1. INTRODUCTION

India is a country with diverse bio-diversity, culture and wide range of ancient traditional medical systems. Traditional medicine has a long history. “It is the sum total of the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health, as well as in the prevention, diagnosis, improvement or treatment of physical and mental illnesses. By WHO”

Traditional Medicine has played an important role in meeting the demands of primary health care in many developing countries and its use has expanded widely in many developed countries.\(^1\) Siddha system of medicine is one among them, which has flourished in the Southern India especially Tamilnadu\(^2\).

Siddha Medical System (SMS) or Indigenous Tamil Medicine is a special, significant and scientific system, being in practice, since time immemorial. It is one of the ancient systems of medicine contemporaneous with Grecian, Egyptian, Mesopotamian, Chinese medicines. It is a unique system which dwelt among the Tamil people of South India rendering service to humanity for more than five thousand years BC era in combating diseases and in maintaining physical, mental, social and spiritual health\(^3\).

Siddha Medical System is based upon the teachings of Siddhas. A great deal of Siddha Medical System comes to us from the selfless work of untiring souls who preferred obscurity and austerity. Ashtama Siddhi i.e eight great supernatural powers, those who attained or achieved the above said powers were known as Siddhas\(^4\). They are perpetual medico-philosophical adepts who had deep knowledge about each and every perspective of life. Siddhas are eminent people who rendered an indefatigable life for the welfare of humanity. They relentlessly rendered service to mankind to cure diseases and for spiritual upliftment to accomplish enlightenment. They have given emphasis for eternity by ways of healthy life without diseases through Kayakarpam practices\(^5\).

Siddhars were the greatest scientists both material and spiritual of ancient days and they were fully acquainted with almost all the branches of science. These Siddhas belongs to a School of great knowledge which originally constituted of eighteen
numbers known as Moolavargasiddhars. Agastya the father of Siddha medical system and author of Tamil language grammar named ‘Agattiyam’ before ‘Tholkappiam’.

Siddha system of Medicine deals not only with the external body but also with the internal soul. The Alma Ata Declaration in 1978 at the WHO international conference on Primary healthcare (PHC) advocated “the importance of integrating traditional practices as primary health care and it also ascertained health is the state of complete physical, mental and social well being not merely the absence of disease or infirmity”. This has striking synergy of Siddha policy of health and wellness as mentioned by the Siddha Thirumoolar some millennia back as

“One that cures physical ailment is medicine
One that cures psychological ailment is medicine One that prevents ailment is medicine and One that bestows immortality is medicine”

The trio systems adopted in practice in India are Ayurveda, Siddha and Unani (ASU) all are based on basic five elements and humoural pathology. The three fundamental principles Vali, azhal and iyam are the basic composition and constitution of the human body which connects between Microcosm or man and Macrocosm or the universe. This humoural pathology defines that all the diseases of human body are caused by the derangements of three basic humours (uyirthatu). The relative proportions of vali, azhal and iyam are responsible for the physical and mental integrity of the human beings and the Siddha materia medica is also based on this humours.

The Siddhas are eminent scholars whose work not only bound to medicine preparations but also played a great part with their contributions in rasavadham(alchemy), kayakarpam, yogam, varmam, medical astrology, panjapatchisastram, saram etc., Being with great treasures encompassing the teachings of philosophy, pharmacology, pathology, medicine, life style management, spirituality and meta physical studies the Siddha medicine had laid foundation to serve the humanity to promote health, prevent sickness, augment longevity, endeavour people to live in harmony with the environment and thus to lead a blissful life from time immemorial.
Siddha medicine uses herbal formulations as a first line drug of choice, which emphasizes to use roots of the plants as medicine primarily and then to use leaves and other parts of the plant and finally to use herbo-mineral preparations in its oxide form, sulphide forms etc.,

Siddha Medical System enlists sixty four kinds of medicine including thirty two kinds of internal medicines and thirty two kinds of external medicines\(^7\). The rich flora of our country is selectively utilized for the welfare of mankind. Siddhas have contributed tremendous work on raw materials from herbal, herbo-mineral, metal, marine and animal origin and formulated many medicines. The unique formulations in Siddha Medicine include Parpam (mineral/metallic oxides), Chendooram (mineral/metallic sulphides), Chunnam (caustic or major oxides) and Pathangam (sublimation)\(^9\) and they used to treat are used in chronic diseases, especially, in cases where in the prognosis is grave. Parpam and Chendooram type of medicines are highly used by the traditional medicine practitioners for its smaller dosage with higher therapeutic values.

Unlike contemporary medicines, Siddha system uses more than one ingredients for preparing medicines, because of their synergistic activity and lower toxicit. These drugs posses increased bioavailability as the mineral drugs are treated with herbal juices which lead to reduction in particle size upto nano level. So very minimum doses also gives increased potency.\(^10\)

Standardization of traditional drugs is a hot topic all over the world as the usage of traditional medicine is become mainstream in the primary health care in most of the countries. But, the methods available for conventional drugs could not be applicable as Siddha drugs uses a number of mixtures.\(^10\)

In this work Sangu Parpam a herbo-mineral Siddha drug is taken which is extensively used by the traditional medicine practitioners. It has high therapeutic value in treating the diseases Peptic Ulcer, Gastro intestinal disorders, Cough, Piles etc.\(^11\)

Due to modern life style habits and fast moving life the quantum of stress and the rate of gastro-duodenal ulcers has increased\(^12\). Peptic ulcers are present in around 4% of the population are found to have peptic ulcer. In 2013 nearly 53 million people were developed peptic ulcer. 10% of people in the world develop peptic ulcer in some
point of time in the air life. In 1990, 327000 deaths were recorded and 2013 nearly 301000 deaths were recorded due to peptic ulcer.\textsuperscript{13}

Peptic ulcer which is also known as Acid Peptic disease is an ulceration of the mucous membrane of the stomach, duodenum or oesophagus. The word ‘ulcer’ refers to any sore or erosion that forms when the lining of the digestive system is corroded by acidic digestive juices\textsuperscript{14}.

It is formed due to the lack of defence mechanism of gastro duodenal mucosa with the gastric acid and pepsin\textsuperscript{15}. Men (10.5\%) are mostly affected then women (9.5\%). The persons infected with H.Pylori have more prevalence than others (15 – 20\%)\textsuperscript{16}. Other factors for peptic ulcers are ingestion of NSAID’s, smoking, stress etc. Prevalence of peptic ulcer is higher in third world countries where it is estimated at about 70\% of the population, whereas developed countries show a maximum of 40\% ratio. The lifetime risk for developing a peptic ulcer is approximately 10\%.\textsuperscript{17}

In recent years the research shows that \textit{H. pylori} infections are decreased, in developed countries. Transmission of \textit{H.pylori} is by food, contaminated water, and through human saliva\textsuperscript{18}.

Even with the advent of many advanced treatments for peptic ulcer posses many side effects like cardiac arrhythmias, hypertension, nephritis and etc\textsuperscript{19}.

In this present scenario, it is high time to search for a potant medicine for peptic ulcer with high efficacy, cost effective and with less or no adverse effects. Loads of research has been conducted in drugs of plant origin but large lacunae remains in research of marine based drugs.

Though the Siddha literatures highly recommend \textit{Sangu Parpam} for Peptic Ulcer disease, the worldwide usage of this medicine will be on hand if the safety, efficacy and mode of actions of the medicine is established by the standard scientific methods. Proper standardization techniques, Toxicological and Pharmacological evaluation on these medicines is required to meet the criteria to support its use worldwide.

Therefore, an attempt has been made to unveil the facts about the herbo-marine Siddha drug \textit{Sangu Parpam}\textsuperscript{20}, a calcined product conch shell from the literature by scientific analysis of its purification and preparation process by evaluating
the Physico chemical characters, Pharmacological actions and toxicological analysis and Standardization.
2. AIM AND OBJECTIVES

AIM:

To scientifically analyze the purification and preparation process of Sangu Parpam (SP) and validate its safety and therapeutic efficacy in Gunma Noi (Peptic ulcer Disease).

The objectives of the present work are as follows:

**Standardization of the study drug**

- Identification and authentication of the ingredients.
- Purification process of the raw drugs as per the literature.
- Preparation of the Medicine Sangu Parpam as per literature.
- Analyzing the drug as per the specified protocol given by PLIM.
- Analysing the drug by ICP-OES, XRD,SEM and EDAX

**Animal experiments**

**Safety studies**

- Evaluation of the safety of the study drug by conducting acute and Sub acute toxicity studies in animals as per OECD guidelines.

**Efficacy studies**

- Effect of SP in Pylorus ligation method
- Effect of SP in Ethanol/HCL induced ulcer method
- Effect of SP in Stress induced ulcer method
3. Review of literature

This section covers the following topics.

1. Drug review
   i) Zoological aspect of conch shell
   ii) Siddha aspect of Sangu
   iii) Botanical aspect of Pergularia daemia
   iv) Siddha aspect of Uthhamani

2. Pharmaceutical review
   Parpam

3. Disease review
   i) Review on Gunmam
   ii) Review on peptic ulcer

4. Scientific validation

3.1 Drug review

Drug as defined by WHO is a substance or product that is used or intended to be used to modify or explore the physiological systems or pathological states for the benefits of the recipient. The comprehensive knowledge of the drug is very important to physician because without knowledge of the drug, the patient cannot be treated properly.

In this way, all siddha classics advocate specific formulation for particular disease. siddha, an eternal source of knowledge has a multi angled textual material including pharmaceutical knowledge. The comprehensive knowledge of the drug is very important to the physician because without knowledge of the drug, the patient cannot be treated properly.

a) Zoological aspect of conch shell:

The phylum Mollusca is the second largest phylum among invertebrates. Majority of molluscs can be recognised by the shell. Molluscs constitute an important
component of marine biodiversity of India on East, West coasts and Lakshadweep and Andaman and Nicobar islands. It is estimated that number of molluscan species varies between 80,000 and 1,00,000\(^2\).

According to Russell-Hunter, Mollusca were derived from Turbellaria like animal\(^2\). Linnaeus adopted the name Mollusca. Pelseneer classified Phylum Mollusca into five classes namely Amphineura, Gastropoda, Scaphopoda, Lamellibranchia and Cephalopoda\(^3\). There are five kinds of Molluscan species found in India. They are Polyplacophora, Gastropoda, Scaphopoda, Bivalvia, Cephalopoda. Out of 586 families found in the world, 279 families were present in India\(^4\).

**An introduction to Molluscs:**

Man has close relation with molluscs since prehistoric times. The mysterious creation of the nature from marine source fascinated man and with time the man attributed magical and mythical powers to shells and started crafting monuments. The excavation of Stone Age cultures found to contain heaps of discarded shells in kitchen. There exists evidence for the shell trade between Protohistoric Iran and Southern Asia\(^5\).

A large number of marine invertebrates are known to provide leads to some biologically active compounds. Biomedical value of molluscs lies in their secretions, which originate from the dermal region or from internal glands. Out of seven classes of Molluscs, Gastropoda and Bivalvia, Nautilus in Cephalopoda produce external shells attractive to human eye\(^6\). They also play a vital role in ecosystem by decomposition of the terrestrial ecosystem and formation of organic detritus in estuaries\(^6\).

**Chanks:**

The history of chank can be dated back to Indus valley civilization. Chank ornaments were also found in excavations of Mohenjadaro and Harappa. According to Tamil literature, chank cutting industry existed 2000 years ago. Though it declined in Tamil Nadu, it is a flourishing industry in West Bengal, Orissa and Bangladesh.
Chanks are of commercial importance due to their varied and unique structure, large size and glittering surfaces when polished. They are used as ornaments like bangles, rings, necklaces and a variety of shell crafts. They are also used as amulet against evil eye. With growing demand in our country, they are being exported to Italy, Spain, France and the USA. Major quantity of quality chanks were obtained from Gulf of Mannar.

Sacred chank is given greater importance in Hindu mythology. The Bhagavad gita refers to different chanks namely Pancha Janya (by Sri Krishna), Ananta Vijaya (by Yudhishtira), Paundra (by Shima), Devadatta (by Arjuna), Sughosha (by Nakula) and Manipushpaka (by Sahadeva), which were used as trumpets in the battlefield of Kurukshetra. Chank blowing is a usual custom to announce auspicious, religious and sacred events and also to last rites. It is blown to invocate God at the time of worship. Water poured from a chank is considered as ‘holy’.

Vernacular names:

<table>
<thead>
<tr>
<th>Language</th>
<th>Tamil</th>
<th>English</th>
<th>Sanskrit</th>
<th>Ben.</th>
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<tbody>
<tr>
<td></td>
<td>Sangu, Sanks</td>
<td>Conch, Conch shell</td>
<td>Shankha</td>
<td>Sankh</td>
<td>Sankhamu</td>
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</tbody>
</table>
The empty shell of *Turbinella pyrum* (or) *Turbinella Rapa* (or) *Xanchus pyrum* Linn is a marine univalve, which is called the sacred conch.

**Source:** Indian Ocean coasts

**Types:**

There are generally three kinds of chanks, namely

i) The sacred chank of India

ii) The Caribbean chank

iii) The Brazilian chank.

Each belonging to a separate species under the genus *Turbinello*.

The Indian chank is distributed from Southeast coast through Sri Lanka to Southwest coast in Kerala and in the Gulf of Kutch, Gujarat. It was also reported from the St. Martin Island, of Bangladesh.

**Zoological Classification**

- **Kingdom:** Animalia
- **Phylum:** Mollusca
- **Class:** Gastropoda
- **Family:** Turbinellidae
- **Sub family:** Turbinellinae
- **Genus:** *Turbinella*
- **Species:** *pyrum*

**Morphology of chank:**

The Indian chank has a strong and spindle shaped shell measuring up to 140 mm in height. Its external surface is covered with a velvety brown periostracum. The aperture is wide with a long and deep anterior canal. There are three to four strong columellar ridges, which give support.

It has a large, massive, elegant shell with a fine pear- shaped spire and a wide opening or mouth which is prolonged into narrow spout. It has an external lustrous yellowish brown horny layer and beneath it a thick layer, chiefly formed by calcium carbonate. A porcelaneous shell is an oblong or conical form. The oblong form is
bulged in the middle and tapering at each end. The conical variety is peculiar. The upper portion is like corkscrew, twisted and tapering at the end. The base is broad. The interior is hollow. The surface is hard of a dull white colour. The upper surface is highly tuberculated, the under surface shining, very brittle and translucent.

*Turbinella pyrum* is a sedentary animal, living mostly in sandy region, have slow growth rate. From the estimate of age and growth of *T. pyrum*, it could be inferred that this species grows much faster up to third year of age and thereafter the growth gets reduced. The onset of first maturity for *T. pyrum* range from 120-140 mm and by this time the animal attains its third year of age.

![Fig 2: Turbinella pyrum](image)

**Valampuri chank**

Chanks are characterised by large shells with five texture and colour and highly valued. Normally, the chank shells are formed in a dextral spiral, occasionally shells with a sinistral spiral are also formed. This peculiar type of chank is called as “*Valampuri chank*”. These have a high value and very rarely caught, almost one in a Lakh$^{27}$. A sinistral chank is very rare and considered very auspicious and deeply venerated by Hindus. *Busycon contrarium* is found very common in Gulf of Mexico and West Florida is imported to India and sold as sacred chank at higher cost$^{21}$. 
Fig. 3. Busycon contrarium

Chank fisheries
The smaller sized chank formed reasonably good fishery. (Siraimetan, 1987). Along Thoothukudi coast, the fishing season for this species starts from October and lasts up to April. The matured chanks (120-140 mm) form a good fishery coinciding with fishing season.

In India, chank fishery is carried out at the following coasts.
- Tuticorin fishery
- Ramnad (Sivagangai fishery)
- Karnatic coast (South Arcot, Tanjore)
- Travancore
- Kothaiyavur

Biomedical potential:
- The shells were used to cure many ailments since many centuries. Molluscan shell is one of the important raw materials for calcium and calcium based industries. It contains 33 to 40% of calcium, of which 90 to 98% occur as calcium carbonate.
Tooth powder from edible oyster shell has been perfected at Render lab of Gujarat Fisheries Aquatic Science Research Institute.

Shell grit is used in production of dental cream, talcum powder, carbide industry.

Chank powder is a panacea for many illnesses like jaundice, general debility, and cough.

Dried visceral mass is efficient in enlargement of spleen.

A remedy for blotches, pimples and other skin troubles on the face and body.

In case of rickets, chank powder mixed with water is rubbed on the breasts.

Internally given to the acute form of dyspepsia.

Given for asthma, cough, constipation, shooting pain and inflammatory conditions in joints.

Used in head ache, general debility and eye diseases.

Institutes like National Institute of Oceanography in Goa, Central Drug Research Institute in Lucknow, Bose Institute of Oceanography in Kolkata are concerned with development of marine medicinal products.
Other varieties of conch:

Marine gastropod shells can be turned into "blowing shells". Blowing of chank requires high respiratory volume and hence it aids pulmonary function.

3.1b) Siddha aspect of Sangu$^{20}$

It is one among the 120 uparasas. Among the five basic natural elements, conch has predominant composition of water.
Synonyms:

Nandhu, Naagu, Kambu, Vandu, Idampuri, Valampuri,

Sangam, Sumbu, Suthhi, Kodu, Surimugam, Panilam,

Devadhathan, Thara, Vandu, Valai, Vellai etc.,

Conch is found abundant in sea shores of Indian Ocean.

1. If conch (Sangu) surrounded by 1000 Chippi (oyster) it is called Idampuri.

2. The conch (Sangu) surrounded by 1000 Idampuri is called Valampuri.

3. The conch (Sangu) surrounded by 1000 Valampuri is called Panilam.

4. The conch (Sangu) surrounded by 1000 Panilam is called Paancha sanniyam.

Although there are many varieties in conchs, oodhu sangu variety (blowing conch) is used for medicinal purposes.

Types:

According to T. V. Sambasivam pillai, there are about 23 types of chanks.

Organoleptic characters:

Taste: Sweet

Nature: Cooling

Division: Sweet

Action:

Diuretic, Nutrient, Anodyne, Carminative, Stomachic, Astringent, Febrifuge, Expectorant.

Indications:

White conch shell is mainly used to treat hypertension, eye diseases, vatha disease, epilepsy and ulcers. It also increases appetite.
**Medicinal uses:**

Raw conch shell prepared as medicines like Parpam and Chunnam shall be used for treatment. The conch has got body strengthening, deflatulant, appetite stimulant, bitter and mucolytic properties.

**Purification methods of conch:**

There are many kinds of methods employed for the purification of conch. These methods are meant for removing the impurities, thereby to reduce the toxicity and also to increase the efficacy of the drug. Few methods are listed below.

**Method 1**:  
Take 35gms of conch shell was soaked in 175 g of juice of *Euphorbia neriifolia* (*Ilaikalli*) and drid in sun light (whole day). The process was repeated for three more days. Finally washed in the water and dried.

**Euphorbia neriifolia:**

**Botanical classification:**

- Division : Magnoliophyta  
- Class : Magnoliopsida  
- Order : Malphigiales  
- Family : Euphorbiaceae

**Habitat:**

It is called milk hedge. It is an erect shrub, tall, fleshy, spiny and slightly succulent, usually with terminal leaves. Spine-shields in 5 distinct rows, grey-brown to blackish. Stipules transformed into spines. Leaves are thick, succulent, 6-12 inches long, sub sessile, obovate in shape, base attenuate, margin entire, apex rounded.

**Part used:**

Whole plant, latex
Chemical constituents:

Triterpenes like nerrifoline, flavonoids, steroidal saponins, sugar, tannins, alkaloids, triterpenoidal saponin, neriifolione.

Pharmacological actions:

Hepatoprotective, anti inflammatory, analgesic, antioxidant, immunomodulatory, radioprotective, wound healing, diuretic, anti diarrhoeal, anti ulcer, anti bacterial (Shaikh Arshad Ahmed et al.).

Therapeutic uses:


Method 2:

The conch was buried in lime stone, soaked and taken out after washing to get it purified.

LIME STONE:

- It is one of the most common types of rock found on the surface of the Earth.
This special type of rock is made by the micro organisms of the sea.

Dissolves in natural water.

It is made up of Calcium Carbonate (CaCO3). Which is arranged in either Calcite form are aragonite form.

Method 3:

Equal quantities of lime stone and fuller’s earth were taken and eight times more than weight of water was added. The conch was put into it and boils well to get it purified.

POONEERU: FULLER’S EARTH

Method of purification:

500 gm of Fullers earth is soaked in dews water (2.5 liter). And allowed to settle, next morning it is churned well and the outer cream layer is removed. The remaining mixture was placed in procelain plates and isolated to obtain purified form. This process is called —thetchai and it is repeated for ten times and stored in the bottle.

UVAR MANN (POONEERU)
The entire philosophers agree that mercury, sulphur, and salt were the chief ingredients used in the alchemical process described in the manuscripts of the ancient civilized world from time immemorial. A super element namely a fifth element of quintessence of creation to transmute the base metal into gold.

This prima material namely — *Muppu*, it is a Tamil word which means the union of three salts namely

1. Pooneeru (Sun)
2. Kalluppu (Moon)
3. Vediyuppu (Fire)

**Pooneeru:**

Andakkal, certain species of limestone is composed of globules are said to be found underneath the fullers earth soil, a white substance that bubbles out from this limestone during the full moon night once in a particular month of a year.

It is collected and purified with the juice of rejuvenative herb or amuri. This may be defined for our purpose here as a special.

**Kinds of fuller’s earth:**

Great virtue is attributed to this lime stone as a fluid derived on the full moon night is reputed to contain certain active principles for alchemical process of kalpha drugs.

