PRECLINICAL SAFETY EVALUATION OF

NAGA RASA PARPAM

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DECLARATION BY THE CANDITATE

I hereby declare that dissertation entitled **"PRECLINICAL SAFETY EVALUATION OF NAGA RASA PARPAM"** is a bonafide and genuine research work carried out by me under the guidance of **Dr. V. Manjari, MD(S), Ph.D.,** Associate Professor, Department of Nanju Maruthuvam, National Institute of Siddha, Chennai – 47, and the dissertation has not formed the basis for the award of Degree, Diploma, Fellowship or and other similar title.

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BONAFIDE CERTIFICATE

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INDEX

1. INTRODUCTION

Siddha system of medicine is a great heritage of India. The Siddha system is the holistic system of codified life style health care perfected many thousands of years ago in Tamil speaking peninsular India. It is developed by Siddhars the ancient super natural spiritual saints of India. The Siddha system of medicine uses a combination of herbs, minerals, metals and to promote good health and longevity.[1] The system worked on the basic concept that a healthy soul can only be developed through a healthy body.

According to Siddha system, the human body is composed of 96 principles (Thathuvam), Among 96 principles, Uyirthathukkal (Vatham, Pitham, Kapam) are constituents which are essential for the normal functions of the body. Any abnormality in the normal statue of these uyirthathukkal can cause pathological changes known as disease.[2]

Metal and mineral based drugs are commonly used in Siddha system of medicine. In Siddha Mercury (Rasam), Gold (Thangam), Tin (Vangam), Iron (Iyam), and Zinc (Nagam) are used as drug in an incinerated (Parpam, Chenthuram, Chunnam, etc.,) form.

Zinc is a nutritionally important nontoxic trace element, which plays a crucial part in the overall balance of the body processes.[3] It's documented as the second most abundant element in the body and as one of the safety mineral to be used for human health. In the advisable body limits, it is neither cytotoxic, mutagenic nor teratogenic.[4,5,] Zinc ion plays a key role in cell proliferation, cell differentiation and programmed cell death (Apoptosis). It is proven that Zinc has involvement in DNA synthesis, reproductive function, vision, taste perception and cognitive function.[6]

The element Zinc is considered as a superior native metal in Siddha medicine[7]. In the traditional approach, the metal Zinc or its formulations are considered as powerful candidates against infection of sexual transmitted and respiratory system.[8]

Mercury is used in therapeutic in a compound form. According to Siddhar's Mercury is the chief of all elements and ubiquitous. Siddhar's believe and prove that mercury can cure most of the incurable disease, protect the body from the disease, gives good health and facilitate to attain eight folds of siddi. The term Panchasootham refers to five types of mercury compounds used to do medicine. Mercury compared to the lord Bhrama, Vishnu and Sivan because of its three power, Birth power, Protective power, and Destructive power respectively.[9]

Mercury containing preparations have been used extensively in Indian system of medicine for treatment of chronic illness like syphilis, high fever, pneumonia, insomnia, nervous disorders, deafness and paralysis of the tongue. Contrary to western medicine, which does not promote the use of mercury due its toxic effects, Indian traditional medical system believe that mercury based formulations have potent therapeutic efficacy, while there is no toxicity due to the unique and repeated purification processes employed during preparation.[9]

In up to date World human beings are suffered by loads of disease. Now days due to the ecological changes and food habits, most of the populations are affected by Ano-rectal disease (Eg: Hemorrhoid and Fissure in Ano) and cancer. Approximately 50% to 66% of people have problem with hemorrhoids at some point in their lives.[10,11] Males and females are both affected with equal frequency. Hemorrhoid affect people most often between 45 and 65 years of age [12]. Globally, every year between 50,000 to 100,000 women are affected by fistula after childbirth.[13]

Cancer is a group of disease involving abnormal cell growth with the potential to invade or spread to other parts of the body.[16,17] Frequency 24million annually (2019)[18], Death 10millionn annually (2019).[18]

Most of the Siddha text clearly mentions that if a drug is used without knowledge of its proper dosage, it would certainly act as a poison. But we will use the poisonous metals like mercury, arsenic, etc in proper dosage act as a drug.[10]

According to Siddha literature ***Naga Rasa Parpam*** is given to Cancer, Gonorrhea, Hemorrhoid and Fistula. Nagam and Rasam are main ingredients in ***Naga Rasa Parpam***[17]

Safety is the major concern of treatment. Till now there is no toxicity assessment research work done in Naga Rasa Parpam. So in this dissertation, Iwill do the scientific validation and toxicity evaluation of ***Naga Rasa Parpam***

2. AIM AND OBJECTIVE

AIM:

To evaluate the safety profile of **Naga rasa parpam (NRP)** through Acute and 28days Repeated dose oral Toxicity studies in Wistar Albino rats.

OBJECTIVE:

- Collecting the Literary evidence (Siddha aspect, Toxicological aspect, Research reports) in detail about the ingredients of Naga rasa parpam.
- Collection of raw drug
- Purification process of raw drugs on the basis of Siddha literature
- Preparation of Naga rasa parpam on the basis of Siddha literature
- To evaluate the physicochemical properties of Naga rasa parpam
- Qualitative analysis on Naga rasa parpam
- Quantitative analysis on Naga rasa parpam
- To do Acute and 28 days repeated oral toxicity study of Naga rasa parpam

3. LITERATURE REVIEW

3.1.நாகம் (SIDDHA ASPECT)

பெயர்காரணம்:

உருக்கு முகத்தில் பாம்பு போல சீறுதலின் இதற்கு இப்பெயர் வழங்கப்படுகிறது.

வேறுபெயர்:

சீறல், பொருமல், பொங்கல், இரைச்சல், ஐம்புகை, சோரம், வாசுகி, வெண்ணாகம், தாம்பிரத்தின் வேதை, வாதத்திற்கு உயிர்.

பண்பு:

- வெண்மையும் நீலமும் கலந்த நிறமாயும், பளபளப்பாயும் கட்டியாயும் இருக்கும்.
- இதனை கம்பியாக நீட்டுதலும், தகடுகலாக தட்டுதலும் செய்யலாம்.
- திரவாகங்களில் கரையும்.
- காற்றில் பட்டால் அதன் மேல் உப்பு பூத்து விடும்.
- இதனுடன் செம்பு சேர்த்து உறுக்க பித்தளையாகும்.

கிடைக்குமிடம்:

- சீனா,
- பர்மா.

வகை:

நாகத்தில் இருவகை உண்டு.

1. பெருங்கண் நாகம்,
2. சிறுகண் நாகம். அணுக்கள் அடங்கப்பட்டது சிறுகண் நாகம்.

நாகத்தினுடைய பகைச் சரக்குகள்:

அண்டம், அபினி, அன்னபேதி, கல்லுப்பு, கிளிஞ்சல், சிங்கி, தங்கம், தரா, நண்டோடு, மிளகு, வளையலுப்பு, வீரம், வெடியுப்பு, வெங்காரம், வெள்ளி, வெள்ளைப்பாஷாணம்.

நட்புச்சரக்குகள்:

அப்பிரகம், இரும்பு, கந்தி, காந்தம், காரீயம், கௌரி, நவச்சாரம், சிலாசத்து, சூதம், செம்பு.

செய்கை:

துவர்ப்பி (Astringent)

குருதிபெருக்கடக்கி (Stypic)

உடல்தேற்றி (Alterative)

பஞ்சபூதக்கூறு:

ஆகாயக்கூறு

பொதுகுணம்:

"**மேகம்கிளர் பேதிவெட்டை யனலைத் தணிக்கும்**
வேகங்கிராணி விலக்குங்காண் – போகா
பரியமுளைப் புண்ணைப் பயித்தியத்தைப் போக்கும்
அரிய துத்தநாகமது"

பொருள்:

துத்தநாகம் மேகம், பேதி, வெள்ளை, உட்சூடு, கிராணி, முனளப்புண், பித்தம் முதலிய நோய்களைப் போக்கும்.

"**சொல்லிமுடியாது துத்தநாகம் பொடியாய்**
மெல்லத்துரத்தும் வியாதிகளை - நல்ல
உரனுடைமை யுண்டாகும் உண்டா வரையென்று
கிருமி மனைமண் மகிமைகேள்"

பொருள்: துத்தநாகம் பிணிகளை நீக்கி, உடலுக்கு வன்மையை அளிக்கும்[20].

சுத்திமுறை:

நாகத்தை உருக்கி இலுப்பை எண்ணெயில் சாய்க்க வேண்டும். இலுப்பை எண்ணெயானது நாகம் நன்றாக அமிழ்த்திருக்கும் படியாக இருக்க வேண்டும். ஒரு தடவைக்கு உபயோகித்த எண்ணெயை மறுதடவைக்கு உபயோகிக்கலாரது ஆறின பின் நாகத்தை எடுத்து உருக்கி முன்போல் மேற்படி எண்ணெயில் சாய்க்க வேண்டும். இவ்விதம் மடக்கி மடக்கி உருக்கி உருக்கி பத்து முறை சாய்த்தால் நாகமானது தூய்மையாகிவிடும்.

- அனுபோக வைத்திய நவநீதம் பாகம் 9, பக்கம் எண் 86[21]

நாகம் சேரும் பெருமருந்துகள்:

நாக செந்தூரம்: 6 வேறு செய்முறைகள்[22], [23], [24], [25], [26]

திராவகமுறை: 2 வேறு செய்முறைகள்[20], [27]

நாகபற்பம் : 6 வேறு செய்முறைகள்([26], [28], [23]. [20], [22], [27]

நாக கட்டு : 3 வேறு செய்முறைகள்[28], [21], [30]

நாகரசம் : : வேறு செய்முறை[29]

3.2 ZINC

Zinc also known as spelter, is a metallic chemical element, it has the symbol "Zn" and atomic number 30. It is bluish white, lustrous, hexagonal crystal structure. Zinc is, in some respects, chemically similar to magnesium, because its ion is of similar size and its only common oxidation state is +2. Zinc is the 24th most abundant element in the Earth's crust and has five stable isotopes. The most exploited zinc ore is sphalerite, a zinc sulfide.

The largest exploitable deposits are found in Australia, Canada, and the United States. Zinc production includes froth flotation of the ore, roasting, and final extraction using electricity (electrowinning). Brass, which is an alloy of copper and zinc, German chemist Andreas Sigismund Marggraf is normally given credit for discovering pure metallic zinc in 1746[31].

VERNACULAR NAMES:

- Sanskrit - Yashada.
- English - Spelter or impure commercial zinc;
- Hindi - Jasta.
- Bengali - Dasta.
- Gujarati - Jasad.
- Duke - Jas.
- Tamil - Tutanagam.
- Telugu - Tuttunagam.
- Malay - Nagam
- Konkani - Tambaku.
- Chinese - Tutenague[32].

VARAITIES OF ZINC:

1. Zinc carbonate and zinc gluconate - It is used as dietary supplement.
2. Zinc chloride - It is used as a deodorants.
3. Zinc pyrithione zinc sulphide - It is used as a anti-dandruff shampoo.
4. Zinc methyl or zinc diethyl - It is used in organic laboratory [31].

PHYSICAL PROPERTIES OF ZINC:

Group: 12[(138)]

Period: 4[(139)]

Atomic number: 30

Melting weight: 55.847

Atomic Mass Average: 65:39 µg.mol^{-1}[(136)]

Boiling Point: 1180K 907°C1665°E

Conductivity:

Electrical: 0.166 10°/cm2

Thermal: 1.16 W/cm K

Density: 7.13g/cc @ 300K

Description: Hard, brittle, shiny bluish-white transition metal

Bulk: 69.4/G Pa

Rigidity: 41.9/G Pa

Heat of Vaporization: 115.3kJ/mol

Melting Point: 692.88K 419.73°C787.51°F

Molar Volume: 9.16cm³/mole

Optical Reflectivity: 80%

Optical Refractive Index: 1.00205

Physical State: (at 20°c & Latm): Solid

Specific Heat: 0.39j/gK

Vapour Pressure: 19.2Pa@419.3°C [(137)].

Density: 7.14g/cm

Boiling point: 1180k

Standard potential: 0.763V

Energy of second ionisation: 1723 Kj.mol^{-1}[(137)]

Discovered: Andreas Marggraf in 1746[(140)].

ISOTOPES:

Five isotopes of zinc occur in nature.

^{64}Zn, is the most abundant isotope[34,35], ^{70}Zn (0.6%), ^{66}Zn (28%), ^{62}Zn (4%) – occur in nature ^{68}Zn (19%)[34]

SOURCES:

- Never occurs free in Nature, but exists variously combined with elements to form salts.
- It exists combined with oxygen as red oxide, with carbon as an impure carbonate, with sulphur as sulphide or sulphuret (Blende) or with Silicate.
- It is obtained by subliming carbonate or oxide of zinc with charcoal[32].
- Zinc is widely distributed in food mainly bound to protein.
- Red meat, fish, wheat germ, whole bran.

PRODUCTION:

Zinc is the fourth most common metal in use, an annual production of about 13 Million tonnes[35].

Two basic methods are used

1) Pyrometallurgy[36]

2) Electrowinning

Pyrometallurgy processing zinc oxide with carbon or carbon monoxide at 950°C (1,740°F) into the metal, which is distilled as zinc vapour. The zinc vapour is collected in a condenser[37]. The below set of equations demonstrate this process[37]:

$$2\ ZnO + C \rightarrow 2\ Zn + CO_2$$

$$2\ ZnO + 2\ CO \rightarrow 2\ Zn + 2\ CO_2$$

Electro winning processing leaches zinc from the ore Concentrate by sulfuric acid[38]

$$ZnO + H_2SO \rightarrow ZnSO_2 + H_2O$$

After this step electrolysis is used to produce zinc metal.

CHARACTERS:

- It is a bluish-white metal of a granulated crystalline structure with considerable lustre, soluble in the weakest acids.
- It is ductile, malleable and can be drawn into wires or rolled into sheets.
- Melted zinc on cooling becomes brittle and may then be reduced to powder. The fused mass if dropped into water, forms granular zinc.
- Pure zinc becomes tarnished by exposure to air.
- When melted with copper it forms an alloy known as Brass[32].

BIOLOGICAL ROLE:

Zinc is an essential trace element, necessary for plants, animals, and microorganisms,

Zinc is found in nearly 100 specific enzymes.

Main→ 14

⇒12

1.5j, serves as structural ions in transcription factors and is stored and transferred in metallothioncins. In proteins, Zn ions are often coordinated to the amino acid side chains of aspartic acid, glutamic acid, eysteme and histamine. There are 2-4 grams of zine distributed throughout the human body.

Most zine is in the brain, muscle, bones, kidney, and liver, with the highest concentrations in the prostatic and parts of the eye. Semen is particularly rich in zinc, which is a key factor in prostate gland function and reproductive organ growth.

In human, zinc plays “ubiquitious biological roles” it interacts with “a wide range of organic ligands” and has roles in the metabolism of RNA and DNA, signal transduction, and gene expression, it also regulates apoptosis.

In the brain, zinc is stored in specific synaptic vesicles by glutamatergic neurons and can “Modulate brain excitability” It plays a key role in synaptic plasticity and so in learning. However it has been called “the brain’s

dark horse" since it also can be a neurotoxin. Suggesting zinc homeostasis plays a critical role in normal functioning of the brain and central nervous system[39].

HEALTH BENEFITS OF ZINC:

- Zinc is necessary to maintain normal serum testosterone, Zinc is essential for the growth of fetus, boost brain activity.
- Zinc plays an important role in regulating the production of cells in the body's immune system, which protects against infection and disease, cures cold.
- Zinc stimulates cell division, healing, proper connective tissue formation and increases the transport of vitamin A from the liver to the skin, helps to protect body tissue from the damage and repair any damage present. Zinc stimulates Taste, Smell, Improves appetite, Mood.
- Zinc may also help in the treatment of pre-menstrual syndrome (PMS). Zinc regulates the secretion of many hormones, including progesterone.

CLINICAL DEFICIENCY:

1. Depressed growth with stunting,
2. Increased incidence of infection,
3. Diarrhoea,
4. Skin lesions,
5. Alopecia.

TOXICOKINETICS:

Oral Zinc is primarily absorbed in the jejunum. Absorption is primarily facilitated by a metallothionein protein complex in the villi of the enterocytes. The majority of Zinc is then bound to the metallothionein, a primary intracellular protein that also regulates levels of many other metals, including copper.

Plasma Zinc is mostly bound to albumin, with a very small fraction found free in the plasma. The body's response to hyperzincemia is to produce more metallothionein to decrease free Zinc concentrations. However, as copper is the

metal with the highest affinity to metallothionein, this will inadvertently lead to decreased copper levels instead. A high level of Zinc will, by this mechanism, always lower the level of copper in a dynamic antagonistic relationship. Homeostasis is, to some extent, controlled by excretion of the metallothionein-Zinc complex via bile and feces. However, this mechanism is not fast enough after large overdose.

FATAL DOSE[40]:

- Zinc phosphate - 5gm
- Zinc sulphate - 10-20gm
- Zinc chloride - 5gm.

FATAL PERIOD[40]:

- Zinc phosphate 24 hours.
- Death from zinc sulphate poisoning, through rare, has occurred in 2hours after taking 3 ounces of Zinc sulphate.

SYMPTOMS OF ZINC TOXICITY[41,42]:

Zinc is an impotant cofactor in the body and is essential for normal function. However, increased level of Zinc can become toxic. It cause,

- Abdominal pain,
- Watery Diarrhoea,
- Nausea,
- Vomiting,
- Muscle cramps
- Hematemesis,
- More than 60mg Zn per day has been known to result in copper depletion by causing blockage if intestinal absorption.
- Certain patient populations may be at higher risk, such as paediatric patients or patients with psychiatric illness who may ingest nutritional supplements in a large volume or a foreign body containing zinc.

- When zinc toxicity is suspected, a full dietary, occupational, and life history is vital because it may reveal hidden sources of zinc exposure.
- Metal fume fever or “Zinc shakes” from acute inhalation causes flu-like symptoms such as cough, fever, chills, headache, malaise, nausea and muscle aches.
- The patient may have symptom free intervals over days when not working, and a detailed occupational history is necessary.
- Chronic ingestion of zinc can manifest as a syndrome referred to as "swayback," leading to a slow progression of neuropathy and anaemia with increasing fatigue, spasticity, gait abnormalities, and sensory ataxia.
- The symptoms of swayback syndrome are related to the superimposed deficiency of copper.
- Copper supplementation and normalizing intake of zinc will reverse hematologic abnormalities within weeks though neurologic deficiencies will remain despite therapy.
- Acute toxic ingestions of zinc sulphate and concentrated zinc chloride will primarily cause GI effects, with abdominal pain, diarrhoea, nausea, vomiting, and hematemesis due to caustic effects. There are also rare reports of renal injury.

TREATMENT:

- Move patient to fresh air if there are poisonous gases or fumes.
- Stomach wash followed by instillation of milk, starch or lavage with 3 to 5% of sodium bicarbonate to neutralise gastric acid.
- Activated charcoal.
- Purgatives.
- Antiemetic and fluids should be given, as well as proton pump inhibitors or H2-blockers.

- PPIs and H2 blockers may help reduce gastric acid production, minimizing the digestion of zinc-containing foreign bodies and resultant zinc release.
- Chelation with DTPA has also been shown to decrease zinc levels in patients with toxicity successfully.

REFERENCE INTERVAL:

- Serum Zinc concentration are generally 5% to 15% higher than plasma because of osmotic fluid shifts from the blood cells when anticoagulants are used. Concentrations decrease after food and are higher in the morning than in the evening.
- A Guidance reference interval is 80 to 120 g/dl. (12 to 18mol/L)

EFFECTS OF ZINC ON THE ENVIRONMENT:

- Zinc occurs naturally in air, water and soil, but zinc concentrations are rising unnaturally, due to addition of zinc through human activities. Most zinc is added during industrial activities, such as mining, coal and waste combustion and steel processing.
- When the soils of farmland are polluted with zinc, animals will absorb concentrations that are damaging to their health. Water-soluble zinc that is located in soils can contaminate Ground water.
- Zinc can interrupt the activity in soils, as it negatively influences the activity of microorganisms and earthworms.

GOVERNMENT HEALTH REGULATION:

The Government has set standards and guidance to protect individuals from the potential health effects of excessive Zinc. So, The Government develops following Guidelines,

1. Food and Drug Administration [FDA]
2. The Occupational Safety and Health Administration [OSHA]
3. Agency for Toxic Substance and Disease Registry [AT SDR]

4. National Institute for Occupational Safety and Health [NIOSH].
5. It denote the limitation up to 11 mg/day [RDA]

Regulation and recommendation can be expressed as "Not to Exceed" level that in level of a toxic substance in water, soil, food that don't exceed a critical value.

SCIENTIFIC VALIDATION OF ZINC:

1. Potential anti - cancer and anti - Candida activity of Zn:

L. M. Marques et al evaluated that the potential of Zn derived foam have been derived from alkaline electrolyte solution by galvanostatic was inhibiting bone cancer cell proliferation-Osteosarcoma cells and important pathogenic fungi responsible for implant related infections – Candida albicans. The invitro behaviour of these Zn-derived foams in stimulated physiological condition was studied. The results revealed that the presence of Zinc oxide was important enough to change invitro behaviour of Candida organism[(46)].

2. Zinc and immune resistance to infection:

E. Mocchegiani et al stated that the catalytic, structural and regulatory roles of zinc mean that this ion is involved in the maintenance of an effective immune response. Infection can cause mortality when the immune system is damaged. The catalytic, Structural (in Zinc finger proteins) and regulatory roles of Zinc means that this ion is involved in the maintenance of an effective immune response. Dietary supplementation with the recommended daily allowance of zinc for one to two months decreases the incidence of infection and increases the survival rate following infection in the elderly[(43)].

3. Blood Sugar lowering effect of Zinc[(44)]:

P. Gunasekaran et al evaluated the effect of zinc and other multi vitamin mineral supplementation on glycemic control and lipid profile in 40 patients with type 2 diabetes mellitus. The result concluded that the supplementation of zinc with other multi vitamins minerals daily, to adult diabetes patients with glycemic and lipid control, for a period of 4 months,

demonstrated favourable changes in metabolic profile, including a better glycemic control and desirable gab changes in lipid profile[44].

4. Effects of Zinc Supplementation in Depression:

Elham Ranjbar et al evaluated the effects of Zinc supplementation in Patients with Major Depression by a randomized clinical trial. This study was a double-blind randomized clinical trail.Forty four patients with major depression were randomly assigned to groups receiving zinc supplementation an placebo. Patients in zinc supplementation with 25mg zinc adjuvant to antidepressant; Selective serotonin Re uptake inhibitors, While the patients in placebo group receive with antidepressants for twelve weeks. ANOVA with repeated measure was used to compare and track the changes during the study. The results of this study reveal that zinc supplementation combined with antidepressant drugs can be effective in the treatment of patients with major depression[45].

3.3 **இரசம் (HYDRARGYRUM)**

வேறுபெயர்:

காரம், சூதம், புண்ணியம், கற்பம், சாமம், சத்து, சூரியவிரோதி, சாதி, சூத்திரன், துள்ளி, ஈசன், வீரியம், சூழ்ச்சி, நீர், விண்ணினீர், விண்மருந்து, இரதம், சுக்கிலம், போகம், மூலம், சிந்தூரம், சிந்து, பக்கிரம், பதினெண்பந்தி, பாரதம், கனல், பூதம் (தசாங்கநிகண்டில்)

"**காரமே சூதம்புண்ணியம் கற்பமாஞ் சாமஞ்சத்து**

சூரியபகையாஞ் சாதிசூத்திரன் துள்ளியீசன்

வீரியஞ்சூழ்ச்சி நீராம் விண்ணினீர் விண்மருந்து

சீர்பெறு மிரதமென்று செப்பினா ரொப்பில்லோரே.

