SAFETY AND PHARMACOLOGICAL PROFILE OF SANJEEVI THEENEER

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INDEX

1. INTRODUCTION

Every form of medicines representing the vast materia medica available in numerous Traditional systems of medicines are based on the modification of formulations to the stage which it can be used in wide category of health conditions in accordance with the age , body constitution, nature of disease and its influence in digestion and metabolism. *Siddha* medicine, which was developed and propounded by ancient supreme spiritual sages called *siddhars* with a vision of not only establishing a medical science for the health wellbeing, also to implement and guide fundamental ethics in each one for their spiritual enrichment towards reaching their ultimate goal of wisdom.

Siddha medicine considers human body as the direct replica of nature. With much praise to the divine, quote *"Andathil ullathey pindam, pindathil ullathey andam "*stated by *Siddhar Sattaimuni* there are so many *Siddhar* school of thoughts in high class T*amizh* which quantifies the theories of human body origin expressed in material anatomy called *Panchabootha Panjeekaranam*, its evolution , Physiology, Transformation and finally degeneration. All this phenomenon comes under the eternal basic principles called 96 *Thathwam* or constituent principles including Physical, psychological and spiritual entities of a human being. The *Siddhars* School fully recognizes these ninety-six *thathwam* and further added that human body is composed of 72,000 blood vessels, 13,000 nerves, 10 main arteries, 10 vital airs all together in the network; and it is, owing to the derangement of the any of the principles one become liable to 4448 types of diseases[(1)].

As a fact of substitution any ailments pertaining to human body there is a healing part in nature. *Siddha* systems, which were developed under the principles of nature, use vast variety of material resources from plant, animal, and mineral kingdom. *Siddhars* had an in-depth knowledge in every aspect of natural resources and how it can be transformed to healing elements of medicine. Understanding the body nature and the sufferings it catches is as tough as understanding the elements of nature. For the effective application these natural elements has to be directly or indirectly modified, processed into a form acceptable or assimilable by the body constituents. Accordingly, they developed lakhs of formulations under different categories each suiting for specific conditions of the body and the disease[(2)].

Theeneer[3] or Distillery medicines is one such unique form of medications under the category of 34 common medications *(Makkal Urai)* and as the first choice in Divine treatment ie, 12 supreme classes of medications called *Deva Maruthvam*[4]**.** Vast commentations on *Theeneer/Dravagam* are attributed to *Siddhars* like *Agasthyar, Nandidevar, Thirumoolar, Pulasthyar, Bohar, Theraiyyar, Konganavar, Yacobe, Ramadevar, Yugimuni* etc.

The sufferers of liver diseases and asthma are day by day increasing and is now very common in our clinical practice. Identifying a novel drug of choice in Liver diseases and respiratory diseases is a challenge to the Researchers. Apart from drug of choice, the factors like the nature of the formulation, its palatability and faster efficacy has to be also considered wisely. As Theeneer is an ideal drug which may cover up all the factors mentioned above, the researcher has selected a classical distillate formulation named "*Sanjeevi Theeneer"* that are indicated for wide range of diseases including Liver diseases, respiratory diseases, skin diseases, Cardiac diseases etc. The drug has not been put into clinical practice or undergone validation. As an initial step Sanjeevi Theeneer will be prepared as per the Siddha literatures, Formulation has been standardized, analytical studies has been performed followed by Toxicity studies and Pharmacological studies in Animal models. The safety and efficacy is established which may pave the way for Clinical evaluation.

II. AIM AND OBJECTIVES

Aim

To evaluate the Safety and Pharmacological profile of the test drug "*Sanjeevi Theeneer"* in Animal Models.

❖ Primary objective

1. Preparation of the Trial drug as per the *Siddha* literature.
2. To standardize the trial drug as per AYUSH guidelines.
3. To Study the Acute, Sub acute and Sub Chronic Toxicity studies of the trial drug as per the OECD guidelines.
4. To Evaluate the Hepato protective activity, Broncho dilator and Anti-Histamine activity of the trial drug.

❖ Secondary objective

1. To Assess the Anti-oxidant property of *Sanjeevi Theeneer* which supports the therapeutic potential of the drug.
2. To Evaluate and assess the Hepato protective activity of the trial drug against Paracetamol induced Hepato toxicity in lower vertebrate like Zebra fish (Danio rerio) model.
3. To compare and study the differences of trial drug prepared from both Traditional apparatus and Glass still through GC-MS Analysis.
4. To collect and document all the literature reviews of *Theeneer*.

III. MATERIALS AND METHODS

DRUG PROFILE

a. Drug selection and Reference

*Sanjeevi Theeneer*is a classical *Siddha* Herbo mineral distillate formulation mentioned in *Chikitsa ratna deepam* and *Siddha* Formulary of India [(5)]

b. Ingredients of *Sanjeevitheeneer* (Table no*:* 1)

S. NO	Ingredient	Botanical/Scientific Name	Parts Used	Quantity
1	*Chukku*	*Zingiber officinale*	Dry Rhizome (Outer skin removed)	60 g
2	*Milagu*	*Piper nigrum*	Dry fruit	60 g
3	*Thippili*	*Piper longum*	Dry Berry	10g
4	*Kadukkai thol*	*Terminalia chebula*	Dry fruit (seed removed)	25g
5	*Nelli vatral*	*Phyllanthus emblica*	Dry fruit (seed removed)	50g
6	*Tantrikkai thol*	*Terminalia belerica*	Dry fruit (seed removed)	25g
7	*Omam*	*Trachyspermum ammi*	Dry fruit	25g
8	*Vaividangam*	*Embelia ribes*	Dry fruit	25g
9	*Chithramoola Verpattai*	*Plumbago zeylanica*	Dry Root Bark	30g
10	*Korai kizhangu*	*Cyperus rotundus*	Dry Tuber	25g
11	*Panam karkandu*	*Borassus flabellifer*	Palm Candy	20g
12	*Irumbu Podi*	Purified Ferrum powder	-- ---------	60 g
13	Water			6 Litres

c. Raw Drug collection and Authentication

All the ingredients (Fig: 1) were purchased locally from reputed raw drug shops in Chennai and Nagercoil. The herbal ingredients were identified and authenticated from the Botany division, National Institute of Siddha (Authentication No: NISMB2472016), the mineral sample were identified from Department of Geology, Anna university (Annexure 2).

Fig: 1 INGREDIENTS OF *SANJEEVI THEENEER*

Chukku (Zingiber officinale)

Milagu (Piper nigrum)

Thippili (Piper longum)

Kadukkai (Terminalia chebula)

Nelli vatral (Phyllanthus emblica)

Thantrikkai (Terminalia belerica)

Omam (Trachyspermum ammi)

Vaividangam (Embelia ribes)

Kodiveli verpattai (Plumbago zeylanica)

Panam karkandu (palm sugar candy)

Method of Preparation

d. 1 Purification of Raw drugs

Table: 2 - Purification *(Suddhi)* Process of Individual Raw Drugs [6]

S.No	**Raw Drug**	**Method of Purification**
1	*Ayam*	Powdered finely soaked in lemon juice for three days. In addition, grinded well, washed and dried (Fig: 2)
2.	*Chukku*	Outer skin removed.
3.	*Milagu*	Cleaned, sorted, and dried.
4.	*Thippili*	Cleaned and dried.
5.	*Kadukkai*	Inner seed removed, washed and dried.
6.	*Nelli Vatral*	Inner seed removed, washed and dried.
7.	*Thantrikai*	Inner seed removed, washed and dried.
8.	*Omam*	Cleaned, sorted and dried.
9.	*Vaividangam*	Cleaned, sorted and dried.
10	*Kodiveli Ver pattai*	Washed thoroughly, cut into pieces, and then soaked in *chunna nee*r (Limewater) for 1 *samam* (3 hours) Finally washed and dried again.
11	*Korai kizhangu*	Cleaned and sorted washed and dried.
12	*Panam Karkandu*	Cleaned and sorted.

Fig: 2 **Raw Iron powder**

Ayam (Raw powder before purification)

Ayam powder soaked in Lemon juice

Ayam completely dried

Ayam powder after Purification

TRIAL DRUG PREPARATION

For the comparative study of distillates prepared from Traditional still (Valai iyanthram) and Glass still denoted as ST (t) and ST (g) respectively. The Comparative study is aimed to identify which make of distillate meets the quality parameters as mentioned in the *Siddha* literature and its scientific perspective through GC-MS Analysis. Apart from the comparative studies, the sample ST (t) will be utilized for all the Drug Standardization, Bio chemical and Preliminary Phyto chemical screening. All the concerned Toxicological and Pharmacological studies will be carried out by using ST (t) sample. Third sample of *Sanjeevi theeneer*, ST(c) is prepared in further concentration, will be subjected to GC-MS studies only for in depth analysis of the Organic compounds.

d.2 Preparation of Distillate sample - ST (t) and ST (C)

- All the dried raw drugs were powdered and mixed with prescribed quantity of water and kept for fermentation upto a period of 7 days. This counts the period of fermentation.
- On the 8^{th} day, the whole mixture is thoroughly stirred and charged in *Valai iyanthram* (Traditional mud still) (Fig: 4).
- Distillation was started in low flame and the *Theeneer* was collected a separate thick glass bottle with tight lid.
- The sample was preserved in dark and under moisture and damp free condition for all the concerned Analytical, Toxicological and Pharmacological studies.
- ST (C) sample is Prepared in the same procedure as mentioned above, but with the difference in the quantity of water added (1.5 litres for the Batch size).

d.3 Preparation of Distillate sample- ST (g)

- This mixture (drug with water) were charged in a glass still which consist of a flat bottomed round beaker (1 litre Capacity) , Cylindrical condenser with coils (glass made) with provision of water inlet, outlet and steam outlet in one side connected to the beaker via ‘U’ bend and distillate outlet connected to the collecting bottle.
- The whole apparatus rests in a heating mantle and temperature set in different intervals up to the maximum 100 ^{0}c (Fig: 5)
- The distillates collected were preserved for studies.

Fig: 2 ST – Fermentation ***Valai iyanthram*** **Glass Still Apparatus**

Labeling:

Name	:	*Sanjeevi Theeneer*
Color	:	Light Lemon Yellow
Dose	:	15 – 30 ml
Adjuvant	:	Diluted with water
Date of manufacture	:	15.09.2016
Date of expiry	:	1 Year

Indication:

- *Kamalai* (Jaundice)
- *Peruvayiru* (Ascites)
- *Gunma katti*
- *Moola noikal* (Hemorrhoids)
- *Venkushttam* (Leucoderma)
- *Sori, sirangu* (Scabies and pruritis)
- *Ilaippu Irumal* (Pulmonary Tb)
- *Iraippu Irumal* (Bronchial Asthma)
- *Marbu noi* (Cardiac ailments)

4.1 a Review on *Theeneer Maruthvam* (Distillation Medicines)

Distillation products are far seen as a special dosage form in all these systems of medicine of Indian, Chinese and Persian origin. Experts belonging to each medical system had an immense knowledge of extracting valuable essences from raw materials primarily using herbal ingredients. The science of distilling materials of herbal origin or non-herbal sources has been elaborately discussed in *Siddha* medicine and the knowledge of chemistry mingling with the medicine preparation is far more superior than any other Traditional systems.

Numerous ancient theories of drug selection and manufacture has been mentioned each in significance with each method of drug preparation. *Siva Sakthi Thathvam*[1] (Core Essence and Energy concept) has been much applied with art of distillation followed in *Siddha*. An ideology contributes the facts about the two major energy existences in each material present in nature and how it is classified based on the energy predominance. *Sivam* or Core Essence is the subtle energy part of a material and the *Sakthi* the subtle material energy surrounding it. Equilibrium of both the energies maintains the stability of the material. *Theeneer* / *Dravaga murai* (Distillation process) is rooted on the concept of separating *Sivam* or the Core extract of a herbal ingredient in case of herbal distillates or separating material energy in the case of mineral or salt distillation thus making the distillate *Siva Veeryam* or *Sakthi Veeryam* or combined *Siva Sakthi Veeryam* distinguished and termed in-respective of their potency.

Theeneer and *Dravagam* more or less similar in conceptology but differs in their process or make (including raw materials used) and its application part. Both are coined as different form of distillates in majority of classical works but the terminologies are commonly used for the same distillates [7, 8].

Theeneer

1. Concept and Terminology

Theeneer[3, 9] is the distilled essence, which contains the volatile constituents or water-soluble constituents of the drugs used in the preparation in a medium of water. They are colloidal suspensions (hydrosol) of essential oils as well as water-soluble components obtained bysteam distillation from plants/herbs.

Theeneer (*Thee* = Fire, *Neer* = Water)

Known by various names like *Vatru marunthu, vaattu*[11], *Aaavi Neer* [10], *Valai Neer* literally denotes distilled water or essence.

These are the common distillation products obtained mainly from plant resources that may be simple or compound formulations with or without adding minerals. The procedures for manufacture are accessible or uncomplicated, and resulting distillates are mainly used as medicines or as neutraceuticals. Herbal distillates are the most commonly practiced form among traditional *siddha* practitioners and as a health supplement among the dravidian peoples truly blended with their culture. These are milder forms of distillates with less concentration, potency and shelf life. The herbal parts after purification, soaking or fermentation in suitable media is charged inside a traditional distillation apparatus (*Vaalai iyanthram*) and subjected to distillation. The steam generated due to boiling of the contents rises up, condensed and released out as purest form of distillates. Essential oils from the herb parts usually escape much faster before the boiling and are collected in the container .These may float as a supernatant layer in the distillates and are termed as *Theeneer ennai*, which are separated and used.

***Theeneer ennai*: (Essential oil obtained from herbal distillation)[13]**

These are the volatile oils collected during the first phase of distillation of herbs. *Theeneer ennai* is widely used internally, externally and as an aromatic additive for oils, topical linaments and lotions.

1. Classification of *Theeneer* (based on formulations)

Siddha system describes the usage of wide range of raw materials (plant, animal or mineral resources) to obtain the distillates and its nature, yield and properties is dependent on the ingredient used. Distillation products can be classified in reference with the type of formulations into:

a. ***Thani sarakku Theeneer/Dravagam*** **(Uni compound distillery formulations**) were only single plant resources or mineral is used.
Egs: *Senkottai Dravagam* [7], *Vasambu dravagam* [8], *Pudina theeneer* [5], *Sombu theeneeer, Oma theeneer* [13].

b. ***Pala sarakku Theeneer/Dravagam*** **(Poly compound distillates**) were a mixture of several herbs, animal products or minerals are used. Egs: *Oma Dravagam*[11], *Mahaguru Dravagam,Sanjeevi theeneer.*

1.Method of Preparation [1-36]

a. Apparatus

The apparatus used for distillation is termed as *Valai iyanthram* [14]*Valai* is defined as the apparatus with provisions or outlet tube to collect the distillates.

b. Types of Distillation apparatus [15]

Different types of distillation apparatus has been mentioned depending on the making or material used for the distillation.

1. *Munn valai* (Traditional *valai iyanthram* made with clay).
2. *Uloga valai* (Apparatus made with metals).
    a. *Irumbu valai* (Iron apparatus).
    b. *Thambira valai* (Copper apparatus).
    c. *Velli Valai* (Silver Apparatus).
3. *Spadika valai* or *Kannadi Valai*(Glass made).
4. *Peenkana Valai* (Porcelain made)

The types will be selected based on raw materials used suited for various distillation methods. Each made has its own peculiarity and it includes the heat tolerability, yield and quality of the distillates. Metallic apparatus are indeed used for specific preparations. As the raw materials to be distilled should not react with the metal used, it is chosen only for herbal distillate production.

Spadika valai (glass made) fails in heat tolerability. With much safety issues, it is not chosen for salt, caustic, concentrated distillates in which the apparatus has to bear extreme heat and pressure[15]. A sustained heat more or less than the boiling point of water can be maintained within a glass made apparatus and is optive for distilling herbal parts. High yield of volatile oils and clarity distillates can be obtained by it. The usage of porcelain made is uncommon in practice and needs further studies.

Among all the apparatus, the traditionally made apparatus stands unique with its high heat tolerability, unreactive to raw drugs, higher alkalies or salts with assured quality and purity of the distillates. There will be mild to moderate yield loss depending on the quality of the clay apparatus.

c. Parts of a Traditional distillation apparatus or stills

The apparatus consist of many components, which are equipped in such a way to form a single unit, which are collectively termed as *Valai Iyanthram* or *Dravaga valai*. So many models of various sizes were used depending on the yield required **(15)**.Clay made apparatus(*Munn valai* [9]) that in vertical installations are most commonly used. The different components of an apparatus serve each purpose and are specific in its make.

Lower vessel (*kalayam*) is used to charge the raw materials with the medium for distillation. Suitable capacity of lower vessel is selected to accommodate the medicine to be distilled. The length of the neck portion should not be less than three finger breadth with narrow mouth region that has to be tightly fit with the upper vessel (*vaalai*). The junction between the *kalayam* and *vaalai is* sealed with clay paste, and after dying of the plaster the entire setup is subjected to heating process.

The upper vessel (*vaalai)* consists of a condenser part to provide a continuous water flow above the vessel. Provisions of water inlet on one side and outleton the other side are long enough to release the heated water away from the apparatus. The openings of the inlet and outlet within the condenser is at different height levels, and the water current is maintained in such a way that the cool water flowing in is proportional to the heated water going out. From inside an outlet is connected to collect the condensed distillates. The outlet tube *(valai iyanthra mookku, keezh kuzhai)* that is slightly curved as a nose is facing downwards in the opposite side of the water outlet with the tip of the outlet called receiver *(Thamar vai, Kathir vai)* kept inside the collecting bottle.

To collect the distillates tightly stoppered thick glass bottles (eg: *Vediyuppu dravagam*, Porcelain bottles *(Peenkan kuppi, Peenkana Kinnam)* with tight lid or stone cork /wooden cork will be used.

Table: 3 Basic comparisons of Traditional and Glass Stills.

	Features	Glass Stills	Traditional Apparatus
1	Make of the Apparatus	Glass	Clay Soil
2	Parts of the Apparatus (full set)	**a**. Glass beaker (Borosil flat bottomed (1no). **b.**'U'Bend (2 nos) **c**. Condenser with Steam inlet, Distillate outlet, water inlet & outlet. **d**. Collecting Bottle (1no). **e**. Heating Mesh.	a. Lower Vessel b. Upper Vessel with condenser part, Water inlet and outlets, Distillate outlet. c. Collecting Vessel or bottle.
3	Working on	Electricity	Conventional Fuels
4	Heat Loss	Minimal	Considerable
5	Steam Loss	Minimal	Depends on apparatus quality and sealing.
6	Safety Issues[5]	Susceptible to breakage during process or inexperienced handling. Safety issues are concerned.	No issues of safety
7	Purpose & Limitations	For distillation of water, alcohol, herbs. *Not suited for Salt distillations or super concentrated distillates. *Best for commercial extraction of Volatile oils.	For distillation of wide range of materials of herbal, herbo-mineral compounds. *Best for salt distillations and concentrated distillates. *Best for Manufacturing Traditional therapeutic distillates.

d. Pre procedures

Before each distillation, preprocedures are done with the aim to make the drug ready for distillation. The extracts of the materials will be easily released into the medium, also it maintains an environment needed for the conversion of the raw material to the stage easier to distillate, or either it purifies or improve its potency of the material used. So many traditional preprocedures that is mentioned before one distillation depending on the processes or raw material nature.

d.1 Raw material purification

d.2 Pounding, powdering or grinding:

The materials become finer, solubility of the raw drugs in the media will be fastened and the essence or compounds will become easily available in the media. Grinding in suitable media ensures proper mixing. For herbal distillation, the purified herbal parts are pounded well before soaking or fermenting for better extraction of essence.

d.3 Soaking in Specific media or to enable Fermentation.

Most of the raw drugs before subjecting to distillation are soaked in suitable media for specific periods. Soaking dissolutes the raw material in the the media and its period may depend on the hardness of the raw material. The main purpose is to soften the material and to enable faster extraction while distillation.

Role of fermentation in distillation

- Fermentation has a notable role in the art of distillation. During the process, it induces natural alcohol formation and a pH, which favors good solubility of the raw material with Simultaneous extraction of wide range of phyto constituents from the herbal parts.
- Detoxification of contaminants or any toxic components from the herbal part also occurs during fermentation.
- Two methods are adopted for fermentation with respect to distillation in *Siddha* system. one is by inducing basic fermentation by adding natural fermenters another by using synthetic acidic distillatesprepared from *omam* (Carum copticum), *padikaram* (Alumen alum) and *vediyuppu* (Potassium nitrate. For

fermentation suitable media like water, plant juices are mixed with coarse or fine powdered raw drugs along with addition of natural fermenters or synthetic acidic distillates in properly sealed vessels preferably wooden or porcelain vessels to maintain a controlled and sterile environment.

- The process of fermentation is considered to improve the power of the mixture(*Siddhi*) before undergoing distillations.

d.4 The raw drugs exposed directly or indirectly to various environments

Powdered raw drugs are mixed with suitable media kept in sunlight (*Soorya pudam*) until complete dehydration (eg: *kadukkai dravagam)*, or upto specific periods (eg: *Kavattam pul dravagam)* and finally distilled. In some preparations, powdered raw drugs are kept exposed to night dew or moon light (*Pani Pudam, Chandra pudam*) prior to distillation.

e. Procedures employed after distillation

After herbal distillations, the mud apparatus (both upper still and lower vessel) are carefully dismantled and soaked in water not less than one *samam* (3 hours) washed thoroughly and sundried. This method prevents crack formation in mud vessels due to constant heat, cross contamination of medicines and makes its ready for the next preparations.

Factors determining quality of *Theeneer*[(1-36)]

The factors that determine the quality and yield of *Theeneer* include:

1. Quality of the raw material used :

Raw materials of superior quality, devoid of pest or rodent contamination and which followed strict purification (if applicable) has to be selected. Expired herbs yield inferior quality distillates.

2. Apparatus used for distillation :

- For traditional mud vessels or apparatus quality of the clay used, thickness of the vessel, and its method of making has an impact in the yield obtained directly proportional to the wastage of medicines.
- Good yield denotes collection of more than 70% of the quantity of the batch volume used.
- Mud vessels showing cracks after heating indicates less heat resistance due to usage of inferior quality clay.Clay pots that have been fired to a point and gazed make them more heat resistant.
- Mud vessels with high porosity will absorb the liquid medicines when kept for a long time this may be due to improper baking in traditional owen or its preprocessing as mentioned below.
- Pretreated mud vessels are used in all distillation process, which include soaking the mud vessel for at least 2 hours to a full overnight in water, pouring rice washed water into the mud vessel, which is kept for a day or upto 4 days.
- For other types of apparatus as mentioned, materials that hinder the quality is avoided. For Distillation the contents are filled upto half of the vessel.

3. Medium for distillation :

Media acts as an effective solvent for most of the raw drugs and as a carrier for the medicine principles of the raw drug, that is undergone distillation, and some were used due to its therapeutic value. In majority of distillations, pure water is used as the medium for distillation. Apart from this dew water (eg: *Pooneerdravagam*), rice washed water (eg: *Pooneerpugai neer*), herbal juices (eg: *Injidravagam*), honey (eg: *Then dravagam*) cow's milk (eg: *Moongiluppathi Theeneer*) animal urine (eg: *kadukkai dravagam*) are also used in special formulations.

4. Quantity used

As a general rule the quantity of water in which the raw drugs completely soaks or two parts of the total raw material weight (eg: *oma dravagam*) or 12 parts of the raw material weight (eg: *Sanjeevi theeneer*). With experience one can ascertain that quantity of the water added which varies with nature of the raw drugs, yield or concentration needed.

5. Plastering

The junction of the two vessels will be properly plastered and dried. This will prevent steam loss and ensure heat retainibilty within the vessel for proper boiling of the contents or its condensation. Therefore, for maintaining heat retention and for considerable distillate yield, proper plastering is crucial.

6. Fuels:

Firewood that does not hinder the quality of *Theeneer* should be used. *Varatti* (cow dung cakes) are also mentioned in special preparations.

7. Mode of heat application

The heating procedures should commence from low flame called *deepakni*, then mid flame (*kamalakni).* The control of heat is very crucial in distillation, as it maintains a slow, steady and maximum extraction or release of phyto compounds and volatile oils in case of herbal distillation The herbs should not come in direct contact with heat. It means the distillation should not start with high heat or *kadakni*, which causes chaaring of the herbal parts, rapid dissolution of volatile contents and alkaloids. This not only reduces the yield, the distillate becomes unpleasant in taste or odour and is considered as inferior quality.

8. **Storage of distillates and expiry**

Bottles should not be kept open after usage. This will prevent the escape of valuable essential oils in case of herbal distillates and reactions due to oxidation in case of dravagam.Shelf life period of *Theeneer* is limited to one year.

5. Traditional quality parameters of distillates[22]

- With reference to traditional experience and literature review, *Theeneer* should have the peculiar color, taste, odor and medicinal property of that of the raw drugs used. Otherwise is considered as inferior quality.
- It is by experience one can know the time for distillation and the quality of the distillates. For herbal distillates occurrence of fine aromatic steam marks the initial part of distillation followed by collection of condensed distillates.

- The aroma, color is unchanged until the end of the middle stage. The final stage of distillation is noticed by reduction of fumes, aroma, color and quantity of the distillates. This is the point at which the distillation has to be arrested, heating beyond this stage causes charring of the herbal contents or raw materials marked by appearance of dark fumes with smoky odor. The distillate collected in this stage is least inferior quality and should be discarded.

6. Mode of Usage[5, 7, 8, 15]

Theeneer /Dravagam are purposed for internal usage.

- **Dose** : For *Theeneer* as the concentration is mild it can be taken in the dosage of 1 *karandi* (1 teaspoon) 1/2, 1 ounce, 2 ounces , 1/4th of *azhakku (1 azhakku = 168ml)* per servings , before or after food diluted with prescribed quantity of water or taken as such.
- **Adjuvant:** Most distillates of internal usage are diluted with prescribed quantity of water or Luke warm water. Adjuvents like ghee, flour (eg: *pooneer dravagm*[17]), *Thrikadugu choornam*, mothers milk, honey, jaggery (eg: *Mantha dravagam*[22]) are specially recommended adjuvants.
- **Course:** Herbal Distillates can be taken on long-term basis as a rejuvenate supplement or as medicine depending on the potency and formulation and is considered as the safest medicines on long term basis[29]
- **Diet Restrictions:** Most of the *Theeneer* indicates mild restrictions as which concerns with the disease. Some salt distillates advices avoidance of sour diet. To reduce medicine heat induced by potent distillates, ghee, milk depending on ones digestive fire is advised.

Fig: 6 PARTS OF A *VALAI IYANTHRAM* (TRADITIONAL DISTILLATION STILL)

Valai (Upper still – Whole view) **Valai (Upper still – Condenser part**

Valai iyanthram (Single Unit Installed) Valai iyanthram (Single Unit Installed) with Kalayam)

சுக்கு (Chukku) [27]

NtWngau; **(Synonyms):** Arukkan, athakam, aardrakam, upakullam, kadupathiram, chundi, sowpannam, sowvarnam, navachuru, naagaram, manoushadam, visabheshajam, vidamoodiya amritham, verkombu.