**Guru medicine- universal salt “Muppu kingdom of Siddhars”**

- Pooneeru is seldom administered as single medicament but used as one of the main ingredient of Muppu. This serves as a catalytic agent. But it is still a matter of great doubt for many that Siddhars might have collected something else in fuller's earth deposit.
- Fuller’s earth is extensively used for commercial purposes such as for cleaning clothes, in purifying edible oils, as a carrier for basic dyes and laboratory absorbent for that determination of the colouring matter in whisky, vinegar etc.
Fuller's earth is one of the ingredients used to check watery diarrhoea and dysentery. The anti-diarrhoeal effects, according to a manual of pharmacology, are explained by the more solid consistency of the stools; by checking of bacterial growth and by absorption of the irritant putrefactive products (Kraus and Barbara 1915).

Hydrous magnesium trisillicate is used as antacid and absorbent in treating gastric hyperacidity and the pain of peptic ulcer.

**Composition:** *(Ref: Thorpe's dictionary of applied chemistry)*

- **SiO** - 53.2
- **Al₂O₃** - 13.3
- **CaO** - 9.8
- **MgO** - 2.0
- **Alkalies** - 1.8
- **H₂O** - 14.2

**Method 4:**

Conch shell is soaked in cow’s butter milk for seven days and then washed.

**Method 5:**

Conch shell is broken into small pieces and boiled well in cow’s milk.

In the present study, first three methods are studied to establish the standard protocol and to find out the more appropriate method for purification.
Preparation of Sangu parpam:

Method 1:

Sangu purified with limestone is made as Parpam by ground with uttamani juice (Daemia extensa) and subjected to pudam process.

**Dosage:** upto 260 mgs

**Adjuvant:** Ghee

**Indications:** Cough, piles, stomach diseases, enlarged tonsils, chest pain, vayu, gunmam etc.,

Method 2:

Break the conch into pieces and soak in the juice of Phyllanthus niruri (Keezha nelli) for three days and subject to puda process using clay smeared ribbons in an earthen ware and cow dung cakes.

**Dosage:** 244 to 488 mg, twice a day.

**Adjuvant:** Ghee

**Indication:** heart attack, burning sensation in chest, chest pain, dysuria and leucorrhoea.

Method 3:

If the paste of lotus leaf (Nelambium speciosum) is applied on the purified conch for one day and dried in sunlight with puda process, it will be a fine Parpam.

**Dosage:** 244 to 488 mgs. Twice a day.

**Adjuvant:** Ghee

**Indications:** Eye diseases and pitha diseases.
Method 4:

Conch calyx prepared from the juice of *Ocimum sanctum* (*Tulasi*), cures delirium caused by kapha and chronic fever.

There is also a practice to prescribe *Sangu Parpam* with crushed snail in a conch for curing tuberculosis. Siddha Theraiyar also reiterates that tuberculosis and kapha diseases shall be cured with conch shell.

**Sangu Chendooram:**

The conch is ground separately with juice of *Cleome viscosa* (*Vaelai*) and (*Aloe vera Katraalai*) and subject to incineration to get the Chendooram.

<table>
<thead>
<tr>
<th>Adjuvants</th>
<th>Indication</th>
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<tbody>
<tr>
<td>Cleome viscose (white)</td>
<td>Body heat</td>
</tr>
<tr>
<td>Sandal paste</td>
<td>Vatha diseases</td>
</tr>
<tr>
<td>Garlic juice</td>
<td>Delirium with perspiration, Pitha diseases</td>
</tr>
<tr>
<td>Leaf juice of <em>Styrax benzoin</em></td>
<td>Swelling with Paandu</td>
</tr>
<tr>
<td>Lemon juice</td>
<td>Leprosy</td>
</tr>
<tr>
<td>Jaggery</td>
<td>Ulcer associated with diabetes</td>
</tr>
<tr>
<td>Fruit juice of <em>Syzygium cumini</em></td>
<td>Patches in the whole body</td>
</tr>
<tr>
<td><em>Cinnamomum verum</em></td>
<td>Delirium due to chillness</td>
</tr>
</tbody>
</table>

**Vellai Mathirai:**

Coral, zinc sulphate, alum and parched copper sulphate are taken (4.2 gms each), Conch (108 gms) is taken, washed with water and dried. The above ingredients are powdered and triturated again. Pills are made and dried. The pills so prepared should be used only after one year which is useful in the treatment of eye diseases.
The conch is rubbed with breast milk or with *Murraya koenigii (Karivepilai)* and applied over the pimples, acne and boils of the eye.

Medicines containing conch:

1. Agnikumara maathirai
2. Agnisoonu rasam
3. Kalyana rasam
4. Sannibadha kulandhaga rasam
5. Kan kaasa maathirai
6. Thurusu maathirai
7. Naga sangu parpam
8. Linga chendooram
9. Puzhuvettu parigaram

c) Botanical review on *Pergularia daemia*\(^ {29, 30} \)

**Tamil Name:** *Uththamani*

**Botanical Name:** *Pergularia daemia*

**Botanical classification:**

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
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<tbody>
<tr>
<td>Sub kingdom</td>
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<td>spermatophyte</td>
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<td>Division</td>
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<td>Asclepiadaceae</td>
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<td>Genus</td>
<td>Pergularia</td>
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</tbody>
</table>
Species: C. daemia

Habitat:

It is a slender climber with foetid smell found wild along fences and shrubberies. It is distributed in tropical and sub tropical areas, found wild on road sides of Tamil Nadu.

Botanical description:

It is a perennial twining herb with milky latex and covered with longer stiff erect hairs. Leaves are thin, broadly ovate and heart-shaped 2-12 cm long. Greenish yellow or dull white sweet scented flowers in axillary long peduncled umbellate or corymbose clusters. Fruits follicles.

Parts used: Whole plant, leaves and root bark.

Chemical constituents:

Coroglaucigenin, uzarigenin, calactin, calotropin, calotropigenin, protouscharin, uscharidin, lupeol, β-sitosterol and its acetate, betaine, β-amyrin, lupeol and its acetate. The plant contains triterpenes and steroidal compounds.

Actions:

Expectorant, anthelmintic, emetic.

Pharmacological actions:

- Antifertility
- Wound healing
- Antidiabetic
- Hepatoprotective
- Antibacterial activity
- Antioxidant
- Analgesic
- Anticancer
Uses:

- Whole plant is anthelmintic, emetic and expectorant.
- Leaves are advocated in catarrhal affections, asthma, infantile diarrhoea.
- Extract of the plant is useful in ulcers, uterine and menstrual complaints.
- Leaves combined with lime is applied to rheumatic swellings.
- Leaves also find its application in veterinary medicine to treat diarrhoea in turkey and eye diseases in cattle.

**d) Siddha aspect on Uththamani**: 41

**Synonyms:**

Vaeliparuthi, uththamakani, uththama kannigai.

**Character:**

- Taste: Bitter
- Nature: Hot
- Division: Hot

**General characteristic:**

- The plant is useful in phlegmatic diseases, delirium, peptic ulcer, seizures, vata diseases, rigor, tremor, indigestion especially in children, asthma etc. It increases appetite.
- This plant is called in Tamil as ‘chunnamakkum kodi’, which explains the usage of the herb to produce highly alkaline medicines of Siddha called chunnam, chiefly from corals and tortoise shell.
- This herb is very vital in Varmam medicine.

**Therapeutic benefits:**

- Leaf decoction act as anthelmintic.
- Bruised leaves are applied on carbuncles.
- Leaf juice is mixed with calcium carbonate and externally applied on swelling of leg.
- The leaf juice is given with honey for dysmenorrhea.
- For bronchial asthma, the following formulation will be effective. Five drops each of leaf juice and honey is taken with 12 g of *Thamira chenduram* (a prepared medicine from copper) and 12 g of Kasthuri.
- Oil prepared from this herb finds its usage in uterine diseases and arthritis.
- Root powder is advocated for vata diseases.

### 3.2 Pharmaceutical review

#### a) Parpam:

The word ‘Parpam’ refers to calcined form of medicines. It is also known as ‘neeru’ which means that which has gone through heat and became white ash-like material. In Sanskrit, it is called ‘bhasma’.

Metals, salts, some minerals, gems and animal products such as bones, horns, hoofs, feathers, corals, shells of oyster, chank, scallops, cowries shall be converted to parpam by soaking and triturating them with selected herbal juices, decoctions or with chemical compounds like theeneer, jeyaneer etc., and then subjecting them to fire.

The raw material is ground with stipulated juice mentioned in each preparation and made into small discs. They are sun dried and placed in single layer in an earthen ware and covered with another earthen ware. The edges are sealed with cotton ribbon smeared with clay and dried well. This is subjected to putam set-up. The process of incineration is executed with cow dung cakes. The variety of dung and quantity of dung cakes used depends upon the raw material which is subjected to incineration. A big pit is dug out and the cow dung cakes are arranged systematically and the above setup is let into the big pit and incinerated. Finally the earthen vessel is taken out and ground to fine powder.
Properties of Parpam:

- Parpams are white in colour, except the one which is made from gold and sulphur.
- They are very fine particles, some proportionate of the medicine will be in nano form.
- They are tasteless, lustreless. Presence of lustre indicates the requirement of incineration.
- They float on stagnant water surface.
- With suitable adjuvant, they possess therapeutic value.
- The finished product is irreversible (incapability to regain its original metallic form)

3.3 Disease review

a) Review on Gunmam

Main features of Gunmam:

- Burning sensation and pain in the abdomen.
- Indigestion.
- Vomiting is the worst symptom there by all the food product ingested are vomited out, as a result, nutritional status and stamina of the body day by day decreases. So patient becomes depressed day by day with severe pain leading to suicidal tendency.
- Some authors called this disease has ‘kulmam’ because one type of this disease, have the feature of forming flatulence, it more like ball from one side to another in the abdomen and produce discomfort.

Causes of peptic ulcer:

- Eating hot and belching substance (foods which increases vatham).
- Eating food mixed with sand, bran, stone and dust.
- Drinking contaminated water like spring water, stagnant water and mixed water.
- Eating food which cannot be digested easily like coconut milk
- Emotional issues like angry, starvation and depression.
• Practising yoga in a wrong manner especially in breath control.

**Prodromal symptoms:**
Anorexia, nausea, frequent flatulence, excessive salitary secretion, regurgitation, abdominal pain, vomiting, flatulence with sourness, increased bowel sounds.

**Types of the disease:**
Siddha yogi classified this disease onto 8 types and they are described as follows,

- Vayu gunnam
- Vatha gunnam
- Pitha gunnam
- Eri gunnam
- Vali gunnam
- Sathi gunnam
- Sanni gunnam
- Sethuma gunnam

Saint Thirukandambigai munivar classified this disease into 8 types and they are described as follows,

- Vatha gunnam
- Pitha gunnam
- Kapha gunnam
- Vatha pitha gunnam
- Vatha kapha gunnam
- Pitha silethuma gunnam
- Tridodha gunnam
- Raththa gunnam

Again this Raththa gunnam is sub divided into ratthha vatha gunnam and ratthha vatha pitha gunnam.

Some ancient physician have classified a disease into 3 types as follows

- Saamaniya vatha gunnam
- Saamaniya pitha gunnam
- Saamania silethuma gunnam.
**Vatha gunmam**

The disease mostly appears during the age of 20 to 30 years which is the ‘Vatha period’. Patient may develop severe abdominal pain and also develop tiredness, giddiness, thirst and dryness of tongue. Vomiting may also follow which will reduce the pain slightly. In this disease the food indigested will not be digested properly and produces stomach pain. Even though the patient will not take food properly, the body will appear as obese. However, patient will loose his strength and will become lean. There may be small quantity of blood in the vomit which appears dark in colour. In later the disease will produce indigestion throughout the life of the patient. Unbearable pain in the abdomen and over the chest below the xiphisternal region.

**Pitha gunmam :**

Pain in the upper abdomen and urine will appear red in colour. The patient will be having burning pain in the stomach and also develop nausea and vomiting. The vomitus may be found mixed with mucus and pitha. Excessive thirst, vertigo are other features of the disease. Patient may become unconscious after vomiting. As the vomiting increases, burning pain in the chest and abdomen proceeds. The pitham causes uncontrollable pain and produce uncontrollable vomiting. It is considered that the disease occurs in the age of 30 -50 years.

Besides, there will be loss of body strength and impaired quality of blood as the food taken are vomited out this causes yellow colouration of skin. As the disease, progresses, the pain in the abdomen will be severe after eating. The abdomen will appear heavy; the appetite will be impaired and patient will dislike eating. Ageusia, dryness of tongue, regurgitation of fluid in the stomach

**Iya gunmam :**

If the disease occurs in the old age period which is the period of ‘Kapha period’. The essential features of the disease are dislike of food, lean body mass due to malnutrition leading to anaemia, vertigo, tremors and frequent fright. The ingested food will not get digested and stays in the stomach; the food will get fermented and come out only in vomitus with a smell of meat. Later, continuous pain in the abdomen and excessive haemetemesis. The body may become lean day by day; these features will make one to suspect that patient might be having cancer in stomach. Patient may also develop vomiting of white coloured substance frequently.
Sanni gunmam:

In this type of disease, there will be excessive salivary secretion; the abdomen will be distended and stools will be passed hot with excessive sound; the mouth will have salty taste; there will be also irritation of the throat. The patient may not have the desire for food. The patient will frequently develop belch, dyspnoea and giddiness; the body also will become cool.

Vayu gunmam:

The excessive activity of vatha dosha in this disease causes damage to the stomach. The disease may be also called as paayuru gunmam and soolai gunmam. Patient may also develop unbearable pain in the stomach due to gas like pulling sensation. The downward directing factor (abana vayu) gets stimulated and it prevents the digestion of ingested food; this results in abdominal pain, irritation of chest, pain as if the intestine is twisted. The patient may also develop dislike to food. Even though the food ingested will be small quantity, abdomen will appear distended like gas filled belly; there will be loss of strength.

Eri gunmam:

The patient will develop pain as if the stomach is twisted. Excessive salivary secretion, pain in the head, sour belch from the stomach, distention of abdomen with excessive bowel sound, diarrhoea and sweating over the root of hair are other features of the disease. In this type of disease, unbearable irritation in the stomach develops within a short time after taking the food. Ultimately, the patient will become lean.

Vaanthi gunmam:

In this disease, the patient will develop indigestion, vomiting, burning pain in stomach, constipation, giddiness, spasm and irritation of stomach, a sensation of heat of fire in the body, inability to walk and ageusia. There will be protuberance of nerves and patient may develop numbness. Ultimately, the patient will develop loss of strength.

Vali gunmam:

As the kaphadosha and vatha doshas are associated in this disease, the ingested food will not be digested and also will not be vomited out. The food stays in the stomach and causes pricking pain in throat and twisting pain in stomach. In this disease, the food ingested will not be digested and the abdomen will be distended with gas. Pain at the rib sites as if it is pricked by thorn, pain in the hip and in the vertebral
region, throbbing pain of the whole body, dislike to food, diarrhoea with excessive bowel sounds are the other features of the disease. Sometimes, the patient may not be in a position to bear the pain and even develop suicidal tendency.

**General features of the disease:**

The disease occurs in women and also in the older age. The disease usually occurs in men in the age group of 25-45 years. The disease progresses gradually and other features such as indigestion, appearance as if the abdomen is distended, rolling pain in the abdomen may also appear. When patients have good body strength and capacity to digest any type of food, may suddenly develop anorexia, nausea, bilious vomiting, belch and sour belch from the stomach. As the disease increases in severity, patient may develop unbearable pain and may put the finger into the throat to inducing vomiting; after vomiting the pain may be reduced slightly.

**Doshas and other factors:**

1. Ancient Siddha Theran said ‘*Thodar vatha bandhamallathu gunnam varadhu*’.
2. Due to the wrong food habit nad bad activities the Vatha dosha worsens.
3. The other dosha will also associated with it and fail to perform their natural junctions. Due to this the downward directional factors and the upward directional factor (*udhana vayu*) malfunctioning; the food ingested will not be digested and purity of blood is also lost.
4. In addition to the above, the downward directional factor controls the passage of stool and causes increase of gas in the stomach the upward directional factors causes vomiting and aggravate the disease.

**Pulse:**

1. If the vatha and pitha pulses appear to be associated and their strength also appears to be integrated as single strength, it can be considered that this tends to erigunnam due to mantham.
2. If the Vatha pulse runs in the left side or by the side may denote Vatha gunnam.
3. The other types of pulses do not move in their proper direction and move to the left or by the side, they may give due to appropriate gunmam disease.

4. In the vatha pulse is felt abnormally like a vibration is tightly held rope which was raised with a finger and has suddenly dropped, it may be suggestive of vali gunmam disease.

b) Review on Peptic ulcer\textsuperscript{43,44}

Ulcers are defined histologically as a “breach in the mucosa of the alimentary tract that extends through the muscularis mucosa into the submucosa or deeper”.

**Peptic ulcers:**

Peptic ulcers are usually solitary, lesions may occur in any part of the gastrointestinal tract which is exposed to acid-peptic juices. The lesions are located in the following sites, in order of decreasing frequency,

- First part of duodenum
- Stomach, usually antrum
- Within Barrett’s mucosa
- Within the margins of a gastroenterostomy (stomal ulcer)
- In the duodenum, stomach or jejunum of patients with Zollinger- Ellison syndrome
- Within or adjacent to an ileal Meckel diverticulum that contains ectopic gastric mucosa.

**Aetiology:**

- H-pylori infection
- NSAIDs
- Cigarette smoking
- Stress
Pathogenesis and pathophysiology:
Clinical features:

Pain:

Pain has got several important features:

**Location:** Usually in the epigastric region

**Character:** Usually burning, sometimes aching or stitching or gnawing in character.

**Relation with Food:** Food minimizes of abolishes pain in 50% cases. Pain comes 2-3 hours after taking food.

**Radiation:** Sometimes pain is radiated to the chest. Post bulbar ulcer pain may be radiated backwards.

**Relieving Factors:** After taking soda, alkali or antacid, pain is relieved. Food also causes relief of pain in about 50% of subjects or by induced vomiting pain is relieved.

**Nocturnal or Hunger Pain:**

Pain appears at midnight of late hours of the night often awakening the patient from the bed. It is very characteristic (and is present in about 60%-70% cases.)

**Complications:**

- Bleeding
- Perforation
- Gastric outlet obstruction

**Investigations:**

- Complete Blood Picture- Tc, Dc, ESR, and Hb.
- Routine Blood Examination- Blood sugar and urea
- Video Endoscopy
- To know the extent of the lesion
- To confirm the diagnosis
- To take biopsy
- EGD -oesophagastroduodenoscopy
- Ultrasound abdomen
Barium Meal Study
Duodenal ulcer - deformed duodenal cap is seen
Gastric ulcer - appears as a Niche in the lesser curvature due to ulcer crater and as a notch on the greater curvature due to the spasm of stomach.
Test for h. pylori
Non-invasive test
- Serology
- Urea breath test
Faecal antigen test
Invasive test -
- Histology (Antral biopsy)
- Rapid urease test
- Microbiological culture

3.4. Scientific validation of conch:

Literature scanning reveals the study on standardization of Sangu parpam using infrared spectrum\(^\text{45}\) (Meena devi) and anti inflammatory activity \(^\text{46}\) of sangu parpam in animal model (Murugan).

Further, one more study to compare the anti ulcer effect of the same drug with Silasathu parpam in animal model was attempted and it proved the anti ulcer effect of the drug\(^\text{47}\). (Thanga Thirupathi).

The review clearly states the lacuna of anti ulcer activity of sangu parpam made with Pergularia daemia.
4. PLAN OF WORK

Raw Sangu

- Purification I
  Sangu with *Euphorbia ligularia* (Spu I)
  - Preparation I
    Purified Sangu processed with *Pergularia damea*

- Purification II
  Sangu with Limestone (Spu II)
  - Preparation II
    Purified Sangu processed with *Pergularia damea*

- Purification III
  Sangu with Limestone & Fuller’s earth (Spu III)
  - Preparation III
    Purified Sangu processed with *Pergularia damea*

- Sangu Parpam I (SP I)
- Sangu Parpam II (SP II)
- Sangu Parpam III (SP III)

Acute toxicity Studies for the three formulation of Sangu Parpam (I, II, III)

Sub Acute toxicity Studies for the three formulation of Sangu Parpam (I, II, III)

Standardization techniques applied on Raw Sangu, Spu I,II,III, and SP I,II,III

Efficacy studies on formulation of Sangu Parpam II
5. MATERIALS AND METHODS

5.1 Authentication of Sangu:

Samples of Sangu were collected from Chennai market and various places of marine resources throughout Tamil Nadu and given for authentication. Authentication done on Marine Biology Regional Centre, Chennai through external morphology and experience.

5.2 Preparation of Sangu Parpam:

Purification of Sangu

a) Process I: 35 gm of Sangu (1 palam) was soaked in 175 gms of Juice of Ilaikkalli (Common Milk Hedge – *Euphorbia ligularia*) and let to dry in sunlight from morning to evening. This process was repeated for another 3 times with fresh juice.

b) Process II:  

Sangu was processed in thaalithal method (Heating process) by covering it with Karchunnam (limestone).

c) Process III:  

Equal parts of Karchunnam (limestone) and Uvarmann (Alkaline earth) was mixed up with 8 parts of water and the clarified water was collected. Sangu was processed by heating with this clarified water. After heating, Sangu was washed with water and dried.

Preparation of process:

100 gms of purified Sangu from each purification process was covered up by ground paste of Uthamani (*Pergularia damea*) and kept in the mud lid and closed by another mud lid. Cotton ribbon soaked in wet clay was winded over the rims of both mud lids and let to dry in sun light for 8 hours. Then this set up was subjected to Gana...
pudam. (100 cow cakes was used). After cooling the set up was taken out and the
calcinated Sangu was taken out, ground well and stored in an airtight container.

**Route of administration:** Oral
**Dose:** 260 mg
**Adjuvant:** ghee
**Indication:** Gunmam (Peptic Ulcer), Stomach diseases, Vayu, Chest pain, Enlarged
tonsils, Piles.

### 5.3 PHYSICOCHEMICAL ANALYSIS:

The physicochemical analysis was done as per the guidelines of WHO

**Organoletic characters:**

“The organoleptic characters of Sangu parpam was identified and tabulated.

