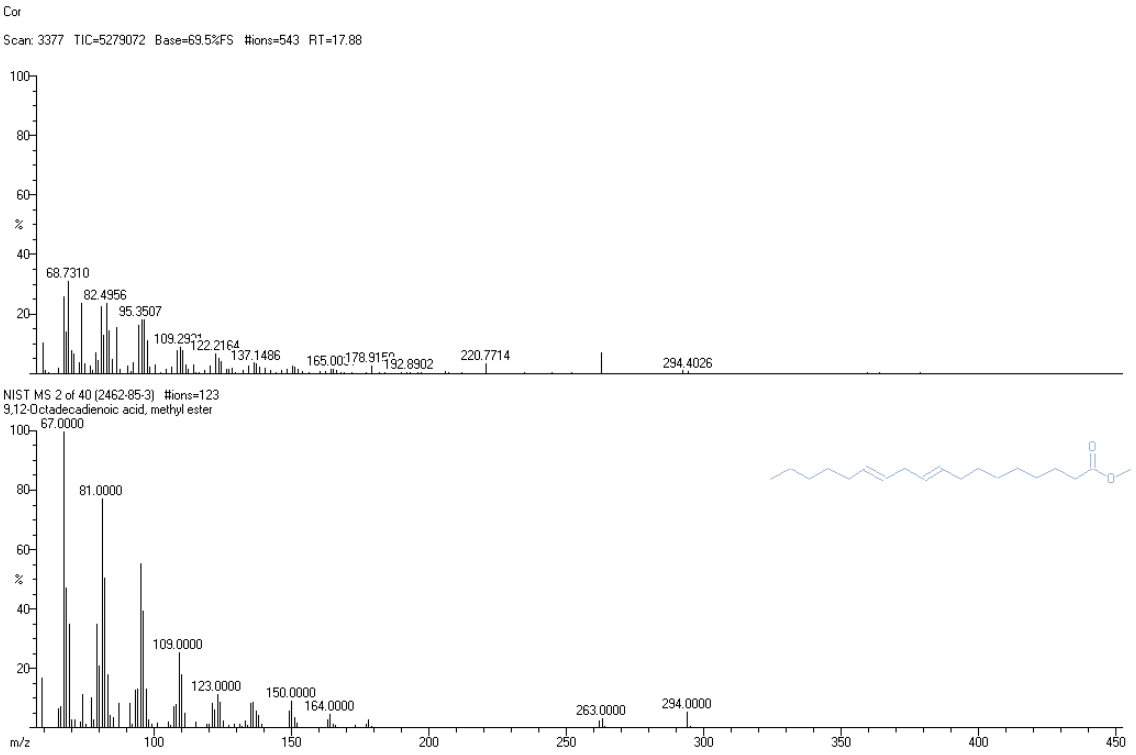
# 4. GCMS Analysis

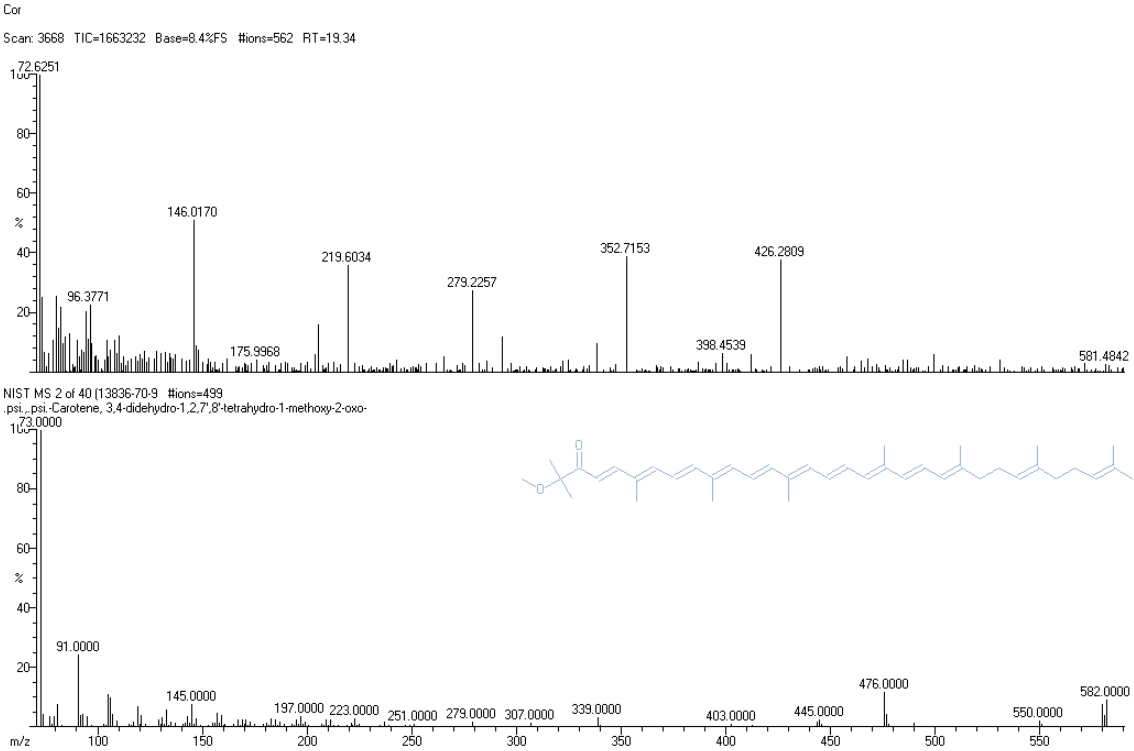
## Graphical representations - Gas Chromatogram Mass Spectrometer Analysis of Aagasagarudan kizhangu Chooranam (AGC)

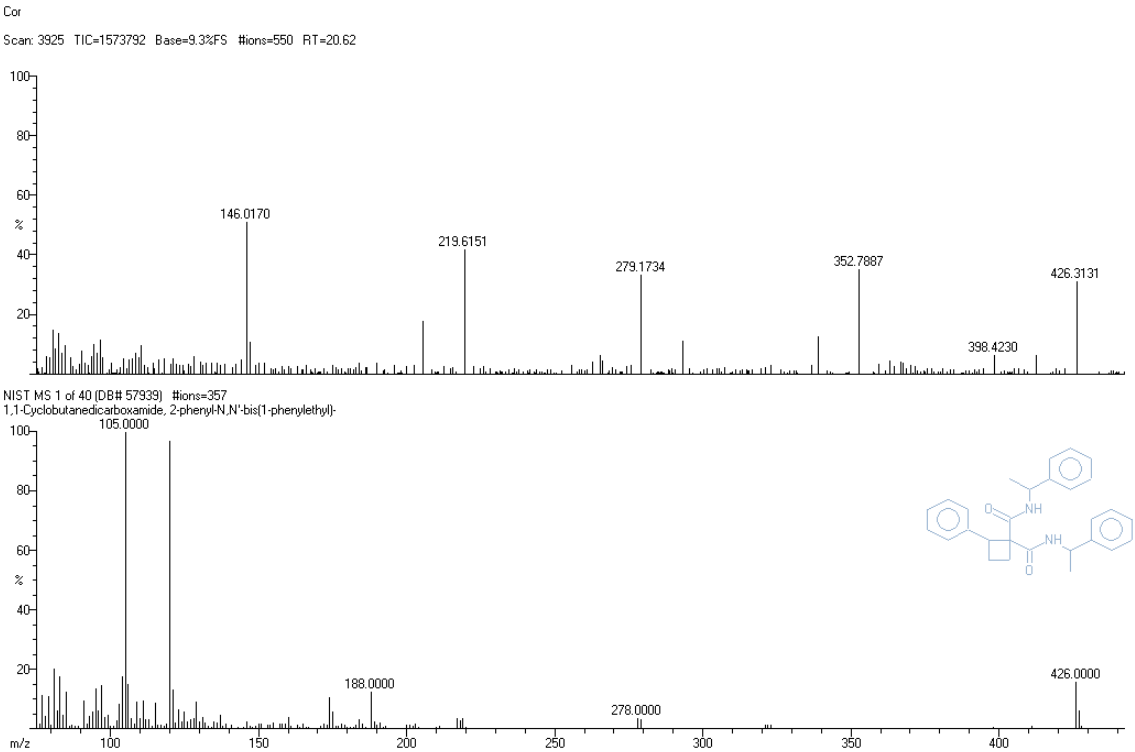



Cor
Scan: 3034 TIC=2857216 Base=43.6%FS #ions=636 RT=16.17
73.5896
225.6740
152.2852
NIST MS 1 of 40 (1731-88-0) #ions=68
Tridecanoic acid, methyl ester
74.0000
228.0000
m/z


Cor
Scan: 3377 TIC=5279072 Base=69.5%FS #ions=543 RT=17.88
68.7310
82.4956
95.3507
122.2164
137.1486
220.7714
294.4026
NIST MS 2 of 40 (2462-85-3) #ions=123
9,12-Octadecadienoic acid, methyl ester
67.0000
81.0000
109.0000
123.0000
150.0000
164.0000
263.0000
294.0000
m/z


Cor
Scan: 3668 TIC=1663232 Base=8.4%FS #ions=562 RT=19.34
72.6251
146.0170
219.6034
352.7153
426.2809
279.2257
96.3771
175.9968
398.4539
581.4842
NIST MS 2 of 40 (13836-70-9 #ions=499
.psi.,.psi.-Carotene, 3,4-didehydro-1,2,7',8'-tetrahydro-1-methoxy-2-oxo-
73.0000
91.0000
145.0000
197.0000
223.0000
251.0000
279.0000
307.0000
339.0000
403.0000
445.0000
476.0000
550.0000
582.0000
m/z


Cor
Scan: 3925 TIC=1573792 Base=9.3%FS #ions=550 RT=20.62
146.0170
219.6151
279.1734
352.7887
426.3131
398.4230
NIST MS 1 of 40 (DB# 57939) #ions=357
1,1-Cyclobutanedicarboxamide, 2-phenyl-N,N'-bis(1-phenylethyl)-
105.0000
188.0000
278.0000
426.0000
m/z

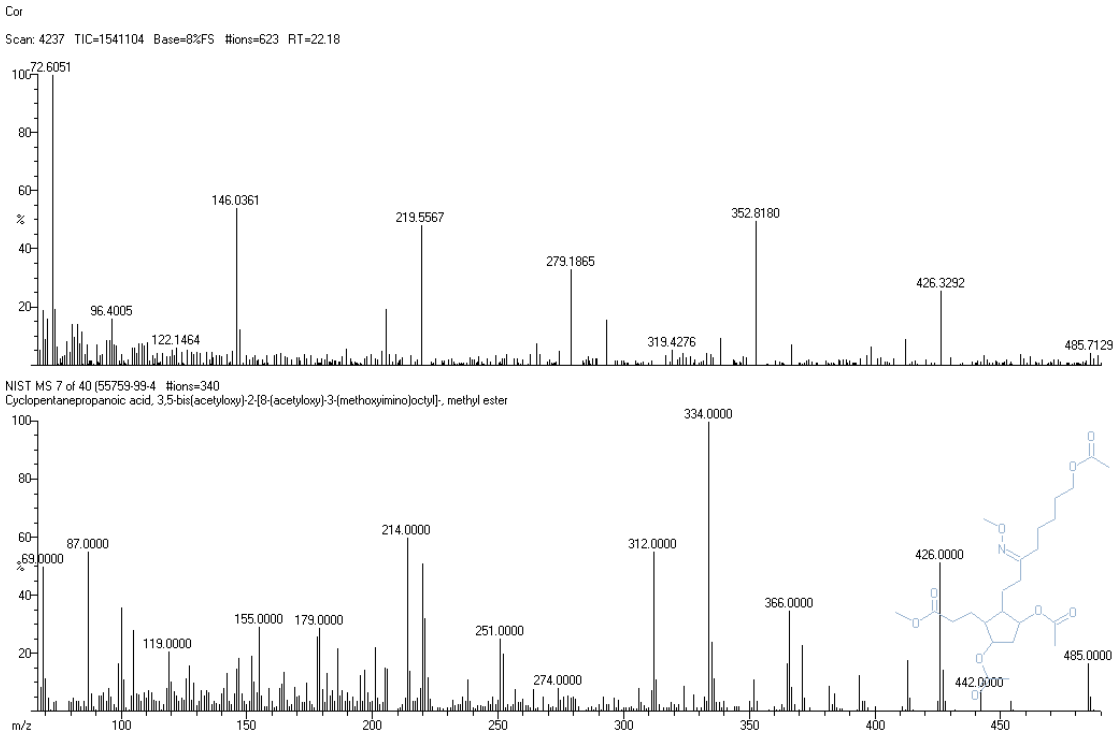



**Table 5: Gas Chromatogram Mass Spectrometer Analysis of Aagasagarudan kizhangu Chooranam (AGC)**

| S.NO | Rt | Name of the compound | Molecular formula | Molecular weight (g/mol) |
|---|---|---|---|---|
| 1 | 18.43 | 9,12-Octadecadienoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis- | $C_{28}H_{44}O_4$ | 444.656 |
| 2 | 16.17 | Tridecanoic acid, methyl ester | $C_{14}H_{28}O_2$ | 228.376 |
| 3 | 17.88 | 9, 12-Octadecadienoic acid, methyl ester | $C_{19}H_{34}O_2$ | 294.479 |
| 4 | 19.34 | Psi, psi carotene, 3,4 didehydro-1, 2, 7'8' – tetrahydro-1-methoxy-2-oxo | $C_{41}H_{58}O_2$ | 582.913 |

| | | | | |
|---|---|---|---|---|
| 5 | 20.62 | 1,1-Cyclobutanedicarboxamide, 2-phenyl-N-N'-bis(1-phenylethyl)- | $C_{28}H_{30}N_2O_2$ | 426.56 |
| 6 | 22.18 | Cyclopentanepropanoic acid, 3,5 bis(acetyloxy)-2-[-(acetyloxy)-3-(methoxyimino)octyl]-, methyl ester | | |

**RESULTS AND INTERPRETATION OF GCMS ANALYSIS:**

Through GCMS Analysis, the identified compounds in Aagasagarudan kizhangu chooranam are 9,12-Octadecadienoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester, cis-, Tridecanoic acid, methyl ester,9, 12-Octadecadienoic acid, methyl ester, Psi, psi carotene, 3,4didehydro-1, 2, 7'8' –tetrahydro-1-methoxy-2-oxo, 1,1-Cyclobutanedicarboxamide, 2-phenyl-N-N'-bis(1-phenylethyl)- 2-phenyl-N-N'-bis(1-phenylethyl)-, Cyclopentanepropanoic acid, 3,5bis(acetyloxy)-2-[-(acetyloxy)-3-(methoxyimino)octyl]-, methyl ester. These identified compounds like 9, 12-Octadecadienoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester having so many medicinal values such as Hypocholesterolemic 5-Alpha reductase inhibitor, Antihistaminic, Anticoronary, Insectifuge, Hypocholesterolemic, Antieczemic, anti oxidant, anti microbial activity[54].