Parts Used: Dried rhizome of Ginger.

சுவை (Taste) : கார்ப்பு (Pungent)

தன்மை (Potency) : வெப்பம் (Hot)

பிரிவு (Division) : கார்ப்பு, (Pungent)

செய்கை (ACTIONS)

- ❖ வெப்பமுண்டாக்கி (Stimulant)
- ❖ பசித்தீத்தூண்டி (Stomachic)
- ❖ அகட்டுவாயுவகற்றி (Carminative)

சுக்கின் பொதுகுணம் (General Properties of Chukku) [11, 27]

"சூலைமந்தம் நெஞ்செரிப்பு தோடமேப்பம்மழலை
மூலம் இரைப்பிருமல் மூக்குநீர் வாலகப
தோடமதிசாரஞ் தொடர் வாத குன்மநீர்த்
தோடம் ஆமம் போக்குஞ் சுக்கு "

- அகஸ்தியர் குண வாகடம் [26]

Description: With Chukku (Dry Ginger), Soolai (painfull conditions), Vayitru mantham (Dyspepsia), Nenjerivu (Retrosternal burning), Dosham, Choodu (Sensation of heat), Azhalai, Moolam (Hemorrhoids), Iraippirumal (Asthmatic affections), Pediatric cough, Athisaaram (Diarrhoel diseases), Gunmam will be cured.

“வாதபிணி வயிறூதர் செவி வாய்

வலி த்தலைவலி க்குலைவலி யிருவிழிநீர்

சீததொடு வரிபேதி ப்பலரோ

சிகமலி முகமக முகமிடி கபமார்

சீதச்சுரம் விரிபேத ச்சுரநோய்

தெறிபடு மெனமொழிகு வர்புவிதனிலே

ஈதுக்குதவு மிதீத்துக்குதவா

தெனும் விதி இலை நவசிறு குணமுனவே”

----தேரன்குணபாடம் [11]

Description: With Chukku Vatha diseases,Vayiroothal (Bloating of the abdomen), Chevi-vai-thalaivali (Pain pertaining to to ear, oral cavity and head region), Soolai vali (Painfull conditions)Excess watery discharge from eyes, Seetham (cold affections) , Bedhi (Diarrhoea),Kapham (Phlegm), Maarbuvali (chest pain), Theeratha suram (Chronic fever) will be relieved. Generally, Chukku can be advised in all diseases.

சுக்கின்பொதுகுணம்

"சுக்கு மிகதாதுவாம் சொல்லரிய தீபனமாம்

மிக்கநல்ல வாதம் போம் மெல்லி தல்லீர்தக்கதொரு

மூக்கு நீர்பாய்ச்சல் மூலரோகத்தினோடு

தக்க தாம் வினைபோகும்தான் "

- பொருள் பண்பு நூல் (மூலிகை வகுப்பு) [11]

Description: Chukku is a Thathu (nutrient).It cures Vatha diseases, rhinorrhea, and moolarogam (Hemorrhoids).

"சுக்கினாலேஅனேகமாம்ரோகங்கள்

தொக்கில்நின்றுவிலகிடுகுன்மமும்

பக்கவாதமோடேயாவும்பித்தமும்

ஓக்கஓடிஒளிந்திடும்உண்மையே "

- பொருள் பண்பு நூல் (மூலிகை வகுப்பு) [11]

Description: Many types of diseases will be benefited with Chukku. Gunmam, Pakkavatham (Hemiplegic conditions) and Pitha diseases.

]

"தீராதவாதம் அதிசாரம் சிறந்த வா த குன்மங்கள்
பாரீர்ஈளை இருமலுடன்பயின்ற சுவாசகாசமும் போம்
ஆறாதீரை அஜீர்ணமும் அநேகவியாதி அகன்றுவிக்கும்
காரர்குழலின் மடமாதே கடிய சுக்கின் குணமாமே"

- பொருள் பண்பு நூல் (மூலிகை வகுப்பு) [11]

Description: Chronic and uncontrolled Vatha diseases (Rheumatic affections), Athisaram (Diarrhoel diseases), Vatha gunmam, Eeelai (Excessive sputum), Irumal (Cough), Swasa Kasam (Asthmatic affections), Ajeernam (Dyspepsia).

"சுக்குதான் கண்ணிலுள்ள தோஷங்கள் பலதும் போக்கும்"

Description : Chukku is very good for Eye related conditions.

மருத்துவபயன் (Therapeutic uses of Chukku)

- **சுக்குகர்ப்பம்**

" சுக்கினை பொடிசெய்து சூரணமாக்கி
இக்கிரத்திலுண்டிட வயிற்றெரிவு போம்
குடிநீர் செய்ததை குடிநித நன்மையாம்"

- அகஸ்தியர் குண வாகடம் [26]

Description: Chukku powder when taken with sugarcane juice at morning period will relieve burning sensation of stomach. Chukku kudineer is very benefical to maintain health.

- Chukku paste locally applied for headache, neck pain and painfull joint affections yield good results.
- Chewing pieces of Chukku will relieve throat congestion, hoarseness of voice.
- Chukku kudineer is very good for dyspepsia, vomiting, chronic fever etc.

மிளகு (Milagu) [27]

Synonyms : Kalinai, Kari, kaayam, kolakam, miriyal, sarumapantham, vallisam, maasam, malaiyaali.

Parts Used : Seed, Stem, root.

சுவை (Taste) : கார்ப்பு (Pungent)

தன்மை (Potency) : வெப்பம் (Hot)

பிரிவு (Division) : கார்ப்பு, (Pungent)

செய்கை (ACTIONS)

- காறலுண்டாக்கி (Acrid)
- முறைவெப்பகற்றி (Anti periodic)
- தடிப்புண்டாக்கி (Rubefacient)
- வெப்பமுண்டாக்கி (Stimulant)
- வீக்கம்கரைச்சி (Resolvant)
- வாதமடக்கி (Anti Vatha)
- நச்சரி (Anti dote)
- அகட்டுவாயுவகற்றி (Carminative)

TYPES

Pepper has 5 main varieties according to siddha literature they are:

1. Nalla Milagu

2. Vaal Milagu

3. Kola Milagu

4. Cheeni Milagu

5. Kaattu Milagu

மிளகின்பொதுகுணம் (General Properties of Milagu) [11, 27]

"சோகை சுரங்களாலும் சூலை கபரோகம் போம்

தாகமுறும் மந்தமதனை போக்கும்போக

சிலந்தி கிருமியும் சில்விஷங்கள் நாசமாகும்

பொருந்தும் மிளகருந்தும் போது"

- பொருள் பண்பு நூல் (மூலிகை வகுப்பு) [11]

Description: Sokai, Suram, soolai, kapham, Mantham will be cured with black pepper. It will induce thirst and is a good antidote for spider poison and other toxicities.

"கோணுகின்ற பக்கவலி குய்யரோகம் வாதம்
சுரோணிதமும் கழுத்திற்குள் தோன்றும்
காதுநோய் மாதர்குன்மம் காமாலை மந்தமென்றி
ஏதுநோய்களிருக்கும்இங்கு "

- பொருள் பண்பு நூல் (மூலிகை வகுப்பு) [11]

Description : Pakka vali, kuyyarogam, vaatham, sronitha noykal, Kathu noi, Maathar gunmam, Kaamalai ,Mantham will be cured by using Milagu.

சீதாசுரம் பாண்டு சிலேத்துமங் கிராணி குன்மம்
வாதமருசி பித்தமாம்மூலம் ஓதுசன்னி
அபஸ்மாரம் அடர்மேகம் காசமிவை
நாசங் கறிமிளகினால் காண்"

- பொருள் பண்பு நூல் (மூலிகை வகுப்பு) [11]

Description : Seetham, Suram , Paandu, Slethmam, Gunmam, Vaatham, Arusi, Pitham, Moolam, Sanni, Megham, kasamwill be prevented with black pepper usage.

"மிளகினுட விரியந்தான் முறைமைய தாய்காந்தி பித்தம்
இளகிய சில்விஷம் இடினாசிகாந்தல் உஷ்ணந்தான்
ஒத்து நிற்கும் எரிப்புட னேபறப்புமிகும்
சத்துதரும் மிளகைகேள் "

- பொருள் பண்பு நூல் (மூலிகை வகுப்பு) [11]

Description : Silvisham, udarchoodu will be relieved and will improve the vigor .

"பழையபிணிவிஷபாண்டுபலதும்போம்கண்நோயும்
தழையவரும்பிசாசுக்கள்தான்போம்தரணியில்
அழகுவடிவாயிருக்கும்அகமுடம்பைநன்றாய்
விழிமயக்கம்பிறத்தில்வெளிப்போம்தான்"

-பொருள் பண்பு நூல் (மூலிகை வகுப்பு) [11]

Description : Chronic Diseases, Vishapaandu, Kannnoi will be controlled with milagu moreover it protect one from Devil spirit attacks. It is good for giddiness associated with uterine diseases.

மருத்துவபயன் (Therapeutic uses of Milagu)

- Milagu decoction is effective for indigestion
- Milagu powder at a dose of 260 – 390 mg is a good appetizer.
- It is included as one of the main ingredients of tooth powder
- It is one of the ingredient of *mukkadugu.*
- Lehyam prepared with Milagu, Perunjeeragam, and Honey is given in the dose of kazharchi alavu for hemorrhoids occurring in old peoples and emaciated ones.
- A mixture of Milagu, venkayam and uppu is a good external application for alopecia areata.

திப்பிலி (Thippili)

Synonyms : Aarkathi, unrasam, ulavai nasi, kaaman, kudari, kolakam, koli, kozhaiyarukki, saram, chaadi, thulavi, maakathi, kanai, chowndi, thanduli, kalini, paanam, pippili, vaideki, ambu, aathi marunthu.

Parts Used	**:**	Berry, root.
சுவை (Taste)	**:**	கார்ப்பு (pungent)
தன்மை (Potency)	**:**	வெப்பம் (Hot)
பிரிவு (Division)	**:**	இனிப்பு (Sweet)

செய்கை (ACTIONS)

- ❖ வெப்பமுண்டாக்கி (Stimulant)
- ❖ அகட்டுவாயுவகற்றி (Carminative)

TYPES

Thippili are of 2 types

1. Arisi thippili
2. Yanai thippili

அரசிதிப்பிலியின் பொது குணம் (General Properties of small variety)

"திப்பிலிக்கு வாதம்மையம் சேர்ந்தவிக்கலுந் தான் போகும்
மற்றும் விஷநீர் பித்தம் வாராது விர்பிரமை
சன்னி குன்மம் சூலை தளர்த்திவிழி நோய் சயம்
பொன்னியாதிசாரம் போம்புகல்"

- பொருள் பண்பு நூல் (மூலிகை வகுப்பு) [11]

Description: With small variety of Long pepper Vishaneer, pitham, prammaipidithal, sanni, gunmam, soolai, vizhinoy, kshyam, athisaram will be cured.

யானை திப்பிலியின் பொது குணம் (General Properties of Large variety)

"வாதமாறும் தீபனமாம் மாறாகபம் கரைப்பான்
ஓது குரல்கம்மல் இவை ஓடுங்காண் பூதலத்தில்
சோனையே நேர்நாசினியில் தோலாசுவாஸவும் போம்
ஆனையென்ற திப்பிலியால்ஆய்"

- பொருள் பண்பு நூல் (மூலிகை வகுப்பு) [11]

Description: Vatham, pasiyinmai, kapham, kural kammal will be reduced with thippili-large variety moreover it cures nasal block also.

திப்பிலியின் பொது குணம்

"இருமல் குன்மம் இரைப்பு கயப்பிணி
ஈளை பாண்டு சந்நியாசம் அரோசகம்
பொருமாலூதை சிரப்பிணி மூர்ச்சைநோய்
பூரிக்கும் நீர்கோவை பீலிகமும்
வருமல பெருக்கோடு மகோதரம்
வாதமாதி முக்குற்றம் சுரங்குளிர்
பெருமலை புரிமேக புடகமும்
பேரும் திப்பிலி பேரங்கு உரைக்கவே "

- பொருள் பண்பு நூல் (மூலிகை வகுப்பு) [11]

Description: Irumal, gunmam, iraippu, kayappini, eelai, paandu, sanni, arosikam, porumal, siarasu noy , moorchainoy, neerkovai, pleekam, malam pidithal, mahodaram, vatham, mukutram, suram, kulir, megham will be relieved with long pepper.

"ஆசனநோய் தொண்டைநோய் ஆவரண பித்த முதல்
நாசிவிழி காதிவை நோய் நட்புருநோய் -வீசிவிடும்
அங்கலாஞ்சனசிதையுமப்பா அழிவிந்தம்
போங்கலாஞ்சனங்கையில் தோல் -போல"

- அகஸ்தியர் குண வாகடம் [26]

Description: Aasana noy, thondai noy, aavarana pitham, nasi-vizhi-kaathu noikal, Vinthu ilaki pothal will be relieved with long pepper.

மருத்துவ பயன் (Therapeutic uses of Thippili)

- Thippili 5 parts, thetran seeds 3 parts are powdered well and given in the dose of 4g with rice washed water for Leucorrhoea, menorrhagia.
- Thippili powder with betel juice and honey is effective for phlegmatic disorders, cough and fever.
- Thippili powder (1/4 palam) is boiled with 350 ml milk and given for delirium, and syncope.

கடுக்காய் (Kadukkai)

Synonyms : *Anganam, akkodam, anthan, abaranam, abaiyan, amritham, amalai, Ammai, amrutha, arabi, arithaki, aliyan, avvyatha, resaki, emavathi, ayavi, haimavathi, kadu, kayastha, siyirutham, sirayahi, sirottam, siva, sethaki, sethanika, seya, divya, devi,*

Parts Used: Tender and mature fruits

சுவை (Taste)	**:**	துவரப்பி, இனிப்பு, புளிப்பு , கார்ப்பு, கைப்பு (Astringent , sweet, sour, pungent, bitter)
தன்மை (Potency)	**:**	வெப்பம் (Hot)
பிரிவு (Division)	**:**	இனிப்பு (Sweet)

- கொட்டை - துவர்ப்பு
- பருப்பு - இனிப்பு
- நரம்பு - புளிப்பு
- காம்பு - கைப்பு
- தோல் - கார்ப்பு

Kadukkai possess all the tastes except salt. So that it is a good remedy for all the diseases pertaining to *thridosham* or *mukutram.* With the sour taste, diseases due to *vatham,* with sweet taste *pitha* diseases, with the bitter, astringent and pungent tastes *kapha* diseases will be cured. Sweet division is due to its 5 elemental conjoince.

கடுக்காயின்- பொதுகுணம் (General Properties of Kadukkai)

"'தாடை கழுத்தக்கி தாலு குறியிலிட
பீடை சிலிபதமுற் பேதிமுடம் - ஆடையெட்டாத்
தூலமிடி புண்வாத சோணிகாமாலை இரண்
டாலமிடி போம் வரிக்காயால்"

- அகஸ்தியர் குண வாகடம் [26]

Description: The diseases of cheek, neck,tongue, male genitalia will be cured with kadukkai. It also cures Silipatham (Elephantiasis), Athithoolam (obesity), Vathasonitham, Kamalai(Jaundice) , thavara-sangama visham (plant toxicity and toxic bites).

"சொல்லுமரிதகிமேற்றோலைமதுவுடனே
யல்லும்பகலுமயில்வே -கல்லும்
கரையவுருமெழுப்புங்காயசித்தியுண்டாம்
நரையுந்திரையுமிலைநை.....

- தேரன்வெண்பா

Description: The outerskin of kadukkai is taken with honey is a tonic to the body moreover its an excellent rejuvinator .one who consumes the same will attain Kayasiddhi.

கடுக்காயின் சிறப்பு [2]

"கடுக்காயும்தாயும்கருதிலொன்றென்றாலும்
கடுக்காய்தாயக்கதிகங்காண்நீகடுக்காய்நோய்
ஓட்டியுடரேற்றும்உற்றவன்னையோசுவைகள்
ஊட்டியுடர்தேற்றுமுவந்து"

- அகஸ்தியர் குண வாகடம் [(26)]

Description : Kadukkai can be compared with ones mother who take care of her child nourishes the body by providing perfect balanced diet with sixtastes. Like wise kadukkai prevent the occurance of diseases , as an alterative it preserves the body, improves ones appetite and taste. So kadukkai is considered superior.

கடுக்காயின் பிரிவுகள் (Classification)

- "ஆதிவிசயன் அரோகிணியோடே பிருதிவி
  தீதில் அமிர்தை சிவந்திமலை-மீதான்
  திருவிருத்த தீயபயன் செப்பிலிவை ஏழாம்
  அரிதகியின் பேதம் அறி"

  - அகஸ்தியர் குண வாகடம் [(26)]

Classification of kadukkai

S. NO	Types	Description	Medicinal Properties
1.	Vijayan kadukkai	Habitat: avanthi land, Appearance: like peichurai.	• Useful in Vatha diseases.
2.	Arohini Kadukkai	Habitat: Kanyakumari Appearance: Globular with 4 streaks Fruit smaller but Seed larger.	• Muppini • Kapha diseases • Can be used externally as an application for ulcers.
3.	Prithvi Kadukkai	Habitat: Sowrashtram Appearance: Thin outerskin	• Controls pitha vitiation
4.	Amritham sethaki	Habitat: Kasi Appearance: Fleshy. Types : Black and white.	• Cures Kapha derangement. • Purgative • Tonic and alterative
5.	Jeevanthi	Habitat: Forest regions Appearance: Golden colored.	• Hemorrhoids
6.	Thiruviruthi	Habitat: Mountains Appearance : 5 colored, 3 streaked.	• Cures ulcers.
7.	Abhayan	Habitat: Pothigai hills Appearance: 5 streaked, black colored, globular, 2 finger length.	• Eye diseases • Expectorant • Laxative

Non advisible conditions for *Kadukkai* Consumption

- Those who taken dry foods
- Dyspepsia
- After fasting
- Those who are sufferers of toxicity and *pitha* diseases
- Pregnant
- Those who are having febrile illness.
- Throat congestion.

Medicinal Uses

❖ **கடுக்காய்கற்பம்**

"உப்புடன் சேர்த்துண்ண ஐயமும்

சர்க்கரையுடன் சேர்த்துண்ண அழலும்

நெய்யுடன் சேர்த்துண்ண வளியும்

வெல்லத்துடன் சேர்த்துண்ண முக்குற்றமும் போம்"

- அகஸ்தியர் குண வாகடம் [26]

Description: As a rejuvintor *kadukkai* can be taken with rock salt for *kapha* derangement, with sugar for *pitha* derangement, with ghee for *vatha* derangement an with jaggery for vitiation of all the three *kutram*.

- கடுக்காய்கற்பம்

  "மாலைகடுக்காய் வருணத்துடனுண்ணகா
  மாலைகடுக்காய் மகோதரம் போம் - மாலையடக்குஞ் "

Description: Kadukkai choornam in the dose of 1 nilakadalai alavu is taken during evenings regularly one will be free from affection of jaundice

- If *kadukka*i is taken with the below prescribed adjuvants during each season, that will prevent the vitiation of any diseases occurring in that season.

S.NO	Seasons	Adjuvant
1.	*Kaarkalam* (Early rainy season)	*Induppu* (Rock salt)
2.	*Koothirkaalam* (Later rainy season)	Sugar
3.	*Munpani Kalam* (Early winter season)	Dry ginger
4.	*Pinpani Kalam* (Later winter season)	Long pepper
5.	*Ilavenil Kalam* (Early summer season)	Honey
6.	*Mudhuvenil Kalam* (Later Summer season)	jaggery

MEDICINAL USES

- *Kadukkai* in the form of decoction or kudineer is useful for polyuria and eye diseases, and also externally used as wash for bleeding hemorrhoids.
- *Kadukkai* can be used as a tooth powder for gingivitis, bleeding per gum and mouth ulcers; moreover, it will strengthen the root.
- Equal proportion of powder of kadukkai and *kasukatti* can be used as a a dusting powder for external ulcers.
- The powder can be used as snuff for bleeding sinus.
- Kadukkai powder stuffed in a cloth and soaked in castor oil kept in sunlight (Soorya pudam), is a useful remedy for Amaram (ophalmia neonatorum).

நெல்லிக்காய் (Nellikkai)

வேறு ngau; (Synonyms): *Aamalakam, aalakam, aambal, aamarikam, thathri, korangam, mrithubala, meethunthu.*

Parts Used: Leaf, flower, bark, root, fruit, and seed.

சுவை (Taste) : புளிப்பு, துவரப்பி, இனிப்பு,
(Astringent, sweet, sour, pungent, bitter)

தன்மை (Potency) : தட்பம் (cold)

பிரிவு (Division) : இனிப்பு (Sweet)

செய்கை (Actions)

Fruit

- குளிர்ச்சியுண்டாக்கி (Refrigent)
- சிறுநீர் பெருக்கி (Diuretic)
- மலமிளக்கி (Laxative)

நெல்லிக்காயின்- பொதுகுணம் (General Properties of Nellikkai)[11, 27]

"பித்தமணலையும் பீனிசம் வாய்நீர் வாந்தி
மத்தமழைக்காடும் மயக்கமு மில்லை -ஒத்தவுறு
வில்லிக்காயம் மாறுங்கமென்னாட்காலந் தேர்ந்தே
நெல்லிக்காயும் மருந்துகண்"

"நெல்லிக்காய்க்குபித்தம்நீங்குமதன்புளிப்பால்
செல்லுமேவாதமதிற்சேர்த்துவரால் - செல்லுமையம்
ஓடுமிதைசித்தத்தில்உன்னஅனலுடனே
கூடுபிறமேகமும்போங்கூறு"

- பொருள் பண்பு நூல் (மூலிகை வகுப்பு) [11]

DESCRIPTION : Nellikkai when taken during daytime will cure verinoi (Psychiatricdiseases), Kaphanoi, Peenisam (Sinusitis), Vaayneer kasappu (bitter taste in tongue),Vanthi (Vomiting), Mayakkam (Giddiness), Thalai suzhalal (Vertigo), Malabhandam (Constipation), Pramegham (Diabetis). With the sour and astringency of the fruit Kapham will be normalised.

நெல்லிமுள்ளியின்குணம்

"நல்ல நெல்லிமுள்ளியது நாவுக்கு ருசிதரும்
மல்லல் விரிபித்தம் அகற்றுமதை
மெல்ல தலைமுழுக கண்குளிரும் தாது பித்தவாந்தி
இல்லை விழிமேகங்களும் போமென்றே சொல்"

- பொருள் பண்பு நூல் (மூலிகை வகுப்பு) [11]

DESCRIPTION: Nelli mulli improves taste,cures viri pitham. Oil processed with Nellimulli is used as a head bath will give cooling effect to the eyes.Strengthens the physical constituents.Pitha vanthi (Emesis), Megham will be controlled.

தான்றிக்காய் (Tantrikkai)

Synonyms : Aksham, Akkaantham, Amutham, ambalathi, aaramam, erikatphalam, kanthakatphalam, koolidrumam, kalathoontri, sakatham, thaabamaari, vaanthiyam, vibheetham, thirilingam, bhootha vasakam.

Parts Used: Leaf, Bark, fruit

சுவை (Taste)	**:**	துவர்ப்பு (Astringent)
தன்மை (Potency)	**:**	வெப்பம் (Hot)
பிரிவு (Division)	**:**	இனிப்பு (Sweet)

செய்கை (ACTIONS)

- துவரப்பி (Astringent)
- கோழையகற்றி (Expectorant)
- மலமிளக்கி (Laxative)
- உரமாக்கி (Tonic)

தான்றிக்காயின் பொதுகுணம் (General Properties of Tantrikkai)

"சிலந்திவிஷம் காமியப்புண் சீழானமேகம்
கலந்துவரும் வாதபித்தம் காலோடலர்துடலில்
ஊன்றிக்காய் வெப்பமுதிர் பித்தம் கரைக்கும்
தான்றிக்காய் கையில் எடுத்தால்"

- அகஸ்தியர் குண வாகடம் [26]

Description: Silanthi visham (spider bite poison), Aankuri pun (Ulcers in male genitalia), Seezhmegham (Chronic purulent discharges (leucorrhoea), Vatha pitham, Veppamuthir pitham (Aggravated Pitha conditions due to excessive heat) will be cured with Tantrikkai.

"ஆணிப்பொன்மேனிக்கழகு மொளிவு மிகும்
கோணிக்கொள் வாதபித்தம் கொள்கை போம் தான்றிக்காய்
கொண்டவர்க்கு மேகம்ஆறுங்க்கூறா அனர்தணியும்
கண்டவர்க்கு வாதம்போம் -காண்"

- பொருள் பண்பு நூல் (மூலிகை வகுப்பு) [11]

Description: Tantrikkai will improve one's complexion and beauty.Vathapitha diseases will be relieved.One who takes regularly will be free from any diseases pertaining to megham. Moreover, it has the power to eliminate Vatham.

மருத்துவபயன் (Therapeutic uses of Tantrikkai)

- Pox Diseases (Ammai noikal): Tantrikkai powder with honey is given.
- Wheezing: Tantrikkai outer skin powder (20 g) added with water (170 ml) boiled and reduced to 1/3rd after honey is used as adjuvant.
- Vision Promoter: Tantrikkai powder with jaggery and required honey is given internally.
- Ulcers: Tantrikkai powder is applied as a dusting powder.
- Throat ulcers, cough and phlegm: Tantrikkai roasted well with pure ghee, covered with wheat flour baked and kept in mouth.

ஓமம் (OMAM)

Synonyms **:** Asamotham, thippiyam

Parts Used **:** Seed.

சுவை (Taste) : கைப்பு (Bitter)

தன்மை (Potency): வெப்பம் (Hot)

பிரிவு (Division) : கார்ப்பு (Pungent)

General dose : ¼ -1/2 Varagan

செய்கை (ACTION)

1. பசித்தீத்தூண்டி (Stomachic)
2. இசிவகற்றி (Antispasmodic)
3. ஆகட்டுவாய்வகற்றி (Carminative)
4. வெப்பமுண்டாக்கி (Antiseptic)
5. உரமாக்கி (Tonic)
6. உமிழ்நீர்ப்பெருக்கி (Sialogogue)

ஓமத்தின் பொதுகுணம் (General properties of Omam)

"சீதசுரங் காசஞ்செறியாமந்தம் பொருமல்
பேதியிரைச்சல் கடுப்பு பேராமம் ஓதிருமல்
பல்லோடு பல்மூலம் பகமிவை நோயென் செயுமோ
சொல்லோடு போம் ஓமமெனசொல் "

- அகஸ்தியர் குண வாகடம் [26]

Description: With Omam Kapha juram (Phlegmatic fever), Irumal (cough), Seriyamantham (Dyspepsia), Kazhichal (diarrhoel diseases), Oozhi (cholera), Iraippu (wheezing), palnoi (Toothache), Kuyya rogam (Genital diseases) e.t.c will be cured.

"பேசுவேன் ஓமம் பேதி கழிச்சலும்
வாசுவும் மந்தம் வாதபித்தங்களும்
கூசுமே அதிசாரம் பொருமல் போம்
நாசமாம் சுரம் காசம் இருமலே"

- பொருள் பண்பு நூல் (மூலிகை வகுப்பு) [11]

Description: With Omam bedhi kazhichil (Diarrhoea & dysentries), vayvu (gaseous disorders), mantham, vatham, pitham, Athisaram, porumal, suram (pyrexia), Kasam(cough) will be cured.

