PRECLINICAL SAFETY EVALUATION OF RASA KARPOORA KULIGAI

The dissertation Submitted by

Dr. R. MURUGAVEL, M.D(S)

Under the Guidance of

Dr.V. MANJARI. M.D(S)

Guide, Department of Nanju Noolum Maruthuva Neethi Noolum

National Institute of Siddha, Ch-47.

Dissertation Submitted to

The Tamil Nadu Dr. M.G.R. Medical University, Chennai – 32

For the partial fulfillment of the requirements to the Degree of

DOCTOR OF MEDICINE (SIDDHA)
BRANCH VI - DEPARTMENT OF NANJU NOOLUM MARUTHUVA NEETHI NOOLUM
2015-2018

NATIONAL INSTITUTE OF SIDDHA
Chennai – 47

DECLARATION BY THE CANDIDATE

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

Date: **Signature of the Candidate**

Place:Chennai-47

Dr.R.Murugavel

BONAFIDE CERTIFICATE

Certified that I have gone through the dissertation submitted by **Dr.R.Murugavel**, (**Reg.No: 321516206**) a student of final year M.D(S), Branch-VI, **Department of Nanju Noolum Maruthuva Neethi Noolum**, National Institute of Siddha, Tambaram Sanatorium, Chennai-47, and the dissertation work has been carried out by the individual only. This dissertation does not represent or reproduce the dissertation submitted and approved earlier.

Place: Chennai-47

Date:

Dr.V.MANJARI. M.D(S)
Name and Signature of the Guide
Department of Nanju Noolum
Maruthuva Neethi Noolum,
National Institute of Siddha
Tambaram Sanatorium,
Chennai – 47.

Dr.R.MADHAVAN. M.D(S)
N**ame and Signature of the HOD(i/c)**
Department of Nanju Noolum
Maruthuva Neethi Noolum,
National Institute of Siddha,
Tambaram Sanatorium,
Chennai-47

Forwarded by Head of the Institution
Prof.Dr.V.BANUMATHI. M.D(S),
Director,
National Institute of Siddha,
Tambaram Sanatorium,
Chennai-47.

ACKNOWLEDGEMENT

- I thank God for giving me this opportunity, providing the strength and energy to fulfill this commitment.
- I express my sincere thanks to the Secretary, Ministry of AYUSH, Health &Family Welfare, New Delhi for giving great opportunity to carry out P.G and this dissertation work in National Institute of Siddha, Chennai-47
- I express my sincere thanks to the Vice-chancellor, The Tamil Nadu Dr. M.G.R. Medical University, Chennai.
- I express my profound sense of gratitude to Prof. Dr. V. Banumathi, M.D(S), Director, National Institute of Siddha, Chennai-47 for granting permission to undertake a study in this dissertation topic and also for providing all the basic facilities in order to carry out this work.
- I express my grateful thanks to my Guide, Dr.V. Manjari, M.D(S), Lecturer, Dept. of Nanju Maruthuvam, National Institute of Siddha, Chennai-47., gave perceptive comments and constructive criticisms at different stages of my research which were thought to provoke and they helped me to focus my ideas.
- I express my sincere thanks to Lecturer Dr. R. Madhavan M.D(S), Head of the Department. i/c, Nanju Maruthuvam, National Institute of Siddha, Chennai-47 for the guidance, memorable support and eternal encouragement in carrying out this work.
- I would like to express my sincere thanks to my Dr. R. Renga Sundari, M.D(S), Associate Professor, Dept. of Nanju Maruthuvam, National Institute of Siddha, Chennai-47 for her encouragement in carrying out this work.
- Dr. P. Shanmugapriya M.D(S), Lecturer, Department of Nanju Maruthuvam, NIS, Chennai -47., for her encouragement in carrying out this work.
- I express my sincere thanks to Dr. S. Murugesan, M.D(S) Lecturer, Dept. of Nanju Maruthuvam, National Institute of Siddha for the guidance and encouragement in carrying out this work.

- I am thankful to Dr. D. Aravind MD(S) Assistant Professor, Dept. Of Botany, National Institute of Siddha, chennai-47.
- I thank Dr. A. Muthuvel, M.Sc., Ph.D. (Biochemistry) Assistant Professor, National Institute of Siddha, and Chennai-47 for his guidance in doing chemical studies.
- My special acknowledgments to Mr. M. Subramanian, M.Sc.,(Statistics), Senior Research Officer, National Institute of Siddha, Chennai-47, for his valuable help in statistical analysis.
- I thank Dr. V. Suba, M.Pharm., Ph.D., Assistant Professor, Dept.of Pharmacology, National Institute of Siddha, Chennai-47 for her interesting teaching of pharmacology and valuable guidance for doing this study.
- I thank the library clerk Mrs. V. Kalpana, Mr. J. Rathinam library attendant of National Institute of Siddha, Tambaram Sanatorium, Chennai-47, from where I derived much of the literary support.
- I gratefully acknowledge the assistance provided by all other faculties, Well-wisher and staffs of NIS, Chennai who rendered their cooperation throughout the course of study.
- I thanks to SAIF, IIT Madras, Chennai-36, to carry out elemental analysis of my test drug and The TN Dr.MGR Medical university to carry out physicochemical and phytochemical analysis. .
- I also thank all my friends who helped me throughout the study, without whom this work will be impossible.
- I am thankful to my parents for the courage they had provided to carry out this dissertation

CONTENTS

1. INTRODUCTION

Siddha System of Medicine is believed to be originated from lord shiva the supreme god of Tamil and also he is considered to be the principal siddhar. Lord shiva preached this grateful science to shakthi, the goddess and the nanthi from them the siddha system was made available to common people by siddhars.

Siddhars were those who were not only a physican but also social reformers. They were well versed in the field of medicine, natural science, alchemy, astrology, etc. Siddhars were the persons who attained siddhi, that perfection and who had overcome death through these siddha Medicines.

They give many excellent medicines which cure many challengeable diseases like diabetes, TB, HIV & Cancer.

It is estimated that at some point during lifetime approximately 39.6% of women and men will be diagnosed of cancer. In India, it is estimated that14.5 lakh people are living with the disease, with over 7 lakh new cases being registered every year and 5,56,400 deaths which are said to be cancer related. An estimated 71% of cancer related deaths are occurring in the age group between 30 and 69 years[1].

As rights said in this modern world the air we breathe, the food we eat and our life styles are all carcinogenic and are ultimately leading us by one way or other to dreadful disease cancer. Everyone in this world rich or poor, men or woman, young or old, and even animals or prone to affect by this disease and that could be prophylatically given against this disease. Also there is no medicine in the world that good completely cure this disease.

The Currently available treatments for Cancer viz Radiation, Chemotherapy and Surgery are all of very Limited Value. The Surgical Procedures are useful only is very early stage and it is miserable that the disease is often detected in advanced stage. The Radiation technique and Chemotherapy are useful only to prolong the survival period[2].

In the pathetic Situation when scientist all over the world struggling to formulate a new medicine to combat the disease. In our Siddha system of medicine we had thousands of medicine with the indications to cure the disease.

Among various medicine mentioned to treat cancer in Siddha literature. Rasa Karpoora Kuligai is one of the Siddha herbomineral formulation mentioned to treat various type of cancer[3]. Rasa Karpoora kuligai shows significant anti oxidant and

anticancer effect against HeLa cell lines studied by in vitro (V.Manjari,et.al ,2016,WJPR,Vol-v,Issue-9). The safety profile of Rasa Karpoora Kuligai is not scientifically validated. The author selected and Studied the safety profile of Rasa Karpoora Kuligai through acute and 28 days repeated oral toxicity studies as per OECD guideline 423 and 407[4].

2. AIM & OBJECTIVES

TITLE OF RESEARCH: Preclinical Safety Evaluavation of **"*Rasa Karpoora Kuligai"***

2.1. AIM:

To evaluate the safety profile of (Acute and 28days Repeated dose oral Toxicity study***) "Rasa karpoora kuligai"*** in Wistar albino rats.

2.2. OBJECTIVES:

- Collect the literature review of ingredients of *Rasa karpoora kuligai*
- Purification and preparation of the Medicine as per literature
- To study the quality parameters for **"*Rasa karpoora kuligai".***
- Acute oral toxicity study & 28days repeated oral toxicity study of "***Rasa karpoora kuligai "***as per OECD Guideline 423 & 407.
- Evaluation of safety of the study drug

3. REVIEW OF THE LITERATURE

3.1. RASA KARPOORAM (இரச கற்பூரம்)

3.1.1 GUNAPADAM ASPECT

Rasakarpooram does not find a place in the list of 64 padanas but it is one of the Pancha sootham. It's prepared by the combination of rasam and salt[3].

SYNONYMS:

"துர்க்கைகளையெலியிடைச்சனிபூரந்

தீட்டுவிடதாலிநக்கிபூடிமதாந்"

- நாமதீபநிகண்டு 29[5]

- Durgai
- Kalai
- Aellidai
- Sani
- Pooram
- Thettu
- Vidathaali
- Nacki
- Buddimaatham

RASA KARPOORA VAIPU MURAI: (Method of preparation)

METHOD I:

“தானென்றகற்பூரமொன்றுசொல்வேன்

சாதகமாய்சூதம்ரெண்டுதூக்கி

வானென்றசட்டிக்குள்மூன்றுபடியுப்பை

வளமாகபொடித்திட்டுநடுவேகேளு

தேனென்றசெங்கல்தூள்கால்படிதானிட்டு

திரமாகக்குளித்ததிலேசூதம்விட்டு

ஏனென்றமறுசட்டிகவிழ்த்துமூடி

இயல்பாகவெழுசீலைமண்ணும்செய்யே

மண்செய்துதொண்ணூறுகடிகையப்பா

வாகாகமூத்தீயுமெரித்துமைந்தா

மண்செய்தமேல்சட்டிக்குள்ளேகேளு

வாகாகஉரைத்திருக்கும்வெள்ளைமெதத்

மண்செய்தநற்பூரங்குழாயில்வைத்து

வாகாகபணவிடைதான்தூக்கிக்கொண்டு”

- **Agasthiyar paripooranam 400[6].**

2 palam sootham will be placed in the mud pot in between 3 padi culinary salt(Nacl) and brick stone powder. Close the pot with another mud pot and seal with clay plaster 7 times (man seelai). It will be burnt for 90 kadikai burn time after that cooled pooram was found deposited on the upper pot and the same was collected.

METHOD II:

மயக்கமுறுமிரதகற்பூரஞ்சொல்வோம்

வியாதியெல்லாந்தீர்ந்துபோம்வரிசைகேளு

மயக்கமுறஉப்புளுக்குச்செங்கல்தூளும்

வரிசைபெறவழித்தெடுத்துவைத்திடாயே

வைத்ததோர்இருவகையுந்தூளாயாட்டி

மைந்தனேசட்டிக்குள்பாதிநீயும்

வைத்திடுவாய்அதிற்குகையைச்சூதமப்பா

வாராடாகழஞ்சுபத்துமாற்றமன்றி

வைத்தங்கேகுகைவாயில்பொடியுமிட்டு

மறுசட்டிகொண்டிட்டுவாயைமூடி

வைத்துநீயேழுசீலைமண்ணும்பூசி

மாயிபதம்பூசித்துஅடுப்பிலேற்றி

அடுப்பேற்றிகமலவன்னிதினந்தானொன்று

அதன்பிறகுகடாக்கினியுந்தான்மூன்று

எரித்தாறியெடுத்துப்பார்சட்டிமேலே

ஏறிநிற்கும்கற்பூரம்எடுத்துவைத்து[5]

10 kalanju of sootham placed in mud pot in between the well powdered culinary salt and brickstone powder. Then close the mud pot with other mud pot then surmounted. The whole set up is burn as deepackini kamalackini and kaadackini for 3 days respectively. After it is cooled with the 7 times of clay plaster. The pooram was found deposited on the upper pot and the same is collected.

METHOD III:

Sulphur 67.2gm is melted in mud pot and mercury 336gm is added to it and grinded well and there forms a black coloured powder (kajali). Brickstone powder is placed up to half of the level of a pot culinary salt (Nacl) 650 gm is placed over it. Mercury sulfer camphor is placed over the salt and sealed with mud paste cloth. It burnt for 12 hours with kadackini after it is cooled the pooram (mercurous chloride) is found deposited on the upper pot and the same is collected.

OTHER CANCER MEDICINES WHICH CONTAIN RASA KARPOORAM AS CHIEF INGREDIENT:

1. CHANDA MAARUTHA CHENDOORAM**:**

Dosage	:	½ - 1 kundri
Adjuvent	:	palm jaggary, tripala legium, panjadepakkini legium.
Indication	:	Aruvagai putru (six types of cancer) Pavuthiram (fistula) Paarisavaayu (hemiplegia) Mugavadham (facial palsy)

2. VAAYU MAATHIRAI**:**

Dosage	:	½-1 maathirai
Adjuvent	:	Palm jaggary, sukku paste.
Indication	:	Uterine cancer (Allgul putru) Penial cancer (Linga putru) Necrotic ulcer (Ali ranam)

3. CHITHIRAMOOLA KULIGAI**:**

Dosage	:	1 Melagu
Adjuvent	:	Palm jaggery
Indication	:	Uterine cancer (Yoni putru),
		Penile cancer (Linga putru),
		8 types of ulcers (Gunmam).

4. KOOROSANAI MEZHUGU**:**

Dosage	:	3-5 kundri
Adjuvantent	:	Sugar, palm jaggery
Indication	:	Uterine cancer (allkul putru)
		Syphilis (kiranthi)
		Amenorrhoea (soodaka kattu)

5. POORA KATTU

Dosage	:	1-4 rice weight
Adjuvantent	:	parangipattai legium, vaalmilaggu legium.
Indication	:	Uterine cancer (allgul putru)
		Syphilis (Meganoi)
		Urinary bladder ulcer (neerpai ranam)

6. VETTAI MEZHUGU**:**

Dosage	:	1/2 – 1 kundri
Adjuvantent	:	palm jaggery, butter
Indication	:	vettai (venelial diseases)
		Aan kurri pan (penear ulcer)
		Neerthaarai pun (ureter ulcer)

PROPERTIES:

Colour	-	White
Appearance	-	Heavy white rhombic crystals
Potency	-	Hot
Taste	-	Salty, Pungent

ACTION:

- Laxative
- Tonic
- Antiseptic
- Diuretic

It is also an after excessive bile producer.

THERAPEUTIC EFFECTS:

"இடைவாதசூலையெரிசூலைகுன்மந்

தொடைவாழைவாதமாஞ்சோணி – யிடையாதோ

வொக்குரசகர்ப்பூரமொன்றேயனவொருதல்

இக்குவெல்லத்தேழுநாளீ".

When calomel is taken along with jaggery for seven days,

- It cures various types of throbbing pains,
- Throbbing pain in the lumbar region,
- Burning sensation,
- Ulcer due to disorders of vatha humours[7],

In siddha system of medicine his compare to sanjeevi because it cures disease like syphilis(kiranthi), vulvar cancer (allgul putru), vulvar ulcer (allgul ranam) and chronic non-healing ulcers.

- AVN 4th vol

PURIFICATION OF CALOMEL:

METHOD I:

The poultice made of betel leaf (piper betel) and pepper (piper nigram) each 8.75gm, is taken and dissolved in 1.3 litre of water. Take 35gm of calomel and tied with a cloth and immersed in the above liquid from the crops bar and heated. When water is reduced to ¾ of its volume the calomel is taken out washed with water and allowed to dry under sunlight[3].

METHOD II:

35 gm of Calomel is consolidified in mother's milk for 3 hours and again it is consolidified in garlic oil(thailam) for 9 hours[3].

SIGNS AND SYMPTOMS OF CALOMEL POISONING:

Medicine prepared from not properly purified calomel will cause Multiple red boils on the face, ache formation, ulcers in the chest, mouth and tongue, diarrhoea and dysentery, scrotal swelling and ulcer in the vulva.

ANTIDOTE FOR POISONING:

Nilapanai kilangu (Curculigo orchides)	-	8.75gm
Vallarai ver (Centella asiatica root)	-	8.75gm
Ponnankanni ver (Alternatheria sessiles)	-	8.75gm
Kanduparangi ver (Clerodendrumserratum)	-	8.75gm

All the above ingredients are mixed and boiled to make a decoction. This decoction is used twice a day for two or three weeks with suitable diet restrictions.

3.1.2 GEOLOGICAL ASPECT

MERCURY SUBCHLORIDE:

Mercury sub chloride is a chemical compound with the formula Hg_2cl_2. Also known as calomel (a mineral form rarely found in nature)

IUPAC name	:	Dimercury dichloride
Other name	:	Mercurous chloride
		Calomel
		Horn mercury
		Mercury (I) chloride
Chemical formula	:	Hg_2cl_2
Molar mass	:	472.09 g/mol
Appearance	:	Heavy white rhombic crystals
Density	:	7.150 g/cm^3
Melting point	:	525°c
Boiling point	:	383 °c
Solubility in water	:	0.2 mg/100ml
Solubility	:	Insoluble in ethanol, ether
Refractive index	:	1.973[8]
LD_{50} median dose	:	210 mg/kg (rat, oral)

OCCURRENCE:

- Haria,
- Obermoscuel,
- Armaden in Spain

PREPARATION OF MERCUROUS CHLORIDE:

It is the most important mercurous compound. In nature it occurs as horn quick silver. It is prepared by

1. $HgSo_4 + 2Nacl + Hg \rightarrow Hg_2cl_2 + Na_2So_4$

Subliming a mixture of mercuric sulphate, mercury and sodium chloride in iron pot

2. $Hgcl_2 + Hg \xrightarrow{\Delta} Hg_2cl_2$

By heating an intimate mixture of Hg_2cl_2 and Hg in appropriate mixture proportions in iron pot.

3. $Hg_2(NO_3) + Nacl \rightarrow Hg_2cl_2 + 2\,NaNo_3$

$Hg_2(NO_3) + 2Hcl \rightarrow Hg_2cl_2 + 2HNO_3$

By adding a soluble chloride, Eg: Nacl or dill Hcl to the solution of $Hg(NO_3)$.

4. $2Hgcl_2 + 2H_2O + SO_2 \rightarrow Hg_2cl_2 + 2Hcl + 2H_2SO_4$

By reducing $Hgcl_2$ solution with SO_2[8]

ACTION:

- Cathartic
- Alterative
- Diuretic
- Antiseptic
- Anthelmintic.

INTERNAL USES:

- Constipation,
- Cholera,
- Dysentery,

- Cardiac dropsy,
- Pleurisy,
- Malign fever
- Malaria,
- Syphilis,
- Gout,
- Worms,
- Cholelithiasis,
- Mitral insufficiency,
- Eclampsia,
- Gravidorum.

EXTERNAL USES:

- Small pox pitting,
- Pruritus,
- Diphtheria,
- Syphilitic ulcer,
- Myiasis,
- Membrane croap,
- Condylomata warts[9]

3.1.3 USAGE OF CALOMEL IN VARIOUS ASPECTS

Calomel was a mercurial compound used extensively by both union confederate doctors to treat wide variety of medical conditions. It came in two main forums. “Blue Pills” contained a mixture of mercury rose water, licorice, powdered rose, honey and sugar. “Blue Mass” was a lump of Mercurous chloride from which dispensing doctors pinched of a place. Doses were never standard.

In 19th century doctors feared constipation and dispensing calomel was one of the main methods of keeping the bowl open. However, strangely enough, calomel was also given to treat diarrhoea and dysentery. Many physicians seemed unaware that the doses of calomel and a related compound, tartar emetic, often caused worse problems that the original conditions.

The large and frequent doses of mercury compounds cause excessive salivation often a pint to a quart a day. Many patients receiving these "heroic" doses, suffered from mercurial gangrene death of cheek and mouth tissue that often led to permanent facial deformities. Lose and lost teeth were common, as was death from mercury poisoning.

In 16th century paracelsian and china physicians are used to treat malaria and yellow fever and a preparation called "worm chocolate" and "worm candy" was given to patients infested with helminthis.

Toxic effects were soon noticed in individuals given large doses for long periods, in which excessive salivation, gum inflammation, loosening of teeth, gastro-intestinal upset and an ashen appearance developed. They had troubling neurological symptom, such as arm and facial tremors, hyperfine, ataxia and erethism, unusual timidity and personality change.

The toxic potential of calomel was highlighted in 1948. A Cincinnati paediatrician discovered that a common infantile and childhood illness called "Aerodynia" or "Pink disease" was caused by the white spread use of calomel in treating childhood teething and constipation. As late as 1950 acrodynia accounted for more than 3% of admissions to children ward's in London hospitals. Official statistics record that 585 children died of pink disease between 1939 and 1948 in England and waves[10].

"Effect of dose not in proportion to size" well – triturated doses better than large coarse one.

3.2ALLIUM SATIVUM –வெள்ளைப்பூண்டு

3.2.1 GUNAPADAM ASPECT

VELLAIPUNDU SYNONYMS:

வெள்ளுள்ளி தன்னுடைய விவரங்கேளு
மேதினிக்குறை சோனா லசு நோலி'டோம்
மள்ளுள்ளி மதுசிய கெந்தாங் கேள் அஸ்தமாம்
மகாகந்தோய்கோ நேர் நியாகு கிரஞ்சனோ
மள்ளுள்ளி தீபத்திரக்கமாகுஞ்
செப்பியதோர் வெள்ளுள்ளிச் செயலுமாமே.