சுக்கிலம் போகஞானம் சுயம்புருவண்டு நாகம்

இக்கியம் விசயம்வேத மூலசிந்தூரஞ்சிந்து

பக்கிரம் பதினெண் பந்திபாரதங் கனலேபூதம்

நக்கனார்க் குள்ளநாம நவிற்றலா மிரதப்பேரே"

-**சட்டமுனி நிகண்டு**

இனிமை, சிவசக்தி, வருணத்தோன், தனிமை, சங்கரன்விந்து, பனிமை, பராபரம், பாய்ந்திடுதூமம், கனிமை, சரக்கிற் கலந்திடு சீவன், சிவன், விந்து, காவன், சிதறிக்காண்போன், கேசரிவேந்தன், பாவன், அந்தரகந்தன், ஆதி, sவராட்டியன், சுந்தரம், சொற்குறி, தூமம். மகாமரம், மந்தரம், மஞ்சி, மாருதம், மகிபன்விந்தரம், சிலை, கணவன், மலைக்குறவன், வாசுகிநாதன், கந்தன், காவக்குடியோன் என்றும் சட்டமுனிநிகண்டில் கூறிப்பிட்டுள்ளது.

சிவம், விந்து, வஞ்சகம், மனவேகி, கமலினி, மகாதேவபலம், அரவீரியம், ரௌத்ராகாரம், கந்தம், சாறு என்றும் வேறுபெயர்கள் கூறப்பட்டுள்ளது.

கிடைக்குமிடம்:

ஸ்பெயின்,

கலிபோர்னியா,

இத்தாலி,

ரஷியா,

சைனா,

ஜப்பான்.

பண்பு:

- இஃது இலிங்கத்திலிருந்து பிரித்தெடுக்கப் படுகின்றது.
- இரசமாகவே கனிமங்களிலிருந்து கிடைப்பதை குறைந்த அளவில் சேகரிக்கின்றனர்.
- நல்ல இரசம் சூரிய ஒளியை வெளிப்புறத்தும் சிறிது நீல ஒளியை உட்புறத்தும் உடைத்தாய் இருக்கும்.
- கடைகளில் வாணிபத்தின் பொருட்டுக் கிடைக்கின்ற இரசத்தில், ஈயம், தகரம், கல், மண், தூசி முதலிய மலினங்கள் கலப்புற்றிருக்கின்றன.
- மலினம் நிறைந்ததைப் பயன்படுத்தினால் நோய்களை உண்டுபண்ணும்.

இரசத்தின் பிரிவுகள்:

பஞ்ச பூதக்கூட்டுறவினால் உற்பத்தியாகின்றமையின் எந்த பூதத்தின் தன்மையையும் அதிகமாய்ப் பெற்றிருக்கின்றதோ அதற்கேற்ப இஃது ஐவகையாய் பிரிக்கப்படும்.

1. இரசம்,

2. இரசேந்திரன்,

3. சூதம்,

4. மிசரகம்,

5. பாரதம்.

சுவை:

''சரக்கிற்கலந்திடுசீவன்"

அறுசுவையையும் சிறப்பாக இனிப்பையும் உடையது.

வீரியம்:

மற்றச் சரக்குகளைப் போலன்றி ஒரு தனித் தன்மை உடையது. வெப்ப. சீத, வீரியங்களிரண்டையும் உடையது.

பிரிவு:

எப்பொருளை இதற்கு துணைமருந்தாக்கி கொடுக்கின்றோமோ அப்பொருளின் உடைய பிரிவை இஃது அடையுமென்று நம்பப்படுகின்றது.

செய்கை:

உடற்தேற்றி,

உடல்உரமாக்கி,

மலம்போக்கி,

பித்தநீர்அகற்றி,

வீக்கமுருக்கி,

உமிழ்நீர்பெருக்கி,

சிறுநீர்பெருக்கி,

மேகநாசினி.

குணம்:

"விழிநோய் கிரந்தி குன்மம் மெய்ச்சூலைபுண்குட்

டழிகாலில் விந்துவினால் அத்தை வழியாய்

புரியு விதியாது புரியினோ யெல்லாம்”

இரியு விதியாது மில்லை."

சிவ வீரிய மென்கிற இரசத்தை முறைப்படி மருந்தாக்கிக் கொடுக்க அது கண்ணோய், கிரந்தி, சூலை, பெரும்புண், தொழுநோய், வளிக்குறைவு முதலிய நோய்களை நீக்கும்.

மகிமை (**பிரபாவம்**):

இரசம் சீதத்தால் உண்டாகும் நோய்கட்கன்றி. வெப்பத்தால் உண்டாகும் பிணிகட்கும் உள்ளாட்சிக்கன்றி, வெளியாட்சிக்கும் எல்லாவற்றிற்கும் தலைசிறந்தது.

தேக நன்னிலைமய உண்டாக்கல்,

உண்டாக்கிய தேக நிலையைக் காத்தல்,

அந்த நிலைக்கு அழிவை உண்டாக்குகின்ற வியாதிகளை அழித்தல்

இரசம் அறம், பொருள், இன்பம் மூன்றையும் அளிக்கும்.

மந்திர பைசாசங்களால் ஏற்படும் வினைகட்கு எமன் போன்றது

இது அட்டமா சித்திகளையும் அளிக்கும்.

இரசம் பவழம் கனிந்தாலொத்த செய்மேனியராம் சிவபிரானை ஒக்கும்.

இது சிவனேயாகும்.

இதனை நவநிதி என்றும் கூறலாம்.

சுத்திமுறை:

மஞ்சள்தூளில் இலிங்கத்தூளைக் கலந்து, வர்த்திபோலச் சீலையிலிட்டுத் திரித்து, ஓர்அகலில் சுற்றி வைத்துக் கொளுத்தி, கீழிருந்து காற்றுப் புகுவதற்காம் இடம்விட்டு, ஒரு பெரியசாலினை, அகலின் மேல் கவிழ்த்து, ஆறினபின் எடுத்து மேல்சட்டியில் படிந்து இருக்கும் பாதரசமானது சுரண்டி எடுத்துக் கொள்ள வேண்டும்.

இரச நஞ்சுகுறிகுணம்:

தூய்மை செய்யாததும் நன்றாக முடிக்கப் பெறாததும், அளவுக்கு அதிகமானதுமான இரசத்தை உண்பாதல் குற்றம் உண்டாகும்.

வாயில் அச்சரத்தைபோல் புண் உண்டாகும்.

பற்சந்துகளில் ஈறுகட்டும், வாயில் நீர் பெருகும்.

பனங்கள்ளைப் போல் வாய் குழம்பும்.

வாய், தொண்டை இவைகள் வெந்து வீங்கும்.

வாய் நாறும்.

பசி மந்தப்படும்.

குடல், வயிறு இவைகள் புண்ணாகும்.

காரம் படமுடியாது.

பக்கத்தில் அடிக்கடி வலித்துக் கொண்டேயிருக்கும்.

வயிற்றின் மீது பட்டை பட்டையாகத் தேமல் படரும்.

கால்களில் வெடிப்பு கண்டு விட நீர் கசியும்.

விக்கல், நீர் அடைப்பு, இரத்த பேதி,

போகங்கெடல், உடல் வெளுத்தல்,

காது செவீடுபடல், கண்பார்வை இழத்தல், சிரங்கு, புண், செம்படை போல் உடம்பில் படை

உண்டாதல், நடுங்கல் ஆகிய குறிகுணங்களுண்டாகும் என தே.ய.வெ.உ கூறிப்பிட்டுள்ளது.

பித்தன் போன்று வாய் பிதற்றும்

துணிகளைக் கிழித்து செய்யும்.

கல்லாலடிக்கவும், மலையேறிக் குதிக்கவும்.

தண்ணீர் விழுந்து முங்கி வெளி வரவும் செய்யும், வியர்வை உண்டாகும்.

நஞ்சுமுறிவு:

1. குண்டியைப் பிடித்தக்கால், சாயப்பட்டையைப் பொடித்து வெல்லத்துடனே கொடுக்கவும்.

2. பல்லுக்கிட்டினால் கோவைத்தண்டுச்சாற்றை நாக்கில் பிழியத் தீரும்.

3. நெஞ்சுவற்றி. உள்வெதும்பி மேல் எரிவு எடுத்து, கைகால் மண்டி, நினைவில்லாமற் கிடந்தால், அறுகங் கிழங்கை ஆய்ந்து எடுத்து அரைத்து, வெள்ளாட்டுப்பால், பசுவின் பால், பருத்திக் கொட்டைப்பால், மோர், இவைகள் ஏதாவது ஒன்றுடன் கொடுக்க வேண்டும்.

4. வெள்ளைமுட்சங்கு இலைச்சாறு அல்லது மிதி பாகலிலைச்சாறு இலைகளில் ஏதாவறு ஒன்றின் சாற்றை 80 மி.லிட் வீதம் காலையிலும், மாலையிலும் மூன்றுநாள் கொடுக்க வேண்டும்.

5. துளசிவேர்ப்பட்டைக் குடிநீர்: துளசிவோப்பட்டை 10 கிராம், தோல் சீவின சுக்கு 10 கிராம்) அளவு எடுத்து 40 மி.லி. தண்ணீர் விட்டு 10 மி.லி.யாக வற்றியவுடன்அதில் 5 கிராம் பொட்டிலுப்பைப் பொடித்துப்போட்டுக் நன்றாக கரைந்தவுடன் குடிக்க வேண்டும். இவ்விதம் 10, 20, 30, 40 நாட்கள்வரை குடிப்பது நலம், இரச நஞ்சு நீங்கும்.

6. சுரைக்கருப்பு: சுரை ஓட்டைச் சுட்டு அக்கரியை வேளை ஒன்றுக்கு 4 கிராம் வீதம் (380 மி.லிட்) புளித்த பசுமோரில் காலை, மாலை இரச நஞ்சு நீங்கும் வரை கொடுக்கவும்[20].

3.4 MERCURY

Mercury exists in elemental, inorganic forms throughout our ecosystem. Its chemical name Hydrargyrum (means silver water), with Hg as its symbol. Elemental mercury is commonly known as quicksilver, the only metal exits in a liquid state at room temperature. Some of the physical properties to mercury include liquid metal with high density, electrical conductance and reflective surface. These characteristics form the basis of its various (Clakson and Magos,2006). Mercury is a component of the earth minerals and can be released into the atmosphere via natural weathering processes, volcanic eruptions and forest fires.

The ancient Egyptian and eastern civilization were acquainted with cosmetic. Medical and all chemical uses of mercury (Clarkson and Magos, 2006). It is believed that mercury and its compounds poses aphrodisiac, anti-ageing and immunomodulatory properties, which aid in eradicating disease even restricting death. The amalgamation of alchemy and spirituality characterizes mercury as seed of Lord Shiva, a Hindu deity (Masur, 2011; Sarkar et a.,2010).

Subsequent to the industrial revolution, there has been a notable increase in atmospheric mercury as a result of anthropogenic activities such as gold mining, Smelting, solid waste incineration and fossil fuel combustion. In addition, production of batteries, electric lights, barometers, thermometers, ect. (mercury containing consumer products) also contribute to mercury release (Guzzi and La Porta, 2008; Risher, 2003; Sun et al., 2016). Once into the atmosphere, mercury is oxidized to Hg^{2+} and Hg^{2+}, mercuric and mercurous ions respectively. These ions can be conjugated with other chemicals to form organic or inorganic mercury compounds. These compounds can be biomagnified via the food chain (ATSDR. 1999; Mergler et al., 2007).

HISTORY:

Discovery - Ancient Egyptians (Discovered 1500 BCE)

Symbol - "Hg": from its Latin name hydrargyrum, itself from Greek hydrargyros, 'water-silver' [(143)]

VERNACULAR NAMES:

- Sanskrit -Parada
- Malayalam- Rassam
- Gujarati-Paro
- Hindi- Para
- Tamil - Padarasam
- Telugu- Padarasam
- English- Mercury, Quicksilver[32].

DESCRIPTION:

Metallic mercury is having bright silvery luster and is volatile at room temperature. Only metal, which is liquid at room temperature. The fumes are odourless and invisible. It is 13.5 denser than water. Metallic mercury is not poisonous if taken by mouth, as it is not absorbed. However, if vapours are inhaled, may exert toxic effects[32].

MERCURY EXITS IN THREE FORMS[32]:

- Elemental mercury-Hgo-vapours are toxic,
- Inorganic mercury,
- Organic mercury[32].

Inorganic salts are of two types:

- Mercuric (bivalent Hg++)- more poisonous
- Mercurous (monovalent Hg+)- less poisonous[32]

APPEARANCE:

- Shiny, silvery liquid[32]

STANDARD ATOMIC WEIGHT:

- 200.592 $\pm$ 0.003[141]

PHYSICAL PROPERTIES:

Phase at STP	- liquid
Melting point	- 234.3210 K (−38.8290 °C, −37.8922 °F)
Boiling point	- 629.88 K (356.73 °C, 674.11 °F)[142]
Density (near r.t.)	- 13.534 g/cm3
Triple point	- 234.3156 K, 1.65×10−7 kPa
Critical point	- 1750 K, 172.00 MPa

Heat of fusion - 2.29 kJ/mol

Heat of vaporization - 59.11 kJ/mol

Molar heat capacity - 27.983 J/(mol·K).

ATOMIC PROPERTIES:

Oxidation states - −2 , +1, +2 (a mildly basic oxide)

Electronegativity - Pauling scale: 2.00

Ionization energies - 1st: 1007.1 kJ/mol

2nd: 1810 kJ/mol

3rd: 3300 kJ/mol

Atomic radius - empirical: 151 pm

Covalent radius - 132±5 pm

Van der Waals radiu - 155 pm[(141)]

OTHER PROPERTIES:

Natural occurrence - primordial

Crystal structure - rhombohedral

Speed of sound - liquid: 1451.4 m/s (at 20 °C)

Thermal expansion - 60.4 µm/(m·K) (at 25 °C)

Thermal conductivity - 8.30 W/(m·K)

Electrical resistivity - 961 nΩ·m (at 25 °C)

Magnetic ordering - diamagnetic[2]

Molar magnetic susceptibility - −33.44×10−6 cm3/mol (293 K)[3]

CAS Number - 7439-97-6

ABSORPTION, METABOLISM AND EXCRETION:

Elemental mercury is readily absorbed through alveolar membrane and enters the blood. Mercury slats are absorbed through skin, GIT mucosa, vaginal mucosa and bladder. Organic compounds can pass placental barrier and cause foetal toxicity. In blood mercury is converted into mercuric ions and causes tubular damage during excretion. In CNS, mercury acts on cerebellum, temporal lobe, basal ganglia and corpus callosum. Mercury gets deposited in liver, kidneys, spleen and bone.

Mercury is excreted in urine, faeces and bile with entero -hepatic circulative[(56)].

MECHANISM OF ACTION:

Mercury compounds act by inactivating sulphydryl enzymes causing interference with cellular metabolism[56].

TOXICITY:

Metallic mercury is used in a variety of household products, such as barometers, thermometers and fluorescent light bulbs. The mercury in these devices is trapped and usually does not cause any health problems. However, when a thermometer will break a significantly high exposure to mercury through breathing will occur for a short period of time while it vaporizes. This can cause harmful effects, such as nerve, brain and kidney damage, lung irritation, eye irritation, skin rashes, vomiting and diarrhoea.

Mercury has a number of effects on humans, that can all of them be simplified into the following main effects:

- Disruption of the nervous system
- Damage to brain functions
- DNA damage and chromosomal damage
- Allergic reactions, resulting in skin rashes, tiredness and headaches
- Negative reproductive effects, such as sperm damage, birth defects and miscarriages

Damaged brain functions can cause degradation of learning abilities, personality changes, tremors, vision changes, deafness, muscle incoordination and memory loss. Chromosomal damage is known to cause mongolism.

FATAL DOSE[56]:

- Mercuric chloride - 1 gm.
- Mercuric cyanide-0.6 to 1.3 gm
- Mercuric nitrate-4 gm
- Mercurous chloride (calomel) - 1.5 to 2 gm

FATAL PERIOD[56]:

3 to 5 days

ACUTE POISONING BY INORGANIC COMPOUNDS[56]:

INHALATION:

Breathlessness, Cough, Fever, chills (metal fume fever), Headache, Blurring of vision, Non-cardiogenic pulmonary oedema, Convulsions. Ataxia, Delirium.

INJECTION:

Subcutaneous or intramuscular injection causes abscess formation with ulceration

Intravenous administration results in thrombophlebitis, granuloma formation and pulmonary embolism

Intra-arterial administration results in peripheral embolism with ischemia and gangrene.

INGESTION:

Metallic taste, Abdominal pain, Vomiting, Shock, Corrosion of mouth and tongue, Hematemesis, Renal failure. Pulmonary oedema, Urine-pinkish in colour. Glossitis and ulcerative gingivitis, Loosening of teeth, Necrosis of jaw, Membranous colitis

POISONING BY ORGANIC COMPOUNDS:

Dysarthria, Ataxia, Paraesthesia, Neuropathy, Diminished visual and auditory activity, Mental deterioration, Chorea, Minimata diseas

CHRONIC POISONING[56]:

Chronic poisoning of mercury also called as hydrargyrism and mercurialism. Chronic poisoning of mercury causes Excessive salivation, Metallic taste, Anorexia, Insomnia, Headache, Gingivitis, Halitosis, Blue line on gums, Lassitude, Visual blurring, Concentric constriction of visual field (tunnel vision), Colitis, Dementia, Shedding of teeth and ulceration of gums.

- Mercuria lentis - opacities of the anterior capsule of the lens of eye due to deposition of mercury, Ataxia- reeling gait, Tremors - classical

manifestation of chronic mercury poisoning and is referred as "Danbury tremo

- The tremors are coarse, intentional type, interspersed with jerky movements, initially involving hands. Later it involves lip, tongue, arms and legs. The advanced condition of tremors is called as "Hatter's shakes"
- As the disease progresses, the most severe form of tremor called as "Concussio mercurialis" which means no activity is possible.
- Mercurial erethism - a classical manifestation of chronic mercury poisoning characterized by cluster of psychiatric symptoms including disturbance in personality, abnormal shyness, timidity, loss of self-confidence, insomnia, excitability, progressing later into delirium with hallucinations (Mad as hatter), Melanosis coli, Renal failure.
- Acrodynia (Pink disease) Seen mostly in children and caused by chronic mercury exposure. It causes anorexia, insomnia, profuse sweating, skin rash, redness, vesiculation and desquamation (peeling) of palm, finger, sole and photophobia. The hands and feet became puffy, pinkish, painful, paraesthesia. perspiring and peeling.

MANAGEMENT:

Gastric lavage with egg white or albumin or milk to bind the mercury, Demulcents, Laxative, Chelation like BAL, DMPS and Supportive care.

ENVIRONMENT EFFECT OF MERCURY:

Mercury from soils can accumulate in mushrooms.

- Acidic surface waters can contain significant amounts of mercury. When the pH values are between five and seven, the mercury concentrations in the water will increase due to mobilisation of mercury in the ground.
- Once mercury has reached surface waters or soils microorganisms can convert it to methyl mercury, a substance that can be absorbed quickly by most organisms and is known to cause nerve damage. Fish are organisms that absorb great amounts of methyl mercury from surface

waters every day. As a consequence, methyl mercury can accumulate in fish and that they are part of the food chains.

- The effects that mercury has on animals are kidneys damage, stomach disruption, damage to intestines, reproductive failure and DNA alteration.

SCIENTIFIC VALIDATION OF MERCURY:

1. Immunomodulatory Activity:

Aruna C et al evaluated the immunomodulatory activity of Rasa chendhuram using Macrophage cell line RAW264.7 using lipo-polysacchrides(LPS)(lug/ml) as a control. When the concentration level is decreased, nitrate level increased. Hence 25µg/ml of Rasa chendhuram has rich level of nitrate and thus proven to be an Immunomodulator[57].

2. Anticancer Activity:

Deepa G et al evaluated the anticancer activity of Rasa parpam in HeLa and SiHa cell line. The experiment was screened at different concentrations to determine the IC50 using MTT assay. The percentage of growth inhibition was found to be increasing with increasing concentrations of test drug. The IC50 of test sample in Hela cell line was found to be 125µg/ml. This confirms that the Siddha formulation Rasa parpam has promising anti-cancerous effect[55].

5. Anti-oxidant activity:

DPPH assay (2, 2-diphenyl-1-picrylhydrazyl). The radical scavenging activity of Rasa parpam extracts was determined by using DPPH assay according to Change tal. (2001). The decrease in the absorption of the DPPH solution after the addition of an anti-oxidant was measured at 517nm. Ascorbic acid (10mg/ml DMSO) was used as reference[58].

Table.1. TOXICOLOGICAL STUDY:

TITLE	AUTHOR	CONCLUSION
A Comparative Acute Toxicity Study of Self Prepared And Market Sample of Tribhuvanakirti Rasa	Dubey Nidhi et al	In case of Acute Toxicity, neither self prepared nor market sample of Tribhuvanakirti rasa show any toxic result at TEDX4 dose level[49].
Safety evaluation of mercury based Ayurvedic formulation (Siddha Makardhwaj) on brain cerebrum, liver & kidney in rats	Kumar Gajendra et al.	In conclusion, the finding of the present study suggest that Siddha Makardhwaj in the doses equivalent to human dose given for 28 days does not have any adverse effects on brain cerebrum, liver and kidney[51].
Assessment of Genotoxic Potential of Hridayarnava Rasa (A herbo-mineralo-metallic Ayurvedic formulation) using Chromosomal Aberration and Sperm Abnormality Assay.	Jagtap Chandrashekhar Yuvaraj et al.	The current study indicates that therapeutic use of Hridayarnava rasa is safe from the genotoxicpoint of view[54].

AUTHOR	TITLE	CONCLUSION
A Study of Subacute Toxicity of Kajjali, A combination of mercury and sulphur on Albino Rats	Therasilin Louis et al.	The Kajjali, at doses investigated, did not provoke toxic effects to the animals liver, heart and kidney[52].
Toxicological Evaluation of Rasa-sindoor in Albino Rats.	Kanojia Anita et al.	The drug formulations of Rasa-sindoor does not have any toxic effect on kidney and its functioning, hence it is safe in animal models[53].
Scientific validation of siddha formulation rasa parpam and its anticancer property in hela cell linean invivo and invitro assay	Dr. G. Deepa et al	The analysis of pharmacological activity through HeLa and Siha cell lines are the novel methods for validation which proves the effective anticancer activity of Rasa Parpam[55].

3.5. **கீழ்க்காய்நெல்லி** – ***Phyllanthus amarus*, Schum & Thonn.**

வேறுபெயர்:

கீழ்வாய்நெல்லி,

கீழாநெல்லி

இஃது, இந்தியாவின் வெப்பநாடுகளில், சதுப்பான எல்லா இடங்களிலும் வளரும்.

பயன்படும் உறுப்பு:

எல்லாம்.

சுவை:

துவர்ப்பு,

கைப்பு,

புளிப்பு,

இனிப்பு.

தன்மை:

தட்பம்

பிரிவு:

இனிப்பு.

செய்கை:

வீக்கமுருக்கி,

சிறுநீர்ப்பெருக்கி,

துவர்ப்பி,

சீதளகாரி,

குளிர்ச்சியுண்டாக்கி.

குணம்:

இம்மூலிகையினால், வயிற்று மந்தம், தீக்குற்றத்தால் விளைந்த கே**டு**, கண்ணில், தோன்றும் நோய்க் கூட்டங்கள், குருதிக் கழிசல், நீரிழிவு, காமாலை, உடலில் உண்டாகும் வெப்பு, உடலில் ஊறிய மேகம், தாது வெப்பம், நாட்பட்ட

மேகப்புண் ஆகிய இவைகள் போகும். மேலும் இஃது, நாவறட்சி, தாபம் இவைகளையும் நீக்கும். வாதத்தைப் பெருக்கச் செய்யும்.

"கீழாநெல்லிக் குணந்தாள் கேளாய் மதுமேகந்
தாழாக் காமாலைகளைச் சண்ணுந்தா – தேழனலுந்
தொக்கிளனலுந் தொலைக்குந் தொன் மேகம்
போக்கிவிடத் தக்கவிரணங் கெடுக்குத் தான்"

"ஆமல கைலாணாஎதி கமளையோடு குறை
யாமல கையுண்ணோயள லெங்கோ"

பல பிணிகளினால் நொந்து உடல் மெலிந்து வெளுத்திருக்குங்கால், கீழாநெல்லியை அரைத்து, நல்ல பசுவின் தயிரில் கலந்து நாளும் கற்பமுறையின்படி உண்ண, மேகவெட்டை முதலிய நோய்கள்யாவும் போம்.

