PRECLINICAL SAFETY EVALUATION OF ANNAPAVALA CHENDHURAM

The dissertation Submitted by

Dr. N. SABARI GIRIJA., M.D (S)

Under the Guidance of

Dr. P. SHANMUGAPRIYA., M.D (s), Ph. D,

Associate Professor and Guide,

Department of Nanju Maruthuvam,

National Institute of Siddha, Chennai - 47.

Dissertation Submitted to

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

For the partial fulfilment of the requirements to the Degree of

DOCTOR OF MEDICINE (SIDDHA)
BRANCH VI – DEPARTMENT OF NANJU MARUTHUVAM
2017-2020

NATIONAL INSTITUTE OF SIDDHA
Chennai – 47.

DECLARATION BY THE CANDIDATE

I declare that the thesis entitled **"Preclinical safety evaluation of Annapavala Chendhuram"** is a bonafide and genuine research work carried out by me under the guidance of Dr. P. Shanmugapriya, M.D(S), Ph.D., Associate Professor, Department of Nanju Maruthuvam, National Institute of Siddha, Tambaram sanatorium, Chennai - 47 and the thesis has not formed previously by the basis for the award of any degree, Diploma, Associate ship, Fellowship titles in this or any other University or similar Institution of higher learning.

Date: Dr. N. SABARI GIRIJA., M.D (S).,

Signature of the candidate

Place:

BONAFIDE CERTIFICATE

Certified that I have gone through the thesis submitted by Dr. N. Sabari Girija., (Reg. No: 321716203) a student of final year M.D (S), Branch VI – Department of Nanju Maruthuvam, National Institute of Siddha, Tambaram Sanatorium, Chennai – 47 and the thesis work has been carried out by the individual only. This thesis does not represent or reproduce the thesis submitted and approved earlier.

Place:

Date:

Dr. P. Shanmugapriya, M.D(S)., Ph.D.,
Name & Signature of the Guide
Associate Professor,
Department of Nanju Maruthuvam,
National Institute of Siddha, .
Chennai – 47

Dr. R. Madhavan., M.D (S).,
Name & Signature of the HOD i/c
Associate Professor,
Department of Nanju Maruthuvam,
National Institute of Siddha,
Chennai – 47.

Forwarded by the Head of the Institute
Dr. R. Meenakumari., M.D (S).,
Name & Signature of the Director,
Professor,
National Institute of Siddha,
Chennai – 47.

TABLE OF CONTENTS

ACKNOWLEDGEMENT

- First and foremost, I would like to thank and invoke the blessings of **god lord Murugan** for giving me the strength, knowledge and ability to undertake the research work on safety evaluation of Annapavala Chendhuram.
- I would like to pay my sincere gratitude to **Ministry of AYUSH** for their financial support.
- I express my sincere thanks to **Vice – Chancellor**, The Tamilnadu Dr. M. G. R medical university, Chennai.
- I express my sincere gratitude to **Prof. Dr. R Meenakumari, M.D (S).,** Director, National Institute of Siddha, Chennai,
- I express my sincere thanks to **Dr. V. Banumathi., M.D (S).,** Former Director, National Institute of Siddha, Chennai for encouraging me to undertaken this study and also for providing all facilities in order to carry out this work.
- It is my privilege to have you as my guide **Dr. P. Shanmugapriya., M.D (S)., Ph.D.,** Associate Professor, Department of Nanju Maruthuvam, National Institute of Siddha, Chennai and my sincere thanks for her encouragement, ideas and guidance in throughout my study.
- I express my deep sense of gratitude to **Dr. R. Madhavan, M.D (S).,** Associate Professor, Department of Nanju Maruthuvam, National Institute of Siddha, Chennai for his valuable support, encouragement and guidance during this work.
- I express my sincere thanks to **Dr. S. Murugesan, M.D (S) and Dr. V. Manjari., M.D(S),** Lecturer, Department of Nanju Maruthuvam, National Institute of Siddha, Chennai for them guidance in my work.
- I thank **Dr. Mariappan, M.D (S).,** Lecturer, Department of Gunapadam, National Institute of Siddha, Chennai for gave the permission to prepare the medicine in Gunapadam Laboratory, National Institute of Siddha, Chennai.
- I would like to thank **Dr. Muthuvel,** Department of Biochemistry, National Institute of Siddha, Chennai for his help in Biochemical analysis of the drug.
- My sincere thanks to **Dr. V. Subha., M. Pharm, Ph.D.,** Assistant Professor of pharmacology and **Dr. Gayathri., M. V. Sc**, Veterinarian, National Institute of Siddha, Chennai for their guidance in animal studies.
- I express my sincere gratitude to **Dr. M. Jeyanthinath., M.S., Ph.D.,** School of Chemistry, Madurai kamarajar university, Madurai for his extreme help in physico

chemical and spectroscopic analysis and also my thanks to **Dr. Ragavendran, Ph.D.,** Madurai Kamarajar University, Madurai.

- I thank **Mrs. V. Kalpana,** library clerk and **Mr. J. Rathinam,** Library attendant, National Institute of Siddha, Chennai for their help in literature collection.
- I express my thanks to animal lab attenders **Mr. Lingam** and **Mrs. Devi** for their help during animal experimental procedures.
- I wish to express my deepest gratitude to **Noble research solutions**, Perambur, Chennai for them valuable work in Hematological, serological, histopathological analysis of animals.
- I would like to pay my pay my special regard to **Dr. Kaaviya** and **Dr. Gomathi,** PG scholars, National Institute of Siddha, Chennai for their best support during preparation of the drug.
- My sincere gratitude to all my seniors, juniors and my friends especially **Dr. L. Sakthimanipriya** and **Dr. K. Prithiviraj** who has helped me in throughout my study.
- Finally, my deep and sincere thanks to my parents **Mr. K. Navaneetha Krishnan, B.A., Mrs. N. Sumathi** and my sister **Er. N. Keerthika, M.E.,** and my friends **Dr. M. A. Sinekha., PG Scholar, Dr. S. Guptaj., B.S.M.S** for their continuous moral support in my research work.
- I wish to thank all the people whose assistance was a milestone in the completion of this research work.

1. INTRODUCTION

"The One is He, the Two His sweet Grace,
In Three He stood, in all the Four witnessed,
The Five He conquered, the Six He filled,
The Seven Worlds pervades, manifests the Eight And so remains".

- *Thirumanthiram* [1]

The basic purpose of any science is to bring solutions to human problems. All sciences since even the most ancient times, as a tradition, have been devoted to freeing the human race from obstacles. Obstacles such as disease; the discomfort of inhospitable environments; drastic weather conditions. [2] Human race in different regions of the world has its unique system of medicine for maintaining health or treatment of diseases. Researchers have documented as many as five thousand 'Folklore medical practices' prevailing in different parts of the world. [3] India has a rich source of traditional medical systems and some of them date back to 5000 years BC. [4]

Siddha medicine**,** thetraditional system of medicine that originated in South India and is considered to be one of India's oldest systems of medicine. [5] The Siddha system is attributed to have originated from the Lord Siva who explained it to his consort Shakthi. The lineage is Nandi, Dhanwanthri, Aswini, Agasthiar, Pulathiyar and Theraiyar.[3] As per the Siddha system of medicine, When the normal equilibrium of three humors (*vatha*, *pitha* and *kapha*) is disturbed, the disease is caused. The factors, which affect this equilibrium are environment, climatic conditions, diet, physical activities, and stress. Siddha system is largely therapeutic in nature.

The drugs used by the Siddhars could be classified into three groups: *thavaram* (herbalproduct), *thathu* (inorganicsubstances)and *sangamam* (anim al products). The *thathu* drugs are further classified as *uppu* (water soluble inorganic substances or drugs that give out vapour when putting into fire), *pashanam* (drugs not dissolved in water but emit vapour when fired), *uparasam* (similar to pashanam but differ in action), *logam* (not dissolved in water but melt when fired), *rasam* (drugs which are soft). The treatment should be commenced as early as possible after assessing

the course and cause of the disease. Treatment is classified into three categories: *devamaruthuvam* (Divinemethod); *manudamaruthuvam* (rational method); and *asura maruthuvam* (surgical method). Accordingly, it is advised to administer first pure herbs in the form of liquid, powder, pill or paste. Then if the ailment is chronic or nonresponsive to herbal medicines the usage of a mixture of herbs, metals, minerals and animal products in addition to herbs is advocated. Likewise, in deva maruthuvam medicines such as *parpam*, *chendooram*, *guru*, *kuligai* madeof mercury, sulphur and *pashanams* are used. [6] In Siddha medicine, the use of metals and minerals are more predominant in comparison to other Indian traditional medicine systems. [7]

Siddhars were poly-pharmacists and their knowledge in the field of Iatro-chemistry is equal to alchemy and medicine. They have described many meticulous processes including calcination, sublimation, distillation, fusion, amalgamation, solidification, fixation i.e. bringing to a state of noncombustible form of an inorganic substance, extraction and so on. These methods are not only useful to prepare medicine but also helpful in alchemical processes (transmutation of basic metals into gold). [8]

There are 32 types of internal and 32 types of external medicine in Siddha system of medicine. Internal medicine comprises Surasam (boiled juice), Chaaru (juice), kudineer (decoction), karkam (herbal paste), utkali (pan cake), adai (toast), chooranam (powder), pittu (baked powder), vadagam (tablet), vennai (medicated butter), manappagu (syrup), nei (medicated ghee), rasayanam (medicated confectionary), ilagam (electuary), ennai (medicated oils), maathirai (pills), kadugu (deep fried granules), pakkuvam (preparation method), thenooral (preserve), theeneer (distillate), mezhugu (waxy pastes), kuzhambu (viscous liquids), pathangam (sublimates), chenduram (red inorganics), parpam (ash/calx), kattu (bonded inorganics), urukku, kalanghu, chunnam, karpam, chathu, gurukuligai.

Chenduram is one of the 32 types of internal medicine. It is described as metallic substances or salts which is made into red-coloured powders, by the process of either burning them or drying them or exposing them to the sunlight or keeping them in specialized tubes by adding decoctions, ceyaneer, dravagam etc. The life period of chenduram is 75 years. [9] The method of preparation of parpam and chenduram shows the mastery of Siddhars in inorganic chemistry and processing herbo - mineral drugs.

The herbo - mineral drugs are made into very fine particles. Finer the particle better is the bioavailability and effect, the minute dose is adequate for treatment. [3]

Annapavala chenduram (APC) is the Siddha internal medicine, comes under the type of chenduram. It is made by the combination of annabedhi and kodipavalam. As per the reference of Siddha pharmacopoeia, *Annapavala chenduram* has the indication for kaasam, shayam, swasam, rathaushnam, pithaushnam, mega ushnam. [10] Clinically this medicine is used for thyroid dysfunction by many practitioners. Among, adult people in India, the prevalence of hypothyroidism has been recently studied. In this population-based study done in Cochin on 971 adult subjects, the prevalence of hypothyroidism was 3.9%. The prevalence of subclinical hypothyroidism was also high in this study, the value is 9.4%. In women, the prevalence was higher, at 11.4%, when compared with men, in whom the prevalence was 6.2% [11].

Many people have done research on annabedhi and kodipavalam based Siddha medicines. Hypolipidemic activity of *Annapavala chenduram* has proven by some research. But till date, there is no data available about the safety evaluation of *Annapavala chenduram.* Nowadays most of the people rely on Siddha medicine and seek medical care in Siddha. However, there are fears about the toxicity of siddha medicines among the people due to the metals in Siddha medicines. So, it is necessary to ensure its safety through animal studies. Hence, the author has chosen the *Annapavala chenduram* for evaluate safety of this particular drug.

2. AIM & OBJECTIVES

2.1. PRIMARY OBJECTIVE:

To Evaluate safety of the drug by conducting acute and long-term toxicity studies in Wister albino rats as per WHO guidelines

2.2. SECONDARY OBJECTIVES:

2.2.1. Standardization of the study drug:

a. To explain the identification and authentication of the ingredients

b. To demonstrate the preparation of the drug.

c. To analyse the Physicochemical properties of the drug as per the specified protocol given by Pharmacopeial Laboratory for Indian Medicines (PLIM) guideline.

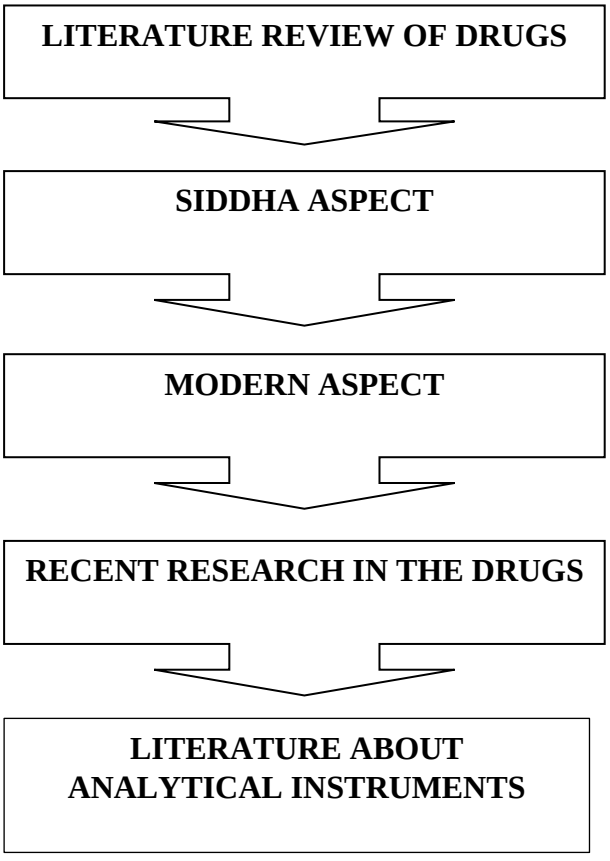
d. To evaluate the particle size analysis by SEM methods.

e. To analyse the chemical nature of the drug by FTIR and XRD.

3. REVIEW OF LITERATURE

In the present work literature review was carried out by using various resources like *Siddha* literatures, modern literatures, internet resources like PubMed, Science direct, google scholar and Scopus journals etc. The review was documented in the following aspects.

Fig: 1 Schematic representation of review of literature



3.1 அன்னபேதி

3.1.1 IN SIDDHA ASPECT:

❖ அன்னபேதி என்பது 120 உபரசங்களில் ஒன்று ஆகும். கட்டிகளாகவும், பச்சை நிறமாகவும் இருக்கும்.[12]

அன்னபேதி தோற்றம்: [13]

வேதையான அன்னமென்ற பேதி தானும்
விளங்கியதோர் உற்பத்தி சொல்லக் கேளு
மாதையான மலையினுட ருதுவு பொங்கி
மாசற்ற கிரணத்தால் பொங்கி நீறும்
பாதையான மலைச்சவுடு என்று பேரு
பாங்கான மூன்றுவித வண்ணமாகும்
காதையான காசீச மென்று பேரு
கருப்புமஞ்சள் வெள்ளைநிற மாகுங் காணே.

- போகர் 7000.

❖ இது அன்னபேதி என்ற காசீசம் மலையில் உற்பத்தியாகின்றது.

- செயற்கையாக இரும்புக் கம்பியுடன் கந்தகத் திராவகம் சேர்த்து செய்யப்படுகிறது.
- இது நீரில் கரையும், சாராயத்தில் கரையாது.
- இதன் மேல் காற்றுப் பட்டால் வெண்மையான தூளாகி விடும்.

வேறு பெயர்கள்: [14]

கல்நாதம், அன்னகாலன், கல்சவடு நாதம், களிம்பு, கல்லுவேகம், மலைவீரியம், திராவகத்துக் கடுங்காரபேதி, மலைருது.

சுவை: துவர்ப்பு

வீரியம்: வெப்பம்

வகைகள்:

- கருப்பு
- வெள்ளை
- மஞ்சள்

செய்கை:

- உடல் உரமுண்டாக்கி
- துவர்ப்பி
- ருது உண்டாக்கி
- நாற்றமகற்றி
- புழுக்கொல்லி
- முறை வெப்பகற்றி

வெளிப்பிரயோகமாக பயன்படுத்தினால்,

- துவர்ப்பி
- வெப்பமுண்டாக்கி

அளவு: ஒன்று (65 மி. கிராம்) முதல் மூன்று அரிசி (195 மி. கிராம் எடை).[12]

பொதுக்குணம்:

"முளைவிரணஞ் சூலைமந்த முட்டாமைக் கட்டி
விளையறன்ம கோதரநோய் வீட்டும் - வளைமலைபோல்
காட்டுமன்னந் தன்னைக் கணத்திற் சலமாக்கிக்
காட்டுமன்ன பேதியது காண்".

- பதார்த்த குணசிந்தாமணி. [15]

தீரும் நோய்கள்:

- முளைக்கட்டி
- சூலை
- அஜீரணம்
- பாய்கின்ற ஆமைக்கட்டி
- வீறிய சலோதரம்.

உட்பிரயோகம்:

- பாண்டு
- சூதகப்பாண்டு
- சூதகக்கட்டு
- கருப்பப் பிரமேகம்
- காய்ச்சல் கட்டி
- முறைச்சுரம்
- எழுஞாயிறு
- நாட்பட்ட கக்கிருமல்
- தட்டைக்கிருமி ரோகம்

வெளிப்பிரயோகம்:

- அக்கி
- மேகவிரணம்

- ❖ சீமூலம்
- ❖ ஆசனவாய் வெளிப்படல்
- ❖ கருப்பவிரணம் முதலிய நோய்களுக்கு மேலுக்கு உபயோகிக்கலாம்.

சுத்தி முறைகள்:

- ❖ தேவையான அன்னபேதியை நீரில் கரைத்து சிறிதளவு கந்தகத் திராவகம் விட்டு வடிகட்டி உப்பு உறையும் பக்குவத்தில் காய்ச்சிக் கொள்ள சுத்தி ஆகும்.
- ❖ அன்னபேதியை நீரில் கரைத்து கந்தகப் புளிப்பு சிறிதளவு விட்டு வடிகட்டிக் காய்ச்சி குழம்பு பதத்தில் இறக்கி உலர விட்டு எடுக்க சுத்தி ஆகும். [12]
- ❖ பழச்சாற்றில் மூன்று நாட்கள் ஊற வைத்து உலர்த்திக் கொள்ளவும். [16]
- ❖ புது ஓட்டிலிட்டு சிவக்க வறுத்தெடுக்க சுத்தி ஆகும். [17]

அன்னபேதியை உட்கொள்ளும் போது கவனிக்க வேண்டுவது: [12]

- ❖ அன்னபேதியை அருந்தி வருங்காலத்து மலம் கறுத்துக் கெட்ட நாற்றத்தோடிருக்கும்.
- ❖ அன்னபேதியைச் சாப்பிடும் பொழுது விடாமுயற்சியாய் வாரத்திற்கொரு முறை விட்டு விட்டுச் சாப்பிட வேண்டும்.
- ❖ அன்னபேதியை அருந்திக் கொண்டு வரும் போது 10 நாளைக்கு ஒருமுறை பேதிக்குக் கொடுத்தல் நல்ல குணமுண்டாகும்.
- ❖ அன்னபேதியைச் சாப்பிடும் போது புளிப்பையும், புளிப்பு உள்ள பழங்களையும் நீக்கி அயத்திற்கு கூறிய பத்தியம் காத்தல் வேண்டும்.
- ❖ குழந்தைகளுக்குக் கொடுக்க வேண்டிய அவசியமிருந்தால் குறைந்த அளவில் கொடுக்கவும்.
- ❖ அன்னபேதியை உணவிற்குப் பிறகே அருந்த வேண்டும்.

வழக்குமுறைகள்: [12]

- ❖ அன்னபேதியைக் கல்வத்திலிட்டு வேண்டிய அளவு நீர் விட்டுக் குழம்புப் பதத்தில் அரைத்து ஆசனம் வெளித்தள்ளல், கருப்பவிரணம், பெண்களின்

உறுப்புத்தள்ளல் முதலியவற்றிற்கு மேலுக்குப் போடச் சுருக்கமடைந்து உள்ளுக்கு இழுத்துக் கொள்ளும்.

- மூலரோகத்தில் காணும் இரத்த ஒழுக்கிற்கு அன்னபேதித் தூள் 1 வராகனெடை (4.2 கிராம்) சுமார் 2 சேர் (280 மி.லி) நீரில் கரைத்து ஒவ்வொரு நாளும் வஸ்தி செய்து வந்தால் இரத்தம் நிற்கும். இந்த நீரை அக்கி, மேகவிரணம், கருப்பப்புற்று, சீமூலம், முதலியவைகளுக்கு மேலுக்கு உபயோகிக்கலாம்.
- அன்னபேதி 2 உளுந்தெடையை (130 மி. கி) ஓர் அவுன்ஸ் (28 மி. லி) நிலவேம்புக் குடிநீரில் அல்லது ஓமத்தீநீரில் கலந்து நாள் ஒன்றுக்கு மூன்று வேளை வீதம் பலக்குறைவு, பாண்டு முதலிய நோய்களுக்குக் கொடுப்பதுண்டு.
- கரியபோளத்தூள் 12 உளுந்தெடை (780 மி. கி) அன்னபேதித்தூள் 30 உளுந்தெடை (2 கிராம்) சேர்த்துப் போதுமான அளவு தேன் கூட்டி அரைத்து 24 மாத்திரைகள் செய்து வேளைக்கு 2 மாத்திரைகள் வீதம் மும்முறை கொடுத்து வர பாண்டுவுடன் கூடிய வெள்ளை, சூதகக்கட்டு, சூதக ஒழுக்கு இவைகள் நீக்கும்.
- அன்னபேதி 12 உளுந்தெடையை (780 மி. கி) மிளகுத்தூள் 15 உளுந்தெடை (975 மி. கிராம்) சேர்த்துப் போதுமான அளவு தேன் கூட்டி அரைத்து 12 மாத்திரைகள் செய்து கொண்டு தினம் வேளைக்கு இரண்டு மாத்திரை வீதம், நிலவேம்புக் குடிநீர் அல்லது சீந்தில் குடிநீரில் இருவேளை கொடுத்து வர முறைச்சுரம் விலகும்.
- அன்னபேதி 1 உளுந்தெடையை (65 மி. கி) இரண்டு சேர் (560 மி. லி) நீரில் கலந்து இதர மருந்துகள் சாப்பிட்டுக் கொண்டு வரும் பொழுதும், தாகம் உண்டாகும் பொழுதும் அருந்தி வந்தால் மகோதரம், சோகை, பலக்குறைவு தீரும்.

சேரும் பிறமருந்துகள்:

- **அய பற்பம்:** [18]

  அளவு: ஒன்று முதல் மூன்று குன்றியளவு.

  தீரும் நோய்கள்: பாண்டு. மகோதரம், குன்மம், சோகை, சயம்.

❖ **வருண லோக செந்தூரம்:** [18]

அளவு: கால் முதல் ஒன்றரை குன்றியளவு

தீரும் நோய்கள்: சகல நோய்களும் தீரும்.

❖ **அன்னபேதிச் செந்தூரம்:** [19]

அளவு: 2 – 4 குன்றியளவு

தீரும் நோய்கள்: சுரம், சீதபேதி, பாண்டு, மூலவாயு, பித்தவாயு, வயிற்றுநோவு, வயிற்று இரைச்சல்.

❖ **பேதிச் செந்தூரம்:** [19]

அளவு: 2 – 4 குன்றியளவு

தீரும் நோய்கள்: நீரெரிச்சல், நீர்கடுப்பு.

❖ **பேதி வீரச்செந்தூரம்:** [19]

அளவு: 1½ - 2 குன்றியளவு

தீரும் நோய்கள்: வாயு சம்பந்தமான நோய்கள், சுரம், சன்னி.

❖ **துருசு செந்தூரம்:** [19]

அளவு: ஒன்று முதல் மூன்று குன்றியெடை

தீரும் நோய்கள்: மூலவாயு, பெருங்கழிச்சல், பாண்டு, சுரம், சயம், இருமல், இரைப்பிருமல், தொடர்கழிச்சல்.

❖ **செந்தூரத் திராவகம்:** [19]

செந்தூரங்களுக்கெல்லாம் குருத் திராவகமாகப் பயன்படுகிறது.

❖ **சோடச மாத்திரை:** [17]

அளவு: தினம் 1 மாத்திரை

தீரும் நோய்கள்: சுரம்.

- **சங்கத் திராவகம்:** [17]

அளவு: 5 – 10 துளி.

தீரும் நோய்கள்:. ஈரல் வீக்கம், குன்மம், வாய்வு, சூதகவாய்வு, வயிற்றுவலி.

- **கபரி மெழுகு:** [17]

அளவு: மிளகளவு

தீரும் நோய்கள்: குளிர்சுரம், சன்னி, சூதகவாயு.

- **தாம்பிர அன்னபேதி செந்தூரம்:** [20]

தீரும் நோய்கள்: குளிர்சுரம், கபசுரம், பித்தசுரம், பாண்டு தீரும்.

- **நாராயண செந்தூரம்:** [20]

தீரும் நோய்கள்: பக்கவாதம், தோள்வாதம், முடக்குவாதம், கெண்டை வாதம், சர்வாங்கவாதம், இடுப்புவாதம், உதிர்வாதம்.

- **செந்தூரச் செயநீர்:** [21]

தீரும் நோய்கள்: சகல நோய்களுக்கும் உள்ளுக்கு உபயோகிக்கலாம்.
மேலுக்குப் பூசுவதால் 18 வகை குட்டமும் தீரும்.

- **வீர பற்பம்:** [22]

அளவு: அரை முதல் ஒரு அரிசியெடை

தீரும் நோய்கள்: எண்வகை குன்ம நோய், முன்னிசிவு, பின்னிசிவு, சூலை, சைத்தியம், தோடம், கிரந்தி.

- **நவ உப்புத் திராவகம்:** [23]

அளவு: 3 – 5 துளி

தீரும் நோய்கள்: பக்க சூலை, மாரடைப்பு, மார்பு நோய், வயிற்று நோய், புளியேப்பம், இடுப்பு வலி, வாந்தி, குடலிரைச்சல்.

3.1.2. IN MODERN ASPECT:

VERNACULAR NAMES:[24]

Tamil: Annabedhi

English: Green vitriol, Green copperas, copperas of commerce, sulphates of iron, ferrous sulphate, iron sulphate, salt of steel.

Sans: Kasisa

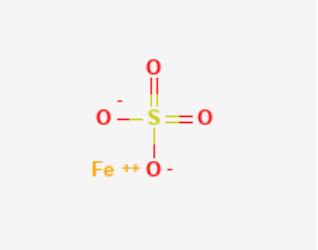
Hindi: haratutia

Tel: Tagramu

Malay: madukalpa

Chemical formula: Ferrous sulphate ($Feso_4$).

Chemical structure: [25]



Iron(II) sulphate or **ferrous sulphate** denotes a range of salts with the formula $FeSO_4 \cdot xH_2O$. These compounds exist most commonly as the heptahydrate ($x = 7$) but are known for several values of x. The hydrated form is used medically to treat iron deficiency, and also for industrial applications. Known since ancient times as **copperas** and as **green vitriol** (vitriol is an archaic name for sulfate), the blue-green heptahydrate (hydrate with 7 molecules of water) is the most common form of this material. [26]

It is a blue-green monoclinic crystalline water-soluble salt. It is prepared commercially by oxidation of pyrite (iron sulphide) or by treating iron with sulphuric

acid. It is used in in the manufacture of inks, in wool dyeing as a mordant, and in water purification as a mordant, and in water purification as a substitute for aluminium sulphate. It melts at 64^{o}C, and at 90^{o}C it loses water of hydration to form the monohydrate, a monoclinic, crystalline powder that occurs naturally as the mineral szomolnokite. The mineral siderotil is iron sulphate pentahydrate. [27] According to Ayurvedic works it is rarely used internally.

PRODUCTION:[28]

It is obtained as a by – product of industrial processes using iron ores that have been treated with sulphuric acid.

ACTION: [24]

- Tonic
- Astringent
- Haematinic

VARIETIES:

It was divided into two varieties by the ancient Hindu chemists.

1) **Valuka kasisa or dhatu kasisa**, the green variety (**ferrous sulphate**)
2) **Pushpa kasisa** the yellowish variety which is probably iron sulphate covered with basic sulphate of sesquioxide from absorption of oxygen.

"**Coppers of commerce**", is produced principally from the so-called **alum** shales from which alum is prepared. As is the case also with alum, copperas is found sometimes as a natural exudation upon alum shales and other rocks which include **"iron pyrites".** Crude greenish-blue crystals of sulphate of iron are available in all the bazaars in India. Its taste is very astringent or styptic and without any odour, acid reaction, soluble in water, insoluble in alcohol.

HYDRATES:

Iron sulphate can be found in various states of hydration, and several of these forms exist in nature.

- $FeSO_4.H_2O$ (mineral: szomolnokite)
- $FeSo_4.4H_2O$

- $FeSo_4.5H_2O$ (mineral: siderotil)
- $FeSo_4.7H_2O$ (mineral: melanterite)

PHYSICAL AND CHEMICAL PROPERTIES: [29]

- Colour – Turquoise / Blue - green crystalline solid
- Density -1.898g /cm3
- Melting point - 680C
- Boiling point - 900C (becomes FeSo4)
- FeSo4.7H2O - 90%
- Mgso4.7H2O - 4%
- pH >1 (10%)
- Other sulphates - 1%
- Insoluble - 0.1%
- Free acid - 0.3%
- Residual moisture - 5%
- Fe (active substance) - 18%
- Dissolves easily in water - 570g/l (20ºC)

PHARMACODYNAMICS: [28]

Ferrous sulphate replaces iron, an essential component in the formation of haemoglobin.

PHARMACOKINETICS: [28]

Absorption:

Ferrous sulphate absorbed from the entire length of the GI tract, but primary absorption sites are the duodenum and jejunum. Up to 10% of iron is absorbed by healthy individuals; patients with iron deficiency anaemia may absorb up to 60%. Food may decrease the absorption by 33% to 50%. Transported through GI mucosal cells directly into the blood, where it's immediately bound to carrier protein, transferrin and transported to the bone marrow for incorporation into haemoglobin.

Metabolism:

Liberated by the destruction of haemoglobin but is conserved and reused by the body.

Excretion:

Healthy people lose very little iron each day. Men and postmenopausal women lose about 1mg/day, and premenopausal women about 1.5 mg/day. The lose usually occurs in nails, hair, faeces, and urine; trace amounts are lost in bile and sweat.

It is advised to consume ferrous sulphate along with ascorbic acid, as this practice has been known to increase its absorption. Reduced absorption with antacids, Zn, Ca, phosphorus, trientine and cholestyramine has been observed.

MECHANISM OF ACTION:

Ferrous sulphate replaces iron stores found in haemoglobin in red blood cells, myoglobin and other heme enzymes in the body. Additionally, ferrous sulphate allows the transportation of oxygen via haemoglobin. Approximately 60% of iron is stored in haemoglobin in RBC, while 9% is stored in myoglobin and other heme enzymes. Additionally, 25% is held in reserve in reticulocytes of the liver, spleen, and bones. Most stored iron is bound to ferritin. While being transferred in the body, Fe2+ iron is converted to Fe3+ by ceruloplasmin. So, it can then be bound to the protein transferrin.

ADVERSE EFFECTS OF FERROUS SULPHATE: [24,28]

- It is apt to irritate the stomach.
- Ferrous sulphate may cause your stools to turn black, an effect that is not harmful and may cause the constipation, diarrhoea.
- Though iron is useful in simple anaemias, it is useful or even harmful in pernicious anaemia.

CONTRA INDICATION: [28]

Hemochromatosis, hemosiderosis and hypersensitivity to any of the ingredients prohibit its use.

MEDICINAL USES: (INTERNAL) [24]

- Preparations made of it was generally bhasma, oil, and solution. Bhasma is prepared by taking equal quantity of iron sulphate and sulphur, reducing them fine powder, mixing and mixture or mass. To this is added tripala, pepper,

honey, ghee and the whole is triturated. A Dose is ¼ to 2 grains a day with honey and milk along with triphala powder and pepper.

- The bhasma is alternative and diuretic and is given in enlargement of the liver, consumption etc.
- In Ayurveda, Chakradatta had recommended a linctus composed of iron sulphate and pulp of wood in hiccup.
- It is used as a lotion in erysipelas, anaemia and constitutional debility, following on malaria, kala-azar.
- Iron sulphate is useful in all diseases, where iron is indicated.
- A grain of ferrous sulphate in an ounce each of omum water and infusion of chiretta thrice a day after food. This is useful in larger doses in case of neuralgic of rheumatic attacks recurring periodically among the weak and anaemic.
- 24 grains of ferrous sulphate and 30 grains of black pepper and cinnamon powder, made into 12 pills in sufficient quantity of honey and given in a dose of 1 pill twice a day.
- For anaemic females suffering from cholera etc leucorrhoea and amenorrhoea purified aloes in equal quantity to ferrous sulphate may be advantageously added.