- Bogar nigandu 1200

SYNONYMS:

- Kuraisonalasu
- Nolistoo
- Madhusiya kaentharam
- Kael astham
- Maha kanthoo
- Koonaerniyaa
- Kiranchunoovaa
- Kaayam
- Ulli
- Pundu
- Vellai pundu
- Velvengayam

VERNACULAR NAME

Eng	:	garlic
Tel	:	thella, gadda , velulli
Mar	:	lohson
Mal	:	vellulli
Kan	:	bellulli
Sans	:	lasuna
Hind	:	lashan

HABITAT:

It is cultivated all over India. It is more pungent than onion. Nilakiri pundu is more popular.

PARTS USED:

Bulb and oil.

ORGANOLEPTIC CHARACTERS:

Colour	-	White
Odour	-	Garlic Odour
Taste	-	Pungent (karppu)
Character	-	Veppam
Pirivu	-	Pungent[11]

- Gunapadam Mooligai Vaguppu

CHARACTERS:

Taste - pungent,

Potency Character-Hot potency

Biotransformation-pungent

GENERAL CHARACTERS:

- Garlic used for deafness chronic cough, asthma dysentery, headache, cyanicites, haemmoroids.
- It also used for vadha diseases, kapha headache and oral diseases.
- Garlic taken along with pepper and karichallai for stomach problems
- 1 to 2 drops of garlic juice droped into ear for ear diseases and deafness
- garlic juice processed along with gingelly oil and used for ear diseases
- 20 to30 drops of garlic juice used 2 to 3 times per a day for chronic cough, asthma.
- Garlic juice processed with mustard or coconut oil then it is externally used for body pain, pricking pain, whezzing, vadha diseases.

ACTIONS:

- Carminative,
- Stimulant,
- Expectorant,
- Anthelminthic,
- Anti oxidant,
- Stomachic,
- Tonic,
- Alterative,
- Diuretic[11].

MEDICINAL USES:

- Clove of garlic was known as a home remedy in olden days in the east and is one of the most useful on account of it s prophylactic and curative properties.
- The garlic oil capsules product the human body from the attacks of bacteria and bacillae in times of epidemics or when the danger of infection is prevalent and containing all the curative properties.
- These capsules renew the blood cleanse if of all impurities regulate the digestion and remove all parasites in the intestines which might be injurious to health
- The capsules recommended for diseases of the lungs arterio-sclerosis high blood pressure, gout, rheumatism, asthma, chronic bronchial catarrh, intestinal complaints, loss of appetite, constipation and worms.
- The oil from seeds is prescribed internally as a febrifuge to prevent recurrence of the cold fits of intermittent fever
- Externally it is used in paralytic and rheumatic affections as resolvent the garlic is applied to indolent tumours.
- Internally garlic given with common salt in affection of the nervous system headache flatulence hysteria cough etc.
- In emergency conditions it is applied like onion to the nose in cases of fainting
- Externally the juice used as a rubefacient, liniment acts very beneficially in infantile convulsions other nervous and spasmodic affections relax sore throat in asthma, general paralysis, facial paralysis, gout and sciatica
- It is used for skin diseases including leprosy.
- When eaten in cold season it is said to ward off attacks of rheumatism and neuralgia
- Garlic produce copious dieresis and therefore it is used in dropsy and anasarca

- Garlic juice mixed with 3-4parts of ordinary or distilled water has been used as a lotion for washing wounds and foul ulcers[11]

OTHER SIDDHA FORMULATIONS:

1. KAALAMEGA NARAYANA CHENDOORAM:

Dosage : 30-100mg

Adjuvant : Thippili powder

Indications : Uterine cancer, Cheek cancer

2. THAMIRA CHENDOORAM:

Dosage : 30-45mg

Adjuvant : Garlic juice

Indications : Weakness

3. PANCHA SOODHA MELUGU:

Dosage : 50-100mg

Adjuvant : Garlic juice

Indications : Vadha diseases, five types of pain

4. PASAANA PARPAM:

Dosage : 1 milagalavu

Adjuvant : Garlic juice

Indications : Many diseases

5. KANTHAGA MELUGU:

Dosage : 2-3 Kundri

Adjuvant : Garlic juice

Indications : Leprosy, Diabetes insipidus[12]

3.2.2. BOTANICAL ASPECTS:

ALLIUM SATIVUM

Taxonomy Classification

Kingdom : Plantae

Division : Angiosperms

Class : Monocots

Order : Asparagales

Family : Amaryllidaecae

Subfamily : Allioideae

Genus : Allium

Species : A.sativum

Vernacular name

Eng : Allium sativum

Sans : Lacuna, Ugragandua, Bhutagua, Mahusudra, Rosanam

Hind&Bom : Lasan

Sind : Thum

Per : Sir

Gug : Lasan, Shutam

Mah : Lasan

Tel : Vellulli, Tellagadda

Tam : Vellapundu, Vallai Pundu, Ulli Pundu

Mal : Vellulli

Can : Bellulli

Ben : Rasan[11]

Parts used:

Bulb

Distribution:

Garlic is among the oldest knowing horticultural crop. In the old world Egyptian and Indian cultures referred to garlic 1000 years ago and there is clear historical evidence for it use by the Barsilonians 4500 years ago.

Garlic grows wild only in Central Asia (Centered in Kyrgyzstan) today. Earlier in history garlic grew wild over a much larger region and, in fact, wild garlic may have occurred in an area from china to India to Egypt to the Ukraine. It's cultivated all over the world in Spain, France, Egypt, Bulgaria, Hungary, USA, Mexico and Brazil.

HABITAT:

Garlic is perennial of the lily family. It grows to a height of about 60 cm. It has short, flat upright leaves 15-30cm. The tall single flower stem bears spherical head of pale pink or greenish- white blooms often mixed with tiny bulbis the subterranean white-skinned bulb or corn is subdivided into numerous "cloves"[13].

ACTION:

- Hot,
- Stimulant,
- Carminative,
- Emmenagogue,
- Antirheumatic,
- Anthelmintic,
- Alterative[11].

Organoleptic properties:

The bulbs are pinkish-white colour and are odoriferous. The size of the bulb varies in between 1.5-2.5 cm.

Morphology \Macroscopical Characters:

- The bulb grows at the base of a perennial plant with an erect flowering stem that grows 2-3 ff long.
- The bulb is made up of several outer thin protective sheaths covering the inner sheathes. The inner sheath covers the swollen leaves called as cloves. The mature bulb has around or more cloves in each bulb.
- The cloves gave no symmetry except for a few present in the centre.

Microscopic characters:

The bulbs are covered by an outer scale. The outers scale is made up of an epidermis which encloses a mesophyll (devoid of chlorophyll), a ground tissue and below it is a layer of lower epidermal cells.

The dry scales also contain about 2-3 layers of rectangular cells.

The rectangular cells may have many rhomboidal crystals of calcium oxalate

The epidermal cells contain parenchymatous cells connected to several rectangular cells and vascular bundles made up of alternating xylem and phloem.

The epidermal cells contain thick pitted walls.

The lower epidermis consists of smaller rectangular cubical cells[14].

Phytochemistry:

Garlic bulbs are made up of numerous minerals, vitamins, carbohydrate, amino acids, volatile oils and other trace elements.

Amongst all the members of the Allium species, garlic is said to have to the highest sulphur content.

Volatile oils are present in about 0.1-0.5% concentration in garlic. These constitute of sulphur containing compounds like diallyl sulphide, diallyl trisulphide, methyl allyl sulphide, allyl propyl disulphide, allin, ajoene etc.

When the garlic clove is crushed allin (s-allyl-1-cysteine sulpoxide) by the action of the enzyme allinase gets converted to 2-propene-2-sulfenic acid which in turn dimerizes to allicin (diallyl thio sulfinate)

Allicin is responsible for the pungent odour of crushed garlic and also for zone of its pharmacological activities of garlic.

Vitamins like vitB, VitA, VitC etc, 17 amino acids including 8 essential amino acids and minerals like Phosphorus, Calcium, Magnesium, Potassium, Iron, Selenium, Germanium etc are present[15].

Garlic medicinal uses

In Hypercholesterolemia– Garlic has said to lower cholesterol levels. The proposed mechanism for this is that the diallyl disulphides and diallyl trisulphides present in garlic oil interfere with the factors normally responsible for lipid synthesis. Garlic can reduce the activity of the thiol group in the enzymes in the body. Garlic oil can also carry out oxidation of NADPH. Thus, both the above activities interfere with normal lipid synthesis and blood lipid levels are reduced. These thiol containing enzymes are HMG-CoA reductase and coenzyme A which are essential enzymes for cholesterol biosynthesis.

Other proposed mechanisms for reducing lipid levels are increased loss of bile salts in the feces. It has also been suggested that mobilization of these lipids into circulation can also reduce lipid levels.

As Antithrombotic agent– **Ajoene** (4, 5, 9-trithiadodeca-1, 6, 11-triene-9-oxide) present in garlic is considered to inhibit platelet aggregation and is the most potent antithrombotic component of garlic. **Methylallyltrisulphide** present also acts as an antithrombotic agent. The suggested mechanism for this is the interference with thromboxane synthesis.

It also shows fibrinolytic properties thereby helping in clot degradation.

Antimicrobial properties– Garlic shows antimicrobial activity against various pathogens such as bacteria including resistant types, fungi, virus etc. It is active against both gram positive and gram-negative bacteria and strains of Mycobacterium. These effects are seen due to ajoene present in garlic. Allicin also shows the antimicrobial properties by inhibiting thiol containing enzymes thereby affecting protein, DNA, RNA synthesis.

Due to the antifungal properties, garlic has been proposed for the treatment of oral and vaginal candidiasis.

Garlic is active against various viruses such as herpes simplex virus type I and II, Influenza B virus, parainfluenza virus, cytomegalovirus, human rhinovirus type 2 etc.

Chemoprotective properties– Animal studies have shown that garlic has positive effects against hepatotoxins. The reaction of allicin in garlic with the sulfhydryl groups contribute to the inhibitory effect. Sulfhydryl groups concentration is high in rapidly dividing cells.

Immunity– Immunity is increased due to a number of factors on consumption of garlic. Selenium and germanium present in garlic are said to be responsible for immunologic activities. Enhanced phagocytosis, increased killer cell activity,

lymphocyte proliferation, increased production of cytokines and reduction of immune suppression are the suggested mechanisms via which garlic increases immunity.

Antioxidant properties– Allicin present in garlic is responsible for the increase of catalase and glutathione peroxidase enzymes which are two important antioxidant enzymes in the body. The other sulphur compounds may also show potential antioxidant properties by inhibiting lipid peroxidation in the liver and preventing a reaction which is considered to be one of the main features of aging in liver cells[16].

3.3. PIPER BETLE (வெற்றிலை)

3.3.1. GUNAPADAM ASPECT

VETRILLAI SYNONYMS

வெற்றிலையின் பேர்தனையே விளம்பக்கேளு
வேண்முயதோர் தாம்புல மாதாவாகும்
சித்திலையாஞ் சாதகலட்சுமி யாகும்
தாம்பூலக் சண்ணி நல நாகவல்லி
ஒத்திலைவோம் புலக்கன்னியாகும்
உறுபல்லுக் கழகிதான் தேகரக்சிகயாம்
நத்தலை நாகத்தை சூரணமாக்கி
நலங்கியதோர் வெற்றிலையின் நாமமாமே

Synonyms:

- Thamboolam
- Thamboola valli
- Thirayal
- Mellilai
- Vellilai
- Melladagu

Vernacular names:

Telugu	:	Tamalapaku
Kannada	:	Vilaya
Duk	:	Thambole
Bengali	:	Pan
Hindi	:	Paan
Sanskrit	:	Tambula and nagavalli
Gujarathi	:	Naagarvel na paan[11]

Types:

Vetrillai	:	Migundha manamum, kaaramum, nivamum illatuattu
Kammaru vettrilai	:	Karuppu niramum, karppum udayatu
Karpoora vettrilai	:	Karpoora manamum siru kaaramumudaiyatuu

Habitat:

In India it's cultivated in hot areas and saduppu nilam. It's a type of climber. It's cultivated for its leaf.

Parts used:

- Leaf

Properties:

Taste(suvai)	:	Uraippu, Pungent (karppu)
Quality(thanmai)	:	Veppam (hot)
Pirivu (postdigstive effect)	:	Pungent (karppu)

Action:

- Stimulant,
- Carminative
- Astringent,
- Aphrodisiac,
- Antiseptic,
- Febrifuge,
- Stomachic,

General properties:

ஐயம் அறுங்கான் அதன் சாரங் கொண்டக்காற்
பையச் சயத்தியம்போம் பைந்தொடியே! – மெய்யெண்
கடியின் குணம் போகும் காரவெற்றி லைக்குப்
படியுமுத் தொடமிதைப் பார்

- Juice of betel leaf cures Iyyam, saytthiyam, kanaakkadi, muppini, urticaria.
- Betel leaf juice cures sinusitis, head ache, stomach pain, sore throat, muppini, maantham and vayitruppisam.

Therapeutic uses:

- For sore throat and thondaiyaddaippu betel leaf is taken along with saambrani pathangam.
- 2-3 drops of betel leaf juice on ear for ear ache.
- Betel leaf juice give along with ginger juice for lung disorders.
- It is externally used for burns.
- Heated betel leaf soaked with gingely oil applied over chest helps to relieve cough, difficulty to breathe.
- Koorosani taken along with betel juice for dyspnoea, cough and cold.
- Mercury purified by betel juice[11].

Other Medicinal uses:

It is useful in bronchitis, asthma, catarrh, cough, leprosy, skin diseases, alcoholism, syncope, otalgia, fever, halitosis, impotency, rheumatism, dyspepsia, indigestion in children, pharyngitis, laryngitis, obesity, conjutivitis, night blindness, glandular swelling oil of betel has been used in the treatment of various respiratory diseases and as a local application either by gargle or by inhalation in diphtheria. It has carminative properties oil shows marked irritant action on skin and mucous membrane. It has an antispasmodic action on involuntary muscle tissue, inhibiting excessive peristaltic movements of intestinal[17].

BETLE LEAF ADDED SIDDHA FORMULATIONS (AS A ADJUVANT):

1. ABRAHA PARPAM:

   Dosage : 100-200mg

   Adjuvant : Honey

   Indication : Diabetes Mellitus and Diabetes Insipidus

2. POORNA CHANTHROTHAYAM:

   Dosage : 100-200mg

   Adjuvant : Betel Leaf juice or Karpoorathi chooranam

   Indication : Tuberculosis, Dysentery, Fever

3. KASTHURI MATHIRI:

   Dosage : 1-2 pills

   Adjuvant : Betel Leaf juice or Honey

   Indication : Heart problems, intermittent fever, fits

4. SAANTHA SANTHROTHAYAM:

   Dosage : 1-2 pills

   Adjuvant : Betel Leaf juice or Honey

   Indication : Liver diseases, constipation

5. SAMBARANI POO MATHIRAI:

   Dosage : 1 pill

   Adjuvant : Betel Leaf or Pepper powder

   Indication : Dysmenorrhea, Amenorrhea[12]

3.3.2 BOTANICAL ASPECT

PIPER BETLE

BOTANICAL ASPECT

Botanical name	:	Piper betel

Taxonomy classification:

Kingdom	:	Plantae
Division	:	Angiospermae
Class	:	Magnoliidae
Order	:	Piperals
Family	:	Piperaceae
Genus	:	Piper
Species	:	Betel
Botanical name	:	Piper betel

Vernacular name:

English	:	Betel Pepper
Ayurvedha	:	Tambula, Nagavallari Nagini, Tambulavalli, Saptashiva,
Siddha	:	Vertillai, Naga Valli, Kanmaru Vetrillai
Unani	:	Pan, Tambool
Telegu	:	Tamala Paku, Tamula Paku
Marathi	:	Pan, Nagvel Vidyachepan
Kannada	:	Veelyade Ele
Common indian name	:	Paan / Pan

Distribution:

- A native of java, it is cultivated in India in Assam, west Bengal, Bihar, Uttar Pradesh, Madya Pradesh, Maharashtra, Karnataka, tramlined and Kerala.

Botanical description:

- Perennial, creeping herbs, stems semi woods climbing by short adventitious root leaves broadly ovate, slightly cordate, acuminate (or) acute entire, globroas, pentiolata, mace spikes dense, cylindrical, female spikes 2.5-5 cm long pendulous fruits rarely produced often sunk in the fleshy spike, forming nodule like structure

Parts used:

- Whole plant and leaf[11]

Pharmacognosy:

- Leaves coriaccous, 10-18 by 5-12.5 cm broadly ovale acuminate, glabrous, 5-9 nerved the suprabasai nerves usually alternate base slightly cordate (or) usually rounded more (or) oblique, pentioles 1.3-2.5 cm.
- Microscopically leaves shows a dorsiventral structure. The cutice is hot striated, the hypodermis contains secretorycells. The petiole shows a discontinuous collenchyma zone below the epidermis. Mucilage canals at the centre, vascular bundles graded in size arranged in arcs and multicellular hairs[18].

Chemical constituents(phytochemical):

- Vitamin A &C, thiamine, riboflavin, nicotinic acid, glucose, fructose, maltose, sucrose malic acid, oxalic acid, amino acids viz leucine phenylalanine, alanine, arginine, threonine, serine aspartic acid, glutamic acid, methionine, valine, tyrosine, asparaginase, glycine, proline, etc[19].

Pharmacological activities:

- Fungicidal
- Hematicidal
- Anti-bacterial

- Anti-fungal
- Hypotensive
- Cardiac and respiratory depressant
- Anti- biotic
- Mild anti infertility
- Anthelmintic
- Anti tuberculous
- Anti-microbial
- Cardiotonic
- Stomach muscle relaxant[20]

Action and uses:

- When leaf is chewed, the mild anti – infective content in the leaf. Freshen breath and cleanse mouth. Its constituents directly enter the blood via buccal mucosa as this is a direct way of entry into blood stream, it is the best way to deliver drugs into the blood stream during sickness.
- The leaf contains several polyphenols that not only fights microbes, but also act as pain relivers and anti-inflammatory agent. Recent studies have shown that the leaf contains tannins, sugar, diastase and an essential oil.
- A particular phenol called chavical present in it has power full antiseptic painful are for temporary relief. Betel leaves possess good diuretic properties. Therefore, it can be mixed with dilute milk and consumed by sweetening as it helps ease urination.
- The oral intake of leaves decreases cough and reducing swelling of throat and throat irritation[11].

3.3.3 SCEINTIFICALLY PROVEN PHARMACOLOGICAL ACTION OF BETEL LEAF

Anti oxidant property:

- The is reported das gupta and de (2004) evaluated the antioxidant activities of aqueous extract of the piper betel leaves by invitro methods - DPPH radicals scavenging activity, Superoxide scavenging activity and Hydroxyl radical scavenging activity. Finally, the piper betel Showed very high antioxidant activity.
- Wong et al (2006) investigated the antioxidant properties of 25 edible tropical plants including piper betel using DPPH and FRAP assays. Total phenolic content was also estimated. Piper betel showed very high antioxidant activity.
- Rathee et al (2006) chevibetol, allyl pyrocatechol and their respective glucosides were isolated from betel leaves. Among the isolated compounds allyl pyrocatechol showed the best results in all the invitro experiments. It could prevent Fe(II) induced lipid peroxidation (LPO) of liposomes and rat brain homogenates as well as gamma ray induced damage of PBR 322 plasmid DNA more efficient then chevibetol.
- Maniguha et al (2009) evaluated the antioxidant of piper betel leaves. Ethanolic extract of piper betel leaf showed strong antioxidant activities like reducing power DPPH radical, superoxide anion scavenging and deoxyribose degradation activities when compared with different standard such as ascorbic acid, DMSO and BHI[21].

Anticancer property:

- The leaf constituents, hydroxyl chavicol and β carotene were found to reduce the number of papilloma per mouse
- Also, the constituents, β carotene and α tocopherol are reported to significantly inhibit DMBA induced skin tumour formation by 83-86 and 86% in swigs mice and 92.94 89% in male swigs bare mice respectively.
- While hydroxyl chavicol showed 90% inhibition in swigs bare mice.
- The constituent eugenol shows minimal protection in both the strains of mice.
- The leaf extract of betel quid when administered simultaneously while mutagenic tobacco specific N nitrosamines (present in the extract of chewed tobacco) viz N-nitrosonornicotive (NNN) and 4 erotogenic affects and reduce the tumour incidence in mice.
- The leaf extract is reported to exhibit antitumor activity in 7, 12, dimethyl benzene and anthracene (DMBA) treated wistar rats.

Anti microbial:

- The betel leaf oil posses' strong antibacterial activity against the gram-positive bacteria bacillus subtills, bacillus pumillus staphylococcus aurous, salmonella typhi, vibrio cholera and several other pathogenic microorganisms.
- The essential oil was also found to be more effective against tape worms (taenia solium) and hook worms (bunostomum trigonocephalum) than the synthetic anthelmintic, piperazine phosphate and hexyl resorcinol[22].