- From Omam, Oma Theeneer and Oma Thailam can be extracted.

1. **Oma Theeneer**

Method of Preparation

For 1440 g of omam add 8400 ml and distilled.
Dose: 30 – 60ml

Indication: *Seriyakazhichal* (dysentries), *Oozhi* (Cholera), *Vayitruvali* (abdominal pain), *Mantham* (Indigestion).

ஓமவாற்றின் குணம்

"போமே வாற்றி எடுத்த தீநீரினால்
பேதி மந்தம் பெரிய கழிச்சலும்
தாமே கூப்பிட்டிரையும் வாய்வுகளும்
சதித்திடும் பித்தம்வாயு கழிச்சலும்
ஆமே நீர்கடுப்பு நீர்தோஷமும்
அதிகமாம் மந்தம் பாலர் நோயும்
வேமே வெப்புகள் நீர்தோஷம் பீனிசம்
வேதனை எழும் நோவுகள் மாறுமே"

- பொருள் பண்பு நூல் (மூலிகை வகுப்பு) [11]

Description: Distillates prepared from *omam*(*oma theeneer*) will cure the following diseases *Bedhi* (Diarrhoel diseases), *mantham* (Indigestion), *seriyakazhichal* (dysentries), *vayu iraichal* (gaseous disturbances), *Pitham* (Bilous disorders), *Vayu* (Gaseous disorders), *kazhichal* (diarrhoel diseases), *Neerkaduppu* (Stranguary), *Neerdosham*, *Balar mantham*, *Veppukal* (Body Heat), *neerthodam*, *Peenisam* (Sinusitis) and other painfull conditions.

2. Oma Thailam

The oil which is collected from the supernatant layer of the *Oma Theeneer* is called *Oma Thailam*

Dose : 1-3 drops

Indication : *Seriyakazhichal* (dysentries), *Oozhi* (Cholera), *Vayitruvali* (abdominal pain), *Mantham* (Indigestion).

வாய்விளங்கம் (Vai vidangam)

Synonyms: *Vayu vilangam, keralam, vaayvilangam, varnanai, vaividangam.*

Parts Used: fruit,seed.

சுவை (Taste) : கைப்பு (Bitter)

தன்மை (Potency) : வெப்பம் (Hot)

பிரிவு (Division) : கார்ப்பு (Pungent)

வாய்விளங்கம் - பொதுகுணம் (General Properties of Vaividangam) [11, 27]

"பாண்டுகுட்டம்குன்மம்பருந்தூலநோய்வாதந்
தீண்டுதிரிவிடஞ்சிரந்துண்டம் - பூண்டமடி
நோய்விளங்ககாட்டாதநுண்கிருமியாசனப்புண்
வாய்விளங்கங்காட்டவிருமார் "

- அகஸ்தியர் குண வாகடம் [26]

Description: With *vaividangam* ,*pandu* (Anaemia), *Gunmam* (Gastro intestinal ailments), *parunthoolanoi* (obesity), *vayu* (gaseous disorders), Poison due to bites (snake bite), *Nunnkrimi*(microbes or minute parasites and worms), *eruvaipun* (ulcers in the anal region) will be relieved.

" வாதகுருவாயுடம்புவாதமறுத்தப்படியே
வேதைஉலோகங்களிலேவேண்டினார் -பாத
விரதமுதற்கையாடலென்றலிசையும்
வர்ணனைநீமனத்தில்வை "

--தேரன்வெண்பா [11]

Description : *Vaividangam* will normalize Vatha kutral(Impaired vatha humour), moreover it will aid in chemistry of consolidating *Rasam*(Mercury) and *vangam*(lead)

மருத்துவபயன்; (Therapeutic uses of Vaividangam)

- Vaividangam is included in so many vermifuge formulations.
- Vermifuge: The seed powder in the dose 4-16 g with adjuvant honey is given for 2-3 times a day followed by castor oil purgation on the next day will evacuate the intestinal worms.
- Digestive disorders: The seed powder , 2-4 g is given along with milk for children will control colic due to indigestion and other gaseous disturbances.
- For headache the seeds are powdered finely, mixed with butter, and applied externally.
- For scorpion stings the seed paste can be applied on the spot.

கோரைகிழங்கு (Korai kizhangu)

Synonyms :முத்தக்காசு (muthakasu)

Two varieties of Korai kizhangu are available,small (siru korai) with tubers and big (perunkorai)without tubers.

செய்கை (Action)

- துவரப்பி (Astringent)
- வெப்பமுண்டாக்கி (Stimulant)
- உரமாக்கி (Tonic)
- சிறுநீர்பெருக்கி (Diuretic)
- வியர்வைபெருக்கி (Diaphoretic)
- உள்ளழல்ஆற்றி (Demulcent)
- ருதுவுண்டாக்கி (Emmenagogue)
- புழுவகற்றி (Vermifuge)

பொதுகுணம்

"சீதசுரந் தீர்க்குஞ் செம்புனல் பித்தம்போகும்
வாதசுரந்தணிக்கும் வையகத்தில் - வேதைசெய்ய
வந்தபிணியை எல்லாம் வாட்டு முத்தக்காசு
கொந்துலாவும் வார்குழல் ! கூறு"

"அதிசாரம் பித்தம் அனற்றதாகம் ஐயங்
குதிவாதஞ் சோபங் கொடிய - முதிர்வாந்தி
யாரைத்தொடர்ந்தாலும் அவ்வர்க் கெலாங் குளத்துக்
கோரை கிழங்கை கொடு "

- அகஸ்தியர் குண வாகடம் [26]

Description: With korai kizhangu seethasuram, kuruthi azhal noi (Hypertension), Suram (pyrexias), neervedkai (Excessive thirst), muppin i(condition to derrangement of 3 humours), kazhichal (diarrhoeal diseases), paithyathodam (psychiatric ailments), pitha daham (Excessive thirst monitered in heat disorders), kapha rogam (Phlegm disorders), Kuthikaal vatham (Calcaneal spur), vanthi (Emesis) will be cured.

"கோல வுணவை குமரநடலிலடு

கோல வுணவைகொடு கயத்தை"

Description: The powder of korai kizhangu tubers is used a regular suppliment for respiratory ailments especially cough.

மருத்துவபயன் (Medicinal Uses of Korai Kizhangu)

- The decoction made with korai kizhangu is effective for diarrhoeal diseases, Gunmam(Gastro intestinal ailments), and vanthi(emesis).
- Inji along with koraikihangu is grinded with sufficient honey and rolled into pills (Chunda sized)this is best given for seetha kazhichil(bacillary dysentry)
- The fresh tubers are grinded into paste and externally applied locally over the breast stimulates milk secretion.The same paste can be applied over the spot of scorpion sting for reducing the symptoms or toxicity.when applied all over the body regularly before bathing it will relieve body odour.
- Decoction made with korai kizhangu,Peipudal,Thriphala, Drakhai, veppu, vettupala is effective for febrile conditions.
- Korai kizhangu is one of the main ingredient for many pedaetric prepartions.
- **Korai kizhangu moozhku neer**(For bathing): Korai kizhangu, thriphala, marukkarai, pungu, kontrai, valuzhuvai, vargothumai, ezhilam palai, koshttam, njaazhal, maramanjal, venkadugu is taken and boiled with water. The water can be used for bathing in skin conditions like kushttam (Leprosy), sori, sirangu scabies, pruritis), paandu (Hypopigmentation).

கொடிவேலி (Kodiveli)

Synonyms: Aninjil, athika naari, athi pathungi, azhal, uthasanan, eri, ezhuna, oli, karunaagam, kanali, kaarimai, agni, thapanan, vanni, vanama, chithrakam, Chithramoolam, thazhal, nekizhi, vanjatharam.

Types

1. Karun Kodiveli (Plumbago capensis)
2. Chen Kodiveli (Plumbago rosea)
3. Ven Kodiveli (Plumbago zeylanica)
   All the 3 varieties possess similar medicinal qualities.

Parts Used: Root, Root bark

சுவை (Taste) : கார்ப்பு (Pungent)

தன்மை (Potency) : வெப்பம் (Hot)

பிரிவு (Division) : கார்ப்பு (pungent)

செய்கை (ACTIONS)

- **முறைவெப்பகற்றி (**Anti Periodic)
- **வியர்வையுண்டாக்கி (**Diaphoretic)

பொதுகுணம் (General Properties of Kodiveli)

"கட்டி விரணங் கிரந்திகால்கள் அரையாப்பு
கட்டி சூலை வீக்கங்காழ் மூலம் - முட்டிரத்த
கட்டு நீரேற்றம் கனத்த பெருவயிறும்
அட்டும் கொடிவேலியாம்"

- அகஸ்தியர் குண வாகடம் [26]

Description: With Kodiveli, Katti, pun, kazhalai, Vathanoi, Aryappukatti, kuththal, sobhai, moolarogam, uthirakattu, neeretram, and peruvayaru will be cured.

“காட்டி யேசூலைகட்டு கருத்திடு குறிப்புண் கிரந்தி
ஓட்டுமே கரணத்தோடு முருமரையாப்புமன்றி
விட்டிடா நெரிச்சுரம் பின்வியன் விடமச்சுரன்தான்
பொட்டென பறந்துபோகும் புகழ்கொடிவேலி கண்டால்"

- ஏடு [27]

Description: Kuripun, meghapun, nanju suram will be cu red with kodiveli.

மருத்துவபயன் (Therapeutic uses of Kodiveli)

- The dried root is made into paste and applied over skin diseases like leprosy.
- The root paste, kottapaakku alavu is given with cow’s milk as an antidote to toxic materials like pashanam.

பனம்கற்கண்டு (Panam Karkandu)

Parts Used : Palm sugar candy prepared from fermented sap of Palm tree.

பனம்கற்கண்டின் பொதுகுணம் (General Properties of *Panam Karkandu*)

" மேகவானலுமிக வீசு மசூரிகையால்
ஆகமுருகனலு மாறுங்கான் - மோகனத்தில்
தங்கிவரு நீர்ச்சுருக்குந் தாகவெப்ப மும்தணியும்
இங்கு பனங்கற்கண்டுகே "

- - அகஸ்தியர் குண வாகடம் [26]

Description: With Panam karkandu, Megha suram, Sensation of heat associated with pox affections (vasoori), Urinary stranguary, excessive thirst will be relieved.

Mineralogical Review

Introduction

Iron is an important trace element of the body, being found in functional form in hemoglobin, myoglobin, the Cytochromes, enzymes with iron sulphur complexes and other iron-dependent enzymes. Iron has the unique ability to alter its oxidation and redox states in response to liganding, which makes it essential for various cellular processes[(39)]

Iron plays an important role in biology, forming complexes with molecular oxygen in hemoglobin and myoglobin; these two compounds are common oxygen transport proteins in vertebrates. Iron is also the metal at the active site of many important redoxenzymes dealing with cellular respiration and oxidation and reduction in plants and animals. Iron forms compounds mainly in the +2 and +3 oxidation states. Traditionally, iron (II) compounds are called ferrous, and iron (III) compounds ferric. Iron also occurs in higher oxidation states. Iron is a necessary trace element found in nearly all living organisms. Iron-containing enzymes and proteins, often containing hemeprosthetic groups, participate in many biological oxidations and in transport. Examples of proteins found in higher organisms include hemoglobin, cytochrome, and catalase [(40)]

The most commonly known and studied "bioinorganic" compounds of iron (i.e., iron compounds used in biology) are the heme proteins: examples are hemoglobin, myoglobin, and cytochrome P450. These compounds can transport gases, build enzymes, and be used in transferring electrons. Metallo proteins are a group of proteins with metal ion cofactors. Some examples of iron metallo proteins are ferritin and rubredoxin. Many enzymes vital to life contain iron, such as catalase, lipoxygenases, and IRE-BP. The cells maintain the free iron concentration to a minimum required level to avoid toxic effects of excess iron. Iron preparations are used in all Indigenous as well as western medicine for a wide range of medical conditions specially for correcting blood parameters.

Iron has been one of the most important agents in the indigenous medical system from time immemorial. Preparations of iron have been extensively employed in different pathological conditions in combination with compounds containing vegetable drugs, spices, aromatic substances, as also the compounds of other metals, in the treatment of different ailments pertaining to blood, bowels and nerves and debilitating

conditions[37].The chemistry and metallurgy of iron were highly developed in south India from very ancient times[2]

Siddha aspect of FERRUM (அயம்) [3]

Elemental iron or ferrum is one among the most widely used Natural metal used in *siddha* medicine. It is included in the category of *Thriloham, panchaloham* and *Pancha bootha ulogam.*

Five Elemental category: *Vayu* (Air element)

Synonyms [35]

" இரும்பினுடேபேர் தனையே இயற்றகேளு
யேசுவுட செயமாகும் சத்துமாகும்
மரும்பான அயசாகும் கறுப்பியாகுங்
மத்திய வாழபூமிநாத மாகுங்
கரும்பி யென்றும் லோகமென்றும் பிண்டதுத்த மென்றும்
கயாச்சு ரோசராமணக்காரி நெகிழந்தான்
திரும்பினும் கிட்டமாங் கிருஷ்ண அயமாகும்
செப்பிய தோர் பேரெல்லை மிரும்புக்காமே"

போகமுனிவர்நிகண்டு -1200

Description:*Sathu, ayasu, karuppi, vaazhboomi naatham, karumbi, loham, pindam, thirumbi, chittam, Krishna ayam,* are the synonyms. Apart from this *aki, ayasu, ayil, idi, eesajeyam, karungkol, karumbu, karumanal, karumpon, kaalil nekilam* are the other names attributed to *Ayam.*

Each one is of different characters.

1. *Pon manal:* Gold placer/ Pyrite ferrous sand.
2. *Karu manal:* Black sand of ilmenite, magnetite, garnet, rutile, zircon etc.
3. *Karunthathu:* Magnetite, ilmenite.
4. *Karumpon:* Titano magnetite, Hematite, marcasite.
5. *Vaazhbhoomi natham:* Marcasite, siderate.
6. *Essacheyum:* Red ochre, lateritic soil, limonite.

சுவை (Taste): துவர்ப்பு, சிறுபுளிப்பு, கைப்பு (Astringent, Mild sour + bitter)

வீர்யம் (Potency): வெப்பவீர்யம் (Hot potency)

செய்கை (ACTIONS)

- உடல்உரமாக்கி (Tonic)
- உடல்தேற்றி (Alterative)
- பசிதீதூண்டி (Appetiser)
- குருதிபெருக்கி (Hemetenic)

பொதுகுணம் [3]

" பாண்டு வெண்குட்டம் பருந்தூலநோய் சோபை
மாண்டிட செய் மந்தங் காமாலை குன்மம்பூண்ட
பெருந்தாதுநட்டமும் போம் பேதி பசியுண்டாங்
கருந்தாது நாட்ட மிடுங்கால்"

Description: With *Ayam, pitha pandu* (Anemia), *Venkuttam* (Leucoderma), *Athithoola noi* (Obesity), *sobhai* (Dropsy), *mantham* (Indigestion), *Kamalai* (Jaundice), *Gunmam* (Diseases of gastro intestinal region), *Suklanattam* (Seminal loss, Oligospermia), *Kazhichal* (Diarrhoea) will be cured. Improves appetite also.

Usage of Elemental Iron Formulations in *Siddha* Medicine

Siddha Medicine uses vast majority of iron and iron compounds to treat numerous ailments. Simpler formulations to complex formulations of iron are in practice. Ayam is one of the crucial drug added in so many preparations like *Kudineer, Lehyam, Rasayanam, Parpam, Chendooram,Theeneer/Dravagam,* As each state of the Ferrum starting from raw ore to the final product, the nature and properties of the element is modified or transformed with numerous processings, the potentiality and therapeutical application with each stage and form various accordingly, and hence such transformed states of the element is been effectively utilized for each disease condition.

Table: 5 *Ayam* Preparations[60]

S.NO	***Ayam* Preparations**	**DOSE/ ADJUVANT**	**INDICATION**
1.	*Aya Chenduram*	Panavedai (488mg), Honey	*Pandu, Pitha vettai, Pitha vayu, Arosakam*
2.	*Aya Chenduram*	100 – 200mg, honey	*Vinthu nattam, Pandu, Peruvayiru*
3.	*Ayakantha chenduram*	100 – 200mg, honey	*Pandu*
4.	*Ayaveera Chenduram*	1 Kuntri (130 mg)	*Soolai, Kuttam, Vaatha neer*
5.	*Aya Mezhugu*	2 – 4 g, *sombu kudineer*	*Pandu, sobhai, Kamalai*
6.	*Aya Bringaraja Panitham*	-	*Pandu, Pitha Kamalai, Peruvayiru.*
7.	*Aya Bringaraja Karpam*	100 – 200mg, honey	*Pandu, Ilaippu,*
8.	*Arumugha Chenduram*	100 – 200mg, honey/*Thrikadu*	*Pandu, Soolai, Andavatham, Vatha noikal*
9.	*Jalamanjari*	200 – 400 mg with lime juice	*Pandu, Sobhai, Athithoolam*
10.	*Karisalai lehyam*	5 – 10 g	*Pandu, Kamalai, Pitham, Mayakkam.*
11.	*Karuppu Vishnu Chakra mathirai*	½- 1 pill, honey	*Sanni, kuttam, Anda vatham, dhanur vatham, gunmam, parisa vatham,*

Usage of Elemental Iron in *Sanjeevi Theeneer*

Out of hundreds of different Ferrum formulations available in *siddha* classical works, some special *Theeneer* and *Dravagam* formulations also uses Iron in various forms. *Sanjeevi Theeneer* is one such medicine.

Distillates cannot yield mineral iron presence directly. The other forms of ionic state in which the iron exist in distillates is unknown and has to be studied in depth. Some preprocedures before undergoing distillation may convert or modify the elemental existence. The most possible mechanism will be the processes of fermentation induced naturally with the addition of fermenters like palm candy in the formulation *Sanjeevi Theeneer,* in which a considerable lowering of pH due to alcohol formation may dissolve or convert into a from in ionic stage to be easily distilled.

Zingiber officinale [37-47]

INTRODUCTION:

Chukku is the dried rhizome of Zingiber officinale belonging to family Zingiberacea widely cultivated in India; rhizomes dug in january-february, buds and roots removed, soaked overnight in water, decorticated, sometimes treated with lime, and dried. Dried ginger is of two kinds, peeled and unpeeled, the latter is the cleaned rhizomes dried in the sun. In the case of dry ginger, the outer skin is scrapped off. When the fresh drug is used is used for extracting the juice, the supernatant fluid alone should be used and the sediment (chunnam) discarded.

SCIENTIFIC CLASSIFICATION	**VERNACULAR NAMES**
Kingdom: Plantae	Tamizh: Sukku, Inji
Clade: Monocots	Malayalam: Chukku, inji
Clade: Angiosperms	Sanskrit: Nagara, adraka.
Clade: Commelinids	Hindi: sonth
Order: Zingiberales	Assam: Adasuth
Family: Zingiberaceae	Beng: Suntha , sunthi
Genus:*Zingiber*	Guj: sunth, Adu
Species: ***Z. officinale***	Tel: Allamu,
	Kan: Sunthi
	Urdu: sonth, zanjabeel.
	Mar: Sunthi, Ale
	English: Ginger root, Ginger.

HABITAT : Ginger is cultivated in many parts of India on a large scale in the warm, moist regions, chiefly in Madras, Cochin and Travancore, and to a somewhat less extent in Bengal and the Punjab.

Odor : Aromatic.

Taste : Pungent and spicy.

Color : Externally pale buff to brownish in color.

Odor : Aromatic and characteristic.

Taste **:** Pungent

Parts Used **:** Scraped and dried rhizomes as well as the green ones.

Major Alkaloids

Phytochemicals of ginger vary depending on its growing place, freshness and dryness of it . Pungency of dry ginger is caused by shogaols, which were dehydrated form of gingerols formed when ginger is dried or cooked. The concentrations of shogaols, which are the major gingerol dehydration products, are more abundant (Jolad et al. 2005) in dry ginger than in fresh ginger. The rhizome contains 1-4% essential oil and an oleoresin (5.3-8.6%). The composition of the essential oil varies as a function of geographical origin, but the chief constituent sesquiterpene hydrocarbons (responsible for the aroma) seem to remain constant. These compounds include Alphazingiberene, ar-curcumene, hexa hydrocurcumin, Beta sesquiphellandrene, Beta bisabolene camphene, phellandrene, citral, citronellol, geranial, linalool, bisabolene, limonene, desmethhexahydrocurcumin, cineole, borneolzingiberole, and cineole. Monoterpene aldehydes and alcohols are also present

The constituents responsible 'for the pungent taste of the drug and possibly part of its anti-emetic properties have been identified as 1-(3’- methoxy-4'-hydroxyphenyI)-5-hydroxyalkan-3-ones, known as gingerols. Apart from this lipid (6.8%), proteins (10%), fats, waxes and starch (40-60%) vitamin B6, vitamin C, calcium, linoleic acid, acetic acid, lignin are present.

Minor Alkaloids: Numerous monoterpene and sesquiterpene hydrocarbons and their corresponding dehydration products (oxygenated derivatives in volatile oil),), paradols, gingerdols, gingerdiacetates, gingerdiones, 6-ginger sulfonic acid, gingerenones and a number of diarylhepataniods, diterpenes, ginger glycolipids A, B &C

Medicinal Uses of Ginger

- Rhizome is highly esteemed as a spice for its characteristic odor and warm pungent taste.
- The rhizomes is useful as an antiemetic, flavoring agent, digestive aid, rheumatic diseases, respiratory disorders, nausea, cardio vascular health and gastrointestinal
- It is used in sprains, sore throats, cramps, muscular aches, pains, constipation, hypertension, dementia, helminthiasias, infectious ailments, bronchitis, stomach disorders, and useful for insect bites.

Piper nigrum [37-40]

Introduction

Piper nigrum (family Piperaceae) is a valuable medicinal plant. It is one of the most commonly used spices and considered as ''The King of spices'' among various spices. Drug consists of dried unripe fruits.

SCIENTIFIC CLASSIFICATION	VERNACULAR NAMES
Order : Piperales Kingdom : Plantae Class: Equisetopsida Subclass: Magnolidae Super order: Magnolianae Family: Piperaceae Genus: Piper Species : nigrum Binomial name : Piper nigrum	Tamizh: Milagu Malayalam: Kurumulaku Sanskrit: Maricha Hindi: Kalimirch, Golmirch Arabic: Fil Fila Siah, Kannada: Karemensu Telungu: Miriyalu Marathi: Kalamirch. Gujarathi: Kalaomirich Bengali: Golmorich, Kalimirch English: Black Pepper

HABITAT: This plant is indigenous in India, Western Ghats, and Malabar Coast growing in the rich soil in the shade of trees. It grows in Kurinji Thinnai.

Color & Appearance: Blackish brown with raised reticulated wrinkles.

Odor **:** Aromatic

Taste **:** Strongly pungent

Parts Used **:** Dried fruit.

The fruits are picked up when fully ripe dried and used in spices and medicines. Roots and leaves are also used in traditional siddha medicines.

Chemical Constituents: Berries contain Volatile oil (1-2.5%), alkaloids/amide (5-9%) and a resin, beta pinene, alpha pinene and humulene. Fruit contains myrcene, piperine, quercetin, kaempferol and eugenol. Many investigators isolated different types of compounds viz Phenolics, flavonoids, alkaloids, amides and steroids, lignans, neolignans, terpenes, chalcones etc and many other compounds. A pungent alkaloid piperine (2-5%) and the resin is responsible for its pungent taste.

Minor Alkaloids: Some of the alkaloids/amides like compounds are Brachyamide B, Dihydro-pipericide, (2E,4E)-N-Eicosadienoyl-pereridine, N-trans-Feruloyltryamine, N-Formylpiperidine, Guineensine, pentadienoyl as piperidine, (2E,4E)- Nisobuty-ldecadienamid, isobutyl-eicosadienamide, Tricholein, Trichostachine, isobutyl-eicosatrienamide, Isobutyl-octadienamide pipericine, piperettine, piperanine, piperamides, pipericide, sarmentine, Sarmentosine, Retrofractamide, piperolein A &B are also considerable.

Black pepper oil is the essential oil obtained by steam distillation of the dried unripe berries of piper nigrum. beta caryophyllene (upto 29%), limonene (upto 17%), The volatile oil contains large amounts of terpenes, alpha pinene, phellandrene, dipentene, sesquiterpenes.

MEDICINAL USES [48]

- Fruits used as a condiment after drying as black pepper or after processing into white pepper.
- It is used with ginger and Piper longum for viral hepatitis.
- Effective in hemorrhoidal affections and rectal prolapses.

Piper Longum

INTRODUCTION

Thippili is the dried immature, catkin like fruits with bracts of Piper longum. It is a slender aromatic climber with perennial woody roots.

SCIENTIFIC CLASSIFICATION	Vernacular Names
Kingdom :Plantae Division : Magnoliophyta Class : Magnoliopsida Order : Piperales Family: Piperaceae Genus : *Piper* Species : ***P. longum***	Tamizh: Thippili
	Malayalam : Thippali
	Sanskrit: Pippali
	Hindi :Pipar
	URDU: Filfil Daraz
	Assam: Pippal
	Kannada : Hippali
	Telungu: Pippalu
	Marathi: Pimpali
	Gujarathi: Pipali
	Oriya: Pipali
	Punjab: Magh Pipali
	Bengal: Pipul
	English: Long pepper

HABITAT

Occurring in hotter parts of india from central himalayas to assam upto lower hills of west Bengal and evergreen forests of western ghats as wild, and also cultivated in North east and many parts of the south. It grows in *kurinji Thinnai.*

Parts Used: Immature berries (dried unripe fruits or fruiting spikes) dried in the sun and stems (roots)

TASTE : Pungent producing numbness on the tongue

Odor : Aromatic

CHEMICAL CONSTITUENTS[1]

Beta caryophyllene, Piperine, Pipernonaline, Piperundecalidine, Piperlatine, Sesamine, Dihydriostifransterol, Piplasterol and Futoamide. Resin, Voltile oil, starch, gum, fatty oil, inorganic matter and alkaloids. Several aristolactams, piperolactam and dioxoaporphines have been isolated from the fruit.

Long pepper contains the long chain isobutyl amide, longamide besides guineesine and lignans, pluviatilol, methyl pluviatilol (fargesin), sesamine and asainine (Koul etal, Photochemistry, 1988, 27, 3523)

Major alkaloids: Piperine (1-2%), piper longumine (piplartine), piperlonguminine and methyl 1-3, 4, 5-trimethoxy cinnamate.

Minor alkaloids: Sesamin. A lignan, dihydrostigmasterol, essential oil consisting of n-hexadecane,n-heptadecane, n-octadecae, n-nonadecane, n-cicosane, n-hencosane, alpha–thujene, terpinolene, zingiberine, p-cymene, p-methoxyacetophenone, dehydrocarveol, and two monocyclic sesquiterpenes. The presence of L-tyrosine, L-cysteine hydrochloride, DL-serine and L-Aspartic acid as free amino acids also have been reported in the fruits. The seeds contain sylvatine, dieudesmin. In addition to plamitic, hexadecanoic, stearic, linoleic, oleic, linolenic, higher saturated acids, arachidic acid and behenic acids are also reported

MEDICINAL USES:

- Old long pepper is more efficacious in medicine then fresh one(U. C. Dutt)
- Powdered long pepper with honey is given for cough, cold, asthma, hoarseness and hiccup.