MEDICINAL USES: (EXTERNAL) [24]

- Iron sulphate is used in skin diseases either alone or with other medicines.
- For painful syphilitic ulcers, ferrous sulphate is dusted over them after washing them. Its stick or solution is applied to foul ulcers and various skin diseases as eczema, pruritus, intertrigo etc.
- Kaisadya taila, as an application to the genitals and the breasts with the view of strengthening them.
- It is applied also in fistula – in – ano for the burning and pain in piles.
- It is made of 16 tolas each of iron sulphate, withania somnifera root, bark of Symplocos racemosa and roots of Pothos officinalis beaten into a paste and it is boiled with 4 seers of sesamum oil and 16 seers of water in the usual way.

- In bleeding piles and prolapse of the rectum, daily enemas of the simple solution of the sulphate are serviceable.
- In chronic skin diseases an ointment made of iron pyrites and ghee is used with benefit.

3.1.3 **SCIENTIFIC VALIDATION OF ANNABEDHI:**

- **Sivaraj et. al., 2018** [30] evaluated the efficacy of Annabedhi Chendhuram (Siddha) and elemental iron (Allopathy) in the treatment of Iron deficiency anaemia to achieve normal function of it. This study shows that for 90% of group I (elemental iron) patients haemoglobin level increases above 2 grams % whereas in group II with annabedhi chenduram only 60% shows improvement. Among group I 24% of cases had unwanted symptoms. In group II only 10% of cases had unwanted symptoms. So, finally group I is slightly more effective than group II in improving haemoglobin level, but group II patients had less unwanted symptoms than group I.
- **Ashwin Ashok Shete et. al., 2018** [31] evaluated the efficacy of kasisa bhasma and annabedhi chenduram in the treatment of Anaemia. These drugs contain number of similarities both in terms of composition and preparation with minimum variations. when compared in between the Kasisa bhasma and Annabhedi chenduram, the effect of Kasisa bhasma is found highly significant.
- **Ashwin Ashok Shete et. al., 2017** [32] evaluated pharmaceutical study of both annabedhi chenduram and kasisa bhasma. Annabedhi chenduram and kasisa bhasma contain number of similarities both in terms of composition and preparation with minimal variation. The pH of kasisa bhasma is more towards basic while that of annabedhi chenduram is acidic.
- **Rajalakshmi et. al., 2017** [33] also studied the analytical characterisation of annabedhi chenduram and raw annabedhi. FTIR spectra of Annabedhi Chendhuram revealed that the iron content was increased compare to the raw
- drug. XRF analytical data confirmed the presence of iron and iron oxide in large portion. XRD pattern explored the formation of crystalline compound which is again due to the impact of incineration processes. The SEM report and particle size analysis data indicated that the raw drug obtained was in nanometre size.

- **Dhiman saha et. al., 2017**[34] was conducted to perform physicochemical characterization for the Kasisa Bhasma as per the procedure mentioned in the literature by using several analytical tools. The SEM study reveals that fine particle of Kasisa Bhasma in the range of 10-100nm and such particles are found in the range of 350nm. Due to the calcination processes agglomerates particles are formed. AAS, EDAX, XRD and elemental analysis result confirmed that Kasisa Bhasma contained iron and sulphur as major element.

- **Satadru Palbag et. al., 2016** [35] was conducted the subacute toxicity study on kasisa bhasma as per OECD guideline 407. The effect of *Shodhita Kasisa* and *Kasisa bhasma* on the weight of vital organs was measured following daily oral administration at different doses for 28 days. *Kasisa Bhasma* is nontoxic and safer compared to *Shodhita Kasisa* at higher dose and did not show any significant change in normal behavior of animals. The toxicity data revealed the efficacy of *bhasma* and established the fact that metals and minerals are preferred in their *bhasma* form in Ayurveda to avoid toxicity and to have enhanced therapeutic potency.

- **Keerthy Unni et. al., 2013** [36] studied that structural characterization of traditional Ayurvedic drug and compare these results with those of raw material used for the synthesis. The PXRD patterns clearly revealed the conversion of the non-haem iron into haem iron. The drying and incineration process converts the Fe2+ into mixed oxides of iron which consists of both +2 and +3 oxidation states. The FTIR spectra clearly confirm the presence of organic moieties along with the formation of iron complexes. BET surface area analysis, SEM and PXRD analysis once again confirms the reduction in particle size of the drug compared to the raw material used for the preparation of iron based traditional drug annabedhi chenduram.

- **Dhiraj Singh Rajput et. al., 2011**[37] was studied the basic concept of *Bhasma Kalpana* by means preparing *Kasisa Bhasma*. This study revealed that the total weight of cow dung cakes, quality of cow dung cakes, time required to reach the maximum temperature and duration of constant maximum temperature can be considered as the parameters for *Bhasma* preparation. During *Bhasma* preparation, heating must be started from a temperature lower

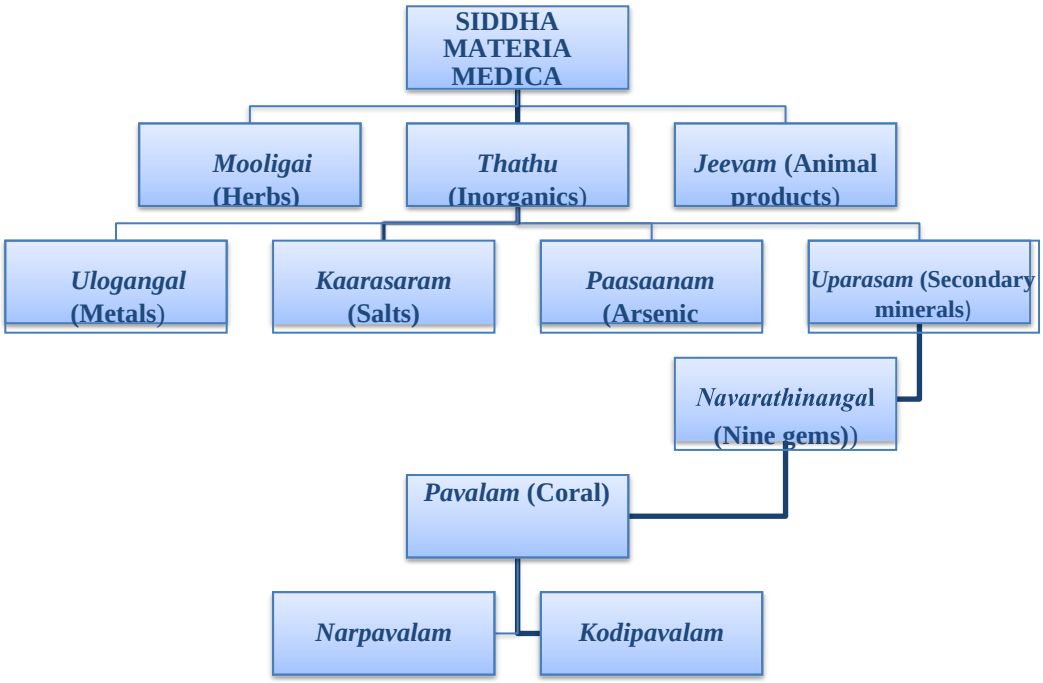
than the required temperature and is gradually increased after each *Puta*, which helps to create perfect *Paka* of *Rasa Dravya*.

- **Dhiraj Singh Rajput et. al., 2011** [38] was conducted a comparative study on Kasisa Bhasma prepared by traditional method and by using MPBNY with special reference to physico-chemical properties. Modified Portable Bhasma Nirman Yantra (MPBNY) was prepared for puta (equipment for calcination) procedure which is easy to handle, portable and facilitate to supply controlled heat. The prepared Kasisa Bhasma was subjected to modern analytical parameters such as AAS (Atomic Absorption Spectroscopy), XRD (X-ray Diffraction) and Ayurvedic analytic parameters. It was observed that Kasisa Bhasma of both methods possesses similar organoleptic as well as physico-chemical properties.

- **Thankamma et. al., 1996** [39] was studied the improved method for the manufacture of annabedhi chenduram. Two samples of Annabedhi chenduram were prepared – one using the classical ayurvedic method and the other using modern methods. The preparation of Annabedhi by the traditional method usually takes more than three days. While the modern method of heating takes only less than three hours. The chemical analysis of these two samples did not show much differences. The same sample of annabedhi when subjected to electrical heating gave a slightly higher value of Fe content than that of traditional method. Final results show that the percentage of iron content is more or less the same in the traditional method and modern method. The traditional method is laborious, time consuming, costly while that of electrical heating is very convenient and cheap. It involves less labour, time and cost.

3.2. பவழம்

3.2.1 IN SIDDHA ASPECT:



- பவழம் என்பது நவமணிகளில் ஒன்றாகும். கொடிப்பவழம் என்பது பவழத்தில் ஒருவகை.

- **“ஓர்க்கோலை சங்கமொளிர் பவழம்வெண் முத்த நீர்ப்படு முப்பினோடைந்து”** என்ற வரிகள் மூலம் பவழம் என்பது கடற்படு திரவியங்களில் ஒன்றென அறியலாம். **[12]**

வேறு பெயர்கள்:

வித்துருமம், துகிர், துப்பு, பிரவாளம், வாரிதித் தண்டு, செந்தண்டு மாலை,[14] கொடியோன்[16]

பவழம் தோற்றம்:

ஆகின்ற பவழத்தின் பிறப்புக் கேளு
ஆதியான வலாசுரன் அரக்கன் என்பான்
தாகின்ற ராட்சசன்தன் தசைக்கோ சந்தான்
சண்டையிட்டுத் தேவரெல்லா மிருக்கும் போது
தோகின்ற கடல்தன்னில் போய்வி ழுந்து
சுத்தமான பவழமது தோற்ற மாச்சு
ஏகின்ற இந்திரன்வச் சிராயு தத்தால்
ஏற்றமான மலைதன்னின் சிறகைக் கேளே.

போகர் கற்பம் 7000.[13]

- வலனுடைய சதை கடலில் வீழ்ந்து நற்பவழமாயிற்று என்றும்,
- தேவேந்திரனின் வஜ்ஜிராயுதத்தினால் மலையின் சிறகுகள் கொய்யப்பட்ட காலத்துக் குருதி கடலில் போய் வீழ்ந்து கொடிப்பவழமாயிற்று என்பதும் புராண வரலாறில் கூறப்பட்டுள்ளது.
- கடலில் வாழும் உயிரினங்களில் ஒருவகை நுண்ணுயிரியின் புறக்கூடே பவழம் என்று அழைக்கப்படுகின்றது.
- இவை மத்திய தரைக்கடல், இந்துமகா சமுத்திரம் போன்ற ஆழ்கடல் பகுதிகளில் காணப்படுகின்றன. [12]

திருவெண்காட்டடிகள் புராணத்தில் **“பவழ நற்குணம்”** என்னும் முதற் குறிப்பையுடைய விருத்தத்தினால் குணங்கள் மற்றும் குற்றம் 6 என்று அறியப்படுகிறது. அவை,

குணங்கள்: (6)

- சிந்தூரம்
- செம்மணி
- செங்காய்
- முசுமுசுக்கைக் கனி
- வீரைக்கனி
- தூதுளைக்கனி

குற்றங்கள்: (6)

- பிளவு
- முடக்கு
- துளை
- கருப்பு
- திருகல்
- வெளிரல்

திருவிளையாடற் புராணத்தில் ‘‘அவ்வழியிற் படுபவன’’ ன்ற அடிகளால் குணம் நான்கு, குற்றம் மூன்று என்று அறியலாம்.

குணங்கள்: (4)

- முருக்கம் பூ
- பசுங்கிளி மூக்கு
- செவ்வரத்த மலர்
- கொவ்வைக் கனி

குற்றங்கள்: (3)

- திருகிக் கோணுதல்
- புழுவரித்தல்
- முகமொடிதல்

பொதுக்குணம்:

"சுரதோடம் ஐயமுத் தோடசுரங் காசம்
அருசிகீ டத்தாலாம் ஆலம் - பெருவிந்து
நட்டம் அதிதாகம் நாவறட்சி போமொளியுங்
கிட்டும் பவழத்தாற் கேள்".

பதார்த்த குண சிந்தாமணி[15]

- சுரத்தில் உண்டாகும் தோடம், கபம், சன்னிபாதசுரம், இருமல், அரோசகம், விட சந்துக்களினால் உண்டாகும் விடம், தாது நட்டம், தாகம், நாவிலுண்டாகும் சுரசுரப்பு நீங்கும்.
- பவழத்தைச் சேவித்தாலே சயம், பித்தம், இரத்த காசம், நேத்திர ரோகம் நீங்குமென்று பெரியோர் கூறுவர்.

செய்கை:[12]

- நரம்பு தளர்ச்சி நீக்கி.
- சிறுநீர் பெருக்கி
- மலமிளக்கி
- துவர்ப்பி.

சுத்தி முறைகள்:

- ஒரு பலம் (35 கிராம்) பவழத்திற்கு ஆறு பலம் (210 கிராம்) பேரீச்சங்கள்ளை காலையில் விட்டு மாலை வரை வெய்யிலில் உலர்த்தி மறுநாள் காலையிலும் புதிதாய் மேற்படி கள்ளை விட்டு வெய்யிலில் வைக்கவும். இவ்விதம் ஐந்து முறை செய்து நீர் விட்டுக் கழுவி எடுக்க சுத்தியாகும். இம்முறையில் செய்தால் பவழம் கரைந்து விடும்.[12]
- சமஅளவு பேரீச்சங்கள் ஊற்றி ஊற வைத்து மறுநாள் கழுவி எடுக்கச் சுத்தியாகும்.[12]

- ஒரு நாள் முழுவதும் பழச்சாற்றில் ஊற வைத்து, மறுநாள் வெந்நீர் விட்டுக் கழுவி எடுக்கச் சுத்தி ஆகும்.[17]
- ஆவின் பால் அல்லது தயிரில் ஒரு சாமம் கொதிக்க வைத்தெடுக்க சுத்தி ஆகும்.[16]
- பசுமோரில் கிழி கட்டி ஒன்றரை மணிநேரம் எரிக்க சுத்தி ஆகும்.[39]
- பழச்சாற்றில் 3 நாட்கள் ஊற வைத்தெடுத்து உலர்த்தி எடுக்க சுத்தி ஆகும்.[16]
- எருக்கம் பாலில் 3 நாள் ஊற வைத்தெடுக்கவும்.[16]

சேரும் பிறமருந்துகள்:

- **பவழ பற்பம்:[19]**

  தீரும் நோய்கள்: வாதபித்தகப சுரங்கள், கடுப்பு வாதம், நடுக்கு வாதம், பிடிப்பு வாதம், பெரும்பாடு, யோனிப்புற்று, கல்லீரல் வீக்கம், சயம், இராசச் சிலந்தி.

- **பவழச் செந்தூரம்:**

  தீரும் நோய்கள்: சூதக சன்னி, நடுக்கல், சத்தி குன்மம், பவுத்திரம், இரத்த மூலச்சூடு, வாயிலைப்பு, தலையிலும் கையிலும் உண்டாகும் எரிச்சல்.

- **கொடிப் பவழச் சுண்ணம்: [19]**

  அளவு: 2 முதல் 3 குன்றியெடை

  தீரும் நோய்கள்: சயம், இருமல், சுவாசகாசம்.

- **நவரத்தின அக்கினிக் குமாரன்:[40]**

  அளவு: குன்றியெடை

  தீரும் நோய்கள்: சன்னிபாத சுரம்

- **இரணிய கற்பரசம்:[40]**

  அளவு: 2 மாத்திரைகள்.

தீரும் நோய்கள்: வாதபித்தகப சன்னிகள், புத்தி கூர்மை உண்டாகும்.

❖ **கபாங்குச சூரணம்:** [17]

அளவு: அரை தோலா

தீரும் நோய்கள்: சயம், உட்டிண இருமல், உட்காங்கை, கை கால் எரிச்சல்.

❖ **கருவங்க யோக பற்பம்:**[21]

அளவு: 1 – 2 குன்றி (260 – 900 மி. கி)

தீரும் நோய்கள்: மூலம், சயம், காசம், குன்மம், மேகம்.

❖ **கருவங்க பவழ செந்தூரம்:**[21]

அளவு: 2 – 4 குன்றி (260 – 900 மி. கி)

தீரும் நோய்கள்: வெட்டை நோய், மேக நோய்.

❖ **பவழ புற்று பற்பம்:**[19]

அளவு: 2 - 3 குன்றி

தீரும் நோய்கள்: இரத்தகாசம், இரத்தங்கக்கல், இருமல்.

❖ **மகாபூபதி பற்பம்:**[17]

அளவு: 1 - 2 குன்றி

தீரும் நோய்கள்: நீரடைப்பு, கல்லடைப்பு, சதையடைப்பு, நீரெரிச்சல், நீர்க்கட்டு, மார்பு நோய், சயம், ஈளை, மேககாங்கை.

❖ **திரிலோக சிந்தாமணி:**[41]

அளவு: 1 வராகன் (3.5கிராம்)

தீரும் நோய்கள்: கவிசை, சூலைக்கட்டு, பாண்டு, பெருவயிறு, பவுத்திரம், வலிப்பு.

- **சுவர்ண மாத்திரை**:[42]

  அளவு: 1 மாத்திரை

  தீரும் நோய்கள்: சுரம், சன்னி, காசம், சுவாசம், வலிப்பு, இசிவு, இருமல்.

3.2.2. IN MODERN ASPECT:

Coral reefs are one of the most ancient and dynamic ecosystems of India. Coral reefs not only provide a sanctuary to a myriad of marine life but also plays key role m protecting the coastline from erosion.

CORAL REEFS IN INDIA:

- All the three major reef types occur in India (atoll, fringing and barrier). Within these habitats are some of the most diverse, extensive and least disturbed reefs in the Indian Ocean. To this day, many of these reefs are largely unstudied.
- The mainland coast of India has two widely separated are as containing reefs: The Gulf of Kachchh in the northwest, which has some of the most northerly reefs in the world and Palk Bay and Gulf of Mannar in the southeast. In addition to these, there are patches of reef growth on the West Coast, for example coral reefs at Malvan.
- Coral reefs on the Tamil Nadu coast (South East Coast) are located in Palk Bay near Rameswaram and in the Gulf of Mannar. Mandapam peninsula and Rameswaram Islands separate Palk Bay from the Gulf of Mannar (between Rameswaram and Tuticorin). [43]

CORAL BIOLOGY:

- Corals are the most conspicuous inhabitants of reefs and provide the habitat amongst which fish and other reef animals exist.
- The term 'coral' has been used to describe a variety of different invertebrate animals from the Phylum Cnidaria including hard corals, soft corals, precious corals and hydro corals.
- Most often the word coral refers to hard corals from the Order Scleractinia.
- Scleractinia corals are divided into **reef-building corals** (hermatypic corals), which form the primary structure of coral reefs, and **non-reef building corals** (ahermatypic corals), which do not contribute significantly to reef formation.

- Hermatypic corals usually contain millions of tiny algal cells, called zooxanthellae, within their tissues.
- These algae are a primary energy source for the reef building activities of hermatypic corals.[43]

CORAL TAXONOMY:[44]

Zoological classification:

- Kingdom - Animalia
- Subkingdom - Radiata
- Phylum - Cnidarians
- Subphylum - Anthozoa
- Class - Anthozoa
- Subclass - Octacorallia
- Order - Alcyonaceae
- Suborder - Scleraxonia
- Family - Coralliidae
- Genus - Corallium
- Species - Corallium rubrum (Linnaeus 1758)

TYPES OF CORAL:

- **Hard corals –** These marine animals in the phylum Cnidaria that build themselves a hard skeleton. The individual animals are known as polyps and have a cylindrical body crowned by an oral disc in which a mouth is fringed with tentacles. Although some species are solitary, most are colonial. The founding polyp settles and starts to secrete calcium carbonate to protect its soft body.[45]

- **Soft corals –** These are an order of corals that do not produce calcium carbonate skeletons, formerly known as **gorgonians** which can bend with the tides.

MORPHOLOGY OF CORAL:

It is a small shrub/ tree like in appearance. It occurs in slender, cylindrical

and generally branched with consistently red in colour. It is made up of numerous minute pieces and longitudinally furrowed. In smell it resembles frankincense. With a crackling sound it easily breaks. In a raw state a cortical substance is covered over the stems and branches of soft small polyp which is the habitat of the polyp.[46]

PHYSICAL PROPERTIES: [28]

- Colour – Deep red to soft pink
- Hardness – 3 to 4 (Mohs)
- Specific gravity – 2.6 – 2.7
- Refractive index – 1.486 – 1.658
- Crystal system – Amorphous
- Double refraction – 0.172
- Transparency- Translucent to opaque
- Lustre – Vitreous, waxy
- Cleavage - None

CONSTITUENTS OF CORAL:

Animal or organic matter, 8 p.c., carbonate of lime 83 p.c., magnesium carbonate 3.5 p.c., and oxide of iron 4.5 p.c. The red colour is due to its containing iron.[24]

THERAPEUTIC USES: [24]

- As a local astringent, it is used in the preparation of tooth powders.
- Its chief use is in cough, phthisis, asthma, low fever, urinary diseases, spermatorrhoea, gleet and gonorrhoea, carbuncle, scrofulous affections, as a nervine tonic in headache, giddiness and vertigo.
- It was found useful in the cases of chronic bronchitis and pulmonary tuberculosis.
- It is given as an antacid to check vomiting and to cure dyspepsia and bilious headache.

CORALS IN ASTROLOGY:[28]

- The first benefit of the red coral is victory over enemies and adversaries.

- The red coral benefits in overcoming procrastination and laziness and gives the impetus and the individual to take tasks to their logical conclusions.
- Another powerful benefit of the red coral is its impact on the mental health. The red coral also imparts courage and helps in overcoming fear and nervousness. It is great gemstone for boosting self- esteem.
- The red coral represents strength of marriage and long life of the spouse. It protects women from widowhood and should be worn after proper astrological analysis.

3.2.3 SCIENTIFIC VALIDATION IN CORAL:

- **Velpandian et al., 2013**[48] evaluated the clinical efficacy of Kodipavala chunnam in hepatitis patients. Kodipavala chunnam normalize the raised liver parameters after a course of treatment.

- **Velpandian et al., 2013**[49] studied the acute and sub - acute toxicity studies on Kodi Pavala chunnam according to OECD guidelines. Kodi pavala chunnam did not cause any toxicity in both acute and sub - acute toxicity study.

- **Velpandian et al., 2013**[50] reported that the hepato protective activity of kodi Pavala chunnam in experimental rats. The liver damage was induced by CCl4 in Wistar rats. The haematological, biochemical parameters are near normal in treated group.

- **Thanigaivelan et al., 2011**[51] evaluated the haemostatic activity of Pavala parpam in Swiss albino mice. Pavala Parpam showed marked reduction in both bleeding and clotting time. The significant reduction in bleeding was comparable to that of standard.

- **Himanshu S et al., 2008**[52] clinically studied the effect of pravala bhasma in hyperacidity patients. It showed better results.

- **Prabhakara N. Reddy et al., 2003**[53] had evaluated the anti - osteoporotic activity of pravala bhasma by inducing progressive bone loss in female Sprague-Dawley rats by ovariectomy followed by low calcium diet. The CT scan

revealed significant increase in combined cortical thickness and cortical and periosteal area ratio. The calcium and phosphorus, excretion in urine is comparatively decreased in treated group.

- **Rosalind Marita et al., 1988** [54,55] had evaluated the anti - atherosclerotic activity of Anna Pavala chendooram, on experimentally induced atherosclerosis on Rabbits. It was evaluated by separation of plasma and aortic phospholipids into individual lipids and incorporating radiolabel from 14C-acetate into phospholipids. The plasma cholesterol level was reduced up to 65% and the HDL level was increased. The atheroma formation and the plasma sphingomyelin levels were also reduced.

3.3. எலுமிச்சை

3.3.1 IN SIDDHA ASPECT: [56]

பயன்படும் உறுப்பு:

இலை, காய், பழம், பழரசம், எண்ணெய்.

இலை, காய், பழம்:

சுவை – புளிப்பு, **தன்மை** – வெப்பம், **பிரிவு** – கார்ப்பு

செய்கை: குளிர்ச்சியுண்டாக்கி

தோல்:

சுவை – புளிப்பு, கார்ப்பு, **தன்மை** – வெப்பம், **பிரிவு** – கார்ப்பு

செய்கை: தடிப்புண்டாக்கி, அகட்டுவாய்வகற்றி.

காயின் பொதுக்குணம்:

தீதெலு மிச்சங்காய் டேர்முத்தோ டத்தையுமுள்
வாதகப சூலையையும் மாகொடிய – சாதியெனுஞ்
சர்த்திகுன் மத்தையுமுள் தங்கமருந் திட்டதையும்
பித்தவெப்பை யுந்தணிக்கும் பேசு.

அகத்தியர் குணவாகடம்.

இது முக்குற்றம், சூலை, வாந்தி, குன்மம், இடுமருந்து, அழல்நோய்கள் இவற்றைப் போக்கும்.

பழத்தின் பொதுக்குணம்:

தாகம் குநகநோய் தாழாச் சிலிபதநோய்
வேகங்கொள் உன்மாதம் வீறுபித்தம் - மாகண்ணோய்
கன்னனோய் வாந்தியும்போங் கட்டுவா தித்தொழிலில்
மன்னெலுமிச் சங்கனியை வாழ்த்து.

அகத்தியர் குணவாகடம்.

இது மயக்கம், வாந்தி, வாய்குமட்டல், நீர்வேட்கை, வெறி, கண்ணோய், காதுவலி போக்கும்.

வழக்குமுறை:

- எலுமிச்சம்பழத்தை ரசமும் ஊறுகாயுமாகக் கற்பமுறையாய்ப் பத்தியத்துடனே ஆறு மாதம் கொள்ள நரை, திரை, பிடிப்பு, பெருவயிறு, பக்கசூலை, முடம், வெறி, மயக்கம், மனசோர்வு தீரும்.
- இப்பழரசத்தோடு சர்க்கரை கூட்டி நீர் சேர்த்துப் பெருக்கி நீர்விடாய்க்கு அருந்தலாம்.
- சுரத்தில் உண்டாகும் வாந்திக்கும், வாய்குமட்டலுக்கும் இப்பழரசத்தால் செய்யப்படும் சாதிசம்பீர குழம்பு நற்பலனைத் தரும்.
- பவள பற்பத்தை இப்பழச்சாற்றுடன் சேர்த்து சீதக்கழிச்சல், பெருங்கழிச்சல், அதிசார கழிச்சல் இவைகளுக்கு கொடுக்கலாம்.
- கழிச்சலுக்காக மருந்துகள் கொடுத்து அடங்காத கழிச்சலும், வாந்தியும் நேரிட்டால், சீரகத்தை தேன் விட்டு வறுத்து, இதன் பழரசத்தை சேர்த்து நீர்விட்டுக் காய்ச்சிக் கொடுக்க உடனே கழிச்சலும் வாந்தியும் நிற்கும்.
- இதன் இலையைக் காடியிலிட்டு நீராகாரமாகப் பருகுவது நம் நாட்டுப் பழக்கம்.
- மேலும் இப்பழரசம் பலவகையான சித்த, ஆயுர்வேத மருந்துகளில் சேரும் சரக்காகவும், சுத்திக்கும் அதிக அளவில் பயன்படுகிறது.

சேரும் மருந்துகள்:

- **சௌபாக்கிய சொண்டி சூரணம்[41]**

  அளவு: வெருகடி

  தீரும் நோய்கள்: அசீரணம், குன்மம், வயிற்று நோய், பேதி, சீதசுரம், மந்தசுரம், சூசிகாவாயு தீரும்.

- **பாவன கடுக்காய்:[41]**

  அளவு: 1 மாத்திரை 2 வேளை

  தீரும் நோய்கள்: எழுசூலை, வாந்தி, குன்மம், முத்தோடம், சுவாசகாசம், சுரம், கிராணி, மூலம், நாசிகாபீடம், வீக்கம், பாண்டு, அருசி, சூலை, வயிற்றுநோய், தாதுவிருத்தி உண்டாகும்.

- **சூலைப்பிடாரம்:[41]**

  தீரும் நோய்கள்: சூலை, வலி, 8 வகை குன்மம், பீலிகை, மகோதரம், நீராமை வலி, வயிற்றில் ஏற்படும் பிணிகள் தீரும்.

- **எலுமிச்சங்கடுகு:[41]**

  அளவு: பாதி எலுமிச்சை

  தீரும் நோய்கள்: காமாலை, பாண்டு, சோபை.

- **கல்யாணி ரசம்: [41]**

  தீரும் நோய்கள்: சன்னி

- **முலைப்பாலெண்ணெய்: [17]**

  அளவு: காலணா எடை தினம் ஒரு வேளை.

  தீரும் நோய்கள்: எலும்புருக்கி, கிராணி, அதிசாரம், சீதக்கடுப்பு, கணச்சூடு, மூலச்சூடு, நாட்பட்ட வெள்ளை.

❖ **நாரங்கத் தைலம்:** [17]

அளவு: ஸ்நானம் செய்யவும்.

தீரும் நோய்கள்: காதில் சீழ் வருதல், மண்டைக் கரப்பான்.

❖ **ஈஸ்வர சூடாமணித் தைலம்:** [17]

அளவு: ஸ்நானம் செய்யவும்.

தீரும் நோய்கள்: நேத்திர நோய்கள்.

❖ **மேக ராஜாங்க கிருதம்:** [17]

அளவு: 1 தேக்கரண்டி

தீரும் நோய்கள்: நீர்ச்சுருக்கு, வெள்ளை, சதை அடைப்பு, பெரும்பாடு, எலும்புருக்கி.

❖ **சங்கு பற்பம்:** [17]

அளவு: ½ அல்லது 1 பணவெடை

தீரும் நோய்கள்: மார்நோய், மார்பு எரிச்சல், மாரடைப்பு, நீர்ச்சுருக்கு, வெள்ளை.

❖ **பஞ்ச லவண பற்பம்:** [17]

அளவு: ½ அல்லது 1 பணவெடை

தீரும் நோய்கள்: சூதகக்கட்டு, வயிற்றுள் உண்டான கட்டி, மகோதரம் தீரும். கருவுயிர்த்த பெண்ணின் அழுக்கை வெளிப்படுத்தும். 5 மாதத்திற்கு உட்பட்ட கர்ப்பிணிகள் இதனை உண்ணலாகாது.

❖ **பாசாண சுண்ணம்:** [17]

அளவு: 2 அரிசியெடை

தீரும் நோய்கள்: சன்னி தோச சுரங்கள்.

❖ **மேகராஜாங்க பற்பம்:** [17]

அளவு: ½ அல்லது 1 பணவெடை

தீரும் நோய்கள்: நாட்பட்ட சயம், காசம், புண்புரை, எலும்புருக்கி தீரும்.

❖ **பவழ பற்பம்:** [17]

அளவு: ½ குன்றியெடை

தீரும் நோய்கள்: மேக சம்பந்தமான உடல் எரிச்சல், காங்கை, நீர்எரிச்சல், நீர்க்கட்டு.

❖ **கருவங்க பற்பம்:** [17]

அளவு: ½ - 1 குன்றியெடை

தீரும் நோய்கள்: அதிசார பேதி, பிரசவத்திற்கு பின் வரும் கிராணி, சயம், ஈளை, இருமல், தீரா விக்கல், எலிக்கடி.

❖ **தாமிர பற்பம்:** [17]

அளவு: 1 அரிசியெடை

தீரும் நோய்கள்: சுரம், குட்டம், பாண்டு, சூலை, வாதரோகம், அக்கினி மந்தம்.

❖ **அயச்செந்தூரம்:** [17]

அளவு: 1 – 1 ½ குன்றியெடை

தீரும் நோய்கள்: சுவாசகாசம், ஈளை, இருமல்.

❖ **மகா மண்டூரச் செந்தூரம்:** [17]

அளவு: 1 – 1 ½ குன்றியெடை

தீரும் நோய்கள்: வீக்க ரோகங்கள் தீரும். இரத்த விருத்தி உண்டாகும்.

❖ **இலிங்கக் கட்டுச் செந்தூரம்:** [17]

அளவு: 2 அரிசியெடை.

தீரும் நோய்கள்: சன்னிபாத சுரங்கள், வாத சம்பந்தமான ரோகங்கள்.

❖ **தாமிர மெழுகு:** [17]

அளவு: ½ - ¾ குன்றியெடை.