## 5. FT-IR ANALYSIS

### Graphical representations - Fourtier Transform Infrared Spectroscopy Analysis of Aagasagarudan kizhangu Chooranam (AGC)

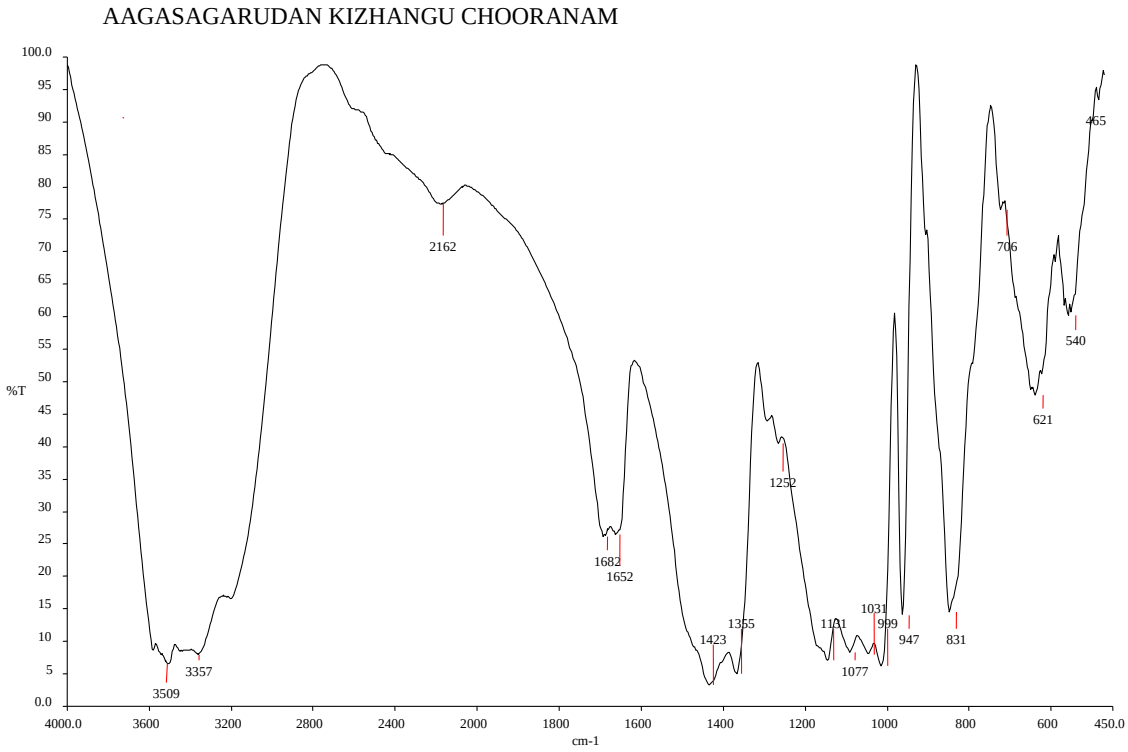



**Table 6: FTIR Analysis of Aagasagarudan kizhangu chooranam (AGC)**

| Sl.no. | Wavelength | Vibrational modes | Functional groups |
|---|---|---|---|
| 1 | 3509 | -OH-Stretch-H-bonded | Alcohols, phenols |
| 2 | 3357 | N-H stretch | 1`, 2` amines, amides |
| 3 | 2162 | -C≡C-stretch | Alkynes |
| 4 | 1682 | -C=C-stretch | Alkenes |
| 5 | 1652 | -C=C-stretch | Alkenes |
| 6 | 1423 | C-C stretch (in-ring) | Aromatics |
| 7 | 1356 | N-O symmetric stretch | Nitro compound |
| 8 | 1252 | C-O Stretch | Alcohols, carboxylic acids, esters, ethers |

| | | | |
|---|---|---|---|
| 9 | 1132 | C-N Stretch | Aliphatic amines |
| 10 | 1077 | C-N Stretch | Aliphatic amines |
| 11 | 1031 | C-N Stretch | Aliphatic amines |
| 12 | 999 | = C-H Bend | Alkenes |
| 13 | 947 | O-H Bend | Carboxylic acids |
| 14 | 831 | C-Cl Stretch | Alkyl halides |
| 15 | 706 | -C≡C-H=C-H bend | Alkynes |
| 16 | 540 | C-Br stretch | Alkyl halides |

**RESULTS AND INTERPRETATION OF FT-IR ANALYSIS:**

In the FT-IR Spectra analysis, this Aagasagarudan kizhangu chooranam sample exhibits the peak value shows in Table 5 at the wave number of 3509, 3357, 2162, 1682,1652, 1423, 1356, 1252, 1132,1077,1031,999,947,465,706,421,540,83. This indicates the presence of some organic functional groups such as alcohols, phenols,1`, 2` amines, amides, Alkynes, Alkenes, Alkyl halides, Nitro compound, Alcohols, carboxylic acids, esters, ethers, Aliphatic amines, alkenes, Carboxylic acids, Aromatics. In this amide linkages in a biochemical context are called peptide bonds when they occur in the main chain of a protein and isopeptide bonds.

Proteins can have structural roles such as in hair or spider silk, but also nearly all enzymes are proteins [55.]

The Aromatic compounds play key roles in the biochemistry of all living things. Aromatic amino acids serve as one of the 20 basic building blocks of proteins. The all 5 nucleotides make up the sequence of genetic code in DNA and RNA are aromatic purines or pyrimidines. The molecule heme contains an aromatic system with 22π electrons and chlorophyll also has a similar aromatic system. Amines and Carboxylic acids possess which enhances the drug effect against the disease.

## 5.3. TOXICOLOGICAL STUDIES ON AAGASAGARUDAN KIZHANGU CHOORANAM

### ACUTE TOXICITY STUDY OF AAGASAGARUDAN KIZHANGU CHOORANAM

In **Acute toxicity study** carried out as per WHO guidelines, there were no treatment related death or signs of toxicity developed in Wister albino rats at therapeutic dose (2000mg/kg b.wt) throughout the study period.

Further, no gross pathological changes have been seen in the internal organs of both control and treated groups.

**Table 7: Effect of Aagasagarudan kizhangu chooranam on Wister albino rats in Acute toxicity study**

| S. 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 | Test Group therapeutic dose (2000mg/kg/b.wt | + | - | - | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

1.Alertness 2.Aggressiveness 3.Pile erection 4.Grooming 5.Gripping 6.Touch response 7.Decreased Motor Activity 8.Tremors 9.Convulsion 10.Muscle spasm 11.Catatonia 12.Muscle relaxant 13.Hypnosis 14.Analgesia 15.Lacrimation 16.Exopthalmos 17.Diarrhoea 18.Writhing 19.Respiration 20.Mortality

(+) Present (-) Absent

## LONG TERM TOXICITY STUDY OF AAGASAGARUDAN KIZHANGU CHOORANAM

**Table 8: Effect of Aagasagarudan kizhangu chooranam on Body weight (gm) changes of Wister albino rats in Long term toxicity study**

| GROUPS | DAY1 | 15 | 30 | 45 | 60 | 75 | 90 |
|---|---|---|---|---|---|---|---|
| CONTROL | 126± 11.41 | 153.6± 53.60 | 220± 73.67 | 233± 73.04 | 251± 73.97 | 270.3± 82.10 | 296.7± 65.6 |
| LOW DOSE | 123.6± 21.5 | 242.6± 51.36** | 245± 55.88 | 266.1± 63.93 | 280.6± 69.64 | 290± 71.28* | 293± 73.28 |
| MID DOSE | 135.9± 23.23 | 248.1± 41.93 | 262.3± 51.71 | 278.2± 54.68 | 290.8± 63.43 | 312.4± 61.81 | 316.2± 60.25 |
| HIGH DOSE | 138± 22.98 | 191.3± 37.98 | 254.3± 52.63 | 266± 56.68 | 279± 59.16** | 289.5± 64.81 | 293.6± 66.28 |

Values are mean of 10 animals ± S.D.(Dunnett's test). **($p < 0.01$),*($p < 0.05$), n = 10.

**Graph 1: Effect of Aagasagarudan kizhangu chooranam on Body weight (gm) changes of Wister albino rats in Long term toxicity study**

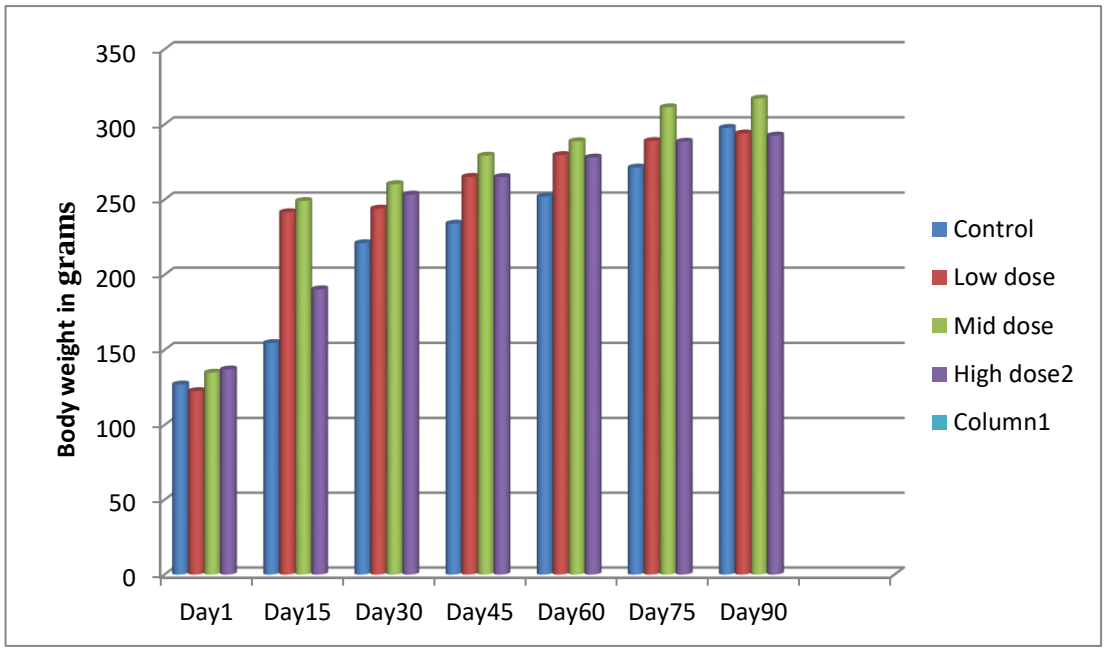
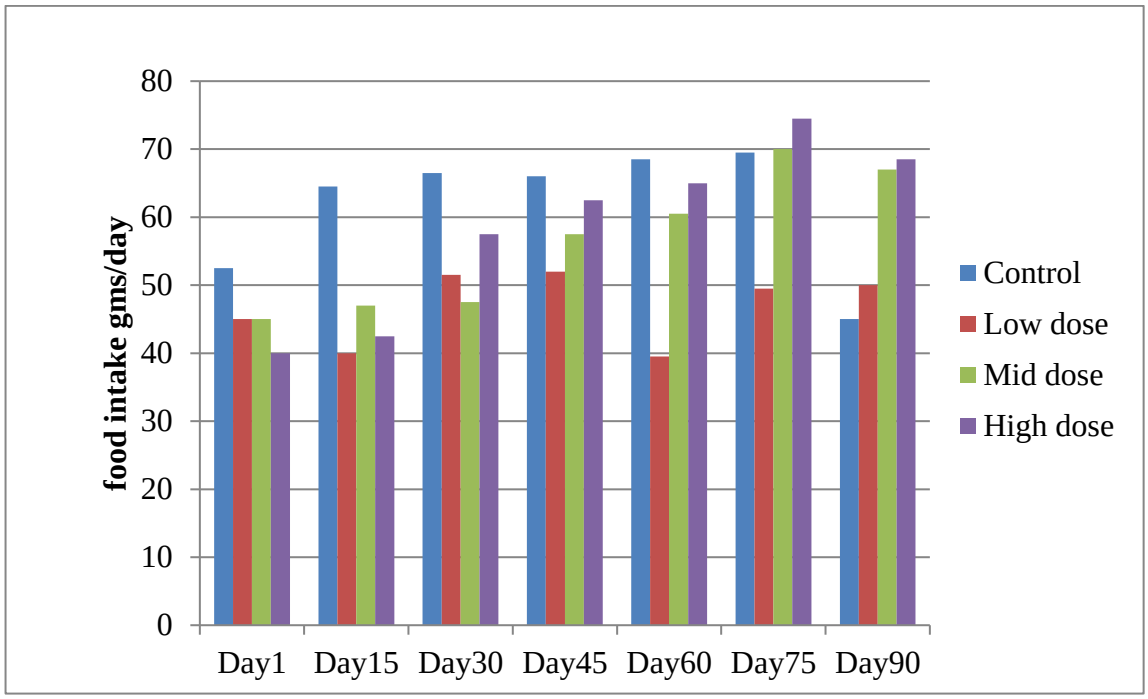