இலை:

இப்பூண்டின் இளங்கொழுந்தைக் குடிநீரிட்டுக் சீதக்கழிச்சலுக்குக் கொடுக்கலாம்.

இலையை உப்பு சேர்த்தரைத்துச் சொறி, சிரங்குகளுக்குப் பூச இவை போகும்.

உப்பில்லாமல் அரைத்துச் சதைச்சிதைவுக்குப் பற்றிடலாம்.

இலையையும் வேரையும் உலர்த்திப் பொடித்து, கழுநீரில் குழைத்து. புண் புரைகளுக்கும் வீக்கங்களுக்கும் பூசலாம்.

இலை, வேர்:

இவ்விரண்டையும் குடிநீரிட்டுச் சுரங்களுக்குச் சூட்டோடே கொடுக்கவும். ஆறின பிறகு குடித்துவர உடல் வலுக்கும். பசித்தீயைத் தூண்டும். இதன் இலை, வேர் முதலியவற்றை அரைத்து மோரில் கலக்கிக் கொடுத்துவரின், மஞ்சட்காமாலை, மேகநோய் இவைகள் போம். உப்பு நீக்கவும்.

வேர்:

வேரைக் கழுநீரில் அரைத்துக் கலக்கிப் பெரும்பாட்டிற்குக் கொடுக்கவும். வேரைப் பச்சையாய் 17 கிராம் எடுத்து அரைத்து, பாலில் கலக்கிக். கொடுக்க காமாலை நோய் நீங்கும்.[59]

3.6 KEEZHANELLI – *Phyllanthus amrus*

TAXONOMICCLASSIFICATION:

Botanical Name	**:**	Phyllanthus amarus L.
Kingdom	**:**	Plantae
Division	**:**	Magnoliophyta
Class	**:**	Magnoliopsida
Order	**:**	Euphorbiales
Family	**:**	Euphorbiaceae
Genus	**:**	Phyllanthus
Species	**:**	Amarus.

VERNACULAR NAMES IN INDIA:

Assamese	**:**	Holpholi
Bengali	**:**	Noar
Hindi	**:**	Chalmeri, Harfarauri, Bhuiaonla.
Kannada	**:**	Kirunelli, Nela Nelli,
Konkani	**:**	Bhuin-avalae,
Telugu	**:**	Ratsavusirike, Nela Usiri,
Tamil	**:**	Arunelli, Keela Nelli,
Malayalam	**:**	Arinelli,Kizhanelli,Nellipuli
Marathi	**:**	Rayavali, Bhuiavli,
Oriya	**:**	Narakoli
Sanskrit	**:**	Amala, Bhumyamlaki, Sukshmadala, Vitunika, Bhoodatri.

PARTS USED:

Whole Plant

ACTION:

- Deobstruent
- Diuretic
- Astringent
- Cooling

BOTANICAL DESCRIPTION:

Phyllanthus amarus is an erect annual herb, growing 40 - 70cm height having ascending herbaceous branching; it is quite glabrous and branching at the base. The genus Phyllanthus means “leaf and flower” because the flower and fruit can be associated with the leaf. It is a plumose leaf that carries flower and fruit.

Leaves:

Numerous, small, green, sub sessile, closely arranged, elliptic oblong shaped, obtuse, having short petiole and stipules present, they are arranged alternatively on each side of the stem

Flowers:

The flowers are yellowish, small, numerous, axillary. These are unisexual, monoecious flowers, male flowers having 1-3 sessile stamens and female. Flowers were solitary in nature.

Fruits:

Fruit is a capsule, very small, depressed globose and more over capsule is smooth, 2-3mm in diameter,

Stem:

It is having horizontal branches and height of 30-60cm, 1-2.5mm width.s

Root:

It is some what branched and large[(60)].

ETHNOBOTANY:

Phyllanthus amarus has extensive medicinal properties and has long history in the health care system of tropical countries.

The plant is known in traditional health care systems. P.amarus is commonly known as “Chanca pedra” (or) “stone breaker”. However there is a lot of confusion about this species identification. Phyllanthus amarus is used as a folk medicine for treating kidney stones, gallbladder stones, liver related diseases such as liver cancer & jaundice, apart from these it is also administered for diuretic, hypoglycemic and hypertension cases and it also shows anti inflammatory, antitumor, antinociceptive and antioxidant property[(60)].

MEDICINAL USES:

- For jaundice: The whole plant juice with 10-20ml of dose is recommended three times daily.
- The fresh roots (10gms) powder is mixed with fresh milk. This is recommended to take in the early mornings for effective cure for jaundice.
- The leaves were crushed with salt and applied for skin diseases.
- The plant decoction was very effective for diabetes and chest pain
- The decoction of leaves or roots is used for ulcers.
- The dried powder of the plant mixed with gruel water is applied over ulcers and wounds.
- The juice of whole plant can be taken as a dose of 45-50 ml in the early morning for leucorrhea, gonorrhea, menorrhea and other urinary complains.
- The extract of this plant can cure Hepatitis very effectively[62,63] and it can be a remedy for HIV-AIDS[62]. P.amarus is having various properties like anti-inflammatory[63], anti-fungal, anti-viral, anti-bacterial[64], anti-oxidant[65,66,68], hepatoprotective[68], hypoglycemic [69,70], hypotensive, analgesic[71,72],inhibitory effect on renal stone formation[67] etc., P.amarus is used as an ingredient of almost 175 Siddha formulations, the fruits of this plant is commonly used in the treatment of hemorrhages, diarrheas, dysentery, jaundice, cough and anaemia.
- The aqueous infusions of the whole plant is employed as a stomachic, appetite, anti-spasmodic, laxative, diuretic[73], carminative, against constipation, fever including malaria, hepatitis B[74], dysentery, gonorrhea, syphilis, tuberculosis, cough, diarrhea, vaginitis[74,75].Majorly scientists focused on hepatoprotective activity of P.amarus, the hepatoprotective effects of crude methanol and aqueous extracts against CCl4 induced liver damage in rats have been investigated . The hexane fractions of extract reported to be hepatoprotective against CCl4 and Gal N induced

cytotoxicity in primary cultured rat hepatocytes[77], radical scavenging activity along with the hepatoprotective activity was found in aqueous extract of this plant[78].

- The Phyllanthus amarus fresh root is believed to be an excellent remedy for jaundice, dropsy and genitor urinary infections[79,80,81,82]. P.amarus promote stone elimination in patients with kidney stones, as well as normalization of Ca levels in hypercalciuric patients[83] so it is best familiar remedy for gall stones & kidney stones in the continent[84]. The extract shows an inhibitory effect on CaOx growth & aggregation in invitro model of crystallization[85]. The fruits are used in treatment of tubercular ulcers, wounds, sores, scabies & ring worms.

- It is having high potential anticancer and antioxidant agents[86] to cure viral hepatitis[87] and increased vinblastin cytotoxicity towards multi drug resistant cancer cells[88]. It also inhibits the endogenous DNA polymerase of Hepatitis B virus in both invitro and invivo models[82].

- The active component of P.amarus is, which has antiviral activity that extends to Human Immuno Deficiency Virus by inhibiting the reverse transcriptase enzyme[62]. P.amarus also shows anti plasmodial activity of the ethanolic and dichloromethane extracts as well as the toxicity of the lyophilized aqueous extract previously reported[89,90].

- Phyllanthus amarus has several bioactive molecules such as lignans, phyllanthin, hypophyllanthin, flavonoids, glycosides, tannins, alkaloids, ellagitannins, triterpenes, phenyl propanoids, steroids, ricinolic acid, niruriside & phyltetralin[91,92,93,94]. The alkaloids have the antispasmodic activity leading to smooth muscle relaxation. It even contains acidic Arabinogalctan[95] and Diterpene[96].

- A protein isolated from the aqueous extract of P.amarus poses protective activity against number of drugs & toxins induced organ pathophysiology. The protein weight about nearly 35K/day, poses antioxidant activity and also radical scavenging activity and it even enhances intra cellular antioxidant property[97]. The seeds of this plant contain Ricinoleic acid, and

Linolenic acid [54%], Fisetin-4-0-glucoside and a new Flavonoids glycoside has been isolated from the aerial parts of this plant

- P.amarus has enormous pharmacological activities such as antiviral activities against hepatitis B, antimicrobial, hepatoprotective, anticancerous and hypocalcemic agent. Methanolic extract of P.amarus exhibited immunomodulatory activity and anti HIV activity. Phyllanthin and hypophyllanthin shows antitumor activities.

SCIENTIFIC VALIATION OF PHYLLANTHUS AMARUS

1. Action of kidney stones & uric acid:

Kidney stone is a common problem that accumulates calcium oxalate crystals, and it includes urinary calculi formation, nucleation, growth, and aggregation of crystals. Phyllanthus amarus's extract interferes in the growth and aggregation of calcium oxalate [CaOx] crystals in the calculi. The extract inhibits CaOx crystal aggregation in the early stages of stone formation in the urine samples of male wistar rats. It is advisable to treat stone formation in the early stages[(99)].

The CaOx metastable limit was decreased by the treatment of P. amarus [5% [v/v]] extract and it can also deprive the CaOx crystals and formation of nucleation.
The extract has the ability to prevent the growth of calculi and also change the shape and texture of the calculi. When treated on the preformed calculi it can form a matrix like material on its surface and it can modify the appearance and texture of the calculus[(99)].

The extract is also administered in hyper calciuric patients; it can decrease the urinary calcium levels and also reduces the excess uric acid in hyperuricemic people by the lignans with uricosuricactionin the extract[(100)].

2. Antispasmodic, pain relieving & anti inflammatory:

The wound healing nature of Phyllanthus amarus has been evaluated by the healing of wounds by oral and topical administration. P. amarus was proved to have a significant role in wound contraction and epithelialisation. When Dexamethasone (suppress the wound healing) suppressed rats were treated with the extract a significant increase in wound contraction was found by both oral and topical administration[(101)].

3. Liver protective, detoxification & antioxidant activity:

The carbon tetrachloride and galactosamine induced cytotoxicity in rat hepatocytes can be decreased by the P. niruri hexane extract. Phyllanthin and hypophyllanthin protects against the CCl4 induced cell lesions and GalN induced Hepato toxicity[102].

Phyllanthus amarus can reduce nimesulide induced hepatic damage. By measuring the levels of glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and alkaline phosphatase (ALP) in serum it was concluded that the levels of three enzymes are decreased in the extract treated group. By these observations intra peritoneal treatment was found to be more effective than oral administration and by combining this data we can conclude that P. amarus protects the liver from nimesulide induced liver toxicity[101]& Oxidative stress[104].

The over dose of paracetamol leads to hepatotoxicity same as viral infection. The glutamic pyryvic transaminase (GPT) levels of serum were decreased in the P. amarus treated group[105].

The serum glutamate pyruvate transaminase (SGPT) and glutamate oxaloacetate transaminase (GOT) was decreased in the invivo studies conducted in rats[106]. The ethanol extract and hexane extract were administered and the serum parameters (serum bilirubin, serum alkaline phosphatase, serum aspartate (AST), serum alanine transferase (ALT), hepatic reduced glutathione (GSH) were analysed and these parameters were controlled after the treatment with hexane extract and hence, it was stated that P.niruri can control the paracetamol induced hepatotoxicity[107].

Protein isolated from this plant was found to enhance cell viability against tertiary butyl hydroperoxide induced cytotoxicity and cell death; and it protects hepatocytes against thioacetamide induced cytotoxicity. The extract prevents the alterations in GSH levels and it also reduces the lipid peroxidation induced by TAA. By the DPPH assay it was found that the isolated protein has radical scavenging activity. This protein protects the liver from the carbon tetra chloride induced hepatotoxicity and this can be measured by the liver enzymes and reduced levels of antioxid antenzymes[108.109,110].Alcohol is a toxin in higher doses and when it is associated with poly unsaturated fatty acids (PUFA) induces oxidative stress & hepatotoxicity. This can be efficiently reduced by P. amarus extract analyzed by the antioxidant potentials of liver enzymes and histopathological studies[111].

4. Anti cancerous & cellular protective actions:

P. amarus has high potential to inhibit the growth and intiation of cancerous cells which were introduced into mouse skin cells with 7, 12 dimethyl benz(a) anthracene (100μg/100ml acetone) and croton oil (1%) and there is drastic increase in the catalase, reduced glutathione and protein levels in the skin. In albino mice the chemopreventive action of P.niruri with DMBA induces skin papillomagenesis[(112)].

5. Immune modulatory actions:

An arabinogalactan(AG) which was obtained from P.amarus tea preparations was found to have immunological properties and is tested with peritoneal mice macrophages. The glycoside showed the same activity when subjected to acidic and neutral gastric conditions using human gastric fluids and aq.HCLsolution[(113)].

6. Antiviral action (Hepatitis B):

The plants of Phyllanthus genus have been used for natural remedy from thousands of years in Asia. (Thyagarajan et al., 1988). P.niruri has been used to inhibit the hepadna virus and it is extensively used to treat jaundice and hepatitis B viru[(114)]. The phyllanthus genus plants inhibit duck hepatitis B virus by inhibiting 50 % of DNA polymerase[(115)].

Hepatitis B is the most prominent disease in emerging era. Phyllanthus niruri extract can prevent Hepatitis B by binding to the endogenous DNA polymerase and even it can bind to the hepatitis B surface antigen in invitro.

Wood chuck hepatitis virus (WHV) was tested against the extract in wood chucks (Marmota monax), it efficiently inhibited the wood chuck hepatitis virus (WHV) and elimination of both surface antigen and DNA polymerase activity was found[(116)].

7. HIV replication inhibition:

The prominent human Immuno Virus replication is inhibited by the alkaloidal extract of P. amarus and tested against virus induced MT-4 cells, it suppressed the activity in strains of HIV 1 cells.

The REV (regulation of virion expression) is an HIV protein that regulates the transport of viral RNA to the cytoplasm and its basic domain is RRE(responsive

element). The niruriside isolated from methanol extract of P.niruri shows inhibitory activity against binding the REV protein to RRE RNA[117].

8. Lipid lowering activity:

The Phyllanthus niruri has the capacity to reduce the serum lipid levels.The extract is fed orally (250 mg/kg b.w) in hyper lipemic rats, results followed by reducing lipid levels[118]. Methanol extract of P. amarus was tested against chlorpyrifos (CPF)-evoked erythrocyte fragility and lipoperoxidative changes in wister rats and observed lipid peroxidative changes and protection from the chlorpyrifos induced erythrocyte fragility.

9. Anti-microbial activity:

The antimicrobial activity of fermented P. amarus by using lactobacillus isolated from the surface of the plant was enhanced. The antimicrobial activity was Anti-methanol extract of P. amarus is strong against Bacillus pumillus, Bacillus microbial activity. The extracts of P. amarus and Piper beetle were tested against food borne & spoilage microorganisms. The ethanolic extracts of dried P. amarus inhibited the growth of microorganisms[119] enhanced 80-170% when compared to the crude extract. The potency was increased by 49% when the extract was fermented with lactobacillus[120] ceraus, E. coli and Vibrio cholera at conc of 750µg/ml/disc. It is tested against standard drug chloramphenicol at conc 10µg/ml/disc shows potential source of antimicrobial agent The phyllanthus amarus extract of alkaloids were tested on rabbits infected with E.Coli. The results examined were found to have increased concentration of WBC, neutrophils and decreased hemoglobin, lymphocytes more over there are no changes in enzyme concentration[121].

10. Antiulcer activity:

The acidic heteroxylan and another polysaccharide showed anti-ulcer activity. These compounds reduced the gastric lesions induced by 65% and 78% ethanol. P. amarus proved to be efficient against peptic ulcers[122].

11. Nematocidal activity:

The two prenylated flavones isolated from the hexane extract of P.amarus showed Nematocidal activity against two nematocides, Meloidogyne incognita and Rotylenchulus

reniformis. The two compounds showed moderate Nematocidal activity against nematodes[123].

TOXICOLOGY:

Phyllanthus amarus is low toxic, and it showed toxicity to batrachians and fishes when extract is alcohol and water based. It is very less toxic to mammals.

3.7 பனை - ***Borassus flabellifer* L**

வேறுபெயர்:

தாலம், கரும்புறம், ஏடகம், காமம், தருவிராகன், தானி

இஃது இந்தியா முழுவதும் தானாக ஓங்கி வளரும் மரம். இலைகள் (ஓலைகள்) மட்டை, பூ, வேர் இவைகள் மருந்துகளுக்குப் பயன்படுகின்றன.

பயன்படும் உறுப்பு:

- குருத்து,
- ஓலை,
- பூ,
- நுங்கு,
- பழம்,
- மட்டைகள்.

இளங்குருத்தைத் தின்னலாம். மிக இனிப்பாக இருக்கும். ஆனால் கழியச்செய்யும்.

இம்மரத்தைப் பீமனுக்கு ஒப்பாகவும், பீமனை இரசத்துக்கு ஒப்பாகவும் கூறியுள்ளதனால், இதன் குருத்துச் சாற்றினால் இரசம் பற்பமாகும்[60].

3.8 PANAI MARAM - *Borassus flabellifer* L.

TOXANAMICAL CLASSIFICATION:

- Botanical Name : *Borassus flabellifer* L.
- Kingdom : Plantea
- Division : Magnoliophyta
- Class : Liliopsida
- Order : Arecales
- Family : Arecaceae
- Genus : Borassus
- Species : Flabellifer

VERNACULAR NAMES IN INDIA:

- Sanskrit : Tala
- English : palmyra palm: Brab tree
- Hindi : Taltar; tal; Tari
- Gujarati : Tad
- Bengali : Tal
- Konkani : Talam; pana
- Telugu : Niti marma

HABITAT:

Grows on dry soils or sandy localities along river banks, throughout tropical India, especially in South India.

PART USED:

Root,

Flowering stalk,

Juice,

bark,

Fruit.

SCIENTIFIC VALIDATION OF *Borassus flabellifer* L:

1. Anti diabetic activity

An extract from Palm tuber - immature endosperm at a concentration of 200ml was administered against randomly selected 30 numbers of type2 diabetic patients for 420 days and discovered that immature endospermisa good diet for diabetic patients. In this study, the positive control used was not mentioned [(128)].

2. Wound healing activity:

One another preliminary human trial was carriedout to determine the efficacy of the local application of *Flabelliferin b.* (FB) at selected 7 volunteers to show the wound healing property of fruit extract at the concentration of 4mg/ml for one week.The prepared FB ointment resulted in wound healing without any adverse effects and the Metronidazole was used as the positive control in this study[(127,128)].

***In-vivo*Studies**

3. Anti arthritic activity:

An ethanolic extract at the dose of 200 mg/kg from the flower was employed using Freund's Complete Adjuvant (FCA) induced polyarthritis model for twenty-one days to screenanti arthriticpotential. Results showed significant anti arthritic activity, as compared to control (Diclofenacsodium) at 100mg/kg[(129)].

4. Anti cancer activity:

Hong et al. studied the anticancer activity of root methanol extract at the concentration of 1 µg/mL in human colorectal cancer cell linesfor twenty-eight days. They found that Trans-Scirpusin. A inhibited the growth of colorectal cancer Her2/CT26 cells in mice and it is not stated the positive control used in this study[(130)].

5. Anti-inflammatory activity:

An extract at the concentration of 150 mg/kg was preparedfrom the flower using ethanol and applied to acetic acid-induced writhes. Eventually, the extract was able to prevent damage to red blood cell membranes and promote the

stabilization of the membrane. In this study, morphine were used as positive control 10mg/kg[125].

6. Antipyretic activity:

An ethanolic extract at a dose of 150 mg/kg from the flowerwas tested on yeast-induced pyrexia in mice and rats and results showed that the extract significantly reversed hyperthermia and this was compared with aspirin at 200mg/kg concentration[131].

7. Diuretic Activity:

The diuretic effect of tuber using the ethanol extract at the concentration of 200 mg/kg was investigated in albino ratand mouse for 5h period and the result was compared with standard drug furosemide(100mg/kg) and extract has show no significant increase in the urinary level of Na^+, K^+, and Cl^-[124].

8. Immunomodulatory Activity:

Ma'Unatin et al. investigated the immunomodulatory effectin mice for the ethanol extract extracted from the flower atthe concentration of 300 μg/ml for twenty-six days. Theresults indicated that the exopolysaccharides produced by two strains of *Leuconostocmesenteroides* have immunomodulatory activity. This was compared with Dextran using the same concentration [125,132].

9. Hypersensitivity:

An ethyl acetate extract of tuber was tested in the mouse atthe concentration of 0.4 mg/kg (effective dose) (*ED50*) to study the potent immunosuppressant activity for five hoursand this was compared with cyclosporin A [126].

***In-vitro*Studies**

10. Anthelmintic Activity:

Jamkhande et al. investigated the anthelmintic property of leaf extract. Extracted using methanolin *Pheretimaposthuma*at the concentration of 10 mg/ml and the results revealed that extract has effective anthelmintic activity against Indian adult earth worms. In this study, albendazole was used as the positive

control at the same concentration [133].

11. Antibacterial Activity:

A methanol extract at the concentration of 100 µg/mL fromleaf was tested for its antibacterial property against Bacillussubtilis, Escherichiacoli, Klebsiellapneumonia, Proteusvulgaris, Pseudomonasaeruginosa, Salmonellatyphi, Staphylococcusaureus and Staphylococcusepidermidis and the extract revealed significant inhibition of growth of selected bacterial strains. In this study, the researchers used amoxicillin and ciprofloxacin as a positive control at the concentration of 100µg/ml [134].

12. Antifungal Activity:

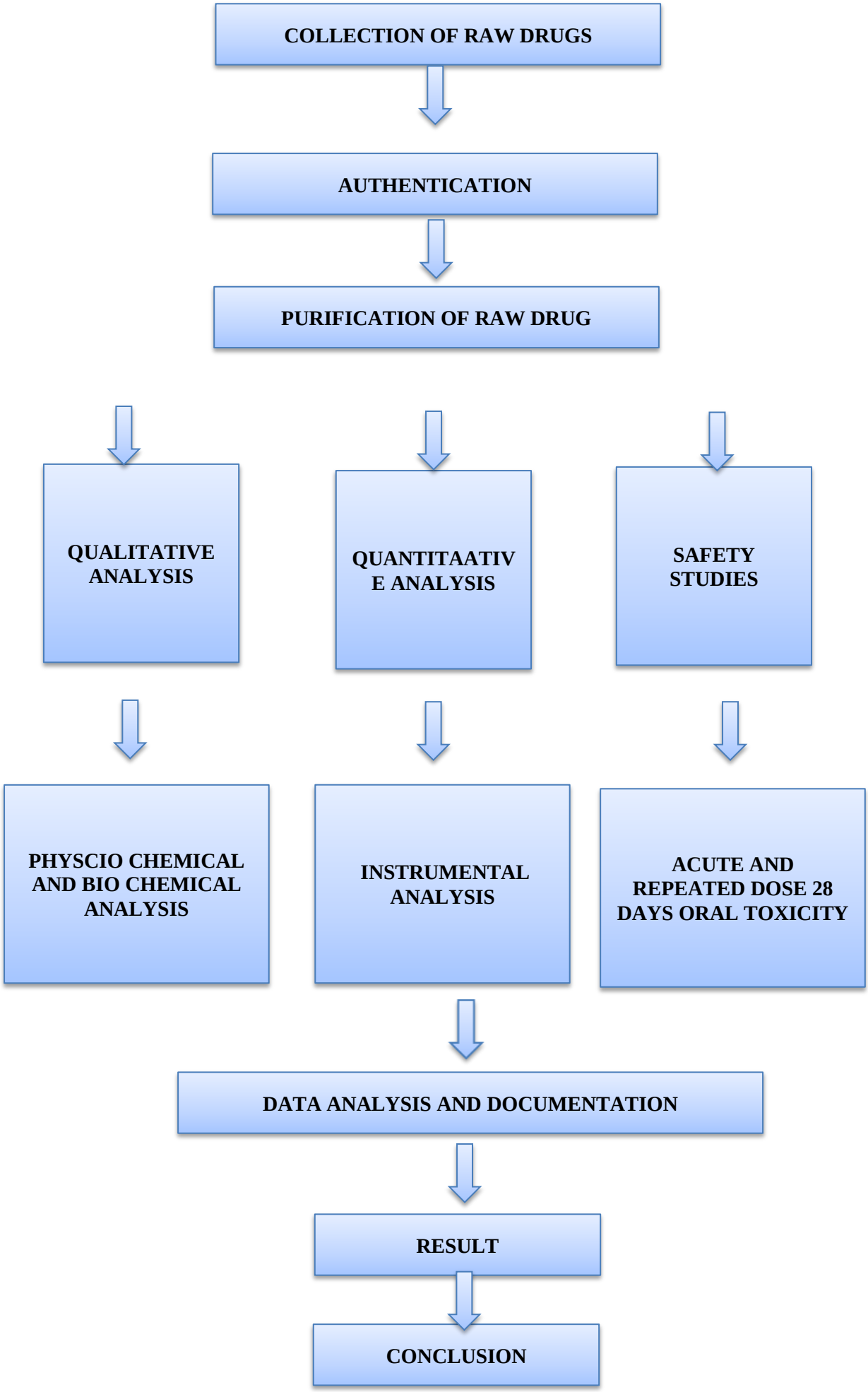
The methanol extract of leaf showed the antifungal property against selected fungal strains (Aspergillusflavus,Aspergillus fumigatus, Aspergillus niger, Candida albicans, Candidablanki, Microsporumcanis, Saccharomycescerevisiae, Vestilago) at the concentration of 100µg/ml and results were compared with standard griseofulvin(100µg/ml) [134].