தீரும் நோய்கள்: குன்மம், பெருவயிறு, பிடிப்பு, விரணம், சுவாசகாசம், திமிர், தடிப்பு, வாத ரோகங்கள்.

❖ **சம்பீர கற்பம்:**[18]

அளவு: 1 – 2 வராகனெடை

தீரும் நோய்கள்: பித்த பாண்டு, சோகை, இரத்தக் குறைவு, அசதி, சோம்பல்.

❖ **அயப்பொடி இளகம்:** [18]

அளவு: 3½ - 5½ கிராம்.

தீரும் நோய்கள்: பாண்டு, சோகை, மஞ்சள் நோய்கள், செரியாமை நோய்கள்.

❖ **லோக மண்டூர மாத்திரை:** [18]

அளவு: 1 மாத்திரை

தீரும் நோய்கள்: பாண்டு, சோகை, காமாலை.

❖ **காந்தச் செந்தூரம்:** [18]

அளவு: 3 – 4 குன்றியெடை

தீரும் நோய்கள்: இரைப்பிருமல், சயம், பாண்டு, சோகை.

❖ **மகா சுயமாக்கினி செந்தூரம்:** [18]

அளவு: 2 – 4 குன்றியெடை

தீரும் நோய்கள்: வளி நோய் 80, குன்மம் 16, வாயு 26 தீரும்.

❖ **ஆறுமுக வடகம்:** [18]

அளவு: 1 – 1½ மாத்திரை

தீரும் நோய்கள்: பாண்டு, சோகை, சுவையின்மை, கக்கல்.

❖ **அஸ்ட பாண்டு விநோத மெழுகு:** [18]

அளவு: 1 மாத்திரை

தீரும் நோய்கள்: எண்வகை பாண்டு, நச்சுப் பாண்டு, காமாலை, சோகை.

❖ **தாம்பிர பற்பம்:** [18]

அளவு: 1 குன்றியெடை

தீரும் நோய்கள்: குட்டநோய்.

❖ **அப்பிரகச் செந்தூரம்:**[21]

அளவு: 2 - 3 குன்றியெடை

தீரும் நோய்கள்: காசநோய், மேகநோய், மதுமேகம், வெட்டை நோய்கள்.

❖ **பளிங்கு அப்பிரகச் செந்தூரம்:** [21]

அளவு: 2 - 3 குன்றியெடை

தீரும் நோய்கள்: பிரமேகம், நீர்க்கடுப்பு, நீரெரிச்சல், வெட்டை, சயம், இரைப்பிருமல்.

❖ **காரீய பற்பம்:** [21]

அளவு: 2 - 3 குன்றியெடை

தீரும் நோய்கள்: காசநோய், மேகநோய், சயம், இரைப்பு, பிரமேகம், பிரமியம்.

❖ **நாக பற்பம்:** [21]

அளவு: 4 - 6 குன்றியெடை

தீரும் நோய்கள்: கை கால் கறுப்பு, ஊரல், தடிப்பு, புடை.

- **வெள்ளி பற்பம்:** [21]

  அளவு: 1 - 2 குன்றியெடை

  தீரும் நோய்கள்: பாண்டு, சுரக்கட்டி, உப்பு நோய், சூலை, கபசுரம், இரத்தபேதி, இரத்தமூலம்.

3.3.2 IN MODERN ASPECT:

VERNACULAR NAMES:[57]

- **Tamil -** Elumichai
- **Sanskrit –** Nimbaka, limbaka, mahajambiram, vijapura
- **English** – Lemon
- **Hindi** – Jambira, paharikaghju
- **Telugu** – Peddanimba
- **Malayalam** – Cerunarakam
- **Kannadam** - Brihat nimbe, dodda nimbe.
- **Panjabi** – Khuttia

HISTORY & HABITAT:[58]

In 1493, first lemon tree in America is planted by the Italian navigator Christopher Columbus. The major producers of lemon today are USA, Italy, Turkey, Israel, Spain and Greece. In India, the cultivation is carried out in UP, Madhya Pradesh, Karnataka and Punjab. In India though the most popular variety of citrus fruits cultivated and used is acid lime. It is grown largely in Andhra Pradesh, Bihar, Gujarat, Maharashtra, Rajasthan, and Tamil Nadu, and to a limited extent in other states of country. Areas with dry climate and low rainfall are best suited for growing limes. Lemons can be grown in heavy rainfall humid regions. The best season of planting is June to August.

TAXONOMY OF LEMON:[59]

- **Kingdom –** Plantae
- **Subkingdom –** Tracheobionta

- **Super division** – Spermatophyta
- **Division** – Magnoliophyta
- **Class** – Magnoliopsida
- **Subclass** – Rosidae
- **Order** – Sapindales
- **Family** – Rutaceae
- **Binomial name -** Citrus limon.

VARIETIES:[60]

Two kinds of limes are found in the Indian market - **Pati** and **Kagzi**. The lemon though belonging to the same stock, differs from the lime fruit in being bigger in size with a rough, thin and loose rind.

PARTS USED:[60]

Rind of the ripe fruit (Limonis cortex, officinal), essential oil of the rind (oleum limonis) and expressed juice of the ripe fruit (Succus limonis).

CONSTITUENTS: [58,60]

A pale-yellow volatile oil derived on either by distillation or by simple expression from the fresh outer part of the pericarp or finely grated rind of the fruit. Lemon is richer in juice and citric acid than lime. The average amount of citric acid available from 100 c. c of lemon juice is 3.7%. Lemon contains many vitamins (niacin, riboflavin, thiamine, choline, pantothenic acid, foliate, vitamin C, vitamin B6) and minerals (calcium, copper, iron, manganese, magnesium, phosphorus, potassium, zinc), which are needed for human body.

ACTION:

- **Rind** – Stomachic, Carminative.
- **Lemon oil** – Bitter, aromatic, stomachic and carminative in doses from 2 to 4 drops, but is rarely employed in this form.
- **Lemon juice** – Antiscorbutic and refrigerant, primarily anti – alkaline and secondarily antacid.
- **Bark** – Febrifuge.
- **Seeds** – Vermifuge.

MEDICINAL USES:[60]

- Lemon oil is used as a local application of in some forms of ophthalmia but with doubtful results.
- Nimba thailam applied is of special use in leprotic ulcers.
- Lemon oil is applied to check postpartum haemorrhage and is highly prized in medicine as a flavouring agent.
- In rheumatic affections such as plerodynia, sciatica, lumbago, pain in the hip joints etc., the administration of lemon juice with the addition of impure carbonate of potash and honey is recommended by sarangadhara.
- Lemon juice and gun powder is applied topically for scabies.
- Juice of the baked lemon is excellent remedy for cough when mixed with an equal quantity of sugar or honey and taken in teaspoonful doses.
- A decoction of the lemon is a very valuable remedy in the treatment of ague.
- Lemon juice is recommended to be taken in the evening for the relief of dyspepsia with vomiting and bilious headaches.
- Preserved with sugar or honey lemons are recommended for sore throat and are considered to act as detergent; they are administered before purgatives to prepare the body for them and afterwards to check excessive action.
- Daily consumption of lemon water helps to purify the blood and also significantly improves mental health. It is a powerful antiseptic and can be used to disinfect cuts, abrasions and scrapes. In order to keep good health.[58]

3.3.3 SCIENTIFIC VALIDATION OF LEMON:

- **Chikako et. al., 2019 [61]** studied the Effects of lifelong intake of lemon polyphenols on aging and intestinal microbiome in the senescence. This result suggested that the lemon polyphenols has anti-aging effects not only on host health but also on the intestinal environment.

- **Yoji kato et. al., 2014 [62]** evaluated the effect of ingestion of lemon and walking in the management of blood pressure. This study suggested that the walking and lemon ingestion have the effect of lowering systolic blood pressure by respectively different action mechanisms. There may be an additive or synergistic effect in movement and lemon ingestion.

- **Elham et. al., 2014**[63] evaluated the Antiviral Activity of Citrus Limon, Matricariarecutita L., Allium ascalonicum L., and Rosa damascene against Newcastle Disease Virus.The results of this test showed Limon had the most antiviral activity and could reduce viral pathogenicity of NDV as 100 - fold. Matricariarecutita L., Allium ascalonicum L. could reduce activity of virus as 3.8 and 40.5-fold respectively, but Rosa damascene did not have any significant effect on virus.

- **Boshtam et. al., 2013**[64] evaluated the impact of fresh lime juice and peel on atherosclerosis progression in an animal model. Based on the results, citrus peel and juice increase plasma antioxidant capacity in rabbits, and can thus prevent or decelerate the process of atherogenesis. However, lime peel is more effective than lime juice.

- **Alshatwi et. al., 2011**[65] conducted the study on methanolic extract of lemon juice was investigated in the anti-tumour activity on the MUF-7 breast cancer cell line by in vitro. The expression of a pro-apoptotic gene, bax was increased and the expression of an anti-apoptotic gene, bel-2 was decreased by LE extract treatment, resulting in a shift in the Box; Bel-2 ratio to one that favoured apoptosis.

- **Ashok kumar et. al., 2011**[66] studied the Antibacterial activity of five different solvent extracts (ethyl acetate, acetone, ethanol, petroleum ether and water) prepared by soxhlet extractor from two citrus fruit peel (Citrus sinensis and Citrus limon) were screened against five pathogenic bacteria Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Klebsiella pneumonia and Salmonella typhi. The highest antibacterial potentiality exhibited by the acetone peel extract of Citrus limon as very potent as the antibiotics such as metacillin and penicillin.

- **Yasmin khan et. al., 2010** [67] studied the hypolipidemic effects of citrus lemon juice in rabbits after high cholesterol diet for four weeks. The citrus lemon juice (1ml/kg/day) revealed a significant reduction in serum cholesterol, triglycerides; low density lipoprotein levels and resulted in an increase in high

density lipoprotein. These results suggest that the hypolipidemic effects of citrus lemon juice may be due to its antioxidant effect.

- **Penniston et. al., 2008** [68] studied the quantity assessment of citric acid in lemon juice, lime juice and commercially available fruit juice products. Lemon juice and lime juice are rich sources of citric acid, containing 1.44 and 1.38 g/oz, respectively. Lemon and lime juice concentrates contain 1.10 and 1.06 g/oz, respectively. The citric acid content of commercially available lemonade and other juice products varies widely, ranging from 0.03 to 0.22 g/oz.

5.4 STANDARDISATION OF DRUG:

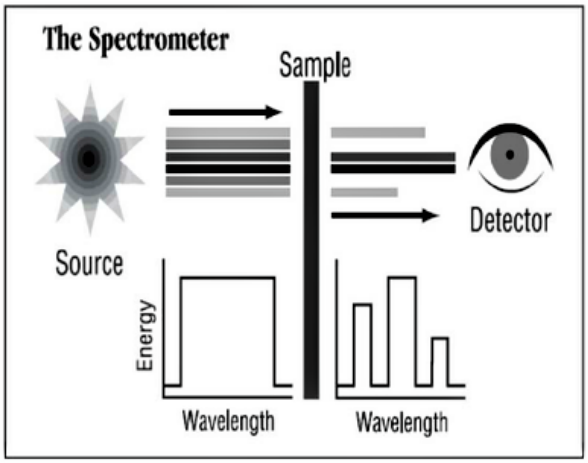
Siddha pharmacopoeia established in recent times have imposed more on standardization aspect of the formulation. Starting from preparatory phase to storage each and individual step involved in formulating Siddha preparation has its own quality check evaluations. Bioactive phytocomponents nanoparticles present in preparations like Chenduram have the unique advantage of multiple modes of action.[69] The measurement of standards for drugs is an important criteria. The modern medicine industry uses standard raw materials and standardized production procedures.[70] Hence the standardisation procedure is essential to check the quality standards of the drug. The standardization process can be carried out chemically, spectroscopically.

5.4.1 FOURIER TRANSFORM INFRARED SPECTROSCOPY: (FTIR) [71]

Fourier transform infrared spectroscopy generates time-domain spectra as the immediately available data, in which the intensity is obtained as a function of time. Fourier transform is introduced to inspect frequency-domain spectra from the corresponding time-domain waveform since direct observation of a time domain spectrum is not possible. It is the only analytical tool which provides both ambient temperature operation and the ability to directly monitor the vibrations of functional groups which characterize molecular structure and govern the course of chemical reactions

5.4.1.1 Principle of FTIR: [72]

In infrared spectroscopy, IR radiation is passed through a sample. Some of the infrared radiation is absorbed by the sample and some of it is passed through (transmitted). The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. An infrared spectrum represents a fingerprint of a sample with absorption peaks which correspond to the frequencies of vibrations between the bonds of the atoms making up the material. Because each different material is a unique combination of atoms, no two compounds produce the exact same infrared spectrum. Therefore, infrared spectroscopy can result in a positive identification (qualitative analysis) of every different kind of material. In addition, the size of the peaks in the spectrum is a direct indication of the amount of material present. With modern software algorithms, infrared is an excellent tool for quantitative analysis.



5.4.1.2 Applications of FTIR:[72]

- It can identify unknown materials.
- It can determine the quality or consistency of a sample.
- It can determine the number of components in a mixture.

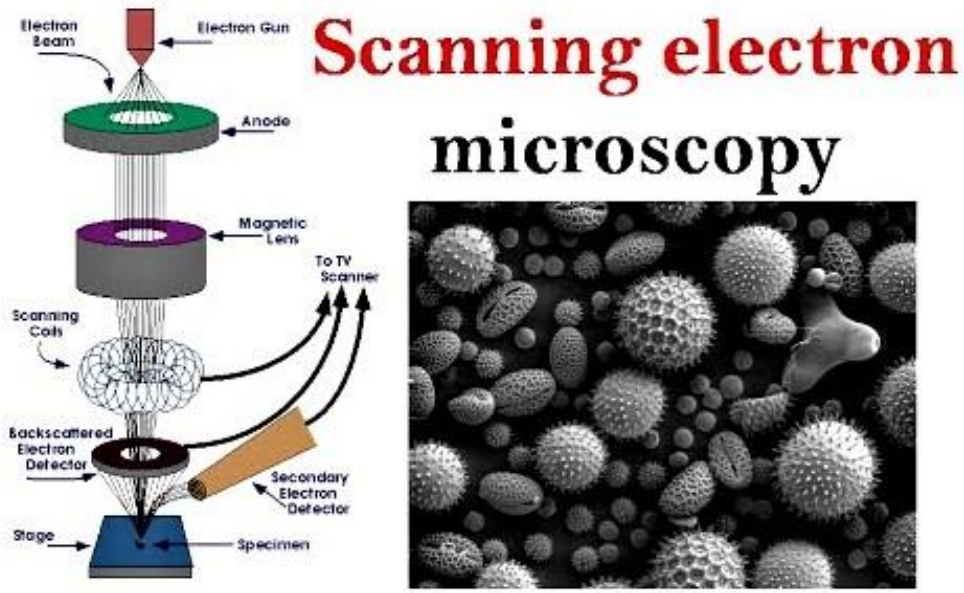
5.4.2 SCANNING ELECTRON MICROSCOPE: (SEM) [73]

A scanning electron microscope (SEM) scans a focused electron beam over a surface to create an image. The electrons in the beam interact with the sample, producing various signals that can be used to obtain information about the surface topography and composition. It is a powerful magnification tool that utilizes focused

beams of electrons to obtain information. The high resolution, three dimensional images produced by SEM, provides topographical, morphological and compositional information, makes them invaluable in a variety of science and industry applications.

5.4.2.1 Principle of SEM:

SEM uses an electron beam instead of a beam of visible light in an optical microscope, which is directed towards the specimen under examination. When a specimen is hit with a beam of the electrons known as the incident beam, it emits X-rays and three kinds of electrons: primary back scattered electrons, secondary electrons and Auger electrons. The SEM uses primary back scattered electrons and secondary electrons. The electrons interact with atoms in the sample, producing various signals that contain information about the sample's surface topography and composition. The electron beam is generally scanned in a raster scan pattern and the beam's position is combined with the detected signal to produce an image.



5.4.2.2 Applications of SEM:

SEM can detect and analyse surface fractures, provide information in microstructures, examine surface contamination, reveal spatial variations in chemical compositions, provide qualitative chemical analyses and identify morphology of the crystalline structures. SEM can be essential research tool in fields such as life sciences, biology, gemmology, medical, forensic science and metallurgy. SEM has practical,

industrial and technological applications such as semiconductor inspection and assembly of microchips for computer.

5.4.3 X – RAY POWDER DIFFRACTION: (XRD) [74]

X-ray – diffraction is a rapid analytical technique used for phase identification of crystalline material and can provide information on unit cell parameters. The atomic planes of a crystal cause an incident beam of X-rays to interfere with one another as they leave the crystal. The phenomenon is called X-ray diffraction. The X-ray diffraction pattern of a pure substance is, therefore, like a fingerprint of the substance. The powder diffraction method is thus ideally suited for characterization and identification of polycrystalline phases.

5.4.3.1 Principle of X- ray diffraction:

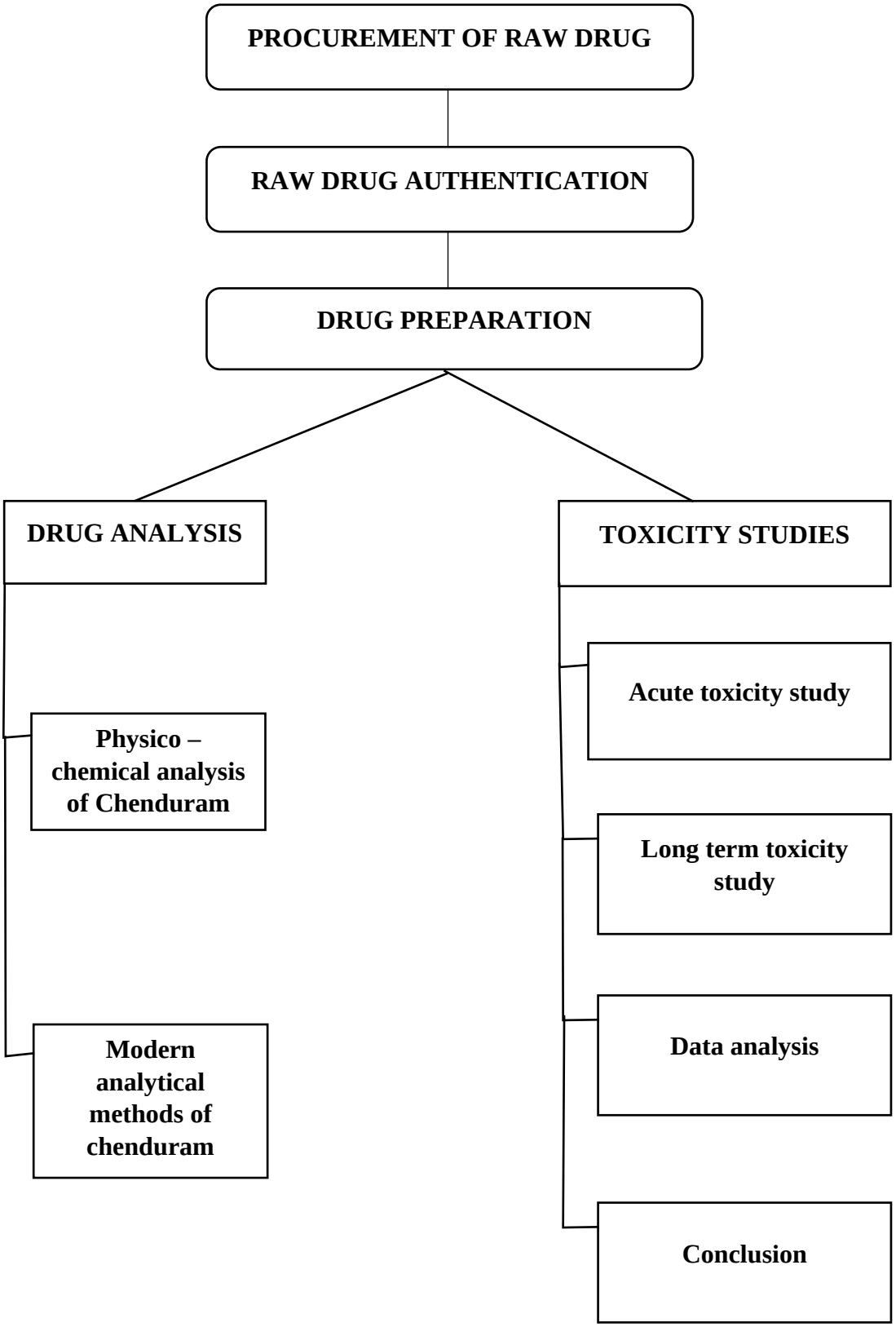
The distance between the plans of atoms that constitute the sample can be measured by applying Bragg's Law.

Bragg's Law is $n\lambda=2d\ sin\theta$, where the integer n is the order of the diffracted beam, λ is the wavelength of the incident X-ray beam, d is the distance between adjacent planes of atoms (the d-spacings), and θ is the angle of incidence of the X-ray beam.

5.4.3.2 Advantages of XRD:

- Measure the average spacings between layers or rows of atoms
- Determine the orientation of a single crystal or grain
- Find the crystal structure of an unknown material
- Measure the size, shape and internal stress of small crystalline regions.

4. WORK PLAN

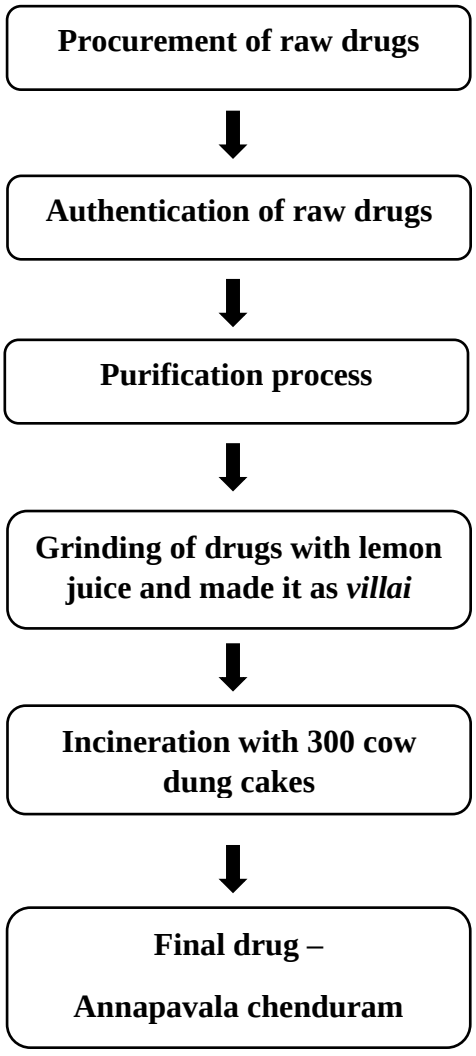

PROCUREMENT OF RAW DRUG
RAW DRUG AUTHENTICATION
DRUG PREPARATION
DRUG ANALYSIS
TOXICITY STUDIES
Physico – chemical analysis of Chenduram
Modern analytical methods of chenduram
Acute toxicity study
Long term toxicity study
Data analysis
Conclusion

Annapavala Chendhuram is the type of chenduram, belongs to the 32 types of internal medicine in Siddha system. The preparation of this drug has treatise by G. D. Naidu in his Siddha pharmacopoeia.

5.1.1 Ingredients of Annapavala chenduram: [10]

S. No	Tamil Name	Botanical/Chemical name	Quantity
1.	Annabedhi	Ferrous sulphate	227 gms
2.	Kodipavalam	*Corallium rubrum*	170 gms
3.	Elumichai	*Citrus limon*	Required amount

5.1.2 Steps involved in the preparation of Annapavala chenduram:



5.1.3 Procurement of the raw drug:

The raw drug Annabedhi and kodipavalam were procured from the authenticated raw drug shop at Chennai. Lemon was procured from the Vegetable market, Tambaram, Chennai.

5.1.4 Authentication of the raw drugs:

Department of Geology, University of Madras, Chennai authenticate the Kodipavalam and Annabedhi samples by analysing the microscopical characters and literature sources.

5.1.5 Purification process of raw drugs:

- **Annabedhi:[16]**

  Annabedhi was soaked in Lemon juice for 3 days and made it dry under the shade in earthen vessel.

- **Kodipavalam:[16]**

  Kodipavalam was soaked in the lemon juice for 3 days in an earthen vessel. It was kept under the sunlight for 3 days. After that, on 4th day kodipavalam was washed with the water and wiped with cloth.

5.1.6 Preparation method of Annapavala chenduram:[10]

The trial drug Annapavala chenduram was prepared in the Gunapadam Laboratory, National Institute of Siddha, Chennai. The purified Kodipavalam and Annabedhi were made into a fine powder separately and both powders were placed in kalvam (mortar). It was ground well with lemon juice for six hours. Then it was made in to villai (pellets) and dried in sunlight. The pellets were kept into an earthen vessel which was closed with another vessel and 7 clay cloth made to the margin of earthen vessels and the set up was dried in sunlight for one day. After that, it was kept in a deep pit and subjected to the pudam process (incineration process) was done with 300 cow dung cakes. Once it cools, the pudam was opened and the pellets were taken out. The collected pellets were weighed, grounded well to make the powder and stored in a clean, dry, airtight glass container.

Dose: 120 – 260 mg

Adjuvant: Honey, Ghee, butter or impural legiyam.

Indication: Kasam (cough), swasakasam (bronchial asthma), Shayam (pulmonary tuberculosis), raktha ushnam (bleeding disorders), pitha ushnam (pitha diseases), mega ushnam (mega diseases) and aththi juram (bone tuberculosis). This chenduram may be given for all diseases with suitable adjuvant as an alternative medicine.

Purification of Raw drugs

Annabedhi – Before purification

Annabedhi – After purification

Kodipavalam – Before purification

Kodipavalam – After purification

Preparation method of Annapavala chenduram

Fine powder of Annabedhi

Fine powder of Kodipavalam

Annabedhi and Kodipavalam

grinding with lemon juice

Villai in clay sealed earthen vessel

Incineration with 300 cow dung cakes

Final drug

5.2 STANDARDISATION OF THE ANNAPAVALA CHENDURAM:

5.2.1 Qualitative analysis of Annapavala chenduram for acid & basic radicals:

Preparation of the extract:

5gm of *Annapavala chenduram* is weighed accurately and placed in a 250ml clean beaker and added with 50ml of distilled water. Then it is boiled well for about 10 minutes. Then it is cooled and filtered in a 100ml volumetric flask and made up to 100ml with distilled water. This preparation is used for the qualitative analysis of acidic/basic radicals and biochemical constituents in it. The biochemical analysis of *Annapavala Chendhuram* was done at Biochemistry Lab, National Institute of Siddha, Chennai-47.

S. No	Experiment	Observation
1.	Appearance of the sample	Brownish red in colour.
2.	*Solubility:* a. A small quantity of the sample was shaken well with distilled water. b. A little quantity of the sample was shaken well with con. HCl / Con. H_2So_4.	Sparingly soluble. Completely soluble.
3.	**Action of heat:** A small amount of the sample is taken in a dry test tube and heated gently at first and then strong.	White fumes evolved.
4.	**Flame test:** A small amount 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 appeared.
5.	**Ash test:** A filter paper is soaked into a mixture of sample and cobalt nitrate solution and introduced into the Bunsen flame and ignited.	No yellow colour flames.

Test for acid radicals of APC:

S. No	Experiment	Observation
1.	**Test for Sulphate:** 2ml of the extract is taken in a test tube to this added 2ml of 4% ammonium oxalate solution.	Cloudy appearance present.
2.	**Test for chloride:** 2ml of the extract is added with dil. Hno_3 till the effervescence ceases. Then 2ml of silver nitrate solution is added.	Cloudy appearance present.
3.	**Test for Phosphate:** 2ml of the extract is treated with 2ml of ammonium molybdate solution and 2ml of Con. Hno_3.	Cloudy yellow appearance present.
4.	**Test for Carbonate:** 2ml of the extract is treated with 2ml magnesium sulphate solution.	Cloudy appearance present.
5.	**Test for Nitrate:** 1gm of the substance is heated with copper turnings and concentrated H_2So_4 and viewed the test tube vertically down.	Characteristic changes present.
6.	**Test for sulphide:** 1gm of the substance is treated with 2ml of con. HCL.	Rotten egg smelling gas evolved.
7.	**Test for Fluoride & Oxalate:** 2ml of extract is added with 2ml of dil. Acetic acid and 2ml calcium chloride solution and heated.	Cloudy appearance present.
8.	**Test for Nitrite:** 3 drops of the extract is placed on a filter paper on that 2 drops of acetic acid and 2 drops of benzidine solution is added.	Characteristic changes present.

S. No	Experiment	Observation
9	**Test for Borate:** 2 pinches of the substance are made into paste by using sulphuric acid and alcohol (95%) introduced into the blue flame.	Bluish green color flame appeared.

Test for Basic radicals of APC:

S. No	Experiment	Observation
1.	**Test for Lead:** 2ml of the extract is added with 2ml of potassium iodide solution.	Yellow precipitate present.
2.	**Test for Copper:** 2ml of the extract is added with excess of ammonia solution.	Blue colour precipitate present.
3.	**Test for Aluminium:** 2ml of the extract is added with excess drops of sodium hydroxide.	Characteristic changes present.
4.	**Test for Iron:** 2ml of the extract is treated with 2ml of ammonium thiocyanate solution.	Mild red colour present.
5.	**Test for Zinc:** 2ml of the extract is added with excess drops of sodium hydroxide.	White precipitate is formed.
6.	**Test for Calcium:** 2ml of the extract is added with 2ml of 4% ammonium oxalate solution.	Cloudy appearance and white precipitate present.
7.	**Test for Magnesium:** 2ml of extract is added with excess drops of sodium hydroxide.	white precipitate present.
8.	**Test for Ammonium:** 2ml of extract is added with few ml of Nessler's reagent and excess drops of sodium hydroxide.	Brown colour appeared.

S. No	Experiment	Observation
9.	**Test for Potassium:** A pinch of the substance is treated with 2ml of sodium nitrite solution and then treated with 2ml cobalt nitrate in 30% glacial acetic acid.	Yellow precipitate present.
10.	**Test for Sodium:** 2 pinches of substance are made into paste by adding HCL and introduced into the blue flame of Bunsen burner.	Yellow colour flame present.
11.	**Test for Mercury:** 2ml of the extract is added with 2ml of sodium hydroxide solution.	Yellow precipitate obtained.
12.	**Test for arsenic:** 2ml of the extract is treated with 2ml of sodium hydroxide solution.	Brownish red precipitate is obtained.

Miscellaneous test for APC:

S. No	Experiment	Observation
1.	**Test for Starch:** 2ml of extract is treated with weak iodine solution.	Blue colour present.
2.	**Test for Reducing sugar:** 5ml of Benedict's qualitative solution is 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.	Brick red colour present.
3.	**Test for Alkaloids:** 2ml of the extract is treated with 2ml of picric acid.	Yellow precipitate obtained.

4.	**Test for Tannic acid:** 2ml of the extract is treated with 2ml of ferric chloride solution.	Black precipitate is obtained.
5.	**Test for unsaturated compound:** 2ml of the extract is added with 2ml of the potassium permanganate solution.	Potassium permanganate is decolourised.
6.	**Test for Amino acid:** 2 drops of the extract are placed on a filter paper and dried well.	Violet colour developed.
7.	**Test for type of compound:** 2ml of the extract is treated with 2ml of ferric chloride solution.	Red colour developed.

5.2.2 ANALYTICAL SPECIFICATION OF ANNAPAVALA CHENDURAM: [75]

The analytical specification of *chenduram* was done according to the protocol given by Pharmacopoeial Laboratory for Indian Medicine (PLIM), Dept of AYUSH, Ministry of Health and Family Welfare, New Delhi. Physico chemical analysis were carried out in Omegaa laboratories (Analytical testing and research center), Namakkal.

Sl. No	Tests
1.	Organoleptic characters: Colour Odour Taste
2.	pH
3.	Loss on drying at 105 degree C
4.	Particle size
5.	Total ash
6.	Acid –Insoluble ash
7.	Water soluble extractive
8.	Alcohol soluble extractive
9.	SEM/XRD/EDX/XRF
10.	Lusterless
11.	Fine powder to enter within lines of finger
12.	Floats on water

13.	Smokeless
14.	Tasteless

5.2.2.1 Physico chemical analysis of Annapavala chenduram:

pH of the APC: [75]

pH value of the Annapavala Chendhuram were determined by using the suitable pH meter.

Acid insoluble ash:

3 gm of Annapavala Chendhuram was incinerated in silica dish at a temperature not exceeding 450º until free from carbon. Then it was taken after cooled.