Terminalia chebula[41, 44]

Introduction

Kadukkai is the pericarp of mature fruit of Terminalia chebula belonging to family Combretaceae.

SCIENTIFIC CLASSIFICATION	VERNACULAR NAMES
Kingdom : Plantae **Subkingdom:** Tracheobionta **Super division:** Spermatophyta **Division:** Magnoliophyta **Class:** Magnoliopsida **Subclass:** Rosidae **Order:** Myrtales **Family:** Combretaceae **Genus :***Terminalia* L. **Species:***Terminalia chebula*	Tamizh: Kadukkai
	Malayalam: kadukka
	Sanskrit: Haritaki
	Hindi: Harad
	Kannada: Alalekai
	Bengal: Harithaki
	Telungu: Karakkaya
	Marathi: Hirda
	Gujarathi: Hirdo
	Oriya: Harida
	Punjab: Halela
	Assam: Shilikha
	English: Myrobalan, chebulic myrobalan

Habitat: This tree is wild I the forests of northern India, central provinces and Bengal, common in madras, Mysore and in the southern parts of the Bombay presidency.

Parts Used: Dried fruits, immature fruits, mature fruits, myrobalans and galls, mostly the outer skin of the fruits.

Taste: Astringent and slightly bitter

Chemical constituents:

Major Alkaloids: Myrobalans contain astringent principles, tannin(tannic acid) 45% and a large amount of Gallic acid (1.21%), chebulagic acid (5%), ellagic acid, mucilage, a brownish yellow coloring matter, chebulinic acid (12.5%) which when heated in water splits up into tannic and Gallic acid. Tannins, which on hydrolysis yield chebulic acid and D-galloyl glucose.

Minor Alkaloids: Fruit possesses corilegin, beta D-Glucogallin, glucose and sorbitol. Polyphenolic compounds, triterpene glycosides, terchebulin (ellagi tannin), terchebin, syringic acid, punicalagin, terflavin A, Flavanoids,reducing sugars and starch .Terpene glycosides, arjungenin and arjunglucoside -1 have been isolated from the fruit.

Medicinal Uses:

- Indigenous medicines use Terminalia chebula for wide range of diseases like asthma, diseases of the heart, ascites, biliousness, spleen diseases, tumors, bleeding piles, leucoderma, itching, anemia, skin diseases.
- Chebulic myrobalans are used in fevers, cough, asthma, urinary diseases, piles, worms and rheumatism and scorpion sting.
- A decoction of chebulic myrobalan is a good astringent wash useful in bleeding piles and some vaginal discharges.
- Coarsely powdered and smoked in pine it affords relief in asthma.
- Chebulic myrobalans are extensively used in combination with belleric and embelic myrobalans under the medicine name of Thriphala and also as an adjuvant for so many medicines.

Phyllanthus emblica [41, 42]

Introduction:

The drug consists of fresh and dried fruits of emblica officinalis belonging to family Euphorbiaceae.

SCIENTIFIC CLASSIFICATION	VERNACULAR NAMES
Kingdom: Plantae **Subkingdom :** Tracheobionta **Super division:** Spermatophyta **Division :** Angiospermae **Class :** Dicotyledonae **Subclass :** Rosidae **Order :** Geraniales **Family**: Euphorbiaceae **Genus :** *Phyllanthus* **Species :** *Phyllanthus emblica*	Tamizh: Nellikkai Malayalam : Nellikka Sanskrit: Amalaka Hindi: Amla Urdu: Amla Kannada: Amalaka Telungu: Usirikai Marathi: Avala Guajarati: Ambala Bengal: Amlaki Oriya: Amla Punjab: Amla Assam: Amlakhi ENGLISH: Indian Gooseberry

HABITAT: It is found both in the forest and cultivated state, common found in mixed deciduous forests in India, ascending to 1300 m on the hills.

Color **:** Grey to black

Taste **:** Sour and astringent followed by delicately sweet taste.

Parts Used **:** Dried fruit, the nut or seed, leaves, root, bark and flowers. Ripe fruits generally fresh, dry also used.

Chemical Constituents:

Primarily contains tannins, alkaloids, phenolic compounds, amino acids and carbohydrates. Its fruit juice contains the highest vitamin C (478.56 mg/100 mL).

Major Alkaloids: Vitamin C(=L.(+).threo.ascorbic acid -2%);tannins(5%), gallic acid, ellagic acid, phyllemblic acid and emblicol, Chebulinic acid, Chebulagic acid, Quercetin, Emblicanin-A, Emblicanin-B, Punigluconin , Pedunculagin, Ellagotannin.

Amino Acids: Glutamic acid, proline, aspartic acid, alanine and lysine constitute 29.6, 14.6, 8.1, 5.4 and 5.3% of the total amino acids present**.**

Minor Alkaloids: Alkaloids, phyllantidine and phullantine, pectin and minerals.

Medicinal Uses **(49-53)**

- Indigenous medicines uses both fresh and dry fruits in so many ailments like biliousness, asthma, bronchitis, cardiac diseases, liver complaints, hemorrhoids.
- It has its beneficial role in cancer, diabetes, liver treatment, heart trouble, ulcer, anemia and various other diseases.
- Fermented liquor prepared from the root is used in jaundice, dyspepsia, and cough.
- Juice of the fresh fruit and ghee mixed together is a good restorative tonic.

Terminalia belerica [44, 45]

Introduction:

Tantrikkai is the pericarp of dried ripe fruit devoid of seeds, of Terminalia belerica.

SCIENTIFIC CLASSIFICATION	VERNACULAR NAMES
Kingdom: Plantae **Subkingdom:** Tracheobionta **Division:** Magnoliophyta **Class:** Magnoliopsida **Subclass:** Rosidae **Order:** Myrtales **Family:** Combretaceae **Genus:** Terminalia **Species:** belerica	Tamil: Tanikkai, Tantrikkai
	Malayalam: Thanikka
	Sanskrit: Bibhitaka
	Hindi :Bahera
	Urdu: Bahera
	Kannada : Tare Kai
	Telungu: Thanikkaya
	Marathi: Baheda
	Guajarati: Bahedan
	Bengal: Bayda
	Oriya: Baheda
	Punjab: Bahera
	Assam: Bhomora
	English: Beleric Myrobalan

HABITAT:

Commonly found in plains and deciduous forests throughout India upto 900m elevation where the climate is not very dry. It is also found in the forests of Burma and srilanka.

Parts Used **:** Fruit, Bark.

Taste **:** Astringent.

Chemical Constituents:

Major Alkaloids : Fruit contain stannins (20-30%) Gallic acid, ellagic acid (0.3%), ethyl gallate, galloyl glucose and chebullagic acid, belleric acid, belerioside, arjungenin and its glycoside, arjunglucoside, cannogenol-3-0-beta-D-galactopyranosyl (1-4), alpha –L-rhamnopyranoside.

Minor Alkaloids : Bellericanin, phyllemblin, termilignan, thaninilignan, 7-Hyroxy-3, 4 (Methylenedioxy) flavan and anolignan B, mannitol, glucose, fructose, rhamnose and Beta –Sitosterol.

Medicinal Uses:

- Fruits are useful in coughs, hoarseness of voice, eye diseases, scorpion sting etc
- Dried ripe fruit is astringent and employed in dropsy, piles, and diarrhoea also occasionally in fever.
- It is a constituent of Thriphala, which is prescribed in liver diseases, gastro intestinal diseases. And a large variety of diseases.

Carum copticum [41, 42]

Introduction:

Omam is the dried fruit of the Trachyspermum ammi, belonging to family Umbelliferae.

SCIENTIFIC CLASSIFICATION	VERNACULAR NAMES
Kingdom: Plantae **Subkingdom :** Tracheobionta **Super division:** Spermatophyta **Division :** Magnoliphyta **Class :** Magnoliopsida **Subclass :** Rosidae **Order :** Apiales **Family**: Umbelliferae **Genus :** *Trachyspermum* **Species :** *Trachyspermum ammi*	Tamizh: Omam Malayalam: Ayamodhakam Sanskrit: Yavani Hindi: Ajwain Urdu: Nankhwah Kannada: Yom Telungu: Vamu Marathi: Onva Guajarati: Ajmo Bengal: yamani Oriya: Juani Arabic: Kamuemulaki Assam: Jain English: Bishops weed

HABITAT: This plant grows largely in eastern India particularly abundant in and around Indore and Nizams dominions. Also in Baluchistan, Afghanistan, Egypt, Europe.

Odor **:** Thymolic.

Taste **:** Pungent.

Parts used **:** fruit

Chemical Constituents:

Major Alkaloids: volatile essential oil (5-6 %%), cumene, terpene, thymene. The seeds of carum opticum contain the antiseptic thymol and they yield 2-3% of an essential oil (ajowan oil) which contains no less than 40-50% of thymol, Carvacrol, alpha and beta pinene, camphene.

Minor Alkaloids: Carvone, limonene, dillapiol, quercetin, kaemferol, fatty acids (oleic and linolieic acid)

Ajowan oil: colorless when recently distilled then turn to yellow tinge and having the odor of the fruit and an acrid burning taste, with a specific gravity 0.896. Oil of ajowan contains about 36% thymol, cymene which is a liquid hydrocarbon.

Medicinal Uses [43]

- The distillates are useful carminatives useful in disguising the taste of disagreeable drugs especially castor oil and reducing the tendency to cause nausea and gripping.
- Powder with buttermilk is effective in difficult expectoration and dried up phlegm.
- In Indigenous medical systems the seed s have been used to cure ascites, abdominal tumors, splenomegaly, piles, chest pain and as a hepatic and cardiac tonic.

Embelia ribes [42, 44]

Introduction

The drug consists of dried mature fruit of Embelia ribes belonging to Myrsinaceae.

SCIENTIFIC CLASSIFICATION	VERNACULAR NAMES
Kingdom: Plantae **Division :** Magnoliophyta **Class :** Magnoliopsida **Order:** Primulales **Family:** Myrsinaceae **Genus:** *Embelia* **Species:** ***E.ribes***	Tamizh: Vai vidangam Malayalam: Vizhalari Sanskrit: Vidanga Urdu: Baobarang Telungu: Vayuvidangalu Assam: Vidang Bengali: Biranga Guajarati: Vavidanga Hindi: Vayavidanga Kannada: Vayuvidanga Marathi: Vavidanga Punjabi: Babrung Oriya: Bidanga

HABITAT: These are perennial herbs & climbers are found in the hilly parts of India from the central and lower Himalayas down to Ceylon and Singapore.

Odor : Slightly aromatic

Taste : Pungent

Chemical Constituents:

Seeds contain benzoquinone compound Embelin ((2, 5-dihydroxy-3-undecyl-2, 5-cyclohexadiene-1, 4-benzo-quinone), quercitol, tannin, christembine, embelic acid, vilangin was isolated from the ripe fruit berries Embelia ribyl ester, Embeliol, Embelinol, Potassium embelate .The plant has also been found to contain quercitol and fatty ingredients, an alkaloid, christembine, a resinoid, tannins and minute quantities of a volatile oil. It has also been studied that the seeds of E.ribes showed the presence of Cr, K, Ca, Cu, Zn and Mn along with high carbohydrates.

Medicinal Uses:

- ❖ Embelin is a useful and safe remedy against tapeworms.
- ❖ The berries enter into the application for ringworm and other skin diseases.
- ❖ In indigenous practice, embellia ribes is widely used in conditions of tumours, ascites, bronchitis, cardiac diseases, jaundice, skin diseases, and to expell worms.

Plumbago zeylanica [38]

Introduction:

Drug consists of the dried root or root bark of Plumbago zeylanica.

SCIENTIFIC CLASSIFICATION	**VERNACULAR NAMES**
Kingdom: Plantae	Tamizh: Kodiveli, Chithramoolam
Subkingdom: Tracheobionta	Malayalam: Vella Kodiveli, Thumbakodiveli.
Super division: Spermatophyta	Sanskrit: Chithraka.
Division: Magnoliophyta	Arabic: Kamuemulaki
Class : Magnoliopsida	Assam: Agiyachit
Subclass: Caryophyllidae	Bengal: Chita
Order : Plumbaginales	Guajarati: Chitrakmula
Family: Plumbaginaceae	Hindi: Chitra
Genus : *Plumbago* L.	Kannada: Vahni, Chithramoolam.
Species : *Plumbago zeylanica* L.	Marathi: Chitraka
	Punjabi: Chitrak
	Oriya: Chita
	Telungu: Chithramulam
	English: Ceylon Leadwort.

Habitat: This garden plant is growing wild in Bengal, up, southern India and ceylon.This is an allied species and is considered cultivated variety of plumbago rosea.

Parts used **:** Root, root bark

Taste **:** Acrid

Odor **:** disagreeable.

Chemical Constituents:

Major Alkaloids: Plumbagin (0.91%), 3-chloroplumbagin, 3,3,-biplumbagin, 12(3)-tetrahydro-3,3'-biplumbagin, plumbagic acid, plumbagic acid glucosidases (3'-0-beta-glucopyranosyl plumbagic acid and 3'-0-beta glucopyranosyl plumbagic acid methyl ester, 3,8-dihydoxy-6-methoxy -2-iso propyl-1-4-naphthaquinone, 5,7-dihydroxy -8-methoxy-2-methyl-1,4-naphthoquinone, zeylinone, isozeylanone, elliptinone, droserone, isoshinanolone, maritinone, seselin, 5-methoxy seselin, suberosin, xanthyletin, xanthoxyletin.

Minor Alkaloids: Catecholtannins, beta sitosterol, vanilic acid, glucose, steroidal gycoside

Medicinal Uses:

- P. zeylanica is used effectively in traditional practices for liver diseases, enlarged liver and spleen and also in ascites.
- It relieves the obstructed phlegm in chronic colds and cough.
- It is a bitter tonic and recommended as a rejuvenator,relieves constipation and alleviates the urticaria- the allergic skin rashes.
- Root has beneficial effect on piles.
- For hemorrhoids an earthern pot or jar is lined with a paste of the root is used for preparing curds which is given for people suffering from piles.
- Indigenous practices indicate its effectiveness in Spleen enlargement, piles.
- Indigenous systems uses root, root bark for leucoderma, piles, itching, ring worm, scabies, bronchitis, liver diseases, spleen diseases, consumption, anemia.
- The root is aid to increase the digestive power and is useful in dyspepsia, piles, anasarca, diarrhoea, skin diseases.

Cyperus rotundus

Introduction:

Drug consists of dried rhizomes of Cyprus rotundus.

SCIENTIFIC CLASSIFICATION	VERNACULAR NAMES
Kingdom: Plantae	Tamizh: Korai kizhangu
Subkingdom: Tracheobionta	Malayalam: Muthanga
Super division: Spermatophyta	Sanskrit: Mustaka,musta
Division: Magnoliophyta	Arabic: Kamuemulaki
Class : *Liliopsida*	Assam: Mutha
Subclass: *Commelinidae*	Bengal: Mutha
Order : *Cyperales*	Guajarati: Moth,nagarmoth
Family: Cyperaceae	Hindi: Mutha
Genus :*Cyprus L*	Kannada: Tungegadde
Species : Cyperus rotundus	Marathi: Motha
	Punjabi: Mutha
	Oriya: Chita
	Telungu: Tungamuste
	English: Nutgrass

Habitat: It is a plentiful species occurring throughout the plains of India especially south India up to 100 m elevation. It grows in moist areas, rice fields and along watercourses.

Parts Used : Tuber or bulbous root.

Chemical Constituents:

Major Alkaloids: 4 alpha 5 alpha oxidoeudesm-11-en-3 apha-ol, cyprene-1 (a tricyclic sesqueterpene) and cyprene-2 (a bicyclic sesquiterpene hydrocarbon), beta –selinene, cyperenone, alpha –cyperone.

Essential oil of Cyprus rotundus : alpha cyperone, cyperene, cyperotundone, cyperol, beta selinene, beta cryphyllene, valerenal, sugeonyl acetate, alpha copaene, patchoulene, trans-pinocarveol, patchoulenenone, aristrol-9-en-3-one, selina-4,11 dienearistrol -9-en-8-one, kobustone, sugetriol, isokobusone, iocyperol, sugeonol and sitosterol.

Medicinal Uses[44, 54]

- The rhizomes are used as a cooling, intellect promoting, nervine tonic, diuretic, antiperiodic, analgesic, anti-inflammatory, antipyretic and to treat diarrhea, dysentery, leprosy, bronchitis, amenorrhea, and blood disorders.
- The tuber part having anti-obesity properties.
- As an infusion or as soup in fever, diarrhea, dysentery, vomiting, and cholera.

Borassus flabellifer [44, 54]

Introduction

The drug consists of Palm sugar prepared from sweet sap of Borassus flabellifer.

SCIENTIFIC CLASSIFICATION	VERNACULAR NAMES
Kingdom: Plantae	Tamizh: Karumpanai, panai maram
***Clade*:**Monocots	Malayalam: Karimpana
***Clade*:**Angiosperms	Sanskrit: Thrinaraja
***Clade*:**Commelinids	Hindi: Tar-ka-jhar
Order : Arecales	Urdu: Drakhte teri
Family: Arecaceae	Kannada: Taalimara
Genus: *Borassus*	Telungu: Taadi chettu
Species: *B.Flebellifer*	Guajarati: Tad
	Bengal: Tal
	Oriya: Talo,trinorajo
	Assam: Tal
	English: Palmyra palm

Panam Karkandu

VERNACULAR NAMES	
Tamizh: Panam Karkandu	Hindi: Tal misri
Malayalam: Panam Kalkandam	English: Sweet palm candy.
Telungu: Taati kalakanda	

HABITAT: The Distribution of flabellifer is so heavily influenced by man. It occurs between sea level and 800 metres though is more abundant at low altitude and is particularly common in coastal areas with sandy or alluvial soils and in areas with permanent soil moisture such as flood plains and river valleys

Parts used: Root, Flowering stalk, juice, bark, and fruit.

Medicinal Uses:

- ❖ It is from the toddy juice jaggery and country sugar is prepared in large quantities in south India.

Palm sugar: The sweet sap is boiled down to prepare palm candy. About 80% by weight of brown sugar can be recovered from palm sap. In the factories, raw jaggery is heaped on platforms for 2 months to drain away most of the molasses, then dissolved in water and refined to obtain brown crystalline sugar.

- o Palm sugar is a nutrient-rich, low-glycemic crystalline sweetener that looks tastes, dissolves and melts almost exactly like sugar, but it's completely natural and unrefined. Brown in color and naturally rich in a number of key vitamins, minerals and phytonutrients, including potassium, zinc, iron, and vitamins B1, B2, B3 and B6.
- o Sugar candy otherwise palm sugar that is produced is widely used in medicine.
- o Palm candy has reputed uses in respiratory affections and as a laxative for children.
- o Palm sugar is anti-bilious and alterative and is used in hepatic disorders.

Drug Standardization [5, 61- 63]

INTRODUCTION

Standardization of the drug complies with confirmation of entire feature of a drug starting from identity, purity and quality through all phases of cycle either it means its shelf life ,storage and use by its various parameters . This is very essential to assess the quality of the distillate and for the scientific justification. As a preliminary step in the standardization of *theeneer* formulations, *Sanjeevi theeneer*, which is a poly herbo mineral distillate, mentioned in the classical text *Chikitsa ratna deepam* and *Siddha* Formulary of *India* (SFI) has been selected. Methods like Heavy metal and microbial load analysis, Physio chemical and Preliminary phyto chemical analysis will be perfomed in the distillate sample. Gas chromatography Mass spectrometry studies (GC-MS) has been executed to screen the biologically active compounds of the distillate.

As per AYUSH Protocol for Standardization, the following parametres were studied.

Table: 6 **Analytical Specifications of Theeneer [63]**

S.NO	**TESTS**
1.	Organoleptic characters
	• Color • Odor • Taste
2.	pH
3.	Volatile matter
4.	Specific gravity at 25^0C
5.	Clarity Test
6.	Microbial Contamination
7.	Test for Specific Pathogen
8.	Test for Heavy metals
9.	Assays
	• Bio chemical analysis • Preliminary Phyto chemical Assays
10.	Identification • Gas Chromatography Mass Spectrometry (GC-MS)

1. Organo leptic Characters of *Sanjeevi Theeneer*

This provides first step information regarding the identity, purity and quality of the drug. Traditional quality parameters of a siddha distillate formulation are mostly expressed in terms of organo leptic characters which include color, consistency and nature , odor and taste of the distillate.

- **Color:** The Distillate was taken in a watch glass or a test tube and placed against white black ground in white tube light. It was observed for its color by naked eye.
- **Odor:** The Distillate was smell individually. The time interval among two smelling was kept 2 minutes to nullify the effect of previous smelling.
- **Taste:** Small quantity of *Sanjeevi Theeneer* was tasted with the tip of the tongue.

2. Determination of pH

pH of the drug is a crucial factor affecting the stability of the product. Stability denotes the product retainibility within the specified limits or throughout its usage and storage period (c)pH determination values specifies the acidity or alkalinity of the solution particularly distillates as the alterations have a strong influence on health.
Five ml of Sanjeevi Theeneer was weighed accurately in a clear 100 ml beaker. Then 50 ml distilled water was added to it and stirred. After 30 min, it was then applied into pH meter as standard buffer solution of 4.0, 7.9, and 9.2. Repeated the test four and the average were recorded.

3.Volatile oil content

Volatile oils or essential oils are considered as the ‘essence’ of the herb material obtained during the first phases of hydro distillation.They are biologically active and are characterized by its peculiar odor or aroma, oil nature, volatility and pungency on taste. They are termed as *Theeneer ennai* that floats as a separate supernatant layer in the distillate collected.

The procedures recommended for analysis of Clarity test, Volatile oil, pH values and Specific gravity are as per the guidelines of WHO .

5.Tests for specific microorganisms (Microbial Load Analysis)

Herbal materials are most viable for microbiological contamination due to large number of bacteria and fungi oftenly sourced from the soil in which it grows or cultivated.Unscientific approaches of harvesting,collection,handling,transportation and storage may dispose to further contamination and microbial growth. These contaminants can be transferred to the finished goods in its various stages.These should not be present in the herbal formulations intended for internal usage.

Distillates are free from most of the impurities and basic contaminants but it is mandatory as per WHO to undergo microbial analysis as one of the steps to validate the genuines of the drug sample.The screening for biological contaminants (Esp bacteria and fungi) in the drug is a very crucial need as it indicates whether the microbial level comply with the limits set in regional or international pharmacopoeias and in assessing its purity. Thereby the sample-Sanjeevi Theeneer has been screened for microbial loads with reference to the standards of WHO.

6. Detection of specific toxic metals(Heavy metal Analysis)

Toxic materials that are hazardous to human health have been categorized widely under chemical contaminants .Out of this, Heavy metals Arsenic,Lead, Cadmium and Mercury are the most common contaminant occurring in herbal resources.Contaminated herbal materials (due to environment pollution or pesticide usage) and its usage in various medications is a serious threat to the authenticity of the classical drugs in terms of its safety. Heavy metal analysis of any given drug sample is as important as drug standardization and safety assessment. The procedures recommended for analysis of Heavy metals are as per the guidelines WHO (1998).

Instrument details:

Thermo FisherM Series, 650902 V1.27 model **A**tomic Absorption Spectrometer (AAS) was used for the Heavy metal analysis. The Hallow cathode lamp for Pb, Cd, Hg and As analysis were used as light source to provide specific wavelength for the elements to be determined.

5.4. Biochemical Analysis – *Sanjeevi theeneer*

S.No	Experiment	Observation	Inference
1.	Physical Appearance of extract	Light lemon yellow	
2.	**Action of Heat :** The sample was taken in a dry test tube and heated gently at first and then strong	No White fumes evolved	Absence of Carbonate
3.	**Flame Test :** The sample was mixed with conc Hcl in a watch glass an introduced into non-luminous part of the Bunsen flame	No Bluish green flame	Absence of Copper
4.	**Ash Test :** A filter paper was soaked into the mixture of drug sample and diluted cobalt nitrate solution and introduced into the Bunsen flame and ignited	No appearance of yellow color flame	Absence of Sodium

Test for Acid radicals			
1.	Test for sulphate: To 2 ml of the drug sample added 2 ml of 4% dil ammonium oxalate solution	No cloudy appearance	Absence of Sulphates
2.	Test for Chloride : To 2 ml of the sample 2 ml dil Hcl was added until the effervescence ceases off	No cloudy appearance formed	Absence of Chlorides
3.	**Test For Phosphate**: 2 ml of the sample was treated with 2 ml of dil. Ammonium molubdate solution and 2 ml of con.Hno3	No cloudy yellow appearance present	Absence of Phosphate
4.	**Test For Carbonate:** 2 ml of the sample was treated with 2 ml of dil.magnesium sulphate solution.	No cloudy appearance present	Absence of Carbonate
5.	**Test For Nitrate:** 1 ml of sample was treated with copper turning and con.H_2So_4 and viewed the tube vertically down.	No brown gas evolved	Absence of Nitrate
6.	**Test For Sulphide:** 1 ml of the drug sample is treated with 2ml of conc Hcl.	No rotten egg smelling gas was evolved.	Absence of Sulphide.

7.	**Test For Fluoride and Oxalate:** 2ml of the sample was added with 2 ml dil.acetic acid and 2ml dil.calcium chloride solution and heated.	No cloudy appearance.	Absence of Fluoride and Oxalate.
8.	**Test For Nitrite:** 3 drops of the sample was placed on a filter paper ,on that 2 drops of dil.acetic acid and 2 drops of dil.Benzidine solution was placed	No characteristic changes were noted	Absence of nitrite
9.	Test For Borate: Sample was made into paste by using dil.sulphuric acid and alcohol (95%) and introduced into blue flame.	No appearance of bluish green color.	Absence of Borate.

II Test For Basic Radicals			
10.	**Test For Lead :** 2 ml of the sample was added with 2ml of dil.potassium iodine solution.	No yellow precipitate was obtained.	Absence of Lead.
2.	**Test For Copper:** Sample was made into paste with conc.HCl in a watch glass and introduced into the non-luminous part of the flame.	No blue color appeared	Absence of Copper.
3..	**Test For Aluminum:** To the 2 ml of sample dil.sodium hydroxide was added in 5 drops excess.	No yellow color appearance	Absence of Aluminium
4.	**Test For Iron** **a.** To 2 ml of sample, added 2 ml of dil.ammonium solution. b. To 2 ml of sample 2 ml thiocyanate solution and 2ml of con.Hno_3 were added.	No red color appeared.	Absence of Iron.
5.	**Test For Zinc:** To 2 ml of sample dil.sodium, hydroxide solution was added in 5drops excess and dil.ammonium chloride was added.	No white precipitate was formed	Absence of Zinc
6.	**Test For Calcium:** 2 ml of sample was added with 2 ml of 4% dil.ammonium oxalate solution.	No cloudy appearance with precipitate	Absence of Calcium.