**Table 9: Effect of Aagasagarudan kizhangu chooranam on food intake changes of Wister albino rats in Long term toxicity study**

| GROUPS | DAY1 | 15 | 30 | 45 | 60 | 75 | 90 |
|---|---|---|---|---|---|---|---|
| CONTROL | 51.5± 3.2 | 64.5± 3.53 | 67± 0.70 | 66± 2.82 | 68.5± 2.12 | 69.5± 0.70 | 45± 7.07 |
| LOW DOSE | 46± 21.2 | 41± 14.12 | 51.5± 12.02* | 52± 22.62 | 39.5± 0.70 | 49.5± 0.70 | 50± 0.70 |
| MID DOSE | 49± 12.72 | 45± 21.2 | 47± 4.24 | 41.5± 4.94 | 57.5± 10.60 | 60.5± 3.53 | 70± 7.07 |
| HIGH DOSE | 40± 7.07 | 42.5± 10.60 | 57.5± 7.77 | 62.5± 7.77 | 65± 14.14 | 74.5± 21.92** | 68.5± 2.12* |

Values are mean of 10 animals ± S.D.(Dunnett's test). **(p <0.01),*(p < 0.05), n = 10.

**Graph 2: Effect of Aagasagarudan kizhangu chooranam on food intake (gm) changes of Wister albino rats in Long term toxicity study**



**WATER INTAKE:**

**Table 10: Effect of Aagasagarudan kizhangu chooranam on Water intake changes of Wister albino rats in Long term toxicity study**

| GROUPS | DAY1 | 15 | 30 | 45 | 60 | 75 | 90 |
|---|---|---|---|---|---|---|---|
| CONTROL | 90± 14.14 | 75± 7.07 | 70± 14.14 | 70± 14.14 | 65± 7.07 | 65± 7.07 | 57.5± 10.60 |
| LOW DOSE | 70± 28.28 | 60± 28.28 | 120± 70.71* | 80± 56.56 | 90± 42.42 | 80± 28.28 | 85± 7.07** |
| MID DOSE | 95± 63.63 | 70± 28.28 | 100± 42.42 | 105± 63.63 | 95± 7.07* | 120± 84.85* | 90± 28.28 |
| HIGH DOSE | 100± 42.42 | 85± 35.35 | 110± 28.28 | 115± 49.49 | 85± 21.21 | 75± 21.21 | 110± 14.14 |

Values are mean of 10 animals ± S.D.(Dunnett's test). **($p < 0.01$),*($p < 0.05$), n = 10.

**Graph 3: Effect of Aagasagarudan kizhangu chooranam on Water intake changes of Wister albino rats in Long term toxicity study**

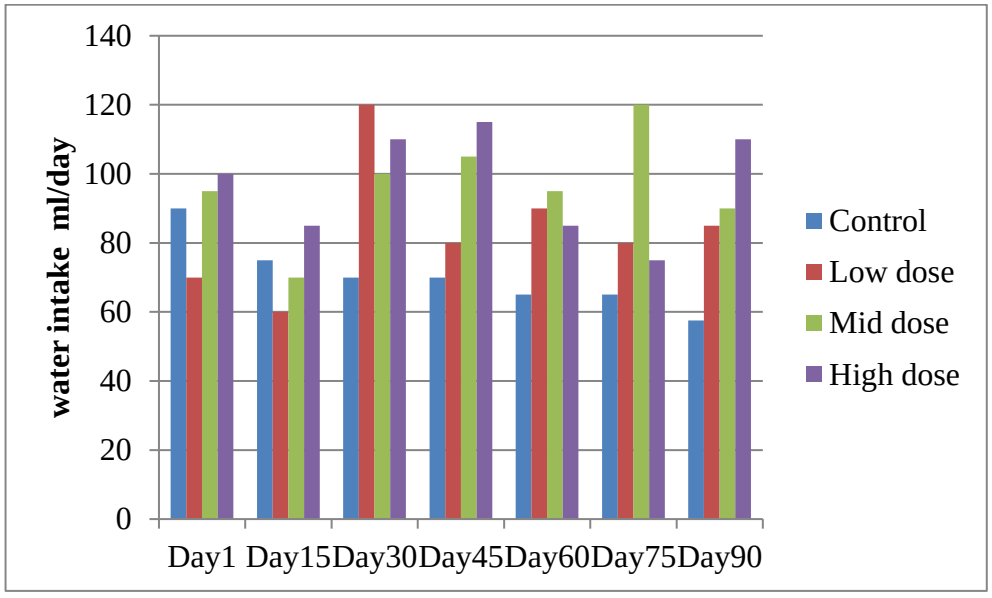
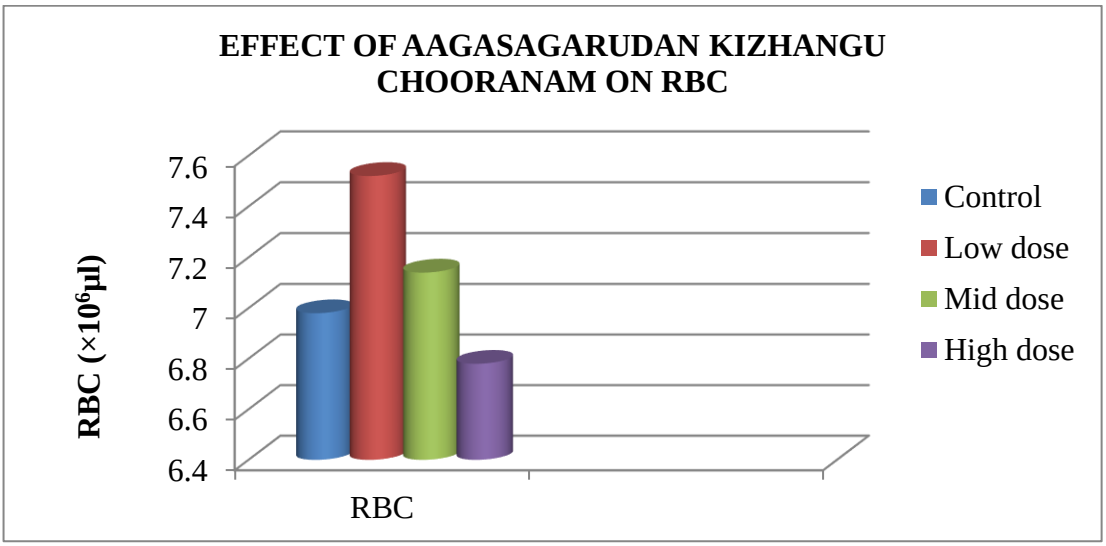


**Table 11: Effect of Aagasagarudan kizhangu chooranam on Hematological parameters of Wister albino rats in Long term toxicity study**

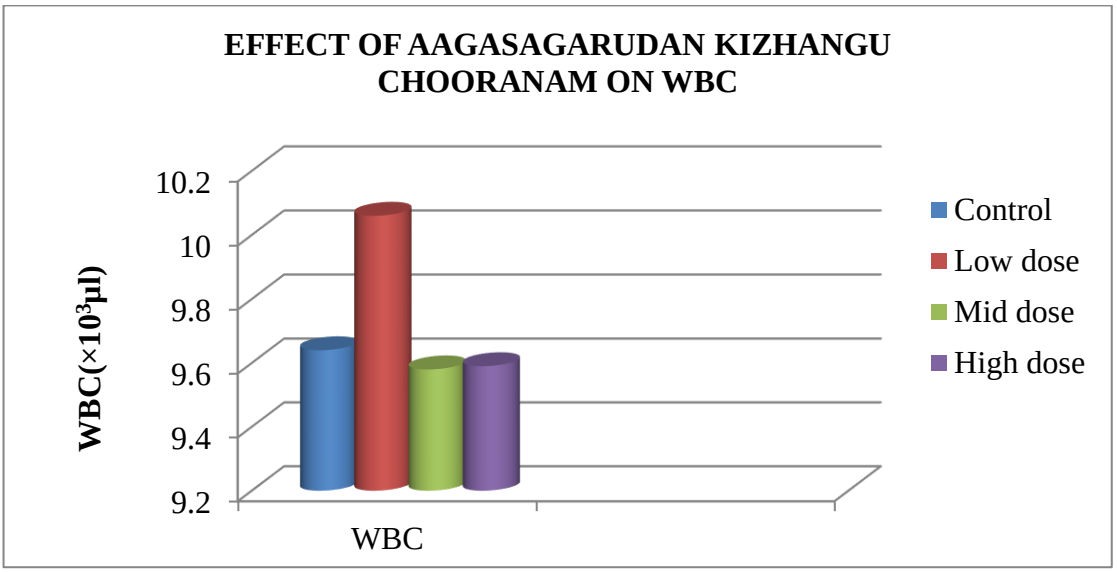
| Parameter | Control | LD | MD | HD |
|---|---|---|---|---|
| RBC ($\times 10^6$µl) | 6.98±3.23 | 7.52±0.62 | 7.14±1.04* | 6.78±1.24 |
| WBC($\times 10^3$µl) | 9.64±1.82 | 10.06±0.96* | 9.58±2.98 | 9.59±1.36 |
| PLT($\times 10^3$µl) | 672.2±154.25 | 683.4±142.78 | 742±129.63 | 731.8±164.64 |
| HGB(g/dl) | 13.52±1.44 | 13.46±2.88 | 13.86±1.95 | 14.14±0.95 |
| Neutrophils $10^3mm^3$ | 1.68±0.35 | 1.6±0.55 | 1.62±0.55 | 1.7±0.48 |
| Eosinophils (%) | 1.27±0.31 | 1.48±0.24 | 1.26±0.004** | 1.46±0.32 |
| Lymphocyte(%) | 71.34±0.84 | 65.2±4.18 | 77.28±5.89 | 79.6±3.43 |
| Monocyte(%) | 2.86±0.95 | 2.94±0.75 | 3.46±1.22 | 2.5±0.77 |
| Basophils (%) | 0.1±0.58 | 0.2±0.45 | 0.1±0.31 | 0.1±0.31 |
| MCH(pg) | 18.03±1.96 | 18.58±2.39 | 19.04±2.24 | 17.34±0.009** |
| MCV (fl) | 58.32±4.26 | 59.92±7.24 | 57.92±0.31 | 61.86±4.57 |

Values are mean of 10 animals ± S.D.(Dunnett's test). **($p < 0.01$),*($p < 0.05$), n = 10**.**

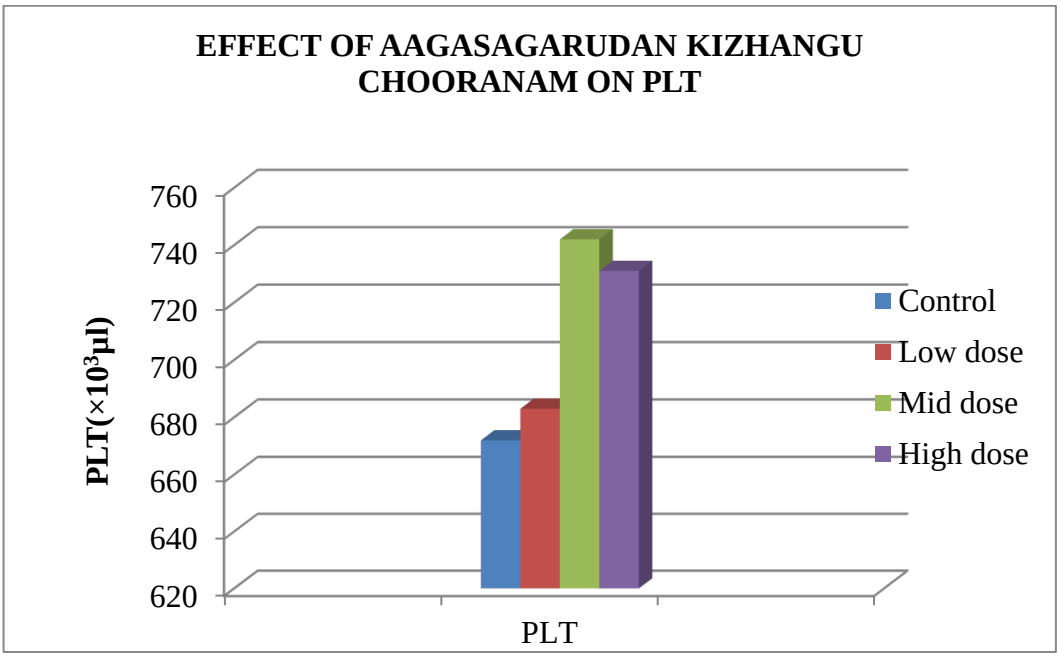
**Graph 4: Effect of Aagasagarudan kizhangu chooranam on Hematological parameters – RBC of Wister albino rats in Long term toxicity study**