The ash was boiled for 5 minutes with 25 ml of dilute hydrochloric acid. The insoluble matter collected by using filter paper. Then it was washed with hot water and ignite to constant weight. Calculate the percentage of acid-insoluble ash with reference to the air-dried drug.

Extract of Ether: (Total fat)

3gm of Annapavala Chendhuram was put in to an extraction thimble, extraction process continued for 6 hours with solvent ether. Filtered the extract quantitatively into a tared evaporating dish and evaporated off the solvent on a water bath. The residue was dried at 105º to constant weight. Then it was calculated and the percentage of ether-soluble extractive with reference to the air-dried drug.

Determination of crude protein: [76]

Transfer 2gm of *Annapavala chenduram* in the kjeldahl flask. Add about 10gm of potassium sulphate or anhydrous sodium sulphate, about 0.5gm of copper sulphate and 25ml of concentrated sulphuric acid. Place the flask in an inclined position, and heat below the boiling point of the acid until frothing ceases. Increase heat until the acid boils vigorously and digest for a time after the mixture is clear or until oxidation is complete. Cool the contents of the flask. Transfer quantitatively to the round bottom flask with water, the total quantity of water used to be about 200ml.

Add a few pieces of pumic stone to prevent bumping. Add carefully the sodium hydroxide solution in quantity which is sufficient to make the solution alkaline by the side of the flask. Assemble the apparatus taking care that the tip of the dip tube

extends below the surface of the standard sulphuric acid in the receiver. Mix the contents of the flask by shaking and distil until all ammonia has passed over into the standard sulphuric acid. Titrate with standard sodium hydroxide solution.

Determination of crude fibre:

2gm of Annapavala Chendhuram weighed accurately and fat extracted for about 8 hours with petroleum ether using a Soxhlet apparatus. Transfer the fat-free dry residue to a one litre conical flask. Take 200ml of dilute sulphuric acid in a beaker and bring to the boil. Transfer the whole of the boiling acid to the flask containing fat free material and immediately connect the flask with a reflux water condenser and heat, so that the contents of the flask begin to boil within one minute. Continue boiling for 30 minutes and filter through a fine linen funnel. Wash with boiling water until the washings are no longer acid to litmus. Bring to the boil some quantity of sodium hydroxide solution under a reflex condenser.

Wash the residue on the linen into the flask with 200ml of the boiling sodium hydroxide solution. Immediately connect the flask with the reflux condenser and boil for exactly 30 minutes. Remove the flask and filtering with cloth. Thoroughly wash the residue with boiling water and transfer to a gooch crucible prepared with a thin but compact layer of ignited asbestos. Wash the residue thoroughly first with hot water and then with about 15ml of 95% ethyl alcohol. Dry the gooch crucible and contents at 105±1°C in the air oven to constant mass. Cool and weigh. Incinerate the contents of the gooch crucible at 600±20°C in a muffle furnace until all the carbonaceous matter is burnt. Cool the gooch crucible containing the ash in a desiccator and weigh.

5.2.2.2 Siddha specification of Chendhuram:

Floats on the water:

A pinch of the chenduram was sprinkled over the water in a vessel. The test drug was float on the water surface. It was noted.

Finger lines test:

Chendhuram should be very fine in manner. While the *Annapavala chenduram* taken between the thumb and index finger, it can fine enough to enter within the lines of fingers.

Tasteless:

Chenduram should be completely tasteless. This test was done by placing a pinch of the *Annapavala chenduram* at the tip of the tongue and then tasted.

Lustreless:

If any shining particles present in the Chendhuram, it indicates the improper medicinal

preparation. There should be no shining particles in the *Annapavala Chendhuram*. The *Annapavala Chendhuram* was taken in a petri dish and evaluated under the sunlight for any shining particles.

5.2.3 SPECTROSCOPIC ANALYSIS:

Spectroscopic analysis for the characterisation of Annapavala Chendhuram were carried out in Avinashi lingam university, Coimbatore.

5.2.3.1 Scanning Electron Microscopy (SEM) of APC:

The morphological features were recorded by FESEM (TESCAN MIRA 3) with an accelerating voltage of ~50 kV and an elemental composition was obtained using an energy-dispersive X-ray spectrometer. It is a fully PC-controlled scanning electron microscopes with a tungsten heated filament.

5.2.3.2 Energy Dispersive X – ray analysis (EDAX) of APC:

Energy Dispersive X-Ray Spectroscopy (EDS or EDAX) is a chemical microanalysis technique used in conjunction with scanning electron microscopy (SEM). SEM uses energy dispersive X-ray spectroscopy (EDS) in the production of elemental maps, which accurately represent the distribution of elements

within the samples. The most typical use is elemental analysis, mineral orientation, morphology and contrast.

5.2.3.3 X- Ray diffraction measurement (XRD) of APC:

The sample was characterized by X'Pert PRO; PANalytical X-ray diffractometer using Cu Kα as the radiation source (λ =1.5406 Å) operated at 30 kV. The Sample was scanned from 10° to 80° at a scan rate of 1°s-1 at 2θ position.

5.2.3.4 Fourier Transform Infrared Spectroscopy (FTIR) of APC:

IRAffinity-1 is a compact Fourier transform infrared spectrophotometer that is housed within an elegant form. FTIR spectrometer (SHIMADZU) was used to analysis samples. The prepared powder samples were mixed with KBr in the ratio of 1:100, and then, the pellets were prepared by subjecting it to a load of 5 tons cm^3 and also a pellet-maker (hydraulic pellet pressure; Kimaya Engineers, India). The spectra were collected from wavelength of 4000 cm-1 to 400 cm-1 with 4cm-1 resolution over 40 scans.

5.3 TOXICITY STUDIES OF ANNAPAVALA CHENDURAM:

To evaluate the safety profile of *Annapavala Chendhuram*, the acute and long-term toxicity study was conducted as per the WHO guidelines 1993.

The complete study procedure of the animal experiments has been approved by the Institutional Animal Ethics committee, National Institute of *Siddha*, Chennai. Institutional Animal Ethics Committee (IAEC) approval number: **(NIS/IAEC-VII/28082018/15).** The animal study was carried out in Animal house of National Institute of Siddha, Tambaram sanatorium, Chennai.

5.3.1 ACUTE TOXICITY STUDY OF ANNAPAVALA CHENDHURAM:

Species	:	Wistar rats
Sex	:	Male and Female
weight at start of test	:	160-200gm b.wt
Acclimatization Period	:	7 days prior to dosing
Housing	:	Polypropylene cages
Husbandry	:	12-hour light/12-hour dark
Room temperature		22°C (±3°)
Relative humidity		30–70%
Feed and Water	:	Rodent pellet feed
		RO purified water *ad libitum*
Identification	:	Animals were kept in individual cages and marked with picric acid.

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 (Honey)
Test group	:	Annapavala Chendhuram.

Grouping of acute toxicity animals:

The total of 20 animals (10 male + 10 female) were included in acute toxicity study. These animals were divided into two groups such as control group and high dose group. Each group consists of 10 animals (5 male + 5 female). Honey was administered to the control group (group I) and high dose of test drug (250mg/kg b. wt) was given to the group II animals.

Table 5.3.1: Grouping of acute toxicity study

Groups	No. of Rat
Group I: Vehicle control (Honey)	10(5M+5 F)
Group II: Test drug (Annapavala chenduram) – 250mg/kg b.wt	10(5M+5F)

Total 20(10M+10F)

Route of administration:

Test drug was administered through oral route.

Administration of Dose:

The animals were fasted (only food was withheld) for 12 hours and weighed prior to dosing. A single dose of the test drug (250mg/kg/body weight) was administered by oral gavage. The food was withheld for another 4hrs after dosing and administration of the drug.

Experiment details of acute toxicity study:

The both sex Albino rats of weighing 160-200 g 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°C and have free access to standard rat pellet diet. The animals were treated with *Annapavala Chendhuram* by oral route for single dose on the 1st day and monitored for mortality and behavioural parameters for the first 4 hours after drug administration. Body weight of the animal was monitored at weekly intervals. After that, on 14th day, animals of both groups were weighed and sacrificed under the injection of thiopental sodium (intra peritoneal). The toxicological effect was assessed on the basis of mortality and behavioural parameters.

Observations:

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

- Mortality, behavioural changes.
- ½ hour, 1 hour, 2 hours,4 hours and up to 24- hour 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, coma.

b. **Gross necropsy**

At the end of the 14^{th} day (15^{th} day), all the animals were sacrificed by using the injection of thiopental sodium. Gross necropsy includes an examination of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents. Brain, lung, heart, spleen, liver, kidney, uterus, testes, ovary of all animals.

5.3.2 LONG TERM TOXICITY STUDY OF ANNAPAVALA CHENDHURAM:

Experimental animals:

- Species : Wistar rats
- Sex : Male and female
- Weight at start of rest: 160 – 250gm
- Housing : Polypropylene cages bedding with husk
- Husbandry : 12 hr light/ 12 hr dark photoperiod,
  22°C (±3º) and relative humidity 30 – 70 %
- Feed and water : Rodent pellet feed
  RO purified water *ad libitum*
- Identification : Animals were kept in individual cages and marked with picric acid.

Experimentation details:

Groups/treatment regimen : Grouped by randomisation

Test guideline : WHO

Length of exposure to test substance : 90 days

No of animas : 10 Male +10 Female/group

Control group : Vehicle (Honey)

Test groups : *Annapavala Chendhuram* (Low, Mid, High dose)

Grouping of animals:

The total of 70 animals were included in this study. 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 *Annapavala Chendhuram* Low dose, Mid dose, and High dose respectively. The number of animals were 10, 20, 20, 20 in the group of control, low, mid and high dose respectively.

Table 5.3.1: Grouping of long toxicity study

Groups	No. of Rats
Group I: Vehicle control (Honey)	10(5M + 5F)
Group II: APC- low dose (25mg/kg b.wt)	20(10M + 10F)
Group III: APC - Mid dose (125mg/kg b.wt)	20(10M + 10F)
Group IV: APC – High dose (250mg/kg b.wt)	20(10M + 10F)

Total 70 (35 M + 35 F)

Dose calculation:

Animal dose = Human dose × Conversion factor (Surface factor for 200gm rat = 0.018)

= 260 × 0.018 = 4.68mg / 200gm rat

Per kg rat = 4.68 × 5 = 23.4mg **(25mg)**

- Low dose = 25mg / kg
- Mid dose = 25 × 5 = 125mg / kg
- High dose = 25 × 10 = 250mg / kg

Experimentation details of long-term toxicity study:

The control animals were administered with honey as a vehicle. The other animals were treated with *Annapavala Chendhuram* by orally for 90 days and it was monitored for behavioural parameters for daily after drug administration. Body weight of the animal was monitored at weekly intervals. The food and water intake were calculated daily. All animals were weighed and sacrificed at the end of the study (91 days) by using the injection of thiopental sodium. Mortality and behavioural parameters were observed throughout the study. Blood was collected from the anaesthetized animals from the abdominal aorta and the following investigations like Haematology, Biochemical analysis and Histopathology were done.

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 a week.
- Feed intake was calculated daily.
- Water intake was calculated daily.

a. 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.

b. Laboratory investigation:

On the 91st day, the animals were fasted overnight, then anaesthetized 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 4^0C for 10 minutes to obtain the serum for biochemical parameters.

c. 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 Lipid profile test (total cholesterol, HDL, LDL, VLDL, triglycerides) Liver function test (AST, ALT, total bilirubin), Renal function test (creatinine, urea).

d. Haematological Investigation:

Blood samples of control and experimental rats were analysed for haemoglobin (Hb), total red blood corpuscles (RBC), White blood corpuscles (WBC) count, platelet, mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), DC were calculated by an auto analyser.

e. Histopathology

The organs included liver, kidney, spleen, brain, heart, lung and stomach of the animals were preserved, and they were subjected to histopathological examination. Control and high dose group animals were initially subjected to histopathological investigation. Various organs such as brain, heart, lung, liver, kidney, spleen, stomach, uterus, testes, ovary were 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.

f. Statistical analysis:

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

6. RESULTS

As per the literature, *Annapavala chenduram* was prepared and the *Siddha* specification of *chenduram* and physicochemical and spectroscopical analysis were done as per the guidelines given by Protocol for testing of Ayurvedic, Siddha & Unani medicines (PLIM guidelines), Department of AYUSH, Ghaziabad. After the preliminary analysis of the test drug, safety study was done on animals with the *Annapavala chenduram.* Results of these studies are as follows.

6.1 Quality control analysis of APC:

6.1.1 SIDDHA SPECIFICATION OF CHENDURAM:

According to literature, the *chenduram* must be reddish [17] in colour, odorless, lusterless, smokeless on heating, micro fine in nature and weightless. The observed *Siddha* parameters of *Chenduram* are listed below in Table 6.1.1.

1.1.1.2 *Table 6.1.1 Organoleptic characters of Annapavala chenduram*

No	Parameter	*APC*
1.	Colour	Brownish red
2.	Odour	No odour
3.	Taste	No taste
4.	Touch	Smooth
5.	Appearance	Powder

6.1.2 Siddha specifications of *Annapavala chenduram:*

Table 6.1.2 Siddha standardization of Chenduram

Parameter	*APC*
Irreversibility	Irreversible
Luster	Nil
Smoke (heating)	Nil
Weight (sprinkle test)	Floats in the water
Fine enough enter within the lines of finger	Enter into the finger lines

6.1.3 Physicochemical properties of APC:

The pH of Annapavala Chendhuram was 8.34. APC sample contains 0.56% acid insoluble ash. Solubility of APC in the water was 6.75%. Crude protein and Crude fiber present in the samples were 0.32% and 5.34% respectively. Energy in APC was 30.8 Kcal. Ether extract of APC was 1.28%. Total carbohydrate and Total organic carbon were 25.40% and 2.14% respectively. Physico chemical properties of the APC have shown in Table 6.1.2.

Table 6.1.3 Physicochemical analysis of *APC*

No	Parameter	*APC*
1	pH	8.34
2.	Acid insoluble ash	0.56%
3.	Solubility in water	6.75%
4.	Crude protein	0.32%
5.	Crude fiber	5.34%
6.	Energy	30.8 Kcal
7.	Ether extract (Total fat)	1.28%
8.	Total carbohydrate	25.40%
9.	Total organic carbon	2.14%

6.1.4 Qualitative analysis of *APC:*

Test for acid and basic radicals is a very basic study which helps to identify the presence of elements qualitatively and helps in the quantitative estimation of the same. The acid radicals present in the APC were sulphate, chloride, phosphate, carbonate and nitrate. The basic radicals present in the drug were Aluminium, zinc, Iron, calcium, magnesium, potassium and sodium.

Table 6.1.4 Test for acid radicals

Sl. No	Test	*APC*
1.	Test for sulphate	+
2.	Test for chloride	+
3.	Test for phosphate	+
4.	Test for carbonates	+
5.	Test for sulphide	-
6.	Test for nitrate	+
7.	Test for fluoride and oxalate	_
8.	Test for nitrite	_
9.	Test for borate	_

Table 6.1.5 Test for basic radicals

Sl. No	Test	*APC*
1.	Test for lead	_
2	Test for copper	_
3	Test for aluminium	+
4	Test for iron	+
5	Test for zinc	+
6	Test for calcium	+
7	Test for magnesium	+
8	Test for ammonium	-
9	Test for potassium	+
10	Test for sodium	+
11	Test for mercury	-
12	Test for arsenic	-

Table 6.1.5 Miscellaneous test for APC

Sl. No	Test	*APC*
1.	Test for starch	-
2.	Test for reducing sugar	-
3.	Test for alkaloids	+
4.	Test for tannic acid	-
5.	Test for unsaturated compound	-
8.	Test for amino acid	-

Acid and basic radicals present in APC:

Table 6.1.6 Acid and Basic radicals in APC

Acid radicals present	sulphate, chloride, phosphate, carbonate and nitrate
Basic radicals present	Aluminium, zinc, Iron, calcium, magnesium, potassium and sodium
Miscellaneous	Alkaloids

6.2 QUANTITATIVE ANALYSIS OF APC:

6.2.1 Scanning Electron Microscopy of APC: (SEM)

The SEM image of *Annapavala Chendhuram* showed the presence of uniform distribution of particles in the lower magnifications. If we closely observe the higher magnification SEM, the particles are agglomerated and form big particles. The particle size is estimated to about 200nm. There is no distinct features or shape is observed in the SEM image.

Fig 6.2.1 SEM image of APC at 2.36 KX magnification

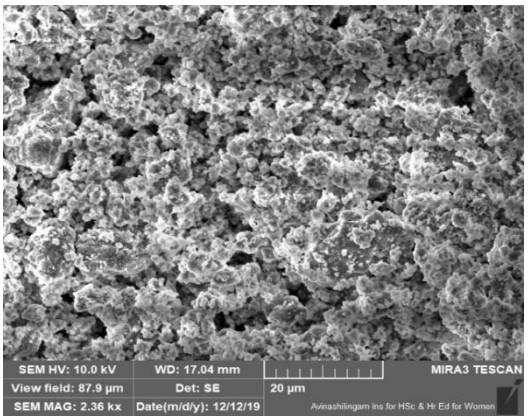


Fig 6.2.2 SEM image of APC at 10.0 KX magnification

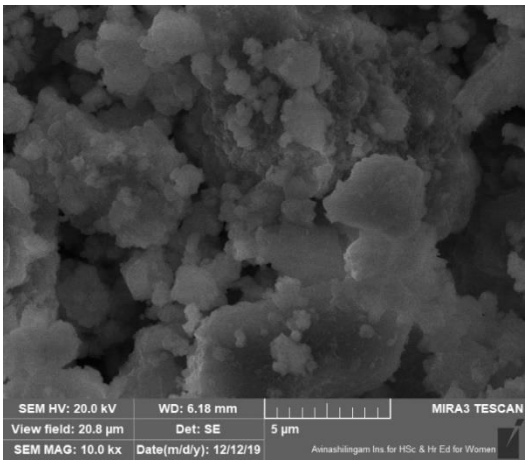


Fig. 6.2.3 SEM image of APC at 20.0 KX magnification

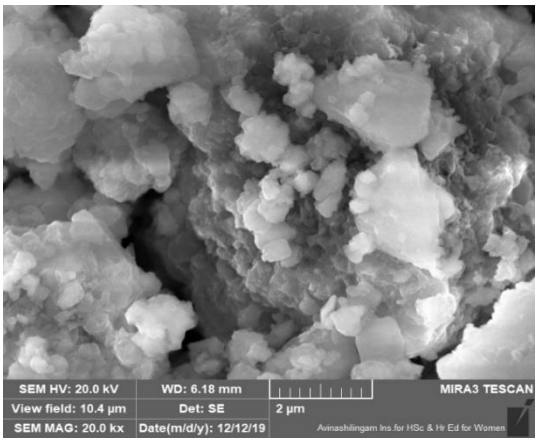
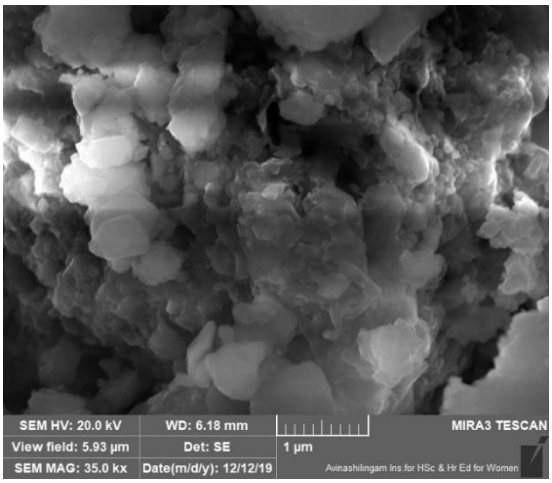


Fig 6.2.4 SEM image of APC at 35.0 KX magnification



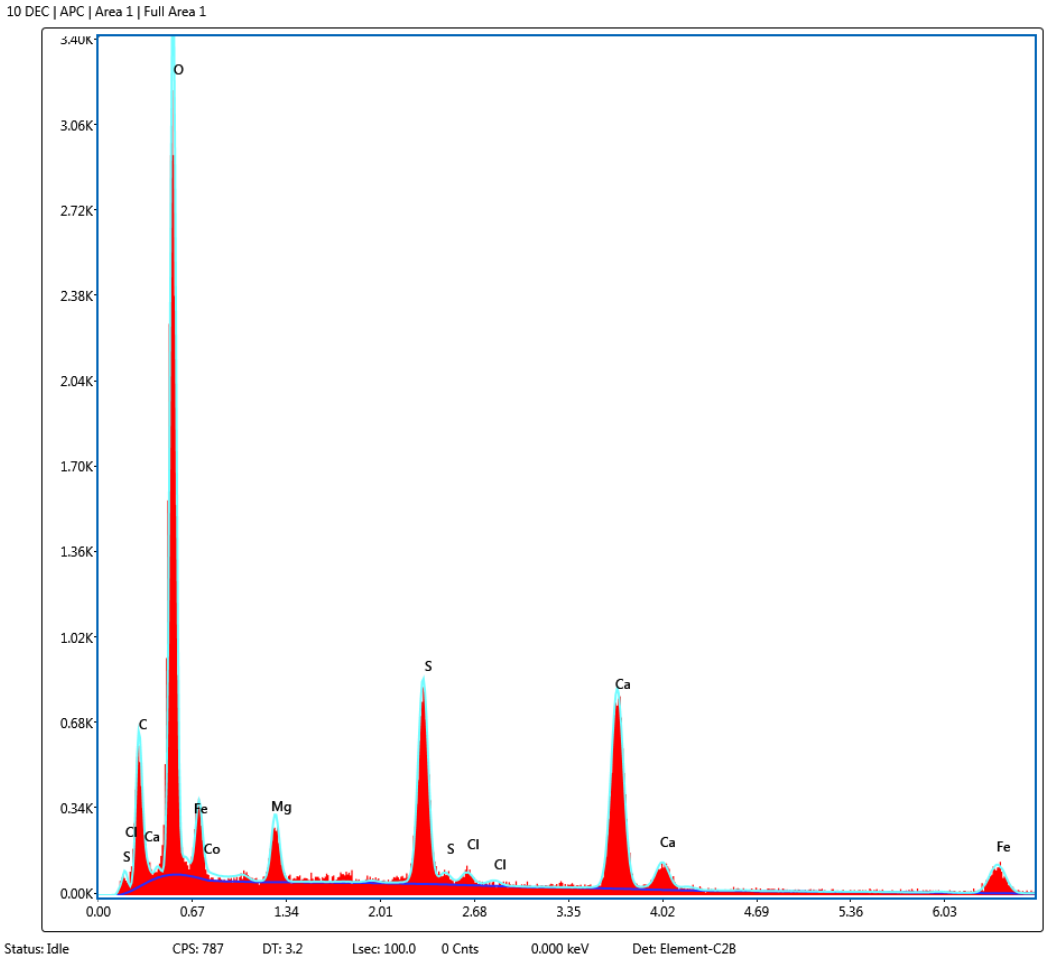
6.2.2 Energy Dispersive X – ray analysis (EDAX) of APC:

In *Annapavala Chendhuram* the mass percentage of carbon, oxygen, iron, magnesium, Sulphur, Chlorine, Calcium are 5.41, 39.75, 5.43, 2.21, 12.29, 0.86, 34.05 respectively. (Table 6.2.1)

Table 6.2.1 Elemental composition of APC

Element	Weight%	Atomic%	Net Intensity	Error%
C	5.41	10.29	25.25	11.15
O	39.75	56.72	204.16	9.34
Fe	5.43	2.22	10.89	13.24
CO	0.00	0.00	0.01	99.99
Mg	2.21	2.08	19.77	8.78
S	12.29	8.75	78.96	4.29
Cl	0.86	0.55	4.41	29.77
Ca	34.05	19.39	93.18	4.54

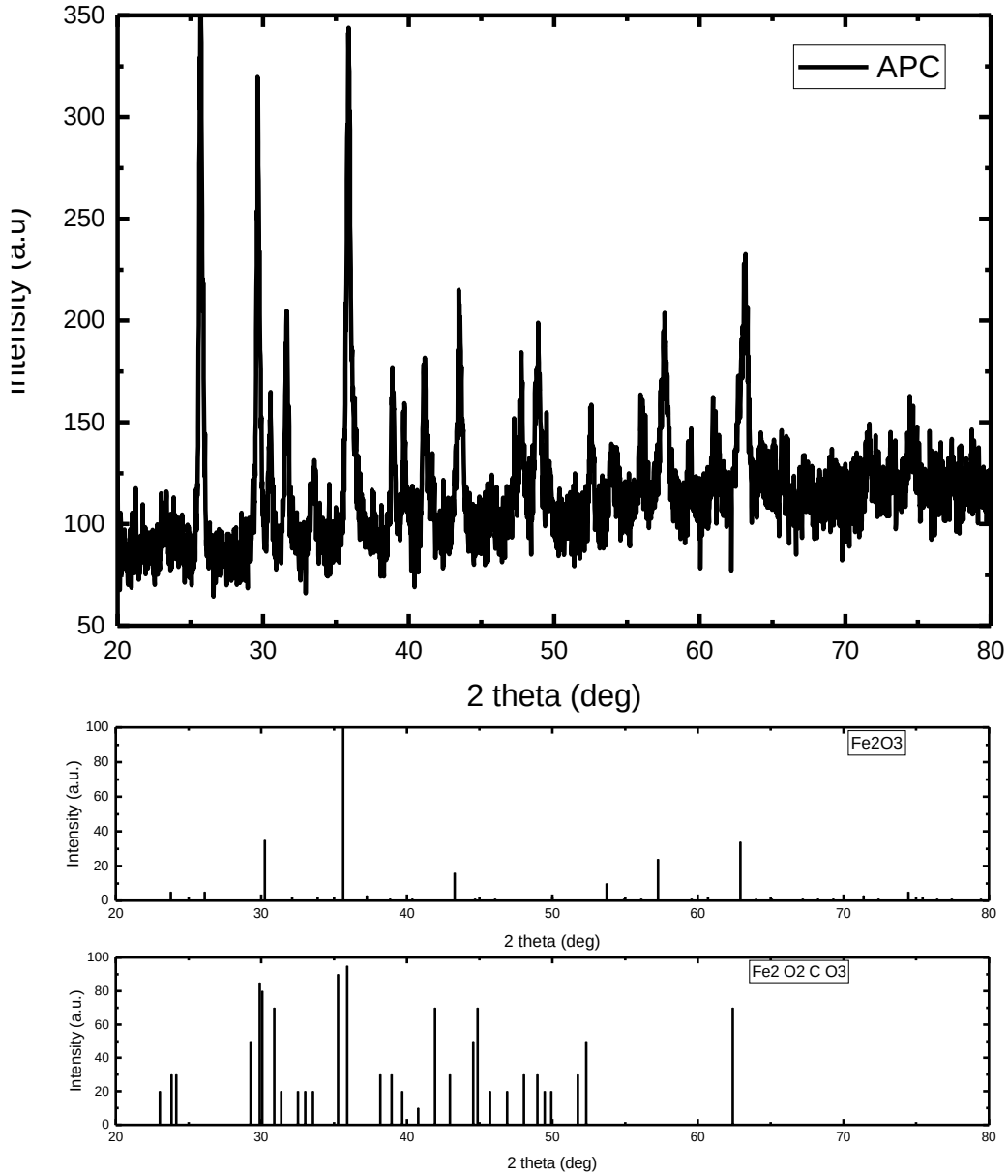
Graph 1: Elemental composition of APC



6.2.3 X – Ray diffraction analysis of APC:

The XRD spectra depicts the structural composition present in the prepared Annapavala Chendhuram. The obtained peaks can be attributed to various possible compounds that may be present in the drug which is produced using the standard procedure as described earlier. Among them here, the peaks are determined and indexed based on the Fe_2O_3 phases extracted from the *JCPDS-card-no.*00-039-1346 and *JCPDS-card-no.* 00-033-0665. The dominating peaks are quite visible and attributed to the Fe_2O_3 and indexed as (040) and (311). The phases such as (211), (102) (511) and (441) are extracted from the $Fe_2O_2CO_3$ and some of the other peaks could also indexed in the form of Fe based oxide with and without silica.

Graph 2: X – Ray diffraction of APC

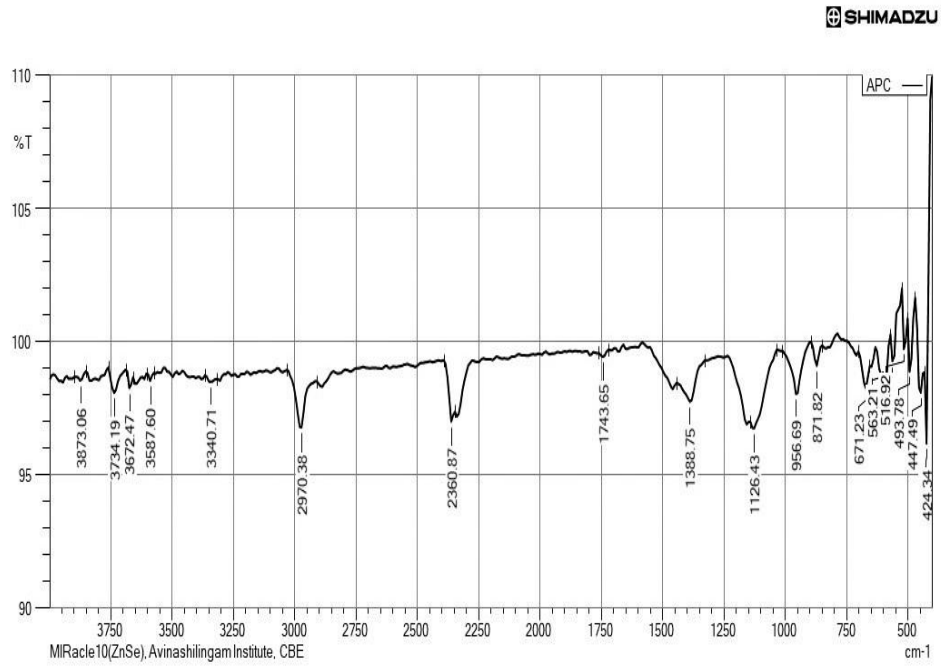


6.2.4 Fourier Transform Infrared Spectroscopy: (FTIR)

In the FTIR spectra analysis, Annapavala Chendhuram exhibits the peaks at wavenumber of 3587 and 3672, 3340, 2360, 2970, 1743, 1388, 1126, 956, 871, 671, 563, 516, 493, 447, 424cm^{-1} having the O-H stretch, N-H stretch, C-CH_3 stretch, C=O stretch, C-H stretch, C-O-C stretch, Fe – O stretch which is mentioned at Table.6.2.2. The expanded region of the FTIR spectra between 4000 - 1500 cm^{-1} looks complex as there are many peaks present in this region.

Table 6.2.2 FTIR spectral details of APC

FTIR peaks (cm^{-1})	Vibrational modes	Functional group
3587, 3672	O-H stretch	Alcohol group
3340	N-H stretch	Amine group
2360, 2970	C-H stretch	Alkane group
1743	C=O stretch	Carbonyl group
1388	C-H stretch	Alkane group
1126	C-O-C stretch	Esters
871 – 671	Fe – O stretch	Alkyl halides
563 – 424	Fe – O stretch	Alkyl halides



6.3 TOXICITY STUDY:

6.3.1 Acute toxicity study:

Acute toxicity study of *APC* was conducted as per WHO guidelines. No mortality or treatment related toxicity was noted in any animals during the experimental period. The animals were gained bodyweight gradually. (Table 6.3.1) There was no significant difference between the control & treated groups in food and water intake throughout the study period. Normal signs were noted in skin, fur, eyes, mucous membranes, salivation, and sleep in control and treated animals. No other signs like tremors, convulsions, lethargy, coma, diarrhea were observed during the study period. (Table 6.3.2) No pathological changes were observed in the vital organs of all groups during necropsy.

Table 6.3.1 Body weight changes (gm) in acute toxicity

Group	0^{th} day	7^{th} day	14^{th} day
Control group (honey)	204±40.21	210±38.50	221.4±43.34
High dose group	206±36.27	215±41.17	223.2±49.60

Table 6.3.2 Effect of Annapavala Chendhuram on behavioral parameters of Wistar albino rats

Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Control group (honey)	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-
High dose group	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-

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. Exophthalmos 17. Diarrhoea 18. writhing 19. respiration 20. mortality

(+) Presence of activity

(-) Absence of activity

6.3.2 Long term toxicity study (90 Days):

There was no mortality and treatment associated toxicity noted in all groups during the experimental period.