**Loss on drying @ 105°C**

Five grams of Sangu Parpam is heated in a hot oven at 105°C to till it reaches
its constant weight.

**Determination of ash value**

Weighed accurately 2 grams of Deepalinga chenduram in tarred platinum or
silica dish and incinerate at a temperature not exceeding 500 – 5500 C until free from
carbon, cooled and weighed.

**Acid in soluble ash**

Boiled the ash 5 minutes with 25 ml of 1:1 dil HCl. Collected the insoluble
matter in Gooch crucible on an ash less filter paper (Whatman No. 41) wash with hot
water and ignite. Cooled in a dessicator and weighed.
The pH of **Sangu Parpam** was estimated as per the method prescribed in Indian Standard (IS) – 6940 (1982). One gram of the **Sangu Parpam** was taken into a 100ml graduated cylinder containing about 50ml of water and filled up to the mark with water. The cylinder was stopped and shaken vigorously for two minutes and the suspension was allowed to settle for an hour at 25 to 27°. About 25ml of the clear aqueous solution was transferred into a 50ml beaker and tested for pH using DIGISUN digital pH meter” (DIGISUN Electronics, Hyderabad, India.)

5.3.2 Sophisticated Analytical Techniques:

5.3.2.a) HR SEM-METHODOLOGY:  

JEOL ASM 3500 SEM was used for the analysis of Particle size and external morphology of the test drug. An SEM is essentially a high magnification microscope, which uses a focused scanned electron beam to produce images of the sample, both top-down and, with the necessary sample preparation, cross-sections. The primary electron beam interacts with the sample in a number of key ways:-

- Primary electrons generate low energy secondary electrons, which tend to emphasise the topographic nature of the specimen
- Primary electrons can be backscattered which produces images with a high degree of atomic number (Z) contrast
- Ionized atoms can relax by electron shell-to-shell transitions, which lead to either X-ray emission or Auger electron ejection. The X-rays emitted are characteristic of the elements in the top few μm of the sample.

**SAMPLE PREPARATION:**

Sample preparation depends on the nature of the samples and the data required. Minimal preparation includes acquisition of a Sangu Parpam that fit into the SEM chamber. And it was analysed.
ENERGY DISPERSIVE X RAY SPECTROMETRY (EDAX) ANALYSIS:

Powder property of the samples was determined by JEOL ASM 3500 EDAX. A minimum quantity of sample was sprinkled onto a double side carbon tape and mounted on aluminum stubs, to get a better quality image.

X RAY DIFFRACTION STUDY (XRD) ANALYSIS:

The powder XRD patterns of the solid samples were recorded on D2 phase XRD instruments. It is used to analyze the characterization of crystalline materials. And this method has been used for analysis of quantity, three dimensional microstructural properties, phase identification, any structural imperfection. It is also used to determine unit cell dimensions. Analysis was done by using High resolution PAN analytical X’pert PRO powder XRD instrument with cu K α radiation (λ = 1.5418) .Samples were prepared and placed for the analysis. Diffraction patterns were recorded at the following range that 2Θ angular range of 10º to 80º with a step size of 0.02 2 Θ and scan step time of 1 sec.

ICP OPTICAL EMISSION SPECTROMETRY METHODOLOGY

Analysis of Test samples was performed by using Optima 5300 DV ICP-OES equipped with a cyclonic spray chamber and Sea Spray concentric nebulizer. When plasma energy is given to an analysis sample from outside, the component elements (atoms) are excited. When the excited atoms return to low energy position, emission rays (spectrum rays) are released and the emission rays that correspond to the photon wavelength are measured. The element type is determined based on the position of the photon rays, and the content of each element is determined based on the rays’ intensity.

To generate plasma, first, argon gas is supplied to torch coil, and high frequency electric current is applied to the work coil at the tip of the torch tube. Using the electromagnetic field created in the torch tube by the high frequency current, argon gas is ionized and plasma is generated. This plasma has high electron density and temperature (10000K) and this energy is used in the excitation-emission of the sample. Solution samples are introduced into the plasma in an atomized state through the narrow tube in the center of the torch tube.
5.4 PRECLINICAL STUDIES:
5.4.1 TOXICITY STUDIES:

Scope of work

Assurance of safety, quality, and efficacy of Indian System of Medicine (ISM) is the key issue that needs to be addressed while conducting toxicity studies. It is an essential step, which will strengthen the acceptance of Siddha medicines by scientific community. Information of toxicity and adverse effects of these formulations are lacking. Some of the formulations are proved to be effective in various animal studies and many more are yet to be tested.

Hence, the present study was carried out to evaluate the acute and repeated oral dose toxicity effects of Sangu Parpam.

Plan of work:

The following studies are carried out on Sangu Parpam
i. Acute oral toxicity study
ii. 28-days Repeated dose oral toxicity study.

INTRODUCTION

1. The acute toxic class method is a stepwise procedure with the use of 3 animals of a single sex per step. Depending on the mortality and/or the moribund status of the animals, on average 2-4 steps may be necessary to allow judgement on the acute toxicity of the test substance. This procedure is reproducible, uses very few animals and is able to rank substances in a similar manner to the other acute toxicity testing methods. The acute toxic class method is based on biometric evaluations with fixed doses, adequately separated to enable a substance to be ranked for classification purposes and hazard assessment.

2. In principle, the method is not intended to allow the calculation of a precise LD50, but does allow for the determination of defined exposure ranges where lethality is expected since death of a proportion of the animals is still the major
endpoint of this test. The method allows for the determination of an LD50 value only when at least two doses result in mortality higher than 0% and lower than 100%. The use of a selection of pre-defined doses, regardless of test substance, with classification explicitly tied to number of animals observed in different states improves the opportunity for laboratory to laboratory reporting consistency and repeatability.

**Acute toxicity study of SP I, II, III:**

The acute toxicity study was conducted as per the OECD 423 Method - Acute toxic class method. As drug is administered through oral route in humans, the same route was chosen for drug administration in animals. The complete protocol of the animal experiments has been approved by the Institutional Animal Ethics committee, National Institute of Siddha, Chennai. The IAEC approval number is 1248/AC/09/CPCSEA -9/Dec 2013/8

**Test Guideline**

Method - OECD 423 - Acute toxic class method.

**Test Item Detail**

Name: SANGU PARPAM I, II, III

**Study Detail**

The study was conducted on Female Wister rats because Wistar rats are the recommended rodent species for oral studies as per followed guidelines. The Animals were purchased from Laboratory Animal Medicine – Centre for Animal Health Studies, TANUVAS, Madhavaram, Chennai. The rats were housed in standard laboratory condition in Polypropylene cages, provided with food and water *ad libitum*

**Husbandry**

The animals were housed in standard Polypropylene cages with a stainless steel top grill. Paddy husk was used as bedding and was changed regularly.
The animals were maintained in a clean environment with artificial photo period. The relative humidity was maintained in the range of 30-70% with air conditioning at 22±3⁰C and 100% exhaust facility. In order to balance the environmental conditions and other external factors, the cages corresponding to each experimental group were disseminated on racks.

**Feed & water**

All through the study period, the animals were fed with standard pelleted diet (Nutrilab rodent, Provimi Animals Nutrition India Private Limited, Bengaluru, India) and purified RO water (Kent RO water filter cum purifier).

**Acclimatization**

The animals were selected after the veterinary examination by the veterinarian and the selected animals were kept for acclimatization for a week.

**Randomization & grouping**

8-12 weeks old Female Wister rats were used for this study and they were subjected to different groups after randomization. One day before the initiation of treatment (days 0- last day of acclimatization), the selected animals were randomly grouped into thirteen different groups containing 3 female rats per group. All groups except control group were treated with SANGU PARPAM I, II, III at various doses.

**Numbering and Identification**

The animals were marked with using picric acid solution. The marking in each group was done as below. The identification of group no., cage no., and the sex of the animals using the cage label and body marking on the animals were as indicated below.
Table 5.1 Numbering and Identification for SANGU PARPAM I, II, III

<table>
<thead>
<tr>
<th>Cage No</th>
<th>Group No</th>
<th>Animal Marking</th>
<th>Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I</td>
<td>H,B,T</td>
<td>Female</td>
</tr>
<tr>
<td>2</td>
<td>II</td>
<td>H,B,T</td>
<td>Female</td>
</tr>
<tr>
<td>3</td>
<td>III</td>
<td>H,B,T</td>
<td>Female</td>
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<td>4</td>
<td>IV</td>
<td>H,B,T</td>
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<td>H,B,T</td>
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<td>6</td>
<td>VI</td>
<td>H,B,T</td>
<td>Female</td>
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<td>H,B,T</td>
<td>Female</td>
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<tr>
<td>8</td>
<td>VIII</td>
<td>H,B,T</td>
<td>Female</td>
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<tr>
<td>9</td>
<td>IX</td>
<td>H,B,T</td>
<td>Female</td>
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<tr>
<td>10.</td>
<td>X</td>
<td>H,B,T</td>
<td>Female</td>
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<tr>
<td>11.</td>
<td>XI</td>
<td>H,B,T</td>
<td>Female</td>
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<tr>
<td>12.</td>
<td>XII</td>
<td>H,B,T</td>
<td>Female</td>
</tr>
<tr>
<td>13.</td>
<td>XIII</td>
<td>H,B,T</td>
<td>Female</td>
</tr>
</tbody>
</table>

H-Head; B-Body; T- tail
Experimental detail

Study schedule:

The animals were fasted overnight with water *adlibitum*. The Sangu parpam I, II, III was administered in four different dose levels i.e 50mg, 300mg, 1000mg & 2000mg/kg b.wt. As OECD guidelines insist the stepwise administration first 50mg/kg b.wt of Sangu parpam I, II, III was given as a single oral dose. As no death was noticed, 300 mg was administered. Likewise, 1000mg and 2000 mg/kg b.wt was administered from group II to Group 13. Group I was served as control which received vehicle i.e ghee (2ml/200gm). After drug administration, all animals were observed for 14 days.

Dose Preparation

The drug Sangu parpam (I, II, III) was freshly prepared with ghee (Aavin, The Tamilnadu Cooperation Milk Producers federation Ltd, Chennai) daily for administration.

Administration

According to the individual body weight and calculated dose levels the drug was administered as a single oral dose.

Observation

The animals were monitored intensively for mortality and morbidity. The animals were observed for all wellness parameters for first one hour and observed continuously with special attention for the next hours. The changes in the appearance or disappearance of clinical signs were recorded at 1.0, 3.0 and 4.0 hours after the dose administration on that day and thereafter for 14 days daily. The cage side observation included changes in fur, eyes and mucous membranes, occurrence of secretions and excretions. Autonomic activity like lacrimation, piloerection, pupil size and unusual respiratory pattern, changes in gait, posture, response to handling, presence of clonic or tonic movements, stereotypes like excessive grooming and repetitive circling or bizarre behavior like self-mutilation, walking backwards etc. were observed. The observations included but were not limited to changes in skin and fur, in the eyes and mucous membranes, in the respiratory, circulatory, central
nervous and autonomous systems, somatomotor activity and behavior. The animals were observed daily for mortality and any signs of toxicity and behavioural changes.

On day 15, the overnight fasted animals (water allowed *ad libitum*) were sacrificed and examined for gross pathological changes in the major internal organs.

**Clinical signs of toxicity**

All the animals were observed at least two times a day to record abnormal behavior. In clinical signs of toxicity, they should be observed daily for 14 days.

**Sub-Acute Toxicity Study Of SP I, II, III 28 days Repeated Dose Oral Toxicity**

‘Sub-Acute Toxicity Study of SANGU PARPAM I, II & III on Female Wister rats’ was to analyze the repeated dose toxicological profile of the test samples. This study put in the picture on the likely health hazards that may arise on a comparatively limited period of time. The observation was done on animals for 28 days after the drug administration. According to LD 50 levels the three different dose levels for subacute toxicity was fixed.

**Test Guideline**

Method - OECD 407- Sub-Acute Toxicity study (Repeated Dose 28-Day Oral Toxicity Study in Rodents) 54

**Good Laboratory Practices**

The study was conducted according to the principles of GLP as defined in the OECD Principles of GLP, OECD, 1998. The complete protocol of the animal experiments has been approved by the Institutional Animal Ethics committee, National Institute of Siddha, Chennai. The IAEC approval number is 1248/AC/09/CPCSEA -9/Dec 2013/8

**Test Item Detail**

Name: SANGU PARPAM I, II, III
Procurement of animal

The Animals were purchased from Laboratory Animal Medicine – Centre for Animal Health Studies, TANUVAS, Madhavaram, Chennai. The rats were housed in standard laboratory condition in Polypropylene cages, provided with food and water *ad libitum*

Husbandry

The animals were housed in standard Polypropylene cages with a stainless steel top grill. Paddy husk was used as bedding and was changed regularly.

The animals were maintained in a clean environment with artificial photo period. The relative humidity was maintained in the range of 30-70% with air conditioning at 22±3⁰C and 100% exhaust facility. In order to balance the environmental conditions and other external factors, the cages corresponding to each experimental group were disseminated on racks.

Feed & water

All through the study period, the animals were fed with standard pelleted diet (Nutrilab rodent, Provimi Animals Nutrition India Private Limited, Bengaluru, India) and purified RO water( Kent RO water filter cum purifier).

Acclimatization

The animals were selected after the veterinary examination by the veterinarian and the selected animals were kept for acclimatization for a week.

Randomization & grouping

8-12 weeks old Female Wister rats were selected after physical and behavioral Examination. The body weight range was fallen within ± 20% of the mean body weight at the time of Randomization and grouping. One day before the initiation of treatment (days 0- last day of acclimatization), the selected animals were randomly grouped into ten different groups containing 5 male and 5 female rats per group.
Numbering and Identification

The animals were marked on their body as H, B, T, HB, NM(Male) and H, B, T, HB, NM(Female) for each group, with picric acid solution prepared in water.

Table 5.2 Dose and grouping of SANGU PARPAM I, II, III in repeated oral toxicity

<table>
<thead>
<tr>
<th>Drug</th>
<th>Group</th>
<th>No of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Ghee (2ml/200gm)</td>
<td>Group I</td>
<td>5/Sex</td>
</tr>
<tr>
<td>S. P I</td>
<td>Group II</td>
<td></td>
</tr>
<tr>
<td>(a) Low dose</td>
<td>100mg/kg b.wt</td>
<td>5/sex</td>
</tr>
<tr>
<td>(b) Mid dose</td>
<td>200mg/kg b.wt</td>
<td>5/sex</td>
</tr>
<tr>
<td>(c) High dose</td>
<td>300mg/kg b.wt</td>
<td>5/sex</td>
</tr>
<tr>
<td>S. P II</td>
<td>Group III</td>
<td></td>
</tr>
<tr>
<td>(a) Low dose</td>
<td>100mg/kg b.wt</td>
<td>5/sex</td>
</tr>
<tr>
<td>(b) Mid dose</td>
<td>200mg/kg b.wt</td>
<td>5/sex</td>
</tr>
<tr>
<td>(c) High dose</td>
<td>300mg/kg b.wt</td>
<td>5/sex</td>
</tr>
<tr>
<td>S. P IV</td>
<td>Group IV</td>
<td></td>
</tr>
<tr>
<td>(a) Low dose</td>
<td>100mg/kg b.wt</td>
<td>5/sex</td>
</tr>
<tr>
<td>(b) Mid dose</td>
<td>200mg/kg b.wt</td>
<td>5/sex</td>
</tr>
<tr>
<td>(c) High dose</td>
<td>300mg/kg b.wt</td>
<td>5/sex</td>
</tr>
</tbody>
</table>

Total number of animals = 100
Dose Preparation

The Sangu parpam I, II, III were freshly prepared daily in ghee (Aavin, The Tamilnadu Cooperation Milk Producers federation Ltd, Chennai) in a fixed concentration (200mg/ml) for oral administration.

Administration

Sangu parpam mixed with vehicle in a standard concentration was administered according to the individual body weight and calculated dose levels. The drug was administered once daily maximum at the same time with usage of feeding tube.

Clinical signs of toxicity

All the animals were observed at least two times a day to record any symptoms of ill health or behavioral changes. The observations included but were not limited to changes in skin and fur, in the eyes and mucous membranes, in the respiratory, circulatory, central nervous and autonomous systems, somatomotor activity and behavior. The observation was continued for 28 days.

Food intake

The food consumption of each cage was recorded prior to the treatment, and daily and thereby the mean weekly consumption per rat was calculated.

Water intake

Water consumption was initially noticed visually and thereafter measured in each cage daily for 28 days.

Bodyweight:

The body weight was recorded for each of the animal a week before the start of the treatment. The body weight was recorded also on the initial day, 3rd, 7th, 10th, 14th, 17th, 20th, 24th and 28th days (the day of sacrifice). From these individual weights, the mean weights of different groups and sexes were calculated.

Blood Collection

On the 29th day after overnight fasting, the blood was collected in tubes containing Heparin/EDTA as an anticoagulant, from the retro-orbital sinus from all the animals of different groups.
Laboratory Studies

During the 4th week of treatment, the blood samples were drawn after application of topical lignocaine anaesthesia to minimize pain to the animals. The blood samples are used to evaluate Hematological parameters like RBC, WBC, and PLATELETS etc.... The collected blood samples also centrifuged 10000 rpm in 10 minutes to separate the serum. The separated serum used to evaluate biochemical parameters like SGOT, SGPT, ALP and BILIRUBIN etc...

Hematology

Auto analyzer was used in analyzing the following Hematological parameters:

- **Hb**: Haemoglobin (g %)
- **PCV**: Packed Cell Volume
- **WBC**: White Blood Corpuscles (x103/cmm)
- **RBC**: Red Blood Corpuscles (x106/cmm)
- **Blood Platelet count**: (x103/cmm)

**Differential WBC count:**

- **N**: Neutrophils (%)
- **L**: Lymphocytes (%)
- **M**: Monocytes (%)
- **E**: Eosinophils (%)
- **RDW**: Red Cell Distribution Width.
- **MPV**: Mean Platelet Volume

Clinical Biochemistry:

Auto analyser was used to investigate the following Biochemical parameters:

- **Total serum protein**: (g/dl)
- **ALT/SGPT**: Alanine amino transferase (U/L)
- **AST/SGOT**: Aspartate amino transferase (U/L)
- **ALP**: Alkaline serum phosphatase (U/L)
- **CHL**: Cholesterol (mg/dL)
- **HDL**: High density lipoprotein
- **TG**: Triglyceride
TERMINAL STUDIES

Sacrifice and macroscopic examination

After the completion of treatment for 4 weeks, rats were sacrificed by ether inhalation and a full autopsy was performed on all animals. After autopsy, the animals were examined for the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents both in situ and after evisceration. At the end of the treatment days, the autopsies were carried out over a period of three successive days as the number of animals exceeded the number of animals that could be sacrificed in one day.

Organ Weights

The organs were initially observed macroscopically followed by weighing the organs after the removal of superficial fat: The organs weighed included Brain, Heart, Spleen, Kidneys, Testes, Liver, Lungs, Pancreas and Stomach. The organs were stored in 10% formal saline for histopathological evaluation.

Histopathology

The various organs collected were fixed in 10% neutral buffer formaline. They were dehydrated through a series of graded alcohol, fixed in paraffin, and routinely processed for histopathological assessment. The tissues cut into 4-5µm thick sections and stained with Haematoxylin-eosin. Tissue slides were examined and photographs were taken by using N-400ME photomicroscope (CELL-TECH diagnostics, Hamburg, Germany) in X40, X100 and X400 objectives.

ESTIMATION OF HEMATOLOGICAL PARAMETERS

Collection of blood for hematological studies:

In order to perform the hematological examination for the animals, they were anesthetized by ketamine hydrochloride and the Retro-bulbar sinus blood was collected using a capillary into a centrifugation tube with EDTA. The haematological parameters were estimated by the following procedures.
**Enumeration of RBC (Red blood cells)**

**Reagents**

- RBC diluting fluid

**Procedure**

"Well mixed blood was drawn upto 0.5 mark with a red blood cell pipette, of hemocytometer and the RBC diluting fluid was taken upto mark II, mixed well and was transferred to a counting chamber. The cells were allowed to settle at the bottom of the chamber for 2 minutes and care was taken not to dry the fluid. The RBCs were counted consistently in the larger corner squares using a 45X or a high power objective.

The cells were expressed as number of cells x$10^{12}$/l

**Estimation of WBCs (White Blood Cells)**

Reagents Turk’s fluid- 2ml of acetic acid and 100ml of distilled water were mixed to prepare the Turk’s fluid and to this mixture, 10 drops of aqueous methylene blue 3% (w/v) was added. Counting of white blood cells now becomes easy as this solution hemolyses the red blood cells due to the acidity of the solution.

Well mixed blood was drawn upto 0.5 mark with a red blood cell pipette, of hemocytometer and the RBC diluting fluid was taken upto mark II, mixed well and was transferred to a counting chamber. The cells were allowed to settle at the bottom of the chamber for 2 minutes and care was taken not to dry the fluid. The WBCs were counted consistently in the larger corner squares using a 10X or a low power objective.

The cells were expressed as the number of cells/10mm.
Differential Platelet Count

Reagent

In order to prepare Leishmann’s stain- 150mg of powdered Leishmann’s stain was dissolved in 133ml of acetone free methanol.

Procedure

The types of WBCs were identified by preparing a blood film and staining it with leishmann’s stain, the film was examined under oil immersion objective and the percentage distribution of these cells was calculated. The blood films were prepared from anticoagulant blood and the slides were preserved for counting the number of lymphocytes and neutrophils per 100 cells.

The absolute lymphocyte and neutrophil count were estimated from the leukocyte and WBC counts.

\[
\text{Absolute neutrophil count} = \frac{\text{Number of neutrophils}}{100} \times \text{TWBC}
\]

\[
\text{Absolute lymphocyte count} = \frac{\text{Number of lymphocytes}}{100} \times \text{TWBC}
\]

MEASUREMENT OF BIOCHEMICAL PARAMETERS ESTIMATION:

The whole blood was used to estimate the Fasting Blood Glucose (FBG) and Hemoglobin (Hb) and serum was used to measure the remaining parameters. Semi-Autoanalyzer was used to analyses the above said biochemical parameters.