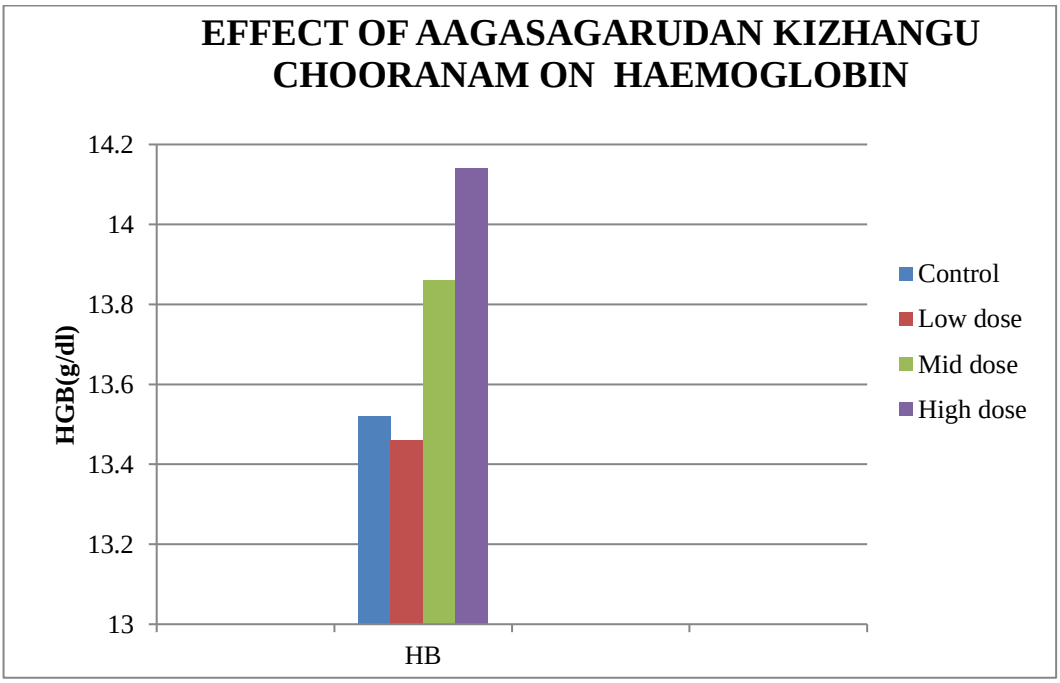

**Graph 5: Effect of Aagasagarudan kizhangu chooranam on Hematological parameters – WBC of Wister albino rats in Long term toxicity study**



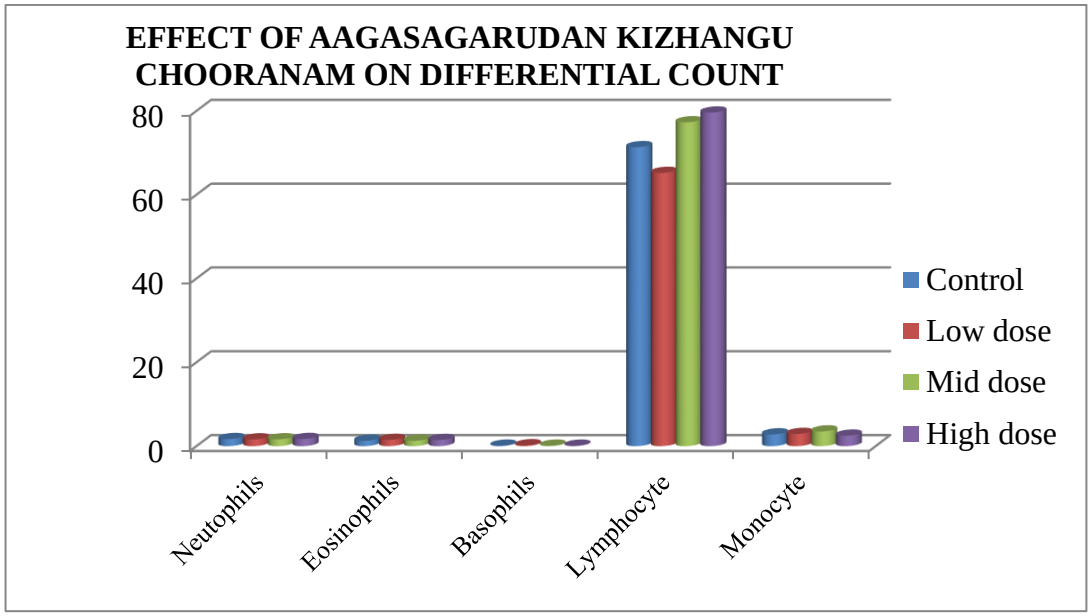
**Graph 6: Effect of Aagasagarudan kizhangu chooranam on Hematological parameters – Platelet count of Wister albino rats in Long term toxicity study**




**Graph 7: Effect of Aagasagarudan kizhangu chooranam on Hematological parameters – Haemoglobin of Wister albino rats in Long term toxicity study**



**Graph 8: Effect of Aagasagarudan kizhangu chooranam on Hematological parameters – Differential count of Wister albino rats in Long term toxicity study**




**Graph 9: Effect of Aagasagarudan kizhangu chooranam on Hematological parameters – MCH & MCV of Wister albino rats in Long term toxicity study**

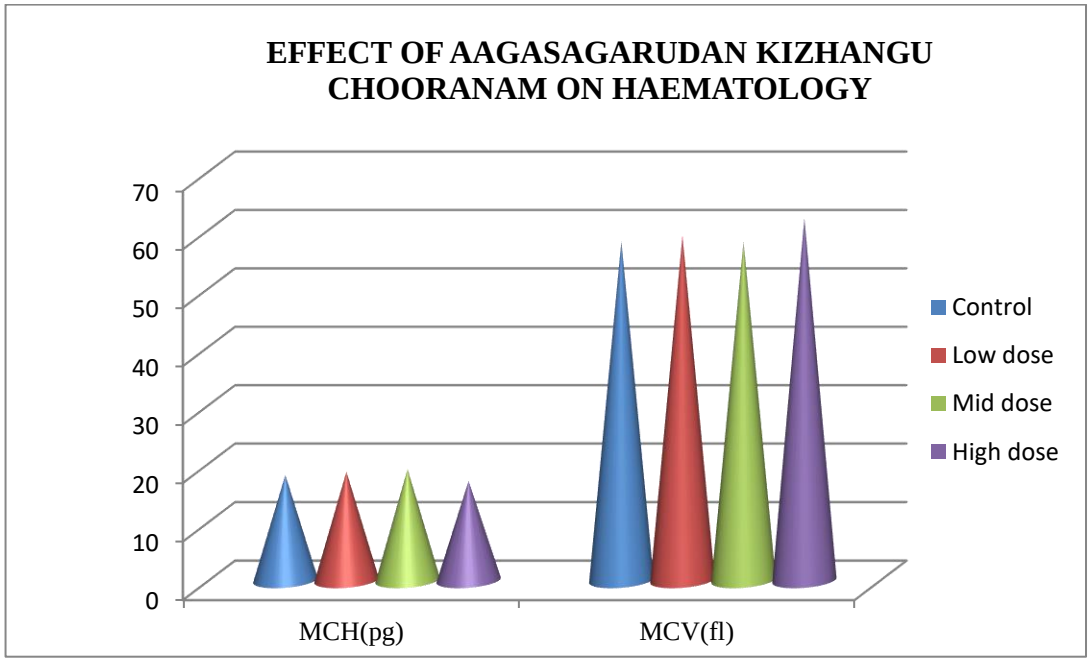


**Table 12: Effect of Aagasagarudan kizhangu chooranam on Biochemical parameters – Lipid profile of Wister albino rats in Long term toxicity study**

| **Dose(mg/kg)** | **Control** | **LD** | **MD** | **HD** |
|---|---|---|---|---|
| Total cholesterol (mg/dl) | 153.67±16.27 | 145.32±23.81 | 164.8±15.38 | 153.04±7.36 |
| HDL (mg/dl) | 59.3±9.06 | 56.3±6.04 | 67.8±11.08 | 61.6±0.04* |
| LDL (mg/dl) | 76.47±11.81 | 74.8±16.85 | 81.6±12.88 | 75.2±14.62 |
| VLDL (mg/dl) | 16.24±2.39 | 17.81±4.68 | 16.74±3.50 | 16.81±4.19 |
| Triglycerides (mg/dl) | 33.53±4.06 | 32.2±5.81 | 33.4±0.14 | 35.4±9.07 |

Values are mean of 10 animals ± S.D.(Dunnett's test). **($p < 0.01$),*($p < 0.05$), n = 10**.**

**Graph 10: Effect of Aagasagarudan kizhangu chooranam on Biochemical parameters – Lipid profile of Wister albino rats in Long term toxicity study**

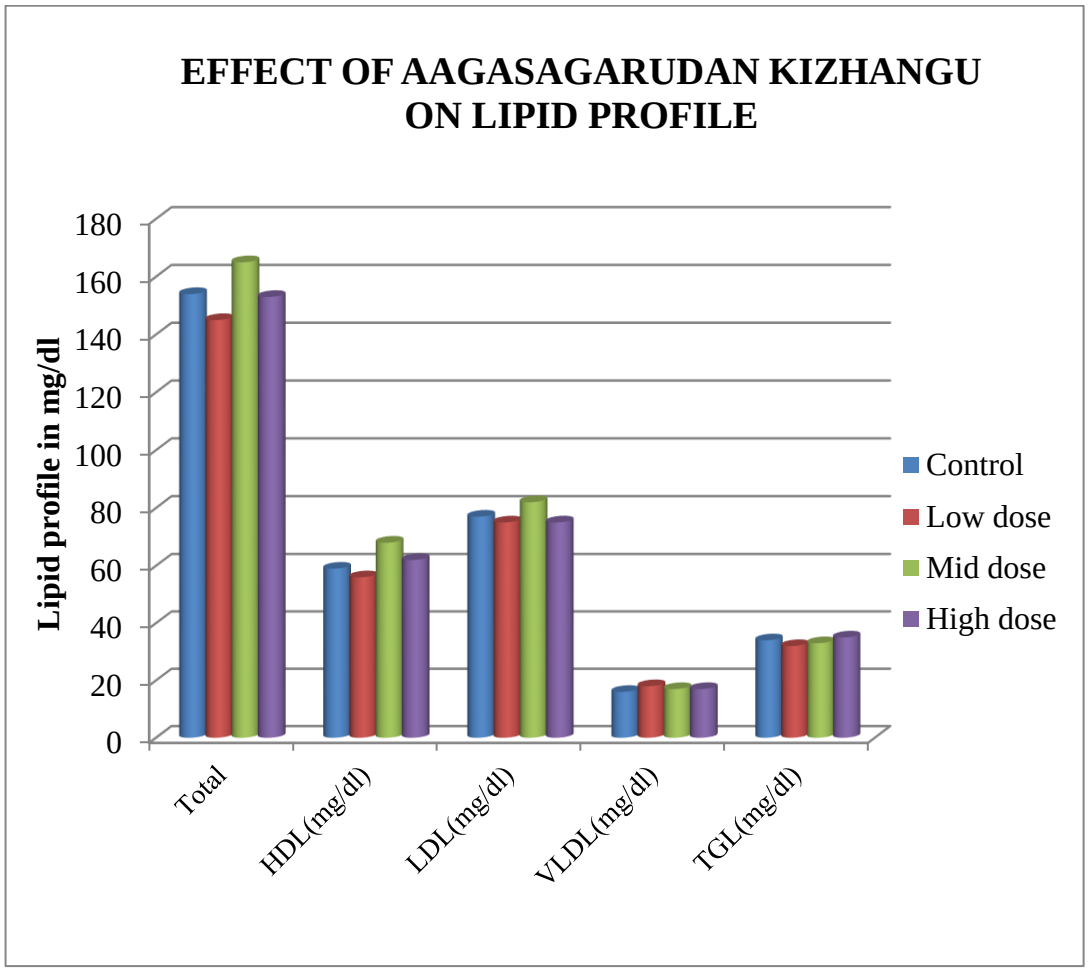



**Table 13: Effect of Aagasagarudan kizhangu chooranam on Renal Parameters of Wister albino rats in Long term toxicity study**

| Dose(mg/dl) | Control | LD | MD | HD |
|---|---|---|---|---|
| BUN(mg/dl) | 14.3±1.69 | 13.5±1.43 | 15.54±1.57 | 15.32±3.62 |
| Creatinine(mg/dl) | 0.68±0.34 | 0.67±0.24 | 0.69±0.16 | 0.71±0.19 |

Values are mean of 10 animals ± S.D.(Dunnett's test). **($p < 0.01$),*($p < 0.05$), n = 10.