Effect of APC on body weight:

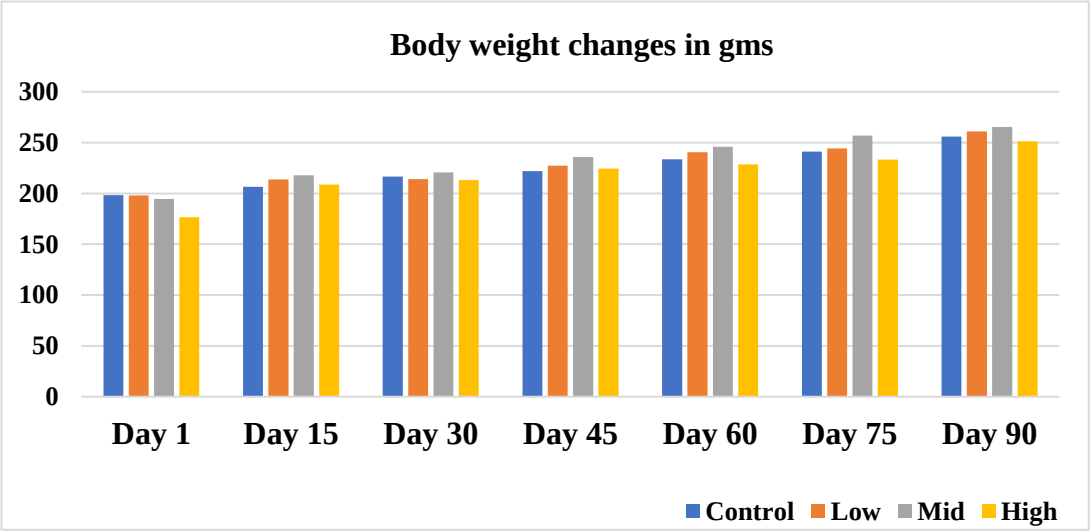
The animals in all groups gained body weight throughout the study period. There were no significant differences observed in the body weight of control and the treated groups. (Table 6.3.3 and Graph 6.3.1)

Table 6.3.3 Effect of Annapavala Chendhuram on Body weight changes of Wistar rats in a long-term toxicity study

	Control	Low	Mid	High
Day 1	198.3±29.74	198±29.77	176.45±24.73	194.35±33.27
Day 15	206.4±33.09	213.6±31.55	208.75±40.64	217.65±46.06
Day 30	216.45±37.49	214±33.83	213.1±40.87	220.65±45.85
Day 45	221.95±37.68	227.4±41.21	224.5±44.31	235.6±45.8
Day 60	233.55±42.94	240.55±48.04	228.65±43.8	245.8±50.2
Day 75	241±46.51	244.15±50.03	233.2±48.26	256.95±52.6
Day 90	256±51.41	260.8±53.95	251.1±59.16	265.45±58.84

Data expressed as Mean ± SD for N = 10 rats in control group and N = 20 in Low, Mid, High dose groups, one – way ANOVA followed by Dunnett's test. Significant indicates that *P<0.05, **P<0.01

Graph 6.3.1 Body weight changes of APC treated Wistar rats



Influence of APC in food and water intake:

Water intake and feed intake of the control and drug treated animals were found to be normal throughout the study period. Feed and water intake of animals was assessed daily for 90 days. APC treatment did not influence any changes in the intake of food and water. Changes of feed intake in APC treated various group animals mentioned in table 6.3.4 and graph 6.3.2. Water intake of Wistar albino rats given in Table 6.3.5 and graph 6.3.3.

Table 6.3.4 Effect of Annapavala Chendhuram on feed intake of Wistar rats

Feed	Control	Low	Mid	High
Day 1	41±1.41	42.5±2.08	43±2.16	43.5±2.38
Day 15	43.5±2.12	45.5±2.08	44.75±1.25	47.75±1.25
Day 30	46.5±2.12	47.25±2.98	48.25±2.36	50±0.81
Day 45	47.5±3.5	50.25±2.87	51.25±2.21	53±0.81
Day 60	54.5±0.70	53.25±2.21	53±1.63	55.75±1.70
Day 75	56±2.82	56.25±1.25	55.5±1.29	57.75±2.64
Day 90	59±1.41	59±2.16	57.5±1.29	60.75±2.21

Data expressed as Mean ± SD for N = 10 rats in control group and N = 20 in Low, Mid, High dose groups, one – way ANOVA followed by Dunnett's test. Significant indicates that *P<0.05, **P<0.01

Graph 6.3.2 Feed intake of APC treated Wistar rats

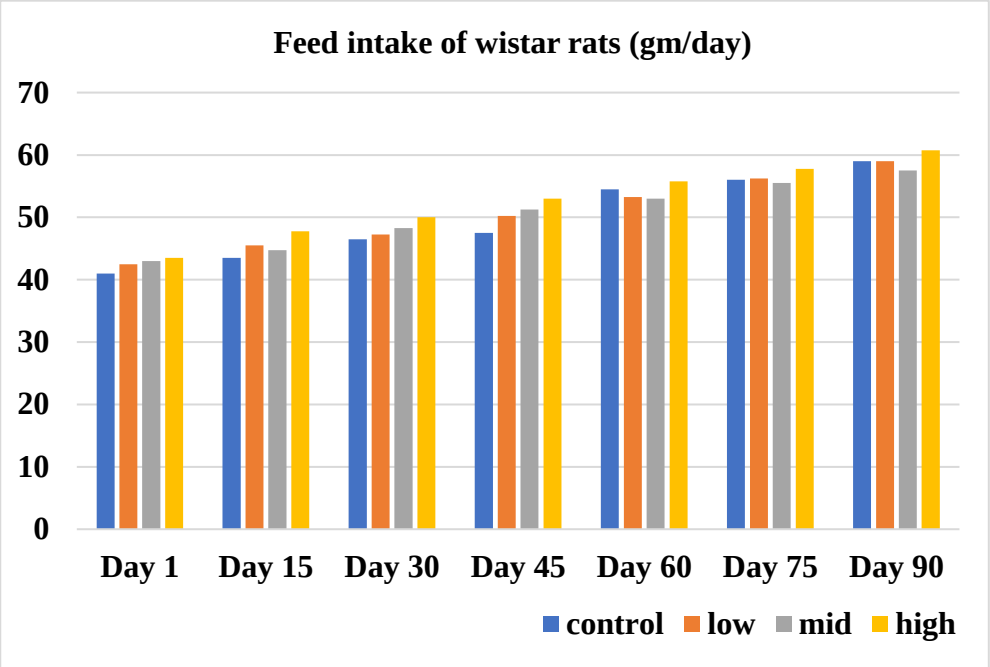
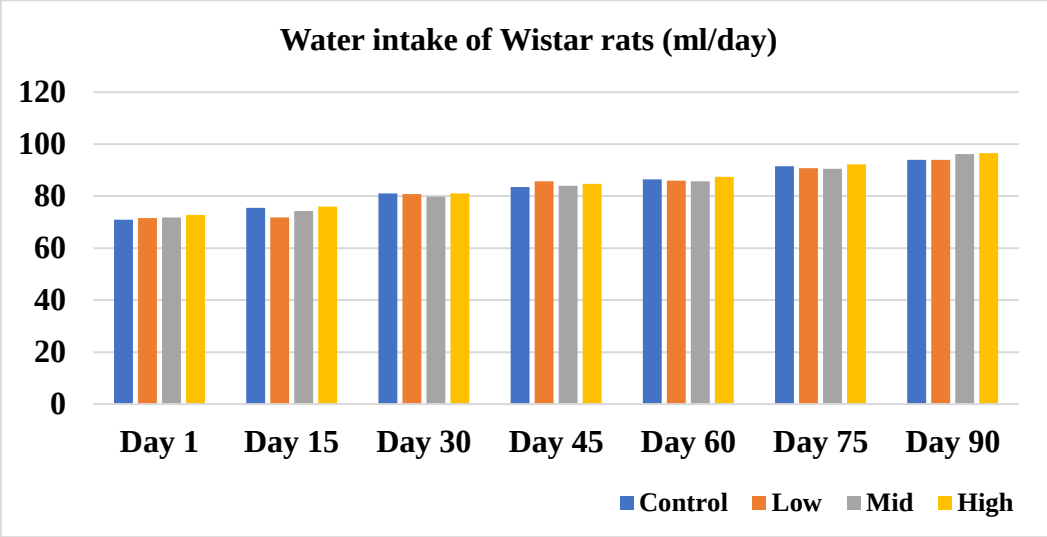


Table 6.3.5 Effect of Annapavala Chendhuram on water intake of Wistar rats

	Control	Low	Mid	High
Day 1	71±1.41	71.5±1.5	71.75±2.06	72.75±1.29
Day 15	75.5±0.70	71.75±0.95	74.25±1.70	76±1.82
Day 30	81±1.41	80.75±0.95	79.75±1.70	81±1.82
Day 45	83.5±0.70	85.75±0.95	84±2.94	84.75±0.95
Day 60	86.5±0.70	86±1.82	85.75±2.5	87.5±1.29
Day 75	91.5±2.12	90.75±1.70	90.5±2.08	92.25±1.70
Day 90	94±1.41	94±2.16	96.25±1.5	96.5±1.29

Data expressed as Mean ± SD for N = 10 rats in control group and N = 20 in Low, Mid, High dose groups, one – way ANOVA followed by Dunnett's test. Significant indicates that *P<0.05, **P<0.01.

Graph 6.3.3 Water intake of APC treated Wistar rats



Haematological parameters:

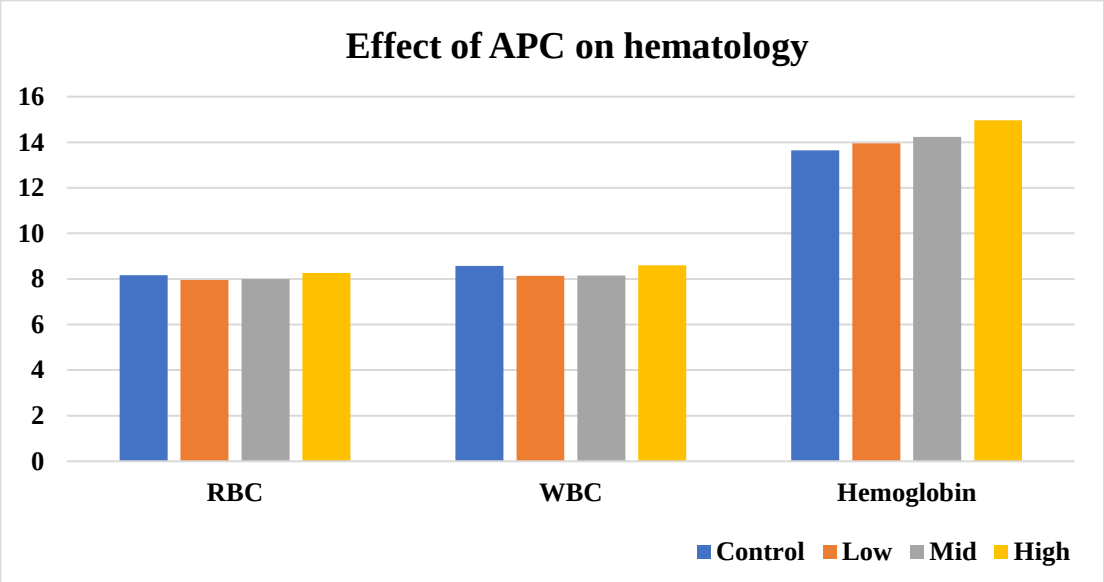
Haematological parameters were assessed in APC treated animals at end of the study and the results were recorded. It has shown in Table 6.3.6.

Table 6.3.6 Effect of APC haematological parameters of Wistar rats

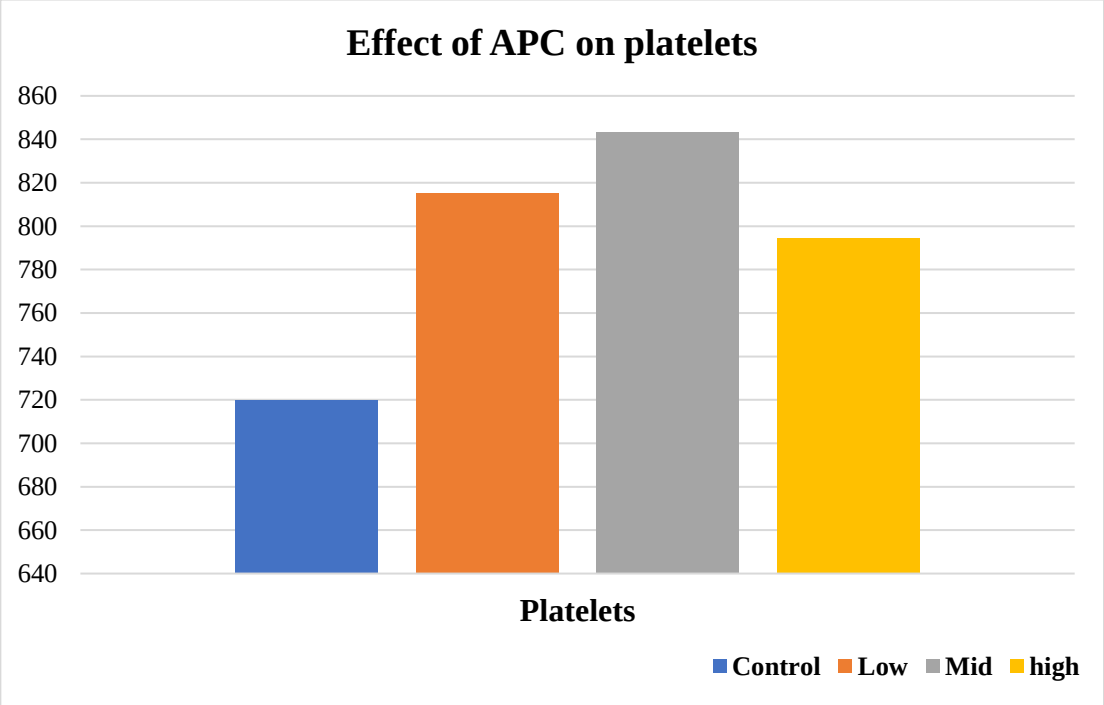
	Control	Low	Mid	High
RBC ($\times 10^6$µ/l)	8.16±0.65	7.96±0.93	8±0.76	8.27±0.83
WBC($\times 10^3$µ/l)	8.57±1.67	8.13±0.95	8.15±0.9	8.60±1.27
Haemoglobin (gm/dl)	13.65±1.62	13.96±1.76	14.24±1.27	14.96±1.50
Platelets($\times 10^3$µ/l)	720.1±133.2	794.35±104.3	843.1±72.22	815.15±103.14
MCH (pg)	18.03±1.19	18.12±1.08	17.94±1	17.88±1.48
MCV (fl)	56.7±4.59	59.48±4.44	60.46±5	58.90±4.51
Neutrophils (%)	2.93±0.63	3.17±0.55	2.85±0.7	3.08±0.53
Eosinophils (%)	1.39±0.18	1.48±0.18	1.49±0.22	1.47±0.20
Basophils (%)	0.1±0.3	0.15±0.35	0.2±0.4	0.15±0.35
Lymphocytes (%)	73.33±5.62	86.69±43.89	77.04±4.87	78.83±4.80
Monocytes (%)	3.07±1.22	3.86±1.19	4±0.99	3.65±1.07

Data expressed as Mean ± SD for N = 10 rats in control group and N = 20 in Low, Mid, High dose groups, one – way ANOVA followed by Dunnett's test. Significant indicates that *P<0.05, **P<0.01

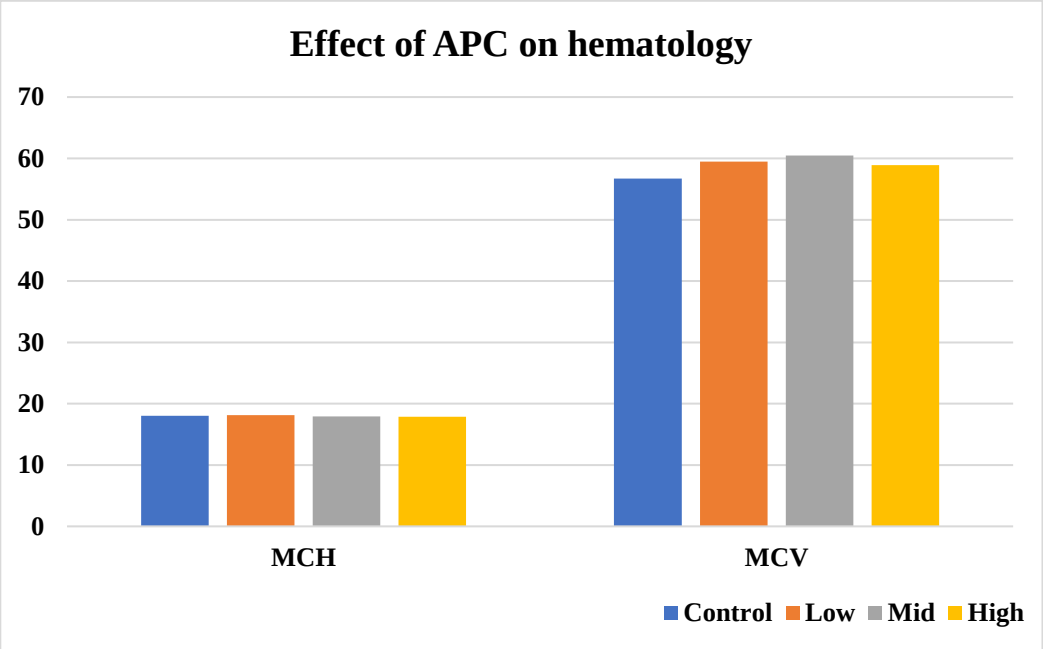
Graph 6.3.4 Hematological parameters of APC treated Wistar rats



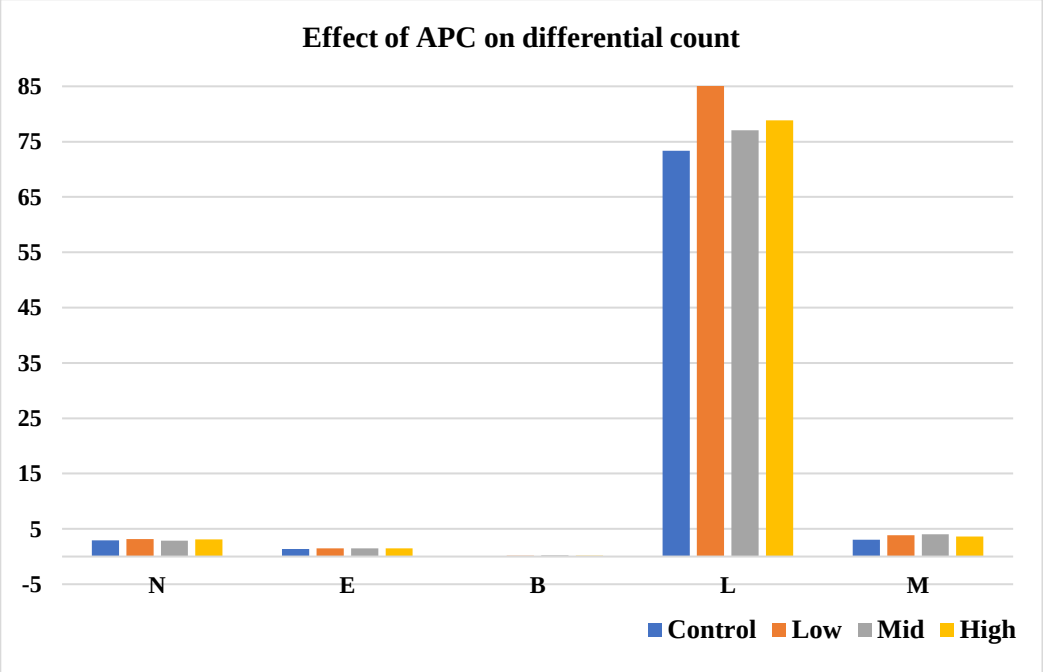
Graph 6.3.5 Hematological parameters of APC treated Wistar rats



Graph 6.3.6 Hematological parameters of APC treated Wistar rats



Graph 6.3.7 Hematological parameters of APC treated Wistar rats



N – Neutrophils; E – Eosinophils; B – Basophils; L – Lymphocytes; M – Monocytes

Biochemical parameters:

Biochemical parameters were assessed in APC treated animals at end of the study and the results were recorded. It indicates the Liver function test, Renal function test and Lipid profile of APC treated animals which was compared with the control group.

Hepatic parameters of APC:

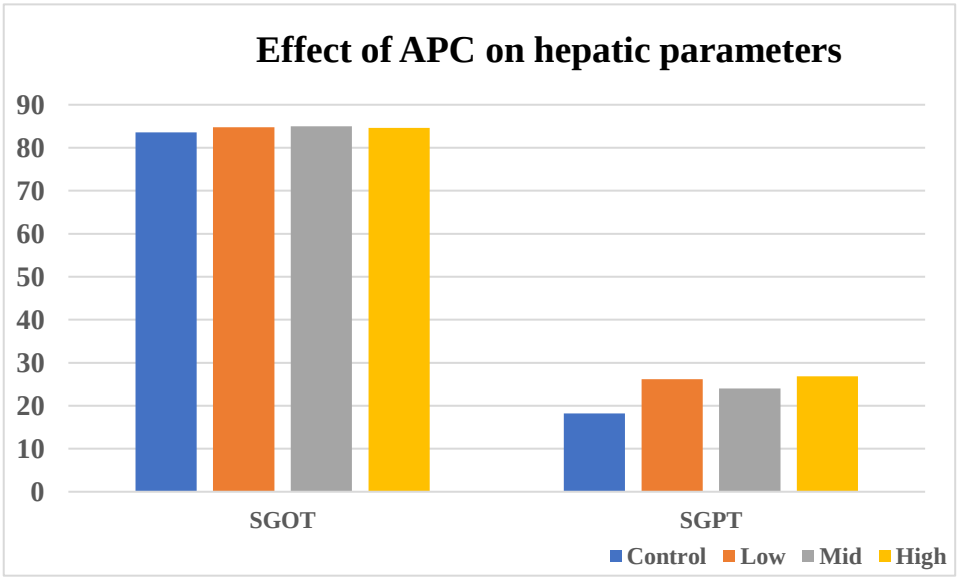
Hepatic parameters were assessed for APC treated Wistar albino rats which was compared with the control group. This result revealed that no significant changes in liver parameters. All the parameters were normal within the limit. Liver function test of APC treated animals have shown in Table 6.3.7.

Table 6.3.7 Effect of APC on Liver function of Wistar albino rats

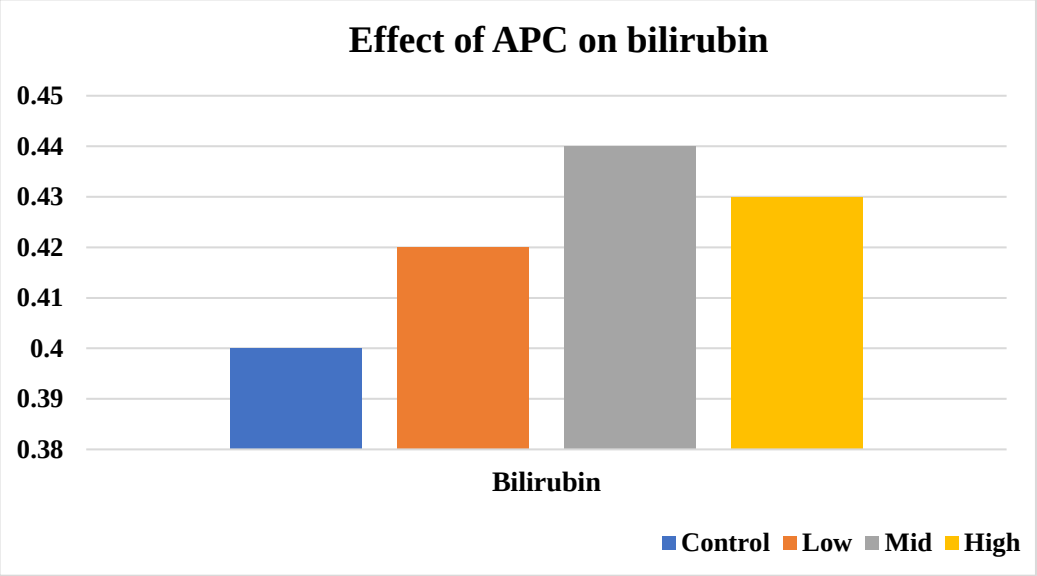
	Control	Low dose	Mid dose	High dose
T. Bilirubin	0.4±0.14	0.42±0.12	0.44±0.13	0.43±0.11
SGOT	83.6±5.73	84.8±5.68	85±6.14	84.6±5.86
SGPT	18.2±7.24	26.15±6.80	24±5.98	26.85±5.42

Data expressed as Mean ± SD for N = 10 rats in control group and N = 20 in Low, Mid, High dose groups, one – way ANOVA followed by Dunnett's test. Significant indicates that *P<0.05, **P<0.01.

Graph 6.3.8 Hepatic parameters of APC treated Wistar rats



Graph 6.3.9 Bilirubin level of APC treated Wistar rats



Renal parameters of APC:

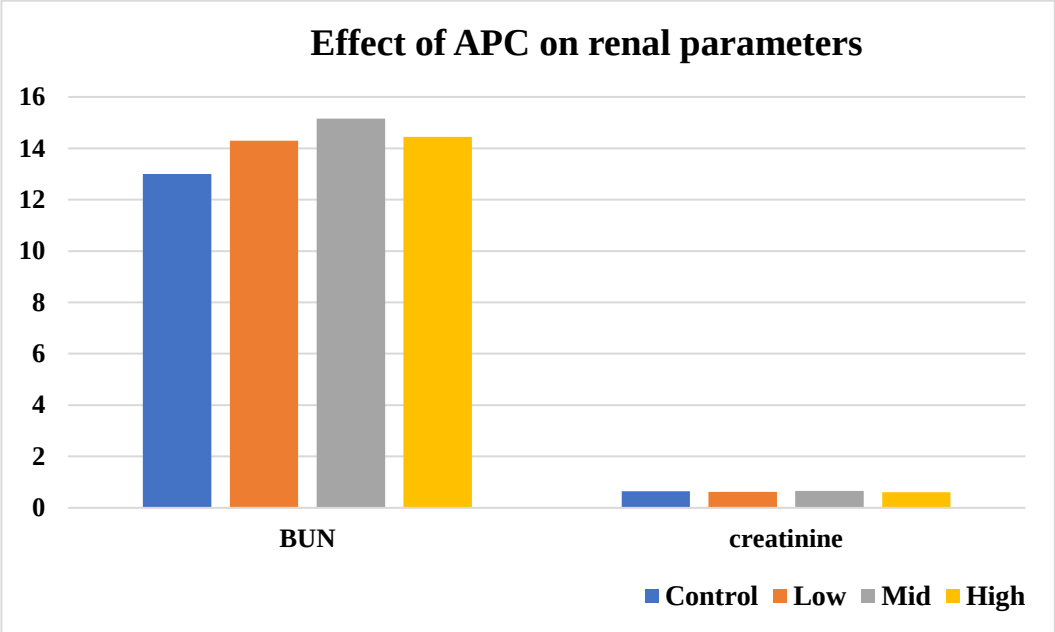
BUN and creatinine are the indicator for the renal function. This analysis was done at the end of the study. Test groups does not have any significant changes in levels of animals when compared to control group. BUN and creatinine levels of APC treated animals were normal compared to control group animals. It has shown in 6.3.8.

Table 6.3.8 Effect of APC on Renal function of Wistar rats

	Control	Low dose	Mid dose	High dose
BUN	13±2	14.3±2.49	15.15±2.1	14.45±2.33
Creatinine	0.64±0.14	0.62±0.12	0.66±0.11	0.61±0.14

Data expressed as Mean ± SD for N = 10 rats in control group and N = 20 in each treat groups, one – way ANOVA followed by Dunnett's test. Significant indicates that *P<0.05, **P<0.01.

Graph 6.3.10 Renal function test of APC treated Wistar rats



Lipid profile of APC:

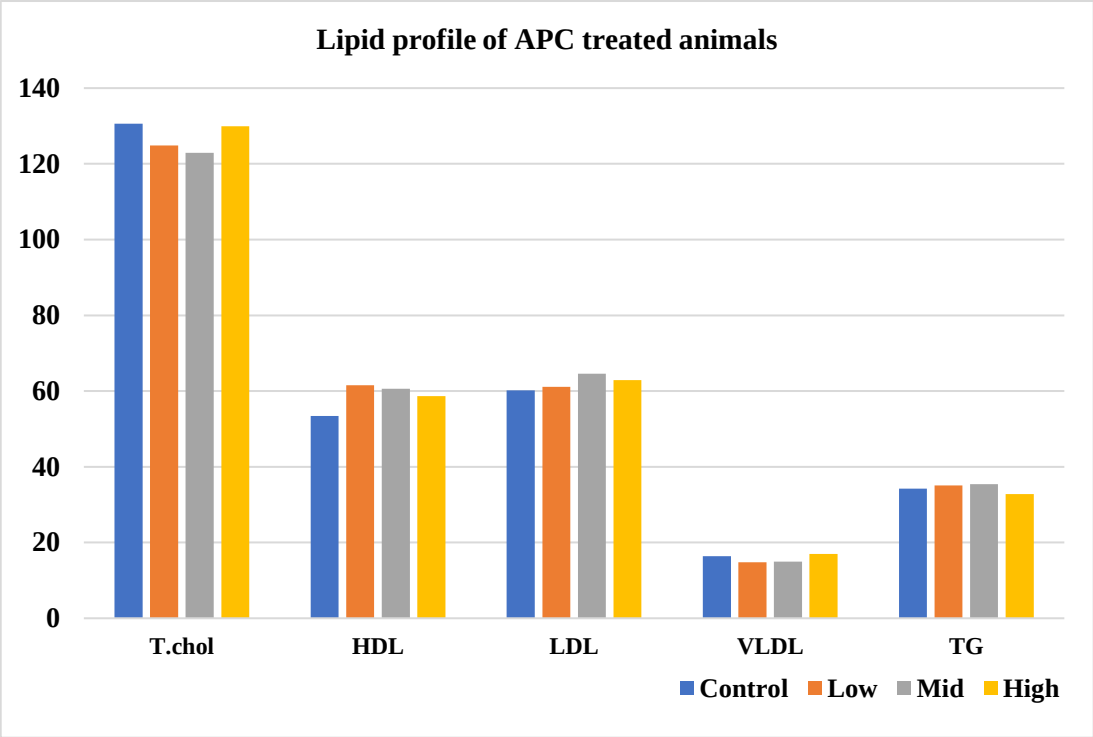
Lipid profile consists of total cholesterol, high density lipoprotein, low density lipoprotein, very low-density lipoprotein and triglyceride levels in blood serum of Wistar rats. Table 6.3.9 shown there was no significant changes in the lipid profile of animals when compared to the control group.

Table 6.3.9 Effect of APC on Lipid profile of Wistar rats

	Control	Low dose	Mid dose	High dose
T. Cholesterol	130.6±18.74	124.85±10.33	122.93±10.06	129.96±7.36
HDL	53.4±7.82	61.5±5.55	60.6±5.48	58.7±5.96
LDL	60.2±12.60	61.15±11.44	64.6±10.74	62.9±10.53
VLDL	16.43±2.39	14.76±1.77	14.95±2.37	16.99±1.98
TG	34.2±6.83	35.1±6.83	35.45±5.63	32.8±6.97

Data expressed as Mean ± SD for N = 10 rats in control group and N = 20 in Low, Mid, High dose groups, one – way ANOVA followed by Dunnett's test. Significant indicates that *P<0.05, **P<0.01.

Graph 6.3.10 Lipid profile of APC treated Wistar albino rats.