3.4. MILAGU *(மிளகு)*

3.4.1.GUNAPADAM ASPECT:

MILAGU SYNONYMS:

மிளகினுடப் பேர்தனையே விளம்பக்கேளு
முதிர்ந்து நின்ற திரை போக்கி மரிசியாகும்
வளகினுட வலசமுமா தீட்சணமாகும்
மகத்தானது வன்மாஞ் சியாமமாகுஞ்
குளகினுட மூணமாம் சத்துவ நே'ங்
கோலக மாஞ்சரதுந் தனியுமாகும்
வளகினுட வாதத்தை யறுக்குகின்ற
மகத்தான மிளகுக்கு நாமமாமே

- Thiraipokki
- Marisi
- Valasam
- Thetsanam
- Thuranam
- Seyamam
- Mooshnam
- Sathuvanesam

-Gunapadam Mooligai Vaguppu

சொல்லியதோர் அருட்டனென்றும் இதற்குப் பேரு
சொற்பெரிய மதங்கன் என்றும் பேருண்டாகும்
அல்லிய தோர் மலைத்திருக்க னென்றும் பேரு
அ'டமாசாதி யென்றும் இதற்குப் பேரு
கல்லியதோர் கத்திரிச னென்றும் பேரு
கருத்துரட னென்றும் நேர்வளந்தா னென்றும்

மல்லயதோர் கெந்தக னென்றிதற்குப் பேரு

வசனித்தோம் மிளகினிட அதீதப் பேரே.

- Panchakaviya Nigandu

- Arutan
- Mathengan
- Malaithirukkan
- Astamasathi
- Kathirisan
- Karvuthurudan
- Nerrvalandan
- Kanthakan

Properties:

Taste	:	Bitter, Pungent
Character	:	Veppam
Class	:	Pungent
used part	:	Seed

Action s:

- Acrid
- Carminative
- Stimulant
- Antiperiodic
- Resolvent
- Rubefacient
- Antivatha
- Antidote.

Vernacular Names:

Tamil	:	Milagu
Eng	:	Pepper
Jehegu	:	Miriyalu
Malayam	:	Kurumilagu
Kannadam	:	Menasy
Sanskrit	:	Maricha
Hindi	:	Kali mirich
Persian	:	Filfliaisiah
Bengoli	:	Glomorich, Morich, Kalamorich
Gujarathi	:	Kalimori
Urdu	:	Fulfil Sioyah, Kalimirich[11]

General Characters:

‘சீதசுரம் பாண்டு சிலே‘மங் கிராணிமூலம்
வாதம் அருசிபித்தம் மாமூலம் ஓதுசன்னி
யாசம் அபஸ்மாரம் அடன்மேகம் காசம் இவை
நாசம் கறிமிளகி னால்’’

‘கோணுகின்ற பங்கவலி குய்யரோ கம்வாதம்
சோணிதங்க ழுத்திற்குள் தோன்றும் நோய் - காணரிய
காதுநோய் மாதர் குன்மங் காமாலை மந்தம் என்றீர்
ஏதுநோய் காய் இருக்கில் ஈங்கு.

It cures the malarial fever, anemia, diarrhoea, piles, ulcer, flatulence, anorexia, diabetes, cough, hemiplegia, vaginal disease, neck and nasal disorders, jaundice, pitham, vatham, vedhasonitha noi, and sanni.

Therapeutic uses

- ❖ It is prescribed in cholerae, dyspepsia, flatulence, aliments.
- ❖ An infusion of black pepper forms a useful stimulant gargle in relaxed sore - throat and hoarseness dependent there on and in toothache also.
- ❖ Piperine is given with much benefit in gonorrhoea, haemorrhoids etc in doses of 3 to 10 grains.
- ❖ In Intermittent fever black pepper in doses of about a drachm is recommended to be given with the juice of leaves of ocimum sanctum or jeucas linifoha.
- ❖ The drug is also used in scorpion bite[11].

MILAGU ADDED SIDDHA FORMULATIONS (AS AN ADJUVANT):

1. Milagu thiravagam

Dosage : Kasu eadai

Indication : Peptic ulcer, Indigestion, Anorexia

2. Milagu Legium

Dosage : Punnai kai alavu

Indication : Peptic ulcer, Vaivu, Diarrhoea.

3. Karuvepilai vadagam

Dosage : Illanthai vithai alavu

Indication : Anorexia, Diarrhoea, Dysentery.

4. Kanthaga Chooranam

Dosage : Verugadi alavu

Indication : Peptic ulcer, Indigestion, Constipation.

5. Sowbagiya sundi Chooranam

Dosage : Verugadi alavu

Indication : Peptic ulcer, Indigestion, Diarrhoea.

6. Pirandai Vadagam

Dosage : Kottaipakku alavu

Indication : Peptic ulcer, Vomitting, Anorexia

7. Thirikadgu Kirutham

Dosage : 1 Spoon

Indication : Piles, Diarrhoea.

8. Pirandai Chooranam

Dosage : Verugadi alavu

Indication : Peptic ulcer, Vomiting, piles, Abdominal, disorders.

9. Thirikadugu thiravagam

Dosage : 5 Drops

Indication : Peptic ulcer, Bronchial Asthma.

10. Muppirandai Chooranam

Dosage : Verugadi alavu

Indication : Peptic ulcer, Indigestion, Piles[12].

3.4.2. BOTANICAL ASPECTS

MILAGU (PIPER NIGRUM)

Taxonomical Classification:

Kingdom	:	Plantae
Subkingdom	:	Tracheobionta
Superdivision	:	Spermatophyta
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Subclass	:	Magnoliidae
Order	:	Piperales
Family	:	Piperaceae
Genus	:	*Piper* L.
Species	:	*Piper nigrum* L.

Common name:

Black pepper, white pepper, green pepper, peppercorn, Madagascar pepper (English); pippali (Sanskrit); kali mirch (Hindi, Urdu); milagu (Tamil)

Synonym:

Muldera multinervis Miq.

Habitat:

Montane tropical evergreen forest.

Geography and distribution

Black pepper is native to the Western Ghats of Kerala State in India, where it grows wild in the mountains.

It is cultivated all over the tropics as a commercial crop. Vietnam, Indonesia, Brazil and India are the major producers.

DESCRIPTION

Overview:

A climber that grows to a height or length of 10 m or more. Once the main stem is established it grows many side shoots to create a bushy column.

The plants form short roots, called adventitious roots, which connect to surrounding supports.

Leaves:

Almond-shaped, tapering towards the tip, dark green and shiny above, paler green below, arranged alternately on the stems.

Flowers:

Borne in clusters along flowering stalks known as spikes. 50–150 whitish to yellow-green flowers are produced on a spike.

Fruits:

Round, berry-like, up to 6 mm in diameter, green at first but turning red as they ripen, each containing a single seed. 50–60 fruits are borne on each spike.

Fruits are picked when green and immature to produce green pepper; when fully grown but still green and shiny to produce black pepper; and when slightly riper to produce white pepper (for which the fruits are also soaked to remove the fleshy outer layer).

Food

The fruits of *Piper nigrum* are used to make black pepper. This hotly pungent spice is one of the earliest known and most widely used spices in the world today. It is used as flavouring, particularly for savoury foods, meat dishes, sauces and snack foods. It is also used as a table condiment.

Black pepper, white pepper and green peppercorns are all produced from *Piper nigrum* fruits but are harvested at different times and are processed differently.

India is a key producer of black pepper and exports much of what is grown. Peppercorns from Malabar and Tellicherry in Kerala, India, are particularly prized for their flavour and pungency[23].

Black peppercorns

Black peppercorns feature as remedies in Ayurveda, Siddha and Unani medicine in South Asia. They are most frequently used as an appetizer and to treat problems associated with the digestive system, particularly to eradicate parasitic worms. Some traditional uses of black pepper are supported by scientific evidence.

In Siddha medicine, black pepper has been used to aid digestion, improve appetite, treat coughs, colds, breathing and heart problems, colic, diabetes, anaemia and piles. Stomach ailments such as dyspepsia, flatulence, constipation and diarrhoea are all treated with black pepper, which may be mixed with other substances such as castor oil, cow's urine or ghee.

Black pepper has been prepared in tablet form as a remedy for cholera and syphilis, sometimes combined with other substances. It has also been used in tooth powder for toothache, and an infusion of black pepper has been suggested as a remedy for sore throat and hoarseness. Black pepper may be chewed to reduce throat inflammation.

Pharmacological activity:

- **Constituents**: Black pepper has been found to contain piperine, alkamides, piptigrine, wisanine, dipiperamide D, and dipiperamide E.

- **Acetylcholinesterase inhibitory activity**: In an *in vitro* study, an extract of *Piper nigrum* L. seeds showed 50-65% inhibitory activity on acetylcholinesterase. **Antibacterial effects**: In an *in vitro* study using 12 different genera of bacterial populations isolated from the oral cavity of 200 individuals, an aqueous decoction of black pepper (*Piper nigrum* L.) exhibited 75% antibacterial activity as compared to aqueous decoction of bay leaf (53.4%) and aqueous decoction of aniseed (18.1%), at the concentration of 10mL/disc
- **Anti-inflammatory effects**: Based on animal study, a polyherbal formulation (Aller-7/NR-A2) containing extracts from seven medicinal plants including Phyllanthus emblica, Terminalia chebula, Terminalia bellerica, Albizia lebbeck, Piper nigrum, Zingiber officinale, and Piper longum demonstrated 31.3% inhibition against carrageenan-induced acute inflammation in Wistar Albino rats, while ibuprofen (50 mg/kg orally) exerted 68.1% inhibition, Aller-7 also exhibited a dose-dependent (150-350mg/kg) anti-inflammatory effect against Freund's adjuvant-induced arthritis in Wistar Albino rats; an approximately 63% inhibitory effect was observed at a dose of 350mg/kg.
- **Gastrointestinal effects**: In a clinical study of intestinal peristalsis in 16 healthy volunteers, consumption of 1.5g of black pepper in capsules increased the orofecal transit time from 90 ± 51 minutes to 122 ± 88 minutes (p=0.09). In an *in vitro* study, piperine inhibited digoxin and cyclosporine A transport in Caco-2 cells with IC_{50} values of 15.5 and 74.1mcM, respectively. The bactericidal and anti-adhesive properties of black pepper have also been investigated against *Helicobacter pylori*, however, aqueous

extracts did not show bactericidal effect on any of the isolates. **Neural effects**: In an *in vitro* study using whole-cell patch-clamp electrophysiology, piperine, a pungent alkaloid found in black pepper, had similar agonist effects on the human vanilloid receptor TRPV1 as capsaicin. However, piperine could induce greater receptor desensitization and exhibit a greater efficacy than capsaicin[24].

3.5. PHARMACEUTICAL REVIEW OF MATHIRAI

3.5.1. SIDDHA ASPECT OF THE FORMULATION OF MATHIRAI

மாத்திரை:

மாத்திரை பாகம்:

கல்க திரவியங்களைத் தனியாகவேனும், பற்பசெந்தூரங்களுடன் சேர்த்தேனும் ஜலம், க'ாயம், மூலகைச்சாறு இவைகளுடன் ஏதேனும் ஒன்றில் நன்கு உறவாகும்படிஅரைத்து குறிப்பிட்ட அளவின்படி மாத்திரை செய்வதாகும்.

அரைப்பு விதி

மாத்திரை செய்வதற்காக நியமிக்கப்பட்ட கல்கங்களை தனித்தனியாகச் சூரணித்து அளவின்படி கல்வத்தில் இட்டு பசையுண்டாக்க கூடிய திரவியமொன்றினால் நனையும்படி பிசறிக் கல்வத்தின் கீழ்க்கையில் வைத்து கொண்டு கொஞ்சங் கொஞ்சமாகக் கல்வத்தின் மேல்கையில் ஏறும்படித் தள்ளி அரைக்க வேண்டும் இப்படி கீழ்க்கையில் இருந்த சரக்கையெல்லாம் மேலேறிய பின் மீண்டும் படிப்படியாக கீழ்கைக்குவரும்படி தள்ளிதள்ளி அரைத்தல் வேண்டும் இங்ஙனம் குறித்த காலம் வரையில் அரைப்பின் நிர்த்தோ'மான மாத்திரையாகும்.

மாத்திரை திரட்டும் பாகம்

அரைக்கும் போது, சரக்கு கல்வத்தில் சிறிதும் பற்றாமல் குழவியுடன் புரள வேண்டுவதுமன்றி அது குழவிக்கும் கல்வத்திற்கும் சம்பந்தமில்லாமல் இருக்க வேண்டும்.

வாசைனை திரவியங்கள் சேர்க்கும் காலம்

கஸ்தூரி, கோரோசனை, அம்பர், குங்குமப்பூ, பச்சைக் கற்பூரம், முதலானவைகளை மாத்திரை திரட்ட 1 நாழிகைக்கு முன் சேர்த்தரைப்பது நன்று. அங்ஙன முடித்த மாத்திரைகளை நிழலுலர்த்தி உடனே கல்கார்க் சீசாவில் பதனஞ்செய்தல் வேண்டும்.

அரைப்பின் முன் பின் சேர்க்க வேண்டுவன

பிரயோகத்தில் அரைப்பதற்கு எது கடினமானதோ அதனை கல்வத்தில் இட்டு அரைத்து, அது பக்குவமானபின் மற்றவைகளைப் படிப்படியாக ஒன்றன் பின் ஒன்றாகச்சேர்த்து அரைத்தல் வேண்டும். வாளம் முதலிய பருப்பினங்கள் சேரும் பொழுதுமாத்திரை திரட்ட 1 சாமத்திற்கு முன் சேர்த்தல் வேண்டும். ஏனெனில்

தாளகத்தைபோன்ற கடின சரக்குடன் மத்திமமான சரக்குசம்பந்தப்படின் தாளகம் எளிதில்அரைபடாது.

வாளம் முதலயவை நீண்ட அரைப்பிற்கு பின் மாத்திரை செய்து வரும்போது, அதனிலிருந்து தைலம் வெளிப்படும் அதனால் அதன் வன்மை குறையும் [25]

3.5.2. MODERN ASPECT OF THE FORMULATION

Tablet (Pill)-*Kuligai*

A tablet is a pharmaceutical dosage form it otherwise called as caplet. Medicinal tablets are called as "pills". Originally "pills" referred specifically to a soft mass rolled into a ball shape, rather than a compressed powder. (wikipedia. org).

As per Indian Pharmacopeia 2007 defined the Tablets are solid dosage forms each containing a unit dose of one or more medicaments. They are anticipated for oral route. A tablet consists an active medicament with excipients which are in powder form are compressed or pressed into a solid dosage form. About two third drugs prescribed are in solid dosage form and tablets include half of them.

Classification:

As per IP2007 tablets are majorly classified into following categories (Indian pharmacopoeia 2007)

1. Uncoated Tablets:

This type of tablets contains single layer or more than one layer tablet consisting of active ingredient with the excipients, no additional cover is applied on to it after the compression.

2. Coated Tablets:

Coated types of tablets have an additional coating layer on it after the tablet was compressed, the coating layer of tablets formed with sugar, gums, resins, inactive or insoluble fillers, plasticisers, polyhydric alcohols, waxes.

3. Dispersible Tablets:

These are the film coated or uncoated tablets because a uniform dispersion when suspended in water

4. Effervescent Tablets:

These type of tablets which are uncoated and are planned to be dissolved and produce an dispersion before they are administered the dissolution is achieved by the reaction between an organic acid and bicarbonate which produce CO2, thus produced CO2 will disintegrate the tablet so which dissolves in the solution to produce an suspension which was rapidly absorbed.

5. Modified-release Tablets:

These types of tablets are the coated or uncoated tablets which are designed in such a way that the rate or location of the active ingredient released is modified. It includes enteric coated tablets, prolong release tablet or delay release tablet.

A) Enteric-coated Tablets:

These are also called as gastro resistant tablets as they resistant to the gastric juices; these are formulated by coating the tablet with anionic polymer of methylacrylic acid and their esters or by coating with cellulose acetyl pthylate.

Ex: erythromycin, NSAIDS

B) Prolonged- release Tablets:

These types are otherwise called as sustain release tablets or extended release tablets was formulated in such a way that the active ingredient is released for a prolong duration of time and is available in systemic circulation after administration.

C) Delayed-release Tablets:

This dosage form was planned to release the drug after some time delay or after the tablet has passed one part of the GIT into another. All enteric coated tablets

are type of delayed action tablet but all delayed action of tablets was not enteric or not intended to produce enteric action.

6. Soluble Tablets:

These are coated or uncoated tablets which are planned to dissolve in water before they are administered.

7. Tablets for Use in the Mouth:

These are the tablet formulations which are planned to be show local action in the buccal cavity. These include buccal tablet, Sublingual Tablets and Troche or lozenges.Buccal tablets are placed in between the cheek and gingival. Sublingual tablets are placed below the tongue Eg: glyceryl trinitrate.

8. Tablets for other routes of administration:

These include implantable tablets and vaginal tablet. These are inserted in to the rectum or vagina for their local or systemic action [26].

4. MATERIALS AND METHODS

4.1.1. PREPARATION OF THE TEST DRUG:

4.1.2. Drug selection

The study drug *"**Rasa Karpoora Kuligai**"* has been selected from the classical siddha literature,***"Gunapadam Thathu Jeeva Vaguppu".***

4.1.3. Ingredients of Rasa Karpoora Kuligai,

1. Purified Pooram - 21gm
   (Calomel/Hydrargyrum subchloride)
2. Betel Leaves (Piper betle) - 168gm
3. Pepper(Piper nigrum) - 126gm
4. Garlic (Allium sativum) - 84gm
5. Betel (Piper betle) leaf juice - Required quantity.

4.1.4. Procurement of Raw drugs:

All the raw materials were obtained from K.Ramasamy chetty country drug shop, No:177, Rasappa chetty street, Park town, Chennai-600003. Betel leaves were procured from Local market, West tambaram, Chennai-600045.

4.1.5. Identification and Authentication:

Pooram (Calomel) was identified and authenticated by the Dept of Gunapadam and herbal ingredients of Rasakarpoora Kuligai were identified and authenticated by the Dept of Medicinal Botany, National Institute of Siddha, Chennnai - 47.

4.1.6. Purification of raw drugs:

Pooram (Calomel):

The karkam (poultice) was made from betel leaf and pepper (each 8.75gm) and the karkam was dissolved in 1.3 liter of water. 35gm of Calomel was tied with a cloth and immersed in the above medicated water and heated by the thula iyanthiram process (calomel tied with cloth immersed in the medicated water from the cross bar without touching the bottom of the vessels and heated). After the water is reduced to ¾ of its volume, the calomel is taken out, washed with water and dried under sunlight. Finally the purified Calomel was stored in a air tight container.

Pepper:

Soaked pepper in the juice of Utthamani(Pergularia daemia) and dry it in sunlight till the juice dries up completely.

Garlic:

Peeled the skin of garlic.

Betel Leaves:

Removed the stalk and midrib of betel leaves.

4.1.7. Rasa Karpoora Kuligai preparation procedure:

Purified 21 gm of Calomel, 84gm of Garlic, 126gm of Pepper, and168gm of betel leaves were taken and grinded with betel leaves juice for 15 hours (5 samam) and then rolled into pills like sundai (Sollanum tuberosum) size(0.798gms). Then it is dried in shade.

4.1.8. Storage of test drug:

Rasa Karpoora Kuligai is stored in an air tight glass container.

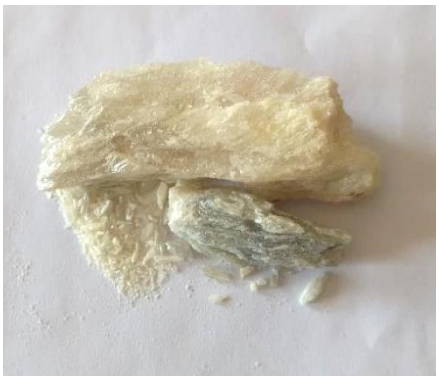
4.1.9. Administration of the drug:

Form of the drug	:	Kuligai
Route	:	Enteral *(oral)*
Dosage	:	One pill- twice a day for seven days
Adjuvant	:	Water.
Shelf Life	**:**	5 years.

4.1.10. Indications:

Scabies, syphilis, Cervical cancer, Penile cancer, Chronic deep ulcers and chronic Pitted wounds.

Ingredients of Rasakarpoora kuligai



1.Rasakarpooram (Purified)

2.*Piper betle*

3.*Allium sativum*

4.Piper nigrum

Prepared medicine

Rasakarpoora kuligai

4.2. STANDARDIZATION OF RASAKARPOORA KULIGAI:

The standardization of test drug is essential to exhibit the purity and quality of drug. This is basically done by physiochemical, phytochemical, biochemical, and instrumental analysis.

The physiochemical analysis have been done at The TN Dr.MGR Medical University, Guindy,Chennai-32. Biochemical analysis were done at National Institute of Siddha and the Instrumental analysis was done at Indian Institute of Technology (IIT), Chennai-36 .