7.	**Test For Magnesium:** To 2 ml of sample dil.sodium hydroxide solution was added in 5 drops to excess	No white precipitate was obtained	Absence of magnesium.
8.	**Test For Ammonium:** To 2 ml of sample 1 ml of Nesslers reagent and excess of dil.sodium hydroxide solution were added.	No brown color appeared.	Absence of ammonium.
9.	**Test For Potassium:** Sample was treated with 2 ml of dil.sodium nitrate solution and then treated with 2 ml of dil.cobalt nitrate in 30% dil.glacial acetic acid.	No yellow precipitate was obtained.	Absence of potassium
10.	**Test For Sodium:** Sample was made into paste by using HCl and introduced into the blue flame of Bunsen burner	No yellow color flame evolved.	Absence of Sodium.
11.	**Test For Mercury:** 2 ml of the Sample was treated with 2 ml of dil.sodium hydroxide solution.	No yellow precipitate was obtained.	Absence of mercury.
12.	**Test For Arsenic:** 2 ml of the sample was treated with 2 ml of dil.sodium hydroxide solution.	No brownish red precipitate was obtained.	Absence of Arsenic.

III.Miscellaneous			
1.	**Test For Starch:** 2 ml of sample was treated with weak dil.Iodine solution.	Blue color was not developed.	Absence of starch
2.	**Test For reducing Sugar :** 5 ml of Benedict's qualitative solution was taken in a test tube and allowed to boil for 2 minutes and added 8 to 10 drops of sample and again boil it for 2 minutes. The color changes were noted	No Green color developed.	Absence of reducing sugar.

4.	**Test For Tannic Acid:** 2 ml of sample was treated with 2 ml of dil.ferric chloride solution.	No Blue -black precipitate was obtained.	Absence of tannnic acid.
5.	**Test For Unsaturated Compound:** To the 2 ml of sample, 2ml of dil.potassium permanganate solution was added.	Potassium permanganate was not decolourised.	Absence of unsaturated compound.
6.	**Test For Amino acid: 2** drops of the sample was placed on a filter paper and dried well 20 ml of Burette reagent was added.	Violet color appeared	Presence of Amino acid.
7.	**Test For Type of Compound:** 2 ml sample was treated with 2 ml of dil.ferric chloride solution.	No green and red color developed No violet color developed.	Absence quinol epinephrine pyrocatecho antipyrine. Aliphatic amino acid and meconic acid, apomorphine salicylate and resorcinol were absent.

5.5 Preliminary Phyto chemical screening[61]

Background:

Plants are the potent reservoir of high class biological compounds with wide range of pharmacological properties and some of it may include alkaloids,flavanoids ,tannins and phenolic compounds.With importance to herbal distillation,*Theeneer* selectively avails the bio active principles of the drug (Volatile and organic). An initial step of research will be very fruitful in finding the presence of active chemical components in the distillate. Thereby it may define the therapeutic outcome of the formulation in a surface level .The Classical poly herbo mineral distillate-*Sanjeevi theeneer* has been selected for phyto chemical screening to support the view.

Phyto chemical Assays

Preliminary phyo chemical studies for all the 12 compounds were carried out on the distillate *Sanjeevi theeneer* as per the standards.

1.Test for the presence of Alkaloid- Mayer's reagent

Principle: Most of the alkaloids are precipitated from neutral or slightly acidic solution by Mayer's reagent (Potassio mercuric iodide solution(HgI_4K_2) The presence of Alkaloids is indicated by a cream or dull white precipitate.To the test drug, about 2ml of Mayer's reagent was added and was observed for the presence of Alkaloids.

2. Test for the presence of flavonoids

Principle : First addition of dilute ammonia to the test sample then followed by the addition of Concentrated sulphuric acid (few drops) will indicate an yellowish coloration that disappear on standing confirms the presence of Flavonoids**.**To 0.1ml of the test sample about 5 ml of dilute ammonia solution were been added followed by addition of few drops of conc. Sulfuric acid.

3. **Test for the presence of Glycosides -Borntrager's Test**

Principle: Test drug is hydrolyzed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydrolysate is subjected to the following tests. To 2 ml of filtered hydrolysate, 3 ml of chloroform is added and shaken, chloroform layer is separated and 10% ammonia solution is added to it. Pink colour indicates presence of Glycosides.

4. Test for the presence of Triterepnoids - Salkowski test

Principle: To 2ml of the test solution 2ml chloroform was added with few drops of conc. Sulphuric acid (3ml) through the side of the test tube. An interface with a reddish brown coloration is formed if Terpenoids constituent is present.

5. Test for the presence of Steroids - Salkowski test

Principle: To the test solution, 2ml of chloroform was added with few drops of conc. Sulphuric acid (3ml), and shaken well. The upper layer in the test tube turns into red and sulphuric acid layer showed yellow with green fluorescence. It showed the presence of Steroids.(Joseph et al., 2013)

6. Test for the presence of Carbohydrates - Benedict's test

Principle: The Benedict's test allows us to detect the presence of reducing sugars (sugars with a free aldehyde or ketone group). The final color of the solution depends on how much of this precipitate was formed, and therefore the color(Green,Orange,Red,and Brown) gives an indication of how much reducing sugar was present. To 0.5 ml of test drug, about 0.5 ml of Benedict's reagent is added. The mixture is heated on a boiling water bath for 2 minutes. A characteristic coloured precipitate indicates the presence of Sugar.

7. Test the presence of Phenol- Lead acetate test

Principle: The test sample is dissolved in of distilled water and to this 3 ml of 10% lead acetate solution is added. A bulky white precipitate indicates the presence of Phenolic compounds.

8. Test for the presence of tannins (Ferric Chloride Test)

Principle: About 0.5ml of test sample is boiled in 20 ml of distilled water in a test tube and then filtered. The filtration method used here is the normal method, which includes a conical flask and filter paper.0.1% $FeCl_3$ is added to the filtered samples and observed for brownish green or a blue black coloration, which shows the presence of Tannins.

9. Test for the presence of Saponins (foaming Test)

Principle: Demonstration of Frothing: Herbal material containing Saponins can cause persistant foam when an aqueous decoction or distillate is shaken.The test drug were shaken vigorously for 10 mins, and observed for lather formation.

10. Test for the presence of Proteins (Biuret Test)

Principle: Equal volume of 5% solution of sodium hydroxide and 1% copper sulphate were added. Appearance of pink or purple colour indicates the presence of proteins and free amino acids (Boxi et al., 2010).

11.Test the presence of Coumarins

Principle: 1 ml of test drug, 1 ml of 10% sodium hydroxide was added. The presence of Coumarins is indicated by the formation of yellow color.

12.Test for the presence of Anthocyanin (Sodium Hydroxide Test)

Principle: About 0.2 ml of the extract was weighed in separate test tube; 1ml of 2N Sodium hydroxide was added, and heated for 5 minutes at 100 ± 2°C. Observed for the formation of bluish green color, which indicates the presence of Anthocyanin.

5.6 GAS CHROMATOGRAPHY MASS SPECTROMETRY (GC-MS) Analysis [67, 68]

INTRODUCTION

GC-MS Plays a key role in the analysis of unknown components of plant origin. GC-MS ionizes compound and measures their mass numbers. Ionization method includes EI (Electron Ionization). The EI method produces ions by colliding thermal electrons emitted from a filament with sample gas molecules. This method provides high stability in ionization and obtained mass spectra show good reproducibility. The EI method provides good result for quantitative analysis as well. Quantitative analysis with GC-MS, in which only ions specific to the compounds are measured, is highly selective method without interfering components. Gas chromatography Technique involves the separation of volatile components in a test sample using suitable capillary column coated with polar or non-polar or intermediate polar chemicals. Elite-1 column (100% Dimethyl polysiloxane) is a non-polar column used for analysis of phyto-components. Elite -5 column (5% phenyl and 95% methyl polysiloxane) is an intermediate column and also used for the estimation of Phytochemical. An inert gas such as hydrogen or nitrogen or helium is used as a carrier gas .The compounds of test sample is evaporated in the injection port of the GC equipment and segregated in the column by absorption and adsorption technique with suitable GC programme.The two samples of Sanjeevi Theeneer were individually analysed through GC-MS

Agilent 7890B GC connected to 5977A MSD, NIST Ver.2.1 MS data library Specification

TOXICOLOGICAL EVALUATION OF SANJEEVI THEENEER

Introduction:

Safety is a fundamental principle in the provision of traditional medicines and herbal products for health care and a critical component of quality control. OECD guidelines provide practical and technical guidance for monitoring the safety of traditional medicines within Pharmacovigilance systems. The safety monitoring of traditional medicines is compared and contrasted with that of other medicines, currently undertaken in the context of the WHO International Drug perspective.

Scope of work:

- Assurance of safety, quality and efficacy of Indian System of Medicines (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 *Siddha* formulations are lacking.
- Some of the formulations are proved effective in various animal studies and many more are yet to be tested.

Hence, the present study was carried out to evaluate the safety of *Sanjeevi Theeneer* in rodents.

Plan of work:

The following studies were carried out on *Sanjeevi Theeneer*

- Acute Oral toxicity Study - OECD 423
- Repeated dose 28 Days Oral Toxicity Study - OECD 407
- Repeated dose 90 Days Oral Toxicity study - OECD 408

6.1 ACUTE ORAL TOXICITY STUDY OF *SANJEEVI THEENEER*

(OECD GUIDELINE – 423)

Introduction:

- 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.
- 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.

Principle of the Test:

It is the principle of the test that based on a stepwise procedure with the use of a minimum number of animals per step, sufficient information is obtained on the acute toxicity of the test substance to enable its classification. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals of a single sex. Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.

- no further testing is needed
- dosing of three additional animals, with the same dose
- dosing of three additional animals at the next higher or the next lower dose level. The method will enable a judgment with respect to classifying the test substance to one of a series of toxicity classes.

Methodology:

Selection of Animal Species

- ❖ The preferred rodent species is the Wistar albino rat
- ❖ Healthy young adult animals or commonly used laboratory strains should be employed
- ❖ Females should be nulliparous and non-pregnant.
- ❖ Each animal, at the commencement of its dosing, should be between 6 to 8 weeks old and the weight (150-200 gm) should fall in an interval within ±20 % of the mean weight of any previously dosed animals.

Housing and Feeding Conditions

- ❖ Animals were housed under standard laboratory conditions.
- ❖ They were maintained in a ventilated room. The temperature in the room should be $22^0(\pm 3^0)$.
- ❖ The relative humidity should be at least 30% and not exceed 70% (50%-60%).
- ❖ Lighting should be artificial; it is maintained as 12h light/dark cycle.
- ❖ Animals were kept in a clean polypropylene cage.
- ❖ Rats were fed with standard pellet diet (Sai Meera Foods, Bangalore) and water *ad libitum.*
- ❖ Animals may be group-caged by dose, but the number of animals per cage must not interfere with clear observations of each animal.

Preparation of animals: The animals were randomly selected, marked on its fur to permit individual identification, and kept in their cages for at least 7 days prior to dosing to allow for acclimatization to the laboratory conditions

Test Animals and Test Conditions:

Sexually mature Female Wistar albino rats (150-200 gm) were obtained from *Tanuvas, Madhavaram, Chennai*. All the animals were kept under standard environmental condition (22±3°C). The animals had free access to water and standard pellet diet (Sai meera foods, Bangalore).

Preparation of animals:

The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 7 days prior to dosing to allow for acclimatization to the laboratory conditions.

Preparation for Acute Toxicity Studies

Rats were deprived of food overnight (but not water 16-18 h) prior to administration of the, ***Sanjeevi Theeneer***.

The principles of laboratory animal care were followed and the Institutional Animal Ethical Committee approved the use of the animals and the study design

IAEC approved Number: IAEC/XLIX/CLBMCP/2016

Test Substance	**: *Sanjeevi Theeneer***
Animal Source	**:** Tanuvas, Madhavaram, Chennai.
Animals	**:** Wister Albino Rats (Female-3+3)
Age	**:** 6-8 weeks
Body Weight on Day 0	**:** 150-200 gm.
Acclimatization	**:** Seven days prior to dosing.
Veterinary examination	**:** Prior and at the end of the acclimatization period.
Identification of animals	**:** By cage number, animal number and individual marking by using Picric acid.
Numberofanimals	**:** 3 Female/group,
Routeofadministration	**:** Oral
Diet	**:** Pellet feed supplied by Sai meera foods Pvt Ltd, Bangalore
Water	**:** Aqua guard portable water in polypropylene bottles.
Housing & Environment	**:** The animals were housed in Polypropylene cages provided with bedding of husk.

Housing temperature **:** Between 22ºC + 3ºC.
Relative humidity **:** Between 30% and 70%,
Air changes **:** 10 to 15 per hour and
Dark and light cycle **:** 12**:**12 hours.
Duration of the study **:** 14 Days

Administration of Doses:

Sanjeevi Theeneer was suspended in water and administered to the groups of wistar albino rats in a single oral dose by gavage using a feeding needle. The control group received an equal volume of the vehicle. Animals were fasted 12 hours prior to dosing. Following the period of fasting, the animals were weighed and then the test substance was administered. Three Female animals are used for each group. The dose level of 5 ml/kg body weight was administered. After the substance has been administered, food was withheld for a further 3 - 4 hours. The principle of laboratory animal care was followed. Observations were made and recorded systematically and continuously as per the guideline after substance administration. The visual observations included skin changes, mobility, aggressiveness, sensitivity to sound and pain, as well as respiratory movements. Finally, the number of survivors was noted after 24 hrs and these animals were then monitered for a further 14 days and observations made daily. The toxicological effect was assessed on the basis of mortality.

Limit test

Number of animals and dose levels

- The limit test is primarily used in situations where the experimenter has information indicating that the test material is likely to be nontoxic, i.e., having toxicity only above regulatory limit doses.
- Information about the toxicity of the test material can be gained from knowledge about similar tested compounds or similar tested mixtures or products, taking into consideration the identity and percentage of components known to be of toxicological significance.
- A limit test at one dose level of 2000 mg/kg body weight can be carried out with three animals per step.

- If the test substance-related mortality was not produced in the experimented animals, further testing at the next lower level need not be carried out.
- Since this test drug is likely to be non-toxic, and the inference from various toxicity studies on distillates a limit test at one dose level of 5 ml/kg body weight will be carried out with 3 animals for each group.

Observations:

Animals are observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days, except where they need to be removed from the study and humanely killed for animal welfare reasons or are found dead. It should be determined by the toxic reactions, time of onset and length of recovery period, and may thus be extended when considered necessary. The times at which signs of toxicity appear and disappear are important, especially if there is a tendency for toxic signs to be delayed. All observations are systematically recorded with individual records being maintained for each animal.

a. Cage-side observation

These include changes in skin and fur, eyes and mucous membranes and also respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior patterns. Attention should be directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. The principles and criteria summarized in the Human Endpoints Guidance Document is taken into consideration.

b. Behavior:

The animals will be observed closely for behavior in the first four hours which includes abnormal gait, aggressiveness, exophthalmos, ptosis, akinesia, catalepsy, convolutions, excitation, head twitches, lacrimation, loss of corneal reflex, loss of traction, piloerection reactivity of touch, salivation, scratching, sedation, chewing, head movements, sniffing, straub, tremor and writhes, diarrhea, leathery, sleep and coma.

c. Food and water Consumption:

Food and water consumed per animal was calculated for control and the treated dose groups.

d. Body Weight:

Individual weight of animals was determined before the test substance was administered and weights will be recorded at day 1, 7, and 14 of the study. Weight changes were calculated and recorded. At the end of the test, surviving animals were weighed and humanly killed.

e. Mortality

Animals will be observed intensively at 0.5, 2.0, 4.0, 6.0, 12.0, 24.0 and 48.0 hour following drug administration on day 1 of the experiment and daily twice thereafter for 14 days.

f. Gross necropsy

All animals (including those which die during the test period are removed from the study), will be subjected to gross necropsy. Gross necropsy includes examination of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents, brain, eye, thymus, lungs, heart, spleen, liver, kidneys, adrenals, testes and uterus of all animals.

g. Mortality:

Animals were observed for mortality throughout the entire period.

Data and reporting

All data were summarized in tabular form, (Table-1-4) showing for each test group the number of animals used, the number of animals displaying signs of toxicity, the number of animals found dead during the test ,description of toxic symptoms,, weight changes, food and water intake.

6.2 REPEATED DOSE 28-DAY ORAL TOXICITY STUDY OF *SANJEEVI THEENEER*

Introduction:

In the assessment and evaluation of the toxic characteristics of a chemical, the determination of oral toxicity using repeated doses may be carried out after initial information on toxicity has been obtained by acute toxicity testing. This Test guidelines is intended to investigate effects on a very broad variety of potential targets of toxicity. It provides information on the possible health hazards likely to arise from repeated exposure over a relatively limited period.

Principle of the Test:

- The test substance is orally administered daily in graduated doses to several groups of experimental animals, one dose level per group for a period of 28 days.
- During the period of administration, the animals are observed closely, each day for signs of toxicity.
- Animals that die or are euthanized during the test are necropsied and at the conclusion of the test, surviving animals are euthanized and necropsied.
- A 28-day study provides information on the effects of repeated oral exposure and can indicate the need for further longer-term studies.
- It can also provide information on the selection of concentrations for longer-term studies.
- The data derived from using the TG should allow for the characterization of the test substance toxicity, for an indication of the dose response relationship and the determination of the No-Observed Adverse Effect Level (NOAEL)

Test Substance	:	*Sanjeevi Theeneer*
Animal Source	:	TANUVAS, Madhavaram, Chennai.
Animals	:	Wister Albino Rats (Male -15, and Female-15)
Age	**:**	6-8 weeks
Body Weight	**:**	150-200 gm.
Acclimatization	**:**	Seven days prior to dose.
Veterinary examination	**:**	Prior and at the end of the acclimatization period.
Identification of animals	**:**	By cage number, animal number and individual marking by using Picric acid
Diet	**:**	Pellet feed supplied by Sai meera foods Pvt Ltd, Bangalore
Water	**:**	Aqua guard portable water in polypropylene bottles.
Housing & Environment	**:**	The animals were housed in Polypropylene cages provided with bedding of husk.
Housing temperature	**:**	between 22ºC $\pm$ 3ºC.
Relative humidity	**:**	between 30% and 70%,
Air changes	**:**	10 to 15 per hour
Dark and light cycle	**:**	12**:**12 hours.
Duration of the study	**:**	28 Days.

Table: 8 Grouping of Animals

Groups	**No of Rats**
Group I Vehicle control (Water)	10(5 male, 5 female)
Group III ST- Mid dose 5X (2.5ml/ kg)	10(5 male, 5 female)
Group IV ST- High dose 10X(5 ml/ kg)	10(5 male, 5 female)

ST: *Sanjeevi Theenee R*

I. Methodology

1. Justification for Dose Selection

The results of acute toxicity studies in Wistar albino rats indicated that*Sanjeevi Theeneer* was non-toxic and no behavioral changes was observed up to the dose level of 5ml /kg body weight. As per OECD, guideline three dose levels usually will be selected for the study. They are low dose (X), mid dose dose (5X), high dose (10X). X is calculated by multiplying the therapeutic dose (30 ml) and the body surface area of the rat (0.018). i.e X dose is 0.54 ml/kg , (rounded to 0.5ml), 5X dose is 2.5 ml/ kg, 10X dose is 5 ml/kg. Here as the trial drug has not shown acute toxicity, Mid and high doses are selected for the study.

2. Randomization, Numbering and Grouping of Animals:

30 Wistar Albino Rats (15 M + 15 F) were selected and divided into 4 groups. Each group consist of 10 animals (5 male, 5 female). First group treated as a control and other two groups were treated with test drug (mid, high) for 28 days. Animals were allowed acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. Each animal was marked with picric acid. The females were nulliparous and non-pregnant.

3. Preparation and Administration of Dose:

Sanjeevi Theeneer suspended in with water, it was administered to animals at the dose levels of 5X, 10X. The test substance suspensions were freshly prepared every two days once for 28 days. The control animals were administered vehicle only. The drug was administered orally by using oral gavage once daily for 28 consecutive days.

4. Observations:

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

a. Body Weight:

Weight of each rat was recorded on day 0, at weekly intervals throughout the course of study and at termination to calculate relative organ weights. From the data, group mean body weights and percent body weight gain were calculated.

b. Food and water Consumption:

Food and water consumed per animal was calculated for control and the treated dose groups.

c. Clinical signs:

All animals were observed daily for clinical signs. The time of onset, intensity and duration of these symptoms, if any, were recorded.

d. Mortality:

All animals were observed twice daily for mortality during entire course of study.

1. Laboratory Investigations:

Following laboratory investigations were carried out on day 29 in animals fasted over-night. Blood samples were collected from orbital sinus using sodium heparin (200 IU /ml) for Biochemistry and potassium EDTA (1.5 mg/ml) for Hematology as anticoagulant. Blood samples were centrifuged at 3000 r.p.m. for 10 minutes. . On 28th day of the experiment, 24 h urine samples were collected by placing the animals in the metabolic cage with free access to tap water but no feed was given.

The urine was free from fecal contamination. Toluene is used as a preservative while collecting the sample. The sediments present in the urine were removed by centrifugation and the collected urine was used for biochemical estimations. On 29th day, the animals were fasted for approximately 18 h, then slightly anesthetized with ether and blood samples were collected from the retro-orbital plexus into two tubes: one with EDTA for immediate analysis of haematological parameters, the other without any anticoagulant and was centrifuged at 4000 rpm at 4 °C for 10 minutes to obtain the serum. Serum was stored at 20 °C until analyzed for biochemical parameters.

2. **Haematological Investigations:**

Blood samples of control and experimental rats was analyzed for hemoglobin content, total red blood corpuscles (RBC), white blood corpuscles (WBC) count and packed cell volume (PCV).

3. Biochemical Investigations:

Serum was used for the estimation of biochemical parameters. Samples of control and experimental rats were analyzed for protein, bilirubin, urea, BUN, creatinine, triglyceride, cholesterol and glucose levels was carried using standard methods. Activities of glutamate oxaloacetate transaminase/ Aspartate aminotransferase (GOT/AST), glutamate pyruvate transaminase/ Alanine amino transferase (GPT/ALT) and alkaline phosphatase were estimated as per the colorimetric procedure.

4. **Urine analysis:**

Urine samples were collected on end of treatment for estimation of normal parameters. The estimations were performed using appropriate methodology.

5. Necropsy:

All the animals were sacrificed on day 29. Necropsy of all animals was carried out and the weights of the organs including liver, kidneys, spleen, brain, heart, and lungs were recorded. The relative organ weight of each animal was then calculated as follows.

$$Relative\ \ organ\ Weight = \frac{Absolute\ organ\ weight\ (g)}{Body\ weight\ of\ animal\ on\ sacrifice\ day\ (g)}\ x\ 100$$

6. Histopathology:

Organs will be collected from all animals, preserved in 10% buffered neutral formalin for 24 h, and washed in running water for 24 h. The organ sliced 5 or 6µm sections and were dehydrated in an auto technicon and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the "L" moulds. It was followed by microtome and the slides were stained withHaematoxylin-eosin red. The organs included Liver, spleen, kidney, Brain and Lung.

7. Statistical analysis:

Findings such as clinical signs of intoxication, body weight changes, water and food consumption, hematology and blood chemistry were subjected to One-way ANOVA followed by Dunnet's multi comparision test using a computer software programme GRAPH PAD INSTAT-3 version. All data were summarized in tabular form.

6.3 REPEATED DOSE 90-DAY ORAL TOXICITY STUDY OF *SANJEEVI THEENEER* (OECD GUIDELINE - 408)

Introduction:

In the assessment and evaluation of the toxic characteristics of a chemical, the determination of sub-chronic oral toxicity using repeated doses may be carried out after initial information on toxicity has been obtained from acute or repeated dose 28-day toxicity tests. The 90- day study provides information on the possible health hazards likely to arise from repeated exposure over a prolonged period. The study will provide information on the major toxic effects, indicate target organs and the possibility of accumulation, and can provide an estimate of a no-observed-adverse-effect level of exposure which can be used in selecting dose levels for chronic studies and for establishing safety criteria for human exposure.

Principle of the test:

- The test substance is orally administered daily in graduated doses to several groups of experimental animals, one dose level per group for a period of 90 days.
- During the period of administration, the animals are observed closely for signs of toxicity.
- Animals which die or are killed during the test are necropsied and, at the conclusion of the test, surviving animals are killed and necropsied.

Test Substance	:	*Sanjeevi Theeneer*
Animal Source	:	Tanuvas, Madhavaram, Chennai.
Animals	:	Wister Albino Rats (Male - 20, and Female-20)
Age	**:**	6-8 weeks
Body Weight	**:**	150-200 gm.
Acclimatization	**:**	Seven days prior to dosing.
Veterinary examination	**:**	Prior and at the end of the acclimatization period.
Identification of animals	**:**	By cage number, animal number and individual marking by using picric acid.
Diet	**:**	Pellet feed supplied by Sai meera foods Pvt Ltd, Bangalore
Water	**:**	Aqua guard portable water in polypropylene bottles.

Housing & Environment	**:**	The animals were housed in Polypropylene cages provided with bedding of husk.
Housing temperature	**:**	between 22ºC + 3ºC.
Relative humidity	**:**	between 30% and 70%,
Air changes	**:**	10 to 15 per hour
Dark and light cycle	**:**	12**:**12 hours.
Duration of the study	**:**	90 Days.

Table: 9 Grouping of Animals

Groups	**No of Rats**
Group I Vehicle control (Water)1ml	20 (10male, 10female)
Group III STR - Mid dose 5X (2.5Ml/kg)	20 (10male, 10female)
Group IV STR - High dose 10X(5ml/kg)	20 (10male, 10female)

I. Methodology

1.Randomization, Numbering and Grouping of Animals:

60 Wistar Albino Rats (20 M + 20F) were selected and divided into 4 groups. Each group consists of 20 animals (Male -10 and Female-10). First group treated as a control and other 2 groups were treated with test drug (mid, high) for 90 days. Animals were allowed acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. Each animal was marked with picric acid. The females were nulliparous and non-pregnant.

2.Justification for Dose Selection:

By following Sub – Acute studies.

3. Preparation and Administration of Dose:

*Sanjeevi Theeneer*was administered to animals at the dose levels of 5X, 10X. The control animals were administered vehicle (WATER) only. The drug was administered orally by using oral gavage once daily for 90 consecutive days.

3.1 Observations:

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

A. Body Weight:

Weight of each rat was recorded on day 1,15,30,45,60,75,90, at biweekly intervals throughout the course of study.

B. Food and water Consumption:

Food and water consumed per animal was calculated for control and the treated dose groups.

C. Clinical signs:

All animals were observed daily for clinical signs. The time of onset, intensity and duration of these symptoms, if any, were recorded.

D. Mortality:

All animals were observed twice daily for mortality during entire Course of study.

E. Necropsy:

All the animals were sacrificed by excessive anesthesia on day 91. Necropsy of all animals was carried out.