1. Estimation of Glucose

“The whole blood sample was drained by capillary action at the end of tip of the test strip. The glucose present in the sample blood reacts with the glucose oxidase and hexaammineruthenium (III) chloride in the test strip, and
produces the product hexaammineruthenium (II) chloride, in proportion to the glucose in the sample. The final product hexaammineruthenium (II) chloride gets oxidized and produces an electric current and it is converted by the meter as glucose concentration which is displayed as the test result.

\[
\text{B-D-glucose} + \text{Hexaammineruthenium (III) chloride} \xrightarrow{\text{GOD}} \text{D-Glucono-lactone} + \text{Hexaammineruthenium (II) chloride}
\]

\[
\text{Hexaammineruthenium (II) chloride} \rightarrow \text{Hexaammineruthenium (III) chloride} + e^{-}.
\]

2. **Total Cholesterol (TC)**

**Principle**

After the enzymatic hydrolysis and oxidation, the estimation of cholesterol was carried out with quinoneimine as the colorimetric indicator. CHOD-PAP: Enzymatic photometric test was used.

**Assay procedure**

a. “1ml (1000 µl) of reagent-1 was taken in a 5ml test tube

b. To this, 0.01ml(10 µl) of the serum was added

c. The solution was mixed well and kept for incubation at 37°C for 5 min

d. The test sample was read”.

3. **Triglycerides**

**Principle**

“The estimation of triglycerides (TG) alters the enzymatic splitting with lipoprotein lipase with the indicator as quinoneimine, obtained from 4-aminoantipyrine and 4-chlorophenol by hydrogen peroxide with peroxidase as the catalyst.
Method

Colorimetric enzymatic test using glycerol-3-phosphate-oxidase (GPO).

Reagents

Components and concentrations in the test Goods buffer pH 7.2, 50 mmol/l

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-chloroPhenol</td>
<td>4 mmol/l</td>
</tr>
<tr>
<td>ATP</td>
<td>2 mmol/l</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>15 mmol/l</td>
</tr>
<tr>
<td>Glycerokinase</td>
<td>&gt; 0.4 Kµ/l</td>
</tr>
<tr>
<td>Peroxidase</td>
<td>&gt; 2 Kµ/l</td>
</tr>
<tr>
<td>Lipoprotein lipase</td>
<td>&gt; 4 Kµ/l</td>
</tr>
<tr>
<td>4-aminoantipyrine</td>
<td>0.5 mmol/l</td>
</tr>
<tr>
<td>Glycerol-3-phosphate- oxidase</td>
<td>&gt; 1.5Kµ/l</td>
</tr>
<tr>
<td>Standard</td>
<td>(2.3 mmol/l)</td>
</tr>
</tbody>
</table>

Assay procedure

a. 1ml (1000 µl) of reagent-1 was taken in a 5ml test tube

b. To this, 0.01ml(10 µl) of the serum was added

c. The solution was mixed well and kept for incubation at 37°C for 5 min

d. The test sample was read”

4. HDL Cholesterol

Principle

The sample was mixed with phosphotungstic acid and magnesium ions so that the Chylomicrons, VLDL and LDL are precipitated from the sample. After centrifugation, the serum analyze for HDL.
**Method**

“Phosphotungstic acid precipitation method.

**Reagents**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphotungstic acid</td>
<td>0.55 mmol/l</td>
</tr>
<tr>
<td>Magnesium chloride</td>
<td>25 mmol/l</td>
</tr>
</tbody>
</table>

**Assay procedure**

A. Preparation of supernatant for the HDL-CHL estimation

About 200 µl of serum was added to 500 µl of HDL-Cholesterol precipitating reagent (from HDL kit) and the mixture was centrifuged in a 1.5ml centrifuge tube at 4000 rpm for 10 min.

B. Preparation of test sample for the estimation of HDL-Cholesterol

a. From the cholesterol kit, 1000 µl of reagent-1 was taken in a 5ml test tube
b. The Supernatant from the above said centrifuged solution was added upto a volume of 100 µl
c. The mixture was incubated at 37°C for 15 min
d. The test sample was read”

5. **Estimation of Serum Glutamate Oxalo Acetate Transaminase (SGOT)**

**Principle**

“In order to avoid low values in samples containing endogenous P-5-P, Pyridoxal-5 Phosphate (P-5-P) was added. e.g., from patients with myocardial infarction, liver diseases and intensive care patients falsely insufficiently
Reagents

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>R 1</td>
<td>TRIS pH 7.8</td>
<td>80 mmol/l</td>
</tr>
<tr>
<td></td>
<td>L- Aspartate</td>
<td>240 mmol/l</td>
</tr>
<tr>
<td></td>
<td>MDH (malate dehydrogenase)</td>
<td>≥600 U/l</td>
</tr>
<tr>
<td></td>
<td>LDH (lactate dehydrogenase)</td>
<td>≥600 U/l</td>
</tr>
<tr>
<td>R 2</td>
<td>2-Oxalocarboxylic acid</td>
<td>12 mmol/l</td>
</tr>
<tr>
<td></td>
<td>NADH</td>
<td>0.18 mmol/l</td>
</tr>
<tr>
<td></td>
<td>Pyridoxal-5-phosphate FS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Good buffer pH 9.6</td>
<td>0.7 mmol/l</td>
</tr>
<tr>
<td></td>
<td>Pyridoxal-5-phosphate</td>
<td>0.9 mmol/l</td>
</tr>
</tbody>
</table>

Method

Optimized UV- test according to IFCC (International Federation of Clinical Chemistry and Laboratory Medicine).

Assay procedure

a. In a 5 ml test tube, 800 µl of reagent-1 was mixed with 200 µl of reagent-2

b. 100 µl of serum was added to this mixture

c. Mixed well and the reading was taken immediately’’
6. Estimation of Serum Glutamate Pyruvate Transaminase (SGPT)

**Principle**

In order to avoid low values in samples containing endogenous P-5-P, Pyridoxal-5 Phosphate (P-5-P) was added. e.g., from patients with myocardial infarction, liver diseases and intensive care patients falsely insufficiently.

**Method**

Kinetic UV test was followed based on the IFCC.

7. Determination of alkaline phosphatase (ALP) \(^{61}\)

At pH 10.0, Alkaline phosphatase present in the serum converts phenyl phosphate to inorganic phosphate and phenol. The phenol thus formed reacts with 4-aminoantipyrine in alkaline medium and forms an orange-red coloured complex with potassium ferricyanide as the oxidizing agent. This orange coloured complex can be read spectroscopically and the intensity of the colour is proportional to the enzyme activity.

**Reagents:**

Buffered substrate

Chromogen Reagent

Phenol Standard, 10 mg%

**Procedure**

“One vial of buffered substrate was mixed with 2.2ml of water to prepare the working solution. The buffered substrate of 0.5ml and purified water of 1.5ml was dispensed in blank, standard, control and test. It was then mixed well and incubated at 37\(^{0}\)C for 3 min. 0.05 ml each of serum and phenol standard were added to test and standard test tubes respectively and it was mixed well and incubated for 15 min at 37\(^{0}\)C. Then, 1ml of chromogen reagent was added to all the test tubes, followed by 0.05ml of serum to the control. The solution was mixed well after adding each of the
reagents and the OD of blank, standard, control and test were read at 510nm against the purified water.

Serum alkaline phosphatase activity in KA units was calculated as follows

\[
[(O.D. \text{ Test} - O.D. \text{ Control}) / (O.D. \text{ Standard} - O.D. \text{ Blank})] \times 10^2.
\]

8. Determination of bilirubin

The bilirubin level is increased in conditions of toxic liver and hyperbilirubinemia results due to the improper uptake of the unconjugated bilirubin by the liver. The predisposing factors for such a situation are generalized liver cell injury, certain drugs (e.g. Rifampicin and probenecid) interrupts the liver uptake of bilirubin in rats and results in a mild unconjugated hyperbilirubinemia.

**Method:**

“The method involves the reaction between Sulfonilic acid and sodium nitrite resulting in the production of azobilirubin and in aqueous solution, it has the maximum absorbance at 546nm. The colour intensity produced is directly proportional to the amount of direct or total bilirubin concentration present in the sample.

**Reagents**

1. Diazo A-(Reagent-R1): Ready to use

2. Diazo B-(Reagent-R2): Ready to use

3. Bilirubin Activator: Ready to use

**Procedure**

Estimation of Bilirubin was done based on Kind & King’s method. 500 µl of working reagent and 50 µl of rat serum was mixed together & incubated for 5 min at 37°C. The Absorbance was measured at 546 nm in a Semi auto analyzer against the standard.

The Bilirubin content was calculated using the following equation:

Total bilirubin (mg/dl) = Abs of the sample blank x 15”. 
9. Estimation of Urea

The nitrogen-containing end product of protein catabolism is Urea. The elevated levels of urea in blood is referred to as hyperuremia or azotemia.

Method

Urease-GLDH: enzymatic UV test was used for the estimation of Urea

Procedure

a. 1000 μl of reagent-1 and 250 μl of reagent-2 was mixed in a 5 ml test tube

b. 10 μl of serum was added to this mixture

c. Mixed well and the test sample was read immediately at 340nm Hg, 334nm Hg, 365nm Hg optical path, 1cm against the reagent blank (2-point kinetic).

d. The values were recorded

Normal range: 10 – 50 mg/dl.

10. Estimation of Uric Acid

“Purine metabolism results in the end products uric acid and its salts. Hyperuricemia is the most common complication in gout, i.e., increased uric acid levels in the serum resulting in the formation of monosodium urate crystal around the joints.

Method

Enzymatic photometric test using TOOS (N ethyl- N (hydroxyl -3- sulfopropyl)-m-toluidin)

Principle

\[
\text{Uric acid} + \text{H}_2\text{O} + \text{O}_2 \xrightarrow{\text{uricase}} \text{Allantoin} + \text{CO}_2 + \text{H}_2\text{O}_2
\]

TOOS + 4 aminoantipyrine + 2H_2O_2 \xrightarrow{\text{POD}} \text{Indamine} + 3\text{H}_2\text{O}”. 
**Procedure**

a. 800µl of reagents -1 was taken in a 2ml centrifuge tube.
b. 20µl of serum was added to this
c. Mixed well and incubate at 30°C for 5 minutes.
d. Further, 200µl of reagent 2 was added to the mixture
e. Mixed well and incubate for 5min at 37°C
f. The values were measured and recorded

**Normal range:** 1.9-8.2mg/dl

11. Estimation of Creatinine:

**Principle:**

“Creatinine when mixed with picrate forms a coloured product in alkaline medium.

The rate of formation of the complex is measured.

**Reagents:**

Reagent 1 Standard Creatinine (2mg/100ml)

Reagent 2 Picric acid solution.

Reagent 3 sodium hydroxide solution

**Procedure**

500 µl of reagent -2 and 500 µl of reagent -3 was taken in a 5ml test tube. 100 µl of serum was added to this and mixed well. The test sample was read immediately at Hg 492 nm 1cm light path and not the values recorded”. 
5.4.2 ANTI ULCER STUDIES:

Anti ulcer study of Sangu parpam II was carried out in three different models. They are

- Pylorus ligation method
- Ethanol/HCL induced ulcer method
- Stress induced ulcer method

Approval of the study

All the protocols of the efficacy studies were approved by Institutional animal Ethics committee, KMCH, Coimbatore. The approval number is KMCRET/MD (S)/11/2014-15

Animals:

Albino Wistar Rats were procured from Sree Venkateshwara Enterprises Pvt. Ltd., Bangalore for the study.

Housing of the animals:

The animals were maintained in a clean environment with 12 alternative hours of light and dark cycles. The relative humidity was maintained in the range of 30-70% with air conditioning at 22±3ºC and 100% exhaust facility.

Feed & water

All through the study period, the animals were fed with standard pelleted diet (Nutrilab rodent, Provimi Animals Nutrition India Private Limited, Bengaluru, India) and purified RO water (Kent RO water filter cum purifier).
PYLORIC LIGATION MODEL: 62,63,64

The ulcer protective effect of SANGU PARPAM II was studied according to the Shay et al., (1945) method. Accumulation of acidic gastric juice was found to cause the ulceration and several parameters can be estimated by this method.

**Method:** Albino Wister rats of either sex weighing between 150 to 200gms were divided into six groups of 6 animals each.

- **Group I:** Control (Ghee 5ml/kg)
- **Group II:** Only pylorus ligation
- **Group III:** pylorus ligation + Ranitidine 30 mg/kg body weight, oral.
- **Group IV:** pylorus ligation + SANGU PARPAM (II) 9.36mg/200gm
- **Group V:** pylorus ligation + SANGU PARPAM (II) 46.8mg/200gm
- **Group VI:** pylorus ligation + SANGU PARPAM (II) 93.6mg/200gm

“According to this method, the Albino Wister Rats were kept under fasting for 24 hours in metabolic cages and was taken care in order to avoid Coprophagy. Control vehicle, three doses of SANGU PARPAM II and the standard drug (Ranitidine 30 mg/kg) were given at different doses for five days orally. At the end of the fifth day, the animals were kept under fasting for 14 hours with water ad libitum. About 30 minutes before the ligation, SANGU PARPAM II was administered to the animals. Under Light ether anesthesia, the abdomen was opened and pylorus ligated. Care was taken in order to avoid bleeding or to occlude blood vessels and the abdomen was sutured. The animals were then sacrificed after 6 hours of pyloric ligation under surplus of anesthetic ether and the stomach was dissected out. Gastric juice was collected from the sacrificed animal and its volume, pH, free acidity and total acidity was measured; Ulcer index was also determined. Evaluation of antioxidant enzymes, SOD, CAT, lipidperoxidation, Myeloperoxidation, and Histopathological evaluation were done from the excised stomach”.
Ulcer Index:

Procedure

“The glandular portion of the stomach was opened along the greater curvature and fixed on a cork plate. A Stereo-microscope was used in order to access the number and the severity of ulcers using the following scores.

Mean ulcer score for each animal was expressed as Ulcer Index.

Calculation:

Ulcer index was calculated as;

\[
\text{Ulcer index (UI)} = \frac{10 \times \text{ulcerated area (mm}^2\text{)}}{\text{total stomach area (mm}^2\text{)}}
\]

The percentage protection was calculated using the formula:

\[
\text{Percentage of ulcer protection} = \frac{U_t}{U_c} \times 100
\]

Where \(U_t\) = Ulcer index of treated group and \(U_c\) = Ulcer index of the control

\% of ulcer protection was calculated by

\% of ulcer protection = \(\frac{M_c - M_t}{M_c}\) x100”

Ulcer scores

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Stomach colours</th>
<th>Ulcer score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal colour</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Red colour</td>
<td>0.5</td>
</tr>
<tr>
<td>3</td>
<td>Red spots</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>Hemorrhagic streaks</td>
<td>1.5</td>
</tr>
<tr>
<td>5</td>
<td>(3 &gt; 5) ulcers</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>(&lt; 5) ulcers</td>
<td>3</td>
</tr>
</tbody>
</table>
1) ESTIMATION OF FREE AND TOTAL ACIDITY:

- “Freshly prepared 0.01N oxalic acid solution (BDH) was used to standardize sodium hydroxide.
- Freshly prepared 0.01N sodium hydroxide
- Topfer’s reagent. It is dimethylaminoazobenzene 0.5% in absolute ethanol available 100ml package.
- Freshly prepared 1% Phenolphthalein (BDH) solution prepared in 50% absolute ethanol.

METHODS FOR BIOCHEMICAL ESTIMATION OF FREE AND TOTAL ACIDITY IN GASTRIC JUICE:

COLLECTION OF GASTRIC JUICE:

From the pylorus ligated rats, the gastric juice was collected, centrifuged and its volume and pH was recorded. It was also subjected for determining the free and total acidity as follows. 62, 63, 65

DETERMINATION OF FREE AND TOTAL ACIDITY

To 1 ml of the gastric juice pippetted into a 100ml conical flask, 2 to 3 drops of Topfer’s reagent was added. It was then titrated using 0.01N Sodium hydroxide solution until the traces of red color vanished and the colour of the gastric juice was changed to yellowish orange. The titration point was noted and this volume corresponds to free acidity. The gastric juice was further titrated after adding 2 to 3 drops of phenolphthalein solution until a red tinge appears. Once again the titration point was noted and this volume corresponds to the total acidity. 63, 64

Acidity was calculated by using the formula:

\[
\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH} \times 100}{\text{mcq/lit/100gm}}
\]

HCL/ETHANOL INDUCED ULCER: 66

Animals:

Albino Wister Rats were obtained from ‘Sree Venkateshwara Enterprises Pvt Ltd’, Bangalore, for the study and they were 150-200gm in weight.
Housing of the Animals:

Before the start of the study, the animals were allowed to acclimatize for a week under the laboratory conditions. They were provided with water and standard rat feed ad libitum but were detained from the feed 12 hours prior to the start of the study.

Method:

Albino Wister rats were divided into 6 groups of 6 animals each. The animals were of either sex and were of nearly 150-200 gms in weight.

Group I: Control (Ghee 5 ml/kg)
Group II: Negative Control (HCL/ETHANOL mixture containing 0.15 N HCL in 70% v/v Ethanol 1.5 ml) p.o
Group III: HCL/ETHANOL+ Ranitidine 30 mg/kg body weight, oral.
Group IV: HCL/ETHANOL+ SANGU PARPAM (II) 9.36mg/200gm
Group V: HCL/ETHANOL+ SANGU PARPAM (II) 46.8mg/200gm
Group VI: HCL/ETHANOL+ SANGU PARPAM (II) 93.6mg/200gm

GASTRIC ULCER INDUCTION BY HCL/ ETHANOL

“The animals were kept under fasting for 24 hours except for drinking water ad libitum until 2 hours before the start of the experiment. Gastric injury was induced with acidified ethanol solution (150 mMCl/ absolute ethanol) 40:60 v/v. (HCl/ethanol solution), as per a modification of the method 67. Ghee was administered orally to the Normal control groups and normal saline was administered to the Ulcer control groups. For the Reference group, 20 mg/kg omeprazole was orally administered and for the experimental groups, oral administration of Sangu parpam 9.36mg, 46.8mg, 93.6mg/200gm was given. After one hour of this pretreatment, ghee and normal saline was orally administered to the normal control group and ulcer control group respectively. Except normal control group all the experimental group were administered with HCl/ ethanol solution (5ml/kg ) orally for inducing gastric ulcers. With an excess of xylazine and ketamine anesthesia, the rats were euthanized 60 minutes after the treatment. Their stomach was immediately excised and the ulcer
index determined. The anti oxidant enzymes SOD, CAT, GPx, Lipid peroxidation, MPO were analysed.  

**Ulcer Index:**

**Procedure**

The glandular portion of the stomach was opened along the greater curvature and fixed on a cork plate. A Stereo-microscope was used in order to access the number and the severity of ulcers using the following scores.

Mean ulcer score for each animal was expressed as Ulcer Index.

**Calculation:**

Ulcer index was calculated as;

\[ \text{Ulcer index (UI)} = \left( \frac{10 \times \text{ulcerated area (mm2)}}{\text{total stomach area (mm2)}} \right) \]

The percentage protection was calculated using the formula:

\[ \text{Percentage of ulcer protection} = \frac{U_t}{U_c} \times 100 \]

Where \( U_t \) = Ulcer index of treated group and  
\( U_c \) = Ulcer index of the control  
% of ulcer protection was calculated by  
% of ulcer protection = \( \frac{M_c - M_t}{M_c} \times 100 \)"

**5.4.2.d) COLD RESTRAINT STRESS-INDUCED ULCERS MODEL:**  

**Animals:**

Albino Wister Rats were obtained from ‘Sree Venkateshwara Enterprises Pvt Ltd’, Bangalore, for the study and they were 150-200gm in weight.
Housing of the Animals:

Before the start of the study, the animals were allowed to acclimatize for a week under the laboratory conditions. They were provided with water and standard rat feed ad libitum but were detained from the feed 12 hours prior to the start of the study.

Method:

Albino Wister rats were divided into 6 groups of 6 animals each. The animals were of either sex and were of nearly 150-200 gms in weight.

Group I: Control (Ghee 5 ml/kg)
Group II: Cold Restraint Stress (CRS)
Group III: CRS + Ranitidine 30 mg/kg body weight, oral.
Group IV: CRS + SANGU PARPAM (II) 9.36mg/200gm
Group V: CRS + SANGU PARPAM (II) 46.8mg/200gm
Group VI: CRS + SANGU PARPAM (II) 93.6mg/200gm

STRESS INDUCTION

“The animals were divided randomly into 6 groups and each group contained 6 rats: Control, CRS, CRS+STD and CRS + SANGU PARPAM II 9.36mg, 46.8mg, 93.6 mg / 200gm b.wt. The control group consists of normal and healthy animals neither pretreated with the sample nor stress induced. 1ml of ghee was administered to this control group intragastrically (ig) using a metal tube for gayage for 14 days. This was the normal healthy group of animals without any pretreatment or stress induction. Ranitidine (30 mg/kg daily, p.o.) dissolved in distilled water for 14 days was given to the second group. The groups IV, V, VI belonging to CRS+SP II received Sangu Parpam (9.36, 46.8, 93.6mg/200gm, p.o. daily) mixed with ghee for 14 days. Furthermore, 120 minutes prior to the induction of CRS, the last dose was administered. The day before inducing the stress, all the animals were kept under fasting for feed for about 24 hours but water, in individual metabolic cages. The animals from CRS and CRS+SP II group were kept under cold stress (4 ±1 °C) for 3.5
hours and were also immobilized in individual restraint boxes to avoid the possibility of visual contact, 16. This type of cold-restraint stress schedule has been reported to produce ulcers in food-deprived rats, 17, 18, and also plasma and hepatic tissue lipid peroxidation, 19. After the treatment regime, the animals were sacrificed under ether anesthesia, their stomach was isolated by midline incision of the abdomen. It was opened along the greater curvature, rinsed gently with water and pinned open for macroscopic examination. Based on the below mentioned rating scale, the number and severity of the gastric lesions were evaluated, 20: 0- no lesion; 1 – mucosal edema and petechiae; 2 – from 1 to 5 small lesions (1-2 mm); 3 – more than 5 small lesions or 1 intermediate lesion (3–4 mm); 4 – 2 or more intermediate lesions or 1 large lesion (greater than 4 mm); 5 – perforated ulcers. The evaluation of Ulcer index was done from the excised stomach. The antioxidant enzymes SOD, CAT, GPx, Lipid peroxidation, MPO were analysed.