4.2.1. ORGANOLEPTIC CHARECTER ANALYSIS:

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

4.2.2. PHYSICO CHEMICAL ANALYSIS

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

1. **Determination of Ash Values:**

1.a. Total Ash:

2g is accurately weighed and incinerated in a crucible dish at a temperature not exceed 600^{o}C until free from carbon. It is then cooled and weighed. The % w/w of ash with reference to the air-dried powder is calculated

1.b. Water Soluble Ash:

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

1.c. Acid insoluble Ash:

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

2. **Determination of Extractive Value:**

2.a. Alcohol Soluble Extractive Value:

2.5gm of test drug powder is weighed and macerated with 100ml of ethanol in a closed container for 24 hours. The resulting solution is shaken continuously for 6 hours and allowed to stand and soak for 18 hours. The solution is filtered and evaporated of the filtrate in a flat bottomed shallow dish and dried at 105^{o}C then cooled and weighed.

2.b. Water soluble Extractive value:

5gm of test drug powder is weighed and macerated with distilled water, respectively, at 80^{o}C for 24 hrs. The resulting solution is shaken continuously for 6 hours and allowed to stand and soak for 24 hrs then filtered. The solution from both

chloroform and water respectively is filtered and evaporated of the filtrate in a flat bottomed shallow dish and dried at 105^{o}C then cooled and weighed.

3. **Loss on Drying:**

2gm of powdered drug is dried in the oven at 100- 105°C to constant weight. The result was noted.

4.2.3. PHYTOCHEMICAL ANALYSIS

Phyto chemical Screening of the test drug have been done using standard procedures

1. Detection of alkaloids:

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

a) Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow colored precipitate indicates the presence of alkaloids.

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

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

d) Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow colored precipitate.

2. Detection of carbohydrates:

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

a) Molisch's Test: To 2 ml of plant sample extract, two drops of alcoholic solution of α- naphthol are added. The mixture is shaken well and few drops of concentrated sulphuric acid is added slowly along the sides of test tube. A violet ring indicates the presence of carbohydrates.

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

3. Detection of glycosides:

Extracts were hydrolyzed with dil. HCl, and then subjected to test for glycosides.

a) Modified Borntrager's Test: Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink color in the ammonical layer indicates the presence of anthranol glycosides.

b) Cardiac glycoside (Keller-Killiani test): Extract was shaken with distilled water (5 mL). To this, glacial acetic acid (2 mL) containing a few drops of ferric chloride was added, followed byH2SO4 (1 mL) along the side of the test tube. The formation of brown ring at the interface gives positive indication for cardiac glycoside and a violet ring may appear below the brown ring

4. Detection of saponins

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

b) Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

5. Detection of phytosterols

a) Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow color indicates the presence of triterpenes.

6. Detection of phenols Ferric Chloride Test:

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

7. Detection of tannins - Gelatin Test:

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

8. Detection of flavonoids

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

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

9. Detection of proteins and aminoacids

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

10. Detection of diterpenes Copper Acetate Test:

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

11. Gum and Mucilage:

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

12. Test for Fixed oils and Fats

a. Spot test :

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

13. Test for Quinones

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

4.2.4. BIO CHEMICAL ANALYSIS:

1. Preliminary Basic and Acidic radical:

Preparation of the extract:

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

1.a. Qualitative analysis for basic radicals:

1.a.1. Test for Calcium:

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

1.a.2. Test for Iron (Ferric):

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

1.a.3. Test for Iron (Ferrous):

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

1.a.4. Test for Zinc:

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

1.b. Qualitative analysis for acidic radicals:

1.b.1. Test for Sulphate:

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

1.b.2. Test for Chloride:

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

1.b.3. Test for Phosphate:

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

1.b.4. Test for Carbonate:

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

1.b.5. Test for starch:

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

1.b.6. Test for albumin:

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

1.b.7. Test for tannic acid:

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

1.b.8. Test for unsaturation:

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

1.b.Test for the reducing sugar:

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

Test for amino acid:

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

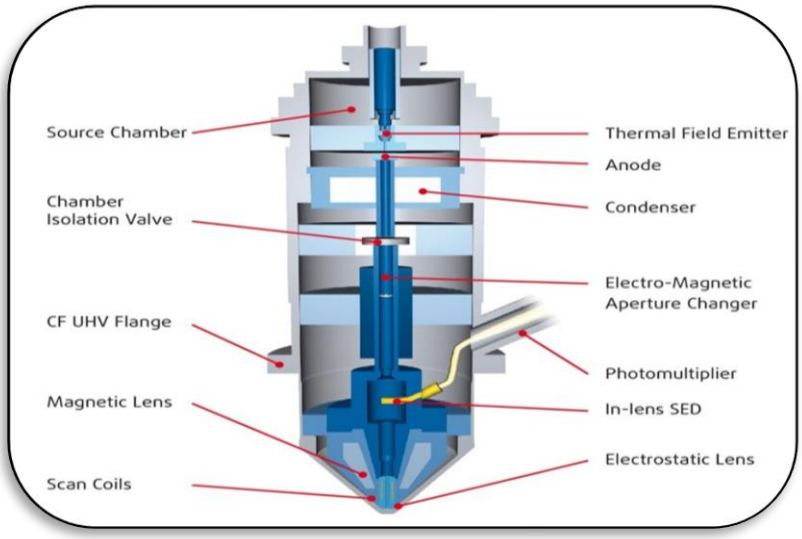
4.2.5. INSTRUMENTAL ANALYSIS:

4.2.5.1. SEM *(SCANNING ELECTRON MICROSCOPE):*

External morphology, texture, crystalline structure, chemical composition and the shape of the test drug – Rasa Karpoora Kuligai was analysed through SEM as per standard procedure.

SEM INSTRUMENT

SEM - SCANNING ELECTRON MICROSCOPE



MECHANISM

In scanning electron microscope high-energy electron beam is focused through a probe towards the sample material. Variety of signals was produced on interaction with the surface of the sample. This results in the emission of electrons or photons and it is collected by a appropriate detector.

The types of signal produced by a scanning electron microscope include

- Secondary electrons
- back scattered electrons
- characteristic x-rays, light
- specimen current
- Transmitted electrons.

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

4.2.5.2. ICP-OES (INDUCTIVELY COUPLED PLASMA OPTIC EMISSION SPECTROMETRY):

Presence of Havy mentals and other elements in the Rasa Karpoora Kuligai was analysed by ICP-OES as per standard procedure.

ICP-OES

Manufacturer **:** Perkin Elmer

Model **:** Optima 5300 DV ICP-OES Inductively Coupled Plasma Spectrometer *(ICP)*

Principle :

An aqueous sample is converted to aerosols via a nebulizer. The aerosols are transported to the inductively coupled plasma which is a high temperature zone *(8,000– 10,000ºC).* The analysts are heated *(excited)*to different (atomic and/or ionic) states and produce characteristic optical emissions *(lights).* These releases are separated based on their respective wavelengths and their strengths are measured *(spectrometry).* The intensities are proportional to the concentrations of analyses in the aqueous sample. The quantification is an external multipoint linear standardization by comparing the emission intensity of an unknown sample with that of a standard sample. Multi-element calibration standard solutions are prepared from single- and multi element primary standard solutions. With respect to other kinds of analysis where chemical speciation is relevant *(such as the concentration of ferrous iron or ferric iron),* only total essential concentration is analysed by ICP-OES.

Application:

The analysis of major and minor elements in solution samples.

Mechanism:

In plasma emission spectroscopy *(OES),* a sample solution is presented into the core of inductively coupled argon plasma *(ICP),* which generates temperature of approximately 8000°C. At this temperature all elements become thermally excited and emit light at their characteristic wavelengths. This light is collected by the spectrometer and passes through a diffraction grating that serves to resolve the light

into a spectrum of its essential wavelengths. Within the spectrometer, this deflected light is then collected by wavelength and amplified to yield an strength of measurement that can be converted to an elemental concentration by comparison with standardizationvalues

The Inductively Coupled Plasma Optical Emission Spectrometric *(ICP-OES)* analysis was done in Saif, IIT Madras, and Chennai-36 using Perkin Elmer Optima 5300 DV.

Sample preparation:

Inductively Coupled Plasma Spectroscopy techniques are the so-called "wet" sampling methods whereby samples are introduced in liquid form for analysis.

100 mg “Rasa karpoora kuligai*”* was occupied in a clean, dry test tube. To this, 3 ml Nitric acid was added and mixed well and allowedfor few minutes untill the reactions were completed. And then, 25 ml of Refined water, was added to prepare digested solution.

4.3. TOXICOLOGICAL STUDIES

PRECLINICAL TOXICITY STUDIES OF *RASA KARPOORA KULIGAI (RKK)* ON WISTAR ALBINO RATS

4.3.1. Acute toxicity study

4.3.2. 28 days Repeated oral toxicity study.

The toxicity studies were carried out after getting IAEC approval from National Institute of Siddha, Chennai-47. (IAEC approval No: NIS/IAEC/13/2016, dated 29.9.2016)

4.3.1. ACUTE TOXICITY STUDY IN FEMALE WISTAR RATS:

Aim:

The aim of this Study is to evaluate the toxicity/ safety of the test substance Rasa Karpoora Kuligai, when administered orally to Female Wistar Rats with different single doses as per OECD guide line 423.

Experimental details:

1.Animal

The female wistar albino rats of weighing 150-200gm were obtained from authorized animal breeders of the animal laboratory in TANUVAS, Madhavaram, Chennai and stocked in the animal house at National Institute of Siddha, Chennai.the animal are acclimatized for seven days prior to do toxicity study.

2.Diet and water

All the rats had free access to a pellet rat diet. The diet was analyzed by the manufacturer to check its composition and to detect possible contaminants. The RO water was offered ad libitum in bottles.

3. Animal selection and Identification

The animals are randomly selected for each group. Each group contain three female animals. They were marked on head, body and tail with picric acid solution prepared in water for identification in each group. The group no., cage no., sex of the animal and animal no. were indicated below the cage in label.

Numbering and Identification of test animals

Group No	Animal Marking
I	Head, Body and Tail (H,B,T)
II	Head, Body and Tail (H,B,T)
Lll	Head, Body and Tail(H,B,T)

4. Dose of Test substance & Dose Preparation:

The doses of the test substance for the study were selected based on OECD guidline-423 and IAEC committee recommendation.

Dose of test substance for each group

GROUP	DOSE
GROUP	DOSE
Group-I	Control
Group-II	300 mg/kg
Group-III	2000 mg/kg

Rasakarpoora kuligai was finely powdered and mixed with twine 80 to make as completely dissolved form for oral administration. The dose was prepared of a required concentration before dosing by dissolving in distilled water. The preparation for different doses was vary in concentrations to allow a constant dosage volume.

The test drug was administered as single dose. After single dose administration period, all animals were observed for further 14 days.

5.Route of administration

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

6. Administration of Dose

The animals were fasted (only food was withheld) for 12hrs and weighed prior to dosing. Three animals were used for each group. A single dose of the test drug solution (300,2000mg/kg) was consecutively administered by oral gavage using intubation cannula. The food was withheld for another 4hrs after dosing. The toxicological effect was assessed on the basis of mortality.

7. Experimental procedure:

Female wistar Albino Rats of weighing 150-200g were used for acute toxicity study. Animals were housed in a cage at 22°C ±3°C and relative humidity 30–70% and have free access to standard rat pellet diet. After seven days of acclimatization the animals are divided into three groups randomly (Group I, II &III). Each group contains 3 female wistar albino rats. Group I served as a control and treated with water.The remaining two groups were treated with 300mg/kg.b.wt and 2000mg/kg.bwt dosage of Rasa Karpoora Kuligai by oral route after 12 hrs fasting with free from water. After drug administration behavioural parameters are monitored for the first 4 hours continuously

(1/2 hr, 1hr, 2 hr, 3 hr,4 hr) and recorded. Then the animals are observed once daily for further 14 days for any mortality and morbidity. The animals are die within this period will be subjected to necropsy. Remaining animals will be weighed and sacrificed under the intra peritoneal injection of Pentothal Sodium on the 15th day of the Study period. The toxicological effect was assessed on the basis of mortality.

8.Observations:

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

½ hour, 1 hour, 2 hours, 4 hours and up to 24 hours observation

All rats were observed twice daily for 14 days

Body weight were Calculated weekly once

Feed & water intake were Calculated daily

Cage side observation

The animals were monitored for behavioral parameters like Alertness, Aggressiveness, pilo erection, Grooming, Gripping, Touch Response, Motor Activity, Tremors, Convulsions, Muscle Spasm, Catatonia, Muscle relaxant, Hypnosis Analgesia, Lacrimation, Exophthalmos, Diarrhea, Writhing, Respiration, Mortality.

Gross necropsy:

At the end of the 14 th day, all the animals were sacrificed by using the intra peritoneal injection of Pentothal sodium. Gross necropsy includes examinations of the external surface of the body, all orifices, cranial, thoracic

and abdominal cavities and their contents. Brain, eye, lungs, heart, spleen, liver, kidneys, adrenals and uterus of all animals.

4.3.2: 28 DAYS REPEATED ORAL TOXICITY STUDY IN WISTAR ALBINO RATS TO EVALUATE TOXICITY PROFILE OF RASA KARPOORA KULIGAI

1. Aim

The aim of this study was to assess the toxicological profile of the test item when treated as a single dose for 28 days. This study provides information on the possible health hazards likely to arise from exposure over a relatively limited period of time.

2. Test Guideline Followed

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

3. Study drug:

Rasa Karpoora Kuligai.

4. Experimental details:

Species: Wistar Albino Rats

Sex: Male and Female.

Age/Weight : 6 weeks/150-200 gm b.wt.

Acclimatization period: 7 days prior to dosing

Housing: Poly propylene cages bedding with Husk.

Husbandary: 12hrs light and 12 hrs dark, 22°C ± 3°C & Relative humidity 30–70%.

Feed: Rodent pellet feed

Water: RO water adlibitium.

Identification: Animals are kept in poly propylene cages and marked- Head,body,tail,head body & Bodytail.

Groups: Randomization

No. Of Animals for each group: 5 Male and 5 Female.

5. Experimental procedure:

150-200 gms of 20 male and 20 female wistar albino rats are used for 28 days repeated oral toxicity study. The animals are divided into four groups. Each group constains 10 animals (5 Female and 5 Male). The first group treated as control and second, third, fourth groups were treated with Low dose 230mg/kg/ b.wt, Mid dose 450mg/kg/b.wt, High dose 600mg/ kg/ b.wt of Rasakarpoorakuligai mixed with twin80 and RO water for 28 days respectively after 12 hrs of fasting with free from water. The low dose, mid dose and high dose of test drug will be calculated from human therapeutic dose based on by using the conversion table Paget and Barnes 1964. The control animals were administered with twin 80 mixed RO water.

The administration was given by oral, once daily for 28 consecutive days. The animals were observed the behavioural parameters for the study period. Body weight of the animal was being monitored at weekly intervals. Food & water intake were Calculated daily. All the animals were sacrificed at the end of the study (29 days) by using the intra peritoneal injection of Pentothal Sodium as prescribed dose level. Blood was collected from the anesthetized animals from the Abdominal aorta for the following investigations like Haematology and Biochemical analysis. Gross pathological changes were monitored in all the organs then the vital organs were preserved and subjected to Histopathological examination.

Observations:

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

- All rats were observed twice daily for 28 days
- Body weight were Calculated weekly once
- Feed & water intake were Calculated daily

a.Cage side observation

The animals were monitored for behavioral parameters like, Alertness, Aggressiveness, piloerection, Grooming, Gripping, Touch Response, Motor Activity, Tremors, Convulsions, Muscle Spasm, Catatonia, Muscle relaxant, Hypnosis Analgesia, Lacrimation, Exophthalmos, Diarrhea, Writhing, Respiration, Mortality.

Laboratory Investigations:

On the 29^{th} day, the animals were fasted overnight, then anesthetized to collect blood samples from the abdominal aorta in two tubes: one with EDTA for hematological parameters, another one without anticoagulant and was centrifuged at 4000 rpm at 4°C for 10 minutes to obtain the serum for biochemical parameters.

Hematological Investigations:

Blood samples of control and drug treated rats were analyzed for haemoglobin (Hb), total red blood corpuscles (RBC), white blood corpuscles

(WBC) count, Platelet, Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), were calculated by auto analyzer.

Biochemical Investigations:

Serum samples of control and experimental animals were analyzed for, Bilirubin, BUN, Creatinine, Triglyceride, Total Cholesterol, HDL, LDL, VLDL, using standard methods. Activities of glutamate oxaloacetate transaminase/Aspartate aminotransferase (GOT/AST), glutamate pyruvate transaminase/ Alanine aminotransferase (GPT/ALT) were estimated as per the colorimetric procedure.

Necropsy:

All the animals were sacrificed on the 29^{th} day and Gross necropsy includes examinations of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents. Brain, eye, lungs, heart, spleen, liver, kidneys, adrenals and sex organs of all animals were recorded.

Histopathology:

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

Histopathological investigation of the vital organs was done. The organ pieces (3-5µm thick) of all the animals (low, mid, high) were preserved and fixed in 10% formalin for 24 hrs. Samples were dehydrated in an auto technic and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at $50^{o}C$ and then in a cubical block of paraffin

made by the “L” molds. It was followed by microtome and the slides were Prepared then stained with Haematoxylin-eosin.

Statistical analysis:

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

5. RESULTS AND DISCUSSION

Many studies have been carried out to bring the efficacy and potency of the drug ***Rasa karpoora kuligai***. The present study includes literary collections, organoleptic character, physicochemical and instrumental analysis, and toxicological analysis of Rasakarpoora kuligai. The drug ***Rasa karpoora kuligai*** has been usefull for treating diseases such as cervical cancer(*yoni putru*), penis cancer (*linga putru*), chronic ulcers and skin diseases in reference with the text ***"Gunapadam Thathu Jeevam".***

5.1. LITRATURE REVIEW:

- Literature collections about the drug from various text books were done. Siddha literatures related to the drug bring the evidence and importance of its utility in treating the cancer.
- Botanical aspect explains the identification, description, active principle and medicinal uses of the plants ingredients of Rasa Karpoora Kuligai.
- Modern and Siddha aspect of the drug was also reviewed.

5.2. STANDARDIZATION OF THE TEST DRUG:

Standardisation of the drug is more essential to derive the efficacy and potency of the drug, which was analysed by the various methods. The results of organoleptic charecters, physicochemical,phyto chemical analysis and Instrumental analysis have been done and tabulated.

5.2.1. Organoleptic characters:

The following characters have been noted in ***Rasa karpoora kuligai***.

Table no:1

Colour in day light	Brown
Smell	Pleasant odour
Taste	Bitter, L.Pungent
Appearance	Round
Touch	Hard

5.2.2. Table No:2. Physicochemical properties:

Parameter	**Result**
Loss on drying	5.71 %
Total Ash content	6.87%
Acid insoluble ash	Less than 1%
Water soluble ash	2.54%
Water sol.Extraction	32.35%
Alcohol sol.Extraction	18.63%

The table no: 2 shows results of Physico chemical properties of RasaKarpoora Kuligai. These suggest:

Total Ash: Total ash value of plant material indicated the amount of minerals and earthy materials present in the plant material. The total inorganic content (ammonium,

potassium, calcium, chloride, iron, etc.,) present in the drug is measured through the Total ash value and it is of 6.84 % for RKK.

Acid insoluble ash: The acid insoluble ash value of the drug denotes the amount of siliceous matter present in the plant. The quality of the drug is better if the acid insoluble value is low. In Rasa Karpoora Kuligai acid insoluble ash is less than 1% . It denotes the quality of RKK.

Water soluble ash: Water-soluble ash is the part of the total ash content, which is soluble in water. It is 2.54 % in RKK.

Water soluble extraction: Water soluble extractive value is 32.35 % in RKK.

Alcohol soluble ash: Alcohol-soluble extractive value is 18.63% in RKK.

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

Loss on drying:

The total of volatile content and moisture present in the drug was established in loss on drying. Moisture content of the drug reveals the stability and its shelf-life. High moisture content can adversely affect the active ingredient of the drug. Thus low moisture content could get maximum stability and better shelf life. Loss on drying of Rasa Karpoora Kuligai is 5.71%. This result suggest stability better shelf life of RKK.

5.2.3. Phytochemical analysis:

The Preliminary phytochemical studies of aqueous extract of ***Rasa Karpoora Kuligai*** were done using standard procedures. The results were presented in tables.

Table No. 3: Result of Phytochemical screening - Qualitative

S.no	Phytochemicals	Test Name	H_2O ext.
1.	Alkaloids	Mayer's Test	-ve
		Wagner's Test	-ve
		Dragendroff's Test	-ve
		Hager's Test	-ve
2.	Carbohydrates	Molisch's Test:	**+ve**
		Benedict's Test	**+ve**
3.	Glycoside	Modified Borntrager's Test	**-ve**
		Cardiac Glycoside- Keller-Killiani test)	**-ve**
4.	Saponin	Froth Test	-ve
		Foam Test	**-ve**
5.	Phytosterol	Salkowski's Test	**-ve**
6.	Phenols	Ferric Chloride Test	**-ve**
7.	Tannins	Gelatin Test	-ve
8.	flavonoids	Alkaline Reagent Test	**-ve**

		Lead acetate Test	**+ve**
9.	Proteins and amino acids	Xanthoproteic Test	**-ve**
10.	Diterpenes	Copper Acetate Test	**+ve**
11.	Gum and Mucilage	Extract + alcohol	**-ve**
12.	Fixed oils and Fats	Spot test	**-ve**
13.	Quinones	NAOH + Extract	**-ve**

Note: +ve/-ve denotes present / absent if component tested.