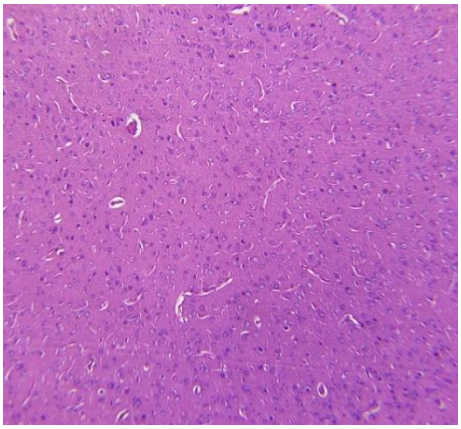


HISTOPATHOLOGY REPORT OF LONG -TERM TOXICITY ANIMALS (90DAYS)

Histopathology of Brain (Male)

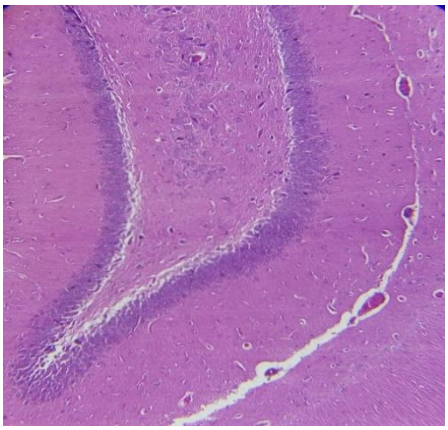
Control group

Low 10X magnification

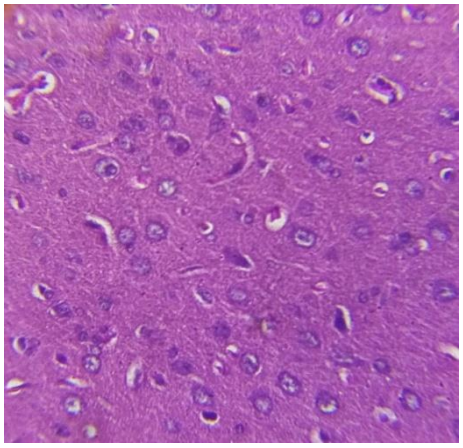


High dose group

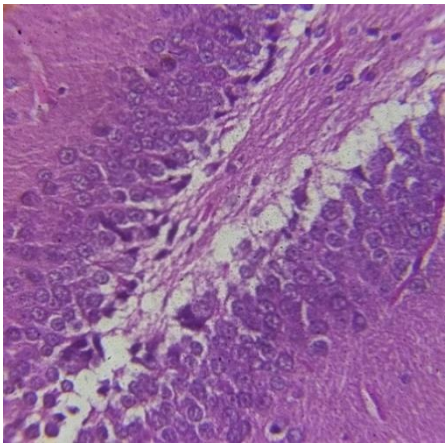
Low 10X magnification



High 40X magnification



High 40X magnification



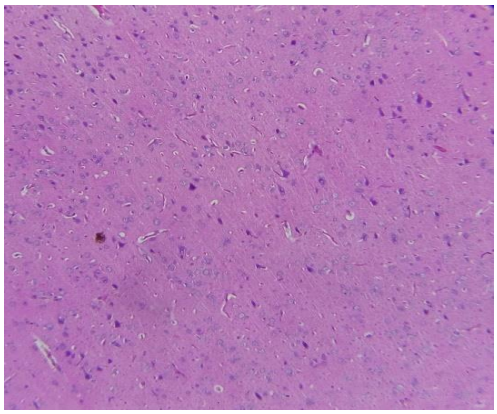
Control group: Regular marginal alignment on the neurons with promising histology were observed

High Dose: No signs of pyknosis and perineural vacuolization.

Histopathology of Brain (Female)

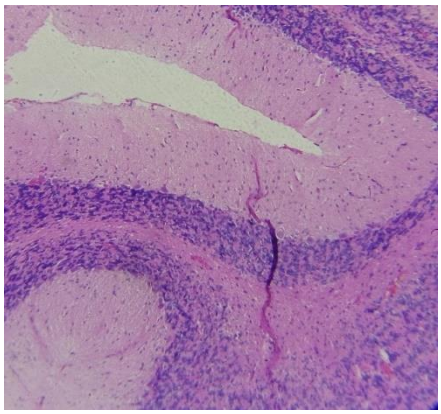
Control group

Low 10X magnification

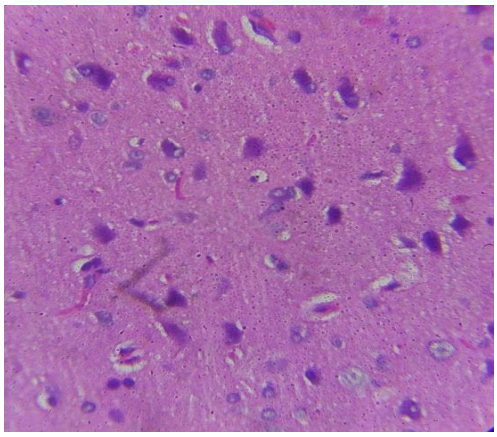


High dose group

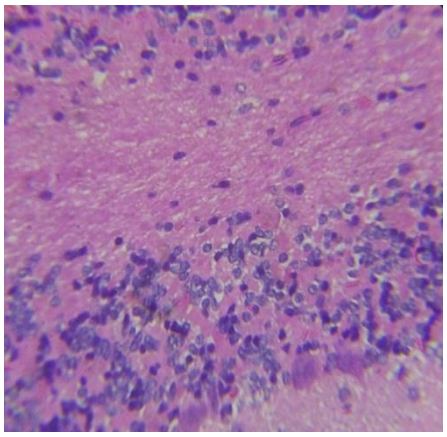
Low 10X magnification



High 40X magnification



High 40X magnification



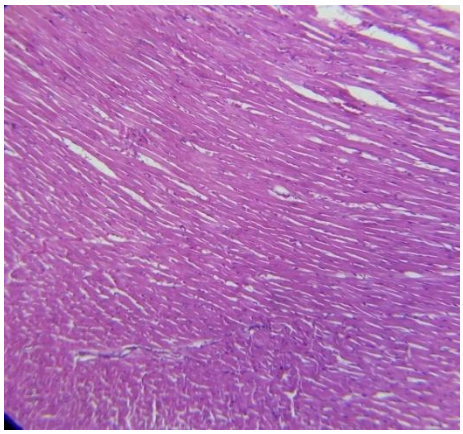
Control group: This report Shows normal histology of striatum.

High Dose: Cerebellar section shows normal architecture of neurons.

Histopathology of Heart (Male)

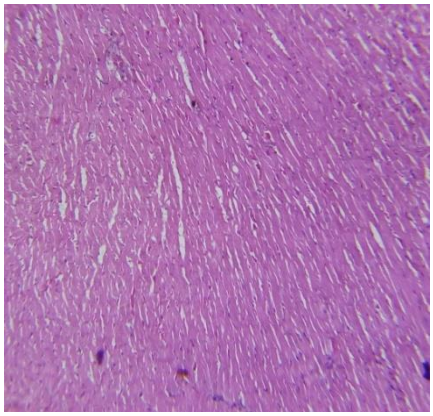
Control group

Low 10X magnification

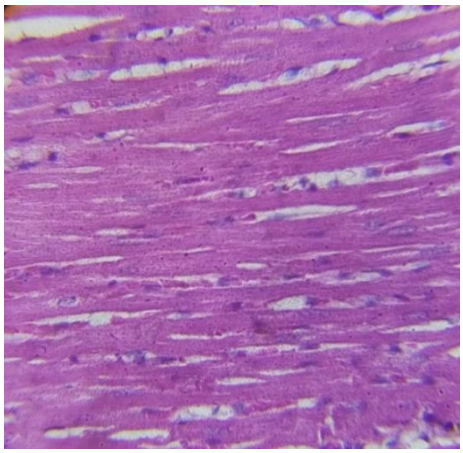


High dose group

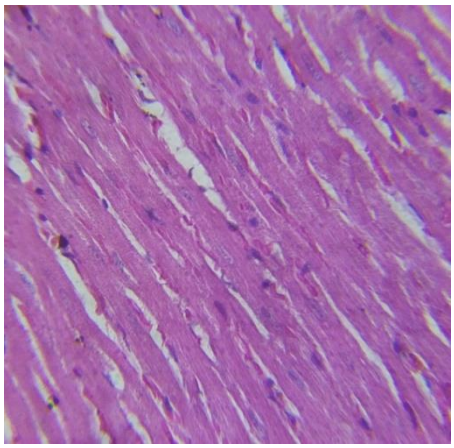
Low 10X magnification



High 40X magnification



High 40X magnification



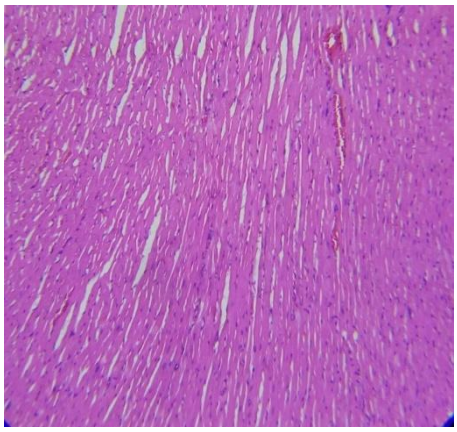
Control group: Showing the normal histological structure of myocardium.

High dose: Fibrils and cross striations are equal distant.

Histopathology of Heart (Female)

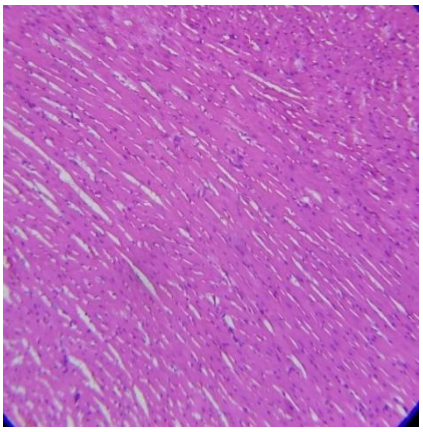
Control group

Low 10X magnification

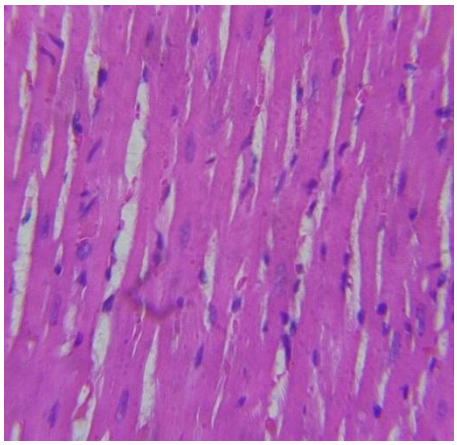


High dose group

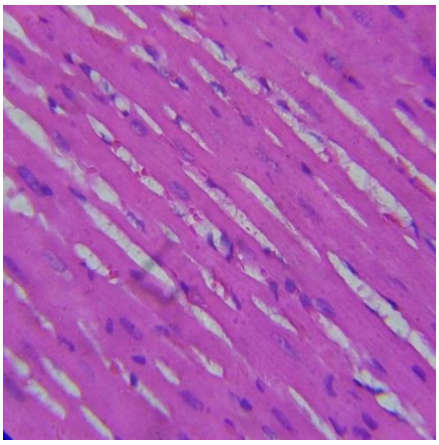
Low 10X magnification



High 40X magnification



High 40X magnification



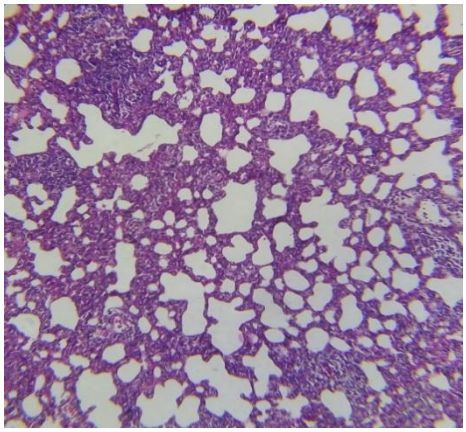
Control group: Appearance of myofibrillar and cytoplasmic zone was normal

High dose: Normal appearance of myocytes.

Histopathology of Lung (Male)

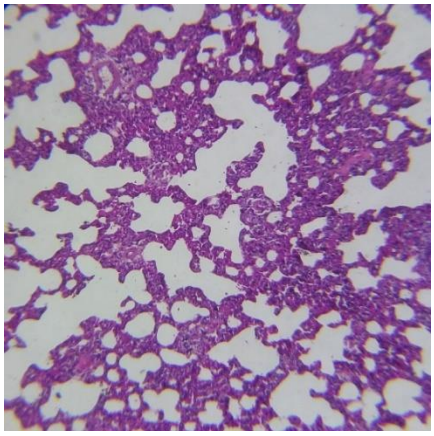
Control group

Low 10X magnification

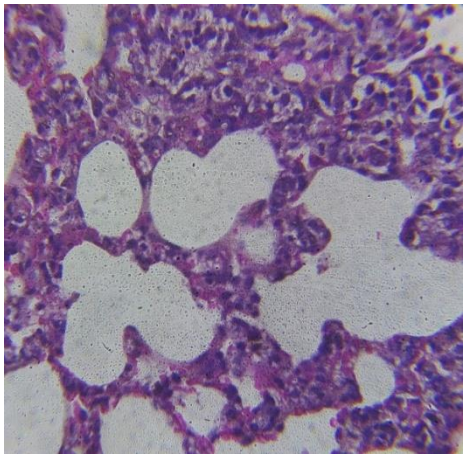


High dose group

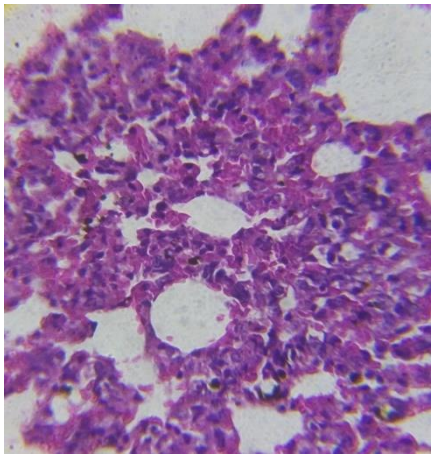
Low 10X magnification



High 40X magnification



High 40X magnification



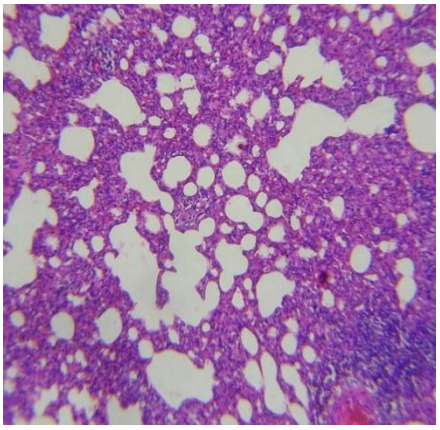
Control group: Arrangement of epithelial and muscular appears normal.

High Dose: Bronchial opening appears regular with no signs of infiltration.

Histopathology of Lung (Female)

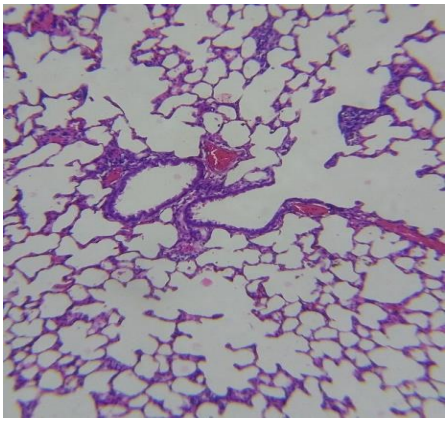
Control group

Low 10X magnification

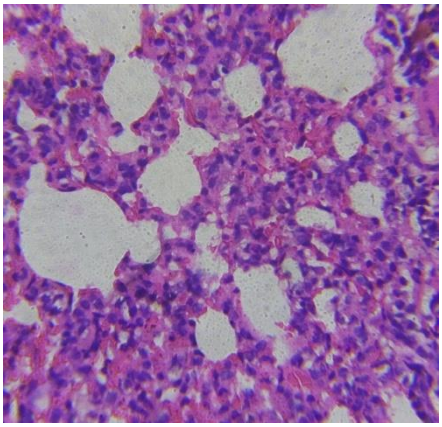


High dose group

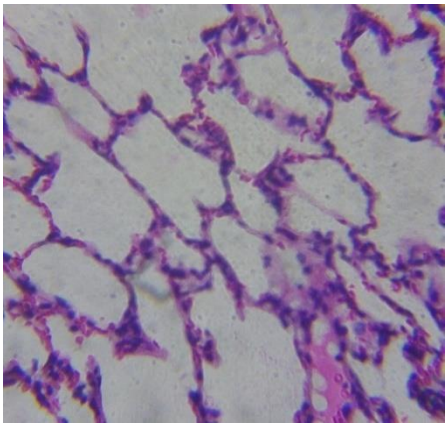
Low 10X magnification



High 40X magnification



High 40X magnification



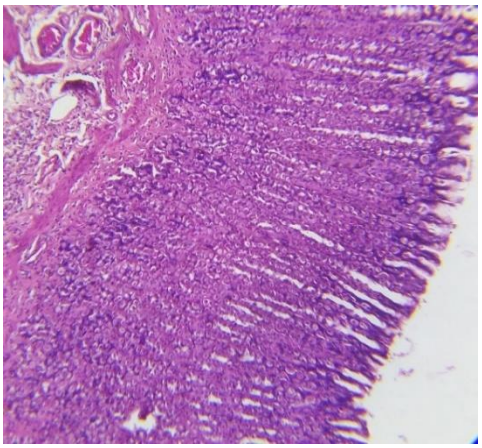
Control group: Alveolar epithelium and capillaries appears normal.

High Dose: Inter alveoli septum and bronchioles appears normal.

Histopathology of Stomach (Male)

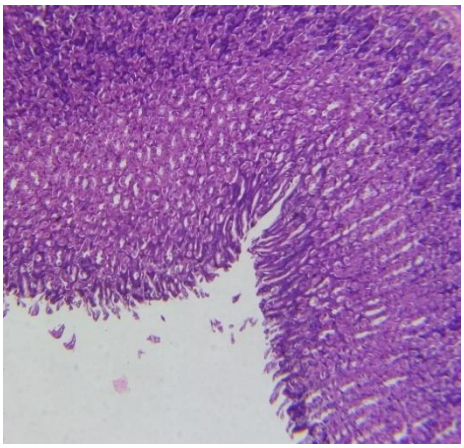
Control group

Low 10X magnification

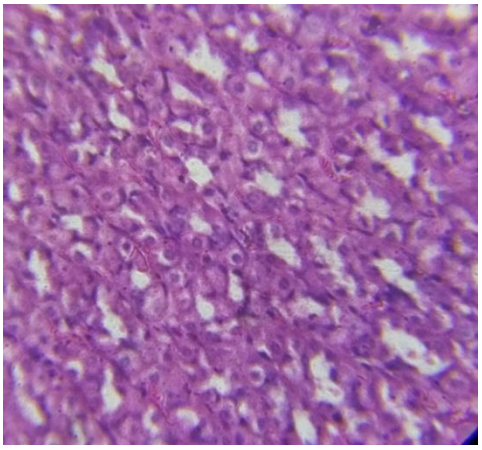


High dose group

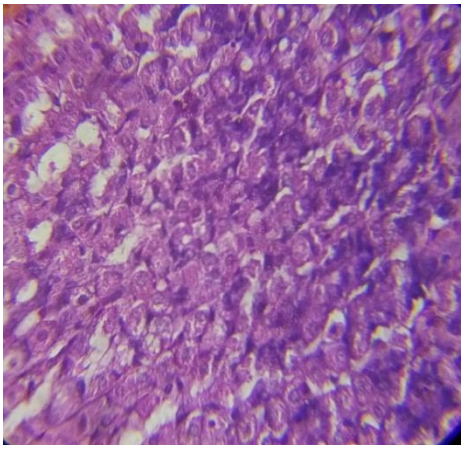
Low 10X magnification



High 40X magnification



High 40X magnification



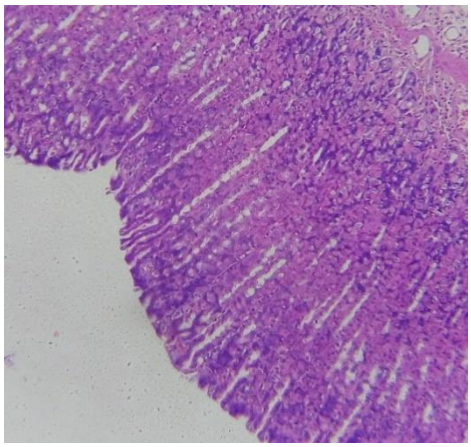
Control group: Appearance of Sub-mucosa and gastric glands appear normal.

High Dose: Appearance of glandular lumen was normal.

Histopathology of Stomach (Female)

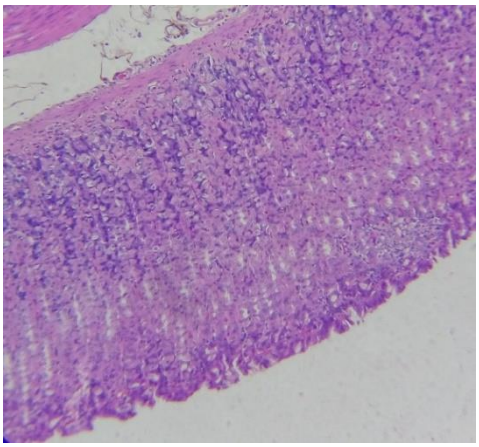
Control group

Low 10X magnification

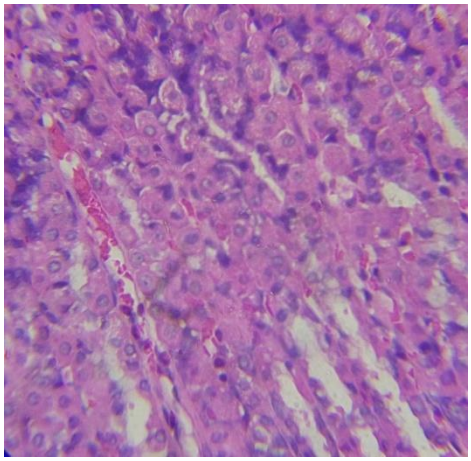


High dose group

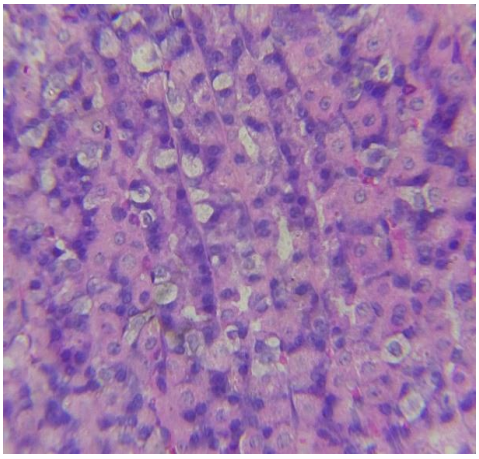
Low 10X magnification



High 40X magnification



High 40X magnification



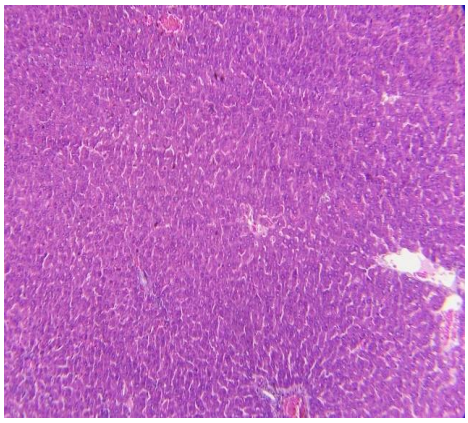
Control group: Mucosal wall appears normal with regular arrangement of connective tissue.

High Dose: Gastric epithelium and mucosa appears normal.

Histopathology of Liver (Male)

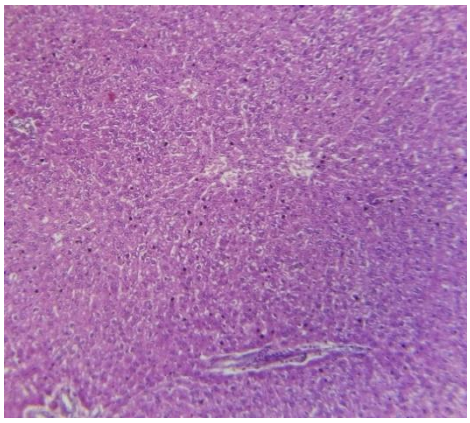
Control group

Low 10X magnification

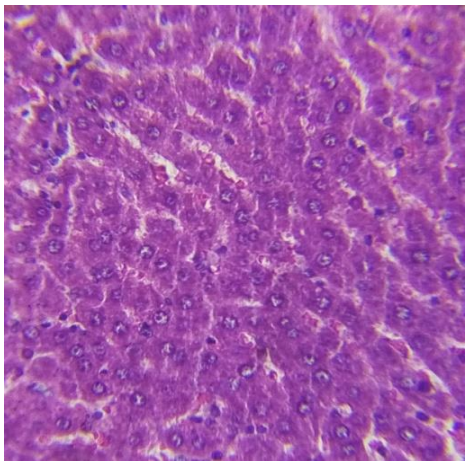


High dose group

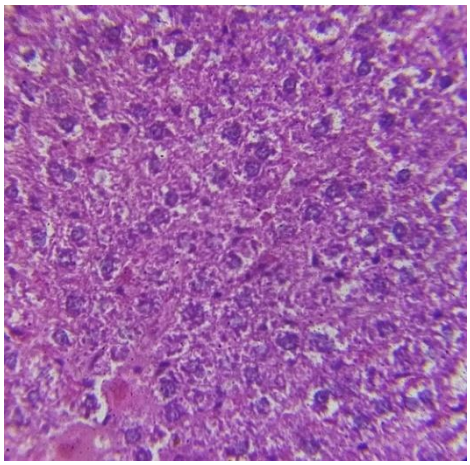
Low 10X magnification



High 40X magnification



High 40X magnification



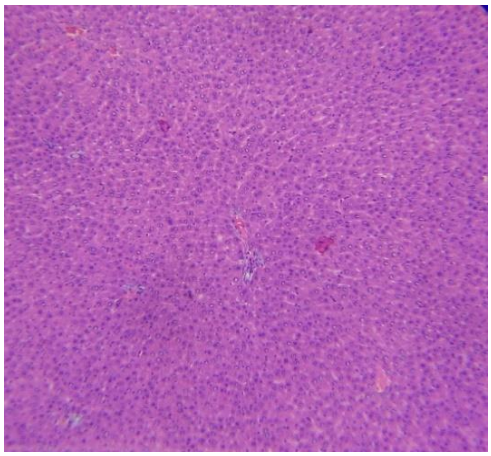
Control group: Normal central vein, sinusoids and hepatocytes.

High Dose: Cytoplasm appears normal with widen portal tract.

Histopathology of Liver (Female)

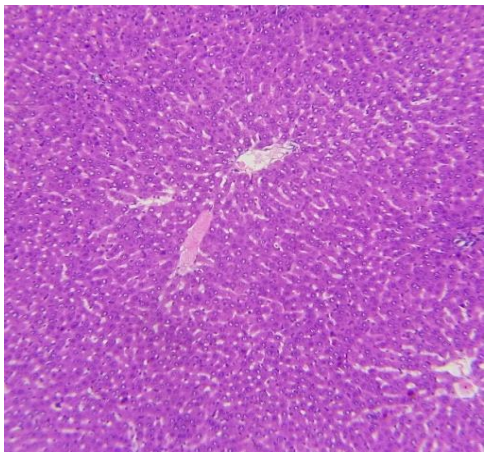
Control group

Low 10X magnification

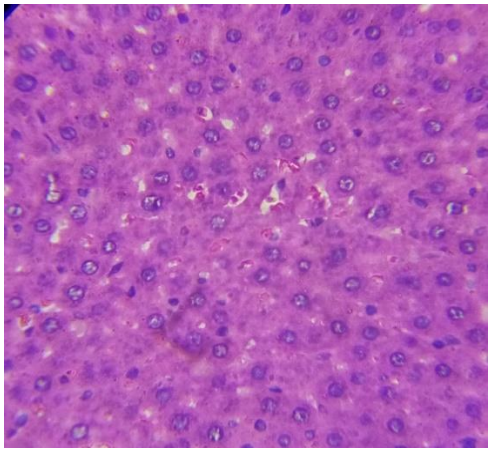


High dose group

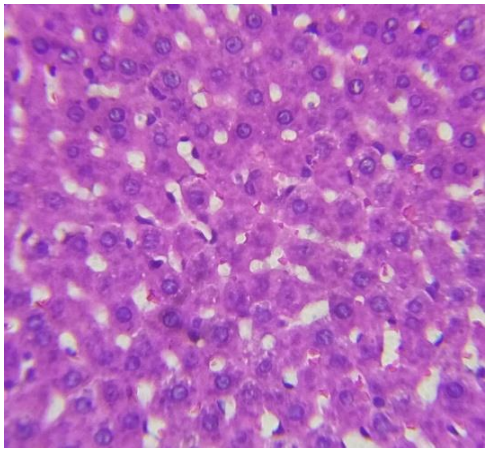
Low 10X magnification



High 40X magnification



High 40X magnification



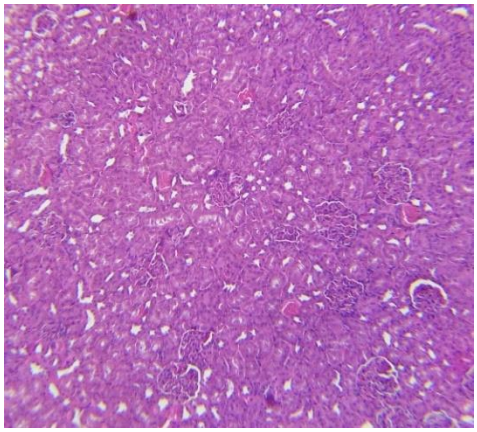
Control group: Showing normal hexagonal hepatic lobules with normal, regular radiated hepatic cords.

High Dose: Normal hepatocytes with occasional, appearance of variably pale necrotic changes was observed.

Histopathology of Kidney (Male)

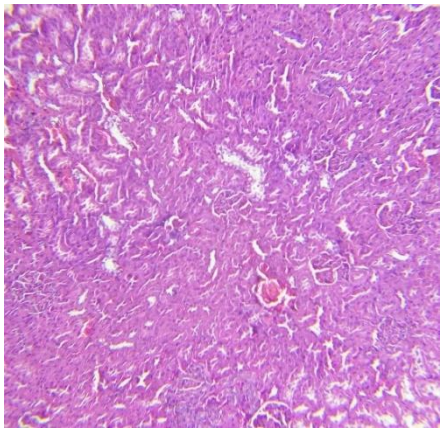
Control group

Low 10X magnification

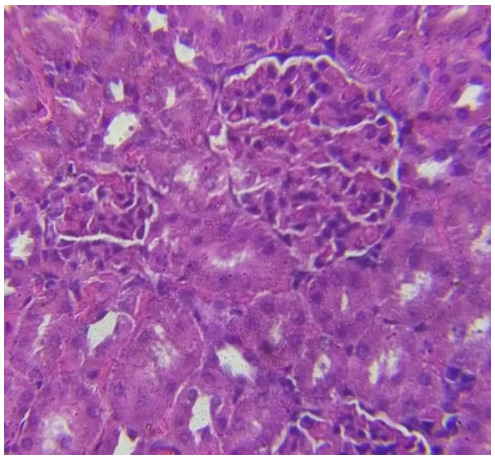


High dose group

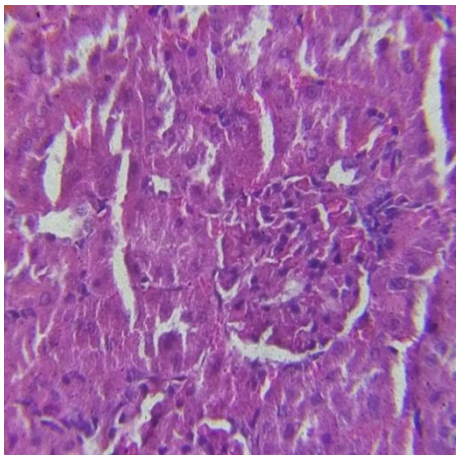
Low 10X magnification



High 40X magnification



High 40X magnification



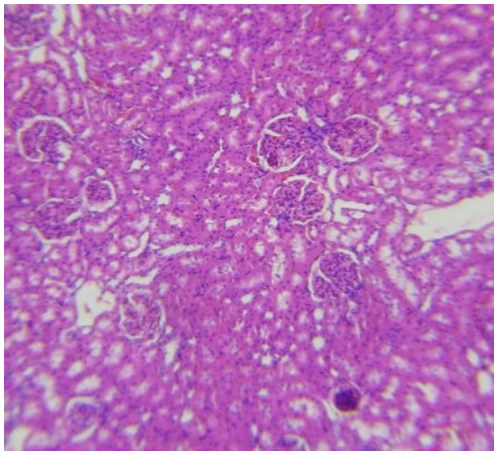
Control group: Showing normal, intact renal tubules as well as renal glomeruli.