The sum of the total scores divided by the number of animals in the group was expressed as the ulcer index ($UI)\pm$standard deviation ($SD$). The percent inhibition of $UI$ in relation to the CRS group was estimated from formula:

$$% \text{Inhibition} = (1-\frac{UI_{SPII + CRS}}{UI_{CRS}}) \times 100$$

$UI_{SPII + CRS}$= Ulcer Index of Sangu parpam II with CRS

$UI_{CRS}$= Ulcer Index of CRS alone

**IN -VIVO ANTI-OXIDANT ACTIVITY**

**Estimation of Protein**

**Principle**

This method involves 2 steps and is a combination of both Folin-Ciocalteau and Biuret reaction.

Step-1- Protein, in alkaline medium, combines with copper and reduces it to Cu++.
Step-2- The oxidation of aromatic amino acid is catalysed by thus formed Cu++ by reducing phosphomolybdo tungstate to polymolybdanum. This results in the formation of blue colour and the absorbance was measured at 640nm.

Reagents

✓ “Alkaline copper reagent
✓ Solution A - 2 % sodium carbonate in 0.1 NNaOH.
✓ Solution B - 0.5 % copper sulphate in 1 % sodium potassium tartarate 50 ml of solution was mixed with 1 ml of solution B just before use.
✓ Folin's phenol reagent (commercial reagent, 1:2 dilutions) bovine serum albumin (BSA).

Procedure

To 0.1 ml of the homogenate, 0.9 ml of water, 4.5 ml of alkaline copper sulphate reagent was added. The mixture was allowed to stand for 10 minutes at room temperature. After 20 minutes of adding 0.5ml of Folin’s reagent to this mixture, the colour produced was measured at 640nm and the protein level was expressed as mg/g tissue or mg/dl.

13. Determination of superoxide dismutase

The first line of defence against the free radical damage is provided by Superoxide dismutase which prevents the damage by scavenging the superoxide radical (O$_2^-$). It is an endogenous enzymatic antioxidant and catalyses the dismutation of superoxide free radical. This method is based according to the principle of inhibition of the spontaneous oxidation of adrenaline to adrenochrome by superoxide dismutase. Superoxide anion (O$_2^-$) interacts with peroxide to form hydroxyl radical (OH’) which causes damage in the absence of superoxide dismutase activity (R’)

Reagents

Carbonate buffer – 0.05M, pH 10.2: 1.14 g of sodium carbonate and 84 g of sodium bicarbonate were dissolved in 100 ml of distilled water.
Ethylene diamine tetra acetate – 0.49M: 14.3 g of EDTA was dissolved in 100 ml of distilled water.

Epinephrine – 3M: 54 mg of epinephrine was dissolved in 100 ml of distilled water.

**Procedure**

The protocol described by Kakkar et al was used for estimating SOD. To a mixture of stomach homogenate (0.5ml) and 0.5ml of distilled water, 0.25ml ethanol and 0.15ml of chloroform, chilled reagents were added. The solution was shaken for 1 minute and centrifuged at 2000 rpm. To 0.5ml of the separated supernatant, 1.5ml of buffer was mixed. The reaction was initiated by adding 0.4ml of epinephrine and a double beam UV-VIS spectrophotometer (UV 1700, Szhimadzhu) was used to estimate the change in the OD per minute at 480nm.

SOD activity was expressed as U/mg. Change in optical density per minute at 50% inhibition to adrenochrome transition by the enzyme is taken as one enzyme unit.

**14. Determination of Catalase**

Catalase is present in all major body organs in animals, especially of more concentration in liver and erythrocytes. Hydrogen peroxide generated during the β-oxidation of fatty acids by flavoprotein dehydrogenase is accepted by catalase present in peroxisomes.

Catalase catalyses the rapid decomposition of hydrogen peroxide to water.

\[
2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2
\]

When the dichromate in acetic acid is heated in the presence of hydrogen peroxide, it was converted to perchloric acid and further to chromic acetate, which is measured spectrophotometrically at 610nm. The reaction is stopped at intervals by adding dichromate-acetic acid mixture and the remaining hydrogen peroxide is measured by estimating chromic acetate.
Reagents

- Dichromate-acetic acid reagent: Five % potassium dichromate was prepared with acetic acid (1:3 v/v in distilled water).
- Phosphate buffer - 0.01M, pH 7.0: 173 mg of disodium hydrogen phosphate and 122 mg of sodium dihydrogen phosphate were dissolved in 200 ml of distilled water.
- Hydrogen peroxide – 0.2M: 2.27 ml of hydrogen peroxide was made up to 100 ml with distilled water.

Procedure

Sinha (1972) method was used to assay the catalase activity. With 0.1ml of the homogenate, 1.0ml of phosphate buffer and hydrogen peroxide was added and the reaction was stopped by adding 0.2ml of dichromate acetic acid mixture. Similarly treatment was given to the standard hydrogen peroxide in the range of 4 to 20 µl. The tubes were kept in a boiling water bath for 10 min and the resulting green color was read in a Double beam UV-VIS spectrophotometer (UV 1700, Szhimadzhu) and the activity was expressed as U/mg.

15. Determination of glutathione peroxidase

Glutathione peroxidase catalyses the following reaction.

\[
\text{R-COOH} + 2\text{GSH} + \text{Glutathione peroxide} \rightarrow \text{H(OH)-COOH} + \text{GSSG} + \text{H}_2\text{O}
\]
Glutathione was measured by its reaction with DTNB to give a compound that absorbs at 412 nm.

Reagents

- Sodium phosphate buffer – 0.32M, pH 7.0: 6.96 g of disodium hydrogen phosphate and 3.89 g of sodium dihydrogen phosphate was dissolved in 200 ml of distilled water.
- Ethylene diamine tetra acetate (EDTA) – 0.8 mM: 233 mg of EDTA was dissolved in 100 ml of distilled water.
- Sodium azide-10mM: 6.5 mg of sodium azide was dissolved in 100 ml of distilled water.
- Reduced glutathione – 4 mM: 122 mg of glutathione was dissolved in 100 ml of distilled water.
- Hydrogen peroxide – 2.5 mM: 0.03 ml of H₂O₂ was made up to 100 ml with distilled water.
- Trichloro acetic acid – 10%: 10 g of TCA was dissolved in 100 ml of distilled water.
- Disodium hydrogen phosphate – 0.3 M: 4.25 g of disodium hydrogen phosphate was dissolved in 100 ml of distilled water.
- DTNB: 40 mg of 5,5'-dithio bis (2-nitrobenzoic acid) was dissolved in 100 ml of 1% w/v sodium citrate.
- Reduced glutathione standard: 20 mg of reduced glutathione was dissolved in 100 ml of distilled water.

Procedure

The Rotruck et al., (1973) method was used to measure the glutathione peroxidase activity. EDTA (0.2 ml each), sodium azide, reduced glutathione, H₂O₂; 0.4 ml of buffer and 0.1 ml of enzyme (liver homogenate) were mixed and incubated at 37°C for 10 min. The reaction was arrested by the addition of 0.5 ml of TCA and the tubes were centrifuged. To 0.5 ml of supernatant, 3 ml of sodium hydrogen phosphate and 1 ml of DTNB were added and the color developed was read at 412 nm immediately in a Double beam UV-VIS spectrophotometer (UV 1700, Szhimadzhu. Glutathione peroxidase activity, in serum is expressed as µg/mg.
16. Determination of lipid peroxidation

The reaction between malondialdehyde and other TBARS with thiobarbituric acid TBA was estimated in acidic condition in this method, to generate a pink coloured chromophore which was read at 535nm.

Reagents:

- TCA-TBA-HCl reagent: 15% w/v TCA, 0.375 w/v TBA and 0.25 N HCl. The solution was heated mildly to assist the dissolution of the TBA.
- 0.25 N HCl
- 3.15% TCA

Procedure

Okhawa et al., (1979) method was used to estimate lipid peroxidation. One ml of stomach homogenate was mixed with 0.2 ml 4 % (w/v) sodium dodecyl sulfate, 1.5 ml 20% acetic acid in 0.27 M hydrochloric acid (pH 3.5) and 15 ml of 0.8% thiobarbituric acid (TBA, pH 7.4). The mixture was heated in a hot water bath at 85°C for 1 h. The intensity of the pink colour developed was read against a reagent blank at 532 nm following centrifugation at 1200 g for 10 min. The concentration was expressed as n moles of MDA per mg of protein using 1,1,3,3,4-tetra-ethoxypropane as the standard. The results were tabulate.

17. Myeloperoxidase assay for quantification of inflammation

Pieces of inflamed tissues of rat ileum (2 cm) were taken. The tissue was then rinsed with ice-cold saline, blotted dry, weighed and excised. Minced tissue was homogenized in 10 volumes of ice-cold potassium phosphate buffer (pH 7.4), using Remi tissue homogenizer (RQ-127A). The homogenate was centrifuged at 3500 rpm for 30 min at 4ºC (Remi centrifuge C23). The supernatant was discarded. 10 mL of ice-cold 50 mM potassium phosphate buffer (pH 6.0), containing 0.5% hexadecyltrimethyl ammonium bromide (HETAB) and 10mM EDTA was then added to the pellet. It was then subjected to one cycle of freezing and thawing and brief period of sonication (15s). After sonication, solution was centrifuged at 15,000 rpm for 20 min
(Remi centrifuge, C23) and the supernatant stored. Myeloperoxidase (MPO) activity in the supernatant was determined by adding 100µl of supernatant to 1.9 mL of 50 mM phosphate buffer (pH 6.0) and 1 mL of 1.5 mol/l 0.167 mg/mL O-dianisidine hydrochloride containing 0.0005% H₂O₂.

The Change in absorbance at 460 nm of each sample was recorded for 3 min using spectrophotometrically (Shimadzu UV 1 60A UV-VIS spectrophotometer). MPO activities of the tissues were expressed as µmol/min/mg tissue (Elson et al., 1995)". 
6. Results

6.1 Authentication of Sangu

Sample of Sangu is to be collected from Chennai market and various places of marine resources throughout Tamil Nadu and authenticated by Marine Biology Regional Centre, Chennai. And other ingredients which are necessary for purification and preparation were authenticated by Department of Medicinal botany, National institute of Siddha, Chennai.

6.2 Preparation process

Purification of ingredients

Three purification processes were done as per the proposal and the samples were collected and named as Process I: Sample - Spu I, Process II: Sample - Spu II, Process III: Sample - Spu III

Preparation of Sangu Parpam

As per the proposal Sangu parpam prepared from purified Sangu Spu I, Spu II and Spu III and named as SP I, SP II and SP III respectively.
### Table 6.3.1: Organoleptic characters:

<table>
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<tr>
<th>S.No</th>
<th>Parameters</th>
<th>Raw Sangu</th>
<th>Spu I</th>
<th>Spu II</th>
<th>Spu III</th>
<th>SP I</th>
<th>SP II</th>
<th>SP III</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>pH at 25 °C (1:10 ratio)</td>
<td>8.06</td>
<td>9.31</td>
<td>8.05</td>
<td>8.83</td>
<td>9.33</td>
<td>9.32</td>
<td>9.12</td>
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<tr>
<td>2</td>
<td>Total ash value</td>
<td>82.77%</td>
<td>72.36%</td>
<td>65.49%</td>
<td>74.54%</td>
<td>76.25%</td>
<td>68.40%</td>
<td>79.86%</td>
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<td>Acid insoluble ash</td>
<td>2.37%</td>
<td>2.28%</td>
<td>0.75%</td>
<td>3.56%</td>
<td>10.40%</td>
<td>7.48%</td>
<td>10.33%</td>
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<td>4</td>
<td>Loss of drying at 105°C</td>
<td>0.25%</td>
<td>0.10%</td>
<td>0.28%</td>
<td>0.12%</td>
<td>0.11%</td>
<td>0.10%</td>
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</table>
Table 6.3.2: SEM Analysis:

Raw Sangu

Sample - Spu I

Sample – Spu II

Sample – Spu III

Sample – SP I

Sample – SP II

Sample – SP III
Table 6.3.3: ICPOES Analysis of samples:

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<th>S.N</th>
<th>Elements in ppm level</th>
<th>Raw Sangu</th>
<th>Spu I</th>
<th>Spu II</th>
<th>Spu III</th>
<th>SP I</th>
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<th>SP III</th>
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<tr>
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<td>BLQ</td>
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<td>BLQ</td>
<td>BLQ</td>
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<td>4</td>
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Table 6.3.4: EDAX Analysis of samples:

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<th>Spu I</th>
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<th>Spu III</th>
<th>SPI</th>
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<th>SP III</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Wt %</td>
<td>At %</td>
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6.4 Preclinical Studies:

6.4.1 Toxicity Studies

Table 6.4.1A - Physical and behavioral examinations for SANGU PARPAM I, II, III

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<thead>
<tr>
<th>Group no.</th>
<th>Drug</th>
<th>Dose(mg/kg)</th>
<th>Observation sign</th>
<th>No. of animal affected.</th>
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<td>Group-I</td>
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</tr>
<tr>
<td>VII</td>
<td>SP II</td>
<td>300 mg/kg</td>
<td>Normal</td>
<td>0 of 3</td>
</tr>
<tr>
<td>VIII</td>
<td>SP II</td>
<td>1000mg/kg</td>
<td>Normal</td>
<td>0 of 3</td>
</tr>
<tr>
<td>IX</td>
<td>SP II</td>
<td>2000mg/kg</td>
<td>Normal</td>
<td>0 of 3</td>
</tr>
<tr>
<td>X</td>
<td>SP III</td>
<td>50 mg/kg</td>
<td>Normal</td>
<td>0 of 3</td>
</tr>
<tr>
<td>XI</td>
<td>SP III</td>
<td>300mg/kg</td>
<td>Normal</td>
<td>0 of 3</td>
</tr>
<tr>
<td>XII</td>
<td>SP III</td>
<td>1000mg/kg</td>
<td>Normal</td>
<td>0 of 3</td>
</tr>
<tr>
<td>XIII</td>
<td>SP III</td>
<td>2000 mg/kg</td>
<td>Normal</td>
<td>0 of 3</td>
</tr>
</tbody>
</table>
TABLE 6.1B: EFFECT OF SUB-ACUTE DOSES (28 DAYS) SANGU PARPAM I,II, III ON BODY WEIGHT (PHYSICAL PARAMETER)

<table>
<thead>
<tr>
<th>GROUP</th>
<th>O day</th>
<th>7th day</th>
<th>14th day</th>
<th>21st day</th>
<th>28th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>125.33±2.76</td>
<td>133.83±3.09</td>
<td>143.5±2.47</td>
<td>152.17±2.86</td>
<td>161.67±2.044</td>
</tr>
<tr>
<td>S.P I-LOW DOSE</td>
<td>118±2.49</td>
<td>126.5±2.45</td>
<td>136±1.69</td>
<td>147.16±1.93</td>
<td>159.17±1.86</td>
</tr>
<tr>
<td>S.P I-MID DOSE</td>
<td>128.5±3.20</td>
<td>136.5±3.22</td>
<td>147.67±3.23</td>
<td>157.67±3.58</td>
<td>165.67±2.52</td>
</tr>
<tr>
<td>S.P I-HIGH DOSE</td>
<td>139.83±2.17</td>
<td>149±2.53</td>
<td>159.67±2.43</td>
<td>168.67±3.26</td>
<td>174.33±2.49</td>
</tr>
<tr>
<td>S.P II-LOW DOSE</td>
<td>146.5±3.79</td>
<td>153.33±5.05</td>
<td>145.17±5.69</td>
<td>147.5±3.93</td>
<td>158.83±2.61</td>
</tr>
<tr>
<td>S.P II-MID DOSE</td>
<td>145.17±2.020</td>
<td>148.5±2.5</td>
<td>149.33±2.82</td>
<td>155.33±5.09</td>
<td>164.16±3.24</td>
</tr>
<tr>
<td>S.P II-HIGH DOSE</td>
<td>147.5±4.23</td>
<td>142±3.14</td>
<td>151.33±2.80</td>
<td>146.16±2.79</td>
<td>159.16±4.35</td>
</tr>
<tr>
<td>S.P III-LOW DOSE</td>
<td>149.5±4.42</td>
<td>146±1.37</td>
<td>151.5±3.30</td>
<td>152.5±2.85</td>
<td>163.83±2.64</td>
</tr>
<tr>
<td>S.P III-MID DOSE</td>
<td>141.33±2.47</td>
<td>141.33±3.41</td>
<td>153±5.43</td>
<td>153±4.55</td>
<td>163±3.087</td>
</tr>
<tr>
<td>S.P III-HIGH DOSE</td>
<td>149.83±2.66</td>
<td>136.83±2.90</td>
<td>158.83±4.003</td>
<td>161.5±1.71</td>
<td>158.83±3.45</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one way ANOVA followed by dunnett’s \(^{a}P< 0.001,^{b}P< 0.01,^{a}P < 0.05\) calculated by comparing treated group with CONTROL group.
EFFECT OF SUB-ACUTE DOSES (28 DAYS) OF SANGU PARPAM I, II, III ON FOOD INTAKE
EFFECT OF SUB-ACUTE DOSES (28 DAYS) OF SANGU PARPAM I, II, III ON WATER INTAKE
Table 6.4.1C – EFFECT OF SUB-ACUTE DOSES (28 DAYS) OF SANGUPARPAM I, II, III ON HAEMATOLOGICAL PARAMETERS

<table>
<thead>
<tr>
<th>GROUP</th>
<th>WBC</th>
<th>POLY MORMS</th>
<th>RBC</th>
<th>HB</th>
<th>PCV</th>
<th>LYMPHO CYTES</th>
<th>MONO CYTES</th>
<th>EOSINO PHILS</th>
<th>MCH</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>10.57±0.79</td>
<td>7±1.53</td>
<td>4.49±1.14</td>
<td>15.83±0.58</td>
<td>48.87±2.41</td>
<td>84.67±1.52</td>
<td>3±0.63</td>
<td>6±1.15</td>
<td>26.63±0.59</td>
</tr>
<tr>
<td>S.P I-LOW DOSE</td>
<td>12.47±0.83</td>
<td>9.33±1.85</td>
<td>6.10±0.27</td>
<td>15.20±0.80</td>
<td>45.9±2.47</td>
<td>81±1.67</td>
<td>3.67±0.76</td>
<td>6±0.58</td>
<td>24.5±0.79</td>
</tr>
<tr>
<td>S.P I-MIDL DOSE</td>
<td>13.3±0.63</td>
<td>4.33±1.85</td>
<td>5.23±0.07</td>
<td>13.53±0.15</td>
<td>41.37±1.70</td>
<td>87.33±1.87</td>
<td>3.67±0.76</td>
<td>4.67±0.88</td>
<td>24.76±0.50</td>
</tr>
<tr>
<td>S.P I-HIGE DOSE</td>
<td>12.27±0.55*</td>
<td>5.67±0.33</td>
<td>5.93±0.22</td>
<td>15.07±0.56</td>
<td>45.77±1.06</td>
<td>84±0.73</td>
<td>4.33±0.56</td>
<td>5.67±0.88</td>
<td>28.2333±0.329309</td>
</tr>
<tr>
<td>S.P II-LOW DOSE</td>
<td>11.8±0.34</td>
<td>5.33±1.20</td>
<td>5.52±0.25</td>
<td>14.63±0.67</td>
<td>40.77±2.17</td>
<td>77.67±3.83</td>
<td>3±0.36</td>
<td>3.67±0.66</td>
<td>22.63±0.67</td>
</tr>
<tr>
<td>S.P II-MIDL DOSE</td>
<td>10.83±0.35</td>
<td>6.33±1.33</td>
<td>5.49±0.24</td>
<td>15.73±0.78</td>
<td>49.23±6.07</td>
<td>73.67±3.15*</td>
<td>4.33±0.21</td>
<td>4±1.15</td>
<td>26.4±1.19</td>
</tr>
<tr>
<td>S.P II-HIGE DOSE</td>
<td>11.7±1.02</td>
<td>6±1.53</td>
<td>5.85±0.15</td>
<td>14.33±0.54</td>
<td>42.8±4.69</td>
<td>77.67±4.20</td>
<td>4.67±0.56</td>
<td>4±1.16</td>
<td>17.9667±1.69c</td>
</tr>
<tr>
<td>S.P III-LOW DOSE</td>
<td>10.67±0.35</td>
<td>7.67±0.67</td>
<td>6.26±0.20*</td>
<td>14.63±0.75</td>
<td>46.17±7.55</td>
<td>81.67±2.01</td>
<td>5±0.36</td>
<td>5.67±0.88</td>
<td>20.96±2.03a</td>
</tr>
<tr>
<td>S.P III-MIDL DOSE</td>
<td>12.23±0.79</td>
<td>6±0.58</td>
<td>6.34±0.21*</td>
<td>12.47±0.52**</td>
<td>41.3±1.88</td>
<td>88±1.26</td>
<td>4±0.36</td>
<td>5.33±1.76</td>
<td>21.66±2.03</td>
</tr>
<tr>
<td>S.P III-HIGE DOSE</td>
<td>12.83±0.59</td>
<td>7.33±0.67</td>
<td>5.59±0.27</td>
<td>15.6±0.73</td>
<td>40.9±1.51</td>
<td>77±3.48</td>
<td>3.67±0.76</td>
<td>4.67±0.88</td>
<td>23.86±1.48</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one way ANOVA followed by dunnett’s; p< 0.001, bP < 0.01, *P < 0.05 calculated by comparing treated group with CONTROL group.
Table 6.4.1D - EFFECT OF SUB-ACUTE DOSES (28 DAYS) SANGUPARPAM I, II, III ON ORGAN WEIGHT IN GMS (PHYSICAL PARAMETER)