Phyto chemical analysis shows (Table No: 3) Carbohydrates, Flavinoids and Diterpenes present in RasaKarpoora Kuligai.

5.2.4. Bio Chemical Analysis:

Table No. 4: Result of basic and acidic radical's studies:

Parameter	Result
Test for Calcium	Present
Test for Sulphate	Present
Test for Chloride	Present
Test for carbonate	Absent
Test for Starch	Present
Test for Iron [Ferric]	Absent
Test for Iron [Ferrous]	Present
Test for Phosphate	Present
Test for Albumin	Absent
Test for Tannic acid	Absent
Test for Unsaturation	Present
Test for the Reducing sugar	Absent
Test for Amino acid	Absent
Test for Zinc	Absent

From the basic and acidic radical studies Calcium, Sulphate, Chloride, Starch, Ferrous iron, Phosphate, Unsaturated compounds are present in Rasa Karpoora Kuligai.

Calcium is a major component of bones and teeth. It also is required for the clotting of blood to stop bleeding and for normal functioning of the nerves, muscles, and heart. Individuals who had a calcium intake of more than 700 mg per day had a 35 percent to 45 percent reduced risk of cancer of the distal (lower) part of the colon.

In a study that included more than 61,000 Swedish women, colorectal cancer risk was approximately 28 percent lower among individuals who had the highest calcium intakes (approximately 800–1000 mg per day). High intakes of total calcium, dietary calcium, and supplemental calcium were associated with an approximately 20 percent lower risk of colorectal cancer.

Chloride regulates the acid base balance of the body fluids, by maintaining the osmotic pressure of the body fluids. In severe diarrhoea vomiting, large amount of water and electrolytes are lost from body. The dehydration has to be treated by administering water and these electrolytes.

Ferrous Iron is easily soluble and readily absorbed from intestine. It is the liquid form of iron. Iron helps to proper function of the human body.

Starches functions much like dietary fibre. They provide nutrition for the beneficial bacteria in the colon, keeping them thriving and health. Dietary fibre in starch reduces effects of haemorrhoids, diverticulosis & controls blood pressure.

Reducing sugars can react with other parts of the food like amino acids to change colour and taste of the food. Reducing sugars like Glucose is essential for brain function and physical energy. Carcinoma of prostate (Ca P), the most common malignancy in men, is also the second most common cause of cancer deaths in men.

Phosphate reduce the risk for the development of prostatic diseases, both benign prostatic hyperplasia and Ca.

Calcium, Sulphate, Chloride, Starch, Ferrous iron, Phosphate, Unsaturated compounds are present in Rasa Karpoora Kuligai suggest this drug have anticancer potency.

5.2.5. INSTRUMENTAL ANALYSIS

a. ICP-OES (Inductively Coupled Plasma Optical Emission Spectroscopy):

The drug *(Rasa karpoora kuligai)* sample was analysed by the Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) to detect the trace elements and other elements quantitatively. The result of ICP-OES is given on the Table.

Table No.5 : ICP-OES of Rasa Karpoora Kuligai

S. no	Elements	Wavelength (nm)	Concentration
1.	Al	396.152	BDL
2.	As	188.979	BDL
3.	Ca	315.807	**11.160 mg/L**
4.	Cd	228.802	BDL
5.	Cu	327.393	BDL
6.	Fe	238.204	BDL
7.	Hg	253.652	BDL
8.	K	766.491	**13.801 mg/L**
9.	Mg	285.213	BDL
10.	Na	589.592	**14.310 mg/L**
11.	Ni	231.604	BDL
12.	Pb	220.353	BDL
13.	P	213.617	**86.321 mg/L**
14.	S	180.731	BDL
15	Zn	206.200	**01.058 mg/L**

BDL:Below Detectable Limit

1% = 10000ppm, 1ppm = 1/1000000 or 1ppm = 0.0001%

ICP-OES results suggest heavy metals like Arsenic,Mercury,Cadmium and Lead are below dedectable limits in RasaKarpoora Kuligai. So the RKK have safe for consumption. Calcium (11.160mg/L), Pottasium(13.801mg/L), Sodium(14.310mg/L), Phosporus (86.321 mg/L) and Zinc(01.058mg/L) present in RKK.

High intakes of total calcium, dietary calcium, and supplemental calcium were associated with an approximately 20 percent lower risk of colorectal cancer

Potassium is one of the major electrolytes that the body carefully controls for proper heart and neuromuscular activity.

Sodium is one of the major electrolytes, carefully maintained within a narrow normal range and necessary for proper functioning of the body's systems. Low sodium levels, or hyponatremia, occur in a wide variety of medical disorders including cancer. Low sodium levels can occur in patients whose cancers produce ADH-like hormones, from other cancer complications and different treatment side effects. Cancers such as small cell lung carcinoma, pancreatic cancer, lymphoma and certain brain tumors can cause due to low sodium level.

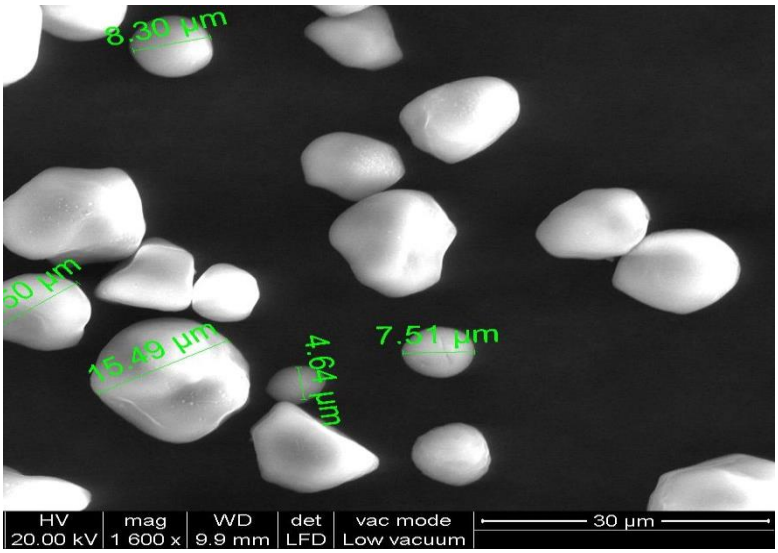
Phosphate reduce the risk for the development of prostatic diseases, both benign prostatic hyperplasia and Ca.

Zinc is essential for growth. It has been used in traditional medicine for everything from healing wounds to preventing blood clots. It can also help increase the blood flow. It has anti-oxidant properties that protect the cells from damage. It can produce healthy veins and arteries that enhance the blood circulation.

The presence Calcium, Sodium, Phosphate and Zinc in Rasa Karpoora Kuligai have the strong evidence for its anti oxidant, wound healing and anticancer potency.

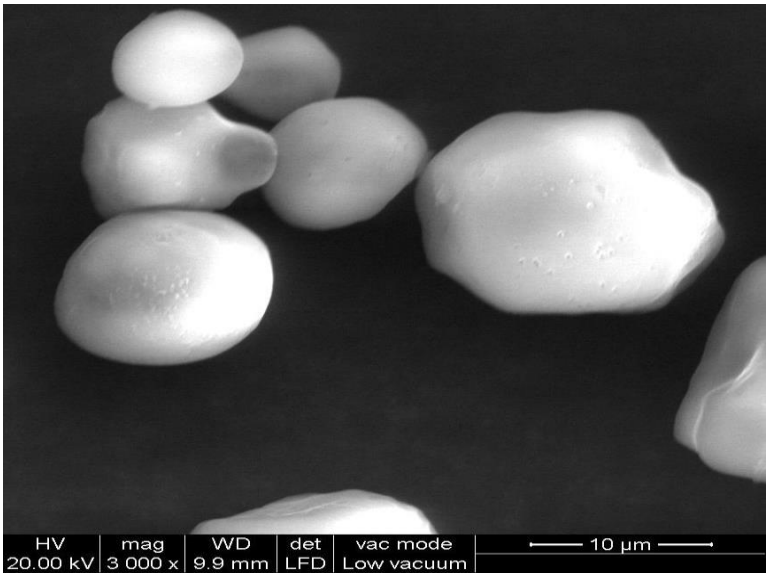
Scanning Electron Microscope *(SEM)* analysis:

FIG-1: SEM analysis of Rasa Karpoora Kuligai



SEM PICTURE at 24,000 MAGNIFICATION.

Figure-2: SEM analysis of Rasa Karpoora Kuligai



SEM PICTURE at 40,000 MAGNIFICATION.

Scanning Electron Microscopy (SEM) is the best method to determine the size distribution of nanoparticles present in the sample. It has been used for this purpose of computer-assisted counting of nanoparticle images. SEM analysis of the ***Rasakarpoorakuligai*** shows that the uniform distribution of particles presents in the entire field. Most of the particles present in the sample is nano size and near nano size, average particle size is 4.64µm - 7.51µm.

Siddhars were the great scientist in ancient times. They used nano technology for the preparation of Medicines. These Nano particles have beneficial properties that can be used to improve drug delivery system. Target cells take up these nano particles quickly because of their smaller size, lesser particles enhance the bio absorption and bio availability resulting better efficacy of the drug. Larger particles could not enter in to the target cell because of their size, resulting they cleared from the body. If a drug is cleared too quickly from the body, this could force a patient to use high dose, which leads to contraindication of the drugs. The Lesser particles from drug delivery system lower the volume of distribution and reduce the effect on non target tissue.

Nano range and uniform distrbution of RKK suggest it has better bioavailability and absorption.

5.2.6. EVALUATION OF ACUTE TOXICITY OF *RASA KARPOORA KULIGAI*

5.2.6.1. Effect of Acute Toxicity of RasaKarpoora Kuligai

Table No.6: Physical and behavioral examinations.

Group no.	Dose(mg/kg)	Observation sign	No. of animal affected.
Group-I	Twin 80 with Ro Water.	Normal	0 of 3
Group-II	300mg/kg	Normal	0 of 3
Group-III	2000mg/kg	Normal	0 of 3

Table No.7: Behavioural Signs of Acute Toxicity Study

S.N	Response in 300mg/kg.bw & 2000 mg /kg b.wt dosage of RKK	Head		Body		Tail	
		Before	After	Before	After	Before	After
1	Alertness	N	-	N	-	N	-
2	Grooming	A	-	A	-	A	-
3	Touch response	P	-	P	-	P	-
4	Torch response	P	-	P	-	P	-
5	Pain response	A	-	A	-	A	-
6	Tremors	A	Present	A	Present	A	Present
7	Convulsion	A	Present	A	Present	A	Present
8	Righting reflux	A	Present	A	Present	A	Present
9	Gripping strength	P	-	P	-	P	-
10	Pinna reflux	P	-	P	-	P	-
11	Corneal reflux	P	-	P	-	P	-
12	Writhing	A	-	A	-	A	-
13	Pupils	N	-	N	-	N	-
14	Urination	N	-	N	-	N	-
15	Salivation	A	-	A	-	A	-
16	Skin colour	N	-	N	-	N	-
17	Lacrimation	A	-	A	-	A	-
18	Hyper activity	A	Present	A	present	A	Present

Note: A- Absent of activity, N- Normal & P- Present of activity.

All the data were summarized in the form of table revealed that there was no abnormal signs and behavioural changes in all animals at the dose level of **300, 2000**

mg/kg body weight administered orally, during the study period. There was no mortality observed after dosing of **Rasa Karpoora Kuligai** upto 2000mg/kg body weight This indicates that LD50 of **Rasa Karpoora Kuligai** is more than 2000mg/kg b.wt.

There were no changes in skin and fur, eyes and mucous membranes of all animals. The eating, drinking habit, sleep pattern, locomotion were normal in all animals and no changes in body weight as compared to control group. At the end of the 14^{th} day, necropsy was performed and there was no abnormality seen in test groups as compared to control group during the examination.

5.2.6.8. 28 DAYS REPEATED ORAL TOXICITY STUDY OF RASA KARPOORA KULIGAI

Table No.8: Body weight changes of test animals in 28 days repeated oral toxicity study of Rasa Karpoora Kuligai

GROUP	CONTROL	RKK. LOW DOSE 230mg/kg	RKK.MID DOSE 450mg/kg	RKK.HIGH DOSE600mg/kg
0,DAY	126.667±1.42984	117.5±0.718795	98.3333±0.954521	95.8333±1.7966
7^{th} DAY	132.667±1.42984	123.5±0.921955	104±1.06458	101.667±1.72562
14^{th} DAY	138.5±1.40831	131±1.41421	110.167±1.27584	107.333±1.7062
21^{st} DAY	144.5±1.40831	137±1.52753	116±1.18322	113±1.59165
28^{th} DAY	150.333±1.52023	143.5±1.82117	121.667±1.22927	118.667±1.68655

Values are expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by dunnett's(N=10); $^{ns}p>0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

Table No.9: Organ weight changes in grams of test animals in 28 days repeated oral toxicity study of Rasa Karpoora Kuligai

GROUP		CONTROL	RKK. LOW DOSE 230mg/kg	RKK.MID DOSE 450mg/kg	RKK. HIGH DOSE 600mg/kg
BRAIN		1.256±0.2052	1.232±0.1547	1.499±0.2023	1.159±0.2025
HEART		0.8727±0.04385	0.8323±0.06207	0.738±0.03329	0.6877±0.02028
LIVER		6.019±0.359	8.448±0.652	6.706±0.07517	5.779±0.338
LUNGS		1.447±0.1242	0.9353±0.03152	1.671±0.1854	1.278±0.2453
KIDNEY	L	0.7613±0.03038	0.8533±0.06263	0.8533±0.05191	0.7467±0.02533
	R	0.7453±0.03374	0.8287±0.06534	0.721±0.01721	0.7333±0.02809
TESTIS		3.095±0.1521	2.517±0.1654	3.295±0.08581	3.11±0.1176
UTERUS		0.736±0.02501	0.9607±0.05447	0.985±0.03436	1.214±0.306

Values are expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by dunnett's(N=10); $^{ns}p>0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

Table No: 10 Effect of 28 days repeated dose of Rasa Karpoora Kuligai on Haematological parameters

GROUP	CONTROL	RKK. LOW DOSE 230mg/kg	RKK.MID DOSE 450mg/kg	RKK. HIGH DOSE 600mg/kg
RBC (X10^6/µL)	4.573±0.1139	5.39±0.3035	4.853±0.6894	5.8±0.3617
WBC(X10^3/µL)	12.5±1.531	11.47±0.5783	13±1.007	11.43±0.3756
HB (g/dl)	10.5±0.5859	13.2±0.8963	11.83±1.683	14.03±0.809
PCV %	32.2±1.833	40.6±2.689	36.17±4.725	43.1±2.427
POLYMORPHS (%)	7.333±0.6667	10±1	8±3.055	7±1.155

LYMPHOCYTES (%)	85.33±2.028	81.33±1.856	79.33±3.528	85.67±2.963
MONOCYTES (%)	5±0.5774	3±0.5774	3.333±0.6667	3.333±0.3333
EOSINOPHILS (%)	3.333±0.3333	4.333±0.3333	4.667±0.8819	4.333±0.3333
MCH (Pg)	23.6±0.611	24.93±0.4702	24.83±0.6566	25.77±0.3283

Values are expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by dunnett's(N=10); p>0.05, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

Table No. 11: Effect of 28 days repeated dose of Rasa Karpoora Kuligai On Biochemical parameters

GROUP	CONTROL	RKK. LOW DOSE 230mg/kg	RKK.MID DOSE 450mg/kg	RKK. HIGH DOSE 600mg/kg
SGOT(IU/ml)	97.2±6.835	116.1±11.08	98.37±10.16	91.5±3.523
SGPT (IU/ml)	44.77±8.151	90.73±17.71	45.3±5.852	48.6±6.012
ALP (IU/ml)	210.1±69.74	175.9±11.87	169.9±27.35	186.6±24.95
TOTAL BILIRUBIN (mg/dl	1.303±0.2452	1.453±0.2822	0.8193±0.3371	0.99±0.194
CREATININE (mg/dl)	0.6133±0.03844	0.58±0.08327	0.4767±0.0441	0.5867±0.02028
URIC ACID (mg/dl)	1.747±0.2761	1.74±0.155	2.06±0.6612	1.983±0.2924

Values are expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by dunnett's(N=10); ^{ns}p>0.05, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

Table No: 12 Effect of 28 days repeated dose of Rasa Karpoora Kuligai on food intake in grams

GROUP	CONTROL	RKK. LOW DOSE 230mg/kg	RKK.MID DOSE 450mg/kg	RKK. HIGH DOSE 600mg/kg
1st DAY	42±4.53137	38±4.64758	54.6667±6.23253	40.6667±4.20053
7th DAY	35.1667±4.13454	39±6.90411	44.5±3.30404	60.6667±7.82588
14th DAY	42±4.14729	42.6667±5.92546	47±7.49667	47.3333±8.40106
21st DAY	52±5.83095	41.1667±6.5341	42.6667±9.04311	34.6667±5.71936
28th DAY	50.5±4.62421	47.3333±6.27517	37.3333±5.3831	42.1667±6.42089

Values are expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by dunnett's(n=6); $^{ns}p>0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated group swith control group.

Table No. 13 : Effect of 28 days repeated dose of Rasa Karpoora Kuligai On Water Intake in ml

GROUP	CONTROL	RKK. LOW DOSE 230mg/kg	RKK.MID DOSE 450mg/kg	RKK. HIGH DOSE 600mg/kg
1st DAY	98.3333±13.5195	89.1667±14.3421	102.5±21.6699	67.5±7.6103
7th DAY	82.5±11.7438	100.833±12.6765	76.6667±9.80363	81.6667±14.4145
14th DAY	58.3333±8.72417	90.8333±14.2838	80±13.9642	89.1667±8.88976
21st DAY	91.6667±12.4944	80±8.46562	65.8333±9.43545	89.1667±8.79552
28th DAY	82.5±11.3835	88.3333±11.4504	75±8.85061	65±7.52773

Values arae expressed as mean ± SEM Statisticalsignificance (p) calculated by one way ANOVA followed by dunnett's(n=6); $^{ns}p>0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groupswith control group.

Table No: 14 : Effect of 28 days repeated dose of Rasa Karpoora Kuligai On Electrolytes

GROUP	CONTROL	RKK. LOW DOSE 230mg/kg	RKK.MID DOSE 450mg/kg	RKK. HIGH DOSE 600mg/kg
Sodium (mg/dl)	4.5±0.6455	4.25±0.6292	6±0.7071	6.75±0.75
Calcium (mg/dl)	1.575±0.137689	3.15±0.170783***	4.2±0.163299***	6.175±0.19311***
Phosphorus (U/L)	0.273±0.022517	0.3005 ± 0.019615^{ns}	0.35625 ± 0.030491^{ns}	5.033±0.32452*

Values arae expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by dunnett's (n=6); NS- non significant, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

Clinical Signs:

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

Mortality:

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

Body weight:

Results of body weight determination of animals Table No: 8 from control and different dose groups exhibited comparable body weight gain throughout the dosing period of 28 days.

Food consumption:

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

Organ Weight:

Group Mean Relative Organ Weights (%of body weight) are recorded in Table No.9 Comparison of organ weights of treated animals with respective control animals on day 29 was found to be comparable similarly.

Hematological investigations:

The results of hematological investigations (Table No:10) conducted on day 29 revealed following significant changes in the values of different parameters investigated when compared with those of respective controls; however, the increase or decrease in the values obtained was within normal biological and laboratory limits or the effect was not dose dependent.

Biochemical Investigations:

Results of Biochemical investigations conducted on days 29 and recorded in Table. No: 11 revealed the following significant changes in the values of hepatic serum enzymes studied. When compared with those of respective control. However, the increase or decrease in the values obtained was within normal biological and laboratory limits.

Histopathology:

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

1) All the animals from control and all the treated dose groups up to 600 mg/kg survived throughout the dosing period of 28 days.

2) No signs of toxicity were observed in animals from different dose groups during the dosing period of 28 days.

3) Animals from all the treated dose groups exhibited comparable body weight gain with that of controls throughout the dosing period of 28 days.

4) Food consumption of control and treated animals was found to be comparable throughout the dosing period of 28 days

5) Haematological analysis conducted at the end of the dosing period on day 29, revealed no abnormalities attributable to the treatment.

6) Biochemical analysis conducted at the end of the dosing period on day 29 no abnormalities attributable to the treatment.

7) Organ weight data of animals sacrificed at the end of the dosing period was found to be comparable with that of respective controls.

8) Histopathological examination revealed normal architecture in comparison with control and treated animal.