13. Antioxidant Activity:

An extract was prepared using seed and methanol exhibited antioxidant effects in 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging at a concentration of 1µg/ml (Thehalf-maximalinhibitory concentration) (IC_{50}), and compared with ascorbic acid at the same concentration[135].

TOXICITY STUDIES:

Determination of median lethal dose (LD50) was done forfixing the therapeutic dose using ethanolic flower extract. This acute toxicity study revealed that even at the higher dose (2000 mg/kg) there were no mortality or any toxic reactions with oral administration of the palm tuber extract [124].


COLLECTION OF RAW DRUGS
AUTHENTICATION
PURIFICATION OF RAW DRUG
QUALITATIVE ANALYSIS
QUANTITAATIV E ANALYSIS
SAFETY STUDIES
PHYSCIO CHEMICAL AND BIO CHEMICAL ANALYSIS
INSTRUMENTAL ANALYSIS
ACUTE AND REPEATED DOSE 28 DAYS ORAL TOXICITY
DATA ANALYSIS AND DOCUMENTATION
RESULT
CONCLUSION

4. METERIALS AND METHODS

4.1 DRUG PROFILE:

The study drug ***"NAGA RASA PARPAM"*** has been selected from the classical Siddha literature – **The Hand Book of Indian Meicine: The Gems of Siddha System**

INGREDIENTS OF NAGARASA PARPAM:

1. Purified Nagam (Zinc)
2. Purified Rasam (Hydrargyrism)
3. Keezhanelli samoolam (Whole plant of *Phyllanthus niruri)*
4. Panai Kuruththu charu.

DOSAGE:

2 Grains. (130mg).

ADJUVANT:

Ghee or Butter.

MEDICAL USES:

1. Moola Noi (Hemorrhoid)
2. Pavuthiram (Fistula)
3. Megavettai (Gonorrhea)
4. Puttru Noikal (Cancer).

PREPARATION OF NAGA RASA PARPAM:

PROCUREMENT OF RAW DRUGS:

Zinc and Lingam obtained from Guruji Siddha medicals and raw drug shop, No: 882/884, GST Road, Tambaram Sanatorium, Chennai - 600 047. Keezhanelli (*phyllanthusniruri)* was collected from National Institute of

Siddha, Herbal Garden, Chennai - 47. Panai kuruththu was collected from Tenkasi district.

IDENTIFICATION AND AUTHENDICATION:

Keezhanelli and Panai kuruththu was identified and authenticated by the Dr. D. Aravind MD(S)., M.Sc., Assistant Professor, Department of Medical Botany, National Institute of Siddha, Chennai -47 and Certificate Number: NISMB4702021. Rasam and Nagam was identified and authenticated by the Department of Gunapadam, National Institute of Siddha, Chennai -47 and Certificate Number: **Gun/Aut/008/21**

PURIFICATION OF RAW DRUG:

Keezhanelli and **Panai kuruththu** are washed with water.

Purification of *Nagam* (Zinc): Iluppai ghee is taken in a mud pot. Two pieces of Ammonium chloride are placed in the above mud pot in such a way that half of the portion of the pieces is immersed in the ghee on opposite direction. The Zinc melted in blacksmith furnace and it is poured to the illupai ghee. Again the same procedure repeated for 20 times. Totally 21 times done this same procedure. After 21 times the purified *Nagam* is finely powdered and stored in a air tight container for medicine preparation.

Preparation of Vaalai rasam:

Vaalai Rasam: Separated *Rasam*(mercury) from *Lingam* (mercury sulphide) called as *Vaalai rasam*. *Vaalai rasam* is the most purified mercury, so the *Vaalai rasam* is used to prepare Naga Rasa Parpam instead of mercury.

Procedure: A cotton cloth dipped in the turmeric powder mixed water and this cloth dried under the shade. Powdered lingam was spread over this dried cloth and rolled as a ball. A elliptical circular shape hole was made in bottom of the mud pot and coconut shell fire charcoal placed over this hole. The ball prepared above was placed top of the charcoal and closed this pot mouth with equal size of another mud pot. The whole setup was taken out after the smoke and heat subside. Then the mercury was collected from the above mud pot and fired ball cloth. The collected mercury was filtered ten times in cotton cloth by

squeezing method. Finally the filtered mercury (*Vaalai rasam)*was stored in a air tight glass container for further usage.

Preparation procedure of Naga Rasa Parpam:

Purified Zinc 3 tolas (36 gms). Melt in an Iron frying pan by placing over the furnace of the smith. Sprinkle over it a handful of the entire plant of the *Phyllanthus niruri*, cut into small pieces, and be turning up and down don't triturate. Zinc will be reduced into flowers by combustion.

Select the flowers, Completely burnt add equal part of purified Mercury and triturate for 10hours with the juice of the tender shooting stems of the *Palmyra palm* by warming the stems over the fire, make Lozenges, dry enclose and seal in a clay pan dishes, burn with 15 cow dung cake. Powder and preserve.

INGREDIENTS OF NAGA RASA PARPAM

Figure 1.Zinc

Figure 2. Mercury

Figre 3. Panai Kuruththu

Figure 4. Keezhanelli

Figure 5.

VARIOUS STAGES OF MEDICINE PREPARATION

Purified Zinc and Mercury grainding with palm tuber juice

Villai's

Dried villai's placed in mud lid and closed with equal mud lid & surmounted by clay plaster (11 times)

Pudam process

Grinding the villa after pudam proc

End product - Naga Rasa Parpam

4.2. STANDARDIZATION OF NAGA RASA PARPAM

4.2.1 QUALITATIVE ANALYSIS:

Qualitative analysis of ***Naga Rasa Parpam*** done as per Protocol for testing of Ayurvedha, Siddha, and Unani Medicines. Organoleptic character and physico chemical analysis of ***Naga Rasa Parpam*** carried at Central council for Research in Siddha, Chennai Ministry of AYUSH, Anna Govt. Hospital campus, Arumbakkm, Chennai- 600106

A.ORGANOLEPTIC CHARACTERS: Organoleptic character for Naga Rasa Parpam was analysed as per standard procedure.

B. PHYSICO CHEMICAL ANALYSIS:

1. Determination of Moisture Content (Loss on Drying):

Accurate weight of 10 gm of Naga Rasa parpam was placed in the tared evaporating dish dry at 105ºC for 5 hours, and weigh. Continue the drying and weighing at one hour interval until difference between two successive weighing corresponds to not more than 0.25 per cent. Constant weight is reached when two consecutive weighing after drying for 30 minutes and cooling for 30 minutes in a desiccator, show not more than 0.01 g difference.

2. Determination of Total Ash:

Incinerate about 2 to 3 g accurately weighed, of the test drug in a silica dish at a temperature not exceeding 450º C until free from carbon, cool and weigh. Calculate the percentage of ash with reference to the air-dried drug.

3. Determination of Acid Insoluble Ash:

Boil the ash of test drug obtained by incineration for 5 minutes with 25 ml of dilute hydrochloric acid; collect the insoluble matter in a Gooch crucible or on an ash less filter paper, wash with hot water and ignite to constant weight. Calculate the percentage of acid -insoluble ash with reference to the air dried drug.

4. Determination of Water Soluble Ash:

Boil the ash of test drug for 5 minutes with 25 ml of water, collect insoluble matter in a Gooch crucible or on an ash less filter paper, wash with hot water, and ignite for 15 minutes at a temperature not exceeding 450º. Substrate the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water soluble ash. Calculate the percentage of water-soluble ash with reference to the air dried drug.

5. Determination of Water Soluble Extractive:

5g of air dried test drug was macerated with 100ml of chloroform-water in closed flask for twenty-four hours, shaken frequently during six hours and allowed to stand for eighteen hours. After filtering the solution 25ml of this filtrate was evaporated in a tared flat bottomed shallow dish, and dried at 105ºC until a constant weight was obtained. Later the percentage of water-soluble extractive with reference to the air-dried drug was calculated.

6. Determination of Alcohol Soluble Extractive:

5g of air dried test drug was macerated with 100ml of Alcohol of the specified strength in a closed flask for twenty-four hours, shaking frequently during six hours and allowing to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tarred flat bottomed shallow dish, and dry at 105º, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug was calculated.

7. Determination of pH values:

1% of the test substance was prepared using distilled water and stored properly. Prior to pH measurement pH electrode was calibrated using buffers of ph 4, 7, 10. After calibration, measurement was taken with a solution of known ph such that the reading should not differ by more than 0.02 from the original value. If the difference is greater than 0.05, the set of measurements was repeated. Later the ph electrode was dipped into the drug to be tested and kept as such until a constant reading was obtained.

C. BIO-CHEMICAL ANALYSIS:

The bio-chemical analysis of Naga Rasa Parpam as done at Biochemistry lab, National Institute of Siddha, Chennai-47.

Table 3.

S. NO	EXPRIMENT	OBSERVATION	INFERENCE
1.	Appearance of the sample	Mild ash in colour	Ash colour
2.	**Solubility:** a. A little of the test sample was shaken well with distilled water. b. A little of the test sample was shaken well with Con.HCL	a.Floating on the water. b.Completely soluble	Not soluble in water. Completely soluble acid
3.	**Action of Heat:** A small amount (500mg) of the test sample was taken in a dry test tube and heated gently at first and then strong.	No white fumes evolved	Absence of carbonate
4.	**Flame test:** A small amount of the test sample was made into a paste with con.HCL in a watch glass and introduced into non luminous part of the Bunsen flame.	No bluish green flame appeared	Absence of Copper
5.	**Ash test:** A filter paper was soaked into		

	a mixture of test sample and cobalt nitrate solution and introduced into the Bunsen flame and ignite.	No yellow colour flame appeared	Absence of sodium

D. PRELIMINARY BASIC, ACIDIC RADICALS AND BIOCHEMICAL SUDIES:

Preparation of extract:

5gms of **Naga Rasa Parpam** is weighed accurately and placed in a 250ml clean beaker and added with 50 ml of distilled water. Then it is boiled well for about 10 minutes. Then it is cooled and filtered in a 100ml volumetric flask and made up to 100ml with distilled water.

Table 4. Test for Acid Radicals:

S. NO	EXPERIMENT	OBSERVATION	INFERENCE
1.	**Test for sulphate:** 2ml of the above prepared extract was taken in a test tube to this added 2ml of 4% dil.Ammonium oxalate solution.	Absence of Cloudy Appearance	Sulphate absent
2.	**Test for chloride:** 2ml of the above prepared extract was added with diluted HNO_3 till the effervescence ceases. Then 2ml of silver nitrate solution was added.	Presence of Cloudy appearance	Chloride present
3.	**Test for phosphate:** 2ml of the extract was treated with 2ml of ammonium molybdate solution and 2ml of con.HNO_3.	Absence of yellow precipitate	Phosphate absent
4.	**Test for carbonate:** 2ml of the extract was treated with 2ml magnesium sulphate solution.	Presence of Cloudy appearance	Carbonate presence

5.	**Test for sulphide:** 1gm of the substance was treated with 2ml of conHCL.	Rotten Egg smelling gas was not evolved	Sulphide absent
6.	**Test for Fluoride and oxalate:** 2ml of extract was added with 2ml of dil. Aceticacid and 2ml calcium chloride solution and heated.	Absence of Cloudy appearance	Fluoride and Oxalate were absent
7.	**Test for nitrite:** 3 drops of the extract was placed on a filter paper, on that 2drops of acetic acid and 2drops of dil. Benzidine solution were placed.	Characteristic changes not appeared	Nitride absent
8.	**Test for borate:** 2 pinches of the substance was made into paste by using sulphuric acid and alcohol (95%) and introduced into the blue flame	Bluish yellow coloured flame not appeared	Borate absent

Table 5. Test for Basic Radicles:

1.	**Test for lead:** 2ml of the extract was added with 2ml of potassium iodide solution.	No yellow precipitate is obtained.	Lead absent
2.	**Test for copper:** One pinch of substance was made into paste with conHCL in a watch glass and introduced into the nonluminuous part of flame.	No Blue colour flame not appeared.	Copper absent
3.	**Test for aluminium:** 2ml of the extract sodium hydroxide was added in drops to excess.	No Characters changes.	Aluminium absent
4.	**Test for iron:** 2ml of extract add 2ml of ammonium thiocyanate solution and 2ml of con HNO_3 was added.	No Blood red colour appeared	Iron absent
5.	**Test for zinc:** 2ml of the extract sodium hydroxide solution was added in drops to excess.	White precipitate was formed.	Zinc present
6.	**Test for calcium:** 2ml of the extract was added with 2ml of 4%ammonium oxalate	Cloudy appearance and white precipitate obtained.	Calcium present

	solution.		
7.	**Test for magnesium:** 2ml of extract sodium hydroxide solution was added in drops to excess.	No White precipitate was obtained.	Magnesium absent
8.	**Test for ammonium:** 2ml of extract few ml of Nessler'sreagent and excess of sodium hydroxide solution were added.	No brown colour appeared	Ammonium absetnt
9.	**Test for potassium:** A pinch of substance was treated with 2ml of sodium nitrite solution and then treated with 2ml of cobalt nitrate in 30% glacial acetic acid	No yellowish precipitate was obtained.	Potassium Absent
10.	**Test for sodium:** 2 pinches of the substance was made into paste by using HCL and introduced into the blue flame of Bunsen burner.	No yellow colour flame appeared.	Sodium absent
11.	**Test for mercury**: 2ml of the extract was treated with 2ml of sodium hydroxide solution.	Yellow Precipitate was obtained.	Mercury present

12.	**Test for arsenic:** 2ml of the extract was treated with 2ml of sodium hydroxide solution	No brownish Red precipitate wa sobtained.	Arsenic absent

Table 6.Miscelloneus:

1.	**Test for starch:** 2ml of extract was treated with weak iodine solution.	No Sky blue Colour developed.	Starch absent
2.	**Test for reducing sugar:** 5ml of benedict's qualitative solution was takenin a test tube and allowed to boil for 2 minutesand added 8 to 10 drops of the extract and again boil it for 2minutes. The colour changes was noted.	No brick red colour developed.	Reducing sugar absent

3.	**Test for the alkaloids:** . 2ml of the extract was treated with 2ml of potassium iodide solution. . 2ml of extract was treated with 2ml of picric acid.	Yellow colour developed.	Alkaloid present
4.	**Test for tannic acid:** 2ml of extract was treated with 2ml of ferricchloride solution.	No black precipitate was obtained	Tannic acid absent
5.	**Test for unsaturated compound:** 2ml of extract 2ml of potassium permanganate solution was added.	Potassium permanganate was not decolourised.	Unsaturated compound absent
6.	**Test for aminoacid:** 2drops of the extract was placed on a filter paper and dried well.	Not violet colour developed.	Amino acid absent
7.	**Test for type of compound:** 2ml of the extract was treated with 2ml of ferric chloride solution.	Red colour developed.	Green/Red/Violet/ Blue colour developed

4.2.2. QUANTITATIVE ANALYSIS

A. INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROMETRY (ICP-OES)[144]:

The analysis ofheavy metal sandtrace elements were estimated by using Inductively Coupled Plasma Optical Emission Spectrometry (ICP- OES).The Experimental Procedure was done at CCRS, Arumbakam, Chennai - 36.

INTRODUCTION:

Inductively coupled plasma optical emission spectrometry (ICP-OES), is an analytical technique used for the detection of trace metals. It is a type of emission spectroscopy that uses the inductively coupled plasma to produce excited atoms and ions that emit electromagnetic radiation at wavelength characteristic of a particular element. The intensity of this emission is inductive of the concentration of the elementwithin the sample.

PROCEDURE:

Take about 20 mg of sample into the Teflon microwave digestion vessel and add 1ml of ultrapure nitric acid to digest about 45 minutes using Anton Paar microwave digestion unit. After that the sample is made up to a 50 mL standard measuring flask. The calibration standard solution is prepared in the range of 0.2µg/mL to 10µg/mL by using ultrapure nitric acid and blank also. Agilent ICP-OES 5100 VDV instrument used with the following operation conditions: a RF powder 1.2 kW, a plasma gas flow rate 12 L min^{-1}, and a nebulizer and spray chamber for the analysis.

EXTRACTION OF IN FORMATION:

Obtaining qualitative information, i.e., what elements are present in the sample, involves identifying the presence of emission at the wavelengths characteristic of the elements of interest. Obtaining quantitative information, i.e., how much of an element is in the sample, can be accomplished using plots of emission intensity versus concentration called curves.

Perkin-Elmer Optima	5300DV
Rf frequency	40MHz
Range	165-782nm
Detectionlimit	Upto ppm level using SCD detector

B. X- RAY POWDER DIFFRACTION (XRD)[144]:

XRD is a powerfulm nondestructive technique for characterizing crystalline materials. It provides information on structure, phases, preferred crystal orientations (texture), and other structural parameters.

XRD analysis of Naga Rasa Parpam was done by as per standard procedure.

C. SCANNING ELECTRON MICROSCOPY WITH ENERGY DISPERSIVE X-RAY ANALYSIS [144]:

SEM provides detailed high resolution images of the sample by ratering a focused electron beam across the surface and detecting secondary or backscattered electron signal. An Energy Dispersice X-Ray Analyzer is also used to provide elemental identification and quantitative compositional information.

SEM with EDX analysis of Naga Rasa Parpam was done by as per standard procedure.

D. FOURIER TRANSFORM INFRARED SPECTROSCOPY:

FTIR analysis is used to obtain the infrared spectrum of transmission or obsorptio of a fuel sample. FTIR identifies the presence or organic and inorganic compounds in the sample.

FTIR analysis of Naga Rasa Parpam was done by as per standard procedure.

4.3. TOXICITY STUDIES

A preclinical toxicity study of ***Naga Rasa Parpam*** was conducted on Wistar albino rats as per OECD guideline 423 & 407after getting IAEC approval. **(IAEC Approval Number is NIS/IAEC-1/13/30092020/13)**.

4.3.1. ACUTE TOXICITY STUDY:

A. Experimental Animals:

Species	: Wister albino Rats
Sex	: Female
Age / Weight	: 8 weeks / 140 – 160 g b.wt.
Acclimatization Period	: 7 Days prior to dosing.
Housing	: 3 numbers in polypropylene cages
Husbandry	:12 hours light/ 12 hours dark cycle.
	Room temperature: 22^{o} c ($\pm$ 3^{o})
	Humidity: 30 – 70 %
Feed and Water	:Rodent pellet feed
	RO purified water
Identification	:Animals will be kept in individually marked with Picric acid (Head, Body and Tail) in each group

B. Animal selection and Identification:

The animals are randomly selected for each group. Each group contains 3 female animals. They were marked in head, body and tail with picric acid solution. Group name, cage number and sex of the animal are mentioned in the front of the each cage cards.

C. Stock solution preparation:

Naga Rasa Parpam 300mg and 2000 mg was take separately and add these in 5 ml of ghee 5ml of RO water. Then mix well to make as completely dissolve form. Then this stock solution is used for acute toxicity study.

D. Experimental procedure:

Acute toxicity study was conducted as per OECD guideline 423. Group I set as control treated with ghee mixed RO water, Group II treated with 300mg/ kg.b.wt and Group III was treated with 2000mg/ kg.b.wt dosage of ***Naga Rasa Parpam***. Each group contains 3 females. Before drug administration animals kept under fasting for 12 hrs with free from water. After drug administration the animals were observed continuously for first 4 hours then one hour once up to 24 hours to record behavioral pattern of the animals, any mortality and morbidity. Observations should include changes in skin and fur, eyes, mucous membrane and also circulatory, autonomic, CNS and somatomotor activity. The animals are observed tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. Food and water intake noted daily and body weight changes noted weekly once for 14 days. At the end of 14^{th} day all the animals were sacrificed under anesthesia and examined any gross pathological changes. It includes examination of the eternal surface of the body, all orifices and organs like brain, lungs, heart, spleen, liver, kidney, adrenals and sex organs of all animals.

E. Cage-side observation

The animals were monitored for behavioural parameters like Alertness, abnormal Gait (rolling and tiptoe), aggressiveness, akinesia, analgesia, catalepsy, convulsions, defecation, excitation, exopthalmos, head twitches, lacrimation, lethality, loss of corneal reflex, loss of righting reflex, loss of traction, piloerection, ptosis, reactivity to touch, respiration, salivation, scratching, sedation, stereotypies (chewing), stereotypies (head movements), stereotypies (sniffing), straub, tremor and writhes..

4.3.2. 28 DAYS REPEATED ORAL TOXICITY STUDY:

A. Experimental Animals:

Species : Wister albino Rats

Sex : Male and Female

Age / Weight : 8 weeks / 140 – 160 g b.wt.

Acclimatization Period: 7 Days prior to dosing.

Housing : 5 numbers in polypropylene cages

Husbandry : 12 hours light/ 12 hours dark cycle.
Room temperature: 22^{o} c ($\pm$ 3^{o})
Humidity: 30 – 70 %

Feed and Water : Rodent pellet feed
Ro purified water

Identification : Animals will be kept in individually marked with Picric acid (Head, Body, Tail, Head Body and Body Tail) in each group.

B. Animal selection and Identification:

The animals are randomly selected for each group. Each group contains 5 male and 5 female animals. They were marked in head, body, tail, head body and body tail with picric acid solution. Group name, cage number and sex of the animal are mentioned in the front of the each cage cards.

C. Stock solution preparation:

Naga Rasa Parpam 12, 60 and 120 mg/ kg bwt (low dose, mid dose, high dose)take separately were add with 5 ml of Ghee mixed 5 ml of RO water. Then mix well to make as completely dissolve form. Then used for 28 days repeated oral toxicity study.

D. Experimental Procedure:

A repeated 28 days oral toxicity study was conducted as per OECD guideline 407. Totally 5 groups of Wister albino rats in both sexes were used in this study. Each group contains 5 male and 5 female. Group I set as a control and received Ghee mixed RO water, Group II to Group IV received ***Naga Rasa Parpam*** daily in Low dose (12mg/kg bwt), Mid dose (60mg/kg bwt), High dose (120 mg/kg bwt) respectively up to 28 days orally via gastric intubation. Low, Mid, High dose was calculated from human effective (HED) dose as per FDA guideline based on body conversion factor. Animals were kept under fasting for 12 hours without food but water prior to dosing first day. All the study animals were observed daily for any behavioral changes, mortality and morbidity after drug administration. Food and water intake noted daily and body weight changes noted weekly once. At the end of the study 29^{th} day all the animals (Low dose,

mid dose and High dose test drug treated) fasted 12 hours without food but water.