<table>
<thead>
<tr>
<th>GROUP</th>
<th>BRAIN</th>
<th>HEART</th>
<th>LUNGS</th>
<th>LIVER</th>
<th>TESTIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>1.12±0.06</td>
<td>0.89±0.02</td>
<td>2.31±0.21</td>
<td>6.86±0.43</td>
<td>2.95±0.29</td>
</tr>
<tr>
<td>S.P I-LOW DOSE</td>
<td>1.20±0.12</td>
<td>0.89±0.03</td>
<td>1.01±0.10</td>
<td>5.04±0.45</td>
<td>2.39±0.23</td>
</tr>
<tr>
<td>S.P I-MIDL DOSE</td>
<td>1.65±0.09</td>
<td>0.82±0.03</td>
<td>1.32±0.16</td>
<td>5.53±0.59</td>
<td>2.00±0.19</td>
</tr>
<tr>
<td>S.P I-HIGE DOSE</td>
<td>1.49±0.23</td>
<td>1.094±0.05*</td>
<td>2.06±0.14</td>
<td>6.05±0.12</td>
<td>2.57±0.21</td>
</tr>
<tr>
<td>S.P II-LOW DOSE</td>
<td>1.52±0.23</td>
<td>1.08±0.08*</td>
<td>1.71±0.19</td>
<td>6.42±0.39</td>
<td>2.44±0.16</td>
</tr>
<tr>
<td>S.P II-MIDL DOSE</td>
<td>1.08±0.07</td>
<td>0.96±0.03</td>
<td>1.69±0.23</td>
<td>5.78±0.55</td>
<td>1.37±0.15</td>
</tr>
<tr>
<td>S.P II-HIGE DOSE</td>
<td>1.02±0.03</td>
<td>0.97±0.02</td>
<td>1.41±0.15</td>
<td>6.78±0.59</td>
<td>3.04±0.20</td>
</tr>
<tr>
<td>S.P III-LOW DOSE</td>
<td>1.29±0.10</td>
<td>0.92±0.04</td>
<td>1.46±0.20</td>
<td>6.43±0.69</td>
<td>3.27±0.19</td>
</tr>
<tr>
<td>S.P III-MIDL DOSE</td>
<td>1.31±0.04</td>
<td>0.87±0.02</td>
<td>1.54±0.20</td>
<td>6.42±0.19</td>
<td>2.79±0.47</td>
</tr>
<tr>
<td>S.P III-HIGE DOSE</td>
<td>1.38±0.09</td>
<td>0.94±0.05</td>
<td>2.06±0.10</td>
<td>6.43±0.93</td>
<td>3.25±0.15</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one way ANOVA followed by dunnett’s *P < 0.001, †P < 0.01, ‡P < 0.05 calculated by comparing treated group with CONTROL group.
Table 6.4.1D (a)- effect of sub-acute doses (28 days) Sanguparpam I, II, III on organ weight in gms (physical parameter)

<table>
<thead>
<tr>
<th>GROUP</th>
<th>KIDNEY (L)</th>
<th>KIDNEY (R) WEIGHT</th>
<th>UTERUS WEIGHT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.05±0.09</td>
<td>1.04±0.04</td>
<td>0.49±0.02</td>
</tr>
<tr>
<td>S.P I - LOW DOSE</td>
<td>0.69±0.014</td>
<td>0.75±0.03</td>
<td>0.56±0.06</td>
</tr>
<tr>
<td>S.P I - MID DOSE</td>
<td>0.69±0.03</td>
<td>0.68±0.01</td>
<td>0.52±0.05</td>
</tr>
<tr>
<td>S.P I - HIGH DOSE</td>
<td>0.95±0.09</td>
<td>0.89±0.03</td>
<td>0.72±0.02</td>
</tr>
<tr>
<td>S.P II - LOW DOSE</td>
<td>0.82±0.08</td>
<td>0.81±0.08</td>
<td>0.51±0.04</td>
</tr>
<tr>
<td>S.P II - MID DOSE</td>
<td>0.98±0.06</td>
<td>0.94±0.03</td>
<td>0.48±0.06</td>
</tr>
<tr>
<td>S.P II - HIGH DOSE</td>
<td>0.81±0.08</td>
<td>0.79±0.08</td>
<td>0.54±0.12</td>
</tr>
<tr>
<td>S.P III - LOW DOSE</td>
<td>0.91±0.08</td>
<td>0.79±0.07</td>
<td>0.43±0.07</td>
</tr>
<tr>
<td>S.P III - MID DOSE</td>
<td>0.79±0.07</td>
<td>0.78±0.07</td>
<td>0.41±0.02</td>
</tr>
<tr>
<td>S.P III - HIGH DOSE</td>
<td>0.95±0.16</td>
<td>0.87±0.11</td>
<td>0.43±0.08</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one way ANOVA followed by Dunnett’s (a)P < 0.001, (b)P < 0.01, (c)P < 0.05 calculated by comparing treated group with CONTROL group.
ORGAN WEIGHT IN GMS (PHYSICAL PARAMETER)

ORGAN WEIGHT IN GMS (PHYSICAL PARAMETER)
Table 6.4.1E - EFFECT OF SUB-ACUTE DOSES (28 DAYS) OF SANGUPARPAM I, II, III ON BLOOD GLUCOSE LEVEL

<table>
<thead>
<tr>
<th>GROUP</th>
<th>BLOOD GLUCOSE LEVEL</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>CONTROL</td>
<td>78±3.12</td>
<td>79±4.02</td>
<td></td>
</tr>
<tr>
<td>S.P I-LOW DOSE</td>
<td>83.33±1.64</td>
<td>95.33±3.45</td>
<td></td>
</tr>
<tr>
<td>S.P I-MID DOSE</td>
<td>75.33±6.63</td>
<td>83.33±1.87</td>
<td></td>
</tr>
<tr>
<td>S.P I-HIGH DOSE</td>
<td>92±7.62</td>
<td>92±1.46</td>
<td></td>
</tr>
<tr>
<td>S.P II-LOW DOSE</td>
<td>92.33±5.93</td>
<td>79.33±4.10</td>
<td></td>
</tr>
<tr>
<td>S.P II-MID DOSE</td>
<td>91.67±8.10</td>
<td>93.33±2.95</td>
<td></td>
</tr>
<tr>
<td>S.P II-HIGH DOSE</td>
<td>90.33±4.10</td>
<td>78.33±4.93</td>
<td></td>
</tr>
<tr>
<td>S.P III-LOW DOSE</td>
<td>87.67±3.31</td>
<td>89.33±4.02</td>
<td></td>
</tr>
<tr>
<td>S.P III-MID DOSE</td>
<td>94.67±9.02</td>
<td>90.33±6.28</td>
<td></td>
</tr>
<tr>
<td>S.P III-HIGH DOSE</td>
<td>89.67±4.39</td>
<td>92.67±2.35</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one way ANOVA followed by dunnett’s $^a$P < 0.001, $^b$P < 0.01, $^c$P < 0.05 calculated by
Table 6.4.1F – EFFECT OF SUB-ACUTE DOSES (28 DAYS) OF SANGUPARPAM I, II, III ON LIPID PROFILE

<table>
<thead>
<tr>
<th>GROUP</th>
<th>TOTAL CHOLESTEROL (mg/dl)</th>
<th>TRIGLYCERIDES (mg/dl)</th>
<th>HDL- CHOLESTEROL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>37.8±2.89</td>
<td>101.87±1.59</td>
<td>8.68±0.99</td>
</tr>
<tr>
<td>S.P I-LOW DOSE</td>
<td>51.2±1.99&lt;sup&gt;a&lt;/sup&gt;</td>
<td>73.17±2.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.65±1.48</td>
</tr>
<tr>
<td>S.P I-MID DOSE</td>
<td>50.17±3.97</td>
<td>85.27±10.89</td>
<td>5.87±0.93</td>
</tr>
<tr>
<td>S.P I-HIGH DOSE</td>
<td>45.67±2.54</td>
<td>95.13±9.59</td>
<td>8.3±2.60</td>
</tr>
<tr>
<td>S.P II-LOW DOSE</td>
<td>30.33±2.69</td>
<td>77.67±3.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.67±0.42</td>
</tr>
<tr>
<td>S.P II-MID DOSE</td>
<td>32.6±3.61</td>
<td>63±2.22&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.23±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>S.P II-HIGH DOSE</td>
<td>37.67±1.65</td>
<td>47.33±1.83&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.93±0.092&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>S.P III-LOW DOSE</td>
<td>36.67±6.38</td>
<td>74.67±2.56&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.57±0.24</td>
</tr>
<tr>
<td>S.P III-MID DOSE</td>
<td>33.33±3.29</td>
<td>66±3.65&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.43±0.40&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>S.P III-HIGH DOSE</td>
<td>31.33±1.52</td>
<td>53.67±3.31&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.2±0.15&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one way ANOVA followed by dunnett’s<sup>c</sup>P< 0.001, <sup>b</sup>P< 0.01, <sup>a</sup>P < 0.05 calculated by
Table 6.4.1G - EFFECT OF SUB-ACUTE DOSES (28 DAYS) OF SANGUPARPAM I, II, III ON SGOT, SGPT, ALP LEVELS

<table>
<thead>
<tr>
<th>GROUP</th>
<th>SGOT (U/L)</th>
<th>SGPT(U/L)</th>
<th>ALP(U/L)</th>
<th>TOTAL BILIRUBIN (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>79.57±5.56</td>
<td>62.02±6.88</td>
<td>297.73±7.87</td>
<td>0.53±0.08</td>
</tr>
<tr>
<td>S.P I-LOW DOSE</td>
<td>116.7±17.53</td>
<td>41.4±2.99*</td>
<td>301.16±13.18</td>
<td>0.75±0.13</td>
</tr>
<tr>
<td>S.P I-MID DOSE</td>
<td>77.27±4.79</td>
<td>56.67±9.44</td>
<td>267±17.32</td>
<td>0.64±0.03</td>
</tr>
<tr>
<td>S.P I-HIGH DOSE</td>
<td>80.53±0.83</td>
<td>59.47±1.56</td>
<td>254.43±2.30</td>
<td>0.69±0.06</td>
</tr>
<tr>
<td>S.P II-LOW DOSE</td>
<td>126.6±15.30</td>
<td>52.43±1.33</td>
<td>322±20.45</td>
<td>0.55±0.09</td>
</tr>
<tr>
<td>S.P II-MID DOSE</td>
<td>82.87±4.74</td>
<td>48.6±1.42</td>
<td>210.7±4.40</td>
<td>0.66±0.09</td>
</tr>
<tr>
<td>S.P II-HIGH DOSE</td>
<td>71.13±0.86</td>
<td>61.89±1.79</td>
<td>260.3±6.07</td>
<td>0.72±0.06</td>
</tr>
<tr>
<td>S.P III-LOW DOSE</td>
<td>85.43±30.4</td>
<td>44.03±3.27</td>
<td>314.7±24.85</td>
<td>0.64±0.03</td>
</tr>
<tr>
<td>S.P III-MID DOSE</td>
<td>69.27±1.08</td>
<td>59.43±5.36</td>
<td>288.6±1.47</td>
<td>0.75±0.02</td>
</tr>
<tr>
<td>S.P III-HIGH DOSE</td>
<td>63.77±4.56</td>
<td>62.5±3.08</td>
<td>268±10.08</td>
<td>0.73±0.08</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± S.D; Statistical significance (p)calculated by one way ANOVA followed by dunnett’s P< 0.001, bP< 0.01, cP < 0.05 calculated by
Table 6.4.1H - EFFECT OF SUB-ACUTE DOSES (28 DAYS) OF SANGUPARPAM I,II, III ON UREA, URIC ACID, CREATININE LEVELS

<table>
<thead>
<tr>
<th>GROUP</th>
<th>UREA (mg/dl)</th>
<th>URIC ACID (mg/dl)</th>
<th>CREATININE (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>33.46±4.42</td>
<td>1.47±0.08</td>
<td>0.33±0.01</td>
</tr>
<tr>
<td>S.P I-LOW DOSE</td>
<td>32.27±2.49</td>
<td>0.94±0.16</td>
<td>0.28±0.04</td>
</tr>
<tr>
<td>S.P I-MID DOSE</td>
<td>38.63±0.61</td>
<td>1.25±0.03</td>
<td>0.19±0.02</td>
</tr>
<tr>
<td>S.P I-HIGH DOSE</td>
<td>29.41±1.91</td>
<td>1.49±0.08</td>
<td>0.31±0.03</td>
</tr>
<tr>
<td>S.P II-LOW DOSE</td>
<td>45±4.75</td>
<td>8.6±4.39</td>
<td>0.36±0.04</td>
</tr>
<tr>
<td>S.P II-MID DOSE</td>
<td>37.33±1.52</td>
<td>0.89±0.10</td>
<td>0.42±0.05</td>
</tr>
<tr>
<td>S.P II-HIGH DOSE</td>
<td>38±5.79</td>
<td>0.61±0.11</td>
<td>0.34±0.04</td>
</tr>
<tr>
<td>S.P III-LOW DOSE</td>
<td>40.6667±2.59058</td>
<td>1.29±0.175385</td>
<td>0.406667±0.0613551</td>
</tr>
<tr>
<td>S.P III-MID DOSE</td>
<td>33±1.31656</td>
<td>1.14333±0.162986</td>
<td>0.37±0.0255604</td>
</tr>
<tr>
<td>S.P III-HIGH DOSE</td>
<td>45±0.966092</td>
<td>0.686667±0.041524</td>
<td>0.266667±0.0183787</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one way ANOVA followed by dunnett’s aP < 0.001, bP < 0.01, cP < 0.05 calculated by
TOXICITY STUDIES:

**Acute toxicity study**

The trial drugs sangu parpam I, II, III were tested for their acute toxicity study as a step wise procedure. The different doses did not produce any mortality and morbidity throughout the study period.

The animals were healthy and they had gained weight throughout the study period. There was no body weight changes noted. No animals in the groups showed significant variation in food and water intake. After the completion period, there was no grass pathological change noted in all the group of animals.

The animals did not show any mortality and morbidity up to 2000 mg/kg body weight. So, the maximum tolerable can be obtained as 2000 mg/kg body weight.

**28 days repeated oral toxicity study**

The repeated oral administration of Sangu parpam II in three different dose levels did not produce any significant changes in the body weight (Table 6.6) when compared to normal control animals.

There was no significant difference was noticed in the food intake as well as the water intake.

The animals treated with sangu parpam of different doses did not produce any significant variations in the haematological parameters like WBC, polymorphs, RBC, HB, PCV & MCH (Table 6.7)

The animals treated with sangu parpam of different doses did not produce any significant variations in the Blood glucose level. (Table 6.9)

The organs which were collected for histopathological studies were weighed and the mean organ weights were tabulated. There was no significant variation noted in the major organs like brain, heart, lungs, liver, testis kidney and uterus (Table 6.8, 6.8(a))

The animals treated with Sangu parpam of different doses did not produce any variation in Total Bilirubin level, SGOT, SGPT and ALP (Table 6.11) when
compared with the control group animals.

Various dose treatment of Sangu parpam after 28 days did not produce any significant deviation in and urea, Uric Acid and Creatinine (Table 6.12) levels when compared to the normal control levels.

The animals treated with sangu parpam I (Low, Mid, High dose) produce a statistically significant ($P < 0.05$) increase in total cholesterol level and decrease in Triglycerides level (Table 6.10), and in Low and High dose of SP I did not produce any significant changes in HDL cholesterol level and Mid dose of SP I decrease in HDL cholesterol level When compared to the normal control group. SP II low dose and Mid dose and High dose ($P < 0.001$) produce a statistically significant decrease in Triglycerides level and Total Cholesterol level and HDL Cholesterol level ($P < 0.001$) when compared to the control group. SP III low dose, mid dose and high dose produce a statistically decrease in Total cholesterol, Triglyceride and HDL-Cholesterol ($P < 0.01$).

**Histopathology**

**Stomach**

**Control**

Section from the stomach showed normal curvatures.

**Group I (100mg/kg)**

Section from the stomach showed normal curvatures.

**Group II (200mg/kg)**

Section from the stomach showed normal curvatures.

**Group III (300mg/kg)**

Section from the stomach showed normal curvatures.
Heart:

Control

Section from the heart showed normal myocardial fibres.

Group I (100mg/kg)

Section from the heart showed normal myocardial fibres.

Group II (200mg/kg)

Section from the heart showed normal myocardial fibres.

Group III (300mg/kg)

Section from the heart showed normal myocardial fibres.

Lung

Control

Section from the lung showed normal bronchioles and alveoli.

Group I (100mg/kg)

Section from the lung showed normal bronchioles and alveoli.

Group II (200mg/kg)

Section from the lung showed normal bronchioles and alveoli.
Group III (300mg/kg)

Section from the lung showed normal bronchioles and alveoli.

Liver

Control

Section from the Liver showed central veins, rows of hepatocytes and portal triad.

Group I (100mg/kg)

Section from the Liver showed central veins, rows of hepatocytes and portal triad.

Group II (200mg/kg)

Section from the Liver showed central veins, rows of hepatocytes and portal triad.

Group III (300mg/kg)

Section from the Liver showed central veins, rows of hepatocytes and portal triad.

Spleen

Control

Section from the spleen showed white pulp and splenic sinusoids.
Group I (100mg/kg)

Section from the spleen showed white pulp and splenic sinusoids.

Group II (200mg/kg)

Section from the spleen showed white pulp and splenic sinusoids

Group III (300mg/kg)

Section from the spleen showed white pulp and splenic sinusoids

Kidney

Control

Section from the kidney showed renal parenchyma with normally appearing glomerulei and tubules.

Group I (100mg/kg)

Section from the kidney showed renal parenchyma with normally appearing glomerulei and tubules.

Group II (200mg/kg)

Section from the kidney showed renal parenchyma with normally appearing glomerulei and tubules.

Group III (300mg/kg)

Section from the kidney showed renal parenchyma with normally appearing glomerulei and tubules.
### 6.4.2 ANTI ULCER STUDIES

**Table 6.4.2** EFFECT OF SANGU PARPAM II ON FREE ACIDITY AND TOTAL ACIDITY ANALYSIS IN PYLORUS LIGATED GASTRIC ULCER IN RATS

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Only pylorus</th>
<th>Pylorus+ Ranitidine 30 mg/kg</th>
<th>Pylorus+ S.P(II) 9.36mg/200 gm</th>
<th>pylorus+ S.P(II) 46.8mg/200 gm</th>
<th>pylorus+ S.P(II) 93.6mg/200 gm</th>
</tr>
</thead>
<tbody>
<tr>
<td>FREE ACIDITY</td>
<td>36.12±1.1</td>
<td>54.67±1.43</td>
<td>39.50±1.3^a</td>
<td>46.72±1.6^a</td>
<td>40.13±1.02^a</td>
<td>45.16±1.12^a</td>
</tr>
<tr>
<td>TOTAL ACIDITY</td>
<td>58.14±1.43</td>
<td>84.32±1.47^a</td>
<td>59.10±1.5^a</td>
<td>59.10±1.5</td>
<td>58.18±1.09</td>
<td>59.38±1.31</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one way ANOVA followed by Dunnett’s^a^p < 0.001, ^b^p < 0.01, ^c^p < 0.05 calculated by
Table 6.4.2B-EFFECT OF SANGU PARPAM II ON GASTRIC PH AND GASTRIC VOLUME IN PYLORUS LIGATED GASTRIC ULCER IN RATS

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Only pylorus</th>
<th>Pylorus+ Ranitidine 30 mg/kg</th>
<th>Pylorus+ S.P(II) 9.36mg/200 gm</th>
<th>pylorus+S.P(II) 46.8mg/200 gm</th>
<th>pylorus+S.P(II) 93.6mg/200 gm</th>
</tr>
</thead>
<tbody>
<tr>
<td>GASTRIC PH</td>
<td>2.3±0.20</td>
<td>1.23±0.16</td>
<td>2.58±0.06</td>
<td>2.35±0.12</td>
<td>1.93±0.1</td>
<td>2.1±0.2</td>
</tr>
<tr>
<td>GASTRIC VOLUME</td>
<td>0.68±0.11</td>
<td>4.83±0.4</td>
<td>2.27±0.12</td>
<td>2.48±0.33</td>
<td>2.86±0.14</td>
<td>2.39±0.32</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one way ANOVA followed by dunnett’s P< 0.001, bP < 0.01, aP < 0.05 calculated by
Table 6.4.2C-EFFECT OF SANGU PARPAM II ON ULCER SCORE AND ULCER INDEX IN PYLORUS LIGATED GASTRIC ULCER IN RATS

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Only pylorus</th>
<th>Pylorus+ Ranitidine 30 mg/kg</th>
<th>Pylorus+ S.P(II) 9.36mg/200 gm</th>
<th>pylorus+S.P(II) 46.8mg/200 gm</th>
<th>pylorus+S.P(II) 93.6mg/200 gm</th>
</tr>
</thead>
<tbody>
<tr>
<td>ULCER SCORE</td>
<td>0±0</td>
<td>7.91±0.19</td>
<td>3.95±0.22</td>
<td>6.07±0.14</td>
<td>4.78±0.18</td>
<td>6.10±0.14</td>
</tr>
<tr>
<td>ULCER INDEX</td>
<td>0±0</td>
<td>8.02±0.39</td>
<td>6.13±0.16</td>
<td>7.66±0.22</td>
<td>3.93±0.10</td>
<td>5.13±0.09</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one way ANOVA followed by dunnett’s \( P < 0.001 \), \( bP < 0.01 \), \( aP < 0.05 \) calculated by