Histopathology of Brain

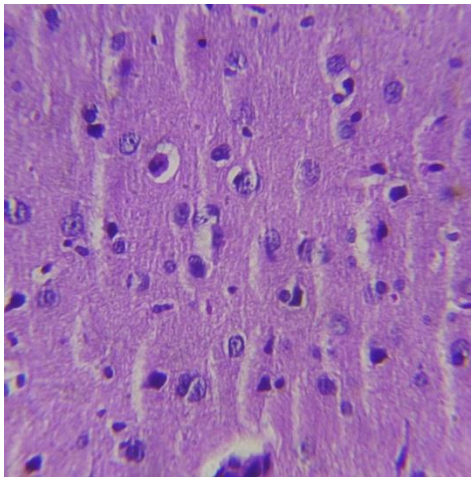


Plate A : Control Male

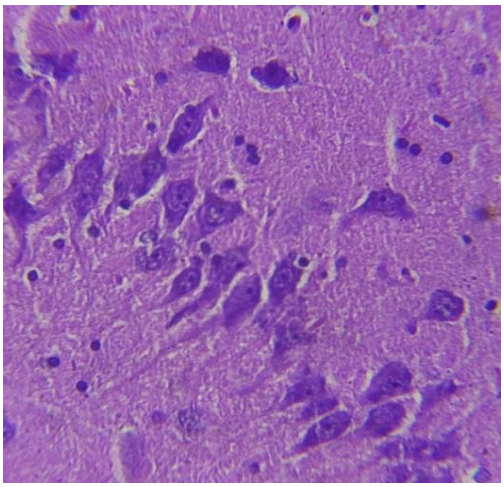


Plate B : Control Female

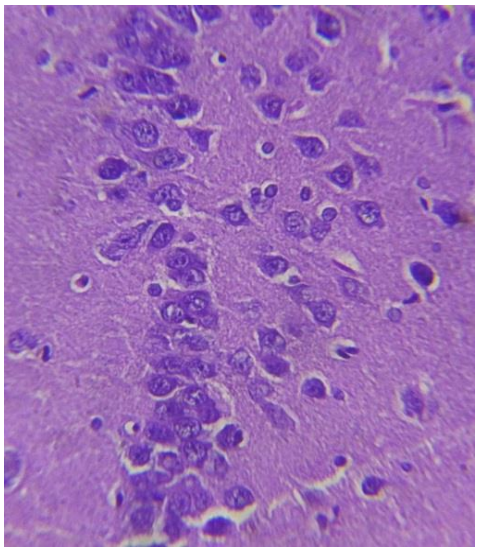


Plate C : High Dose Male

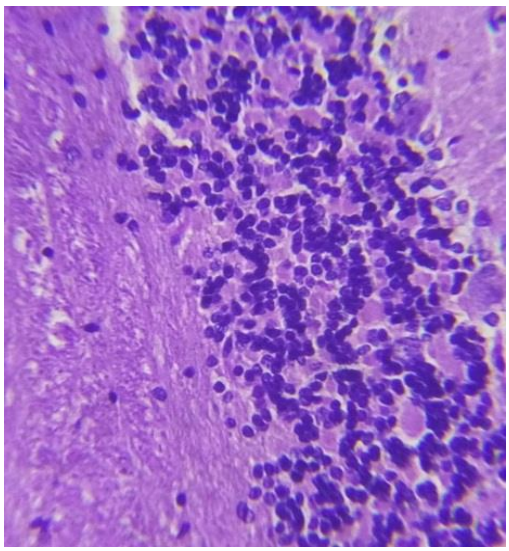


Plate D : High Dose Female

Plate A: Regular marginal alignment on the neurons with promising histology was observed.

Plate B: The CA zones of brain hippocampi are filled with densely packed Pyramidal cells.

Plate C: Arrangement of neurons on cerebral cortex appears normal and dense.

Plate D: Three layers of cerebellar cortex, the molecular, purkinje and granular layers, appeared clear and distinct without any changes in their cells

Histopathology of Heart

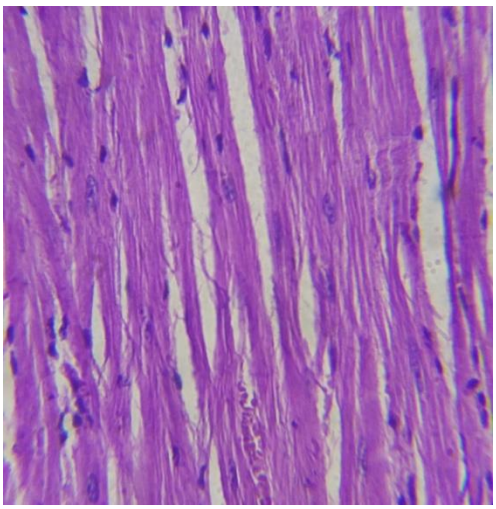


Plate A : Control Male

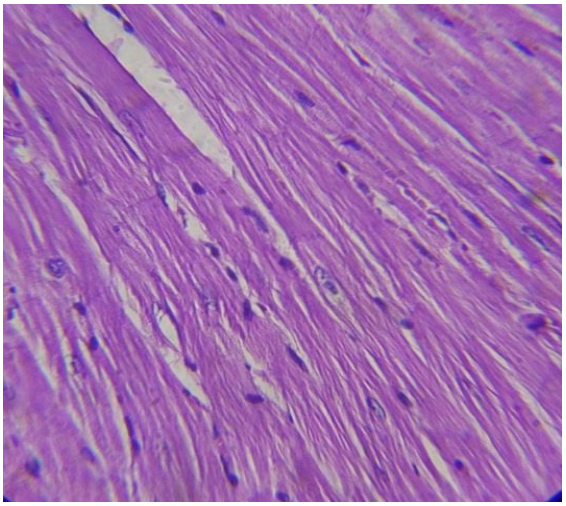


Plate B : Control Female

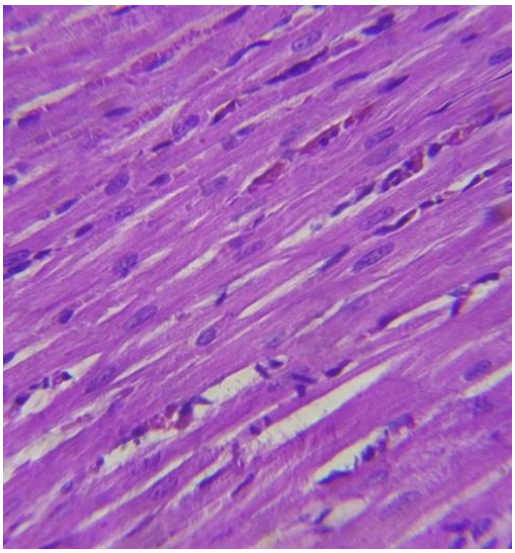


Plate C : High Dose Male

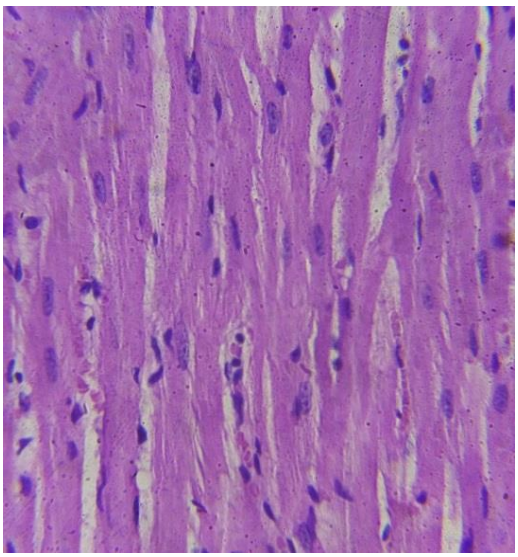


Plate D : High Dose Female

Plate A: Nucleus appears prominent with regular arrangement of fibres. No evidence of pyknotic nucleus.

Plate B: Myocardial cells appears normal with well-defined mycoplasma and prominent nucleus and nucleolus.

Plate C: Appearance of fibrils and cross striations are normal and equidistant.

Plate D: Appearance of cardiac myocyte was normal.

Histopathology of Lung

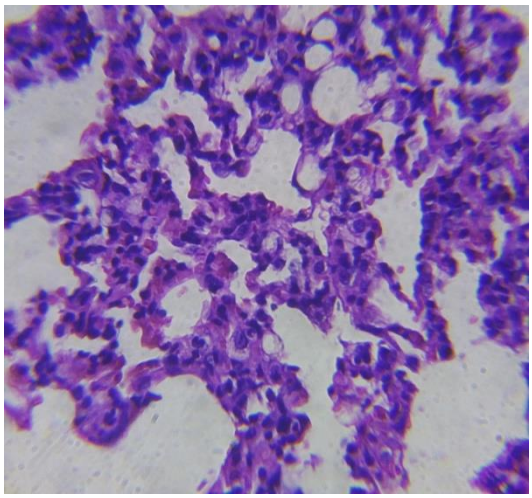


Plate A : Control Male

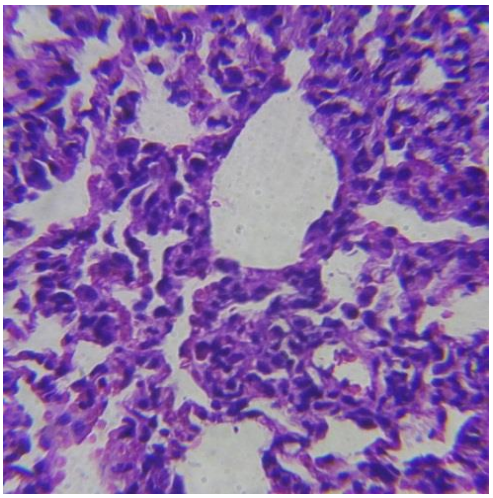


Plate B : Control Female

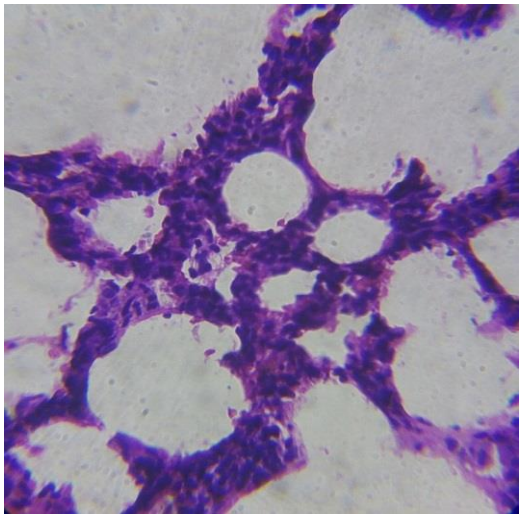


Plate C : High Dose Male

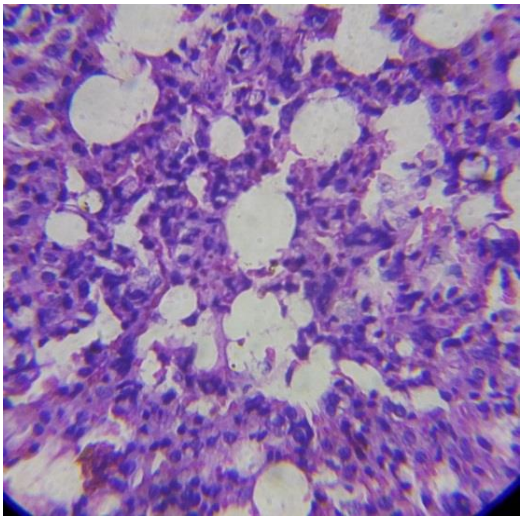


Plate D : High Dose Female

Plate A: Arrangement of epithelial and muscular appears normal.

Plate B: Lung parenchyma appears normal with regular arrangement of alveoli and alveolar sac with no signs of lymphocyte infiltration and pulmonary fibrosis.

Plate C: Perfect network of simple squamous epithelium were observed. Inter alveolar septum and alveolar capillary appears normal.

Plate D: Pneuomocyte and capillary appears normal.

Histopathology of Stomach

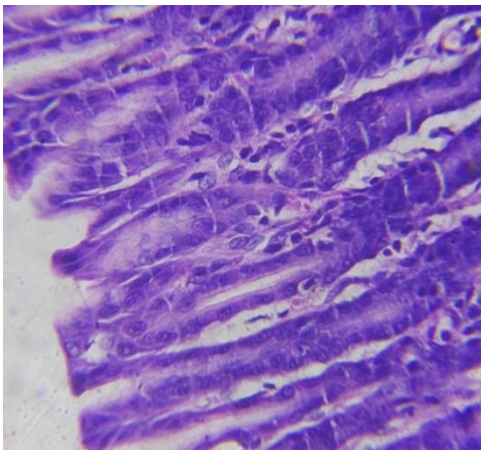


Plate A : Control Male

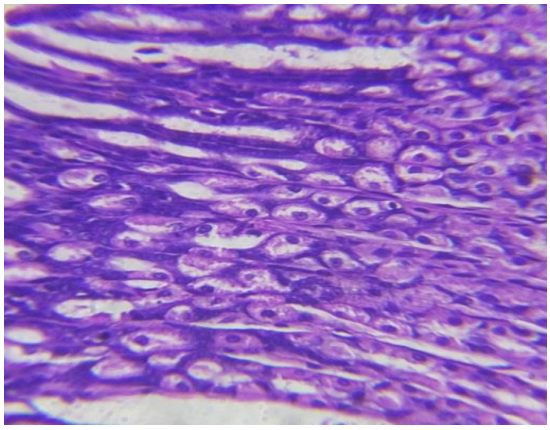


Plate B : Control Female

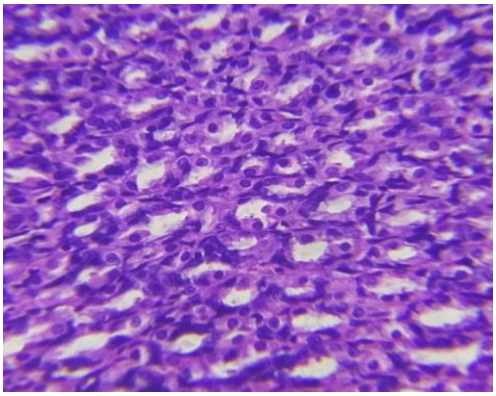


Plate C : High Dose Male

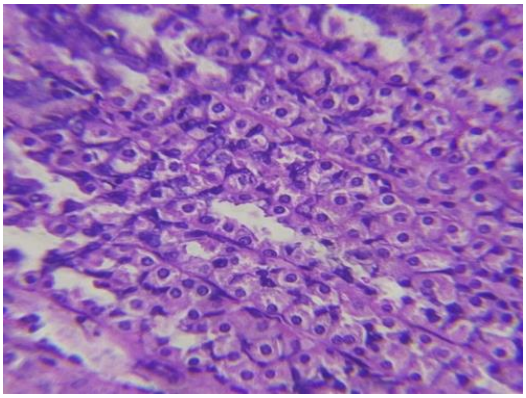


Plate D : High Dose Female

Plate A: Lumina of blood vessels appears normal. Appearance of glandular lumen was normal

Plate B: Regular arrangement of muscularis externa and outer longitudinal muscle were observed.

Plate C: Gastric glands including secretary sheath appears normal.

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

Histopathology of Liver

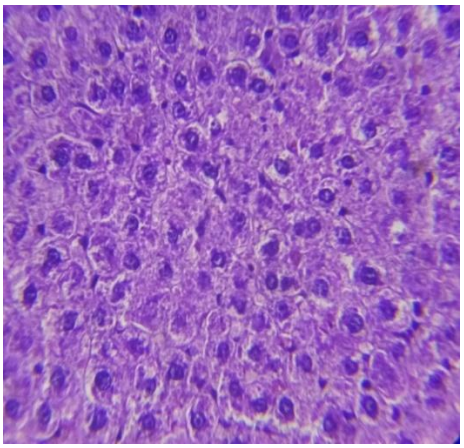


Plate A : Control Male

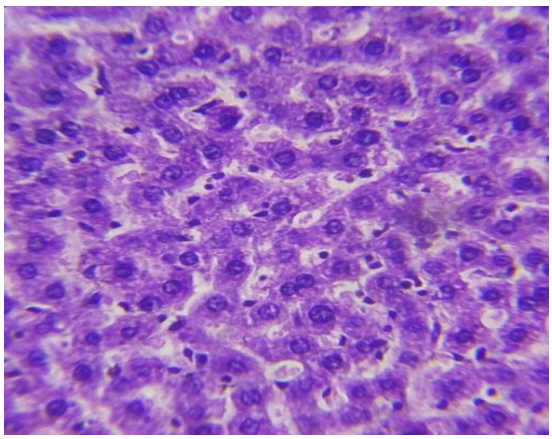


Plate B : Control Female

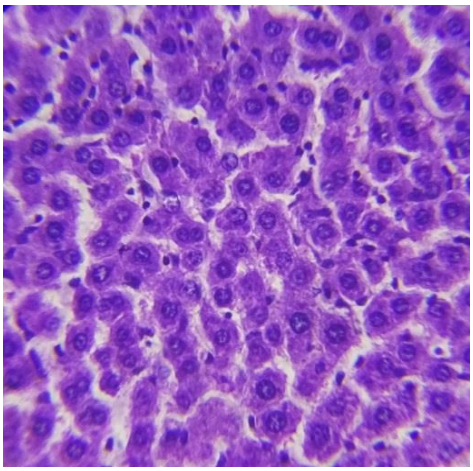


Plate C : High Dose Male

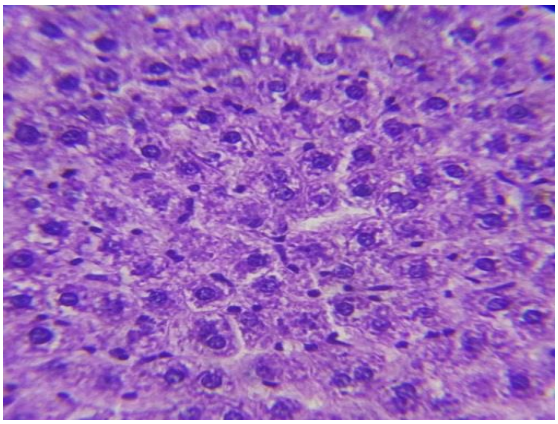


Plate D : High Dose Female

Plate A: Cytoplasm appears normal with widen portal tract.No signs of nodular degeneration and cirrhosis.

Plate B: The walls of the lumen appears normal with no evidence of ischemic changes .

Plate C: Liver parenchyma appears normal with no evidence of necrosis. Rare appearance of Kupffer cells with no evidence of phagocytosis in intracytoplasmic region.

Plate D: The centrilobular hepatocytes appears normal with stained cytoplasm.

Histopathology of Kidney

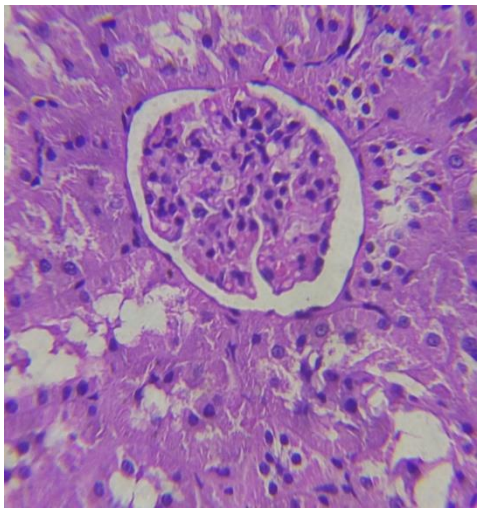


Plate A : Control Male

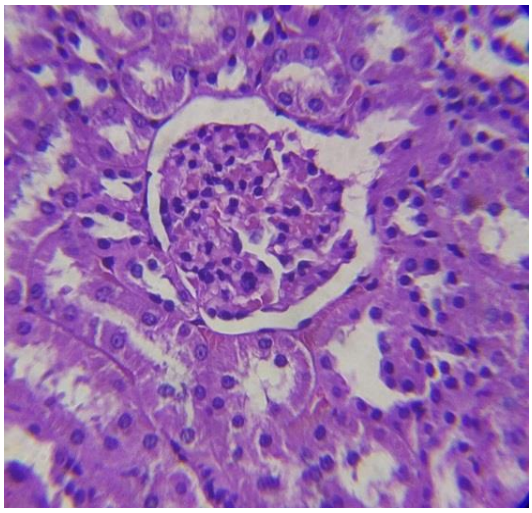


Plate B : Control Female

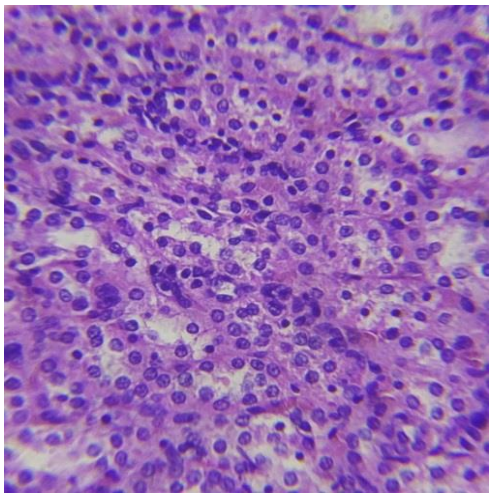


Plate C : High Dose Male

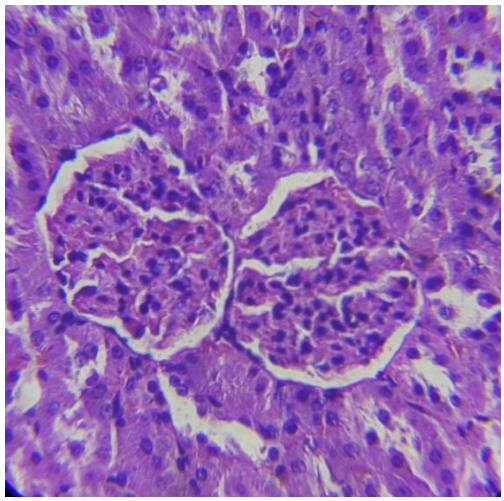


Plate D : High Dose Female

Plate A: Appearance of central artery and marginal sinus are normal. No abnormalities found in lymph node

Plate B: Appearance of glomerular basement membrane was normal.