3.2 Laboratory Investigations:

Following laboratory investigations were carried out on day 91 in animals fasted over-night. Blood samples were collected from orbital sinus using sodium heparin (200 IU/ml) for Biochemistry and potassium EDTA (1.5 mg/ml) for Hematology as anticoagulant. Blood samples were centrifuged at 3000 r.p.m. for 10 minutes.

a. Haematological Investigations:

Haematological parameters were determined using Haematology analyzer.

b. Biochemical Investigations:

Biochemical parameters were determined using auto-analyzer

c. Histopathology:

Control and highest dose group animals will be initially subjected to histopathological investigations. If any abnormality found in the highest dose group than the low, then the mid dose group will also be examined. Organs will be collected from all animals and preserved in 10% buffered neutral formalin for 24 h and washed in running water for 24 h. The organ sliced 5 or 6µm sections and were dehydrated in an auto

technicon and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the "L" moulds. It was followed by microtome and the slides were stained withHaematoxylin-eosin.

e. Statistical analysis:

Findings such as clinical signs of intoxication, body weight changes, food consumption, hematology and blood chemistry were subjected to One-way ANOVA followed by dunnet test using a statistics software Graph Pad version 7. All data were summarized in tabular form Table ()

7.1 Evaluation of Anti-Oxidant Activity of Sanjeevi Theeneer Using Dpph (2, 2-Diphenyl 1-2 picrylhydrazyl) Free radical Scavenging Assay

Procedure:

The antioxidant activity of test drug sample ST was determined using the 2,2-diphenyl 1-2 picrylhydrazyl (DPPH) free radical scavenging assay . Sample ST was mixed with 95% methanol to prepare the stock solution in required concentration (100µg/ml). From the stock solution 10 µg/ml, 20 µg/ml, 40µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml of this solution were taken in five test tubes and by serial dilution. Ascorbic acid were used as standard was prepared in concentration 10 µg/ml, 20 µg/ml, 40µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml respectively by using methanol as solvent. Final reaction mixture containing 1 ml of 0.3 mM DPPH methanol solution was added to 2.5 ml of sample solution of different concentrations and allowed to react at room temperature. Absorbance in the presence of ST at different concentration of (10 µg/ml, 20 µg/ml, 40µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml) was noted after 15 min incubation period at 37^0C. Absorbance was read out at 517 nm using double-beam U.V Spectrophotometer by using methanol as blank.

% scavenging = [Absorbance of control - Absorbance of test sample/Absorbance of control] X 100

The effective concentration of test sample ST required to scavenge DPPH radical by 50% (IC_{50} value) was obtained by linear regression analysis of dose-response curve plotting between %inhibition and concentrations.

7.2 Evaluation of Hepatoprotective activity of *Sanjeevi Theeneer* against paracetamol induced Hepato toxicity in zebra fish Danio rerio model [77, 78]

Background

The liver may be considered as the most important organ in drug toxicity because it is functionally interposed between the site of absorption and the systemic circulation and is a major site of metabolism and elimination of foreign substances; these features render it a preferred target for drug toxicity. Drug-induced liver injury (DILI) therefore poses a major clinical problem. DILI is initiated by direct hepatotoxic effects of a drug, or a reactive metabolite of a drug.

Paracetamol, a widely used analgesic and antipyretic drug, produces acute liver damage in high doses in both animals and in humans. Paracetamol administration causes necrosis of the centrilobular hepatocytes characterized by nuclear pyknosis and eosinophilic cytoplasm followed by large excessive hepatic lesion. The covalent binding of Nacetyl- P-benzoquinoneimine, an oxidative product of paracetamol to sulphydryl groups of protein, result in lipid peroxidative degradation of glutathione level and thereby, produces cell necrosis in the liver.

Zebra Fish Model (Danio rerio)

- Zebra fish is good translational model to assess drug induced toxicity , Hepato protectivity or hepatic regeneration.
- Can accurately model human physiology.
- Have similar molecular/ cellular/ drug metabolism/ CYP genes/ Histopathological changes.
- Can reduce and replace higher order animals for Drug studies.

❖ **Materials and Methods**

- Purchase : Local Aquarium facility.
- Acclimatization : four weeks prior to the start of experimentation.
- Laboratory condition : 28 °C $\pm$ 1°C , 14:10 h light/dark cycle photo period.
- Grouping : four groups of 10 fish each.

- Weight per ml calculation of the drug ST = 0.015 gm (15000 micro gram or 15 mg)/ ml
- Drug administration : Dilution of drug in water .
- Drug Exposure period : 7 days.

Table: 10 Grouping of Zebra Fishes for Study Trial

Groups	Exposure	Period
Group I Control	Untreated	7 Days
Group II Toxicant	Paracetamol 5mM (755.8mg) per litre concentration	7 Days
Group III: **Test group (ST Low dose)**	Paracetamol 5mM + ST Low Dose 150 mg/ litre.	7 Days
Group IV: **Test group (ST High dose)**	Paracetamol 5mM + ST High Dose 300 mg/ litre.	7 Days

Procedure:

Animal belongs to group I left untreated and group II treated with Paracetamol at the concentration of 5mM (755.8mg) per liter concentration for the period of seven days. Animal belongs to group III received test drug Sanjeevi Theeneer (ST) at the concentration of 150 mg/liter and group IV received test drug Sanjeevi Theeneer (ST) at the concentration of 300 mg/liter along with paracetamol 5mM for the period of seven days.

Histopathology

After a one-week exposure period, the livers of zebrafish were dissected and fixed in 10% formalin at 4 °C for 24h. Subsequently, the fixed liver tissues were dehydrated in gradient ethanol, hyalinized in xylene, and embedded in paraffin wax at 56 °C. Then, the paraffin blocks were sectioned at 4-μm thickness. The sections were collected on glass slides and stained with hematoxylin and eosin (H&E) using an H&E Staining Kit. Histologic lesions were observed using an optical microscope equipped with a digital camera.

7.3 Evaluation of Hepatoprotective activity of *Sanjeevi Theeneer* on paracetamol induced hepatotoxicity in rat's model[76]

AIM:

To study the Hepato-Protective study of *Sanjeevi Theeneer* in Wistar albino rats by Paracetamol induced hepatotoxicity method.

Materials and Methods:

Test Substance	*:*	*Sanjeevi Theeneer.*
Animal Source	:	TANUVAS, Madhavaram, Chennai.
Animals	:	Wister Albino Rats (Male -30).
Age	**:**	6-8 weeks.
Body Weight	**:**	150-200gm.
Acclimatization	**:**	14 days prior to dose.
Veterinary examination	**:**	Prior and at the end of the acclimatization period.
Identification of animals	**:**	By cage number, animal number and individual marking by using Picric acid
Diet	**:**	Pellet feed.
Water	**:**	Aqua guard portable water in polypropylene bottles
Housing & Environment	**:**	The animals were housed in Polypropylene cages provided with bedding of husk.
Housing temperature	**:**	between 22ºC $\pm$ 3ºC.
Relative humidity	**:**	between 30% and 70%,
Air changes	**:**	10 to 15 per hour
Dark and light cycle	**:**	12**:**12 hours.

I. Experimental design:

Paracetamol induced hepatotoxicity in rats model was used for evaluation of hepato-protective activity for the *Sanjeevi Theeneer*. Animals were divided into five groups, each group containing six animals (Table: 1)

a. Grouping of Animals

- **Group I(normal):** received distilled water only for 7 days.
- **Group II(Toxicant):** received distilled water for 7 days and Paracetamol 1ml/kg, i. p. 1:1 dilution with coconut oil on 7th day.
- **Group III (Test group ST 1):** received *Sanjeevi Theeneer*(0.5ml/kg per day, p.o.) for 7 days and paracetamol induction on 7^{th} day.

  Groups IV (Test group ST 2): received *Sanjeevi Theeneer* (1ml/kg per day p.o) for 7 days and paracetamol induction on 7th day
- **Group V (Standard):** received standard marketed drug silymarin (25mg/kg per day, p.o.) for 7 days and Paracetamol induction on 7^{th} day. (Dr. Sanjay R. Arote et. al.)

Table: 11 Grouping of animals

Group	Treatment	Dose	No : of Animals
A	Normal control	Distilled water	6 Rats
B	Toxicant Control	Partacetamol 1ml/kg, i. p. 1:1 dilution with coconut oil	6 Rats
C	Test Drug ST - 1	0.5ml/kg, *p.o.*+ paracetamol	6 Rats
D	Test Drug ST - 2	1ml/kg, *p.o.*+ paracetamol	6 Rats
E	Standard (Silymarin)	25mg /kg, *p.o.*+ paracetamol	6 Rats

b. Blood Sample Collection and Analysis

After 14 days of experimental period, blood sample has been collected individually for all the animals by retro-orbital puncture method and the blood was allowed to clot for 30 min; serum was separated by centrifuging and was used for various parameter estimations. Later all the animals were sacrificed by cervical dislocation for histopathological study.

c. Serum Biochemistry

The whole blood serum was separated by centrifuging for the following analysis. The activities of serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT) were estimated using standard methods. Estimation of serum ALP, serum bilirubin and electrolytes were also carried out to assess the acute hepatic damage caused by paracetamol.

d. Histopathology study

The animals were sacrificed by decapitation method and the abdomen was cut open to remove the liver. , and the individual weights of the livers were estimated. Liver tissue was quickly removed after autopsy and fixed in 10% formalin in saline. Initially the materials were fixed in 10% buffered neutral formalin and then with Boucins solution (mixture of 75 ml of saturated picric acid, 25 ml of 40% formaldehyde and 5 ml of glacial acetic acid) for 12 hr, then embedded in paraffin and cut into 5 µm thick section and stained using hematoxylin-eosin dye and finally mounted in di-phenyl-xylene were then observed under microscope for histopathological changes in liver architecture and their photomicrographs were taken for the evaluation of histopathological changes

e. Statistical analysis

The experimental results were expressed as the Mean ± SEM for animals in each group. The biochemical parameters were analyzed statistically using one-way analysis of variance ANOVA, followed by Dunnetts multiple comparison test. P value of < 0.05 was considered as statistically significant

7.4 Evaluation Of Bronchdilator Activity of *Sanjeevi Theeneer*- Isolated Guinea Pig Tracheal Chain Preparation.

Introduction: Histamine plays an important role in the symptomatology of allergic reactions. Histamine can provoke bronchoconstriction, it may also be responsible for bronchial hypersensitivity which is a common feature of asthma. Targeting histamine, either prevention of its release from mast cells or use of histaminergic receptor antagonists becomes part of anti histaminic therapy in allergic diseases. Drugs which have the capacity to control the histamine release and its further effects can be called as antihistaminic or antiallergic drugs [(80)]**.** The tracheal muscle has histamine H1, M3.B2 receptors . The stimulation of histamine H1 receptors cause contraction of bronchiole smooth muscles. In present study is to evaluate the effectiveness of Sanjeevi Theeneer in inhibition of histamine induced contraction of Guinea pig tracheal chain preparation, indicating histamine H1 receptor antagonist activity

Aim:

To study the Broncho dilator of Sanjeevi Theeneer in guinea pig Tracheal chain [(79)] preparation

Materials and methods:

Test Substance	:	***Sanjeevi Theeneer***
Animal Source	:	TanuvaS, Madhavaram, Chennai.
Animal	:	Albino Guinea pig (Male -1)
Body Weight	**:**	700 gms
Acclimatization	**:**	14 days prior to dosing.
Veterinary examination	**:**	Prior and at the end of the acclimatization period.
Diet	**:**	Pellet feed
Water	**:**	Aqua guard portable water in polypropylene bottles.
Housing & Environment	**:**	The animal was housed in Polypropylene cage provided with bedding of husk.
Housing temperature	**:**	25±2°C
Air changes	**:**	10 to 15 per hour
Dark and light cycle	**:**	12**:**12 hours.

Selection of animals:

Healthy albino guinea pig weighing 700 gms of male sex was used in this study with the approval of the Institutional Animal Ethics Committee and obtained from the animal laboratory IAEC approved no: IAEC/XLIX/CLBMCP/2016

The animal kept in plastic cage and maintained under controlled environment (temperature 25±2°C and 12hrs dark and light cycle) with standard diet, water and lipitum during experiment. The animal was allowed an acclimatization period of 14 days before actual experiment. The animal experiment was performed with accordance legistation on welfare.

Procedure:

The guinea pigs (overnight fasted weighing 300-500g) were sacrificed. The trachea was cut at suitable length (approximately 2 cm long) was mounted in an organ bath containing Krebs solution of the composition; Nacl 5.9, kcl 0.35, cacl2 O.28, Mgso4 0.11, Nahco3 2.1, KH2 PO4 O.16 and glucose 2.0gm per liter which will be continuously aerated and maintained at 37 ± 0.5.c, one end tracheal chain will be attached to an S shaped aerated tube and other attached to an isotonic frontal wrighting lever to smoked drum. Histamine was added in different concentrations and contractions were recorded. In order to observe the broncho dilator effect of the test substance on induced contractions, the test material Sanjeevi Theeneer was added in a cumulative fashion (15 ml and 30 ml) to obtain the concentration-dependent inhibitory responses. The relaxation of the tissue preparation was expressed as percentage of inhibition in contraction. Percent of maximum contractile responses were plotted to record dose response curves of histamine in the absence and presence of Sanjeevi Theeneer.

7.5 EVALUATION OF ANTI-HISTAMINE (H_1 RECEPTOR ANTAGONISM) OF *SANJEEVI THEENEER*- Isolated Guinea Pig Ileum Preparation.

Introduction:

Guinea pig ileum is used for screening of antihistaminic activity. The stimulation of H1 receptors produces graded dose related contraction of isolated guinea pig ileum [(81)]

Aim:

To study the Anti- Histamine activity of Sanjeevi Theeneer in guinea pig ileal preparation..

Procedure:

The guinea pigs (overnight fasted weighing 300-500g) were sacrificed. The abdomen was cut open a suitable length of the ileum (approximately 2 cm long) was mounted in an organ bath containing Tyrode solution. The composition of the tyrode solution in Mm was NaCl 137Mm, $NaHCO_3$, 12 Mm, NaH_2PO_4 0.3mM, KCl 2.7 Mm, MgCl 1.0Mm, $CaCl_2$ 1.0mM and d-glucose 5.6 Mm.

Experiment were performed in a 30 ml organ bath containing Tyrode solution maintained at 37 ^{0}C under a tension of 0.5 gm which was continuously aerated (air mixture – O_2 + $C0_2$) at 37±0.5°. Isometric Contractions were recorded in a smoked kymograph paper with frontal writing lever. After an equilibration period of 30 min during which the Tyrode solution was changed intervals of 10 mins, contractile responses were recorded for histamine (10 micro gram/ml). The contact time of 30 sec recorded at 5 min time cycle is kept for proper recoding of the responses. The *Sanjeevi Theeneer* tissue contact time was 5 min (Kulkarni, 2003) before the addition of histamine. Dose response curve of histamine in plain Tyrode solution and in Tyrode solution containing 15 ml, 30 ml of Sanjeevi Theeneer were performed. Percentage maximum contractile response was plotted to generate dose response curve of histamine, in the absence and presence of the drug Sanjeevi Theeneer.

8. RESULTS

Table: 12 –Organo leptic Reports of *Sanjeevi Theeneer*

S. No	Parameters	Results &Remarks
a.	**Description**	
1.	**Color**	Light lemon yellow.
2.	**Odor**	Pleasant and highly aromatic.
3.	**Taste**	Pleasant and mild pungent.
b.	**Clarity test**	Confirmed / Complies
c.	**Volatile Oil**	1.320%, 1.332%, 1.324%
d.	**Specific gravity at 25ºC**	0.1622, 0.1624, 0.1626
e.	**pH values**	7.6

Interpretation: The light lemon yellow color with pleasant odor and taste truly complies the Traditional Quality parametres of a distillate and it is directly related with the bioactivity of the drug. The presence of Volatile content (1.32%) supports the claim of its efficacy. The pH of the drug *Sanjeevi Theeneer* is 7.6 which proves the alkaline nature of the drug, and it is essential for its bioavailability and effectiveness.

Table: 13 Heavy Metal Analysis (HMA) - Reports of *Sanjeevi Theeneer*

S. No	Heavy metal	Reference Limits as per API- Vol.-I	Results
1.	Lead	Not more than 10ppm	**Not detected**
2.	Arsenic	Not more than 3.0ppm	**3.1408ppb**
3.	Cadmium	Not more than 0.3ppm	**0.0073ppm**
4.	Mercury	Not more than 1.0ppm	**14.2894ppb**

Table: 14 Microbial Load Analyses - Reports of Sanjeevi Theeneer.

S.No	Parameters	Reference Limits as per WHO (2007)	Results	Remarks
1.	**Total Bacterial Count (TBC)**	10^5CFU/gm	Less than 10 cfu/ml	Within permissible limits
2.	**Total Fungal Count (TFC)**	10^3CFU/gm	Absent	
3.	**Enterobacteriaceae**	10^3	Absent	
4.	***Escherichia coli***	10	Absent	
5.	**Salmonella Spp**	Absent	Absent	
6.	***Staphylococcus aureus***	Absent	Absent	

Interpretation: The heavy metal and microbial load presence were within the permissible limits. Both the results indicate the nontoxicity and purity of the distillate-*Sanjeevi theeneer*.

Table: 15: Bio chemical Analysis of Sanjeevi *Theeneer*

S.NO	Parameters	Results
1.	**Silicate**	Absent
2.	**Sulphate**	Absent
3.	**Chloride**	Absent
4.	**Phosphate**	Absent
5.	**Carbonate**	Absent
6.	**Nitrate**	Absent
7.	**Sulphide**	Absent
8.	**Oxalate**	Absent
9.	**Nitrite**	Absent
10.	**Borate**	Absent
11.	**Lead**	Absent
12.	**Copper**	Absent
13.	**Aluminium**	Absent

14.	**Iron**	Absent
15.	**Zinc**	Absent
16.	**Calcium**	Absent
17.	**Magnesium**	Absent
18.	**Ammonium**	Absent
19.	**Potassium**	Absent
20.	**Sodium**	Absent
21.	**Mercury**	Absent
22.	**Arsenic**	Absent
23.	**Starch**	Absent
24.	**Reducing sugar**	Absent
25.	**Alkaloids**	**Present**
26.	**Tannic acid**	Absent

Interpretation: The Bio chemical analysis reported the presence of Alkaloids which are active principles possessing Alkaloids possess antispasmodic, analgesic, bactericidal effects. A synergistic effect of all these flavonoids, alkaloids, glycosides, tannins, phenols, saponins, increases the potency of the drug against Hepatic damages.

Table: 16 Results of Phytochemical Analysis of Sanjeevi Theeneer.

S.NO	Variable	Test	Observation	Result
1	**Alkaloids**	ST+ Mayers Reagent (2 ml)	- No dull white precipitate	Absence of alkaloids
2	**Flavonoids**	ST (0.1 ml) + Dil Ammonia (5 ml)+ Conc H_2SO_4	- NO yellowish Discolouration	Absence of Flavonoids.
3	**Glycosides**	H2SO4 hydrolyzed ST(2 ml) +Chloroform (3 ml)+10% Ammonia	-No Pink color	Absence of Glycosides.
4	**Steroids**	ST+ Chloroform+ Conc H2SO4 (few drops)	-No Reddish or yellowish Color formation	Absence of Steroids.
5	**Sugar**	ST(2 ml)+ Benedicts Reagent (0.5 ml), Heated for 2 mins in boiling water bath.	-No coloured precipitate.	Absence of Sugars
6	**Triterepnoids**	ST+ Chloroform (2 ml)+ Conc H2SO4(few drops)	-No reddish Brown discoloration	Absence of Terpenoids
7	**Coumarins**	ST(1 ml)+ 10% NaOH (1 ml)	-No Yellow color formation	Absence of Coumarins.
8	**Phenols**	ST dissolved in distilled water+10% lead acetate(3 ml)	+A bulky white Precipitate Present	Presence of Phenols
9	**Tannins**	ST(0.5 ml) is boiled in distilled water(20 ml) ,filtered (with filter paper) then fecl3 (0.1%) is added	-No Brownish green or Blue Black coloration	Absence of Tannins
10	**Saponins**	ST mixed with water and shaken vigorously for 10 mins	-No Lather formation	Absence of Saponins
11	**Proteins**	ST+ NaOH (5%)+ CuSO4	-No Pink or Purple color	Absence of Proteins
12	**Anthocyanin**	ST(0.2 ml)+ NaOH (1 ml),Heated for 5 mins (100 ± 2°C)	-No formation of Bluish green color	Absence of Anthocyanin

ST = Sanjeevi Theeneer. (+) = Positive, (-) = Absence

Interpretation: The preliminary phyto chemical analysis shown the presence of Phenolic compounds in the drug. Phenolic compounds are considered as the most active and predominant group of metabolites derived from the herbal resources (Singh 2007). Natural compounds having Anti-oxidant properties are mainly derived from the plants as phenolic compounds, phenolic acids, tocopherols and flavonoids ((Ali *et al.*, 2008). Phenols exhibit wide range of pharmacological and biological functions especially considering the cardiovascular health. Their role in prevention of atherosclerosis (Anti-atherosclerotic) and inflammation (Anti-inflammatory) and also in improving endothelial function has been well established [23, 24] various studies prove it as an Anti-carcinogenic, Ant apoptosis and Anti-ageing compound.

REPORTS – GC-MS OF Sanjeevi Theeneer (ST(t), ST(g), ST (c)

Fig: 7 GC-MS chromatogram of ST (t)

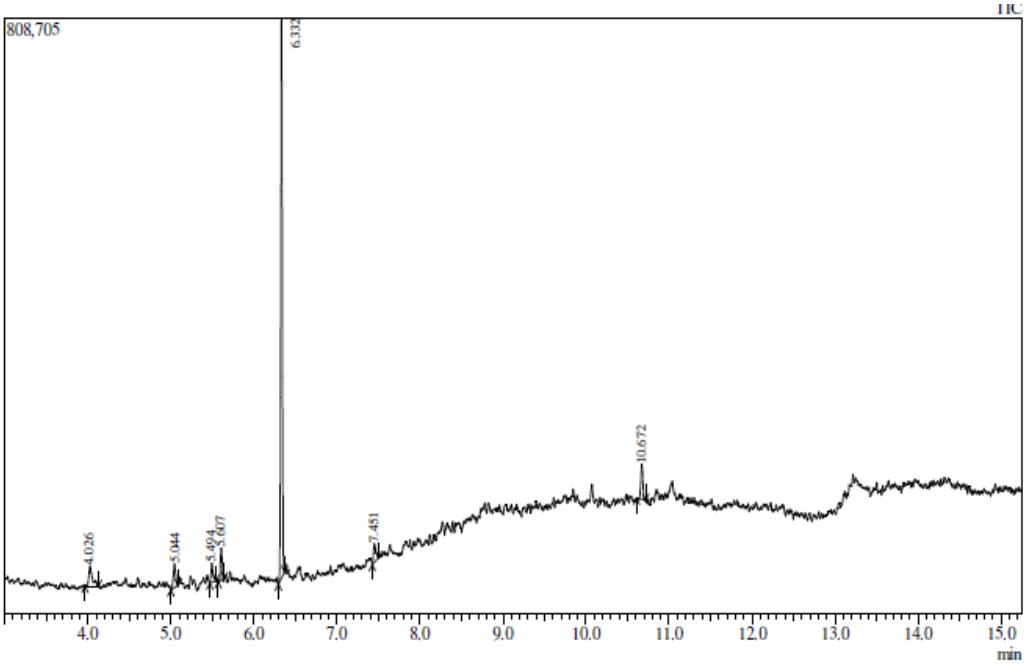


Table: 17 - GC-MS chromatogram of ST (t)

Peak No	Retention Time (RT)	% Peak Area	Peak Intensity Rank
1	4.026	5.71	2
2	5.044	4.60	4
3	5.494	3.17	7
4	5.607	4.40	5
5	**6.332**	**72.97**	1
6	7.451	3.74	6
7	10.672	5.40	3
	Total	**100**	

Fig: 8 GC-MS chromatogram of ST (g)

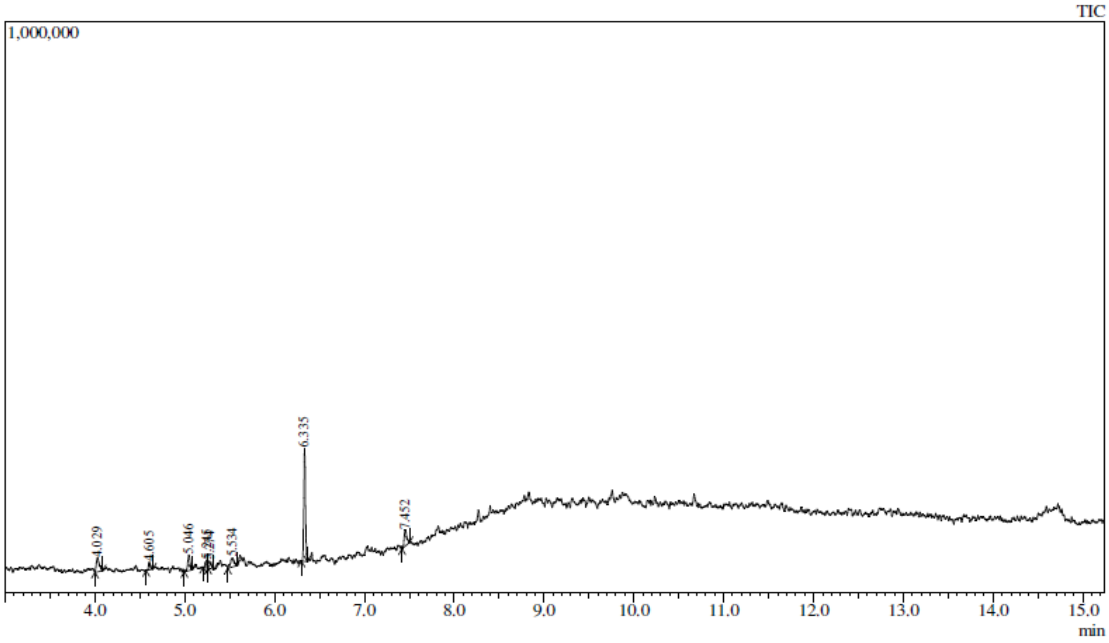
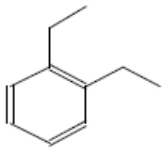
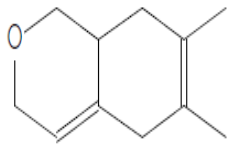
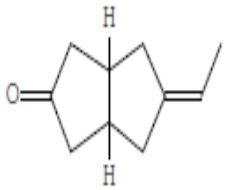
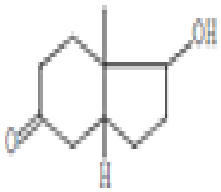
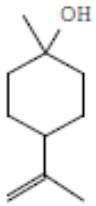
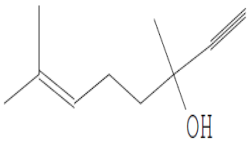
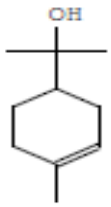
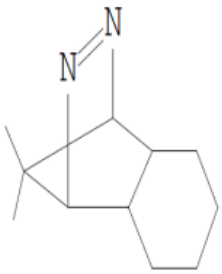
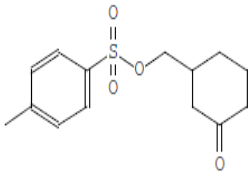
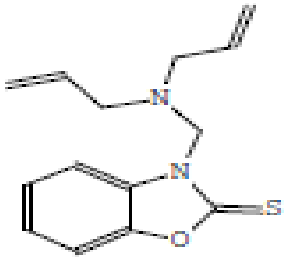
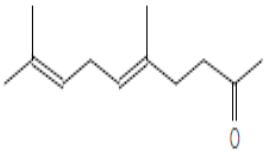