![Graph showing Ulcer Score and Ulcer Index in Pylorus Ligated Gastric Ulcer in Rats](image-url)
Table 6.4.2D-EFFECT OF SANGU PARPAM II ON TOTAL PROTEIN IN PYLORUS LIGATED GASTRIC ULCER IN RATS

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Only pylorus</th>
<th>Pylorus+ Ranitidine 30 mg/kg</th>
<th>Pylorus+ S.P(II) 9.36mg/200 gm</th>
<th>pylorus+S.P(II) 46.8mg/200 gm</th>
<th>pylorus+S.P(II) 93.6mg/200 gm</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOTAL PROTEIN(g/dl)</td>
<td>0.76±0.0004</td>
<td>0.72±0.0009</td>
<td>0.47±0.00002^a</td>
<td>0.71±0.0007</td>
<td>0.82±0.0007</td>
<td>0.78±0.0001</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one way ANOVA followed by dunnett’s^cP< 0.001, ^bP< 0.01, ^aP < 0.05 calculated by
Table 6.4.2E - EFFECT OF SANGU PARPAM II ON ANTIOXIDANT PARAMETERS IN STOMACH OF PYLORUS LIGATED GASTRIC ULCER UN RATS

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Only pylorus</th>
<th>Pylorus+ Ranitidine 30 mg/kg</th>
<th>Pylorus+ S.P(II) 9.36mg/200 gm</th>
<th>pylorus+S.P(II) 46.8mg/200 gm</th>
<th>pylorus+S.P(II) 93.6mg/200 gm</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SOD (Unit/min/mg protein)</strong></td>
<td>0.65±0.005</td>
<td>0.33±0.002</td>
<td>0.55±0.002</td>
<td>0.48±0.001</td>
<td>0.52±0.002</td>
<td>0.55±0.002</td>
</tr>
<tr>
<td><strong>CAT (µmol of H$_2$O$_2$ consumed /min/mg protein)</strong></td>
<td>0.90±0.0002</td>
<td>0.61±0.0005</td>
<td>0.81±0.007</td>
<td>0.76±0.0004</td>
<td>0.80±0.0002</td>
<td>0.82±0.0004</td>
</tr>
<tr>
<td><strong>GPX (µmoles of glutathione oxidized/min/mg protein)</strong></td>
<td>0.69±0.0005</td>
<td>0.47±0.0004</td>
<td>0.59±0.001</td>
<td>0.51±0.001</td>
<td>0.53±0.0009</td>
<td>0.51±0.0005</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one way ANOVA followed by dunnett’s $^a$P < 0.001, $^b$P < 0.01, $^c$P < 0.05 calculated by

![Antioxidant parameters in stomach of pylorus ligated gastric ulcer un rats](chart.png)
**Table 6.4.2F - EFFECT OF SANGU PARPAM II ON LIPID PEROXIDATION IN STOMACH OF PYLORUS LIGATED GASTRIC ULCER IN RATS**

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Only pylorus</th>
<th>Pylorus+ Ranitidine 30 mg/kg</th>
<th>Pylorus+ S.P(II) 9.36mg/200 gm</th>
<th>pylorus+S.P(II) 46.8mg/200 gm</th>
<th>pylorus+S.P(II) 93.6mg/200 gm</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPO (nmol of MDA/mg protein)</td>
<td>0.69±0.021</td>
<td>0.83±0.004c</td>
<td>0.67±0.0005ns</td>
<td>0.52±0.002</td>
<td>0.53±0.0008</td>
<td>0.51±0.0005</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one way ANOVA followed by dunnett’s \(^{a}P<0.001\), \(^{b}P<0.01\), \(^{c}P<0.05\) calculated by
Table 6.4.2G - EFFECT OF SANGU PARPAM II ON MYELOPEROXIDATION IN STOMACH OF PYLORUS LIGATED GASTRIC ULCER IN RATS

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Only pylorus</th>
<th>Pylorus+ Ranitidine 30 mg/kg</th>
<th>Pylorus+ S.P(II) 9.36mg/200 gm</th>
<th>pylorus+S.P(II) 46.8mg/200 gm</th>
<th>pylorus+S.P(II) 93.6mg/200 gm</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPO(µmol/min/mg tissue)</td>
<td>0.87±0.00  4</td>
<td>1.06±0.082</td>
<td>0.75±0.022&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>0.75±0.022&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>0.60±0.031</td>
<td>0.77±0.019</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one way ANOVA followed by dunnett’s<sup>a</sup>P < 0.001, <sup>b</sup>P < 0.01, <sup>c</sup>P < 0.05 calculated by
Table 6.4.2H- EFFECT OF SANGU PARPAM II ON ULCER SCORE IN HCL/ETHANOL INDUCED ULCER IN RATS

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Only Hcl/Ethanol</th>
<th>Hcl/Ethanol+ Ranitidine 30 Mg/Kg</th>
<th>Hcl/Ethanol+ S.P-II 9.36mg/200gm</th>
<th>Hcl/Ethanol+ S.P-II 46.8mg/200gm</th>
<th>Hcl/Ethanol+ S.P-II 93.6mg/200gm</th>
</tr>
</thead>
<tbody>
<tr>
<td>ULCER SCORE</td>
<td>0±0</td>
<td>11±0.36&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.33±0.211&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.33±0.42&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.33±0.56&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.33±0.42&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>ULCER INDEX</td>
<td>0±0</td>
<td>15±0.36&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3±0.36&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4±0.36&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.33±0.56&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.33±0.42&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one way ANOVA followed by dunnett’s<sup>a</sup>P < 0.001, <sup>b</sup>P < 0.01, <sup>c</sup>P < 0.05 calculated by
Table 6.4.2I - EFFECT OF SANGU PARPAM II ON TOTAL PROTEIN LEVEL IN HCL/ETHANOL INDUCED ULCER IN RATS

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Only Hcl/Ethanol</th>
<th>Hcl/Ethanol+ Ranitidine 30 Mg/Kg</th>
<th>Hcl/Ethanol+ S.P-II 9.36mg/200gm</th>
<th>Hcl/Ethanol+ S.P-II 46.8mg/200gm</th>
<th>Hcl/Ethanol+ S.P-II 93.6mg/200 gm</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOTAL PROTEIN (g/dl)</td>
<td>50.67±3.60</td>
<td>74±9.89</td>
<td>67±1.67m</td>
<td>48.67±2.56m</td>
<td>44.67±2.56m</td>
<td>34.33±2.0m</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one way ANOVA followed by dunnett’s *P < 0.001, †P < 0.01, ‡P < 0.05 calculated by
Table 6.4.2J - EFFECT OF SANGU PARPAM II ON ANTI OXIDANTS ENZYMES IN HCL/ETHANOL INDUCED ULCER IN RATS

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Only Hcl/Ethanol</th>
<th>Hcl/Ethanol + Ranitidine 30 Mg/Kg</th>
<th>Hcl/Ethanol + S.P-II 9.36mg/200 gm</th>
<th>Hcl/Ethanol + S.P-II 46.8mg/200gm</th>
<th>Hcl/Ethanol + S.P-II 93.6mg/200gm</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (Unit/min/mg protein)</td>
<td>0.4±0.07</td>
<td>0.14±0.01 c</td>
<td>0.49±0.08 ns</td>
<td>0.39±0.07ns</td>
<td>0.38±0.02 ns</td>
<td>0.44±0.006 ns</td>
</tr>
<tr>
<td>CAT (µmol of H2O2 consumed/min/mg /protein)</td>
<td>5.31±1</td>
<td>2.59±1</td>
<td>4.20±1</td>
<td>4.59±1</td>
<td>4.09±1</td>
<td>3.39±1</td>
</tr>
<tr>
<td>GPX (µmoles of glutathione oxidized /min/mg protein)</td>
<td>7.2±0.06</td>
<td>3.49±0.10 c</td>
<td>6.15 ±0.11 b</td>
<td>5.18±0.09 ns</td>
<td>5.38±0.90 a</td>
<td>3.66±0.27 c</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± S.D; Statistical significance \( p \) calculated by one way ANOVA followed by dunnett’s c \( P < 0.001 \), b \( P < 0.01 \), a \( P < 0.05 \) calculated by

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**ANTIOXIDANT PARAMETERS IN STOMACH OF HCL/ETHANOL INDUCED ULCER IN RATS**
Table 6.4.2K - EFFECT OF SANGU PARPAM II ON LIPID PEROXIDATION LEVEL IN HCL/ETHANOL INDUCED ULCER IN RATS

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Only Hcl/Ethanol</th>
<th>Hcl/Ethanol + Ranitidine 30 Mg/Kg</th>
<th>Hcl/Ethano l+ S.P-II 9.36mg/200gm</th>
<th>Hcl/Ethano l+ S.P-II 46.8mg/200gm</th>
<th>Hcl/Ethano l+ S.P-II 93.6mg/200gm</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPO (nmol of MDA/mg protein)</td>
<td>4.49±0.21</td>
<td>13.63±0.36c</td>
<td>5.03±0.48ns</td>
<td>5.03±0.13ns</td>
<td>4.67±0.63ns</td>
<td>5.15±0.11ns</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one way ANOVA followed by dunnett’s cP < 0.001, bP < 0.01, aP < 0.05 calculated by
Table 6.2 L - EFFECT OF SANGU PARPAM II ON MPO LEVEL IN HCL/ETHANOL INDUCED ULCER IN RATS

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Only Hcl/Ethanol</th>
<th>Hcl/Ethanol + Ranitidine 30 Mg/Kg</th>
<th>Hcl/Ethanol + S.P-II 9.36mg/200 gm</th>
<th>Hcl/Ethanol + S.P-II 46.8mg/200 gm</th>
<th>Hcl/Ethanol + S.P-II 93.6mg/200 gm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>MPO (µmol/min/mg protein)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.37±0.061</td>
<td>0.47±0.055&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>0.29±0.017&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>0.35±0.021&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.41±0.036&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>0.26±0.05&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one way ANOVA followed by dunnett’s<sup>a</sup>P< 0.001, <sup>b</sup>P< 0.01, <sup>c</sup>P < 0.05 calculated by
Table 6.4.2M - EFFECT OF SANGU PARPAM II ON MUCUS WEIGHT AND PGE2 IN HCL/ETHANOL INDUCED ULCER IN RATS

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Only Hcl/Ethanol</th>
<th>Hcl/Ethanol + Ranitidine 30 Mg/Kg</th>
<th>Hcl/Ethanol + S.P-II 9.36mg/200g m</th>
<th>Hcl/Ethanol + S.P-II 46.8mg/200g m</th>
<th>Hcl/Ethanol + S.P-II 93.6mg/200g m</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucus weight (g)</td>
<td>0.52± 0.022</td>
<td>0.27± 0.028&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.39± 0.084&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.29± 0.011&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.27± 0.011&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.42± 0.013&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>PGE2 (Pg/ml)</td>
<td>132±1.46</td>
<td>46.67±1.84</td>
<td>87±1.67</td>
<td>70.67±1.12</td>
<td>64.33±1.17</td>
<td>47±2.03</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one way ANOVA followed by Dunnett’s<sup>c</sup> P< 0.001, <sup>b</sup>P< 0.01,<sup>a</sup>P < 0.05 calculated by.
Table 6.4.2 N - EFFECT OF SANGU PARPAM II ON ULCER SCORE AND ULCER INDEX IN COLD RESTRAINT STRESS INDUCED ULCER IN RATS

<table>
<thead>
<tr>
<th>Group</th>
<th>Control (Ghee)</th>
<th>Cold Restraint Stress(CRS)</th>
<th>CRS+ Ranitidine 30 mg/kg</th>
<th>CRS + S.P(II) 9.36mg/200gm</th>
<th>CRS + S.P(II) 46.8mg/200gm</th>
<th>CRS + S.P(II) 93.6mg/200gm</th>
</tr>
</thead>
<tbody>
<tr>
<td>ULCER SCORE</td>
<td>0±0</td>
<td>8.5±1.87c</td>
<td>2.67±1.75a</td>
<td>6.67±2.065c</td>
<td>5.67±1.21c</td>
<td>4.17±2.14c</td>
</tr>
<tr>
<td>ULCER INDEX</td>
<td>0±0</td>
<td>7.5±3.48b</td>
<td>3.67±1.69a</td>
<td>4±1.86a</td>
<td>2.67±1.20a</td>
<td>2.17±1.046a</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one way ANOVA followed by Dunnett’s: ^cP<0.001, ^bP<0.01, ^aP<0.05 calculated by
Table 6.4.2 O -EFFECT OF SANGU PARPAM II ON TOTAL PROTEIN IN COLD RESTRAINT STRESS INDUCED ULCER IN RATS

<table>
<thead>
<tr>
<th>Group</th>
<th>Control (Ghee)</th>
<th>Cold Restraint Stress(CRS)</th>
<th>CRS+ Ranitidine 30 mg/kg</th>
<th>CRS+ S.P.(II) 9.36mg/200gm</th>
<th>CRS + S.P.(II) 46.8mg/200gm</th>
<th>CRS + S.P.(II) 93.6mg/200gm</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOTAL PROTEIN (g/dl)</td>
<td>0.56±0.03</td>
<td>0.69±0.02^c</td>
<td>0.47±0.001^a</td>
<td>0.54±0.02^m</td>
<td>0.58±0.02^m</td>
<td>0.71±0.02^c</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one way ANOVA followed by dunnett’s^cP< 0.001, ^bP< 0.01, ^aP < 0.05 calculated by.
PARAMETERS IN STOMACH OF COLD RESTRAINT STRESS INDUCED ULCER IN RAT

<table>
<thead>
<tr>
<th>Group</th>
<th>Control (Ghee)</th>
<th>Cold Restraint Stress(CRS)</th>
<th>CRS+ Ranitidine 30 mg/kg</th>
<th>CRS+ S.P(II) 9.36mg/200gm</th>
<th>CRS + S.P(II) 46.8mg/200gm</th>
<th>CRS + S.P(II) 93.6mg/200gm</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD(Unit/min/mg protein)</td>
<td>0.64±0.01</td>
<td>0.26±0.019</td>
<td>0.58±0.019</td>
<td>0.47±0.001</td>
<td>0.51±0.002</td>
<td>0.53±0.002</td>
</tr>
<tr>
<td>CAT (µmol of H₂O₂ consumed/min/mg protein)</td>
<td>0.41±0.09</td>
<td>0.19±0.03c</td>
<td>0.42±0.04cc</td>
<td>0.29±0.001</td>
<td>0.31±0.033</td>
<td>0.39±0.032</td>
</tr>
<tr>
<td>GPX(µmoles of glutathione oxidized /min/mg/protein)</td>
<td>0.07±0.002</td>
<td>0.03±0.001c</td>
<td>0.06±0.002cc</td>
<td>0.04±0.003c</td>
<td>0.05±0.002c</td>
<td>0.05±0.002c</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± S.D; Statistical significance (p)calculated by one way ANOVA followed by dunnett’s^cP< 0.001, ^bP< 0.01, ^aP < 0.05 calculated by.
Table 6.4.2Q - EFFECT OF SANGU PARPAM II ON LIPID PEROXIDATION IN STOMACH OF COLD RESTRAINT STRESS INDUCED ULCER IN RATS

<table>
<thead>
<tr>
<th>Group</th>
<th>Control (Ghee)</th>
<th>Cold Restraint Stress(CRS)</th>
<th>CRS+ Ranitidine 30mg/kg</th>
<th>CRS+ S.P(II) 9.36mg/200g m</th>
<th>CRS + S.P(II) 46.8mg/200g m</th>
<th>CRS + S.P(II) 93.6mg/200g m</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPO (nmol of MDA/mgprotein)</td>
<td>0.65±0.00</td>
<td>0.72±0.00</td>
<td>0.66±0.00</td>
<td>0.43±0.00</td>
<td>0.55±0.024</td>
<td>0.28±0.020</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one way ANOVA followed by dunnnett’s P<0.001, bP<0.01, aP < 0.05 calculated by

![Lipid Peroxidation in Stomach of Cold Restraint Stress Induced Ulcer in Rats](image-url)
Table 6.4.2 R - EFFECT OF SANGU PARPAM II ON MYELOPEROXIDATION IN STOMACH OF COLD RESTRAINT STRESS INDUCED ULCER IN RATS

<table>
<thead>
<tr>
<th>Group</th>
<th>Control (Ghee)</th>
<th>Cold Restraint Stress (CRS)</th>
<th>CRS + Ranitidine 30 mg/kg</th>
<th>CRS + S.P(II) 9.36mg/200gm</th>
<th>CRS + S.P(II) 46.8mg/200gm</th>
<th>CRS + S.P(II) 93.6mg/200gm</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPO (μmol/min/mgtissue)</td>
<td>0.70±0.007</td>
<td>1.04±0.085</td>
<td>0.71±0.008</td>
<td>0.80±0.013&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.47±0.019&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.33±0.018&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one way ANOVA followed by Dunnett’s<sup>c</sup>P < 0.001, <sup>b</sup>P < 0.01, <sup>a</sup>P < 0.05 calculated by...
Table 6.4.2 S - EFFECT OF SANGU PARPAM II ON PGE2 IN STOMACH OF COLD RESTRAINT STRESS INDUCED ULCER IN RATS

<table>
<thead>
<tr>
<th>Group</th>
<th>Control (Ghee)</th>
<th>Cold Restraint Stress (CRS)</th>
<th>CRS+ Ranitidine 30 mg/kg</th>
<th>CRS + S.P(II) 9.36mg/200gm</th>
<th>CRS + S.P(II) 46.8mg/200gm</th>
<th>CRS + S.P(II) 93.6mg/200gm</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPE2 (Pg/ml)</td>
<td>122.7±6.63</td>
<td>61±1.32c</td>
<td>75.7±0.92c</td>
<td>50±2.63c</td>
<td>53.3±2.76c</td>
<td>116.7±6.38n</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one way ANOVA followed by dunnett’s aP< 0.001, bP< 0.01, cP< 0.05 calculated by
ANTI ULCER STUDY:

**Pyloric Ligation Method:**

The animals treated with all the dose levels did not produce any significant weight variations and weight throughout the study period.

The animals treated with SP II at the dose of 9.36, 46.8 and 93.6 mg showed a statistically significant increase (P < 0.5) in the few acidity level when compared to the normal control group. (Table 6.13)

The pyloric ligation group alone showed a marked increase in the total acidity level when compared to normal control group which is statistically significant (P < 0.05)

All the animals treated with sangu parpam in different doses showed a statistically significant variation in gastric PH (P < 0.05) and total volume of gastric juice when compared to normal control animals (P < 0.05) (Table 6.14). The ulcer score as well as the ulcer index of the sangu parpam also showed a significant variation (P<0.001) (Table 6.15) compared with control group.

There is no significant variation in the total protein(Table 6.16) level of the sangu parpam treated group with control group. The anti oxidant enzymes SOD, CAT, GPX, LPO and MPO did not produce any statistically significant variation when compared to the normal control group. (Table 6.17,6.18,6.19)

**Hcl / Ethanal Induced Method:**

The ulcer score was found significant increase when compared to control group (P < 0.001). The ulcer index also showed a significant increase when compared to control group. (Table 6.20)
The animals treated with sangu parpam did not produce any significant variation in total protein level. (Table 6.21)

In antioxidant enzyme SOD level was not significantly changed. But the animals treated with 46.8 mg / 200 gm showed a significant increase (P < 0.01) in catalase and GPX levels while 93.6 mg/200gm group also showed a significant increase in there values (P < 0.001). But the LPO and MPO level did show any significant variation. (Table 6.22,6.23,6.24,6.25)

The animals treated with sangu parpam as well as standard drug showed a significant increase in mucus weight.

**Stress induced Ulcer:**

Sangu parpam produce a significant variation in Ulcer score (P < 0.001) when compared to the normal control group. The ulcer index also showed significant variation. (Table 6.26)

There is no significant variation in the total protein level except the negative control group showed a increase in total protein level (P < 0.001) when compared to control. (Table 6.27)

In antioxidant enzymes. Animals treated with sangu parpam 9.36 mg 46.8 mg/200gm showed a significant increase in catalase level (P < 0.01of P < 0.05) respectively. The animals treated with all the doses of sangu parpam showed a significant increases in GPX level ( P < 0.001) when compared to normal control. The lipid peroxidase , myeloperoxidase level were also found the significant ( P < 0.001) when compared to control. (Table 6.28,6.29,6.30,6.31)
7. DISCUSSION

The oldest form of healthcare in the world is the use of herbs as a medicine. During the past decade, developed and developing countries were highly accepting the natural therapy. Because of poverty and limited access to allopathy medicine, about 80% of the world’s population, uses traditional system of medicines as their source of healthcare. Most of the herbal preparations are safe for consumption, some herbs like most biologically active substances could be toxic. Mostly, without any proper scientific evaluation herbal products are launched into the market. And also there is no effective tool to regulate manufacturing practices and standards of drug.

Standardization of traditional medicines is the process of prescribing a set of standards and constant parameters that carry an assurance of quality, efficacy, safety and reproducibility of the drugs. Standardization is a tool in the quality control process.

Several problems often influence the quality of herbal drugs. For instance:
1. Herbal drugs are mostly mixtures of many ingredients.
2. The active principle is unknown.
3. Analytical methods may not be available commercially.
4. Plant materials are chemically and naturally variable place to place.
5. The quality of the raw material is variable place to place.

The standardization of herbal drug is very essential for the global acceptance. In this study Sangu Parpam which is highly recommended for the disease Peptic Ulcer in Siddha Literature is taken up for standardization methods. Proper standardization techniques, Toxicological and Pharmacological evaluation on these medicines to meet the criteria to support its use worldwide. Therefore, in this study an attempt has been made to evaluate the Physico chemical characters, Pharmacological actions and toxicological analysis and Standardization of a Herbo-marine Siddha drug Sangu Parpam. Sangu (Conch) is a Marine origin drug. Loads of research has been conducted in drugs of plant origin but very little amount research work done in the marine origin drugs. This may helps to reveal the quality and safety of the drug and
will lead to universal acceptance of the drug Sangu parpam for the disease Peptic Ulcer.