**Graph 11: Effect of Aagasagarudan kizhangu chooranam on Renal Parameters of Wister albino rats in Long term toxicity study**

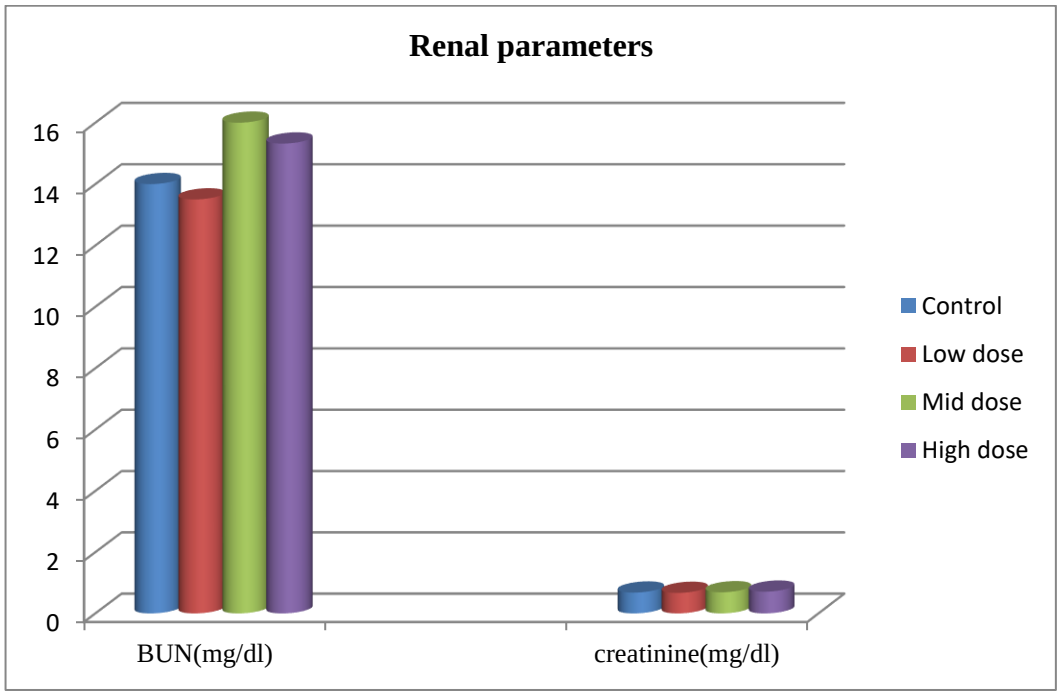
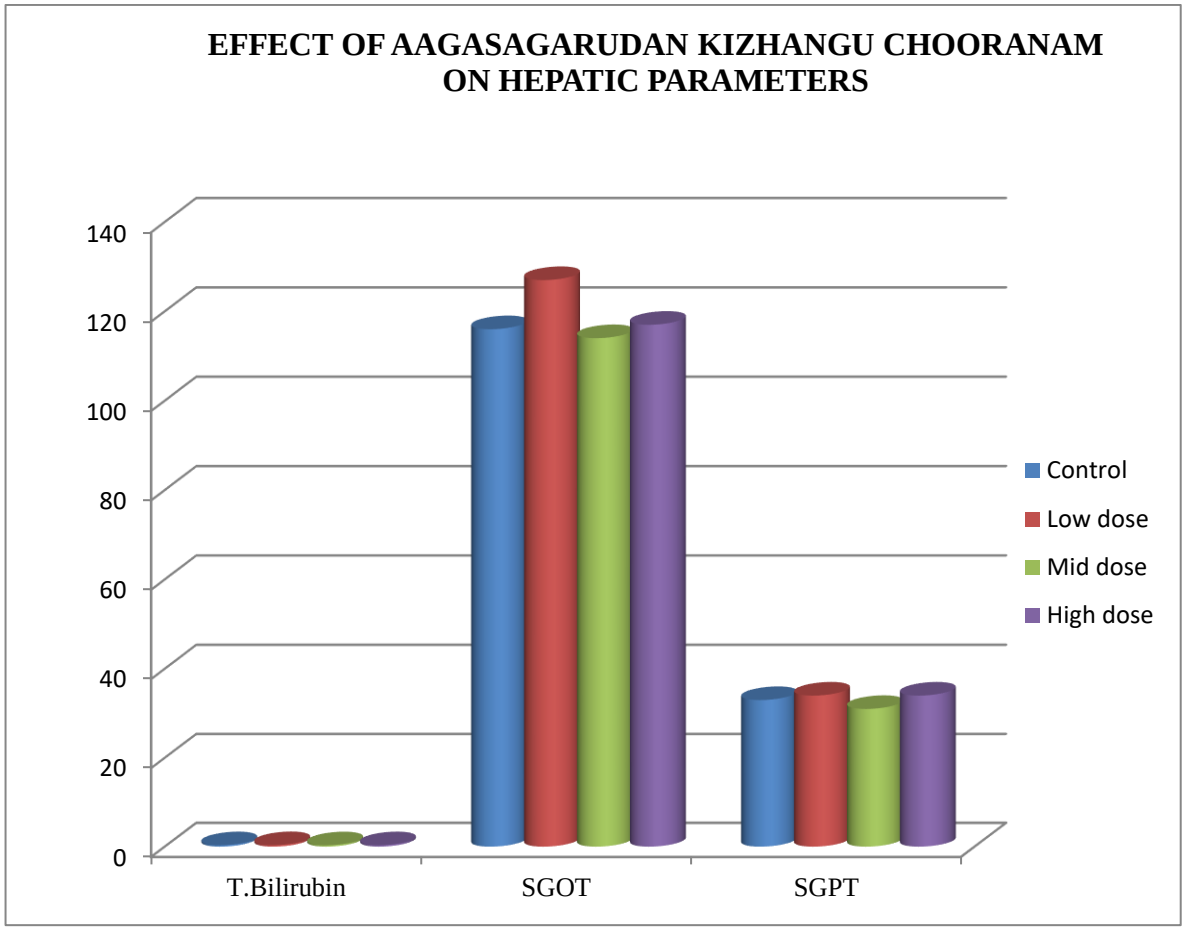



**Table 14: Effect of Aagasagarudan kizhangu chooranam on Hepatic Parameters of Wister albino rats in Long term toxicity study**

| **Dose(mg/dl)** | **Control** | **LD** | **MD** | **HD** |
|---|---|---|---|---|
| Total Bilirubin (mg/dl) | 0.32±0.19 | 0.34±0.11 | 0.31±0.17 | 0.32±0.09 |
| SGOT(U/L) | 115.6±32.21 | 126.6±28.97 | 113.6±24.08* | 117.4±34.35 |
| SGPT(U/L) | 33.45±3.54 | 34.4±4.62 | 31.2±5.81 | 34.2±9.13 |

Values are mean of 10 animals ± S.D.(Dunnett's test). **($p < 0.01$),*($p < 0.05$), n = 10.

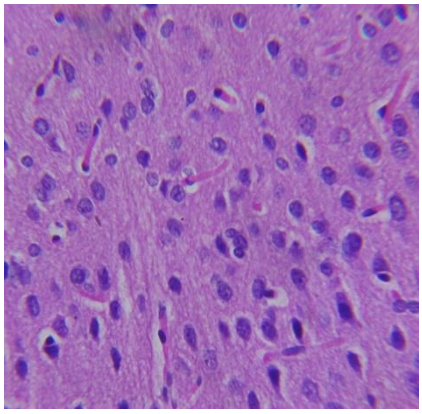
**Graph 12: Effect of Aagasagarudan kizhangu chooranam on Hepatic Parameters of Wister albino rats in Long term toxicity study**



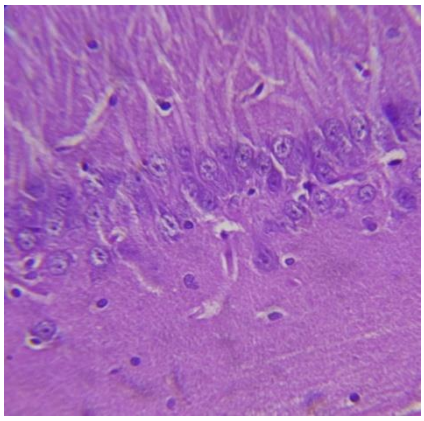
**HISTOPATHOLOGICAL INVESTIGATION OF CONTROL AND AGC TREATED ANIMALS UNDER MAGNIFICATION POWER 40X FOR 90 DAYS LONG TERM TOXICITY STUDY**

## BRAIN

Slide 1. CONTROL



Slide 2. HIGH DOSE



BRAIN

CONTROL:

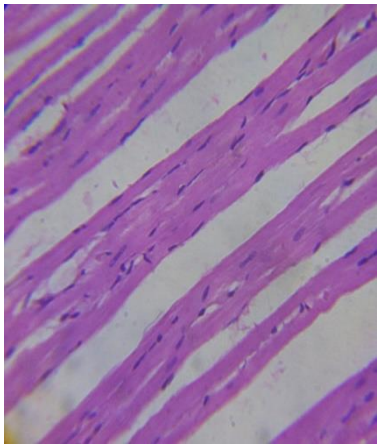
Arrangement of the neurons appears intact with no signs of degeneration or apoptotic changes in both the sample so cortex region showed normal neurons with polygonal to round cell bodies containing dense cytoplasm.

HIGH DOSE:

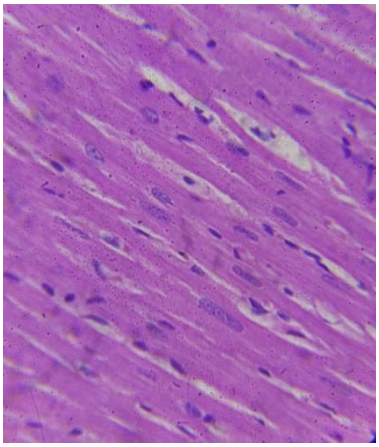
Appearance of Hippocampal neurons was normal with dense network o No signs of ischemic changes in the cerebral hemisphere

**HEART**

Slide 1. CONTROL



Slide 2. HIGH DOSE



HEART

CONTROL:

Perfectly -arranged myocardial fibers, clear transverse striation and normal structure were observed.

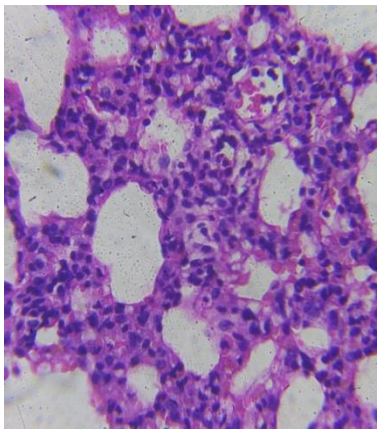
Appearance of cardiomyocyte was normal with dark nuclear region. The nuclei of muscle fibers appear oval arrangement

HIGH DOSE:

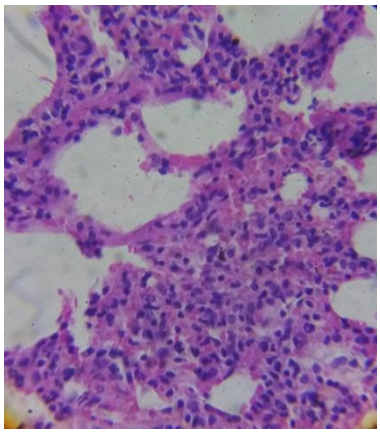
Myocardial cells appears normal with well-defined mycoplasma and prominent nucleus and nucleolus

# LUNGS

Slide 1. CONTROL



Slide 2. HIGH DOSE



LUNGS:

CONTROL:

Bronchial opening appears regular with no signs of infiltration

Appearance of alveolar network was normal

Nucleus of type I and II alveolar cells looks normal

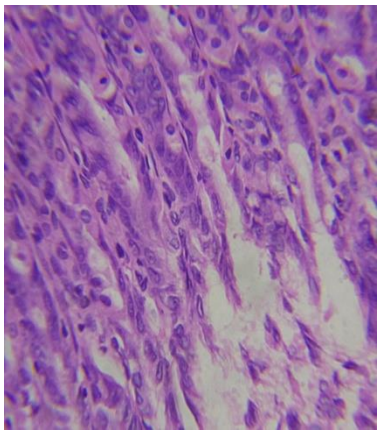
HIGH DOSE:

Perivascular region appears normal, Alveolar septa and wall appeared widen and normal

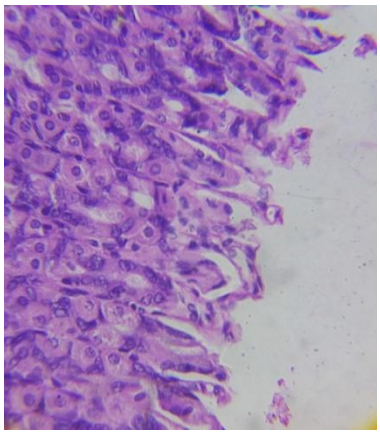
No signs of lymphocyte cuffing

**STOMACH**

Slide 1. CONTROL



Slide 2. HIGH DOSE



STOMACH:

CONTROL:

Gastric glands, gastric glands including secretary sheath appears normal

Normal gastric mucosa containing intact gastric gland cells, parietal cells which are spherical cell with deeply stained dark nucleus