High Dose: Glomerular loop was normal with regular interstitium.

Histopathology of Kidney (Female)

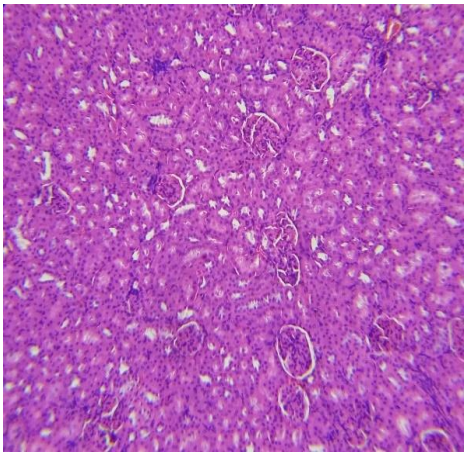
Control group

Low 10X magnification

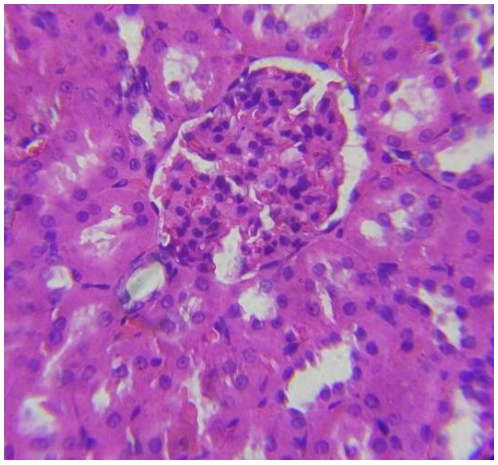


High dose group

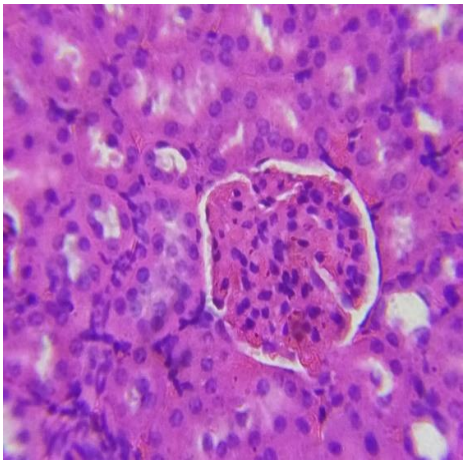
Low 10X magnification



High 40X magnification



High 40X magnification



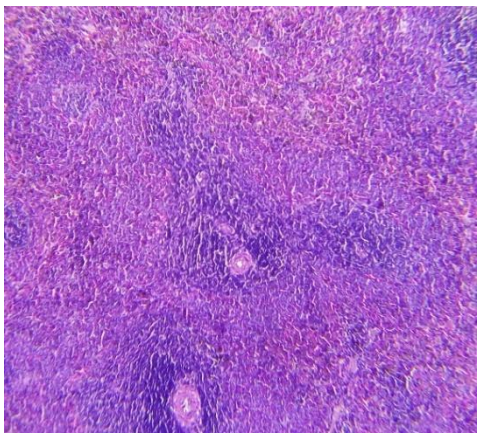
Control group: Showing normal, intact renal tubules as well as renal glomeruli.

High Dose: Glomerular loop was normal with regular interstitium.

Histopathology of Spleen (Male)

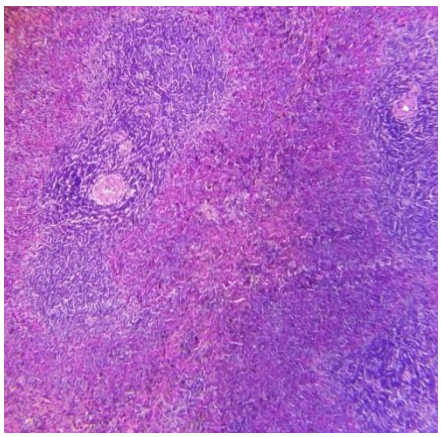
Control group

Low 10X magnification

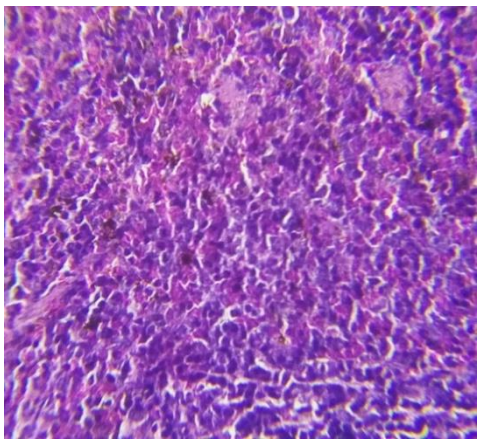


High dose group

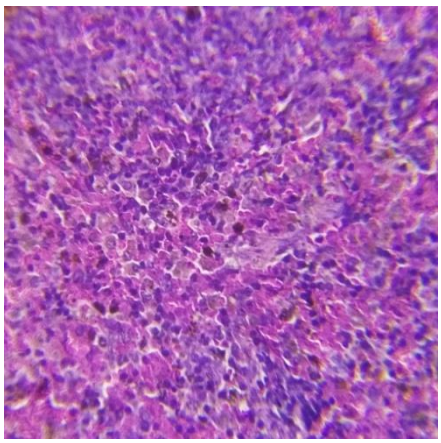
Low 10X magnification



High 40X magnification



High 40X magnification



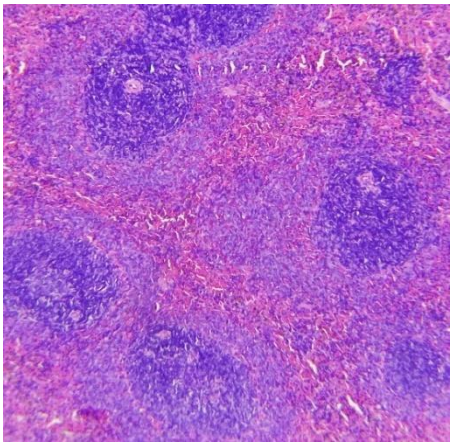
Control group: PALS – periarterial lymphoid sheath was normal with no significant signs of enlargement.

High Dose: Lymphoid follicles appears normal.

Histopathology of Spleen (Female)

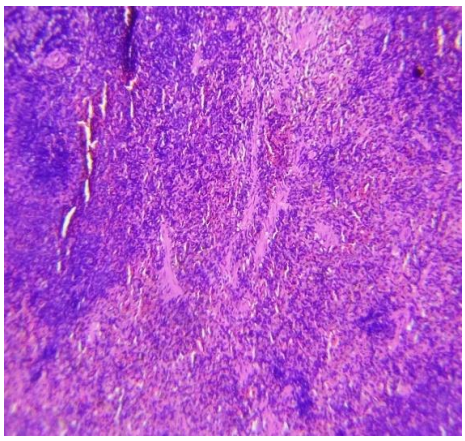
Control group

Low 10X magnification

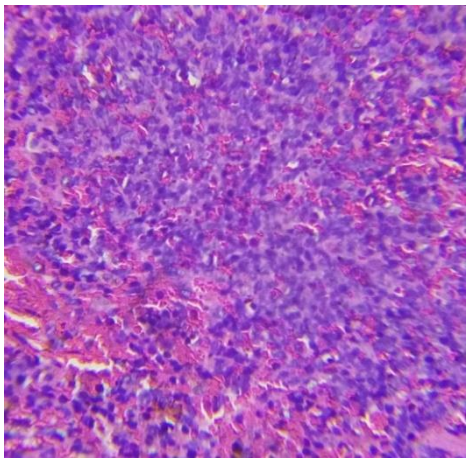


High dose group

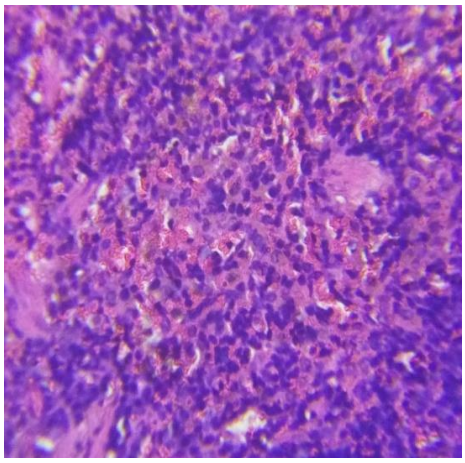
Low 10X magnification



High 40X magnification



High 40X magnification



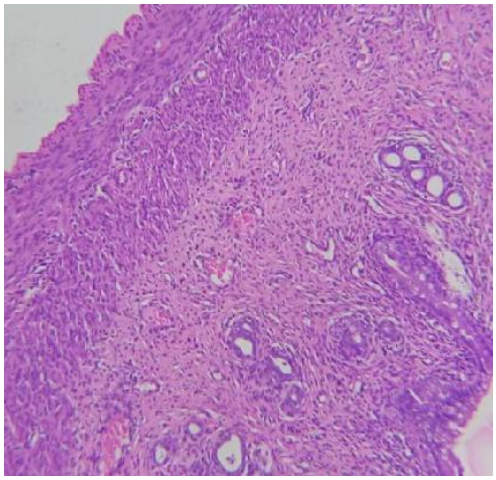
Control group: PALS – periarterial lymphoid sheath was normal with no significant signs of enlargement.

High Dose: Marginal sinus (MS) of the spleen and its sinus lining cells appears normal.

Histopathology of Uterus

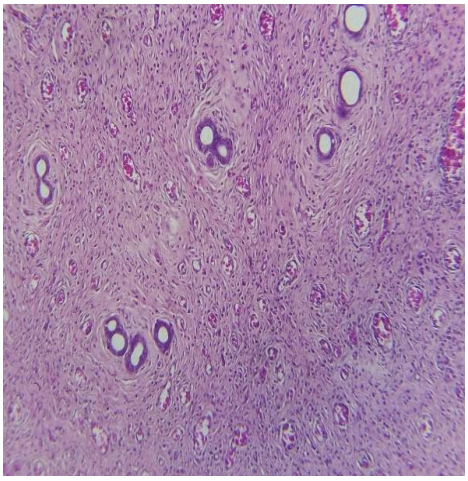
Control group

Low 10X magnification

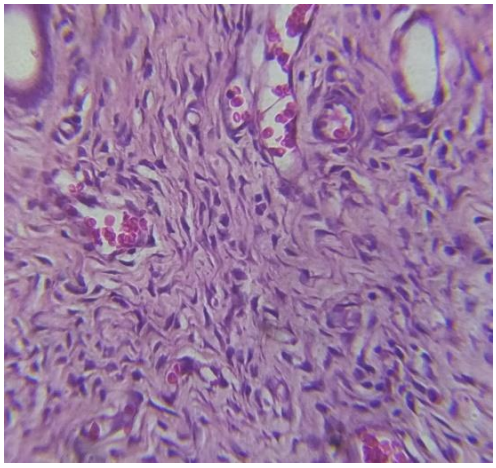


High dose group

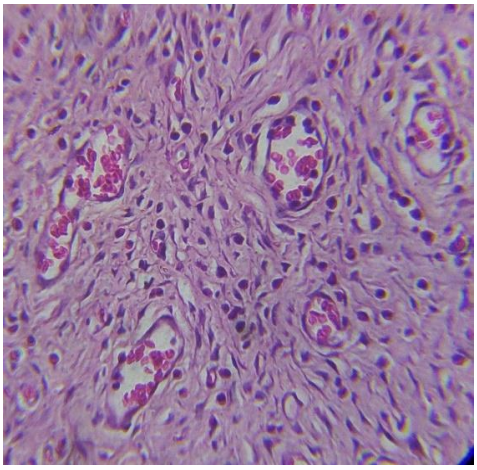
Low 10X magnification



High 40X magnification



High 40X magnification



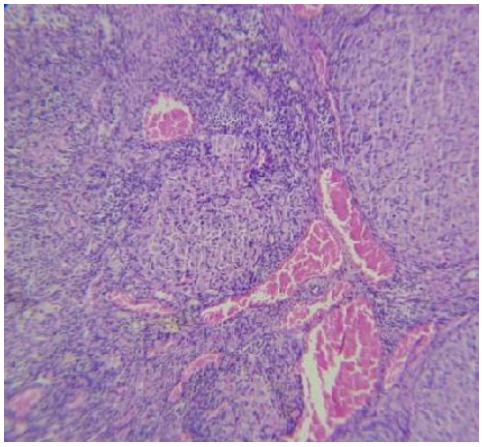
Control group: Regular histology of uterine epithelium and endometrial glands.

High Dose: Arrangement of stratum basale, functionale and surface epithelium seems normal.

Histopathology of Ovary

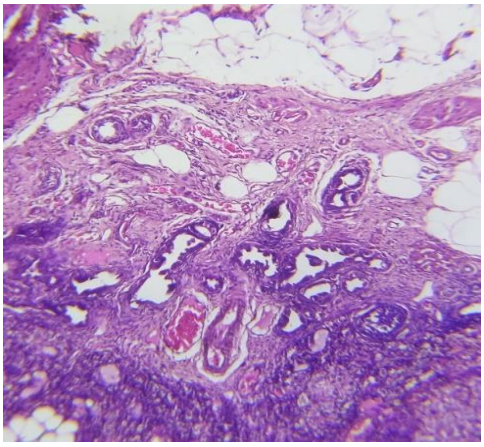
Control group

Low 10X magnification

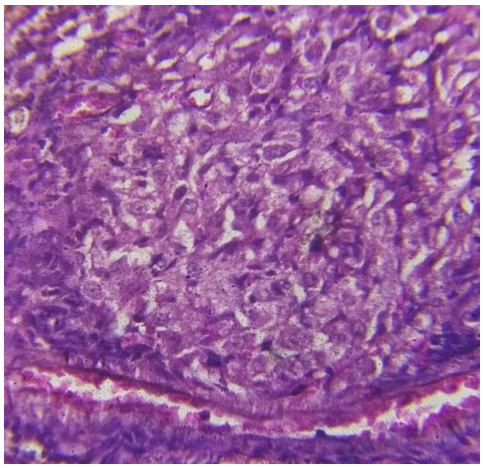


High dose group

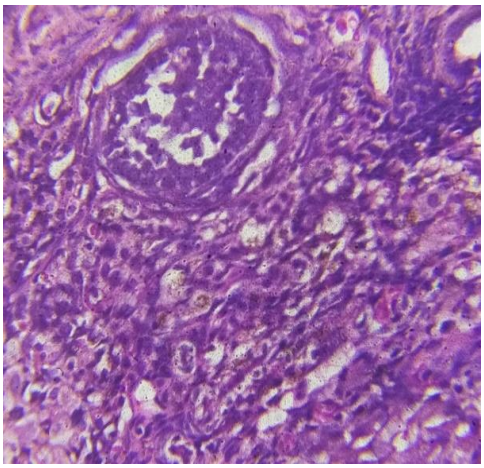
Low 10X magnification



High 40X magnification



High 40X magnification



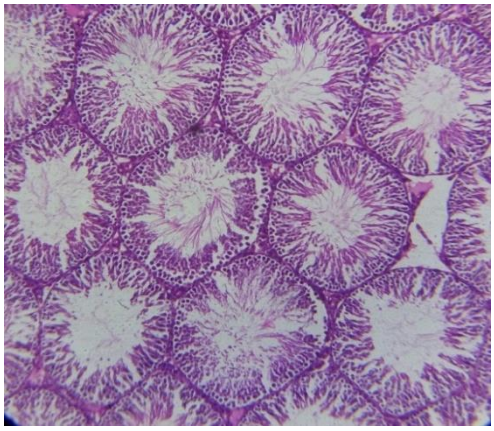
Control group: Granulosa cells around oocyte was normal and regular.

High Dose: Appearance of antral follicle, primary oocyte and secondary follicles are normal.

Histopathology of Testes

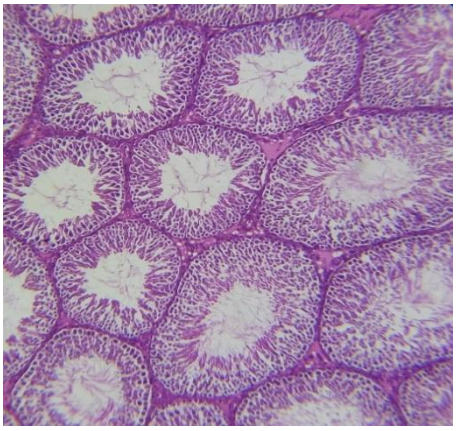
Control group

Low 10X magnification

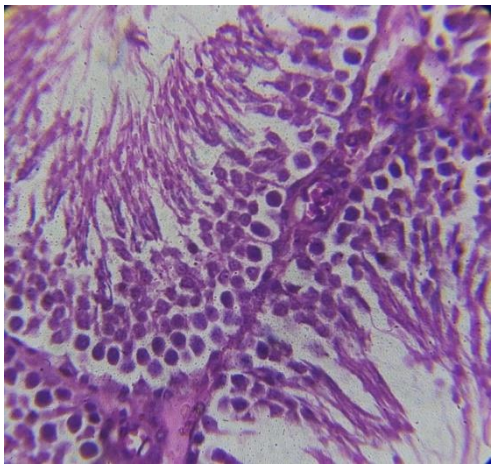


High dose group

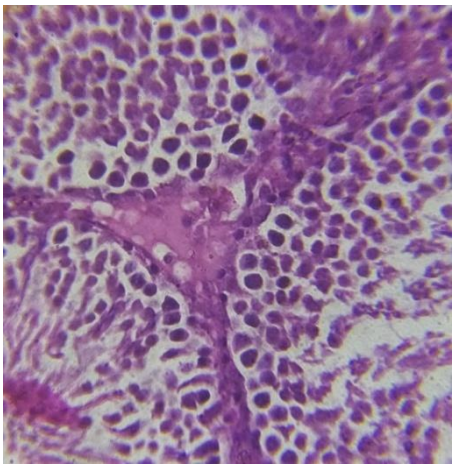
Low 10X magnification



High 40X magnification



High 40X magnification



Control group: Presence of mature somatic cells project the perfect histomorphology of testicular cells were observed.

High Dose: Primary spermatocytes with large centred nucleus and dense chromatin were observed.

7. DISCUSSION

Rising popularity of Siddha system of medicine in recent years have increased the demand of Siddha preparations. It is challenging for the Siddha pharmaceuticals to produce the standard, genuine and safe drug in required quantity with utmost quality. Raw drug quality plays main role in the quality of preparatory medicines. The raw drugs of Annapavala Chendhuram such as annabedhi and Kodipavalam comes under the category of minerals. So, authentication for these minerals got from the Department of geology, University of Madras, Chennai.

Preparation of Chenduram and Parpam comprise the three procedures. (i.e.) Suddhi (Purification), Araippu (Grinding) and Pudam (Incineration). Purification process removes the impurities as well as enhances the drug activity.[75] The raw material annabedhi and kodipavalam purification process was done with lemon juice. After the purification, Annabedhi and kodipavalam loses its classical green and pink colour respectively. Lemon juice is a rich supplier of vitamin C and vitamin B complex, which acts as an intrinsic-factor in the absorption of iron in the body providing a synergistic effect. Acidic nature of lemon juice removes the impurity in raw materials and loss of water.[77] This reaction may be reason for colour changes after purification.

Standardisation of traditional medicine is the process of prescribing a set of standards and constant parameters that carry an assurance of quality, efficacy, safety and reproducibility of the drugs. [78] The analytical study was carried out with a view to know particular chemical configuration of the final product. Organoleptic characters of Annapavala chenduram have mentioned in Table 6.1. On organoleptic evaluation, Annapavala Chendhuram was brownish red in colour with no specific odour and taste. Specific colour of Chendhuram indicates the formation of specific compounds because each chemical compound possesses specific colour. Touch indicates physical properties such as smoothness, softness and fineness of Chendhuram. All the Siddha classical analytical parameters have definite significance. Siddha standardisation for Chendhuram given in Table 6.2. Floating of the test drug APC on the surface of the water indicated the weightless of the drug and Chendhuram enters into the creases of

fingers showed the micro fineness of the drug.

Physico chemical analysis of APC mentioned in the Table 6.3. pH value represents alkalinity and acidic nature of formulation, pH of APC was 8.34. It was weak alkaline. pH of the drug plays main role in the drug absorption. The weak base is absorbed at a faster rate from the intestine (pH 7.50 – 8), this is because the basic substances can't be ionized in basic medium. So, the uncharged substances can be passed easily due to its lipid solubility. This alkaline nature revealed that it would be absorbed well in the intestine. [79]

The physical constant evaluation of the drug is an important parameter in detecting adulteration or improper handling of drugs. A high ash value is indicative of contamination, substitution, adulteration or carelessness in preparing the medicine. Acid insoluble ash in APC was 0.56%. This result gives an inference that the drug was prepared without any external contamination as this may affect the absorption of drug. Lower value of the acid insoluble ash suggests the greater physiological availability of the drug and gets easily digested in alimentary canal. [80] Crude protein and crude fibre present in the APC was 0.32% & 5.34% respectively. It indicated the protein and fibre amount in the Annapavala Chendhuram.

Presence of acid radicals in APC was sulfate, chloride, phosphate, carbonate and nitrate showed in Table 6.7. Chloride plays an important role for regulation of water balance, osmotic pressure, as well as acid base equilibrium. [81] Presence of nitrate stimulates the production of nitric oxide. Nitric oxide is important physiological signalling molecule that is used in, among other things, regulation of muscle blood flow and mitochondrial respiration.[82] Sulfate is a major contributor to the ionic strength of urine and alters the equilibrium constants governing saturation and precipitation of calcium salts.[83] Carbonate ions in the APC revealed that, pH and acid balance are regulated by the carbonate, as bicarbonate ions. [84] Presence of phosphate ions which maintain blood sugar level, normal heart contraction, bone growth, and kidney function when consumed by human beings. [85]

Presence of basic radicals in APC was Aluminium, zinc, Iron, calcium, magnesium, potassium and sodium was mentioned in Table 6.7. Sodium and potassium

of regulate the acid – base balance of the body fluids. Potassium ions are essential for contraction of cardiac and skeletal muscles. It is essential for the activity of nerves. Calcium present in body in great abundance. It is necessary for muscle contraction and normal transmission of nerve impulses. It is also played a vital role in the maintenance and regulation of acid-base balance. [86] Zinc is necessary to maintain the normal levels of Vitamin A in serum and the storage, secretion of insulin from the β – cells of pancreas require Zn. Iron mainly exerts its functions through the compounds in which it is present. Haemoglobin and myoglobin are required for the transport of O_3 and CO_2. Iron is associated with effective immune competence of the body. [87]

Annapavala Chendhuram was scanned under FESEM to know the shape, size, arrangement and texture of particles. Chendhuram was well agglomerated. Most of the particles were very tiny (size 200nm), unarranged and irregular in shape with amorphous in nature. (fig 6.1 – 6.4) The SEM report and particle size analysis data indicated that the test drug obtained was nanometre in size. EDAX report showed the presence of carbon, oxygen, iron, magnesium, Sulphur, Chlorine, Calcium in different proportions, which proves that APC is a multi-mineral compound mentioned in Table 6.8. Calcium is rich in the APC due to the presence of kodipavalam which contains 85% calcium carbonate in raw drug. [88]

For the identification of the compounds present in the APC, XRD was carried out in which prominent peaks of ferric oxide were seen which confirms that final product is oxide form of ferrous.[33] Ferric means the iron atom has lost three electrons to form Fe+3, and ferrous means the iron atom has lost two electrons to form Fe+2. Studies done in ferric oxide revealed the same effect in iron metabolism compared with the ferrous form.[89] In the FTIR spectra, peaks range from 400 to 4000cm^{-1}. The probable functional groups which can be correlated to some of the peaks present in the region are given Table 6.9. The region between the 400 to 1500cm^{-1} looks complex and many peaks (Fe-O) were exhibited in this range.

This qualitative analysis on APC revealed that the medicine had prepared with authenticated raw drug and free from contamination. It revealed the combination of APC.

Annapavala chenduram is widely prescribed by siddha practitioners. Although, In the current literature no systematic information on toxicity is available on APC. Therefore, it was decided to conduct a toxicity study with WHO guideline, the research finding will not only add value to its ethnopharmacological profile, but also provides information on possible health hazards likely to arise from repeated administration over a limited period of time. In the acute toxicity study of APC, all the animals were found to be normal throughout the study periods. The high dose of APC (250mg/ kg) was administered as a single dose through oral route with honey. The animals were monitored for any behavioral abnormalities and mortality for 14 days. No mortality or morbidity was observed after the administration and also up to the end of the study period. The gross necropsy did not show any abnormality.[90] Body weight of these animals were observed 7 days once. There was gradual increase in the body weight of acute toxicity animals but no statistical significance was found between control and treated groups in body weight gain. [91]

In the Long - term toxicity study, the effect of APC on body weight during 90 days treatment in rats was mentioned in Table 6.3.3. There was gradual increase in body weight of APC treated animals when compared to control during the experimental period. But no statistical significant was found in the body weight of the treated groups when compared with the control groups. The effect of feed and water intake of APC treated animals was mentioned in Table 6.3.4 and Table 6.3.5. There were no significant changes in feed and water intake when compared to the control group.

The effect of APC on hematological parameters shown in Table 6.3.6 & 6.3.7. It reveals that there were no significant changes in the hematological and biochemical parameters. Serum enzymes such as Aspartate transaminase (SGOT), Alanine transaminase (SGPT) are useful biomarkers of liver injury. These are the enzymes found mainly in the liver, red blood cells, pancreas, heart, kidneys and biliary ducts of the liver. The levels of AST and ALT in serum are used to diagnose body tissues especially the heart and the liver is injured or not. [92] Research suggests that, when body tissues are damaged, additional AST and ALT are released into the bloodstream and raise the serum enzyme level. As a result, the amount of AST and ALT in the blood is

directly associated with the amount of tissue damage. Parameters of liver function were within the normal limits when compared with the control group.

The kidney, an important organ of the renal system, plays a principal role in homeostasis by excreting urine. It achieves this by filtering waste products from the blood stream and converting the ultra-filtrate to urine. Measurements of some biological (biochemical) markers can demonstrate beneficial or harmful changes in kidney function, thereby serving as indices of renal function. Important biochemical markers that are often used for routine analysis of kidney function include creatinine, urea, uric acid. [93,94] The renal parameters not showed significant changes in treated groups when compared to the control group. The histopathological study on the organs such as brain, heart, lung, kidney, spleen, liver, stomach, uterus, ovary and testes were normal in high dose groups when compared to control. Normal levels of liver function and kidney function tests revealed that the safety of Annapavala chenduram.

8. SUMMARY

- Raw drug of Annapavala chenduram was procured from the reputed shop in Chennai and got authentication from Department of Geology, University of Madras, Chennai.
- As per the literature, APC was prepared in Gunapadam Laboratory, National Institute of Siddha, Tambaram sanatorium, Chennai.
- Standardisation was done as per PLIM guidelines.
- Physico – chemical analysis of APC revealed its quality standard.
- Qualitative analysis of acid and basic radicals for APC showed the acid radicals such as sulphate, chloride, phosphate, carbonate and nitrate. The basic radicals present in the drug were Aluminium, zinc, Iron, calcium, magnesium, potassium and sodium.
- The SEM analysis of the drug APC revealed that irregular shape and uniformity of the drug which was 200nm in size.
- The EDAX associated with SEM showed the presence of elements like carbon, oxygen, iron, magnesium, Sulphur, Chlorine, Calcium.
- The XRD patterns of samples indicated the presence of dominating peaks Fe_2O_3.
- The FTIR analysis of APC revealed that the presence of alcohol, amine, alkane, carbonyl and alkyl halides (Fe- O) group which is ranged from 400 to 4000cm^{-1}.
- Animal toxicity was done as per WHO guideline.
- There was no mortality or drug related toxicity during acute toxicity study.
- The long-term toxicity study did not show any toxic effects in the animals.
- Hematological, Serological and histopathology reports revealed the no toxic effects during throughout the study period.

9. CONCLUSION

Annapavala Chendhuram has been used for thyroid dysfunction by many practitioners. The qualitative and quantitative analysis of Annapavala Chendhuram was done as per PLIM guidelines. It revealed that the quality standard of drug. From the animal study, it is concluded that Annapavala chenduram along with proper adjuvant is not toxic on acute administration at a maximum oral dose level of 2000 mg/kg in Wistar rats. There was no mortality and drug related toxicity noted. In long term toxicity, hematological, biochemical and histopathological studies showed no significant adverse effects during the 90 days of the study. Hereby, safety of Annapavala chenduram has been proven through this study. Further efficacy studies and clinical trials on Annapavala Chendhuram will be more beneficial for the researchers and world.

10. BIBILIOGRAPHY

1. Thirumoolar, Thirumanthiram English translation of classical text (verses 1 - 112), pg. no: 5.
2. Palpandian, Siddhas – Masters of the basics, Published by Achala Siddha. Pg. no: 1,
3. Thirunarayanan T, Introduction to Siddha Medicine Published by Centre for Traditional Medicine and Research, Chennai, 2012, pg. no: 7,13,139
4. Prasad LV, Indian system of medicine and homoeopathy, Editor: Ranjit Roy Chaudhury, Uton Muchtar Rafei, WHO, -Regional office for South East Asia, New Delhi 2002. P 283.
5. Siddha medicine: https://www.britannica.com/science/Siddha-medicine.
6. National health portal – Treatment methods https://www.nhp.gov.in/treatment-methods_mtl
7. Lalit Tiwari, Siddha medicine: Its basic concepts http://www.infinityfoundation.com
8. Chemistry in Siddha http://ayush.gov.in/about-the-systems/siddha/chemistry-siddha
9. Thiyagarajan. R, Gunapadam Thadhu – Jeeva vaguppu, volume II, 4th edition, Directorate of Indian medicine and Homeopathy, Chennai, 2004.
10. G.D.Naidu and M. Shanmugavelu, The pharmacopoeia of Siddha research medicines, chapter II, The Industrial welfare association limited, Coimbatore – 18, 1973, p.no:7.
11. Unnikrishnan AG, Menon UV. Thyroid disorders in India: An epidemiological perspective. *Indian J Endocrinol Metab*. 2011;15(Suppl2): S78–S81. doi:10.4103/2230-8210.83329.
12. Thiyagarajan. R, Gunapadam Thadhu – Jeeva vaguppu, Volume II & III, 8th edition, Pub - Directorate of Indian medicine and Homeopathy, Chennai, reprint 2013, pg. no: 525, 526, 527, 462, 463, 465.
13. Sage bogar, Bogar 7000 – 3 kaandam, 3rd edition, Pub – Thaamarai noolagam, Chennai, 2003, pg. no: 227, 165.