Plate C: Foot processes of podocytes are separated from one another by a regular narrow Filtration Slit .

Plate D: Bowman's capsule appears normal and surrounded with Proximal Convoluted Tubule , Distal Convoluted Tubule and Collecting Duct.

Histopathology of Spleen

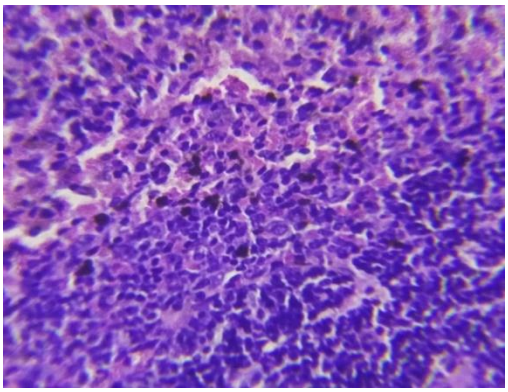


Plate A : Control Male

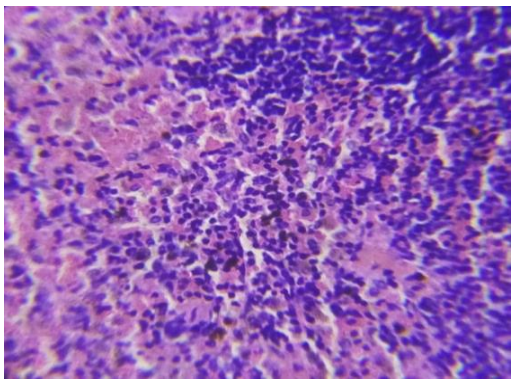


Plate B : Control Female

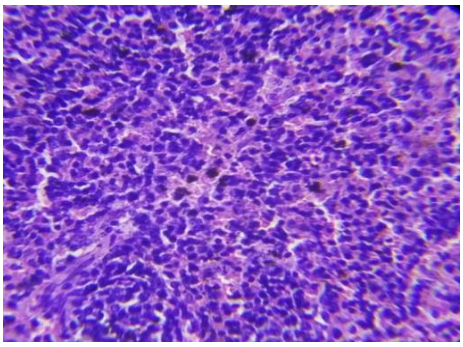


Plate C : High Dose Male

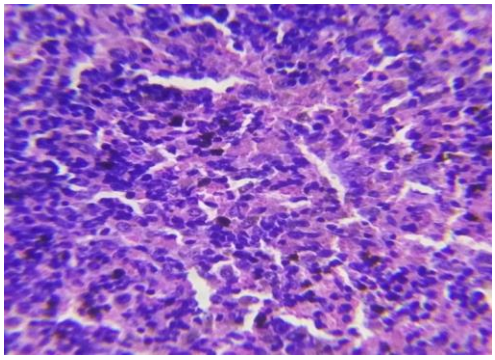


Plate D : High Dose Female

Plate A: Normal renal structure with rounded renal corpuscles formed of the Glomerulus. Increased bowman space around glomeruli

Plate B: Marginal vascular zone radiated in between red and white pulp. Appearance of splenic red pulp was normal.

Plate C: Lymphoid follicles appears normal.

Plate D: Erythropoietic cells (EP) are scattered throughout the red pulp with increased number of megakaryocytes.

Histopathology of Testes

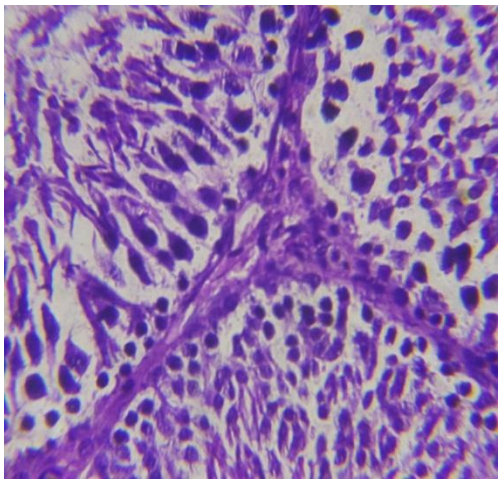


Plate A : Control Male

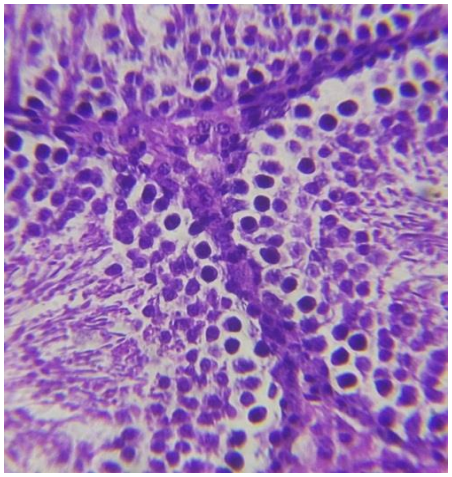


Plate B : High Dose Male

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

Plate B: Normal sertoli cell aligned properly on the basement membrane with oval dome shaped nucleus.

Histopathology of Uterus

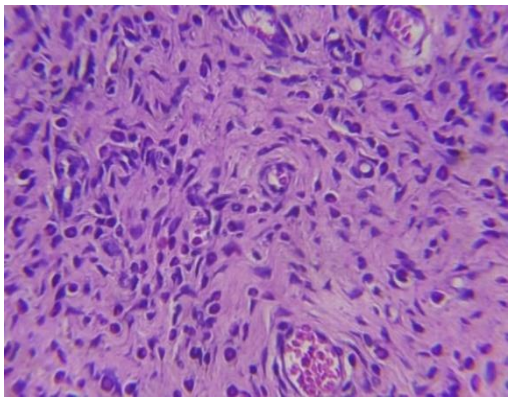


Plate A : Control Female

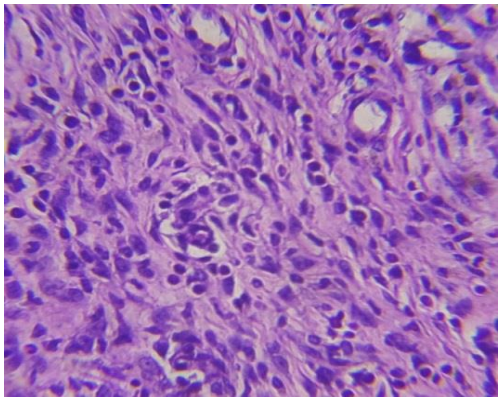
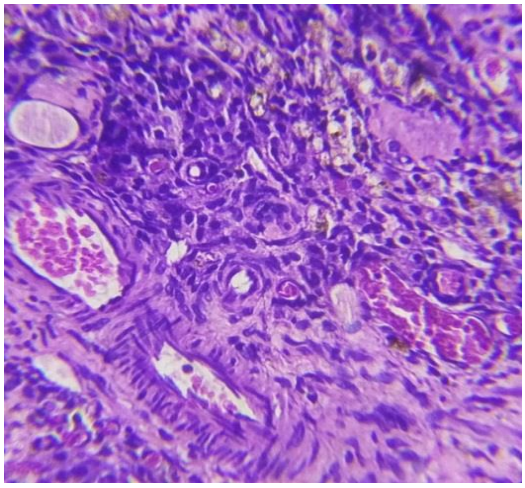


Plate B : High Dose Female.

Plate A: Appearance of endometrium, myometrium and uterine glands was normal.

Plate B: Endometrial stroma; G, gland; M, myometrium; P, perimetrium; L, lumen exhibits normal histological aspect of endometrium and myometrium

Histopathology of Ovary



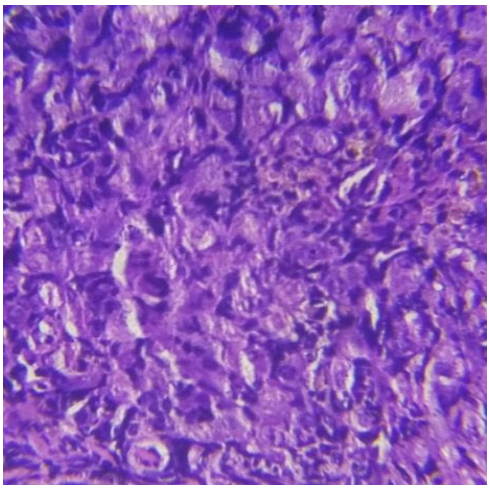


Plate A : Control Female **Plate B** : High Dose Female

Plate A: Follicular cells, cytoplasm and nucleus appear normal.

Plate B: Corpora lutea , atretic follicles and interstitial tissue appears normal.

Body weight changes of test animals in 28 days repeated oral toxicity study of Rasa Karpoora Kuligai

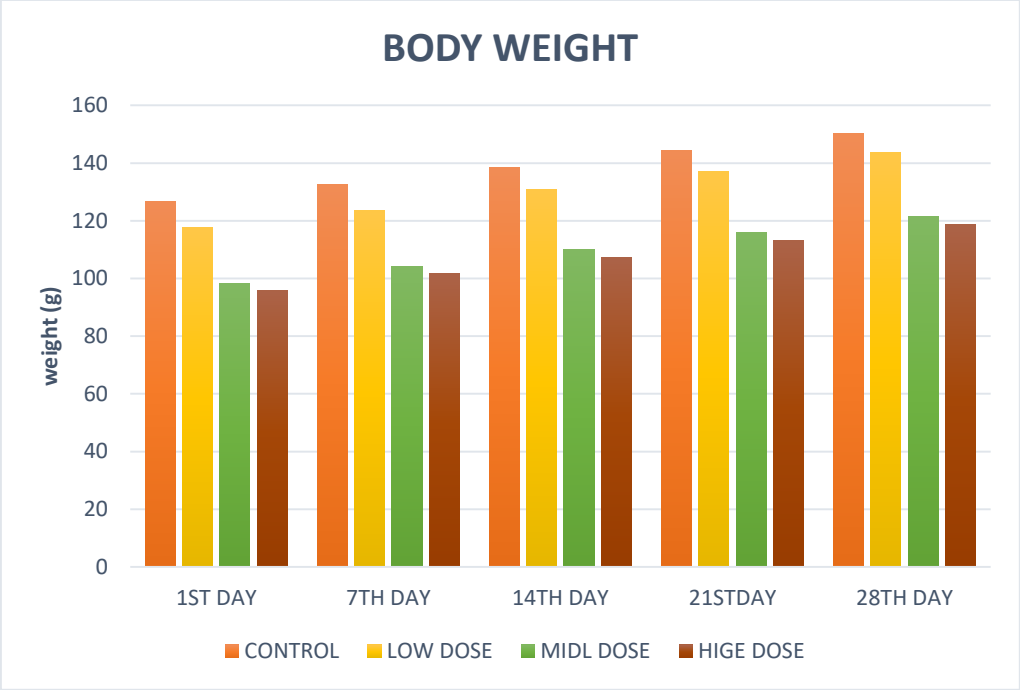


Fig 3

Fig:4 Organ weight changes in grams of test animals in 28 days repeated oral toxicity study of Rasa Karpoora Kuligai

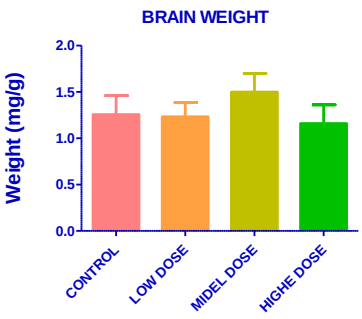


Fig 4.1

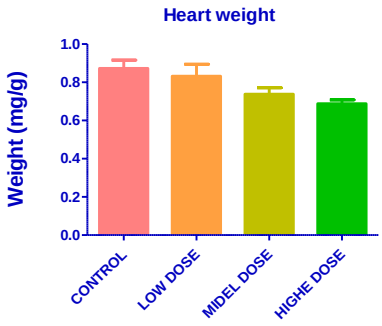


Fig 4.2

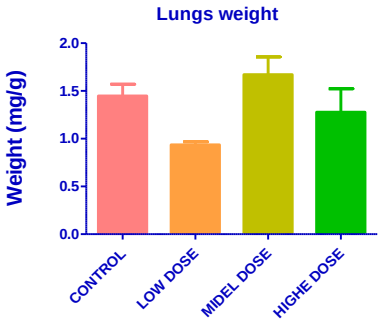


Fig 4.3

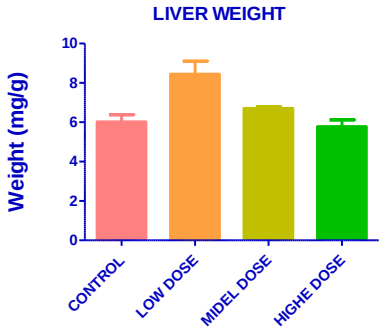


Fig 4.4

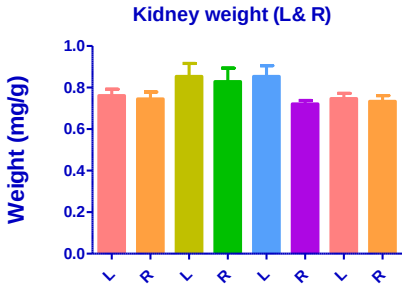


Fig 4.5

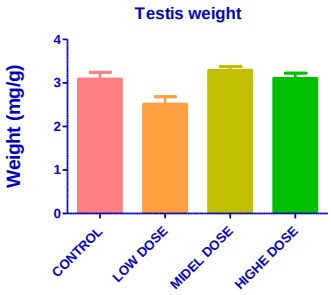


Fig 4.6

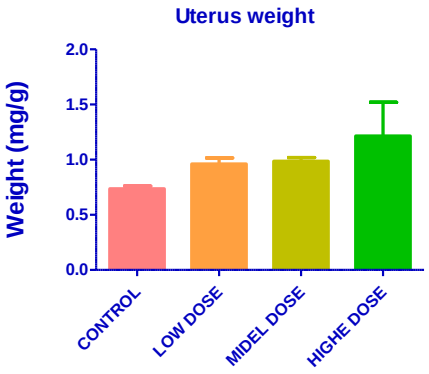


Fig 4.7

Fig 5: Effect of 28 days repeated dose of Rasa Karpoora Kuligai on Haematological parameters

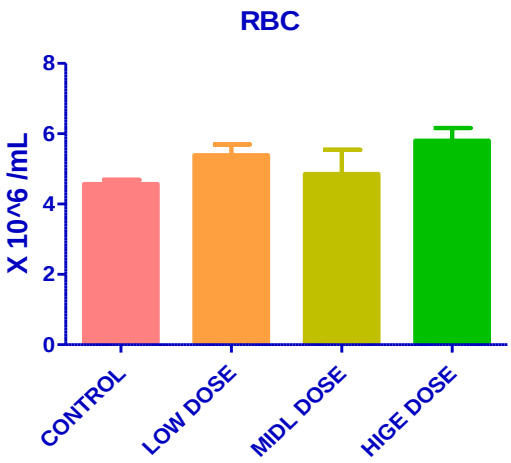


Fig 5.1

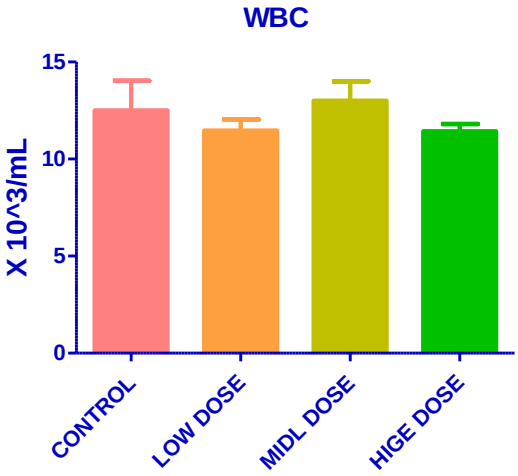


Fig 5.2

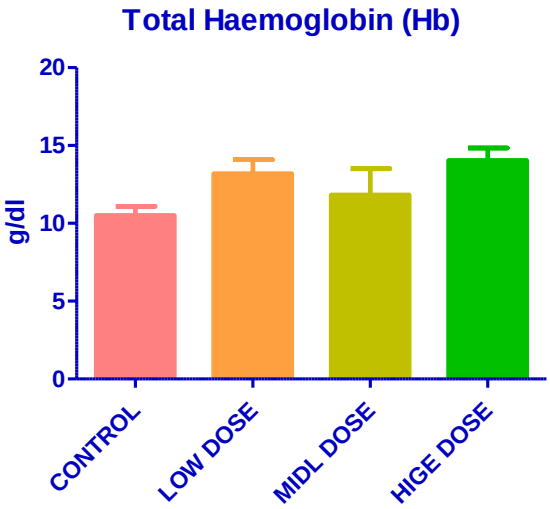


Fig 5.3

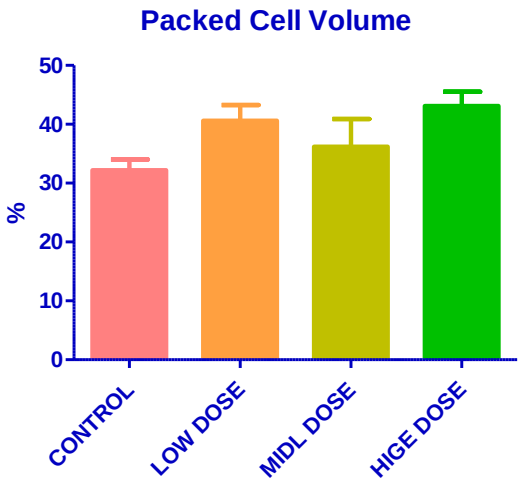


Fig 5.4

Fig: 6

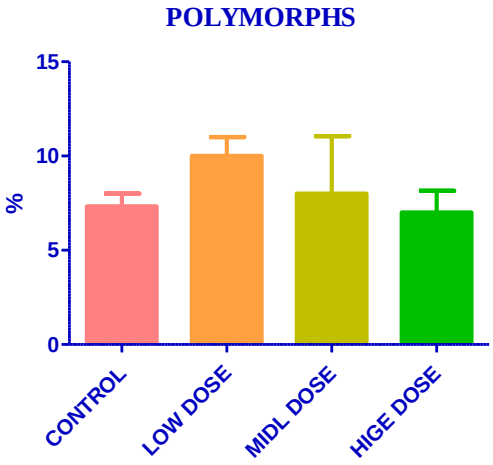


Fig 6.1

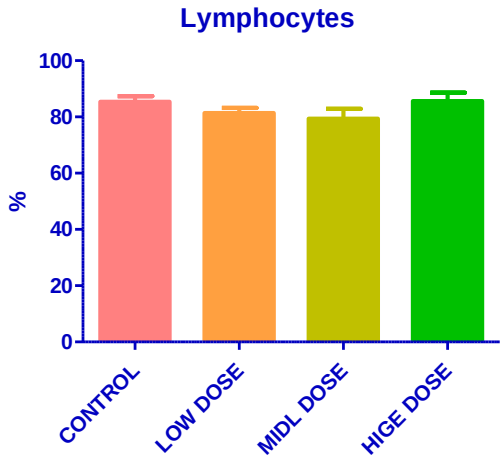


Fig 6.2

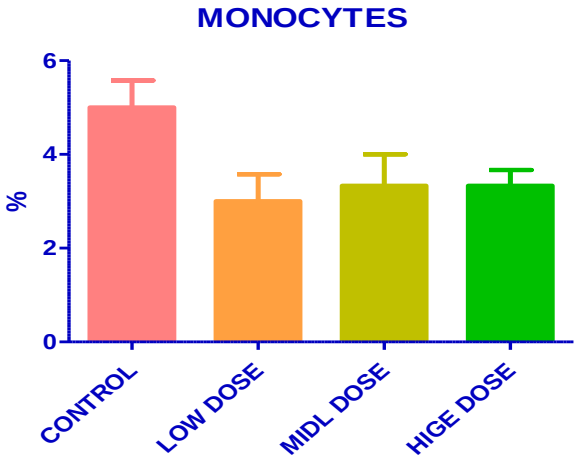


Fig 6.3

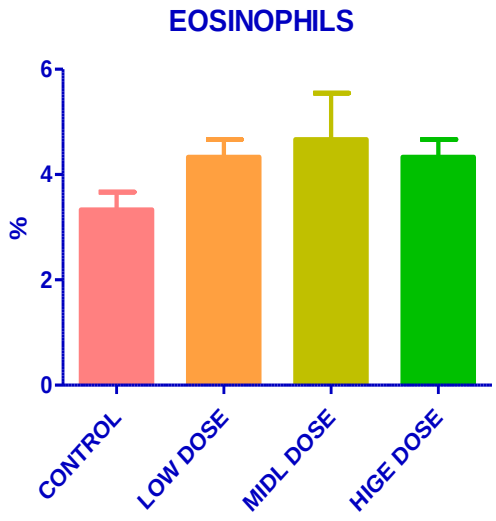


Fig 6.4

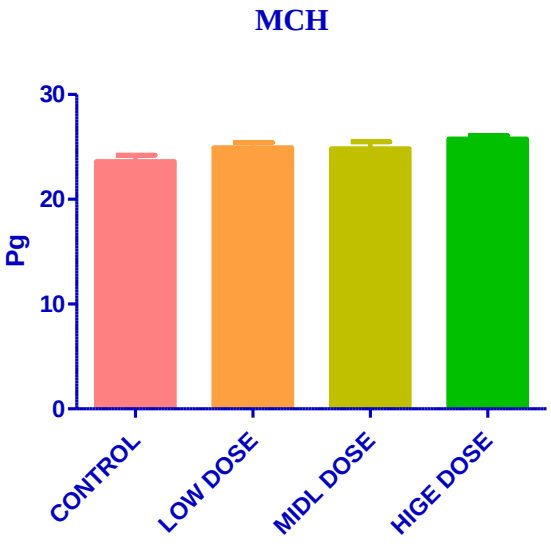


Fig 6.5

Fig:7 Effect of Subacute toxicity of Rasa Karpoora Kuligai on Biochemical parameters