Then blood samples were collected by cardiac puncture in under anesthesia to access biochemical (Glucose, triglycerides, cholesterol, SGOT, SGOT, total bilirubin, total proteins, albumin, urea, creatinine, potassium, sodium and chloride) and hematological parameters (Total erythrocyte, total leucocytes, Hb, PCV, MCV, MCH, MCHC, platelet count). Then all the animals were sacrificed on the day of 29. After blood collection, to observe gross pathological changes in all organs such as brain, heart, lungs, liver, kidney, stomach, spleen, uterus, ovary and testis and these organs were collected from all the animals for histopathology investigation. If any toxicological changes noted in high dose treated group vital organs, then only histopathology of low and mid dose treated group vital organs will be studied [0].

5. Statistical analysis:

All data were expressed as mean ± Standard Deviation (SD). The treatment groups were compared with control for testing significance by one way analysis of variance (ANOVA), Dunnett's multiple range tests was applied by using GraphPad Instat 3.0 version. Values of $p < 0.05$ will be considered to be statistically significant.

5. RESULTS

5.1 LITERATURE REVIEW

Literature evidence were collected about the study drug and this ingredients form various Siddha books and Reasearch articles. Literature results support and strenghthen the Naga rasa parpam is used to treat hemorrhoids, fistula in Ano, gonorrhoea and Cancer mentioned in Siddha literature.

5.2. STANDARDIZATION:

Standardization of the drug is more essential to derive the efficacy and potency of the drug which has analyzed by the various methods. The results of Physiochemical analysis, Biochemical analysis, Instrumental analysis of Naga Rasa Parpam is tabulated below.

QUALITATIVEANALYSIS:

Table.7: Organoleptic characters analysis of Naga Rasa Parpam:

S.No	**Charecters**	**Results**
1.	Luster	Lusterless
2.	Nature	Fine Powder
3.	Colour	White colour
4.	Odour	Odourless
5.	Taste	Tasteless
6.	Solubility a. Distilled water b. Ghee c. Ghee + Distilled water(equal qty)	1.Not soluble 2.Completely soluble 3.Completely soluble
7.	Test of float on water	Naga Rasa Parpam floated on water

The organoleptic characters showed that Naga Rasa Parpam is lusterless, colorless, odourless, tasteless and white colour fine powder. It is completely soluble in Ghee & Ghee with distilled water (equal quantity) and not soluble in distilled water.

Table8: Physicochemical analysis of Naga Rasa Parpam:

S.No	Parameters	Percentage
1	Loss on drying	Lessthan 1%
2	Total ash value	99.67%
3	Acid insoluble ash	22.84%
4	Water soluble ash	16.42%
5	Water soluble extraction	31.68%
6	Alcohol soluble extraction	5.1%
7	pH Value	8.02

Table 9: Biochemical analysis of Naga Rasa Parpam(NRP):

S.No	PROCEDURES	RESULTS
1	Test for Sulphate	-
2	Test for Phosphate	-
3	Test for Carbonate	+
4	Test for Nitrate	-
5	Test for Chloride	+
6	Test for Sulphide	-
7	Test for Fluride and Oxalate	-
8	Test for Nitrite	-
9	Test for Borate	-
10	Test for Lead	-
11	Test for Copper	-
12	Test for Aluminum	-
13	Test for Iron	-
14	Test for Zinc	+
15	Test for Calcium	+
16	Test for Magnesium	-
17	Test for Ammonium	-
18	Test for Potassium	-

19	**Test for Sodium**	**-**
20	**Test for Mercury**	**+**
21	**Test for Arsenic**	**-**
22	**Test for Starch**	**-**
23	**Test for reducing sugar**	-
24	**Test for alkaloids**	+
25	**Test for Tannic acid**	-
26	**Test for Unsaturated compounds**	-
27	**Test for amino acid**	-
28	**Test for type of compound**	-

(+)- Present; (-) -Absent

Biochemical analysis of Naga rasa parpam revealed the presence of Zinc, Mercury, Calcium, Chloride, Carbonate and Alkaloids.

QUANTITATIVE ANALYSIS:

A. INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROMETRY (ICP-OES):

Table 10: Results of ICP-OES analysis of Naga Rasa Parpam:

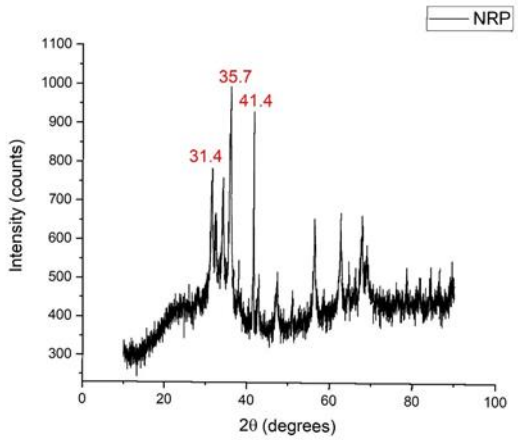
S.No	Elements	Wave Length In(nm)	Results
1.	Arsenic	As 188.90	99.02 mg/L
2.	Calcium	Ca 396.847	BDL
3.	Zinc	Zn 213.857	42.92% w/w
4.	Copper	Cu 327.395	31.17 mg/L
5.	Iron	Fe 238.204	0.021% w/w
6.	Mercury	Hg 184.887	25.78% w/w
7.	Potassium	K 766.491	0.196% w/w

8.	Magnesium	Mg 279.553	0.037% w/w
9.	Sodium	Na 589.592	BDL
10.	Manganese	Mn 257.610	2.88 mg/L
11.	Lead	Pb 220.353	169.04 mg/L
12.	Cobalt	Co 238.892	8.90 mg/L
13.	Chromium	Cr 267.716	BDL
14	Cadmium	Cd 214.439	1.93 ma/L

BDL – Below Detection Limit

B. X-RAY DIFFRACTION PATTERN OF NAGA RASA PARPAM

Graph No.1



NRP – Naga Rasa Parpam

Result:

Using a Bruker D8 diffrarctometer. The X- ray diffraction method was used to examine the phase purity and crystallinity of the product metal drug samples. The characteristic peaks located at 31.4, 35.7 and 41.4. The crystalline nature and grain size were calculated from the full width half maximum data of the XRD peaks. The 2 –Theta range from 10^0 -90^0.

C. SCANNED ELECTRON MICROSCOPY AND EDAX ANALYSIS OF NAGA RASA PARPAM:

Figure 8: SEM Image of NRP

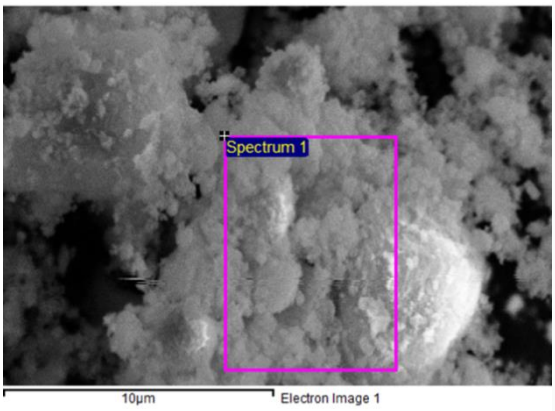
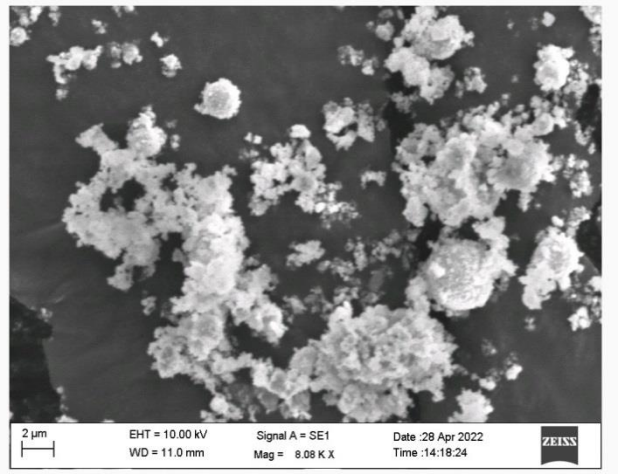
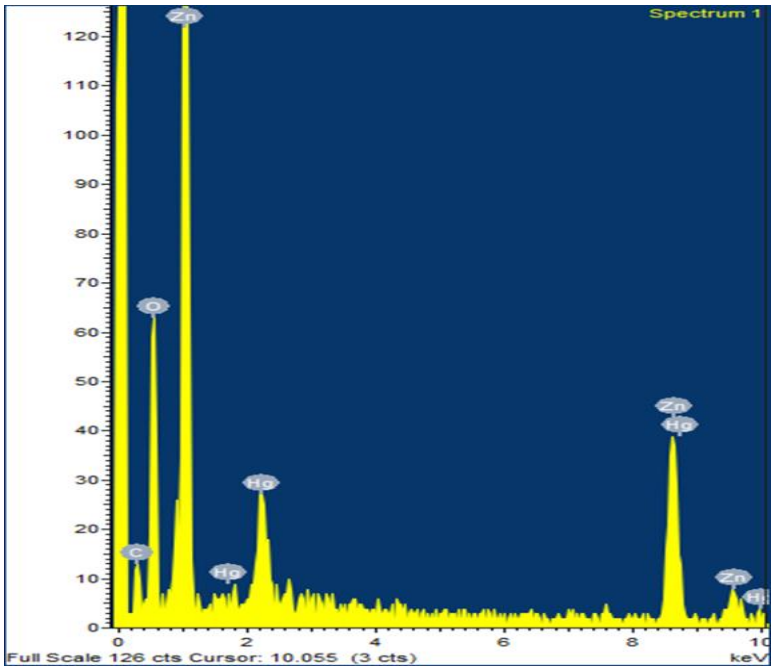


Figure9 :SEM Image of NRP



Graph 2: EDAX analysis of Medicine sample:



Result:

Particle Size : 10 - 60 µm.

Shape : Cluster, Spongy appearance.

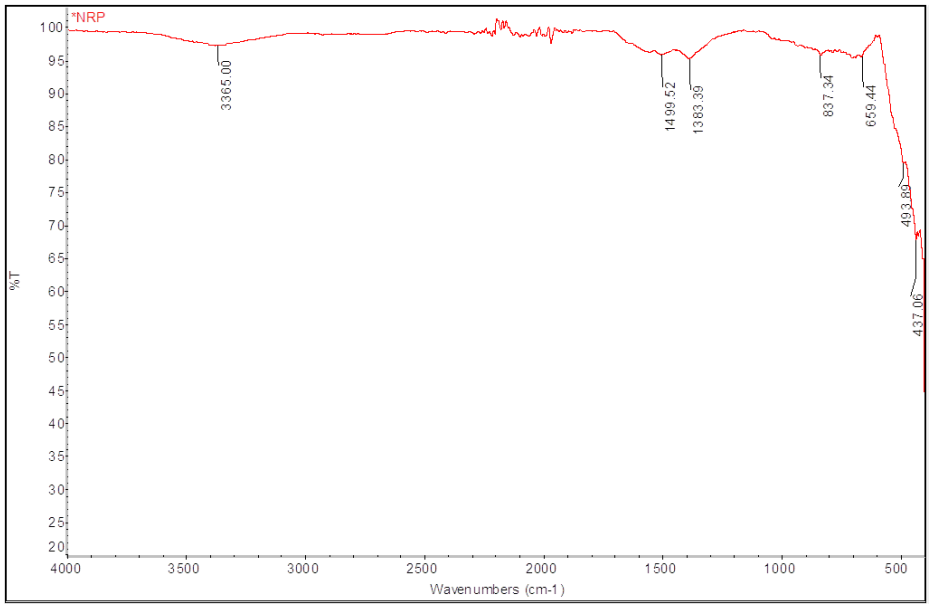
Surface : Smooth.

Table 11: The weight and atomic percentage of elements present inNaga Rasa Parpam:

Element	Weight%	Atomic%
CK	13.27%	29.36%
OK	30.47%	50.62%
ZnK	45.84%	18.64%
HgM	10.42%	1.38%

D. FT- IR ANALYSIS OF NAGA RASA PARPAM:

Graph No.3



Result:

Infratred absorbtion pattern of Aliphatic secondary amine stretching was observed in the region of 3365 cm^{-1}. Sharp absorption peak abserved in the region of 437.06^{-1} indicate the Aryl disulfide S=S bond. Absorption peak at 493.89 cm^{-1} corresponds to Poly disulfide S=S bond.Wide absorbtion peaks at 1499.52 cm^{-1} may be due to presence of Aromatic Nitro compounds. 1383 cm^{-1} and 837.34 cm^{-1} corresponds to carboxylate (Carboxylix acid salt) and Alkyne C=H bond.

5.3. RESULTS OF TOXICITY STUDIES:

ACUTE TOXICITY STUDY:

Acute toxicity study results showed, there was no Mortality, Behavioural changes and morbidity observed at 300 mg/Kg b.Wt and 2000 mg/Kg b.Wt test drug treated animal groups. No gross pathological changes had been seen in the internal organs of both control and test drug treated groups in Acute Toxicity study.

BEHAVIORAL CHANGES IN ACUTE TOXICITY OF NAGA RASA PARPAM AT 300, 2000mg/kg DOSAGE

Table 12:

No	Dose mg/ kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	300	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
2	2000	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N

(+)-Present; (-)-Absent; (N)-Normal

1.Alertness, 2.Aggressiveness, 3.Piloerection, 4.Grooming, 5.Gripping, 6.Tough response, 7.Decreased Motor Activity, 8.Tremors, 9.Convulsions, 10.Muscle Spasm, 11.Catatonia, 12.Muscle relaxant, 13.Hypnosis, 14.Analgesis, 15. Lacrimation, 16.Exophthalmos, 17.Diarrhoea, 18.Writhing, 19.Dyspnoea, 20.Mortality, 21.Respiration.

RESULTS OF 28DAYS REPEATED ORAL TOXICITY STUDY:

Table 13: Body weight changes of Male Wistar Albino Rats in 28 Days Repeated Oral Toxicity Study of Naga Rasa Parpam

GROUP	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
1st WEEK	236±38.30	240.2±21.48	228±14.85	212.4±28.45
2nd WEEK	240.4±41.67	239±14.78	250.8±17.24	230.4±32.14
3rd WEEK	248.4±40.86	246.4±16.20	257.2±15.90	238.2±31.24
4th WEEK	253.8±41.79	266.4±16.90	276.2±31.15	243.6±34.96

Values are expressed as mean SEM statistical significance (P) calculated by one way ANOVA followed by dunnett's (N=5)^{ns} p>0.05, * p<0.05,** p<0.01, ***p<L0.001 calculated by comparing treated groups with control groups.

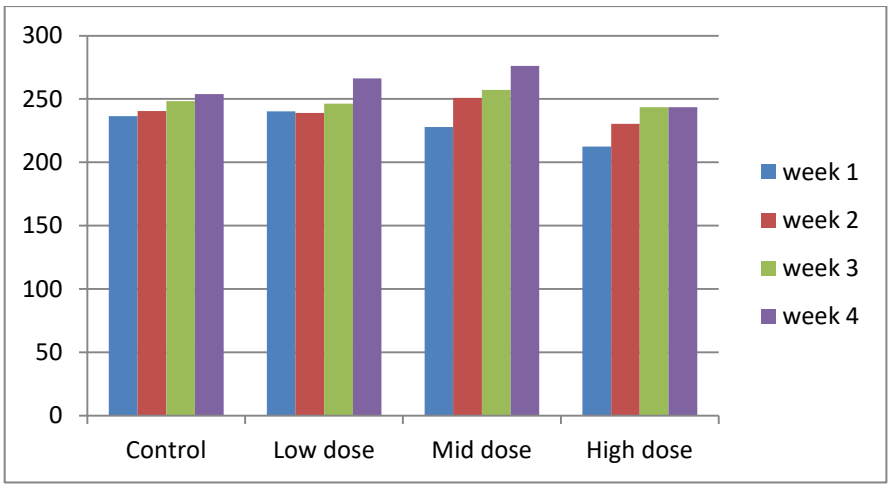


Figure10. Body weight changes of Male Wistar Albino Rats in 28 Days Repeated Oral Toxicity Study of Naga Rasa Parpam.

Table 14: Body weight changes of Female Wistar Albino Rats in 28 Days Repeated Oral Toxicity Study of Naga Rasa Parpam

GROUP	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
1st WEEK	155±8.57	185.2±18.71**	173.2±16.84	168±19.70
2nd WEEK	165.4±9.04	196.6±15.32	182.6±14.9	180.4±21.82
3rd WEEK	180.6±7.70	206.4±14.50	196.2±13.65	196.2±17.4
4th WEEK	169.4±12.01	198.6±25.36	186.6±15.07	185±24.3

Values are expressed as mean SEM statistical significance (P) calculated by one way ANOVA followed by dunnett<s (N=5)[ns] p>0.05, * p<0.05,** p<0.01, ***p<0.001 calculated by comparing treated groups with control groups.

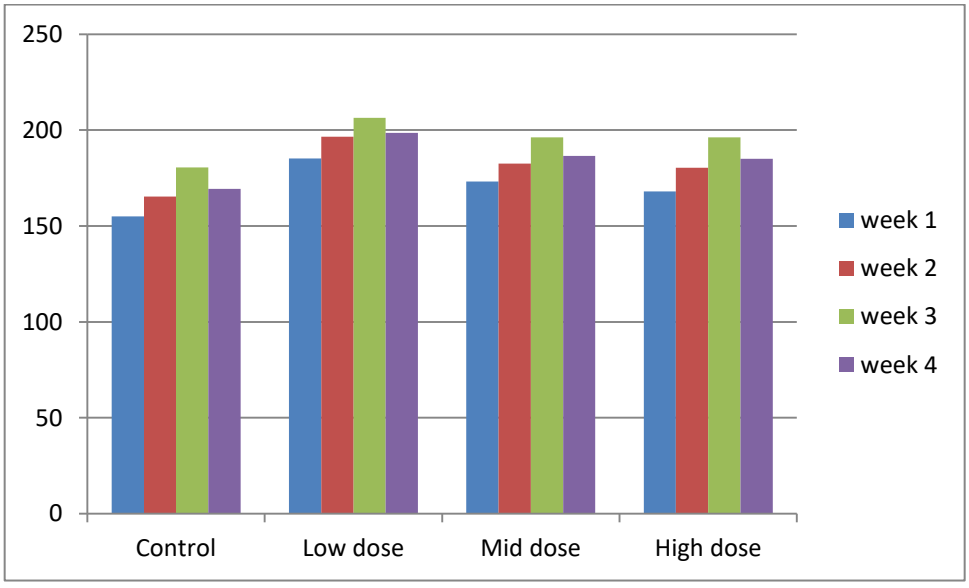


Figure 11. Body weight changes of Female Wistar Albino Rats in 28 Days Repeated Oral Toxicity Study of Naga Rasa Parpam.

The results suggest body weight of all the treated animals male and female were gradually increased during the study period and there were no significant changes observed between control and test drug treated group at 12 mg/kg.b.wt, 60 mg/kg.b.wt, 120 mg/kg.b.wt. But Significantly increased at first week in low dose treated females.

Table 15: Feed intake of Male Wistar Albino Rats in 28 Days Repeated Oral Toxicity Study of Naga Rasa Parpam

GROUP	**CONTROL**	**LOW DOSE**	**MID DOSE**	**HIGH DOSE**
1st WEEK	41.57±3.40	40.51±3.82	41.57±5.06	45.85±4.18
2nd WEEK	48.71±2.29	41.29±3.35	44±2.65	41±4.90
3rd WEEK	49.1±7.71	44.29±9.55	47.86±9.40	44.71±7.41
4th WEEK	49±9.49	45.14±7.08	43.17±7.62	42.43±2.30

values are expressed as mean SEM statistical significance (P) calculated by one way ANOVA followed by dunnetts (N=5)^{ns} $p>0.05$, * $p<0.05$,** $p<0.01$, ***$p<0.001$ calculated by comparing treated groups with control groups.

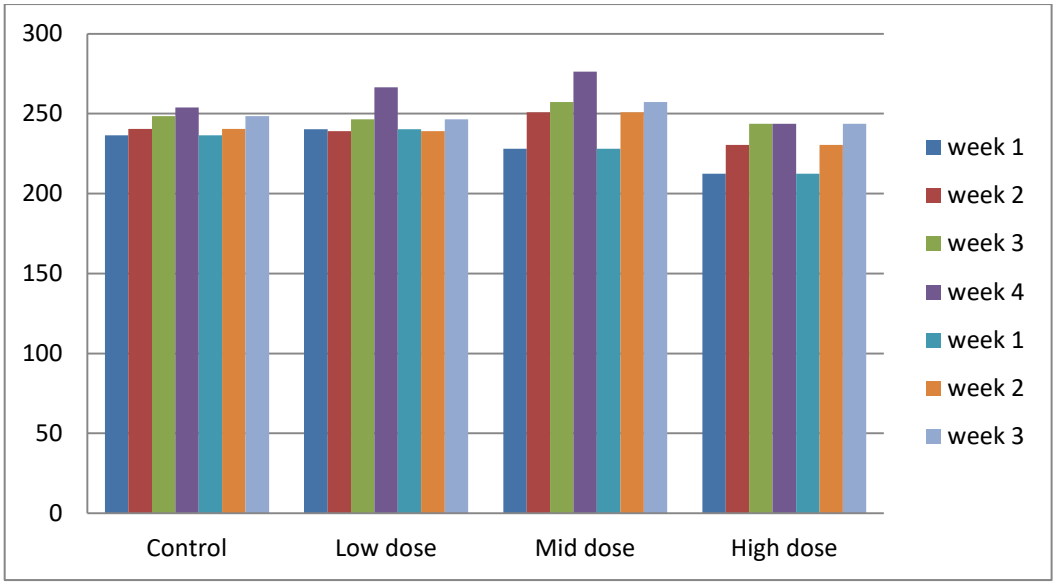


Figure12. Feed intake of Male Wistar Albino Rats in 28 Days Repeated Oral Toxicity Study of Naga Rasa Parpam.

Table16: Feed intake of Female Wistar Albino Rats in 28 Days Repeated Oral Toxicity Study of Naga Rasa Parpam

GROUP	**CONTROL**	**LOW DOSE**	**MID DOSE**	**HIGH DOSE**
1st WEEK	32.7±4.46	33.29±4.64	35.86±4.26	45.7± 6.32
2nd WEEK	31.71±5.25	38.29±3.73	35.86±5.67	38.2±3.73
3rd WEEK	35.43±4.89	40.14±7.33	39.43±7.96	47.3±9.21
4th WEEK	39.71±5.65	41±5.71**	39.8±5.27	52.7±9.32

Values are expressed as mean SEM statistical significance (P) calculated by one way ANOVA followed by dunnett<s (N=5)ns p>0.05, * p<0.05,** p <0.01, ***p< 0.001 calculated by comparing treated groups with control groups.

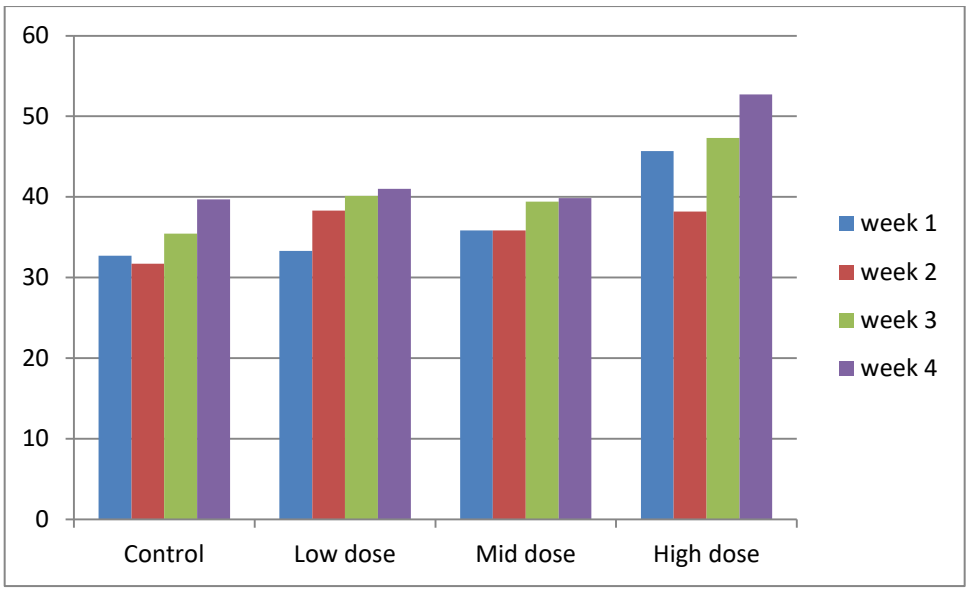


Figure 13 Feed intake of Female Wistar Albino Rats in 28 Days Repeated Oral Toxicity Study of Naga Rasa Parpam.