14. Sage bogar, Bogar nigandu attavanai, 4th edition, Pub – Saraswathi mahal noolagam, Thanjavur, 2001, pg. no: 13,11
15. Anaivari R. Anandan, Pathartha guna cinthamani, 1st edition, Pub - Directorate of Indian medicine and Homeopathy, Chennai, 2009, pg. no: 328, 350.
16. Judge Balaramaiyya, vadha vaidiyathukkadhi, 3rd edition, Pub – Aruljothi padhippagam, Chennai, 1988, pg. no: 103, 107,94.
17. Kannusamy Pillai S, Sikitcha Rathna Deepam, pub - B.Rathinanayakar & sons, Chennai, 2011, pg. no – 147, 249, 278, 299, 40,107,235, 184, 199, 221, 223, 226, 228, 233, 237, 239, 241, 243, 260, 270,70.
18. Hakkim P. M. Abdullah sahibu, Anuboga vaidhiya navaneetham, part 1, 1st edition, Pub – Thaamarai noolagam, Chennai, 1995, pg. no: 19,29, 35, 38, 47, 62, 86, 100, 101, 117.
19. Hakkim P. M. Abdullah sahibu, Anuboga vaidhiya navaneetham, part 3, 2nd edition, Pub – Thaamarai noolagam, Chennai, 2006, pg.no: 81, 96, 102, 103,127,139,140
20. Kannusamy pillai S, Kannusamy Parambarai Vaithiyam, 5th Edition, Pub - B.Rathinanayakar & sons, Chennai, 2015, pg. no: 326, 327.
21. Hakkim P. M. Abdullah sahibu, Anuboga vaidhiya navaneetham, part 2, 2nd edition, Pub – Thaamarai noolagam, Chennai, 2006, pg. no: 31,75,83,37, 42, 70, 91, 111, 134.
22. Hakkim P. M. Abdullah sahibu, Anuboga vaidhiya navaneetham, part 4, 1st edition, Pub – Thaamarai noolagam, Chennai, 1995, pg. no: 148,
23. Kannusamy pillai S, padhartha guna vilakkam (Thadhu – jeeva varkkam), 2nd edition, Pub - B.Rathinanayakar & sons, Chennai, 2011, pg. no: 45,46.
24. Nadkarni K.M. Indian Materia Medica, volume 2, 2nd reprint of 3rd revised and enlarged edition, Pub - popular prakasham, Bombay, 2010, pg.no: 63,64, 157.
25. Ferrous sulfate - https://pubchem.ncbi.nlm.nih.gov/compound/Ferrous-sulfate
26. Iron sulphate - https://en.wikipedia.org/wiki/Iron(II)_sulfate
27. Ferroussulphatehttps://www.infoplease.com/encyclopedia/science/chemistry/elements/ferrous-sulfate

28. G. Essakkypandian, Gunapadam Thathu jeevam with medicinal chemistry, 1st edition, Pub – postgraduate department of gunapadam, Thirunelveli, 2019, pg. no: 146.
29. Ferroussulphatehttps://www.softschools.com/formulas/chemistry/ferrous_sulfate_formula/397/
30. Sivaraj R, Sivaraj U, Choudhary RAK, Abirami R. Clinical comparison of Annabedi Chendhuram (siddha system of medicine) and elemental iron in the treatment of iron deficiency anaemia. Int J Comprehensive Adv Pharmacol 2018;3(4):142-145.
31. Dr Ashwin Ashok Shete, Dr. Avinash Ashok Shete, Dr. PVNR Prasad, Clinical study of Kasisa bhasma and Annabhedi chenduram with reference to Hb%, The Pharma Innovation Journal 2019; 8(1): 285-289.
32. Ashwin Ashok Shete, et. al., comparative study of kasisa bhasma and annabedhi chenduram with reference to their pharmaceutical study, Volume 6, Issue 12, 674-684.
33. Rajalakshmi, et al.: Analytical studies on Annabethi chenthuram, Indian J Pharm Sci 2017;79(6):987-993o
34. Dhiman Saha et al. Pharmaceutical and analytical study of Kasisa. Int. Res. J. Pharm. 2017;8(5):108-114 http://dx.doi.org/ 10.7897/2230-8407.08583.
35. Palbag S, Saha D, Gautam DS. Toxicity studies of iron-containing ayurvedic drug Kasisa Bhasma. BLDE Univ J Health Sci 2016:39-43
36. Keerthy Unni, Sheela Karalam B., A.V. Vijayasankar. Scientific basis for the preparation and characterization of Iron based traditional drug Annabhedi sindooram: A materialistic approach. Int. J. Res. Ayurveda Pharm. 2013; 4(2):149-152
37. Rajput DS, Tekale GS. Study on Bhasma Kalpana with special reference to the preparation of Kasisa Bhasma. *Ayu*. 2011;32(4):554–559. doi:10.4103/0974-8520.96133
38. Rajput D, Tekale GS, Patgiri B. Comparative study on Kasisa Bhasma prepared by two different methods. *Anc Sci Life*. 2011;31(2):62–72.

39. Thankamma, An Improved Method for the manufacture of Annabhedi Sinduram, Ancient Science of Life, Vol No. 17 (2), October 1997 Pages 135 – 140.
40. Sarakku suthi seimuraigal, First edition, Pub – Directorate of Indian medicine & homeopathy, Chennai, Pg.no: 93, 2008
41. Prema. S, Agasthiyar mani 4000 ennum vaithiya cinthamani venba 4000 – 1st part, 1st edition, Pub – Thaamarai noolagam, Chennai,1996, pg.no: 352,371, 176, 203, 228, 230, 374
42. Prema S, Eliya vaithiya murai, Pub – Thaamarai noolagam, Chennai, pg. no: 248.
43. Koshaayi – Anuboga vaithiya bramma ragasiyam, part 2, first edition, Pub – Thaamarai noolagam, Chennai,1999, pg.no: 92.
44. Venkataraman, K. and Ch. Satyanarayana. 2012. Coral Identification manual: Published by the Director, Zoological Survey of India, Kolkata. Pg. no: 1,3,
45. Integrated Taxonomic System Information Centre online data base system. http://www.itis.gov/servlet/SingleRpt/SingleRpt?search_topic=TSN&search_valu e=20251
46. Scleractinia - https://en.wikipedia.org/wiki/Scleractinia
47. GFCM Secretariat A brief report o Red coral (Sardinia coral)
48. Velpandian V, Pitchiahkumar M, Gnanavel IS, Anbu N, Abdul Kadher AM. Clinical evaluation of *Kodipavala chunnam* in the treatment of infective hepatitis, drug induced hepatitis and alcoholic hepatitis. International Research Journal of Pharmacy 2013; 4(4): 152-157.
49. Velpandian V, Ashwini anjana, Anbu J, Prema S. Acute and sub - acute toxicity studies of *Kodi pavala chunnam i*n rodents. Asian journal of pharmaceutical and clinical research 2012; 5(4): 36-41.
50. Velpandian V, Anbu J, Prema S. Evaluation of hepatoprotective activity of *Kodi pavala Chunnam* in carbon tetrachloride induced Liver damage in rats. Int J Pharm Bio Sci 2013; 4(1): 829- 839.
51. Thanigaivelan V, Victor Rajamanickam G, Kaliyamurthi V, Lakshmana Kumar V,et.,al, Antibacterial and haemostatic activities of a Siddha formulation – P*avala parpam* Pharmacology online 2011(cited on 2012 Aug) 1: 613-624

52. Himanshu S. Tiwari, Patgiri B, Prajapati PK. A Comparative study on *Pravala mula bhasma* and *Pravala shakha bhasma* in *amlapitta* J.R.A.S 2008; 31(3): 123- 128.
53. Prabhakara N. Reddy, Lakshmana M, Venkatesh Udupa U. Effect of *Praval bhasma* (*Coral calx*), a natural source of rich calcium on bone mineralization in rats. Pharmacological Research 2003; 48: 593–599.
54. Rosalind Marita and Radha Shanmugasundaram K. Effect of *Anna Pavala sindhooram* on plasma and aortic lipids in experimental atherosclerosis. Atherosclerosis 1982; 45: 331-343.
55. Rosalind Marita, Shanmugasundaram KR *Anna pavala sindhooram*, A novel hypolipidemic agent reduces phospholipid levels in atherosclerosis. Pharmacological research communications 1988; 20 (7): 591-600.
56. Murugesa mudhaliyar, Gunapdam Mooligai, 9th edition, pub by Directorate of Indian medicine and Homeopathy, Chennai, 2013, pg. no: 158.
57. Plant details for citrus limon, http://envis.frlht.org/plantdetails/0312585bf680296c924c21e572f035c0/2f7b4662f320889a2dd890f54b4915a0
58. Mahendra Pal (2017), Lemon: A versatile root of multiple uses, Agriculture world, 3, 52 – 54.
59. Plants database, Natural resources conservation service, https://plants.usda.gov/java/ClassificationServlet?source=display&classid=CITRU2
60. Nadkarni K.M. Indian Materia Medica, volume 1, 2nd reprint of 3rd revised and enlarged edition, Pub - popular prakasham, Bombay, 2010, pg.no: 347.
61. Chikako Shimizu et al, Effects of lifelong intake of lemon polyphenols on aging and intestinal microbiome in the senescence accelerated mouse prone 1 (SAMP1), Scientific reports, (2019) 9:3671.
62. Kato Y, Domoto T, Hiramitsu M, et al. Effect on blood pressure of daily lemon ingestion and walking. *J Nutr Metab*. 2014; 2014:912684. doi:10.1155/2014/912684.
63. Rezatofighi Seyedeh, Elham & Ebrahimian, & Lordifar, & Mehravar, Neda & Sirnejad,. (2014). ., Antiviral Activity of Citrus Limon, Matricariarecutita L

. Allium ascalonicum L., and Rosa damascene against Newcastle Disease Virus .Journal of Veterinary Microbiology. 10. 1-7

64. Boshtam M, Asgary S, Moshtaghian J, Naderi G, Jafari-Dinani N. Impacts of fresh lime juice and peel on atherosclerosis progression in an animal model. *ARYA Atheroscler*. 2013;9(6):357–362.
65. Alshatwi AA, Shafi G, Hasan TN, et al. Apoptosis-mediated inhibition of human breast cancer cell proliferation by lemon citrus extract. *Asian Pac J Cancer Prev*. 2011;12(6):1555–1559.
66. Kumar, Ashok & Narayani, M. & Subanthini, A. & Jayakumar, M. (2011). Antimicrobial Activity and Phytochemical Analysis of Citrus Fruit Peels - Utilization of Fruit Waste. International Journal of Engineering Science and Technology. 3. 5414-5421.
67. Khan, Yasmin & Khan, Rafeeq & Afroz, Syeda & Siddiq, Afshan. (2010). Evaluation of hypolipidemic effect of citrus lemon. J. basic appl. Sci. 6. 39-43.
68. Penniston KL, Nakada SY, Holmes RP, Assimos DG. Quantitative assessment of citric acid in lemon juice, lime juice, and commercially-available fruit juice products. *J Endo urol*. 2008;22(3):567–570. doi:10.1089/end.2007.0304.
69. Kartik Ch.patra .Standardisation of siddha formulation. Indian Journal of Traditional Knowledge .2009:8.
70. Finar, I.L. Organic Chemistry ELBS Longman 767 (1989).
71. P. R. Griffiths and J. A. de Haseth, Fourier transform infrared spectrometry (Wiley, New York, 1986).
72. Introduction to Fourier transform infrared spectroscopy, Thermo Nicolet corporation, 2001, pg. no: 1-8.
73. D. M. C. Mullan, Scanning. 17, 175 (2006).
74. Basics of X-ray Diffraction, Scintag, inc,1999
75. Protocol for testing of Ayurvedic, Siddha & Unani medicines, Department of AYUSH, Ghaziabad.
76. Indian standard 7874 -1: Methods of test for animal feeds and feeding stuffs, part I.

77. Shrestha N, Shrestha S, Bhattarai A. Determination of Ascorbic Acid in Different Citrus Fruits of Kathmandu Valley. Journal of Medical and Biological Science Research 2016;2(1):9-14.
78. Varma, Radhika & Harisha, Channappa & Shukla, Vinay. Pharmacognostical and physicochemical analysis of triphaladi yoga: an ayurvedic poly-herbal formulation. Journal of pharmaceutical and scientific innovation. 1. 9-12.
79. Padmaja udaykumar, Medical pharmacology, 5th Edition, CBS publishers & Distributors, New Delhi, 2015, pg. no: 18.
80. Jagtap CY, Prajapati P, Patgiri B, Shukla VJ. Quality control parameters for Tamra (copper) Bhasma. Ancient Sci Life 2012; 31:164-70.
81. Herry JB, Statland BE. Chemical Pathology and Clinical Chemistry. Philadelphia, PA: W.B. Saunders Company; 1979.
82. Maughan, Ronald J (2013). *Food, Nutrition and Sports Performance III*. New York: Taylor & Francis. p. 63.
83. Cole, David & Evrovski, Jovan. (2000). The Clinical Chemistry of Inorganic Sulfate. Critical reviews in clinical laboratory sciences. 37. 299-344. 10.1080/10408360091174231.
84. Moses EA, Ogugbuaja VO, Ogarawu VC. Enrichment of element of Nigerian bituminous coal fly ash and their effects on hematological parameters of exposed rabbits. Nig J Exp Appl Biol 2002; 3:95-100.
85. Johns T, Duquette M. Deficiency of phosphorus in man. Am J Clin Nutr 1991;53: 448-56
86. Ambika Shanmugam, fundamentals of Biochemistry for Medical students. Seventh edition 1998. Pub: Author, Chennai, India. p 647.
87. Sathyanarayana U, Chakrapani U, Essentials of Biochemistry, revised print, pub: Books and allied pvt ltd, Kolkata, 2012, p.no 218, 223.
88. Rathinamala Rathinam, Murugesan moonandi; Pavalam, A valuable Siddha mineral drug. Int. J. Res. Ayurveda Pharm. 2014;5(3):367-371.
89. Kheiri, Reyhaneh & Koohi, Mohammad & Sadeghi-Hashjin, Goudarz & Nouri, Hadiseh & Khezli, Noushin & Hassan, Mohsen & Hoomani, Fatemeh & Shams, Gholamreza & Rasouli, Ali & Motaghinejad, Majid. (2017). Comparison of the Effects of Iron Oxide, as a New Form of Iron

Supplement, and Ferrous Sulfate on the Blood Levels of Iron and Total Iron-Binding Globulin in the Rabbit. Iranian Journal of Medical Sciences. 42. 79-84.

90. Lavekar, Gandhidas & Ravishankar, Basavaiah & Rao, S & Gaidhani, S & Bk, Ashok & Shukla, Vinay. (2009). Safety / Toxicity studies of ayurvedic formulation-Navratna rasa. Toxicology International. Vol-16. 37-42.
91. Kazi Md. Mahmudul Hasanab1, NasrinTamannaa1, Md. Anwarul Haquea Biochemical and histopathological profiling of Wistar rat treated with Brassica napus as a supplementary feed, Food Science and Human Wellness, Volume 7, Issue 1, March 2018, Pages 77-82
92. G. Kasarala, H.L. Tillmann, Standard liver tests, Clin. Liver Dis., 8 (2016), pp. 13-18
93. F.B. WalterMedical Physiology: A Cellular and Molecular Approach (1st ed.), Elsevier, Saunders (2004)
94. F. Stuart, Human Physiology (12th ed.), McGraw-Hill (2011)

11. ANNEXURE

The following certificate are enclosed,

- Institutional animal ethical committee certificate
- Workshop on Research methodology certificate
- Workshop on laboratory animal care and basic research technique
- Authentication certificate
- Journal publication

IAEC CERTIFICATE

(15)

CERTIFICATE

This is certify that the project title Preclinical Safety Evaluation of **"Annapavala chendooram** – A Siddha formulation **has been approved by the IAEC.** Total No animal approval: 90 Rats (45M+45F)

Approval No: NIS/IAEC-VII/28082018/15

V. Banumathi

Prof. Dr. V. Banumathi MD(S),
Chairman IAEC,

Prof .Dr. K. Nachimuthu
CPCSEA Nominee

Chairman/Member Secretary of IAEC:

CPCSEA Nominee:

Name of the principle investigator: Dr. N. Sabari girija,
Department of Nanjumaruthuvam

Name of the Guide : Dr. P. Shanmuga priya
Lecturer ,

Received the original certificate.

N. Sabari

20/11/18

(Dr. N. Sabari Girija)

RESEARCH METHODOLOGY WORKSHOP CERTIFICATE

CERTIFICATE

NATIONAL INSTITUTE OF SIDDHA

Ministry of AYUSH, Government of India

Tambaram Sanatorium, Chennai - 600 047.

Ministry of AYUSH

WORKSHOP ON

RESEARCH METHODOLOGY & BIOSTATISTICS

This is to certify that

Dr. M. SABARIGIRIJA

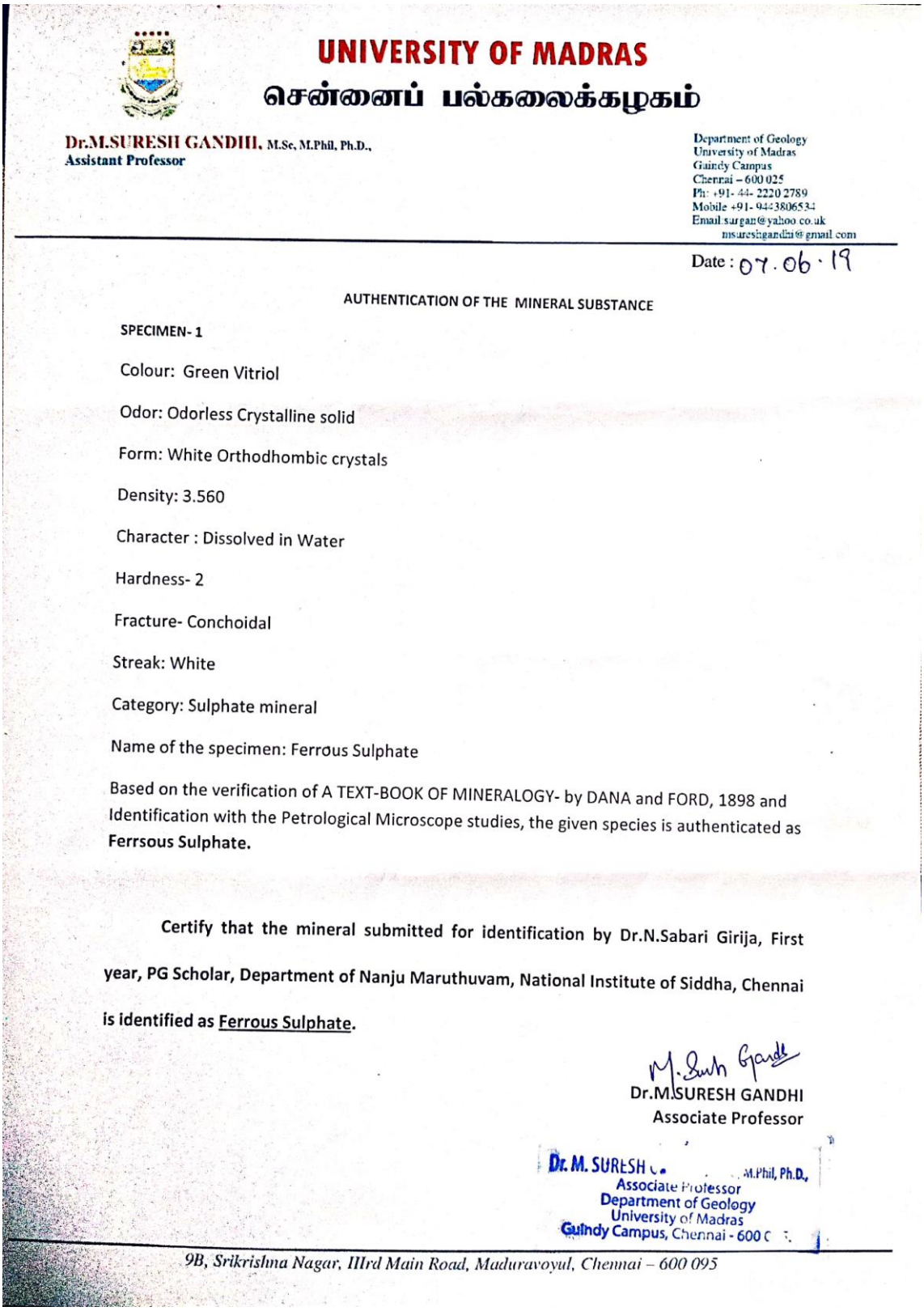
has participated in the above Workshop held from 16.04.2018 to 20.04.2018 conducted by the Dept. of Noi Naadal, at National Institute of Siddha, Tambaram Sanatorium, Chennai-600 047.

Dr. G.J. Christian
Coordinator
HoD, Dept. of Noi Naadal,
National Institute of Siddha

Prof.Dr.V.Banumathi
Director
National Institute of Siddha
Chennai - 600 047

WORKSHOP ON LABORATORY ANIMAL CARE AND BASIC RESEARCH TECHNIQUES

AUTHENTICATION CERTIFICATE



UNIVERSITY OF MADRAS

சென்னைப் பல்கலைக்கழகம்

Dr.M.SURESH GANDHI, M.Sc, M.Phil, Ph.D.,
Assistant Professor

Department of Geology
University of Madras
Guindy Campus
Chennai – 600 025
Ph: +91- 44- 2220 2789
Mobile +91- 9443806534
Email:sargan@yahoo.co.uk
msureshgandhi@gmail.com

Date : 07.06.19

AUTHENTICATION OF THE MINERAL SUBSTANCE

SPECIMEN- 1

Colour: Green Vitriol

Odor: Odorless Crystalline solid

Form: White Orthodhombic crystals

Density: 3.560

Character : Dissolved in Water

Hardness- 2

Fracture- Conchoidal

Streak: White

Category: Sulphate mineral

Name of the specimen: Ferrous Sulphate

Based on the verification of A TEXT-BOOK OF MINERALOGY- by DANA and FORD, 1898 and Identification with the Petrological Microscope studies, the given species is authenticated as **Ferrsous Sulphate.**

Certify that the mineral submitted for identification by Dr.N.Sabari Girija, First year, PG Scholar, Department of Nanju Maruthuvam, National Institute of Siddha, Chennai is identified as Ferrous Sulphate.

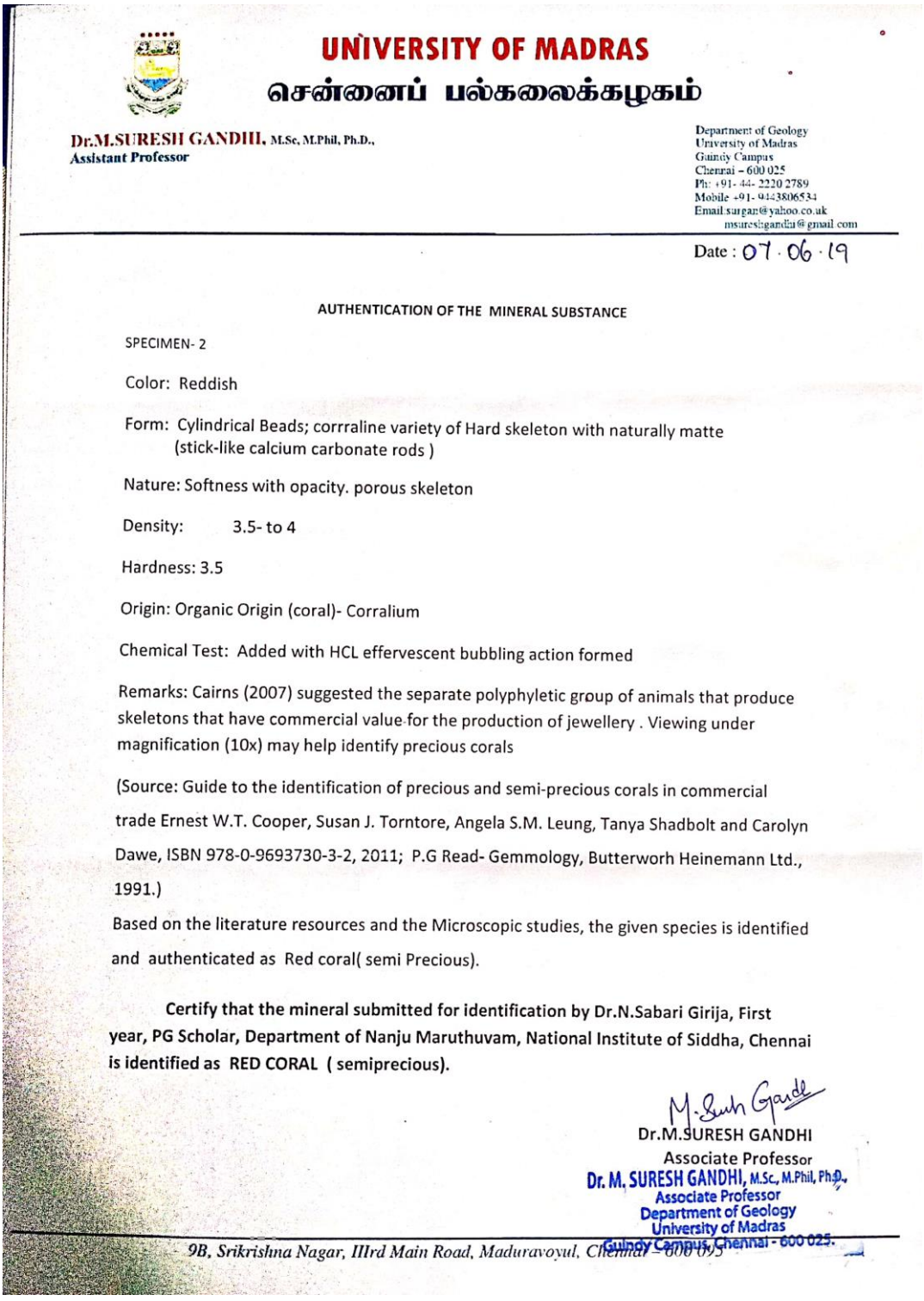
M. Suresh Gandhi

Dr.M.SURESH GANDHI
Associate Professor

Dr. M. SURESH G... M.Phil, Ph.D.,
Associate Professor
Department of Geology
University of Madras
Guindy Campus, Chennai - 600 0...

9B, Srikrishna Nagar, IIIrd Main Road, Maduravoyal, Chennai – 600 095

AUTHENTICATION CERTIFICATE



UNIVERSITY OF MADRAS

சென்னைப் பல்கலைக்கழகம்

Dr.M.SURESH GANDHI, M.Sc, M.Phil, Ph.D.,
Assistant Professor

Department of Geology
University of Madras
Guindy Campus
Chennai – 600 025
Ph: +91- 44- 2220 2789
Mobile +91- 9443806534
Email:sargar@yahoo.co.uk
msureshgandhi@gmail.com

Date : 07 · 06 · 19

AUTHENTICATION OF THE MINERAL SUBSTANCE

SPECIMEN- 2

Color: Reddish

Form: Cylindrical Beads; corrraline variety of Hard skeleton with naturally matte (stick-like calcium carbonate rods)

Nature: Softness with opacity. porous skeleton

Density: 3.5- to 4

Hardness: 3.5

Origin: Organic Origin (coral)- Corralium

Chemical Test: Added with HCL effervescent bubbling action formed

Remarks: Cairns (2007) suggested the separate polyphyletic group of animals that produce skeletons that have commercial value for the production of jewellery . Viewing under magnification (10x) may help identify precious corals

(Source: Guide to the identification of precious and semi-precious corals in commercial trade Ernest W.T. Cooper, Susan J. Torntore, Angela S.M. Leung, Tanya Shadbolt and Carolyn Dawe, ISBN 978-0-9693730-3-2, 2011; P.G Read- Gemmology, Butterworh Heinemann Ltd., 1991.)

Based on the literature resources and the Microscopic studies, the given species is identified and authenticated as Red coral(semi Precious).

Certify that the mineral submitted for identification by Dr.N.Sabari Girija, First year, PG Scholar, Department of Nanju Maruthuvam, National Institute of Siddha, Chennai is identified as RED CORAL (semiprecious).

M. Suh Gandhi

Dr.M.SURESH GANDHI
Associate Professor

Dr. M. SURESH GANDHI, M.Sc., M.Phil, Ph.D.,
Associate Professor
Department of Geology
University of Madras
Guindy Campus, Chennai - 600 025.

9B, Srikrishna Nagar, IIIrd Main Road, Maduravoyal, Chennai – 600 095

JOURNAL PUBLICATION ABOUT ANNAPAVALA CHENDURAM

WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 6, 593-602. Review Article ISSN 2277– 7105

REVIEW ON ANNAPAVALA CHENDHURAM – A SIDDHA HERBO – MARINE FORMULATION

Sabari Girija N.[1]*, Guptaj S.[2], Sinekha M. A.[3], Shanmugapriya P.[4] and Madhavan R.[5]

[1]PG Scholar, Department of Nanju Maruthuvam, National Institute of Siddha, Chennai, Tamilnadu, India.

[2]Resident Medical Officer, Sri Sairam Siddha Medical College and Research Centre, Chennai, Tamilnadu, India.

[3]PG Scholar, Department of Noinaadal, National Institute of Siddha, Chennai, Tamilnadu, India.

[4]Associate Professor, Dept. of Nanju Maruthuvam, National Institute of Siddha, Chennai.

[5]Associate Professor, Head of the Department, Dept. of Nanju Maruthuvam, National Institute of Siddha, Chennai.

Article Received on
25 March 2020,
Revised on 15 April 2020,
Accepted on 05 May 2020
DOI: 10.20959/wjpr20206-17560

***Corresponding Author**
Dr. Sabari Girija N.
PG Scholar, Department of Nanju Maruthuvam,
National Institute of Siddha,
Chennai, Tamilnadu, India.

ABSTRACT

Siddha medicine is one of the oldest medical systems in the world. This system is most commonly practicing in India especially in southern regions. Annapavala chenduram is the herbo marine Siddha formulation belongs to the category of chenduram. Annapavala chenduram is the combination of Annabedhi and kodipavalam. Annabedhi is one of the uparasam (hydro chemicals) and pavalam is comes under the navamanigal (nine gems). As per Siddha literatute, Annapavala chenduram has the indication for kasam, swasakasam, Shayam, raktha ushnam, pitha ushnam, mega ushnam and aththi juram. This chenduram may be given for all diseases with suitable anubanam as an alternative medicine. Here, an attempt has been made to review the explored ethno pharmacological activities of the ingredients of Annapavala chenduram to strengthen the scientific facts favouring this formulation.

KEYWORDS: Annabedhi, kodipavalam, annapavala chenduram, review, siddha.

JOURNAL PUBLICATION ABOUT ANNAPAVALA CHENDURAM

AYUSHDHARA
ISSN: 2393-9583 (P)/ 2393-9591 (O)
An International Journal of Research in AYUSH and Allied Systems

Research Article

ANGIOTENSIN CONVERTING ENZYME INHIBITION POTENTIAL OF *ANNAPAVALA CHENDHURAM* FOR THE TREATMENT OF HYPERTENSION: AN IN-VITRO ASSAY

Sabari Girija N[1]*, Sinekha M A[2], Guptaj S[3], Shanmugapriya P[4], Madhavan R[5]

*[1]PG Scholar, [4]Associate professor, [5]Associate professor, Head of the department, Department of Nanju Maruthuvam, National Institute of Siddha, Chennai, Tamilnadu, India.

[2]PG Scholar, Department of Noinaadal, National Institute of Siddha, Chennai, Tamilnadu, India.

[3]Resident medical officer, Sri Sairam Siddha medical college and research center, Chennai, Tamilnadu, India.

KEYWORDS: *Annapavala chendhuram*, ACE inhibition, hypertension, in-vitro assay, Siddha.

***Address for correspondence**
Dr. N. Sabari Girija
Department of Nanju Maruthuvam, National Institute of Siddha, Chennai, Tamilnadu, India.
Mobile number: 9944895785
Email: drgirijakrish@gmail.com

ABSTRACT

Hypertension is the most noteworthy risk factor for cardiovascular diseases and stroke. Dietary and lifestyle changes play the foremost part to decrease the hazard of hypertension and other related wellbeing complications. Angiotensin Converting Enzyme (ACE) inhibitors play a major role in treating hypertension. *Annapavala chendhuram* is a herbo – mineral Siddha formulation comes under the type of 32 internal medicines of Siddha. Hypolipidemic activity of *Annapavala chendhuram* has been proven by some research studies. Hence, the purpose of the present study was to evaluate the ACE inhibition activity on *Annapavala chendhuram* by using an in-vitro assay. The ACE inhibition assay was evaluated by UV Spectrophotometry technique based on the hydrolysis of histidyl-hippuryl-leucine (HHL) by ACE. About 50µL test sample with varying concentration (100- 500 µg/ml) along with standard captopril (100µg/ml) added with 50µL of ACE and some process had continued. The present study indicates that the test drug *Annapavala chendhuram* was effective in inhibiting the enzyme ACE dose-dependently. Maximum percentage inhibition of about 53.24±8.403% was observed at 500µg/ml when compared to that of the Captopril, a standard ACE enzyme inhibitor agent with the maximum inhibition 86.98 ± 6.375 at the concentration of 100µg/ml. It was concluded that the test drug *Annapavala chendhuram* possess significant anti-hypertensive property in protein denaturation assay. So, further in-vitro evaluation of ACE inhibitory activity on Siddha herbal preparations and clinical trials will be the need of the hour.

INTRODUCTION

One of the largest single risk factors of deaths worldwide is hypertension. Cardiovascular disease and stroke are the world's biggest killers, attributing for a combined 15.2 million deaths in 2016. Globally these diseases have remained the leading causes of death within the last 15 years. Hypertension is that the most noteworthy risk factor for cardiovascular diseases and stroke.[1] Dietary and lifestyle changes play the foremost part to decrease the hazard of hypertension and other related wellbeing complications. However, therapeutic treatment may prove essential for patients whose lifestyle changes prove ineffective and inadequate.[2] Hypertension treatments have widely evolved from low sodium dietary regimens to an unlimited arsenal of contemporary pharmaceuticals. There are many choices for the treatment of hypertension. Some treatments include diuretics, β-blockers, calcium channel blockers and hypertension receptor blockers, the most common of which are angiotensin-converting enzyme inhibitors.[3]

The Angiotensin-Converting Enzyme (ACE), the component of the renin-angiotensin-aldosterone system (RAAS) plays a major role in regulating the blood pressure. Regulation of blood