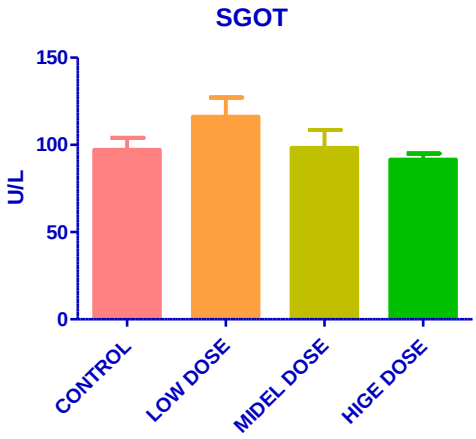


Fig 7.1

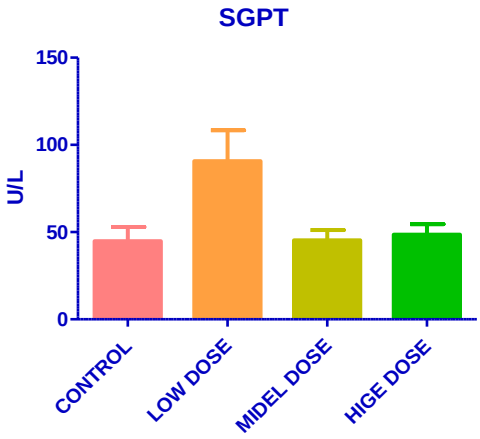


Fig 7.2

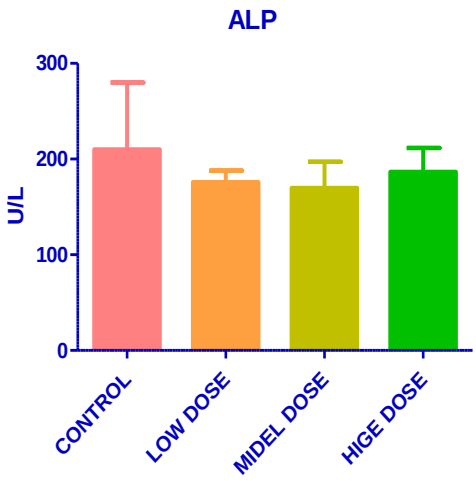


Fig 7.3

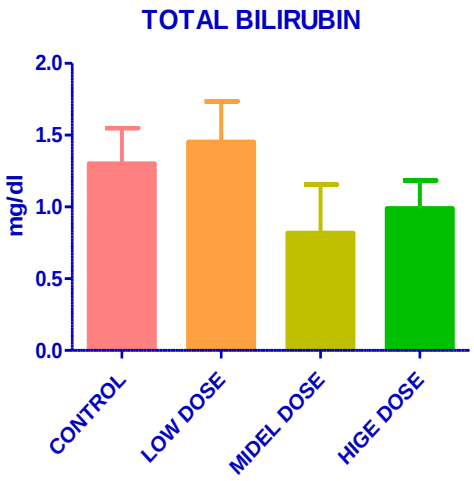


Fig 7.4

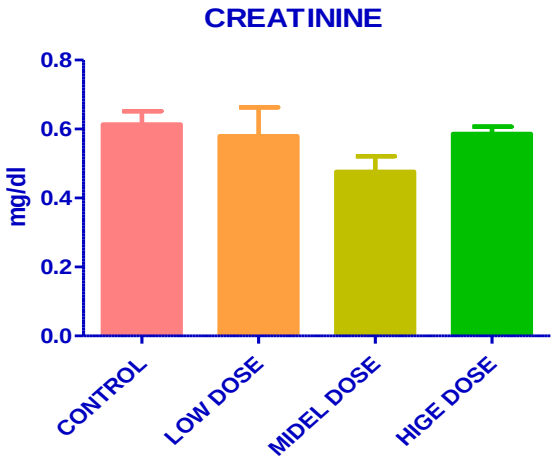


Fig 7.5

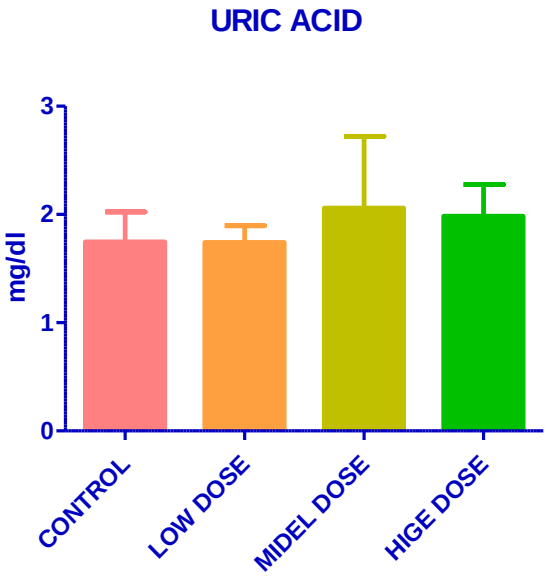


Fig 7.6

Fig- 8: Effect of 28 days repeated dose of Rasa Karpoora Kuligai on food intake in grams

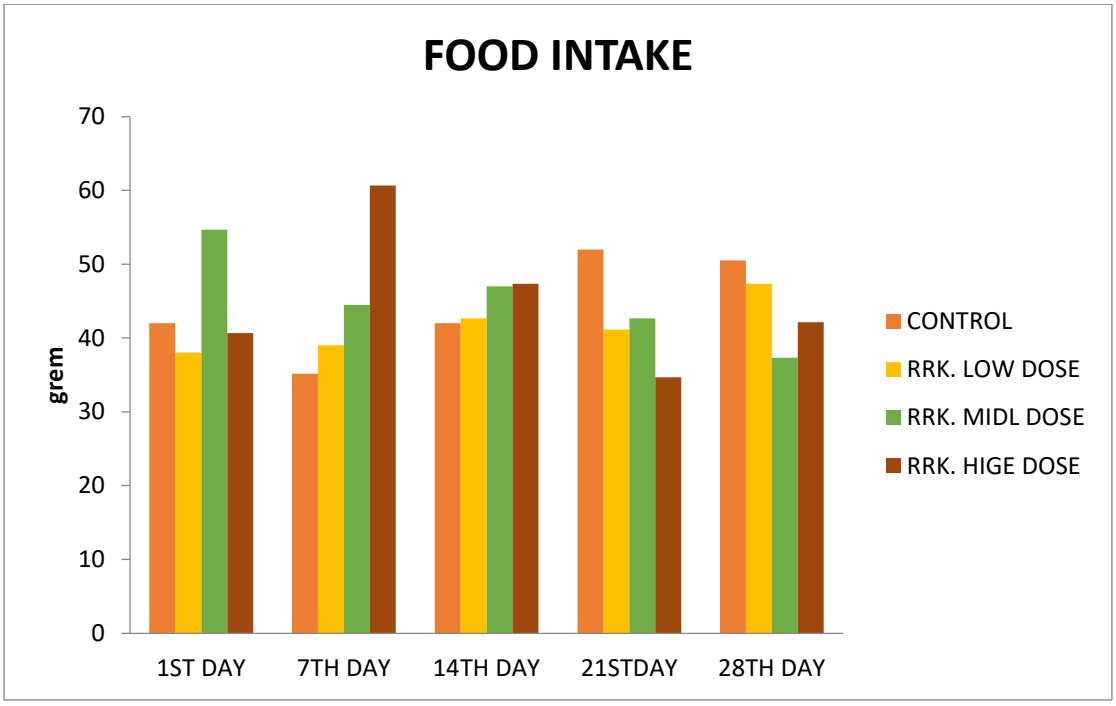


Fig 8

Fig -9 : Effect of 28 days repeated dose of Rasa Karpoora Kuligai on Water intake in ml

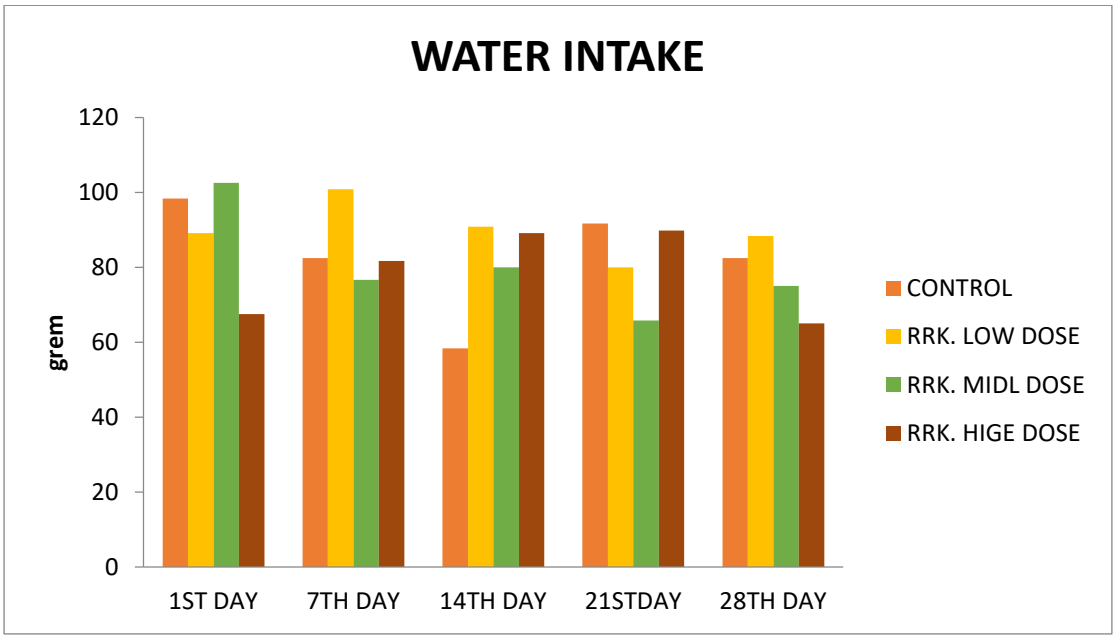


Fig 9

6. SUMMARY

The test drug **Rasa Karpoora Kuligai** is selected from the text, **Gunapadam Thathu Jeevam** for the evaluation of safety, efficacy and therapeutic potency.

Aim of the dissertation is to study the toxicity of the test drug Rasa Karpoora Kuligai by universal accepted scientific methods.

The review of the literatures and scientific research reveals pepper, garlic, betel leaf, mother milk that, are having anti cancer, anti-oxidant, anti-inflammatory activities.

The test drug was prepared properly by the given procedure all the ingredients were identified and authenticated by the concerned departments.

The preparation of trial drug was standardized primarily by physicochemical and biochemical analysis

The physicochemical analysis the drug shows brown in colour with pleasant odour and bitter mixed light pungent in taste.

In physiochemical analysis the total Ash value of test drug is 6.87 which shows the total inorganic content (ammonium, potassium, calcium, chloride, iron) present in the drug. These contents are having important role in physiological functions of the body.

Biochemical analysis shows the presence of calcium, sulphate, chloride, starch, phosphate, ferrous iron these compounds are protect the body from the risk of cancer.

In instrumental analysis the ICP-OES result shows the toxic heavy metals such as As, Hg, Cd, Pb, Al, Cu, Ni are in Below Detection Limit(BDL) . The main

ingredient of the drug is mercuric II chloride, but the final product RKK shows below detection limit of the mercury.

SEM analysis of the ***Rasa karpoora kuligai*** shows that the uniform distribution of particles presents in the entire field. Most of the particles present in the sample is nano size and near nano size, average particle size is 4.64µm - 7.51µm which increase the efficacy and bio availability of the test drug.

The acute toxicity study shows that ***Rasa Karpoora Kuligai*** did not produce any toxic effect at dose of 300mg/kg, and 2000 mg/kg to rats. So No-Observed-Adverse-Effect-Level (NOAEL) of ***Rasa karpoora kuligai*** is above 2000mg/kg.Bw.

In sub acute toxicity study test drug Rasa *Karpoora Kuligai* can be considered safe, as it did not cause either any lethality or adverse changes with general behaviour of rats and also there were no observable detrimental effects **(230 ,450 & 600 mg/kg body weight)** over a period of 28 days. These results have demonstrated that the ***Rasa Karpoora Kuligai*** is relatively safe when administered orally in rats.

In organs of control group and drug treated groups no abnormality was detected. Histopathological examination revealed normal architecture in comparison with control and treated animal.

7. CONCLUSION

From the results of analytical evaluation of the test drug ***Rasa Karpoora Kuligai,*** it is inferred that quality and stability was good when prepared under the standard protocol mentioned in this study. Qualitative analysis of RKK reveals the purity and bioavailability of the drug. As heavy metals were found to be with in the permissible limit so the drug is safe for oral consumption. The particle size of the test drug was determined by SEM analysis. In vivo toxicity study reveals the drug **RKK** shows no mortality and signs of toxicity upto 2000 mg/Kg bodyweight in acute oral administration. In 28 days repeated oral toxicity study there was significantly changes in haematological, biochemical parameter in **RKK (230mg, 450mg &600mg /Kg bodyweight)** treated group but the levels were with in physiological limits. The histopathology report also confirms that there are no remarkable cellular changes at all the dose level. Based on the results, it can be concluded that, the dose level of ***Rasa Karpoora Kuligai*** is *sundai alavu* (0.798mg) mentioned in G*unapadam Thathu Jeevam* is a safe dosage for human consumption.

8. BIBLIOGRAPHY

1. Rise and prevalence of cancer in India, Times of India. 4 Feb 2017
2. A.K.Gupta,Wealth of India,vol-IV ,Publication and Information Directorate ,New delhi,1988 edition,Pg-21, 22.
3. Dr.R,Thiyagarajan, Gunapadam Thathu jeeva vaguppu,part-II&III, 8th edition, M.L.M printers,Chennai. Pg-285-287.
4. OECD guidelines 423 and 407.
5. Namadeepa nigandu
6. Agasthiyar paripooranam 400, Thamarai noolagam publications
7. S.P.Ramachandran,Agathiyar yemmathathuvam Ennum panchakaviya Nigandu –Mahalakshmi offset,Chennai. pg.142
8. https://en.wikipedia.org/wiki/mercury(II)-chloride,June 8,2018,2.30 pm.
9. Rustomjee, Naserwanjee Khory, Mateira medica of India and their therapeutics,1999 edition,BDH printers,New Delhi.pg-255
10. Anil kumar dhiman, Purushotam kausik,Medicinal plants and Raw drugs of India,1999 edition,shiva offset press,Dehradun. Pg.no.255, 1020, 1021
11. Murugesa Mudaliyar, Gunapadam Siddha mooligai vaguppu-I part, 3rd edition, Pg.710, 201, 174, 406, 460, 806, 787.
12. C.Kannusami pillai, Sikicha Ratna Deepam Enum Vaidya Nool, – published in 1991,Thirumagal printers, Chennai. pg.106, 111, 118, 124, 126, 132, 212, 217, 220, 269, 284.
13. R.Kritikar and B.D. Basu, Indian medicinal plants ,Vol –IV,1989 second edition , pg:2627.
14. Rao shahib,M.Rama rao,Flowering plants of Travancore,International book distributor,Dehradun.pg:162.
15. S.N.Yoganarasimahan, Medicinal plants of India, volume-II,2000 edition,Research Regional Institute,Bangalore, Pg-541
16. Database of Medicinal plants used in Ayurveda,vol-III,2005 edition,Pearl offset press pvt ltd,New Delhi, Pg.no-282-286.
17. Ivan A.Ross,Medicinal plants of the world,vol-3,2007 edition,Rajkamal electric press,Delhi,pg:228,229.

18. Dr.yoganarashiman,Medicinal plants of India ,part-II,2000 edition,mangala graphics,pg:19
19. T.E.Wallis, Text Book of Pharmacognosy,1985, 5th edition,CBS Publishers&distributors,New Delhi.pg:396,397
20. Dr.k.m.Nadkarni,Indian materia medica, popular prakasan private Ltd,1993 edition,pg:108,537,538.
21. T.Pulliah, Medicinal plants in India Vol-I,2002 edition,Regency publications,New Delhi. pg.251
22. Prof.S.K.Bhatiacharjee, Hand book of Medicinal plants,5th revised enlarged edition 2008,pointer publishers,Jaipur,India. Pg.no-344
23. A.K.Gupta,Madhu sharma,Indian Medicinal plants,vol-V,2007 edition,Mehta offset,New Delhi.pg:578,579.
24. Ram.P.Rastogi, B.N.Mehrota,compendium of medicinal plants Volume-I,2004 edition,NISCAIR Press,council of scientific and Industrial research,New Delhi.Pg. 406, 407
25. S.P.Ramachandran,Gunapadam kaiyedu,Thamarai noolagam, creative offset,Chennai-26, pg..76
26. Padmaja udayakumar, Textbook of Pharmacology,Fourth edition, 2013, CBS Publishers, pp 7-9

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

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

This Certificate is awarded to Dr/~~Mr/Mrs~~....R.:..MURUGAVEL.....

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

"RESEARCH METHODOLOGY & BIOSTATISTICS"

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

Organized by the Department of Siddha

The Tamil Nadu Dr. M.G.R. Medical University From 06th to 10th June 2016.

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

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

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

CERTIFICATE

This is certify that the project title. Pre clinical Safety evaluation of the Siddha formulation Ragakarpoora kuligai in Rat

Has been approved by the IAEC. model - 49 Rats (20M + 29F)
Approval No: NIS/IAEC-II/13/2016

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

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

V. Banumathi 29/9/2016
Signature with date

29/9/2016

Chairman/~~Member Secretary~~ of IAEC:

CPCSEA nominee:

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

NATIONAL INSTITUTE OF SIDDHA

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

Workshop on

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

06 -10 February 2017

CERTIFICATE

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

Dr. V. Suba
Organizing Secretary

Dr. P. Muthusamy
Veterinary Consultant

Prof. Dr. V. Banumathi
Director / Chairperson

NATIONAL INSTITUTE OF SIDDHA
MINISTRY OF AYUSH
GOVERNMENT OF INDIA
TAMBARAM SANATORIUM, CHENNAI - 600 047
Tele : 044-22411611 Fax : 22381314
nischennaisiddha@yahoo.co.in www.nischennai.org

14.07.17

AUTHENTIFICATION CERTIFICATE

Certified that the sample submitted for identification by **Dr. R. Murugavel**, II year PG scholar, Dept. of Nanju noolum Maruthuva neethi noolum, National Institute of Siddha, Chennai - 47, is identified as **RasakarPooram- Merucry subchloride** on the basis of macroscopic character.

This certificate is issued for the purpose of preparing her dissertation medicine in Gunapadam laboratory, NIS.

Dr. S. Visweswaran, M.D (s)
Head of Department
Department of Gunapadam
National Institute of Siddha
Tambaram Sanatorium, Chennai-47.

NATIONAL INSTITUTE OF SIDDHA, CHENNAI – 600047

BOTANICAL CERTIFICATE

Certified that the following plant drugs used in the Toxicity studies of **Rasakarpoora Kuligai** taken up for Post Graduation Dissertation studies by **Dr.R.Murugavel** M.D.(S), III year, Department of Nanju Maruthuvam, 2018, are identified through Visual inspection, Experience, Education & Training, Organoleptic characters, Morphology and Taxonomical methods as

Piper betle Linn. (Piperaceae), Leaf
Piper nigrum Linn. (Piperaceae), Fruit
Allium sativum Linn. (Liliaceae), Bulb

DEPARTMENT OF MEDICINAL BOTANY ★ CHENNAI 600 047 ★ National Institute of Siddha

rtificate No: NISMB3432018

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