Table: 18 - GC-MS Chromatogram of ST (g)

Peak No	Retention Time (RT)	% Peak Area	Peak Intensity Rank
1	4.029	10.87	2
2	4.605	3.45	8
3	5.046	9.06	4
4	5.245	4.18	7
5	5.274	4.96	6
6	5.534	8.44	5
7	**6.335**	**48.30**	1
8	7.452	10.73	3
	Total	**100**	

Table: 19 - GC-MS screened compounds of ST (t) and ST (g)

ST (t) compound details	ST (g) compound details
1,2-Diethylbenzene Mw: 134 	6,7-Dimethyl-3,5,8,8atetrahydro-1H-2 benzopyran Mw: 224 
Bicyclo [3.3.0] octan-3-one, 7 ethylidene- Mw: 150 	Z,Z-8,10-Hexadecadien-1-ol Mw: 238 
Beta.-Terpineol Mw: 154 	Octen-1-yn-3-ol Mw: 152 

Terpineol Mw: 154  	1,4-Methanophthalazine Mw: 178 
p-toluene sulfonate Mw: 282  	Benzoxazol Mw: 260 
Z,Z-3,13-Octadecadien-1-ol Mw: 266  	5,8-Decadien-2-one Mw: 180 

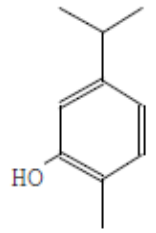
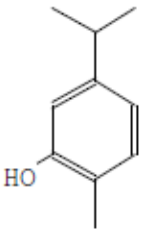
Isothymol Mw: **150**  	Isothymol Mw: **150** 

Table: 20 - Difference between ST (t) and ST (g) Distillates

A	**Traditional Parameters**	**Traditional Distillate (ST(t)**		**Glass Still Distillate (ST(g)**
a	Nature & Appearance	Moderate clarity ,good Purity		Good Clarity and purity
	1. Color	Light lemon yellow		Colorless
	2. Aroma	Pleasant Aroma		Mildly aromatic
	3. Taste	Pleasant taste, slightly pungent		No pleasant Taste, Slightly pungent
b	Yield	Less than Average		Good (>75%)
c	Effect on Long storage			
	Nature & Appearance	Clarity +, Sediments +		No change in clarity ,no sediments
	1. Color	Color Fading+		No change in color
	2. Aroma	Mildly aromatic		Aroma absent
	3. Taste	Slight pleasant taste, very less pungent		No pleasant taste, very less pungent
B	**GCMS Analysis**			
		1	Isothymol (**72.97%)**	Isothymol (**48.30%)**
		2	1,2-Diethylbenzene	6,7-Dimethyl-3,5,8,8a-tetrahydro-1H-2-benzopyran
		3	Bicyclo[3.3.0]octan-3-one, 7-ethylidene-	Z,Z-8,10-Hexadecadien-1-ol
		4	Beta.-Terpineol	1, 4 Methano phthalazine
		5	Terpineol	Octen-1-yn-3-ol
		6	p-toluene sulfonate	5,8-Decadien-2-one
		7	cis-Linoleic acid	Benzoxazol
		8	----------	Z,Z-3,13-Octadecadien-1-ol

Interpretation: There will be difference in the nature and percentage value of the original chemical compounds during the various stages of distillation. The most possible known mechanisms will be the loss or alteration during various purification procedures that is specific to the raw drug or general (including washing, excess drying or sun drying), bio degradation due to pH variation while fermentation, inter drug molecular interactions- synergist or antagonist (in compound formulations), heat interaction (due to the nature, thickness and quality of the apparatus), heat degradation (during boiling, evaporation and re-distillation). As the process up to the fermentation is similar to both the distillates, the factors of heat interaction and heat degradation can be coined to explain the differences in quality of the distillates.

Glass stills offer purity of the compounds, yield and is suited for commercial purpose. The quality of the distillate as when considering the traditional quality parameters like colour, odour, aroma traditional made distillates have priority over glass still .Traditional Stills can be tuned to higher temperatures and are way ahead in obtaining maximum extraction of organic compounds. For therapeutic purposes, distillates made from traditional stills are better one.

There is much difference in the compounds screened in both samples by GC-MS. by comparing the Extend of component extraction (peak %) and most of biologically active constituents spotted through GC-MS, distillate prepared from traditional still was far superior than distillate made in glass still.

Table: 21 - GC-MS CHROMATOGRAM ST (c)

Peak No	Retention Time	% Peak Area	Peak Intensity Rank
1	2.4	8.44	3
2	5.7	7.46	4
3	8.9	1.53	5
4	11.6	0.91	7
5	11.8	4.8	8
6	17.6	34.32	1
7	33.0	1.9	6
8	36.5	40.85	2
	TOTAL	**100**	

Fig: 9 GC-MS CHROMATOGRAM OF NIS

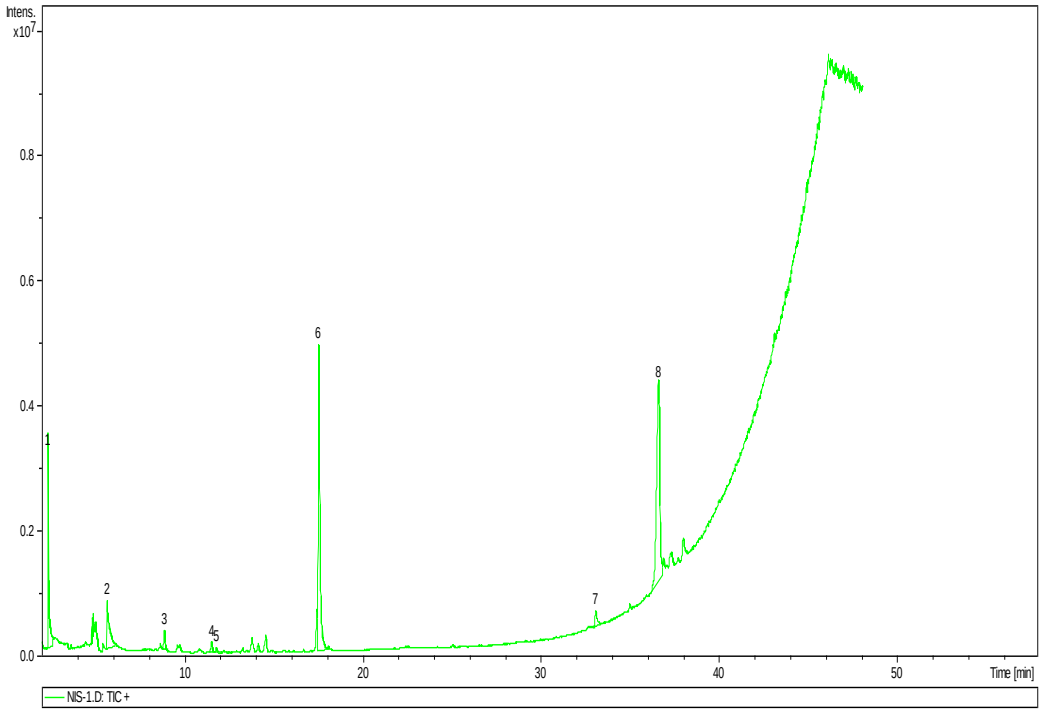


Table: 22 - GC-MS screened compounds of ST (C)

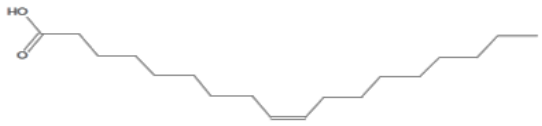
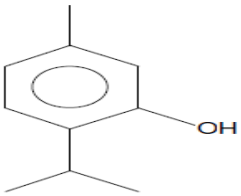
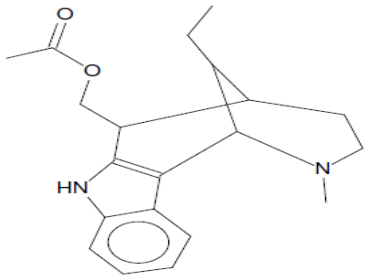
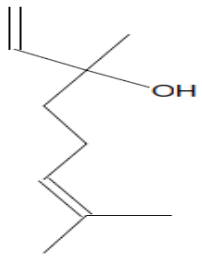
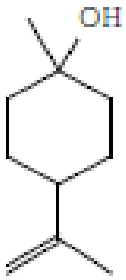
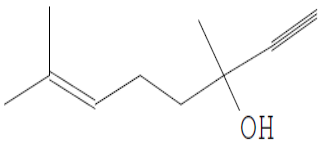
ST (t) compound details	ST (g) compound details
Oleic Acid Mw: 282 	**Thymol** Mw: 150 
Dasycarpidan-1-methanol, acetate Mw: 130 	**1,6-Octadien-3-ol, 3,7-dimethyl- (Linalool)** Mw: 154 
Beta.-Terpineol Mw: 154 	**Octen-1-yn-3-ol** Mw: 152 

Table: 22 Compounds reported from GCMS Analysis -*Sanjeevi Theeneer and its pharmacology*

S.NO	Compounds	Chemical formula	Pharmacological Importance /Uses.
1.	**Oleic Acid** [(70)]	$C_{18}H_{34}O_2$	*** Cardio vascular Effects** 1. Prevention of Ischemic heart diseases/ Cardio protective 2. Inhibition of platelet function and aggregation 3. Hypocholestremic. 4. High density lipoprotein (HDL) enhancer. (Ruiz-Gutiérrez et al. 1996). 5. Vaso dilator effects 6. Cardiac Tonic (Lahey et al. 2014). 7. Protection against cardio vascular insulin resistance 8. Anti-atherosclerotic. *** Effects on body fat** (Lim et al. (2013) 1. Increases fat oxidation in muscle cells. 2. Increases expression of genes responsible for fat oxidation. *** Effects in GIT** (de Silva et al. 2014). 1. Prevention of Ulcerative colitis *** Inhibitor of inflammatory cytokine TNF-ALPHA**(Yudkin 2007, Vassilious et al. 2009) *** Effects in Brain** 1. Reduce age related changes in brains mitochondria (Ochoa et al. 2011). *** Anti-tumor effects**

2.	**Thymol** [71, 72]	$C_{10}H_{14}O$	1. Anti-inflammatory 2. Anti-asthmatic 3. Anti-microbial 4. Neuro protective 5. Anti-fungal 6. Anti-bacterial 7. Hepato protective 8. Gastro protective 9. Cardio protective 10. Anti-hyperglycemic 11. Anti-hyperlipidemia 12. Nephro protective 13. Anti-apoptosis 14. Anti-oxidant
3.	**Dasycarpidan-1-methanol, acetate (ester)**	$C_{20}H_{26}N_2O_2$	1. Anti-oxidant 2. Anti-microbial 3. Anti-inflammatory
4.	**1,6-Octadien-3-ol, 3,7-dimethyl- (Linalool)**	$C_{10}H_{18}O$	1. Anti-inflammatory 2. Anti hyperalgesic 3. Anti nocioceptive 4. Anti bacterial 5. Anti-fungal 6. Spasmolytic 7. Anti-oxidant 8. Hypolipidemic
5.	**Benzene, 1-methyl-4-(1-methylethenyl)-**	$C_{10}H_{12}$	Commercial significance
6.	**Benzoic acid, 5-methyl-2-trimethylsilyloxy-, trimethylsilyl ester.**	$C_{14}H_{24}O_3Si_2$	Commercial significance

Interpretation: The compounds screened from the distillate samples like Thymol, Iso thymol, Linalool, Dasycarpidan all are scientifically validated for their Excellent Anti-oxidant, Hepato protective, Anti-inflammatory and Bronchodilator activities.

Scientific Reviews

- **Thymol** [73]
  - Thymol, are naturally occurring monocyclic phenolic compounds.
  - A study suggested that thymol ameliorated airway inflammation in OVA-induced mouse asthma, possibly through inhibiting NF-κB activation. These finding indicates that thymol may be used as an alternative agent for treating allergic asthma.
  - In another study,done by palabiyik thymol and Carvacrol protected against Paracetamol induced toxicity in HepG2 cells (Hepato cellular carcinoma cell lines) by increasing antioxidant activity and reducing pro-inflammatory cytokines, such as tumor necrosis factor α and interleukin 1β [72]
  - One study confirmed that thymol has strong ameliorative effect against hydrocortisone-induced oxidative stress injury in hepatic tissues.
  - Carvacrol, thymol and their mixtures were studied for its anti-oxidant property to protect cells against the damage induced by the H2O2.
  - A study of Thymol on High Fat Diet (HFD) induced obesity in murine model proves it as a good Anti-Oxidant, Hypolipidemic and Anti-obesity compound. The Study showed significant reduction in lipid peroxidation and a marked elevation of Anti-oxidants ,improved Insulin & Leptin sensitivity in HFD induced obese rats
  - Animal studies proves that Thymol has typical effect on Tracheal and ileal smooth muscles in which a dose dependent Anti-spasmodic property and increased mucosal clearance was noted.

- **Iso thymol:**
  - Iso thymol otherwise termed as Carvacrol with the molecular formula $C_{10}H_{14}O$ is an organic compound defined as the structural isomer of Thymol belonging to the class of Monoterpenes or can be viewed as a Monoterpenic phenol having vast Therapeutic potential and commercial importance.
  - It naturally occurs in so many aromatic plants mostly as a main component in the seed oil of Carum copticum. The peculiar aroma of the ajowan seeds is due to the presence of this compound.
  - It is mentioned in British Pharmacopoeia as a strong antiseptic agent.

- Carvacrol is a very potent Anti-Microbial agent that exhibits strong activity against Salmonella typhimurium.

❖ **Linoleic acid**

- (LA) is a polyunsaturated omega-6 fatty acid.
- Linoleic-acid is proven for its activities like Ant arteriosclerotic, Anticoronary, Antifibrinolytic, Antihistaminic, Anti-inflammatory, Hepatoprotective.

❖ **Terpineol**

- The Monoterpenes are natural products belonging to the chemical group of terpenes and the main constituents of essential oils. They are found in many bioactive essential oils and medicinal plants. Terpineol is a naturally occurring monoterpene alcohol
- Terpineol is antibacterial and antiviral, an immune system stimulant, a good general tonic.
- Borneol and terpineol are two Monoterpenes extracted from Wu Hu Tang, a Chinese formulation which consists of seven crude drugs that has been used for the treatment of asthma for hundreds of years. The study of their effect on isolated tracheal smooth muscle in guinea pig showed that both compounds prevented histamine-induced in vitro bronchoconstriction of guinea pig, thus indicating its Broncho dilator property.

❖ **Benzoxazole:**

- Benzoxazole is an aromatic organic compound.
- Benzoxazoles belong to the group of well-known antifungal agents with antioxidant, antiallergic, antitumoral and antiparasitic activity.

❖ **6,7-Dimethyl-3,5,8,8a-tetrahydro-1H-2-benzopyran**

Benzopyrans and their derivatives, in particular have shown several biological and pharmacological properties, such as spasmolytic, Diuretic, Antisterility, Anticancer.

The Pharmacological effect of Sanjeevi theeneer as a Hepato protective, Broncho dilator, Anti-Histamine and Anti-Oxidant drug is may be due to the presence of these Organic compounds which further be confirmed through Animal studies.

Acute oral toxicity study of*Sanjeevi Theeneer*

Table: 8 Dose finding experiment and its behavioral Signs of acute oral Toxicity

S.No	**Observation**	**Group (CONTROL)**	**Group (TEST GROUP)**
1	Body weight	Normal	Normally increased
2	Assessments of posture	Normal	Normal
3	Signs of Convulsions	Absence of sign (-)	Absence of sign (-)
4	Body tone	Normal	Normal
5	Lacrimation	Normal	Absence
6	Salivation	Normal	Normal
7	Change in skin color	No significant color change	No significant color change
8	Piloerection	Normal	Normal
9	Defecation	Normal	Normal
10	Sensitivity response	Normal	Normal
11	Locomotion	Normal	Normal
12	Muscle gripness	Normal	Normal
13	Rearing	Mild	Mild
14	Urination	Normal	Normal

Table: 9 Observational study Results

No	**Dose mg/kg**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
1	**Control**	**+**	**-**	**-**	**+**	**-**	**+**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
2	**Test group (5ml /kg)**	**+**	**-**	**-**	**+**	**-**	**+**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**

1. Alertness 2. Aggressiveness 3. Pile erection 4. Grooming 5. Gripping 6. Touch Response 7. Decreased Motor Activity 8. Tremors 9. Convulsions 10. Muscle Spasm 11. Muscle relaxant 12. Hypnosis 13.Lacrimation 14. Diarrhoea 15. Writhing 16. Respiration 17. Mortality.

   (+ Present, - Absent)

Interpretation of Acute toxicity Studies

- The acute oral toxicity potentials of ST in Wistar albino rats were studied effectively.
- From the maximum tolerable dose, 5ml/kg of *ST* .The, treated animals were observed for mortality, untoward clinical/toxic signs, and alterations in body weight gain and necropsy findings during the study.
- The treated animals survived throughout the study period and did not reveal any treatment related major abnormal clinical signs at the test dose levels.
- Morphological characters like changes in skin, eyes, fur, nose appeared normal.
- The rats did not reveal any observable signs of central nervous system.
- The overall percentage of body weight gain in rats treated with the drug every weekly was found to be normal indicating that the test animals were in a healthy condition during the days of observation period. The changes in water and food intake recorded did not show any distinct deviations.
- On necropsy, no abnormalities were observed. In conclusion, acute oral toxicity testing of screened drug did not produce any treatment-related adverse effects. This indicates that the dosages administered were below toxic level and proves the safety of the drug.

Repeated Dose 28- day oral toxic study of*Sanjeevi Theeneer*

Table: 10 Body weight of wistar albino rats group exposed to Sanjeevi *Theeneer*

DOSE	DAYS				
	0	7	14	21	28
CONTROL	220.6±33.673	221.4 ± 40.114	221.7 ± 39.661	222.6 ± 39.73	222.7 ± 41.311
MID DOSE	176.6± 10.64	176.3 ± 22.74	176.4 ± 38.12	178.1 ± 33.36	179.7 ± 23.12
HIGH DOSE	187.4± 36.74	187.6 ± 32.72	187.6 ± 32.46	187 ± 22.78	186.92 ± 26.49
P value (p)*	NS	NS	NS	NS	NS

NS- Not Significant, **(p > 0.01),*(p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

The above results showed that the body weight did not differ and remained within the normal limits. The overall percent body weight gain in rats treated with the drug was found to be normal showing a steady increase in weight indicating that the test animals were in a healthy condition during the 28 days of observation period.

Chart 1: Sub acute Toxicity – Mean value of Body **weight of Wistar albino rats group exposed to Sanjeevi *Theeneer***

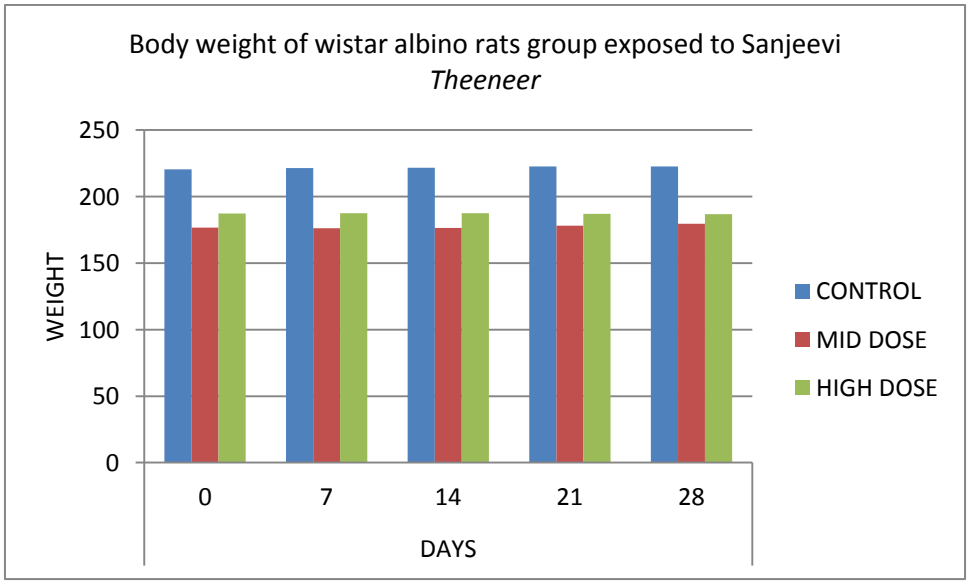


Table: 11 Water intake (ml/day) of Wistar albino rats group exposed to *Sanjeevi Theeneer*

DOSE	DAYS				
	0	7	14	21	28
CONTROL	61.5 ± 8.95	61±6.23	58.5±6.23	59±8.196	61.5±3.96
MID DOSE	55.7±4.33	56.3±2.11	57.1±2.43	58.4±2.11	58.4±2.34
HIGH DOSE	60.1±1.32	60.2±2.13	60.7±2.13	65.2±1.73	63.4±2.65
P value (p)*	NS	NS	NS	NS	NS

N.S- Not Significant, **(p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

Chart 2: Sub acute Toxicity - Water **intake (ml /day) of Control and treated groups exposed to *Sanjeevi Theeneer*.**

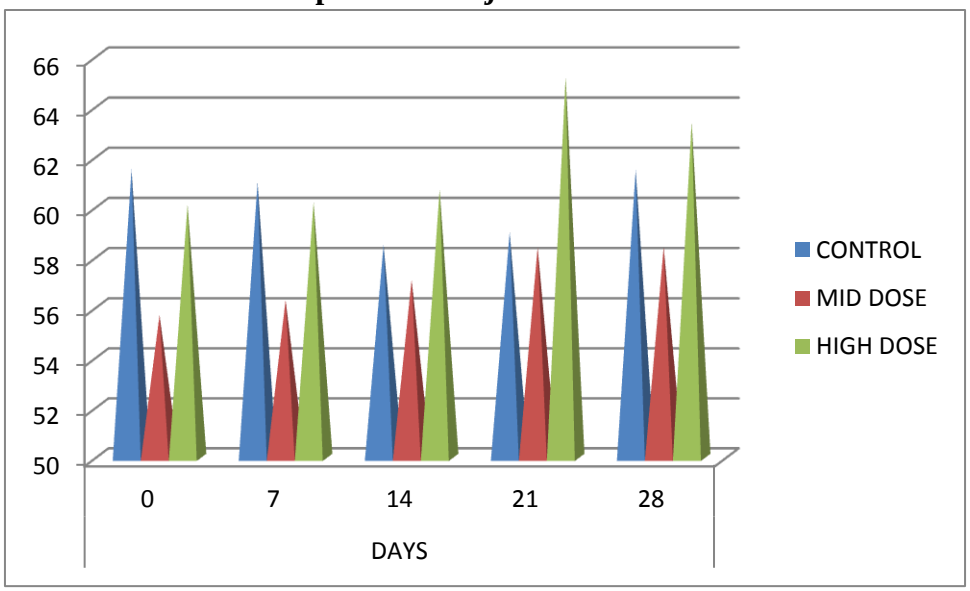


Table: 12 Food intake (gm/day) of Wistar albino rats group exposed to *Sanjeevi Theeneer*

DOSE	DAYS				
	0	7	14	21	28
CONTROL	37±5.37	38.5±3.22	39.5±3.37	38.5±3.37	37±3.12
MID DOSE	47.2±3.75	47.2±3.60	47.2±4.25	47.4±2.68	49.2±2.44
HIGH DOSE	46.2±2.34	46.2±2.64	49.6±2.66	48.2±3.20	48.0±3.62
P value (p)*	NS	NS	NS	NS	NS

N.S- Not Significant, **(p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test

Chart 3: Sub acute Toxicity - Food intake (gms /day) of Control and treated groups exposed to *Sanjeevi Theeneer*

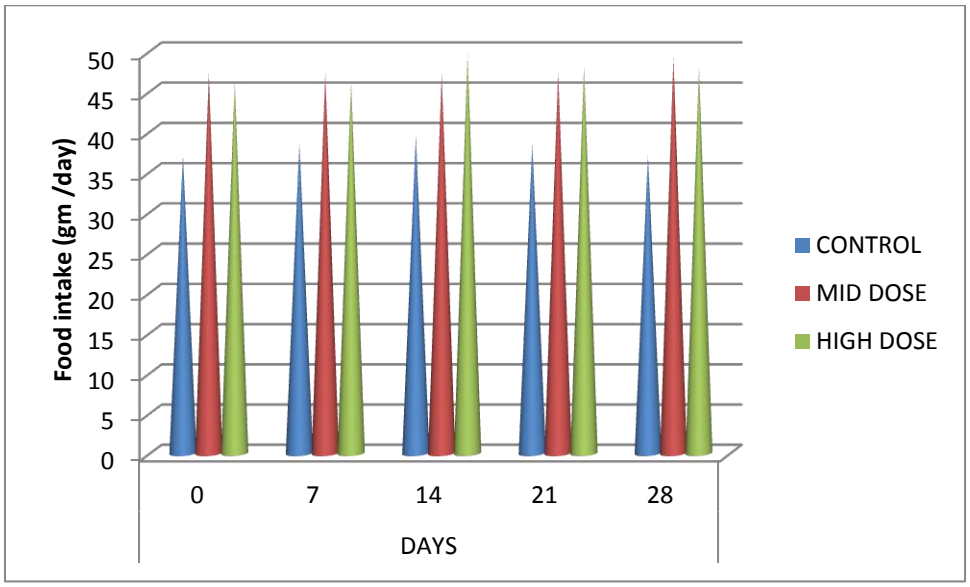


Table: 13 Hematological parameters of Wistar albino rats group exposed to *Sanjeevi Theeneer*

Category	Control	Mid dose	High dose	P value (p)*
Hemoglobin (g/dl)	13.8±0.88	14.14±0.66	13.28±0.96	N.S
Total WBC ($\times 10^3$ l)	11.91±0.59	11.48±0.91	11.20±1.17	N.S
Neutrophils (%)	33.65±0.06	35.41±1.36	35.20±2.20	N.S
Lymphocyte (%)	70.24±1.48	70.20±2.66	70.10±2.16	N.S
Monocyte (%)	0.86±0.07	0.82±0.03	0.81±0.06	N.S
Eosinophil (%)	0.54±0.09	0.56±0.06	0.57±0.04	N.S
Basophil (%)	0.14±0.04	0.20±0.07	0.19±0.06	
Platelets cells	687.17±8.76	683.18±9.0	687.16±9.74	N.S
Total RBC 10^6/µl	7.99±0.12	7.82±0.59	8.05±0.72	N.S
PCV%	37.79±0.6	43±1.68	45.82±2.54	N.S
MCHC g/dL	33.6±2.23	36.98±1.22	34.03±1.24	N.S
MCV fL(µm^3)	49.07±3.64	51.20±1.24	52.24±1.44	N.S

N.S- Not Significant, **($p > 0.01$), *($p > 0.05$), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

Chart 4:Sub acute Toxicity – The mean value of HB and T.RBC of Control and treated groups.