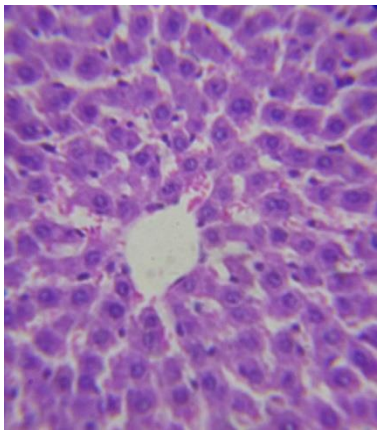
HIGH DOSE:

No signs of ulcer and glandular degeneration were observed

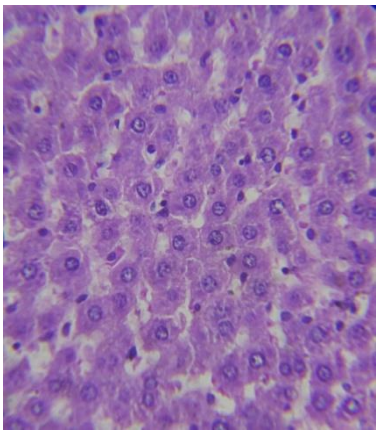
Appearance of Sub-mucosa and gastric glands appear normal

## LIVER

Slide 1. CONTROL



Slide 2. HIGH DOSE



LIVER:

CONTROL:

Rare appearance of Kupffer cells with no evidence of phagocytosis in intracytoplasmic region

Liver parenchyma appears normal with no evidence of necrosis

Appearance of terminal hepatic venules (central veins) to the portal tracts was Normal

HIGH DOSE:

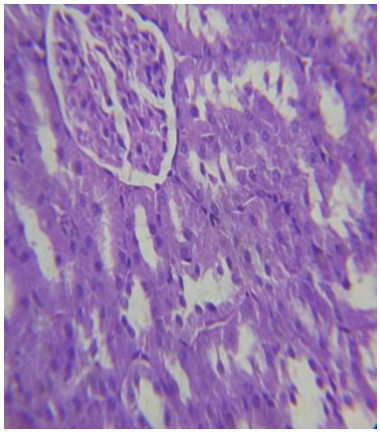
Apparent loss of liver parenchyma were observed

Increase distant of liver sinusoids were observed

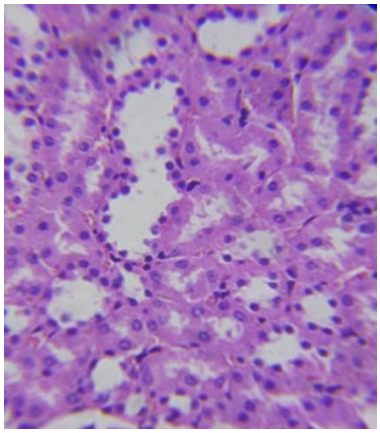
Occasional presence of Kupffer cells with no evidence of phagocytosis in intracytoplasmic region

## KIDNEY

Slide 1. CONTROL



Slide 2. HIGH DOSE



KIDNEY:

CONTROL:

Appearance of Podocytes and parietal epithelium in the glomeruli appears normal

Proximal and distal convoluted tubule appears normal

No signs of lesion or inflammation were observed

No signs of cellular necrosis

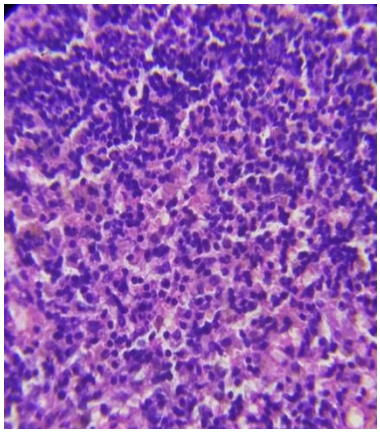
HIGH DOSE:

Some renal tubules appears hypertrophic

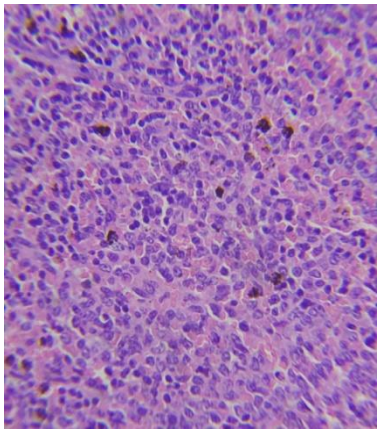
Appearance of Podocytes and parietal epithelium in the glomeruli appears normal

**SPLEEN**

Slide 1. CONTROL



Slide 2. HIGH DOSE



SPLEEN:

CONTROL:

No signs of perivascular inflammation

Appearance of splenic sinuses, Splenic cord and endothelial orientation was normal

Appearance of LF – lymphoid follicle; PALS – periarterial lymphoid sheath was normal with no significant signs of enlargement

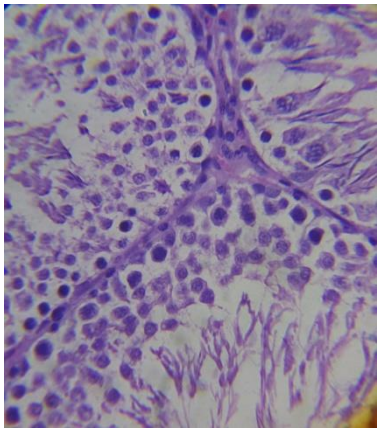
HIGH DOSE:

Marginal vascular zone radiated in between red and white pulp

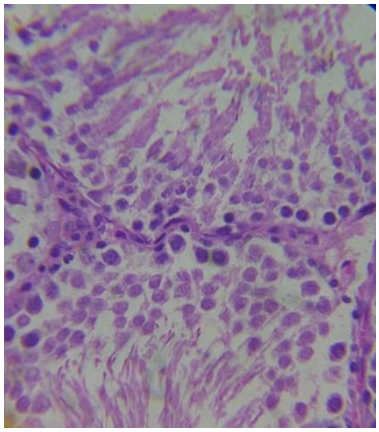
Appearance of spleenic red pulp was normal

**TESTES**

Slide 1. CONTROL



Slide 2. HIGH DOSE



TESTES:

CONTROL:

Histocytology of testicular tissue shows well differentiated germ cells with respect of spermatogonia includes spermatid and sperm were observed

Appearance of leydig cells, interstitial tissue, seminiferous tubule, Sertoli cells and spermatogonia were normal

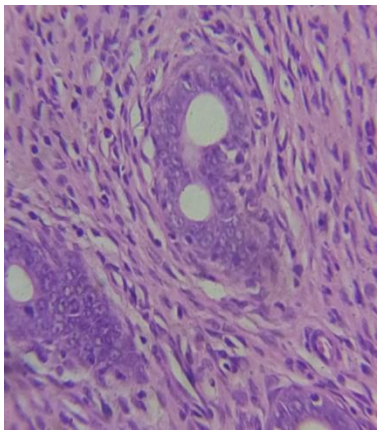
HIGH DOSE:

Histo cytology of testicular tissue shows well differentiated germ cells with respect of spermatogonia includes spermatid and sperm were observed

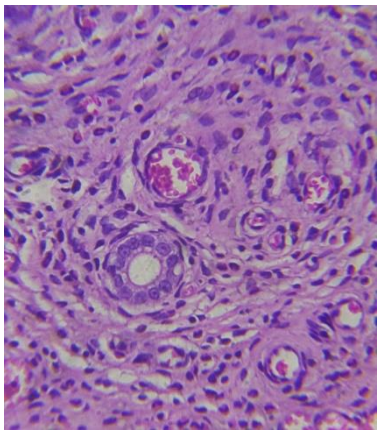
Appearance of leydig cells, interstitial tissue , seminiferous tubule, Sertoli cells And spermatogonia were normal

## UTERUS

Slide 1. CONTROL



Slide 2. HIGH DOSE



UTERUS

CONTROL:

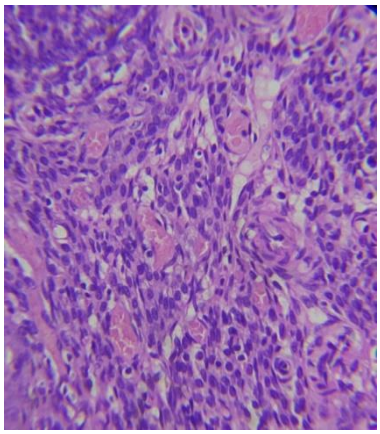
Appearance of endometrium,
myometrium and
uterine glands was normal.

HIGH DOSE:

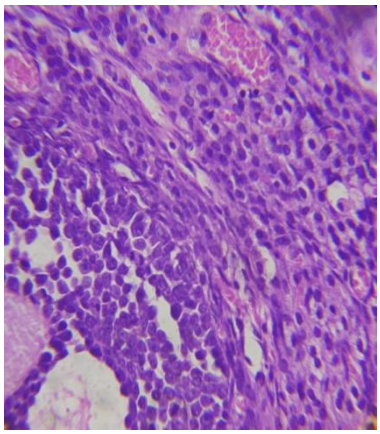
Appearance of endometrium,
myometrium and
uterine glands was normal

## OVARY

Slide 1. CONTROL



Slide 2. HIGH DOSE



OVARY

CONTROL:

Histopathological analysis of ovary showing normal corpus luteum (CL) and primordial follicles with few mature ovarian follicles with no signs of abnormality.

HIGH DOSE:

Appearance of antral follicle,

primary oocyte and

secondary follicles all were normal

# 6. DISCUSSION

I have selected ***Aagasagarudan kizhangu chooranam*** to evaluate the safety profile. First with the test drug I have done the process of standardization with qualitative and quantitative analysis**.** The following are the analysis

- Physico-chemical analysis
- Biochemical Analysis
- ICP-OES Analysis
- GC-MS Analysis
- FT-IR Analysis

The safety profile is evaluated by Acute and Long Term toxicity studies on Wister Albino rats as per WHO guideline.

The **Physico-chemical analysis** of AGC (Table: 1) concludes the following results. The pH of AGC is 6.5. Being weak acidic, the drug is more readily absorbed in an acid medium like stomach which enhances the bioavailability of the drug.

The loss on drying test is designed to measure the amount of water and volatile matters in a sample when the sample is dried under specified condition. Low moisture content is always desirable for higher stability of drugs. The percentage of loss on drying of AGC was 6.19% (Normal range: 1-20%). So Low moisture of AGC could get maximum stability and better shelf life.

The Ash limit Tests are designed to measure the amount of the residual. A high ash value is indicative of contamination, substitution, adulteration or carelessness in preparing the drug.The total Ash content and Acid Insoluble Ash values of AGC were 3.6% (Normal range: 1-25%) and 0.4% (Normal range: 0.1 – 10%). This indicates the purity of the test drug.

Extraction value determines the amount of active constituents in a given amount of the formulation when extracted with a solvent media such as water and alcohol. The water soluble and alcohol soluble extract values provide an indication of the extent of polar and non polar compounds respectively present in AGC .The extract values of Alcohol in AGC is 11.69% and water is 14.09%. Decreased Water soluble ash value (2.8 %) indicates easy facilitation of diffusion and osmosis mechanisms.

**Biochemical Analysis** of Aagasagarudan kizhangu chooranam for Acid radicals, Basic radicals, and other constituents demonstrates the presence of **Silicate, Phosphate, Zinc, Iron, Calcium, Magnesium, Ammonium, Potassium, Starch and Alkaloids.** (Table3)

Phosphate is a charged particle that contains the mineral phosphorus. The mineral phosphorus is primarily used for growth and repair of body cells and tissues. It reduces the histamine release by activated mast cells. Zinc is deeply involved in regulation of immune system. Deficiency of zinc leads to development of inflammatory and autoimmune disorders. So the presence of zinc in AGC cures the inflammatory disorders thereby regulating the immune system.

The **Quantitative Analysis** of Aagasagarudan kizhangu chooranam through **ICP-OES** results showed that the Heavy metals like lead, mercury, nickel, copper, arsenic and cadmium were found in below detection level. Hence it may be safe for human consumption. It also shows the presence of physiologically important minerals like Arsenic, Calcium, Cadmium, Copper, Iron, Mercury, Potassium, Manganese, Sodium, Nickel, Lead, Phosphorus and Zinc which are within ppm limit. (Table-4)

Through GCMS Analysis, the identified compounds in Aagasagarudan kizhangu chooranam are 9,12-Octadecadienoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester, cis-, Tridecanoic acid, methyl ester,9, 12-Octadecadienoic acid, methyl ester, Psi, psi carotene, 3,4didehydro-1, 2, 7'8' –tetrahydro-1-methoxy-2-oxo, 1,1-Cyclobutanedicarboxamide, 2-phenyl-N-N`-bis(1-phenylethyl)- 2-phenyl-N-N'-bis(1-phenylethyl)-, Cyclopentanepropanoic acid, 3,5bis(acetyloxy)-2-[-(acetyloxy)-3-(methoxyimino)octyl]-, methyl ester. These identified compounds like 9, 12-Octadecadienoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester having so many medicinal values such as Hypocholesterolemic 5-Alpha reductase inhibitor, Antihistaminic, Anticoronary, Insectifuge, Hypocholesterolemic, Antieczemic, anti oxidant, anti microbial activity[54].(Table 5)

In the **FT-IR** Spectra analysis, this Aagasagarudan kizhangu chooranam sample exhibits the peak value indicates the presence of some organic functional groups such as alcohols, phenols,1`,2`amines, amides, Alkynes, Alkenes, Alkyl

halides, Nitro compound, Alcohols, carboxylic acids, esters, ethers, Aliphatic amines, alkenes, Carboxylic acids, Aromatics.