Feed intake results suggest, the feed intake of all the test animals was gradually increased. But significantly feed intake was increased in Low dose group at 4^{th} week when compared with control group Females.

Table 17: Water intake of Male Wistar Albino Rats in 28 Days Repeated Oral Toxicity Study of Naga Rasa Parpam

GROUP	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
1st WEEK	40±4.08	41.43±3.78	40±7.07	37.86±2.67
2nd WEEK	47.14±6.36	47.14±6.36	47.14±6.36	46.88±4.87
3rd WEEK	50.71±10.58	47.14±4.88	50±4.88	51.42±7.48
4th WEEK	52.14±6.36	52.86±6.36	55±6.36	55.57±6.27

Values are expressed as mean SEM statistical significance (P) calculated by one way ANOVA followed by dunnett's (N=5)^{ns} $p>0.05$, * $p<0.05$,** $p<0.01$, ***$p<0.001$ calculated by comparing treated groups with control groups.

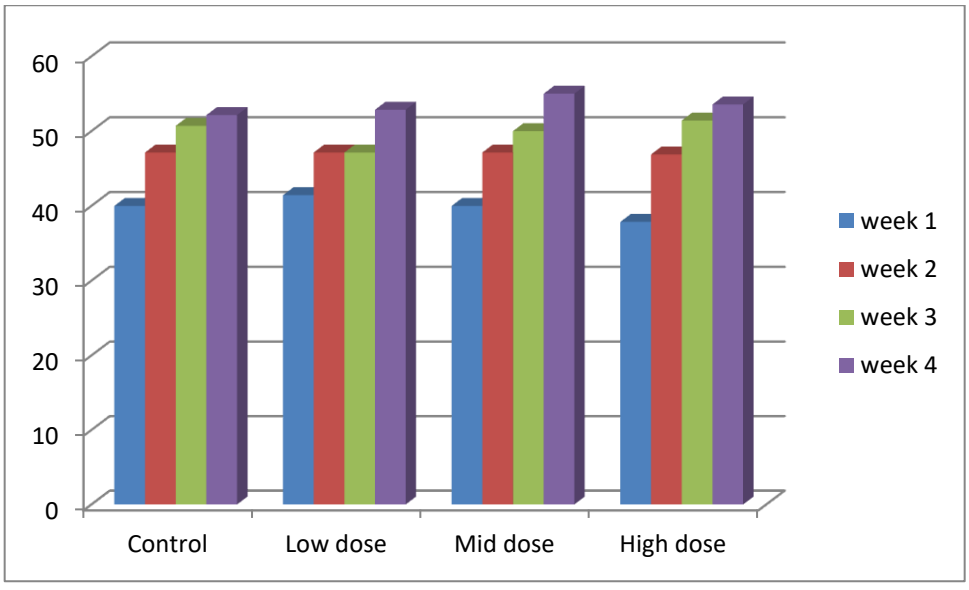


Figure14 Water intake of Male Wistar Albino Rats in 28 Days Repeated Oral Toxicity Study of Naga Rasa Parpam.

Table 18: Water intake of Female Wistar Albino Rats in 28 Days Repeated Oral Toxicity Study of Naga Rasa Parpam

GROUP	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
1st WEEK	39.29±3.45	58.57±5.56	53.57±5.56	54.29±8.86
2nd WEEK	42.14±4.08	41.43±6.90	47.14±6.36	50.71±10.18
3rd WEEK	56.42±9.45	56.43±13.45	62.14±9.94	65±7.07
4th WEEK	60.71±7.87	50.71±6.07	62.86±14.68	55.71±10.18

Values are expressed as mean SEM statistical significance (P) calculated by one way ANOVA followed by dunnett<s (N=5)^{ns} p>0.05, * p<0.05,** p<0.01, ***p<0.001 calculated by comparing treated groups with control groups.

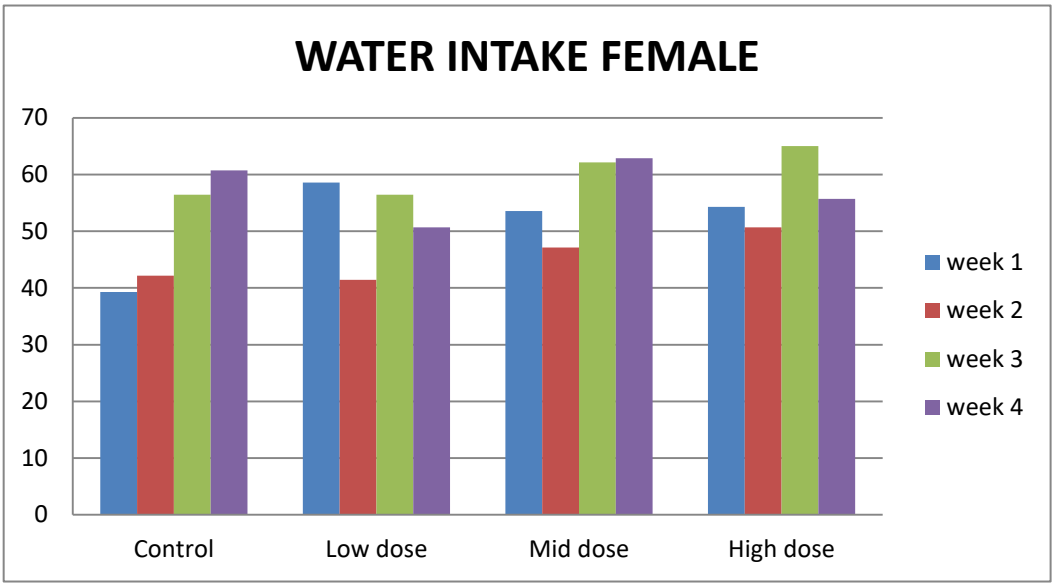


Figure 15. Water intake of Female Wistar Albino Rats in 28 Days Repeated Oral Toxicity Study of Naga Rasa Parpam.

No significant changes observed in water intake of test drug treated group animals compared with control group animals

EFFECT OF 28 DAYS REPEATED ORAL TOXICITY STUDY OF NAGA RASA PARPAM ON HEMATOLOGICAL PARAMETERS

Table 19: Effect of 28days repeated oral toxicity study of Naga Rasa Parpam on Hematological parameters

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
RBC ($X10^6$ l)	7.71±0.47	7.54±0.36	7.95±0.51	7.91±0.42
WBC($X10^6$ l)	14.93±4.02	16.47±3.81	13.90±4.81	11.80±3.77
PLT($X10^6$ l)	592.6±51.2	610±45.5	627±97.46	712±97.46
HGB(g/dl)	14.44±0.69	13.74±0.75	14.64±0.71	13.5±0.99
MCH(pg)	21.88±0.19	21.98±0.34	21.64±0.15	21.72±0.31
MCV(fl)	84.92±1.81	82.84±1.08	84.4±1.89	85.16±2.27
N(fl)	9.44±3.54	**9.58**±1.66	10.74±2.24	9.92±1.08
E(%)	0.82±0.24	0.9±0.43	1.96±0.93	1.18±0.33
B(%)	0±0	0±0	0±0	0±0
L(%)	89.18±3.76	88.44±2.04	86.42±3.56	87.34±1.13
M(%)	0.56±0.14	1.08±0.43	0.88±0.66	1.56±0.46

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 groups.

Figure. 16 . Effect of 28days Repeated oral Toxicity study of Naga Rasa Parpam on Hematological Parameters.

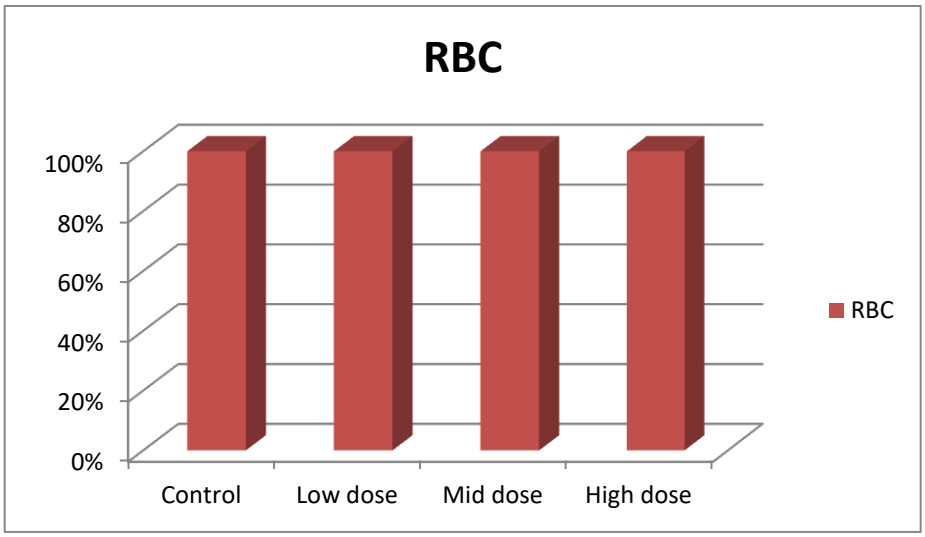


Figure17 Effect of 28 Days Repeated Oral Toxicity Study of Naga Rasa Parpam on Hematological Parameters.

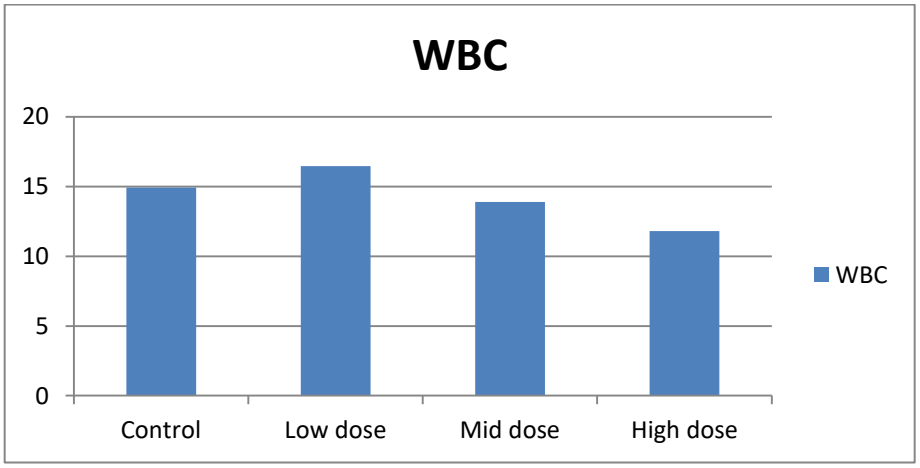


Figure18. Effect of 28 Days Repeated Oral Toxicity Study of Naga Rasa Parpam on Hematological Parameters.

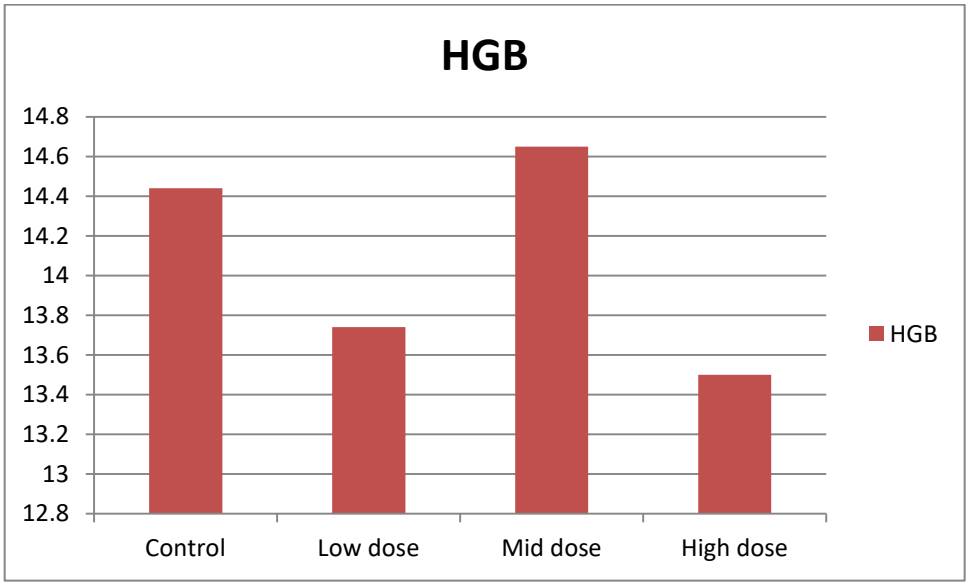


Figure 19 Effect of 28 Days Repeated Oral Toxicity Study of Naga Rasa Parpam on Hematological Parameters.

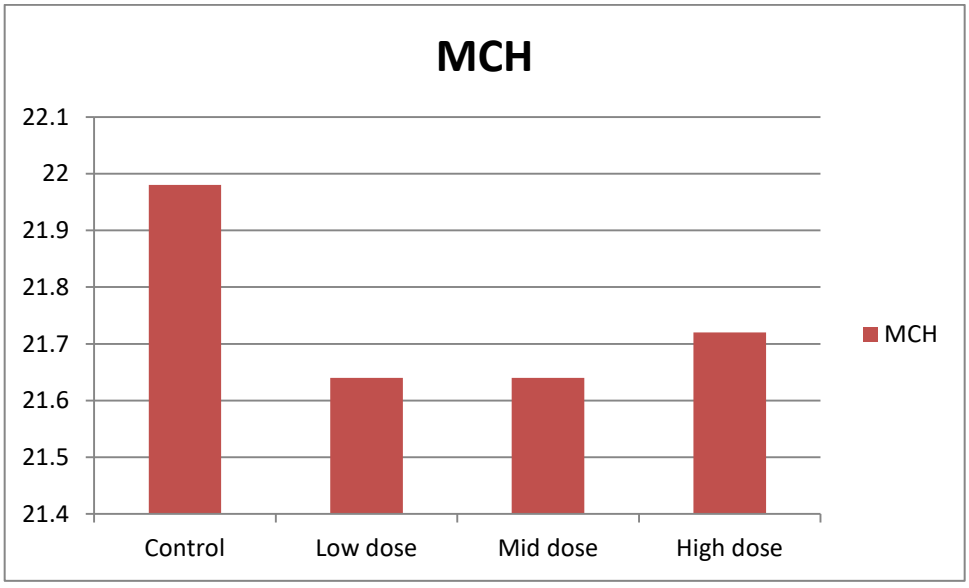


Figure 20…. Effect of 28 Days Repeated Oral Toxicity Study of Naga Rasa Parpam on Hematological Parameters.

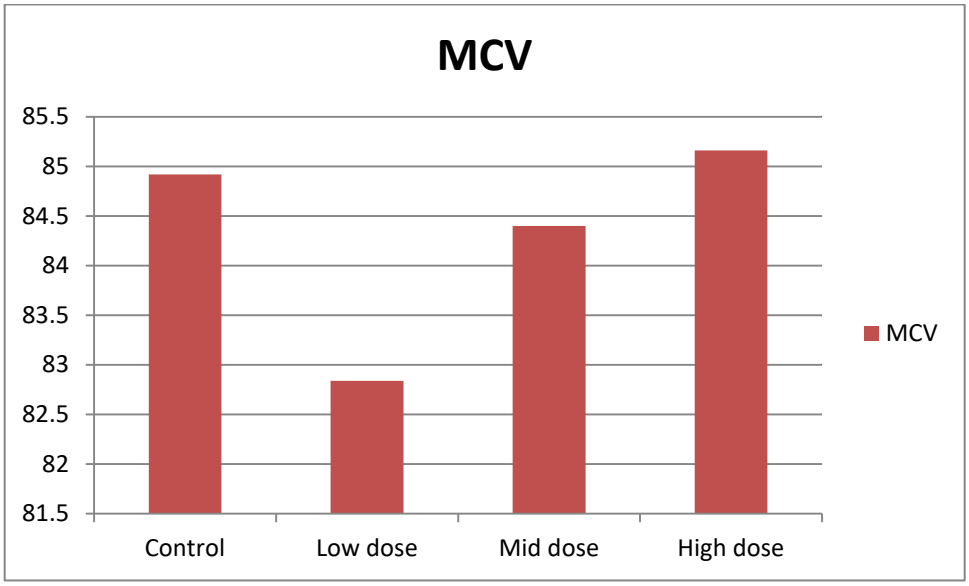


Figure 21…. Effect of 28 Days Repeated Oral Toxicity Study of Naga Rasa Parpam on Hematological Parameters.

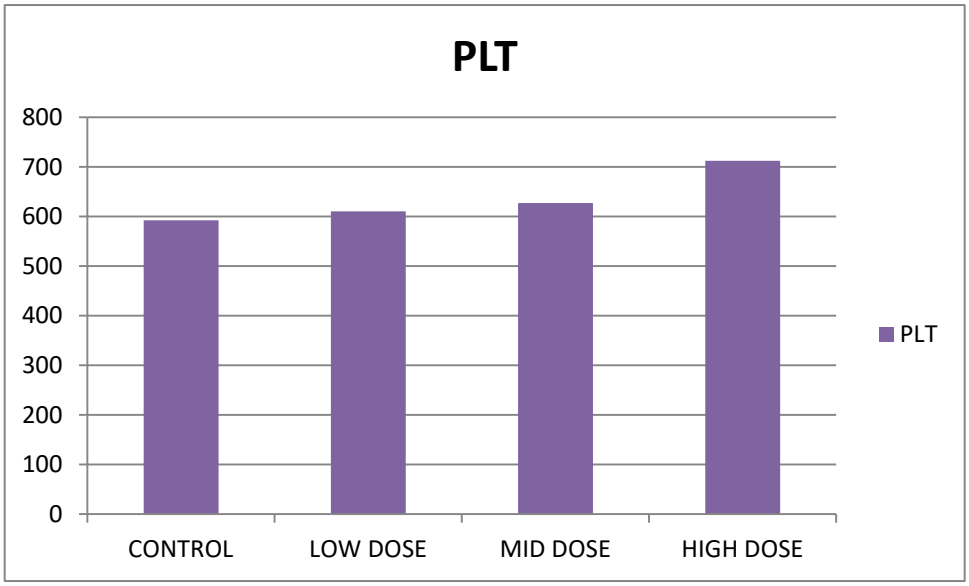
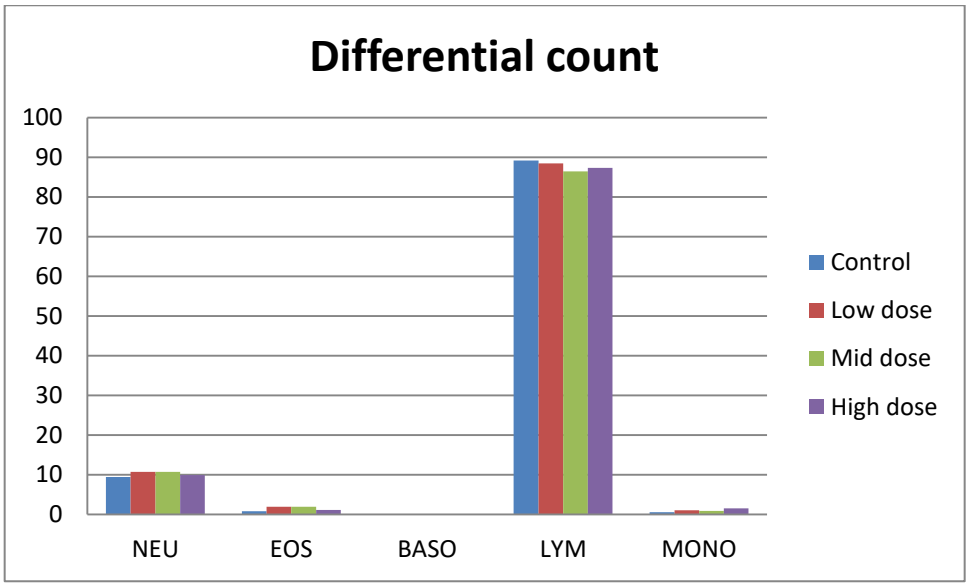


Figure 22. Effect of 28 Days Repeated Oral Toxicity Study of Naga Rasa Parpam on Hematological Parameters.



Haematological parameters results suggest, the parameters of all the test animals were normal level when compared with compared with control group.

EFFECT OF 28 DAYS REPEATED ORAL TOXICITY STUDY OF NRP ON SEROLOGICAL PARAMETER – RENAL FUNCTION TEST

Table 20. Effect of 28 days repeated oral toxicity study of Naga Rasa Parpam on Serological parameters – Renal Function Test.

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
BUN	22.62±6.85	27.54±11.43	25.64±5.42	28.72±13.24
S.Cr	0.41±0.13	0.42±0.11	0.50±0.15	0.70±0.18

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 groups.

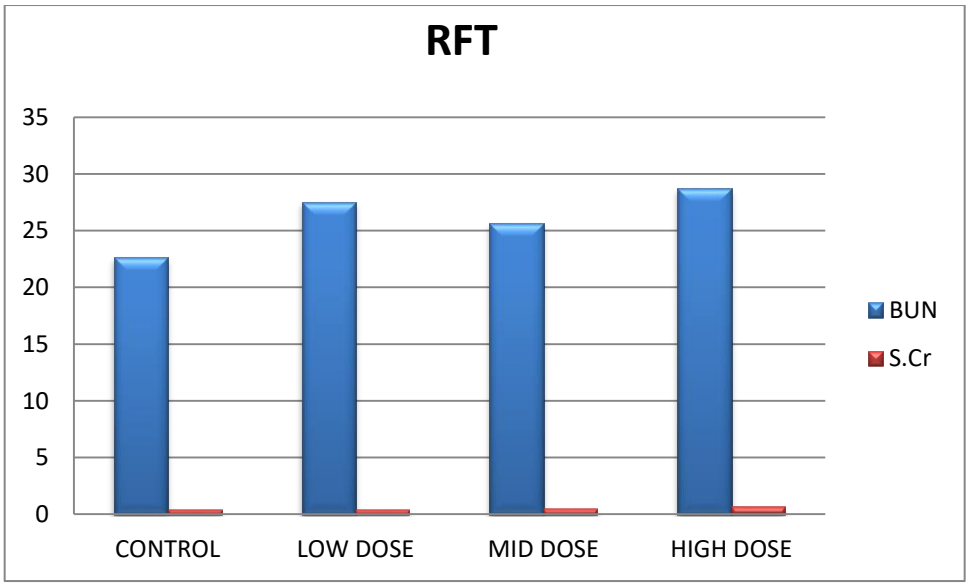


Figure23. Effect of 28 Days Repeated Oral Toxicity Study of Naga Rasa Parpam on Serological Parameter – Renal Function Test

Renal function test results suggest the parameters of all the tests animals was normal level compared with control group

EFFECT OF 28 DAYS REPEATED ORAL TOXICITY STUDY OF NAGA RASA PARPAM ON SEROLOGICAL PARAMETER – LIVER FUNCTION TEST

Table21. Effect of 28 days repeated oral toxicity study of Naga Rasa Parpam on Serological parameters – Level Function Test.