**Preparation processes:**

**Purification of Sangu**

Three purification processes were mentioned in our Siddha literatures and done as per the proposal and the samples were collected and named as Process I: Sample - **Spu I**, Process II: Sample - **Spu II**, Process III: Sample - **Spu III** and taken up for the preparation of Sangu parpam.

**Preparation of Sangu Parpam**

100 gms of purified Sangu from each purification process is covered up by ground paste of Uthamani (*Pergularia damea*) and kept in the mud lid and closed by another mud lid. Cotton ribbon soaked in wet clay is wound over the rims of both mud lids and let to dry in sun light for 8 hours. Then this set up is subjected to Gana pudam. After cooling the set up is taken out and the calcinated Sangu is taken out. Dose is 260mg and the adjuvant is ghee and indicated for Cough, Piles, Stomach diseases, Enlarged tonsils, Chest pain, Vayu, Gunmam(Peptic Ulcer). And highly recommended for Peptic Ulcer disease. After the preparation standardization techniques were applied to the samples Spu I,II,III and SP I,II,III.

**Physico chemical Analysis:**

**Organoleptic characters:**

In analytical specifications (Table 6.1) the total ash value of every samples found to be with in the range of 65% to 80% this indicating a less amount of organic matter $^{78}$ and a high amount of minerals present in the Sangu parpam samples. This indicates purification and preparation process is the key to addition of concentration of minerals and some organic compounds. The drug possesses a low value in samples SP I (10.40 %) SP II (7.48%) SP III (10.33%) of acid insoluble ash indicating that the preparation did not contain any siliceous matter $^{78}$ and the medicine was prepared in a hygienic condition signifying a better quality of drugs. The loss on drying test of every sample at 105°C indicates that only of less than 0.25 % .This moisture content prevents reduction of efficacy and degeneration. So the shelf life has been dated up to
100 years for parpam type of medicines which is mentioned in Siddha literature. The pH value of every samples at 25°C was found to be within the range of 8.00 to 9.50 which indicates the alkalinity of the drug. The pH of every Sangu parpam samples were above 9 is a good indication that the drug is alkaline and recommended for the treatment of Gunmam.

**ICP-OES analysis**

ICP-OES analysis (Table 6.3) indicates the presence of Heavy metals like Lead, Mercury, Copper, Silica and Zinc in Raw Sangu sample. After purification concentration of the heavy metals are highly reduced in Spu I, II, III. And in the final purification process the presence of heavy metals in SP I, II, III are in Below the limit of quantification as per WHO guidelines. Other minerals Calcium, Sodium, Chloride, Zinc and Magnesium which are identified in the Raw sangu. After Purification and preparation the concentration was increased. This may responsible for therapeutic value of the medicine and also indicates the quality of the purification and preparation processes of Sangu. Overall analysis indicates the purification process II that is Spu II and Prepared Sangu parpam SP II from Spu II shows better quality than other Samples.

**XRD analysis**

X-ray diffraction study of the Sangu sample showed a sharp peak indicating its crystalline nature. Whereas the Sangu Parpam did not give sharp peaks indicating the reduction of crystalline nature, thus the sharp crystalline structure of the Sangu parpam reflects light rays whereas reduction of crystalline nature in the Sangu parpam prevents it from doing so. The XRD high intensity peaks confirm the presence of Calcium oxide as the major crystalline phase in the samples. Other low intensity peaks were observed which may be due to presence of trace element. After the process of purification strongest peaks of samples corresponded to Calcium oxide and few weak peaks corresponds to Na, Si and C peak of very low intensity was observed.

**SEM analysis:**

SEM images (Table 6.2) of Raw Sangu indicates the size of particle is 5µm to 10 µm and the particles are crystal in shape. Surface of the particle is rough in Raw
sangu. After purification the samples surfaces were slightly smooth and particles moderately get agglomerated and particle size was reduced to 3µm to 5 µm. After preparation of Sangu parpam sample’s are highly agglomerated due to repeated incineration (Pudam Process) process.  

And the surface of every sample is smooth and particle size ranging from 1µm to 2 µm in samples SPI, II and SP III. Of these samples SP II is better because of it’s smooth surface and the particle size is 1µm to 2 µm and the shape of the sample is Oval, this is because the flowability of the drug is possible and absorption is possible. Shape of the samples SP I, III are changed and mostly agglomerated and oval in shape. As the preparation involves crunching of raw materials with herbal juices, heat processes and subsequent cooling of product, it tends to agglomerate the drug particles, which causes the particle size variation.

**EDAX Analysis:**

The EDAX spectra (Table 6.4) showed the presence of Calcium, Sodium, Carbon and Oxygen in higher percentage compared with other nutrients. The nutrients are responsible for the therapeutic action of the drug. This analysis confirmed the presence of various elements viz. Ca, Na in their oxide form. The major percentage of spectra was Calcium oxide. The source of the other elements can be attributed to the fact that various processes involving different herbal drugs were used in the purification process and preparation processes of Sangu parpam. The putam process (calcination) may also have contributed to the addition of these elements.

**Toxicity Studies:**

**Acute toxicity**

SANGU PARPAM I, II, III was administered single time at the dose of 50 mg/kg, 300 mg/kg, 1000 mg/kg and 2000 mg/kg and observed for consecutive 14 days after administration. Doses were selected based on the pilot study and literature review. All animals were observed daily once for any abnormal clinical signs. Weekly body weight and food consumption were recorded. No mortality was observed during the entire period of the study. Data obtained in this study indicated no significance
physical and behavioral signs of any toxicity due to administration of SANGU PARPAM I, II, III at the doses of 50 mg/kg, 300 mg/kg, 1000 mg/kg and 2000 mg/kg. At the 14th day, all animals were observed for functional and behavioral examination. In functional and behavioral examination, home cage activity, hand held activity were observed. Home cage activities like Body position, Respiration, Clonic involuntary movement, Tonic involuntary movement, Palpebral closure, Approach response, Touch response, Pinna reflex, Sound responses, Tail pinch response were observed. Handheld activities like Reactivity, Handling, Palpebral closure, Lacrimation, Salivation, Piloerection, Papillary reflex, Abdominal tone, Limb tone were observed. Functional and behavioral examination was normal in all treated groups.

**Subacute toxicity**

All the animals from control and all the treated dose groups up to 300 mg/kg survived throughout the dosing period of 28 days. No signs of toxicity were observed in animals from different dose groups during the dosing period of 28 days. Animals from all the treated dose groups exhibited comparable body weight gain with that of controls throughout the dosing period of 28 days. Food consumption of control and treated animals was found to be comparable throughout the dosing period of 28 days. Haematological analysis conducted at the end of the dosing period on day 29, revealed no abnormalities attributable to the treatment. Biochemical analysis conducted at the end of the dosing period on day 29, no abnormalities attributable to the treatment. Organ weight data of animals sacrificed at the end of the dosing period was found to be comparable with that of respective controls. Histopathological examination revealed normal architecture in comparison with control and treated animal.
**Anti ulcer activity Studies:**

**Hcl-Ethanol induced Ulcer Model:**

Peptic ulcers are caused by an imbalance between the protective and the aggressive mechanisms of the mucosa, and are the result of the association of several endogenous factors and aggressive exogenous factors that are related to living conditions. Sanguparpam II could significantly protect the gastric mucosa against Hcl-Ethanol induced injury on comparing the control group, test drug shows significant increase in protection of gastric wall mucosa and also in ulcer area by inhibiting oedema and leukocyte infiltration of submucosal area. PGE2, SOD and MDA levels of tissue homogenate reveals that SOD decreased the level of MDA in the treated group. This study provides complete evidence that the SP II possesses an anti ulcer activity.

**Stress Induced Ulcer:**

Here the study conclude that the Sangu parpam II shows good anti secretary via anti ulcer action towards cold resistant stress induced model on rats. It reduces the average number of ulcers, both superficial and deep ulcer when compared with standard drug like ranitidine and it may be due to its antioxidant and antisecretory activity on the stomach. Ranitidine and Sangu parpam II significantly decreased the ulcer index. It shows significant anti ulcer activity in dose dependant manner when compare to that of standard drug like ranitidine. Its effect was further confirmed by histological examination showing prevention of mucosal lesions and sub-mucosal edema in stomach. The study shows that it provide complete evidence of the SP II towards anti ulcer effect.

**Pyloric Ligation Model:**

Here the study conclude that the Sangu parpam II shows anti ulcer action towards Pyloric Ligation Model on rats. The antiulcer property of Sangu parpam II in pylorus ligation model is evident from its significant reduction in free acidity, total acidity, number of ulcers and ulcer index. Moreover, this S.P II significantly suppressed the formation of the ulcers. The significant inhibition of gastric ulcer in rats pre-treated with S.P II was comparable with ranitidine which is a standard drug used for curing gastric ulcer. Sangu parpam II treated animals decreased both the concentration and
increased the pH, and increased the gastric wall mucus and protein content of the gastric mucosa so it is suggested that Sangu parpam II can suppress gastric damage induced by aggressive factors. As per the study SP II Shows the significant anti ulcer activity.
8. SUMMARY AND CONCLUSION

- The raw drug sangu purchased from various part of Tamil Nadu.
- Raw drug and ingredients were authenticated by Marine Biology Regional Centre, Zoological survey of India, Chennai
- Raw Sangu was purified as per the literature in three different methods and named as Spu I, Spu II and Spu III.
- By using purified samples Spu I, Spu II and Spu III, Sangu parpam (SP I, SP II, and SP III respectively) were prepared as per the proposal.
- Then the samples, Sangu before purification, sangu after purification and the three different preparation of sangu parpam were analysed for standardization.
- The physicochemical analysis of the samples revealed the quality of purification process and preparation of the medicine.
- Sophisticated instrumental analysis reveals the quality and concentration of minerals and metals and powder properties of Raw Sangu and purified samples Spu I, II, III and prepared Sangu Parpam samples SP I, II, III.
- ICP-OES analysis of samples revealed that heavy metals like As, Hg, Pb, C concentration was reduced after purification and found to be in normal limits as per WHO permissible limit. Other Minerals like Ca, Na, Mg concentration was increased in purification and preparation processes and is responsible for its therapeutic values. And there is no evidence of presence of heavy metals in samples and elevated concentration of minerals in Sample SP II when compared with other samples.
- SEM analysis of the samples indicates the particle size which varies from 1 µm to 2µm. Particles are crystalline in raw Sangu sample and in Spu I, Spu II, and Spu III and SP I, SP II, and SP III samples the particles were agglomerated because of purification and repeated incineration processes (Pudam process). Surface of SP I, II, III was smooth. Among purified and Sangu Parpam samples, Powder property of SP II revealed moderately smooth surface and low particle size and particles were agglomerated when compared with other samples.
- Acute toxicity study revealed Sangu Parpam I, II, III at the doses of 50 mg/kg, 300 mg/kg, 1000 mg/kg and 2000 mg/kg to the rats did not produce drug-related toxicity. No mortality was observed during the entire period of the
study. Data obtained in this study indicated no significance physical and behavioral signs of any toxicity.

- The Maximum tolerable dose is about 2000mg/kg b.wt.
- Sub acute toxicity study revealed **Sangu Parpam I, II, III** can be considered safe, as it did not cause either any lethality or adverse changes with general behaviour of rats and also there were no observable detrimental effects (100 to 300 mg/kg body weight) over a period of 28 days.

- Both acute and Sub acute toxicity studies of various preparation of Sangu parpam revealed safe in animals tested.
- Sangu parpam Sample SP II was taken for Anti ulcer studies as per above mentioned studies, and anti ulcer activity revealed Sangu Parpam sample SP II had a significant anti ulcer activity. The treatment with SP II sample shows reduction in the gastric lesion area, and promoting significant regeneration of the gastric mucosa. Thus in Pylorus ligation method, Ethanol/HCL induced ulcer method and Stress induced ulcer method Sangu Parpam sample SP II confirm it’s anti ulcer activity.

- And based upon physico chemical and sophisticated instrumental analysis SP II was validated for the Anti ulcer activity studies and the study revealed that SP II has significant anti ulcer activity in animal models tested. **Thus this entire research work justifies and confirms the traditional claim Sangu parpam is one of the important medication for Peptic Ulcer.**
9. RECOMMENDATION

As the drug was found to be safe and effective, a pilot study can be planned in Gunmam (Peptic Ulcer) patients.

As this study lacks pharmacokinetic studies. A detailed pharmacokinetic and pharmacodynamic studies can be planned and will helps to understanding the mechanism of action of the drug.
Fig 6: Raw Sangu Samples
Fig 7: Plants used for purification and preparation process of Sangu parpam I, II, III

Euphorbia ligularia

Euphorbia ligularia

Pergularia damea

Pergularia damea
Fig 8: PREPARATION PROCESS OF SANGU PARPAM I, II, III

Cow Dung Cake used for incineration process

Incineration process
Fig 9: RAW SANGU, PURIFIED SANGU (Spu I, II, III), SANGU PARPAM (SP I, II, III)

<table>
<thead>
<tr>
<th>Raw Sangu</th>
<th>Raw Sangu</th>
<th>Raw Sangu</th>
</tr>
</thead>
<tbody>
<tr>
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<td><img src="image3" alt="Raw Sangu" /></td>
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</table>

<table>
<thead>
<tr>
<th>Sangu Purified SPU I</th>
<th>Sangu Purified SPU II</th>
<th>Sangu Purified SPU III</th>
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<tbody>
<tr>
<td><img src="image4" alt="Sangu Purified SPU I" /></td>
<td><img src="image5" alt="Sangu Purified SPU II" /></td>
<td><img src="image6" alt="Sangu Purified SPU III" /></td>
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</table>

<table>
<thead>
<tr>
<th>Sangu Parpam SP I</th>
<th>Sangu Parpam SP II</th>
<th>Sangu Parpam SP III</th>
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<tr>
<td><img src="image7" alt="Sangu Parpam SP I" /></td>
<td><img src="image8" alt="Sangu Parpam SP II" /></td>
<td><img src="image9" alt="Sangu Parpam SP III" /></td>
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</tbody>
</table>
Fig 10: SIDDHA SPECIFICATION OF TEST FOR PARPAM

Sangu Parpam Sample

Floating Test

Presence of SP (I, II, III) in Crevices of finger
Fig 11: EVALUATION OF ANTIULCER ACTIVITY OF SANGU PARPAM II ON PYLORIC LIGATION INDUCED ULCER IN RAT

MACROSCOPICAL VIEW OF PYLORUS LIGATION INDUCED ULCER

Control (saline) Normal Mucosal Layer

Only P.L shows severe damage of mucosal layer

PL+ Ranitidine 30 mg/kg

Shows mucosal layer

PL+ Sangu parpam III 9.36mg/200gm

Protected mucosal layer

PL+ Sangu Parpam II 46.8mg/200gm

Moderately damage of mucosal layer

PL + Sangu parpam II- 93.6/200gm

Shows Protected mucosal layer
Fig 12: GROSS APPEARANCE OF THE GASTRIC MUCOSA IN HCL/ETHANOL TREATED RATS

CONTROL

HCL/ETHANOL

HCL/ETHANOL + RANITIDINE

HCL/ETHANOL +
SANGU PARPAM II 9.36mg/200Gm

HCL/ETHANOL +
SANGU PARPAM II 46.8mg/200gm

HCL/ETHANOL +
SANGU PARPAM II 93.6mg/200gm
Fig 13: HISTOPATHOLOGY SLIDES OF TOXICITY STUDIES ON SANGU PARPAM SAMPLES SP I, II, III:

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<tr>
<th>Control</th>
<th>SP I Higher Dose (300mg/kg)</th>
<th>SP II Higher Dose (300mg/kg)</th>
<th>SP III Higher Dose (300mg/kg)</th>
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<tr>
<td><img src="image" alt="Stomach Control" /></td>
<td><img src="image" alt="Stomach SP I" /></td>
<td><img src="image" alt="Stomach SP II" /></td>
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<td><img src="image" alt="Heart Control" /></td>
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<td><img src="image" alt="Heart SP II" /></td>
<td><img src="image" alt="Heart SP III" /></td>
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<tr>
<td><img src="image" alt="Kidney Control" /></td>
<td><img src="image" alt="Kidney SP I" /></td>
<td><img src="image" alt="Kidney SP II" /></td>
<td><img src="image" alt="Kidney SP III" /></td>
</tr>
<tr>
<td>Liver</td>
<td>Lung</td>
<td>Spleen</td>
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<tr>
<td>---------------</td>
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<td><img src="image8.png" alt="Lung Image" /></td>
<td><img src="image9.png" alt="Spleen Image" /></td>
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</tbody>
</table>
Fig 14: HISTOPATHOGY SLIDES OF PYLORIC LIGATION MODEL

GROUP- I CONTROL (Ghee 5 ml/kg)

10x submucosal edema

40 x shows cryptitis

GROUP II: ONLY PYLORUS LIGATION

10x decreased submucosal edema

40x lymphocytic infiltrations

GROUP- III PYLORUS + RANITIDINE 30 mg/kg BODY WEIGHT, ORAL

10x submucosal edema

40x mild inflammation
GROUP – IV PYLORUS LIGATION + S.P (II) 9.36mg/200gm

10x mild submucosal edema
40x shows few lymphocytic infiltrations

GROUP – V PYLORUS LIGATION + S.P (II) 46.8mg/200gm

10x submucosal edema
40x inflammatory cell in lumen
BIBLIOGRAPHY


5. Thiagarajan R., Sirappu maruthuvam, Chennai: Dept. of Indian Medicine & Homoeopathy 2006, Pg No.1


9. Thiagarajan, Gunapadam thathu jeeva vaguppu Part II & III 4\textsuperscript{th} reprint, Pub directorate of Indian medicine and Homeopathy. Chennai. 1990, Pg 1.


11. Dr.R.Thiagarajan , Siddha Materia Medica Mineral & Animal Sections Page no 469 – 472

12. MP Sharma, Vineet Ahuja, Current Management of Acid Peptic Diseases, JIACM 2003; 4(3): 228-33


20. Thiagarajan, Siddha material medica (Mineral & Animal Kingdom) Published by Directorate of Indian medicine and Homeopathy. Chennai.106.Editor-Dr. Anaivaari R. Anandan, 2008, Pg 471.

    Macmillan, London
centre, Zoological Survey of India, Calcutta.
25. Durante. S, 1979, Marine shells from Balakat, Shahr-i-Sakhta and Tepe Yahya,
their significance for trade and technology in ancient Indo- Iran, South Asian
Archeaology, 1977. Fourth International Conference of South Asian Archealogists
in Western Europe, Naples 1979, P. 317-344.
from India, 1-40, Zoological survey of India, Kolkata.
27. Council of Scientific and Industrial Research, Wealth of India, Fish and Fisheries,
p.no.126,
28. Mohamed Sayab, Therayar yamaga venba, 1973, Dept of Indian Medicine and
Homeopathy, Chennai.
29. Asima chatterjee, satyesh Chandra prakash, The treatise on Indian medicinal
plants, National Institute of science communication, 1997, vol -5
30. Wealth of India, Raw Materials, National Institute of Science Communication and
Information Resources (CSIR publication).
Distributors; 1993; 3:1616-1617.
32. Golam S, Gafur MA, Shah MAB, Khurshid AHM, Biswas MHU, Hassan P et
33. Kumar B, Yadav DK, Govindarajan R, Pushpangadan P. Wound healing activity of


35. Sureshkumar SV, Mishra SH. Hepatoprotective activity of extracts from


37. Kandan Karthikeyan, Sankaran Mirunalini, Assessment of the antioxidant potential of *Pergularia daemia* (Forsk.) extract in vitro and in vivo experiments on hamster buccal pouch carcinogenesis, Asian Pacific Journal of Tropical Disease, 2012; s 509- 516.


40. Kandan Karthikeyan, Sankaran Mirunalini, Therapeutic potential of

_Pergularia daemia:_ The Ayurvedic wonder, International Journal of
Pharmacology 6 (6): 836- 843, 2010

41. Murugesan mudhaliar KS, Gunapadam part-1, 2002, Dept of Indian Medicine
and Homeopathy, Chennai.

42. Shanmugavelu, Noi nadal Noi mudhal nadal, part 2, 2003, Dept of Indian Medicine
and Homeopathy, Chennai.

43. Robins Robbins and Cotran Pathologic basis of disease 7th edition by Kumar,
Abbas, Fausto, Churchil Livingstone.

44. Davidson’s Principles and Practice of Medicine. 20th Edition by Nicholas A.
Boon. International Editor John A.A. Hunter

45. Standardization of Siddha drug Sangu Parpam using infrared spectrum. V. N. Meena
devi, P. Nagendra Prasad, K. Kalirajan, IJPT | Sep-2010 | Vol. 2 | Issue No.3 | 634-641

46. Pharmacological evaluation of the anti-inflammatory activity of Sangu parpam,
V. Murugan, Thomas M. Walter, Sulfin Nihar, B. Sampath Kumar,
https://www.researchgate.net/profile/DrThomas_M_Walter/publication/43158336

47. Pharmacological validation of two Siddha drugs (parpam) for antiulcer
effect in albino rats. A. Thanga Thirupathi, R. Vekatanarayanan, R.

48. Thiagarajan, Siddha material medica (Mineral & Animal Kingdom) Published by
Directorate of Indian medicine and Homeopathy. Chennai.106.Editor-Dr.Anaivaari
R.Anandan, Ph.D., 2008, Pg 468.

49. Thiagarajan, Siddha material medica (Mineral & Animal Kingdom) Published by
Directorate of Indian medicine and Homeopathy. Chennai.106.Editor-Dr.Anaivaari
R.Anandan, Ph.D., 2008, Pg 471.


56. The total white blood cells were enumeration, John., 1972.


78. R.Moovendan, S.Vidhya, N.Saravanan, A.Kingsly, G.Essakky pandian,