In this amide linkages in a biochemical context are called peptide bonds when they occur in the main chain of a protein and isopeptide bonds. Proteins can have structural roles such as in hair or spider silk, but also nearly all enzymes are proteins. The Aromatic compounds play key roles in the biochemistry of all living things. Aromatic amino acids serve as one of the 20 basic building blocks of proteins. The all 5 nucleotides make up the sequence of genetic code in DNA and RNA are aromatic purines or pyrimidines. The molecule heme contains an aromatic system with 22π electrons and chlorophyll also has a similar aromatic system. Amines and Carboxylic acids possess which enhances the drug effect against the disease

In **Acute toxicity study** there was no abnormal signs reported at the dose level of (2000 mg/kgb.wt) within 24hours in Wistar Albino Rats. No mortality and No pathological changes have been seen in the internal organs of both control and treated groups in the 14 days study period. And the Body weight, food intake and water intake of animals are normal.

**Long term Toxicity Study** was conducted for about 90 days as per WHO guideline in 3 doses Low dose (360mg/kg b.wt), Mid dose (720mg/kg b.wt), High dose (1440mg/kg b.wt). Animals were observed throughout the period. There was no significant change in body weight (Table: 8), food intake (Table: 9), and water intake (Table: 10). After 90 days animals were sacrificed and blood samples were collected and investigated. The results revealed that there were significant in RBC, WBC count and more significant changes in Eosinophils (Table 11). In hepatic parameter significant changes in SGOT (Table: 14) and in Biochemical parameter significant changes in HDL (Table: 12). The histopathological study on the organs such as brain, heart, lungs, kidney, spleen, liver, stomach, uterus, ovary and testes was normal in control, low dose, mid dose and high dose groups.

## 7. SUMMARY

*Aagasagarudan kizhangu chooranam* was taken as dissertation drug for the evaluation of its toxicity profile. This is a single drug formulation which was prepared by purification of Aagasagarudan kizhangu through Pittaviyal process and administered with Naattu sarkkarai as adjuvant. The drug was chosen from the Siddha text Gunapadam mooligai vaguppu. The above drug is indicated for virulent poison, Anemia, Pruritus, Thyroidism, Leprosy and Herpes zoster.

The raw drugs were procured from Rajan Herbals shop; Broadway, Chennai and the drugs were identified and authenticated by Asst.Professor of Botany, National Institute of Siddha. The drug was underwent physicochemical, biochemical analysis, heavy metal analysis using ICP-OES, Phytocompound analysis using GC-MS and organic functional group analysis using FT-IR techniques. Acute and long term toxicity studies were conducted as per WHO guideline.

The weak acidic pH of AGC enhances the bioavailability of the drug. Decreased Water soluble ash value indicates easy facilitation of diffusion and osmosis mechanisms.

The Ash value of the test drug shows the purity,

ICP-OES results shows the heavy metals such as lead, mercury, nickel, copper, arsenic and cadmium were found in below detection level.

The Biochemical analysis of the drug contains Silicate, Phosphate, Zinc, Iron, Calcium, Magnesium, Ammonium, Potassium, Starch and Alkaloids.

Through GCMS analysis, the identified compounds like 9,12-Octadecadienoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester, having so many medicinal values such as Hypocholesterolemic 5-Alpha reductase inhibitor, Antihistaminic, Anticoronary, Insectifuge, Hypocholesterolemic, Antieczemic, antioxidant and anti microbial activities.

The FT-IR Spectra analysis indicates the presence of some organic functional groups such as alcohols, phenols,1`,2`amines, amides, Alkynes, Alkenes, Alkyl halides, Nitro compound, Alcohols, carboxylic acids, esters, ethers, Aliphatic amines, alkenes, Carboxylic acids, Aromatics. Amines and Carboxylic acids possess which enhances the drug effect against the disease.

The Acute toxicity study results revealed that no mortality in dose level 2000mg/kg body weight group animals. It concludes that LD 50 cut-off of *Aagasagarudan kizhangu chooranam* may be above 2000mg//kg body weight as per WHO guideline. Long term toxicity study was conducted for about 90 days as per the WHO guideline. The different dose levels of *Aagasagarudan kizhangu chooranam* selected were Low dose (360mg/kg b.wt), Mid dose (720mg/kg b.wt) and High dose (1440mg/kg b.wt) respectively. The control animals were administered normal saline only.

The haematology and biochemical parameters were subjected to one way ANOVA analysis which shows no significant changes in CBC, Lipid profile, RFT and LFT in all test groups compared to control group in blood. The histopathology report of organs reveals that all organs such as Brain, Heart, Lung, Stomach, Liver, spleen, kidney, testes, uterus and ovary were normal in low, mid and high dose groups when compared to control group.

# 8. CONCLUSION

From the results of this study, the qualitative analysis of (AGC) reveals the Purity and Bioavailability of the drug. As heavy metals were found in Aagasagarudan kizhangu chooranam are within the permissible limit, the drug is safe enough for oral consumption. Toxicity studies states that there was no mortality and signs of toxicity observed for acute oral administration of AGC with the therapeutic dose (2000 mg/kg b.wt) in the prescribed manner. In long term toxicity study there was no significant changes in haematological, biochemical parameter in AGC treated groups when compared to control group. The histopathology report also confirms that there are no remarkable cellular changes at all the dose levels. It clearly demonstrates that there was No Observed Adverse Effect Level (NOAEL) upto the high dose level (1440 mg/kg b.wt). Based on these results it can be conclude that, the dose level of Aagasagarudan kizhangu chooranam 2 to 4gm (Arai muthal oru varaagan alavu) mentioned in the Siddha literature Gunapadam mooligai vaguppu is safe dosage for human consumption.

In upcoming days it should be carried out to study the pharmacological activity and clinical trial to prove the efficacy of the drug.

BIBILIOGRAPHY


1. Thiyagarajan R, Gunapadam thathu jeeva vaguppu, Indian Medicine & Homeopathy department, second edition revised 2006, Pg.no:57
2. Sambasivampillai TV, Tamil-English dictionary of Medicine, Chemistry, Botany and allied science, Vol 1. The research institute of Siddhar's science; 1931. Pg.no:550.
3. Murugesa mudhaliyar KS, Gunapadam mooligai vaguppu, Indian Medicine & Homeopathy department, Sixth edition 2006, Pg.no:60-61
4. Sakthivel G .et al ,in vivo and vitro analysis of poly herbal formulation against Russell's viper and cobra venom and screening of bioactive components by Docking studies, hindaw publishing corporation vol2013 article i.d781216, Pg.no:12.
5. Somasundaram S, Angiospermgalin vagaippaadu (Maruthuva thavaraviyal-paguthi 2), Elangovan pathippagam, 3rd revised edition, September 2006, Pg.no: 110
6. Kannusami pillai C, Sikitcharatna deepam ennum vaithiya nool, B.Rathina naickar & sons, 2007, Pg.no:32
7. Pandit Murugesa mudhaliyar KS, Nanju Murivu Nool, Indian Medicine and Homeopathy deparment, Chennai-106, 4th revised edition, 2006, Pg.no: 91-149 (Ref Text)
8. The Wealth of India, National Institute of science communication and Information resources, First supplement series-Raw materials Vol II, First reprint 2004, Pg 194.
9. Chopra *et al*, I, 410; Chopra *et al*, 1958, 544; Sharma *et al, Indian For,* 1970, 96, *678;* Godbole, *Poona Agric Coll Mag*, 1959, 50, 91
10. Kattamanchi G., Kontham Ramakanth R., Gudur Pavan K., Bheemanapally K., Karka Srinivas R. and Avvari Sanjeeva K., *Int. Curr. Pharm. J.*, 2013; 2(3): 53-56. DOI 10.3329/icpj.v2i3.13581.
11. Kirtikar K.R.and Basu B.D., *Indian Medicinal Plants*, International Book Distributors, Dehradun, 1988.
12. Heinrich M., Barnes J., Gibbons S. And Williamson E.M., *Fundamentals of Pharmacognosy and Phytotherapy,* Churchill Livingstone, Elsevier Science Ltd, 2004.

13. https://herbpathy.com/Uses-and-Benefits-of-Corallocarpus-Epigaeus-Cid539
14. Available at https://www.flowersofindia.net/catalog/slides/Redfruit%20Creeper.html
15. Available at https://en.wikipedia.org/wiki/Corallocarpus
16. Available at https://npgsweb.ars-grin.gov/gringlobal/taxonomydetail.aspx?id=459876
17. WHO traditional medicine strategy: 2014-2023; December 2013, available at *www.who.int/iris/.../9789241506090_eng.pdf*
18. Dhanapal, R; Chandanam, Sridhar; M, Vrushabendra Swamy B; L, Ashoka Babu V; Gupta M et al. Asian Journal of Chemistry, 18.2 (2006): Pg 1013-1016.
19. Nutritional Properties and Chemical Composition of *Corallocarpus epigaeus* (Arn.) Cl : As Remedy to Control Diabetes mellitus Kalpesh Ishnava* [a], Ruchi Kotadia [a] and Sandip Patel[b]. Chiang Mai J. Sci. 2015; 42(4): 806-815. Available at http://epg.science.cmu.ac.th/ejournal/Contributed Paper. 3 October 2014.
20. Formulary of Siddha Medicines, Indian medical practitioners cooperation pharmacy and stores, Lattice bridge road, Thiruvanmiyur, Chennai-600041. 1993, Pg:38
21. Krishnamoorthy JR, et al, Egyptian Dermatology Online Journal 7 (1): 1; Effective Management of Allergy by a Siddha preparation drug G7 - An In Vitro Study
22. Kalpesh Ishnava et al, Nutritional Properties and Chemical Composition of *Corallocarpus epigaeus* (Arn.) Cl: As Remedy to Control Diabetes mellitus, Chiang Mai Journal Science. 2015; 42(4) Pg 806-815
23. Kim H.J et al, *BMC Biology*, 2005; 3: 23. DOI 10, 1186/1741-7007-Pg 3-23
24. Saranya U et al, Evaluation of Haemopoietic Activity of the Rhizome of Corallocarpus epigaeus Benth. Ex Hook-A Pilot Study, International Journal of Advanced Ayurvedic and Herbal Medicine 2017, Volume 3, Issue 1, Pg 18-25, Article ID Med-355
25. Venkata.Naga.Anantha. Sandhya R Nandyala, Evaluation of *In-vitro* Anti-cancer activity of Ethanolic Extract of *Corallocarpus epigaeus* on Chronic

Myeloid Leukaemia K562 Cell lines Journal of Comprehensive Pharmacy 2015;2(1): Pg 8-13

26. Saranya et al, Screening of Functional Groups, DNA Quantification and Determination of Antimicrobial Potency of Corallocarpus epigaeus Tubers, International Journal of Pharmaceutical and Clinical Research 2015; 7(1): Pg 1-4
27. Jayaseelan M et al, Biochemical Quantification and antibacterial properties of *Corallocarpus epigaeus,* International Journal of Pharmaceutical And Biomedical Research 2014, 5(1), Pg 18-24
28. Bhavani and Leelavathi, Investigation On In Vitro Cytotoxic Activity of a Selected Wild Cucurbitaceae Plant Corallocarpus Epigeaus Against Cancer, International Journal of Pharmaceutical Science and research, 2015; Vol. 6(8): Pg 3554-3557
29. Vijaya ponna *et al*, Impact of antidote medicinal plant- *corallocarpus epigeus* extract on lipid peroxidation induced by *naja naja*- snake venom in albino rat, International Journal of Medicine and Pharmaceutical sciences (IJMPS), vol. 3, issue 5, dec 2013, Pg 21-32
30. Narendra et al, Studies on Chemical and Biological properties of *Bryonia epigaea* (Rottler), Journal of Medicinal Plants Research, Vol. 9(22), June, 2015, Pg 664-673
31. Hemalatha S *et al*, Anti-snake venom activity of root and rhizome of *corallocarpus epigaeus*, International Journal of Experimental Pharmacology, Vol 4|Issue 3| 2014 | 145-147.
32. Gnananath et al., Evaluation of antidiabetic activity in *Corallocarpus epigaeus* rhizomes, International Current Pharmaceutical Journal, February 2013, 2(3): Pg 53-56
33. T. Shri Vijaya Kirubha *et al*, Anthelmintic activity of roots and rhizomes of Corallocarpus Epigaeus, Journal of Natural Product and Plant Resources, 2011, 1 (1): Pg 81-84
34. Vidyathri, R.D., "A Textbook of Zoology", 14th Edn., S.Chand and Co., New Delhi, 1997,Pg.329