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
SGOT	63.97±9.08	65.93±28.86	81.79±12.71	70.48± 18.15
SGPT	46.43±27.68	35.47±12.45	35.05±9.56	56.18± 26.73

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 groups

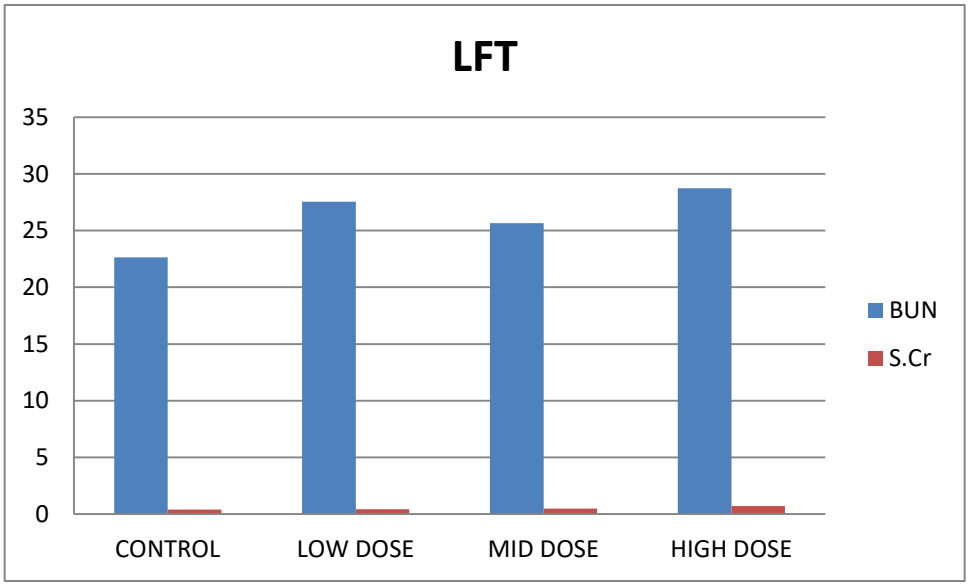


Figure24. Effect of 28 Days Repeated Oral Toxicity Study of Naga Rasa Parpam on Serological Parameter – Liver Function Test

Liver function test results suggest, the parameters of all the animals was normal level.

FFECT OF 28 DAYS REPEATED ORAL TOXICITY STUDY OF NAGA RASA PARPAM ON SEROLOGICAL PARAMETER – LIPID PROFILE

Table 22. Effect of 28 days repeated oral toxicity study of Naga Rasa Parpam on Serological parameters – Lipid profile.

PARAMETER	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
T.Chol	53.45±18.58	40.53±19.42	78.69±34.86	48.16±21.40
HDL	53.45±13.10	55.93±6.13	44.4±6.66	63.33±25.2
LDL	47.76±3.34	38.2±2.21	36.68±2.03	40.88±2.54
VLDL	16.97±4.90	13.4±3.47	15.3±3.58	14.4±3.84
TGL	44.44±30.75	101.1±64.28	46.01±11.87	60.34±15.42

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 groups.

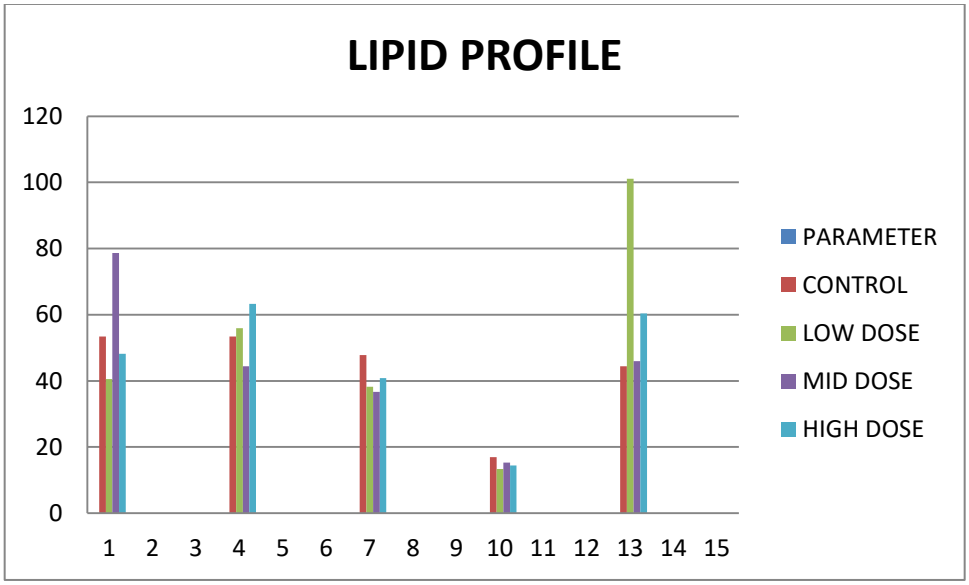


Figure25. Effect of 28 Days Repeated Oral Toxicity Study of Naga Rasa Parpam On Serological Parameter – Lipid profile

In lipid profile results suggest, the parameters of all the test animals was normal level.

No significant changes observed in Hematological parameters of test drug treated group animals (G-II,III & IV) compared with control group animals (G-I)

HISTOPATHOLOGICAL FINDINGS AND RESULTS OF 28 DAYS REPEATED ORAL TOXICITY STUDY OF NAGA RASA PARPAM

HISTOPATHOLOGY OF BRAIN

Figure:26 CONTROL MALE

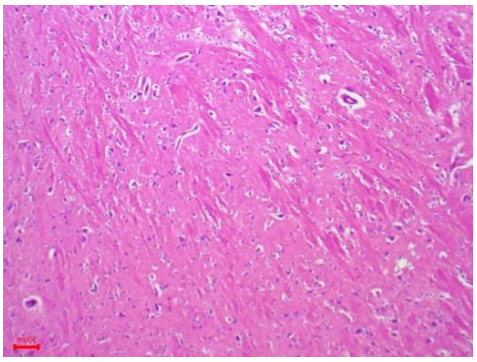


Figure:27 CONTROL FEMALE

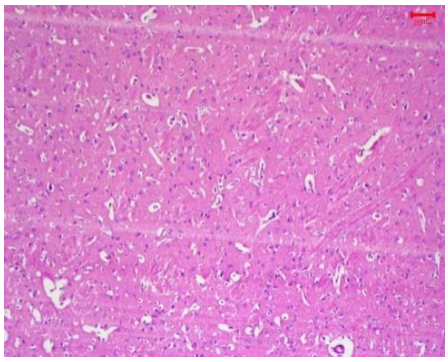


Figure:28 GROUP IV MALE

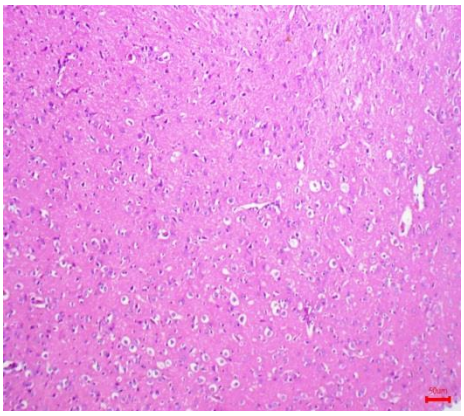
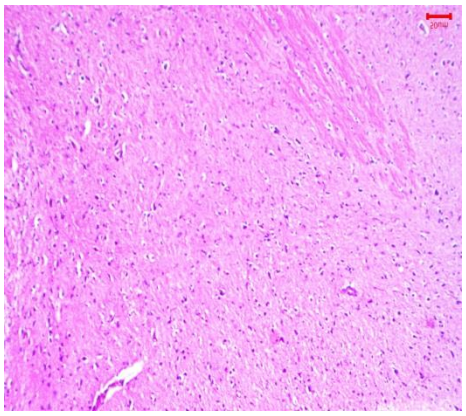


Figure:29 GROUP IV FEMALE



HISTOPATHOLOGY OF HEART

Figure:30 CONTROL MALE

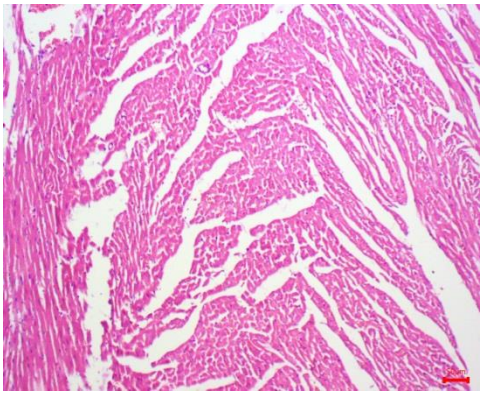


Figure:31 CONTROL FEMALE

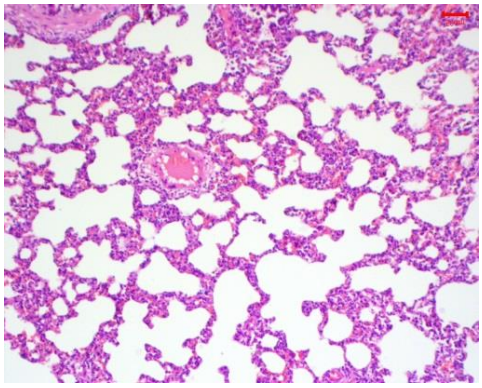


Figure32: GROUP IV MALE

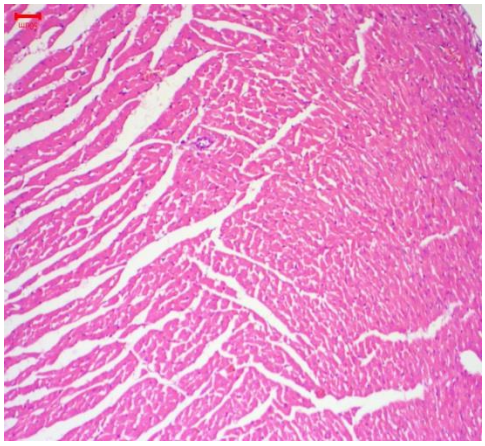
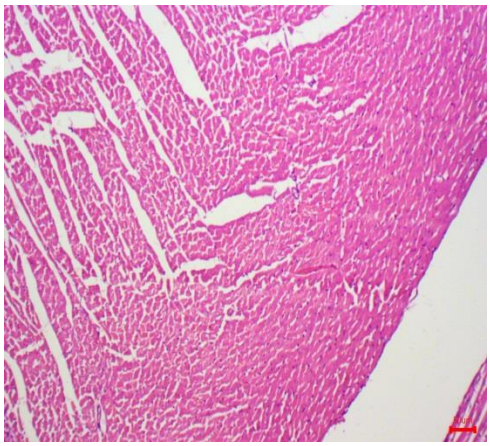


Figure:33 GROUP IV FEMALE



HISTOPATHOLOGY OF KIDNEY

Figure:34 CONTROL MALE

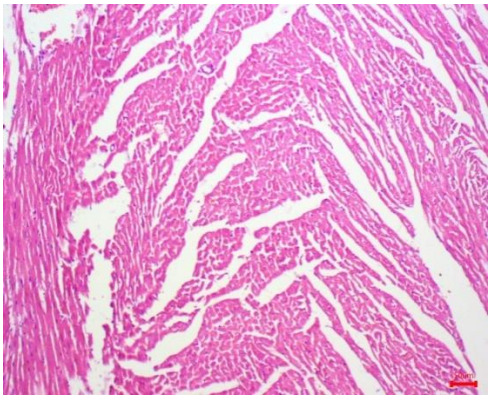


Figure:35 CONTROL FEMALE

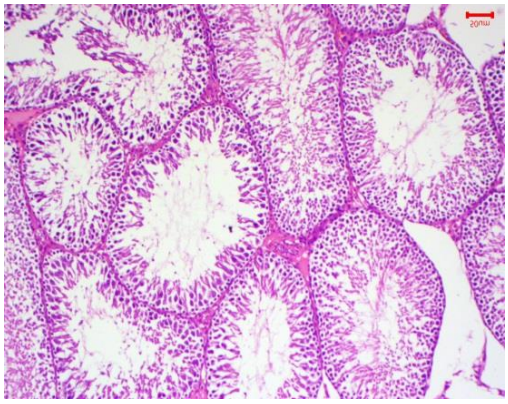


Figure:36 GROUP IV MALE

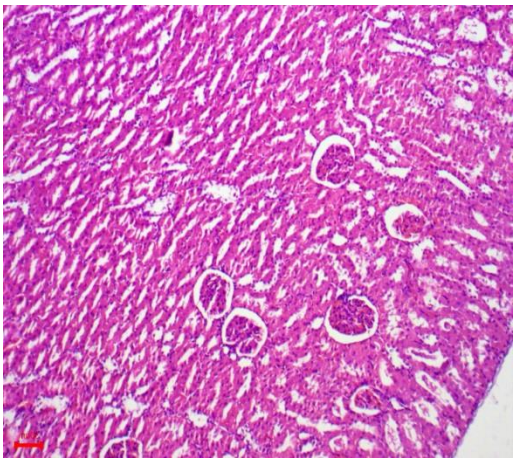
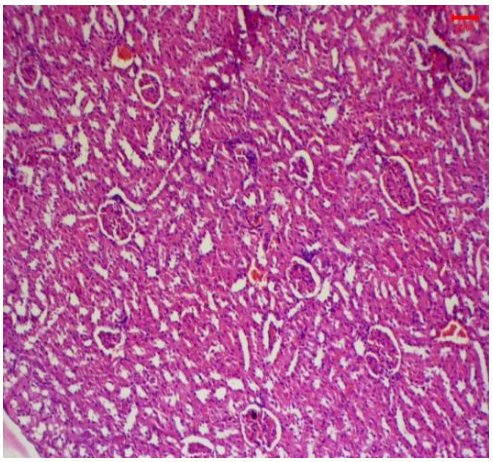


Figure:37 GROUP IV FEMALE



HISTOPATHOLOGY OF LIVER

Figure:38 CONTROL MALE

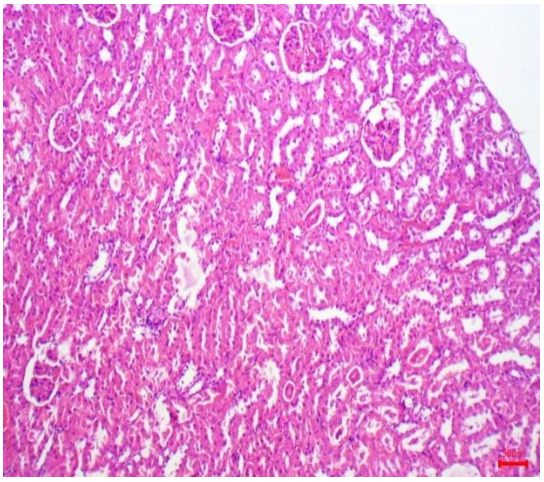


Figure:39 CONTROL FEMALE

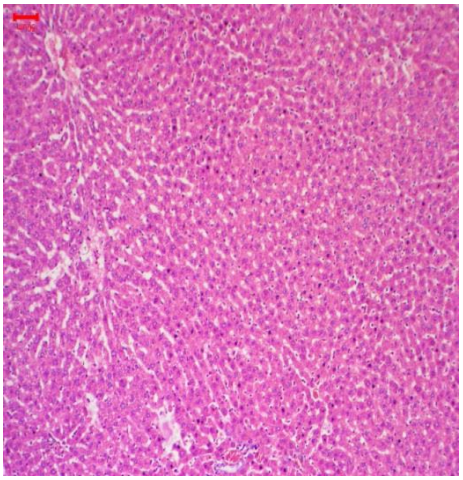


Figure:40 GROUP IV MALE

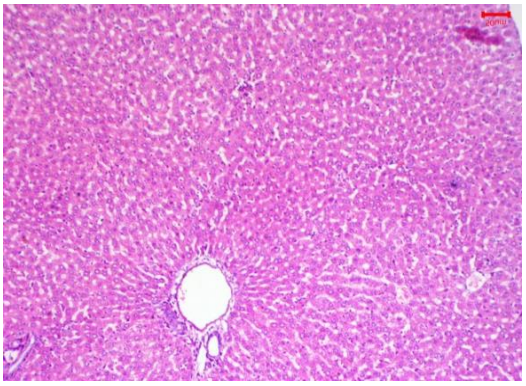
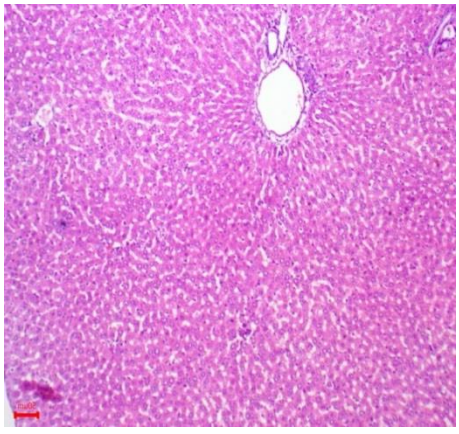


Figure:41 GROUP IV FEMALE



HISTOPATHOLOGY OF LUNGS

Figure:42 CONTROL MALE

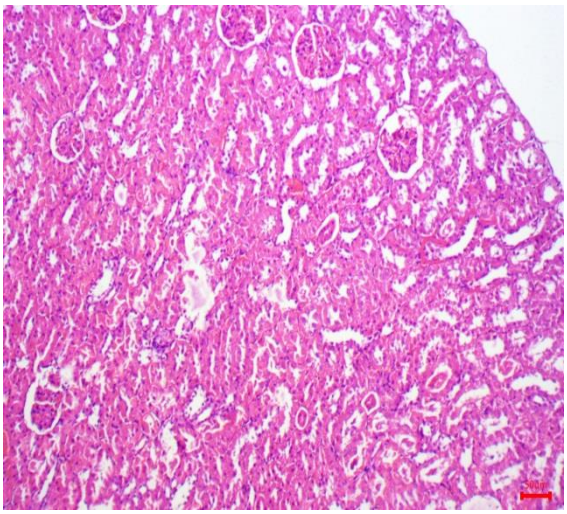


Figure:43 CONTROL FEMALE

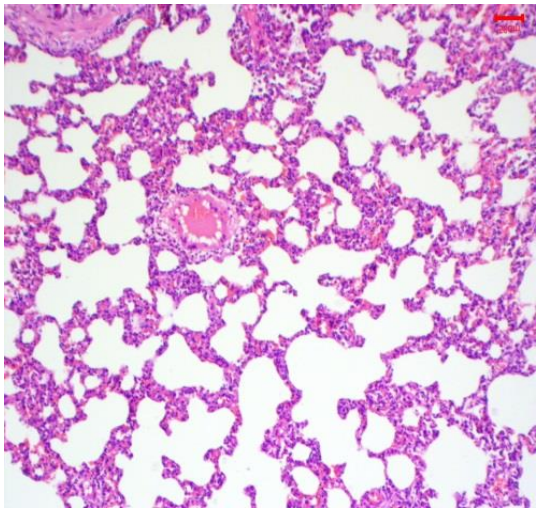


Figure:44 GROUP IV MALE

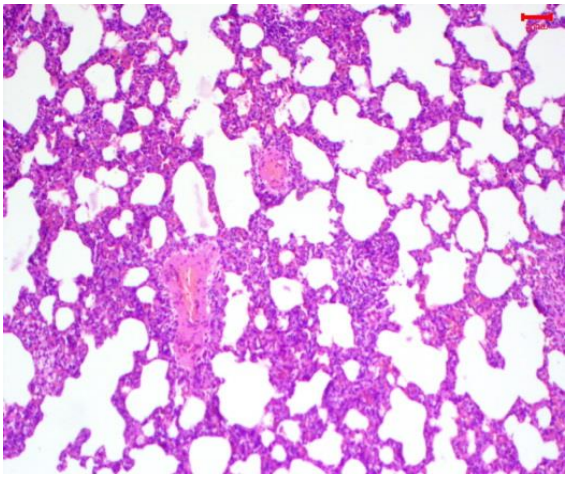
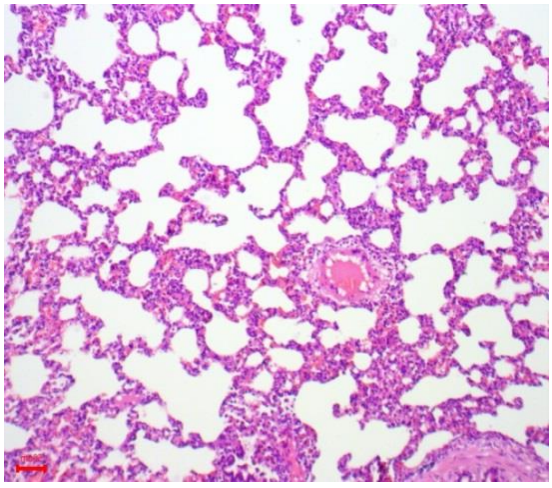


Figure:45 GROUP IV FEMALE



HISTOPATHOLOGY OF STOMACH-GLANULAR

Figure:46 CONTROL MALE

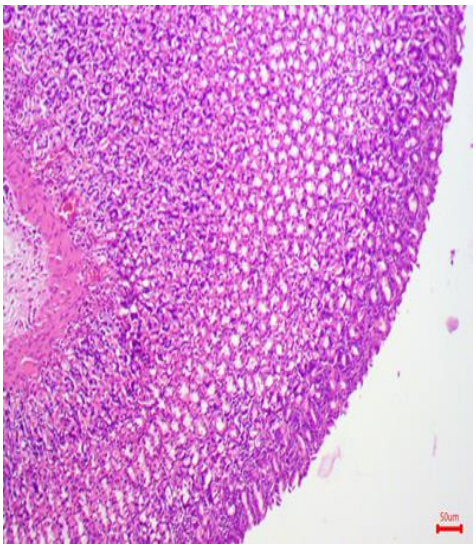


Figure:47 CONTROL FEMALE

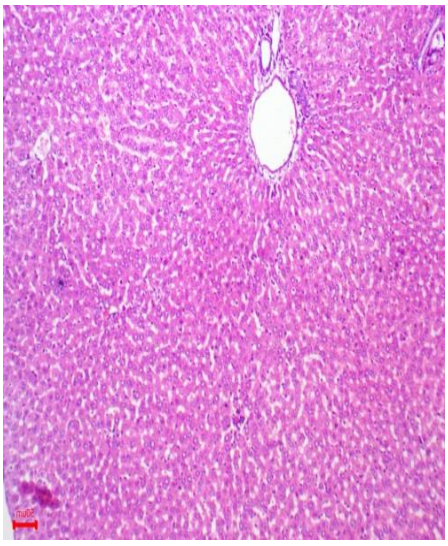


Figure:48 GROUP IV MALE

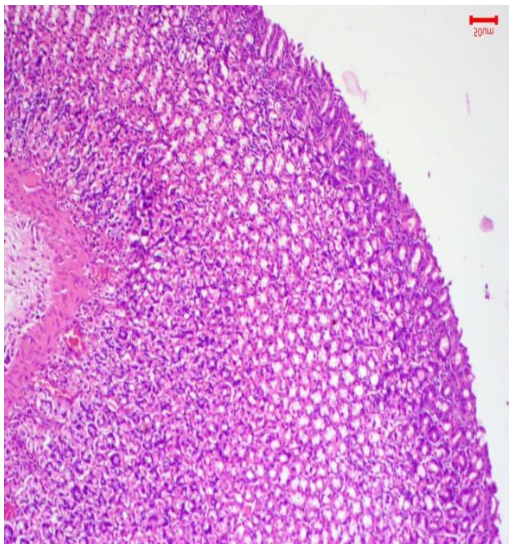
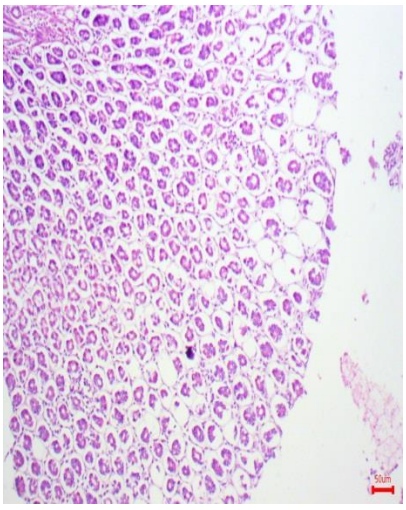


Figure:49 GROUP IV FEMALE



HISTOPATHOLOGY OF STOMACH- NON-GLANDULAR

Figure:50 CONTROL MALE

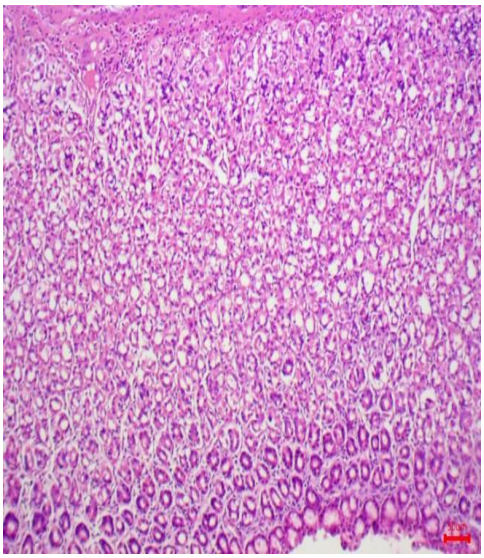


Figure51: CONTROL FEMALE

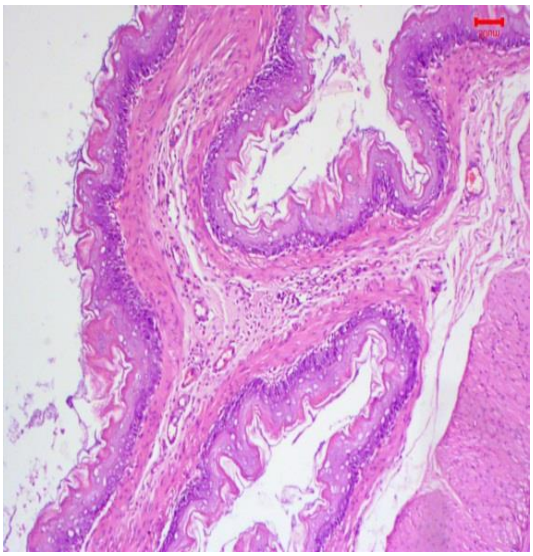


Figure:52 GROUP IV MALE

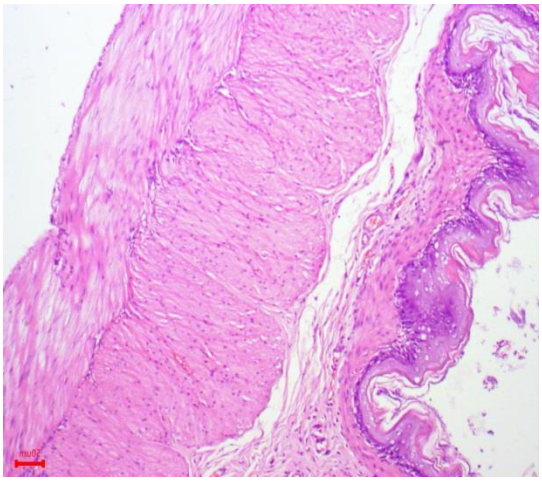
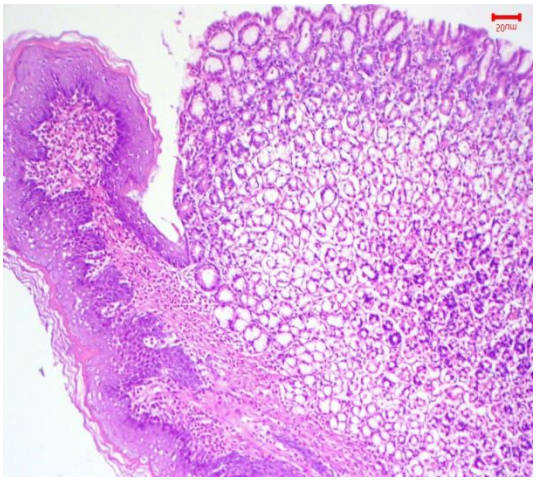
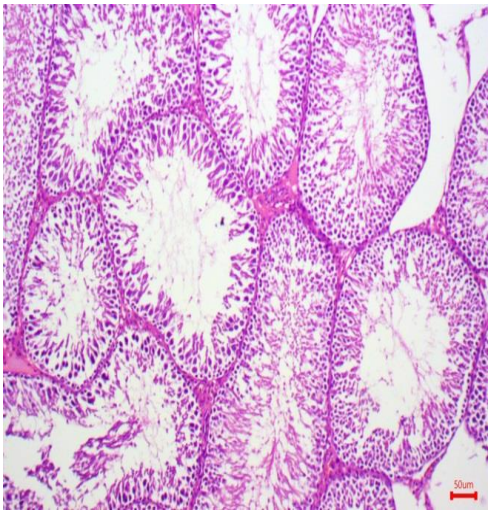


Figure:53 GROUP IV FEMALE



HISTOPATHOLOGY OF TESTIS

Figure:54 CONTROL



HISTOPATHOLOGY OF UTERUS

Figure:55 CONTROL

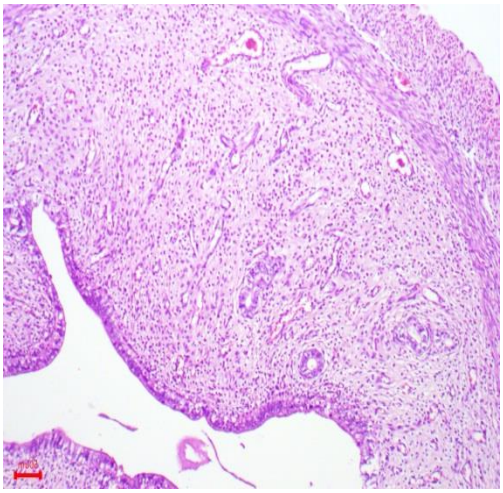


Figure:56 GROUP IV

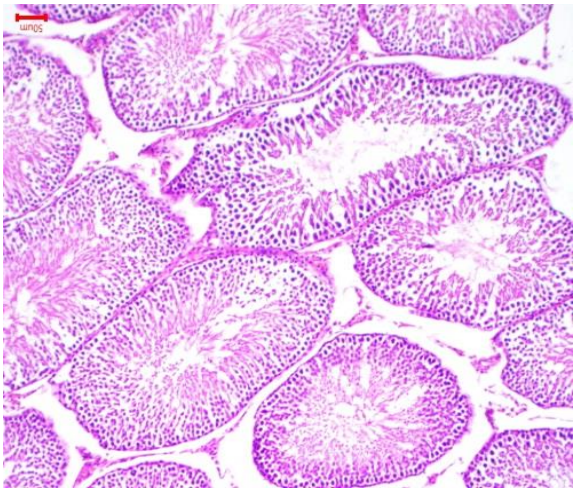


Figure:57 GROUP IV

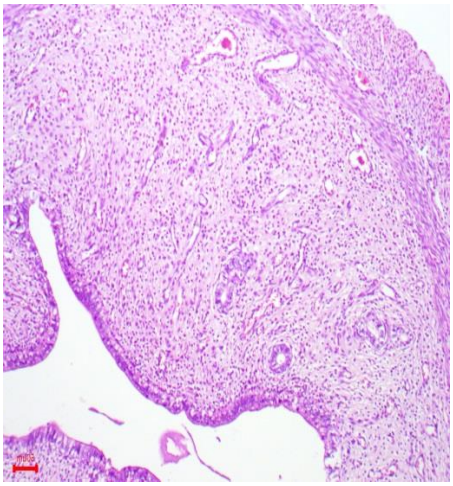


Table.23: HISTOPATHOLOGICY FINDINGS OF CONTROL GROUP ANIMALS

TISSUES	FINDINGS MALE	FINDINGS FEMALE	RESULT
BRAIN	No abnormalities detected	No abnormalities detected	Normal
HEART	No abnormalities detected	No abnormalities detected	Normal
LUNGS	No abnormalities detected	No abnormalities detected	Normal
LIVER	No abnormalities detected	No abnormalities detected	Normal
STOMCH-GRANDULAR	No abnormalities detected	No abnormalities detected	Normal
KIDNEY	No abnormalities detected	No abnormalities detected	Normal
STOMCH-NON GRANDULAR	No abnormalities detected	No abnormalities detected	Normal
TESTIS	No abnormalities detected	-	Normal
OVARY	-	No abnormalities detected	Normal

Table.24: HISTOPATHOLOGICAL FINDINGS OF GROUP- IV NAGA RASA PARPAM TREATED TEST ANIMAL

TISSUES	FINDINGS MALE	FINDINGS FEMALE	RESULT
BRAIN	No abnormalities detected	No abnormalities detected	Normal
HEART	No abnormalities detected	No abnormalities detected	Normal
LUNGS	No abnormalities detected	No abnormalities detected	Normal
LIVER	No abnormalities detected	No abnormalities detected	Normal
STOMCH-GRANDULAR	No abnormalities detected	No abnormalities detected	Normal
KIDNEY	No abnormalities detected	No abnormalities detected	Normal
STOMCH-NON GRANDULAR	No abnormalities detected	No abnormalities detected	Normal
TESTIS	No abnormalities detected	-	Normal
OVARY	-	No abnormalities detected	Normal

RESULTS:

Histopathological finding of Brain, Heart, Lungs, Liver, Stomach,Kidney Testis and Uterus in control group and high dose treated group showed there was no pathological changes observed.

6. DISCUSSION:

Naga Rasa Parpam is used to treat Cancer, Hemorrhoid, Fistula and Gonorrhoea.[18] Literature review of all the ingredients of Naga Rasa Parpam were collected in Siddha aspects, evaluated pharmacological actions and toxicological aspects. It's revealed all the ingredients of Naga Rasa Parpam have properties to treat Cancer, Ano rectal disease and Gonorrhea.

Standatation of Naga Rasa Parpam was done by through Organoleptic analysis, Physico-chemical analysis, Biochemical analysis, ICP-OES, SEM and EDX analysis and FTIR analysis.

The Organoleptic characters **(Table 7)** of Naga Rasa Parpam showed it's White in colour, lusterless, tasteless, odourless fine powder form of drug. Physico chemical analysis results showed, the pH is 8.02%. The pH value denotes that Naga Rasa Parpam is Alkaline in nature and better absorption through oral administration. Moisture is one of the major factors responsible for deterioration of the drug.[148]The percentage of loss on drying of Naga Rasa Parpam is less than 1% (Normal range 1-20%). Low moisture content is always desirable for higher stability of drug.[148]. So Naga Rasa Parpam if higher stability drug.The total ash value is useful in determining the quality and purity of drugs, especially in powder form.[148] Total ash value of Naga Rasa Parpam is 99.67%, due to calcination process.The acid insoluble ash test is designed to measure the amount of ash of the drug insoluble dilute hydrochloride acid. The acid insoluble ash value of Naga Rasa Parpam was 22.84% and it shows the purity of test drug. The percentage of soluble matters present in the drug is determined by the values of water extractive and ethanol extractive. Water soluble extractive value plays an important role in evaluation of drug. The alcohol soluble extractive value serves the same purpose as the water-soluble extractive value. The alcohol soluble extract of Naga Rasa Parpam is 5.1% (normal range 4-85%) and water is 31.68% (normal range 4-85%).

The Biochemical analysis of Naga Rasa Parpam indicates **(Table 9,10)** the presence of Alkaloid, Calcium, Carbonate, Zinc, Mercury and Chloride. Zinc and Mercury had Anti-Cancer, Anti-Inflammatory and Anti-Oxidant activity. Calcium is help reduce the risk of certain cancer. Calcium also to help prevent the weakening of

bones that occur with certain chemotheraphy medications and its supplement is strenghthen bones and prevent osteoporosis, or thinning of the bones. Bone loss is of special concern to women who are taking aromatase inhibitors to treat breast cancer, Since bone loss is a side effect of all three aromatase inhibitors. Calcium treatment provides an efficient, fast, and safe symptomatic relief from acute symptoms of Haemorrhoidal disease. Calcium Carbonate nanoparticles stimulate cancer cell reprogamming to suppress tumor growth an invasion in an organ. The presents of Alkaloid in Naga Rasa Parpam have Anti-inflammatory, Anti-oxidant, Anti-hemorrhoidal, Anti-cancerous and Anti-ulceric activity. These indicate Naga Rasa Parpam is stutable to treat Hemorrhoid, Fistula in Ano, Cancer and Gonorrhea.

The **Inductive Coupled plasma Optical Emison Spectrometry (ICP-OES)** analysis of Naga Rasa Parpam showed the presence of Zinc (42.92% w/w), Iron (0.021% w/w), Mercury (25.78% w/w), Potassium (0.196 % w/w), and Magnesium (0.037% w/w), Arsenic (99.02 µg/g), Potassium (0.196 % w/w), Cadmium (1.93 µg/g), Lead (169.04 µg/g), and Copper (31.17 µg/g). Zinc and Mercury are the main constituent in Naga Rasa Parpam. The presence of trace elements like Iron, Copper, Cadmium, Arsenic, Magnesium and potassium may be due to purification and medicine process.

The **SEM** analysis of Naga Rasa Parpam showed that particles are Cluster and spongy in shapes and sizes are in the range from 10 – 60 µm. Thus, the size of the particle is capable to encourage the efficacy.

EDAX analysis showed the elements present in the Naga Rasa Parpam. This shows the weight and atomic percentage of sample. The presence of Mercury is 10.42%, Zinc is 45.84%, C is 13.27% and O is 30.47%. The presence of Carban and Oxygen are due to calcination process.

The **X-ray Differaction** analysis showed the element present in Naga Rasa Parpam is crystalline nature.

The **Fourier Transform Infra Red Spectroscopy (FT-IR)** analysis of Naga Rasa Parpam shows the presence of vibrational band observation around 493^{-1} - 1383^{-1} confirms is attributed to the presence of Carbonate, Sulphide and Nitrate compound.

Acute and 28 days repeated oral toxicity study of Naga Rasa Parpam was

carried out as per OECD guideline 423 and 407.

In Acute Toxicity study there was no abnormal behavioural changes noted at the dose level of 300 mg / kg b.wt and 2000 mg / kg b.wt of Naga Rasa Parpam treated groups within 24 hours observation in female wistar albino rats **(Table 12)** No mortality and morbidity was observed throughout the study period (14 days). And also there was no pathological changes seen in all the internal organs of sacrifised test animals at the end of the study period. These results showed LD_{50} of Naga Rasa Parpam is greater than 2000 mg / kg b.wt. and Naga Rasa Parpam classified under category 5 as per Globally Harmonized Classification System.

In Repeated 28 days oral toxicity study was conducted as per OECD guideline 407. Totally 4 groups of Wistar albino rats in both sexes were used, each group contain 5 males and 5 females. Group I received RO water with mixed Ghee and Group II. III and IV received 12, 60 and 120 mg / Kg b.wt of Naga Rasa Parpam respectively by oral administration. All the test animals were observed throughout the study period, Naga Rasa Parpam did not produce any behavioural changes in all groups of animals. The body weight of the all the test drug treated animals were gradually increased during the study period(**Table 13**) and significantly body weight was increased in low dose group (185.2±18.71**, $p<0.01$) at first week when compared with control group Female (**Table 14**). Feed intake results suggest, the feed intake of all the male and female test drug treated animals were gradually increased (**Table 15**). But significantly feed intake was increased (41±5.7**, $p<0.01$) in Low dose group (4^{th} week) when compared with control group Female(**Table 16).** Water consumption result suggest, the water intake of all the male and female test drug treated animals not significantly changes when compared with control group.(**Table 17,18)**.

Hematological parameters results suggest that there were no significant changes in complete blood count (**Table 19**) when compared with control group. It results suggests that no alteration in their components and all parameters within physiological level. Bio – chemical parameters (LFT, RFT, Lipid profile) results also no significant changes when compared with control group. And it suggests there is no alteration in the serum components. (**Table 20,21,22**)

At the end of the study period all the animals sacrificed and all the vital organs

and cavities were observed. There were no gross pathological changes noted. The histopathology (**Table 23,24**) of Brain, Heart, Lungs, Liver, Kidney, Stomach, Testis and Uterus were done in control and High dose treatment group. The histopathology results of revealed all the studied organs are normal. Finally the 28 days repeated oral toxicity study of Naga Rasa Parpam reveals No observed Adverse Effect Level (NOAEL) of Naga Rasa Parpam is higher than 120 mg/Kg b.wt.

7. SUMMARY:

The Siddha formulation ***Naga Rasa Parpam*** has been choosen for my dissertation work from The Hand Book of Indian Medicine- The Gems of Siddha System. Naga Rasa Parpam prepared with the ingredients of purified Zinc, Hydrargyrum, Keezha nelli (*phyllanthus amarus),* Panai kuruththu (palm tuber). It's used to treat Hemorrhoid, Fistula-in-ano, Gonorrhea and Cancer.

The aim of the research work is to study the safety of Naga Rasa Parpam by acute and 28 days repeated oral toxicity in the wister albino rat as per OECD Guidelines 423 and 407.

The Zinc and lingam were purchased from raw drug market, Chennai. Keezha nelli collected from Nationa Institute of Siddha, Herbal Garden,. Panai kuruththu collected from Tenkasi district. Zinc, Lingam, Keela nelli and Panai kuruththu are identified and authenticated by Gunapadam Department and Botany department, National Institute of Siddha. All the ingredients were purified and meicine was prepared in Gunapadam Lab at National Institute Siddha as per Siddha Literature.

Naga Rasa Parpam was analyzed by qualitatively and quantitatively by Physico-chemical analysis, Biochemical analysis and Instrumental analysis.

Intially the trail drug - Naga Rasa Parpam was subjected to organoleptic character and physico-chemical analysis. It reveals the purity and quality of the drug.

Then the Naga Rasa Parpam was analyzed for chemical constituents. It showed the presence of Zinc, Mercury, Calcium, Chloride, Carbonate and Alkaloids.

SEM results showed the presence of evenly distributed Cloudy and spongy shaped particles ranging 10 – 60 µg with smooth surface.

EDAX analysis confirm the present of Zinc and Mercury, Carbon, Oxygen in the sample.

ICP-OES analysis of Naga Rasa Parpam showed that the presence of Zinc, Mercury, Iron, Copper, Lead, Magnesium, Pottasium, Cobalt, Cadmium and Arsenic.

FTIR analysis confirms the presence of corbonate, Nitrate and Sulfide compounds.

In Acute toxicity there was no behavioural changes, Mortality and Morbidity observed all test animals. A acute toxicity study revealed the LD_{50} of Naga Rasa Parpam is greater than 2000 mg / Kg b.wt.

28 days Repeated oral toxicity study results showed Naga Rasa Parpam did not produced any behavioural changes, mortality and morbidity with in study period. The feed intake, water intake and Body weight of all the test drug treated animals were gradually increased during the study period but no significant changes observed when compared with control group. But significantly increased feed intake at first week (41±5.7**, $p<0.01$) and body weight at 4^{th} week (185.2±18.7**, $p<0.01$)in low dose treated group.

Hematological parameters revealed that all the test drug treated animals were normal when compared with control group. Bio-chemical analysis also ithin normal level when compare with control group. Histopathological examination of Naga Rasa Parpam treated animal results revealed that Naga Rasa Parpam did not induce any lesion of toxicological significance in all the vital organs examined under the experimental conditions.

This 28 days repeated oral toxicity study suggest the No Observed Effect Level (NOAEL) of Naga Rasa Parpam is greater than 48 ml/ Kg b.wt.

8. CONCLUSION:

The quality parameters assessment of ***Naga Rasa Parapam*** results showed purity and bio availability of the drug. The results obtained by quality assessment of Naga Rasa Parpam will be used as standard for future research.

Safety profile of ***Naga Rasa Parpam*** was analyzed by acute and 28 days repeated oral toxicity stuy as per OECD guidelines 423 and 407.

In acute toxicity study the LD_{50} of ***Naga Rasa Parpam*** is greater than 2000mg / Kg b.wt. So ***Naga Rasa Parpam*** classified under category – 5 in Globally Harmonised Classification System.

The 28 days repeated oral toxicity study of (260 mg / day) was studied in wistar albino rats at different dose levels such as 12 mg / kg.b.wt, 60 mg / kg.b.wt and 120 mg / kg.b.wt. This study results showed the NOAEL of ***Naga Rasa Parpam*** is greater than 120 mg / kg.b.wt. The animal effective dose of 120 mg/kg b.wt is four fold of human effective dose of ***Naga Rasa Parpam.***

In conclusion the human effective dose of ***Naga Rasa Parpam*** 130mg twice a day is safe for consumption.

Efficacy of ***Naga Rasa Parpam*** will be analyzed by invivo methods in future is necessary to strengthen the therapeutic usage of Naga Rasa Parpam.

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AUTHENTICATION CERTIFICATE

Certificate No: Gun/Aut/008/21 Date: 15.07.2021

Certified that the following minerals/ metals/ animal products used in the Siddha formulation ***Naga Rasa Parpam*** taken up for the Post Graduate Dissertation study by **Dr. M. NITHYA KALYANI** Department of Nanju Maruthuvam, National Institute of Siddha, Chennai-47 are correctly identified and authenticated through visual inspection/ experience, organoleptic characters, morphology,etc.

1. *Nagam* - **Zinc**
2. *Lingam* - **Mercury Sulphide**

15/07/2021

Head of the Department

Chennai-47

15.07.2021

प्रो.डॉ.आर. मीनाकुमारी / Prof. Dr. R. Meenakumari
निदेशक / Director
राष्ट्रीय सिद्ध संस्थान / National Institute of Siddha
आयुष मंत्रालय, भारत सरकार
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Tambaram Sanatorium, Chennai-600 047.

NATIONAL INSTITUTE OF SIDDHA, CHENNAI – 600047

BOTANICAL CERTIFICATE

Certified that the following plant drugs used in the Siddha formulation **"Nagarasa parpam"** (Internal) taken up for Post Graduation Dissertation studies by **Dr.M.Nithyakalyani** M.D.(S), II year, Department of Nanju Maruthuvam, 2021, are identified through Visual inspection, Experience, Education & Training, Organoleptic characters, Morphology and Taxonomical methods as

Phyllanthus amarus Schum. & Thonn. (Euphorbiaceae), Whole plant

Borassus flabellifer Linn. (Arecaceae), Terminal shoot

MEDICINAL BOTANY CHENNAI 600 047 National Institute of Siddha

Certificate No: NISMB4702021

Date: 26-07-2021

Authorized Signatory

Dr. D. ARAVIND, M.D.(s),M.Sc.,
Assistant Professor
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Institutional Animal Ethics Committee (IAEC)

NATIONAL INSTITUTE OF SIDDHA

(An autonomous body under Ministry of AYUSH, Govt. of India)
Tambaram Sanatorium, Chennai 6000 47.

CERTIFICATE

This is to certify that the project proposal No. **NIS/IAEC-1/13/30092020/13** entitled "Preclinical Safety Evaluation of *Nagarasa Parpam*" submitted by **Dr.M.Nithyakalyani** has been approved/recommended by the IAEC of National Institute of Siddha in its meeting held on 30.09.2020 and **52 Rats (32 Female + 20 Male)** have been sanctioned under this.

Authorized by	**Name**	**Signature /Date**
Chairperson	Prof.Dr.R.Meenakumari	30/09/2020
Main Nominee of CPCSEA	Prof.Dr.Geetha Ramesh	30/9/2020
Member Secretary	Dr.B.R.Senthilkumar	30 Sept 2020

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

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

This certificate is awarded to Dr. M. NITHYA KALYANI

for participating as Resource Person / Delegate in the 34th Workshop on

"How To Do a Good Dissertation & Publish? (Research Methodology and Biostatistics)"

(Virtual mode) for AYUSH Post - Graduates & Researchers organized by the

Department of Siddha, The Tamil Nadu Dr.M.G.R. Medical University

from 26 - 07 - 2021 to 30 - 07 - 2021.

Dr.N. KABILAN
PROFESSOR & HEAD, DEPT. OF SIDDHA

Dr.M.B. ASWATH NARAYANAN
REGISTRAR

Dr.SUDHA SESHAYYAN
VICE-CHANCELLOR

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