35. Thorn, G.W., Adams, R.D. Braunwald, E., Isselbacher, K.J. and Petersdorf, R.G., "Harrison's Principles of Internal Medicine", Mcgraw Hill Co., New York, 1997, Pg.1088
36. Vigar, N., "Atlas of Medical Parasitology", 2nd Edn., P.G. Publishing House, Singapore, 1984, Pg: 216
37. Vasantha K et al, Efficacy of Phytochemical and Antibacterial activity of Corallocarpus epigaeus Hook.f., /International Journal Pharm Tech Research., 2012, 4(1)
38. Rajalakshmi.A et al, Scientific validation on Siddha sashtric herbomineral formulation "Gendhaga Vallaathi" for Rheumatoid arthritis – A review
39. V. Sambasivampillai: Tamil English Dictionary. Indian medicine andHomoeopathy Chennai ; 1998; Volume 4: P. 213- 251
40. Pradeep Kumar, Govindprasad mangal, pharmacutial study of Jayapala sedd, IAMJ, ISSN 2320.
41. Rajalakshmi et al, Physico-chemical analysis of Gandhagam before and after purification.2010, pages 32-35
42. Ramachandran S.P, Agasthiyar Vaithya Rathinachurukam, Thamarai Noolagam, May1994,Page no.35
43. Dr. D.R.Lohar, Protocol for Testing Ayurvedic, Siddha and Unani Medicines published by: Government of India, Department of AYUSH (PLIM Guidelines
44. Prashant Tiwari, Bimlesh Kumar, Mandeep Kaur, Gurpreet Kaur, Harleen Kaur. Phytochemical screening and Extraction: A Review. Internationale Pharmaceutica Sciencia 2011; 1: 98-106
45. Mathew.S Wheal, Terasa O fowles et al.A Cost effective acid digestion method using closed polypropylene tuber for ICP-OES analysis of plant essential elements.Analytical methods Issue 12, 2011
46. Northern Illinois University, Chemistry Analytical lab, FT-IR sample preparation.
    2007.http://www.niu.edu/ANALYTICALLAB/ftir/samplepreparation.shtml (06 Oct 2013)
47. Fourier Transform Infrared Spectroscopy (FT-IR) Analysis and testing chemical compound available at http:// www.intertek.com/analysis/ftir

48. FT-IR sample preparation, Northern Illinois University, Dept of Chemistry & Biochemistry. Availableat http;//www.niu.edu/ANALYTICALLAB/ftir/samplepreperation.shtml
49. Langes's Handbook of Chemistry, 8th edition, handbook publisher.Inc, 1952 Flim Scientific Inc, manual 2004
50. Phosphate in Blood, WebMD. Available from http://www.webmd.com/a-to-z-guides/phosphate-in-blood.
51. Sue Roberts, What is the Main function of Phosphorus in the body. Available from http://healthyeating.sfgate.com/main-function-phosphorus-body-5789.html
52. Treatment of disease with fructose-1,6-diphosphate. Available from http://www.google.com/patents/US5858985
53. Available from https://www.ncbi.nlm.gov/pmc/articles/PMC5852775
54. Never Zekeya, Musa Chacha, Francis Shahada and Abdul Kidukuli, Analysis of phytochemical composition of Bersamaabyssinica by gas chromatography – mass spectrometry, Journal of Pharmacognosy and Phytochemistry 2014; 3(4): 246-252
55. http://en.m.wikipedia.org/wiki/Amide

# CERTIFICATE

This is certify that the project title. Pre-clinical Safety evaluation of 'Akarasagarudan kizhangu choornam (AGC)'

has been approved by the IAEC. 100 Rats (50 M + 50 F) Approval No: NIS/IAEC-II/11/2016

Prof. Dr. V. Banumathi
Name of Chairman/~~Member Secretary~~ IAEC:

Prof. Dr. K. Nadimuthu.
Name of CPCSEA nominee:

V. Banumathi 23/8/16
Signature with date

DIRECTOR
NATIONAL INSTITUTE OF SIDDHA
Tambaram Sanatorium,
Chennai - 600 047.

Chairman/~~Member Secretary~~ of IAEC:

23/8/2016
CPCSEA nominee:

(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by Office)

# NATIONAL INSTITUTE OF SIDDHA, CHENNAI – 600047

## BOTANICAL CERTIFICATE

Certified that the following plant drug used in the Siddha formulation **"Aagasagarudan Kizhangu Choornam"** (Internal) for taken up for Post Graduation Dissertation studies by **Dr.V.M.Karthic**, M.D.(S), II year, Department of Nanjunool Maruthuvam, 2017, are identified through Visual inspection, Experience, Education & Training, Organoleptic characters, Morphology and Taxonomical methods as

*Corallocarpus epigaeus* Benth. Ex Hook.f. (Cucurbitaceae), Root tuber

MEDICINAL BOTANY ★ CHENNAI 600 047 ★ National Institute of Siddha

Certificate No: NISMB2692017

Date: 07-01-2017

Authorized Signatory

Dr. D. ARAVIND, M.D.(s),M.Sc.,
Assistant Professor
Department of Medicinal Botany
National Institute of Siddha
Chennai - 600 047. INDIA

# International Conference on Advances in Biotechnology and Biotherapeutics

## ICABBS - 2017

*08th - 10th March, 2017*

*www.icabbs2017.com*

Organized by

**SATHYABAMA UNIVERSITY**

(Established under section 3 of the UGC Act,1956)

**Declared as Category 'A' University by MHRD, Government of India**

Jeppiaar Nagar, Rajiv Gandhi Road,
Chennai - 600119, India

Supported by

**INDIAN NATIONAL SCIENCE ACADEMY**
New Delhi - 110 002

## CERTIFICATE OF PRESENTATION

*This is to certify that Dr./~~Mr./Ms.~~* V.M. Karthic *of* National Insititute of Siddha, Chennai. *has presented a paper entitled* Phytochemical & heavy metal analysis of Purified Corallocarpus epigaeus. in the aspect of Siddha System of medicine. *in the International Conference on "Advances in Biotechnology and Biotherapeutics" (ICABBS-2017) organised by Sathyabama University, Chennai India during March 08th - 10th, 2017.*

**Dr. T. SASIPRABA**
**Organizing Secretary**
**ICABBS-2017**

**Dr. MARIAZEENA JOHNSON**
**PRO CHANCELLOR**
**Sathyabama University, Chennai**

**Dr. MARIE JOHNSON**
**VICE PRESIDENT**
**Sathyabama University, Chennai**

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

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

*This Certificate is awarded to Dr/~~Mr~~/~~Mrs~~.....V.M.:..KARTHIC.....*

*For participating as ~~Resource Person~~ / Delegate in the Twenty second 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 06th to 10th June 2016.*

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

Prof.**Dr.S.PUSHKALA,** M.D.,
REGISTRAR (FAC)

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

# NATIONAL INSTITUTE OF SIDDHA

(An Autonomous body under Ministry of AYUSH, Govt. of India)
Tambaram Sanatorium, Chennai- 600 047

Workshop on

## "BASIC RESEARCH TECHNIQUES AND PRACTICES INVOLVED IN LABORATORY ANIMAL CARE"

**06 -10 February 2017**

## CERTIFICATE

This is to certify that Dr. V. M. Karthic has participated as Delegate/~~Resource~~ Person in the workshop on "Basic Research Techniques and Practices involved in Laboratory Animal Care" held on 06-10 February, 2017 at National Institute of Siddha, Chennai-47, Tamilnadu.

**Dr. V. Suba**
Organizing Secretary

**Dr. P. Muthusamy**
Veterinary Consultant

**Prof. Dr. V. Banumathi**
Director / Chairperson

CLINICAL RESEARCH & HOSPITALS (P) LTD

## *National Conference on*

# HERBAL MEDICINE AND ETHNOPHARMACOLOGY

Date: 06.04.2017; Venue: TICEL Biopark.

*This is to certify that ~~Ms./Mr.~~/Dr.* V.M. KARTHIC *from* DEPT. OF NANJU NOOLUM, NARUTHUVA NEETHI NOOLUM, NATIONAL INSTITUTE OF SIDDHA, CHENNAI *attended the National Conference on "**Herbal Medicine and Ethnopharmacology**" conducted in V.S. Clinical Research & Hospitals (P) Ltd., Chennai, Tamil Nadu. He/She presented a paper/poster in the topic* PURIFICATION PROCESS OF NERVAALAM

T. Mathangi

Dr. T. Mathangi
Scientist & Coordinator

Dr. L. Lokoranjan
Chairman & Managing Director

# SIDDHA MEDICINAL PLANTS GARDEN
# CENTRAL COUNCIL FOR RESEARCH IN SIDDHA

Mettur Dam - 636 401.

**National Workshop**
**on**
**"Conservation and Cultivation of Medicinal Plants"**

## CERTIFICATE

This is to certify that Dr./Mr./Ms. V. M. Kavitha, NIS Chennai

has participated / presented a paper entitled "Ethnovalue of Medicinal Plants"

in the **National Workshop on "Conservation and Cultivation of Medicinal Plants"** organized by Siddha Medicinal Plants Garden, Mettur Dam during 16th and 17th December 2017.

**Dr. M. Padma Sorna Subramanian**
Organizing Secretary & Convener

**Prof. Dr. R. S. Ramaswamy**
Director General, CCRS

NATIONAL SEMINAR ON

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

## (RM & PHISSM – 2018)

6TH & 7TH APRIL 2018

# प्रमाण पत्र
# CERTIFICATE

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

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

This is to certify that Dr./Shri/Smt. Karthic V.M. NIS., Chennai has participated/presented a paper entitled Ancient Cultural and Health Habits as the Aspect of Siddha System in the National Seminar on "Research Methodology and Public Health Initiative through Siddha System of Medicine" (RM & PHISSM – 2018) organized by Siddha Regional Research Institute, Thiruvananthapuram on 6th & 7th April 2018 at Dr. M R DAS Convention Centre, Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, Kerala.

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

RM & PHISSM 2018

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

தேசிய கருத்தரங்கம்

சித்த மருத்துவத்தில் புற மருத்துவ முறைகள்

# SIDDHA REGIONAL RESEARCH INSTITUTE

(Under Central Council for Research in Siddha, Chennai.
Ministry of Ayush, Government of India)
**Kuyavarpalayam, Puducherry - 605 013.**

Certificate No : SRRI/NCPM/2017/ 339

## Certificate

*This is to certify that Dr./~~Sh./Km./Smt~~.* **Dr. V.M.KARTHIC**

*has Presented a ~~Pape~~r/Poster entitled* **A Literature reveiw of Kalikkam( Collyrium- An External Therpy in Siddha Medicine**

*in the National Conference on Pura Maruthuvam - External Therapies in Siddha System of Medicine organized by Siddha Regional Research Institute, Puducherry held on 9th & 10th December, 2017 at Dr. APJ Abdul kalam JIPMER Auditorium, Puducherry.*

**Organising Secretary** **Convenor** **Chairman**

SASTRA UNIVERSITY
ENGINEERING · MANAGEMENT · LAW · SCIENCES · HUMANITIES · EDUCATION
A UNIVERSITY U/S 3 OF THE UGC ACT, 1956

# Centre For Advanced Research In Indian System Of Medicine (CARISM)

## Certificate of Participation

This is to certify that Dr. **V.M.KARTHIC** of **National Institute of Siddha, Chennai** participated in Ministry of AYUSH supported training programme on **"Characterization Techniques in the Standardization of Ayurvedha & Siddha Herbo-Metallic Preparations"** held during 28 to 30 march 2016.

P. Brindha
Convener
Prof. **P. Brindha**

G. Balachandar
Registrar
**SASTRA University**

SHANMUGHA ARTS, SCIENCE, TECHNOLOGY & RESEARCH ACADEMY

# SRI RAMACHANDRA UNIVERSITY

(Declared under section 3 of UGC Act, 1956)
**Accredited by NAAC with 'A' Grade**
Porur, Chennai - 600 116.

## FACULTY OF PHARMACY

## CERTIFICATE

*This is to certify that Dr./~~Mr./Ms~~.....V.M. KARTHIC.....*
*participated as a ~~Resource person~~ / ~~Presented e-poster~~ / Delegate in the National Seminar on "**Pharmacovigilance of AYUSH Drugs**" held on 19th January 2016, organized by the Faculty of Pharmacy, Sri Ramachandra University, in association with the Society for Ethnopharmacology, Chennai Chapter.*

*This seminar carries four credit hours.*

**Dr. D. Chamundeeswari**
Principal
Faculty of Pharmacy

**Dr. P.V. Vijayaraghavan**
Director (Academic Administration) &
Dean - Education

**Dr. S.P. Thyagarajan**
Professor of Eminence &
Dean (Research)

# புதுவைப் பல்கலைக்கழகம்

## சுப்பிரமணிய பாரதியார் தமிழ்மொழி & இலக்கியப் புலம்

பல்கலைக்கழக மானியக் குழு நிதி நல்கையுடன் நடத்தும்

(SAP-DRS-Phase-III)

# "சித்தர் இலக்கியம்"

## பன்னாட்டுக் கருத்தரங்கம் - 2016

திரு / செல்வி / திருமதி Dr. வே. மு. கார்த்திக் அவர்கள்

24-03-2016 முதல் 26-03-2016 வரையில் நடைபெற்ற "சித்தர் இலக்கியம்" பன்னாட்டுக் கருத்தரங்கில் கலந்துகொண்டு "சித்த மருத்துவத்தில் நாக முறிஞ்சி பயன்பாடு அகவி (மூலிகை) பற்றிய நூல் ஆய்வு" என்னும் தலைப்பில் கட்டுரையளித்தார் எனச் சான்றளிக்கப் பெறுகின்றது.

புலமுதன்மையர் (பொறுப்பு)
(ஆ. திருநாகலிங்கம்)

துணைவேந்தர் (பொறுப்பு)
(அனிசா பஷீர்கான்)

ஆய்வுத்திட்ட ஒருங்கிணைப்பாளர்
(க. இளமதி சானகிராமன்)