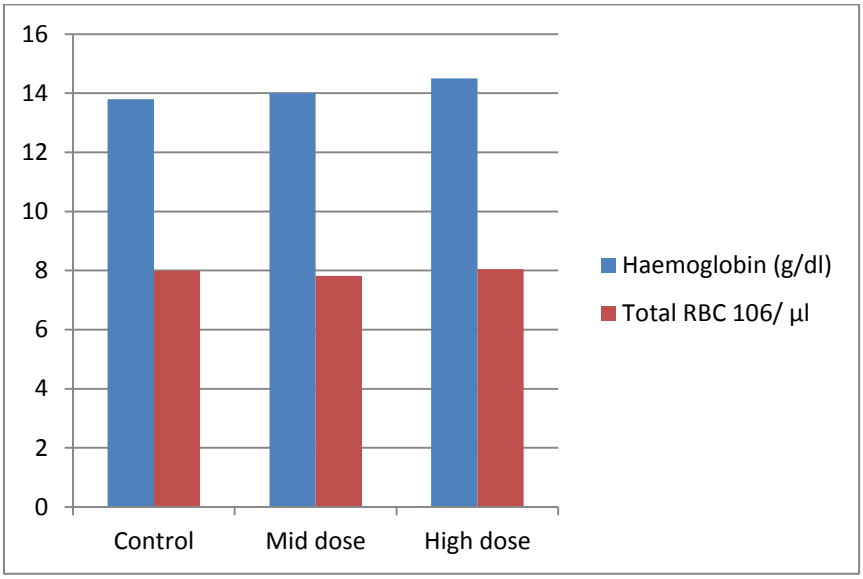


Chart 5:Sub acute Toxicity – The mean value of T.WBC of Control and treated groups.

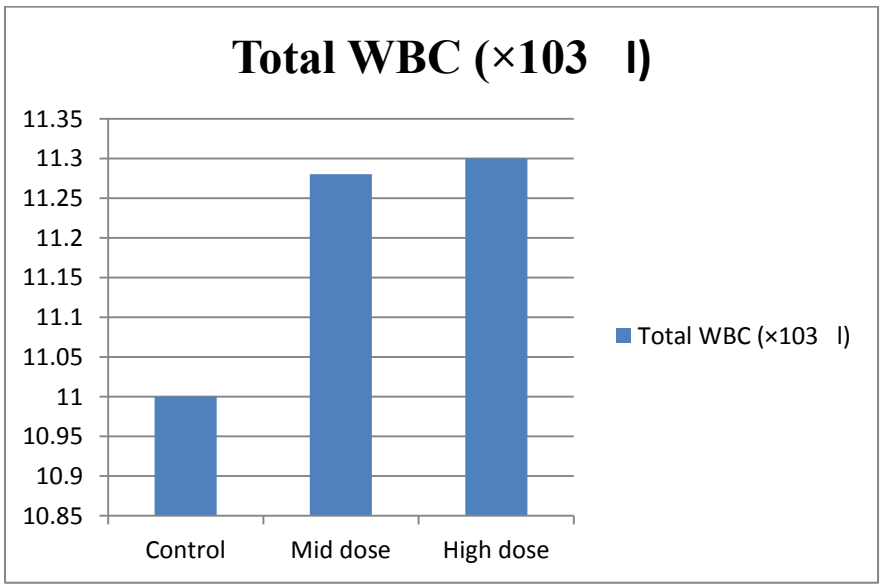


Chart 6:Sub acute Toxicity – The mean value of Neutrophils and Lymphocytes of Control and treated groups.

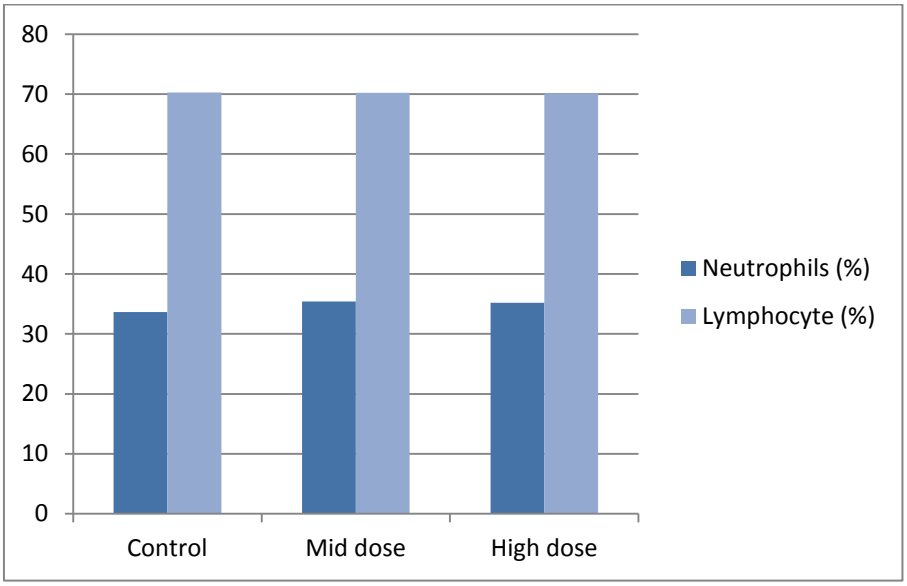


Chart 7:Sub acute Toxicity – The mean value of Monocytes, Eosinophil and Basophils of Control and treated groups.

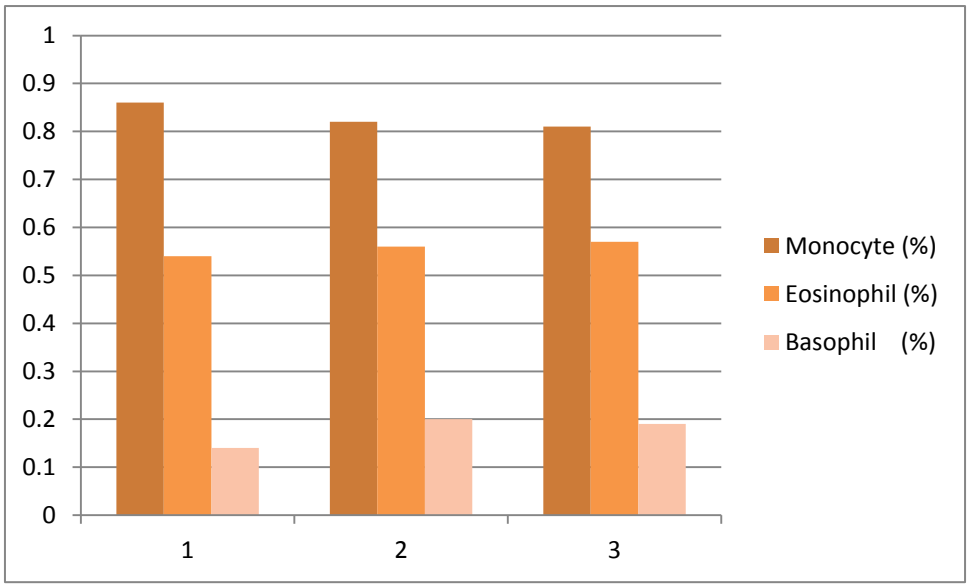


Chart 8:Sub acute Toxicity – The mean value of Platelet cells of Control and treated groups.

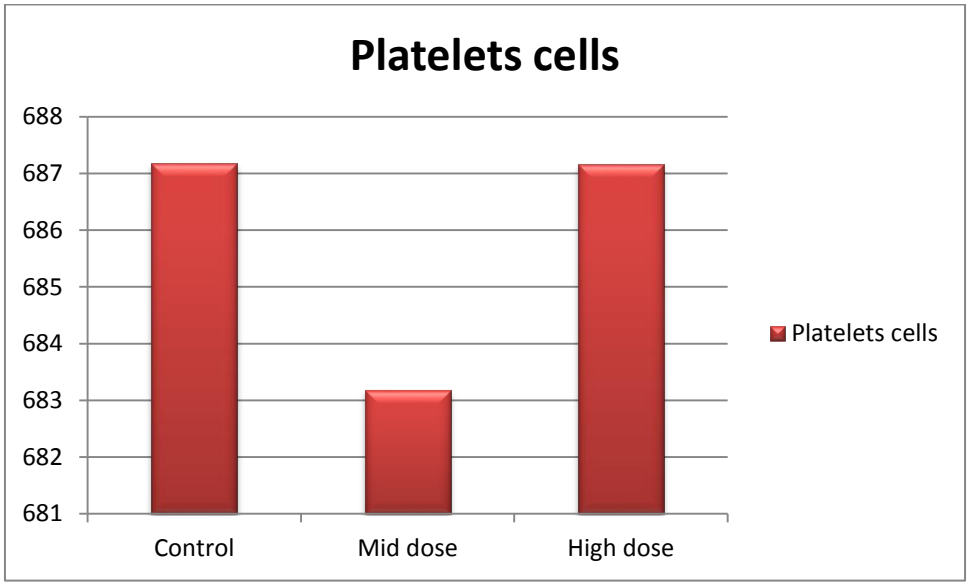


Chart 9:Sub acute Toxicity – The mean value of PCV, MCHC, MCV of Control and treated groups.

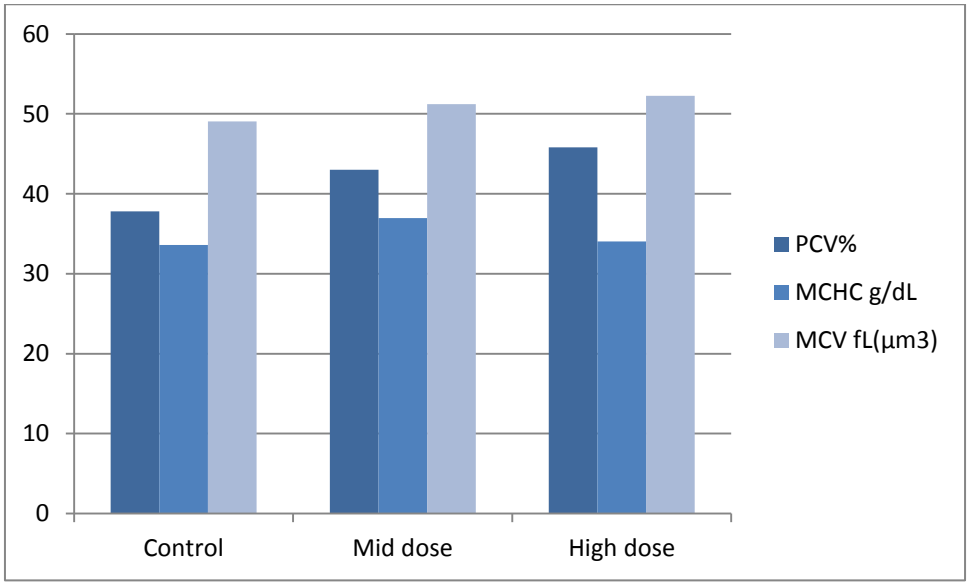


Table: 14 Biochemical Parameters of of Wistar albino rats group exposed to *Sanjeevi Theeneer*

BIOCHEMICAL PARAMETERS	CONTROL	MID DOSE	HIGH DOSE	P Value (p)*
GLUCOSE (R) (mg/dl)	74.45±13.4	75 ±11.20	76.42±11.6	N.S
T. CHOLESTEROL(mg/dl)	115.26±1.83	116.42±1.78	116.22±1.73	N.S
TRIGLY(mg/dl)	46.35±1.48	44.58±1.30	45.66±1.33*	N.S
LDL	73.8±2.43	73±2.44	73.64±24.32	NS
VLDL	15.2±2.44	15.44±6.64	15.64±34.36	NS
HDL	26.66±6.88	26.68±4.66	26.78±21.22	NS
Ratio 1(T.CHO/HDL)	4.42±2.44	4.44±8.44	4.46±22.22	NS
Ratio 2(LDL/HDL)	2.83±24.22	2.86±2.20	2.66±46.02	NS
Albumin (g/dL)	3.3±0.17	3.34±22.02	3.54±6.86	NS

NS- Not Significant, **($p > 0.01$), * ($p > 0.05$), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

Chart 10:Sub acute Toxicity – The mean value of Blood sugar of Control and treated groups.

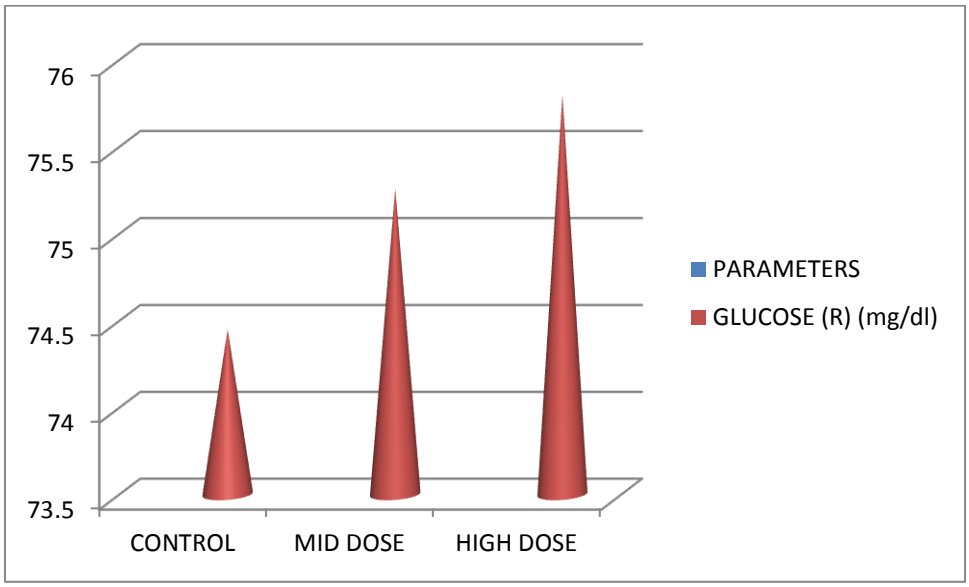


Chart 11:Sub acute Toxicity – The mean value of of Sr.TC, Sr.TG and treated groups.

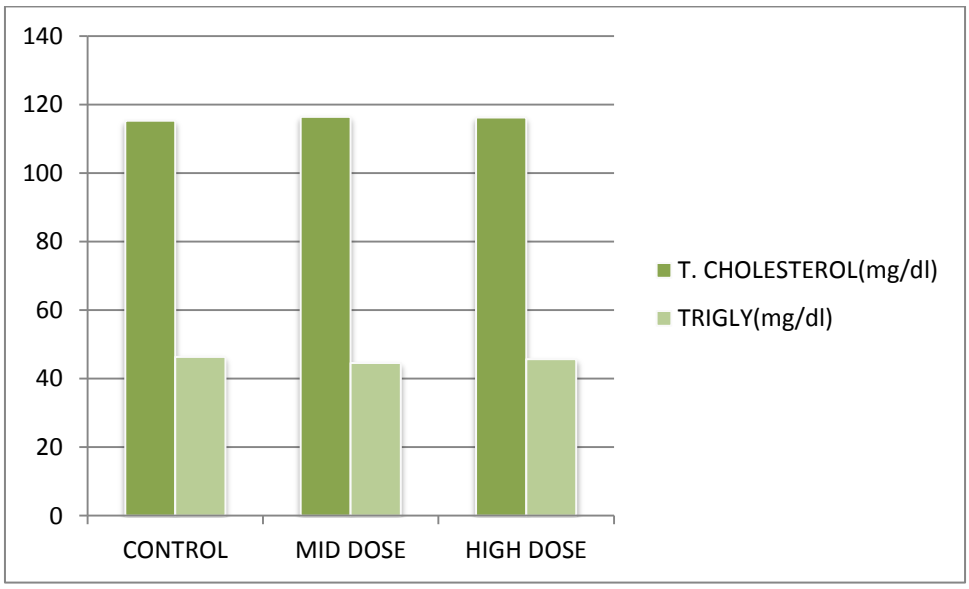


Chart 12:Sub acute Toxicity – The mean value of LDL, VLDL, HDL and treated groups.

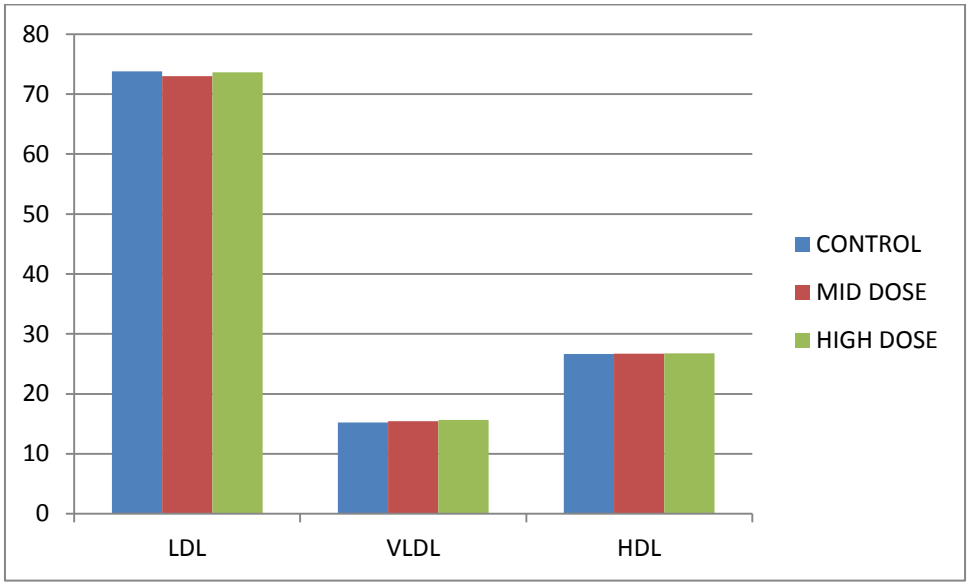


Chart 13:Sub acute Toxicity – The mean value of Serum Albumin of Control and treated groups

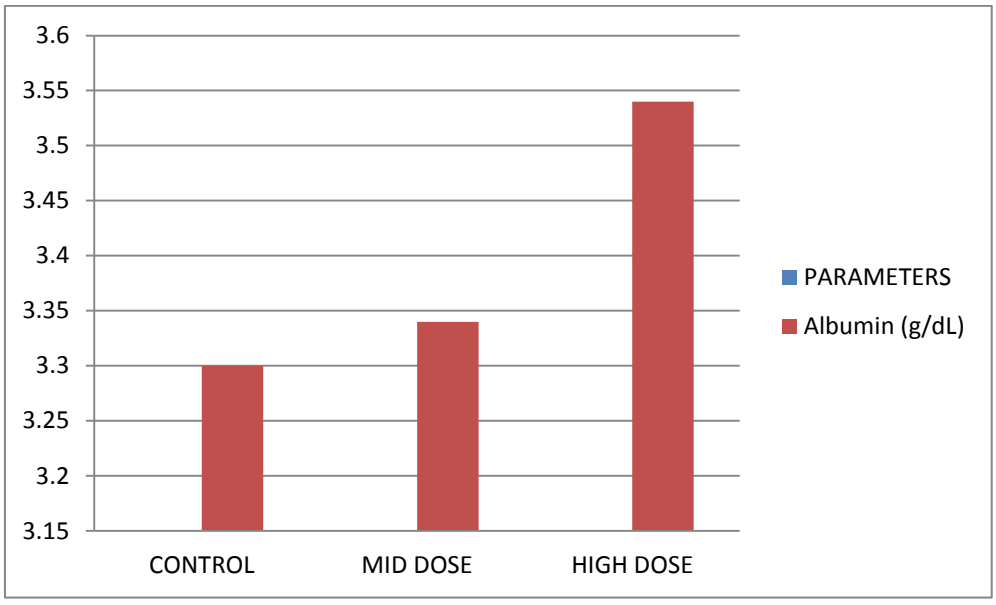


Table: 15 Sub acute Toxicity – Renal function test of of Wistar albino rats group exposed to *Sanjeevi Theeneer*

PARAMETERS	CONTROL	MID DOSE	HIGH DOSE	P Value (p)*
UREA (mg/dl)	13.35±0.99	14.06±1.38	14.48±1.42	N.S
CREATININE(mg/dl)	0.58±0.08	0.57±0.04	0.55±0.02	N.S
BUN(mg/dL)	15.12±0.10	16±0.44	16.10±2.12	NS
URIC ACID(mg/dl)	5.37±0.35	5.36±1.25*	5.35±0.23	N.S

NS- Not Significant, **(p > 0.01), * (p >0.05) , n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test

Chart 14:Sub acute Toxicity – The mean value of Urea, BUN of Control and treated groups

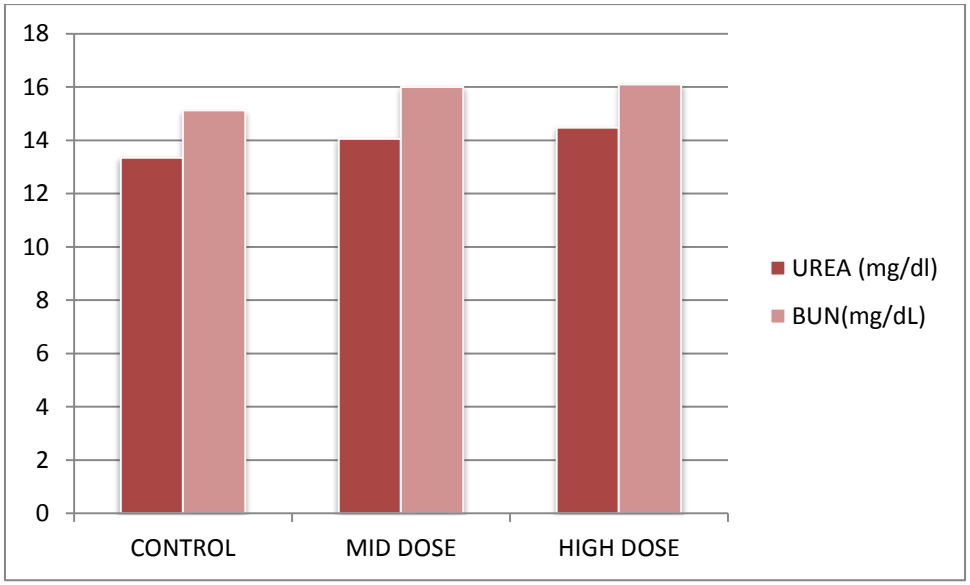


Chart 15:Sub acute Toxicity – The mean value of Creatinine of Control and treated groups

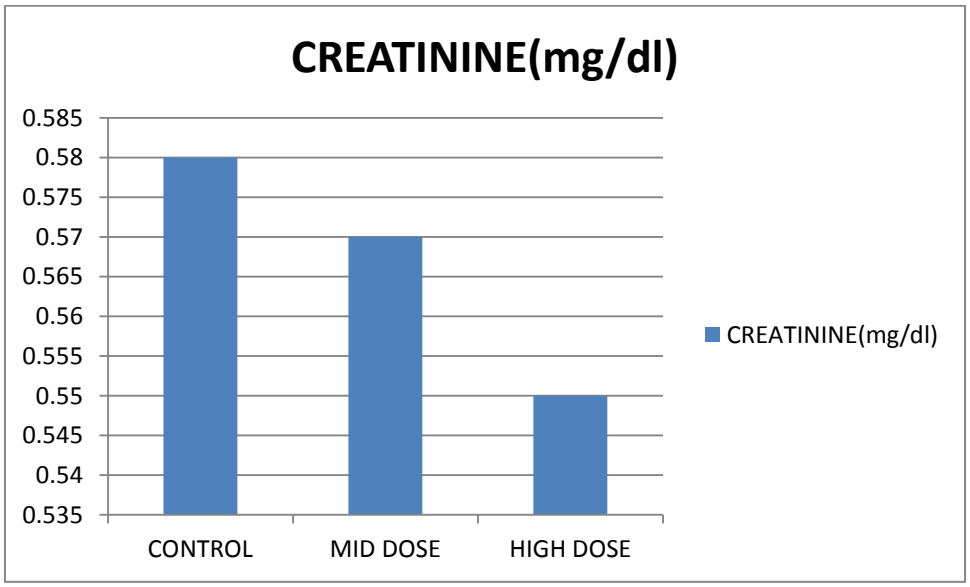


Chart 16:Sub acute Toxicity – The mean value of Creatinine of Control and treated groups

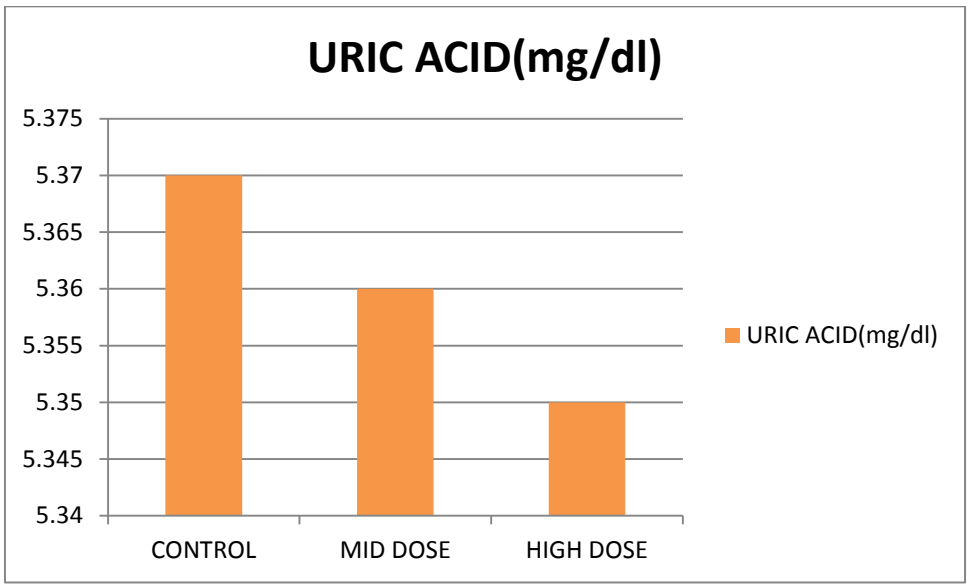


Table: 16 Liver Function Test of of Wistar albino rats group exposed to *Sanjeevi Theeneer*

PARAMETERS	CONTROL	MID DOSE	HIGH DOSE	P Value (p)*
T BILIRUBIN (mg/dl).	0.50±0.07	0.59±0.08	0.56±0.05	N.S
SGOT/AST(U/L)	114.95±1.39	117.01±1.53	116.55±1.03	N.S
SGPT/ALT(U/L)	71.23±1.28	75.34±1.48	74.32±0.68	N.S
ALP(U/L)	146.25±8.77	148.16±24.07*	149.33±14.65*	N.S
T.PROTEIN (g/dL)	6.32±0.38	7.016±0.23	6.53±0.46	N.S

NS- Not Significant, **(p > 0.01), * (p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

Chart: 17Sub acute Toxicity – The mean value of Total Bilirubin of Control and treated groups

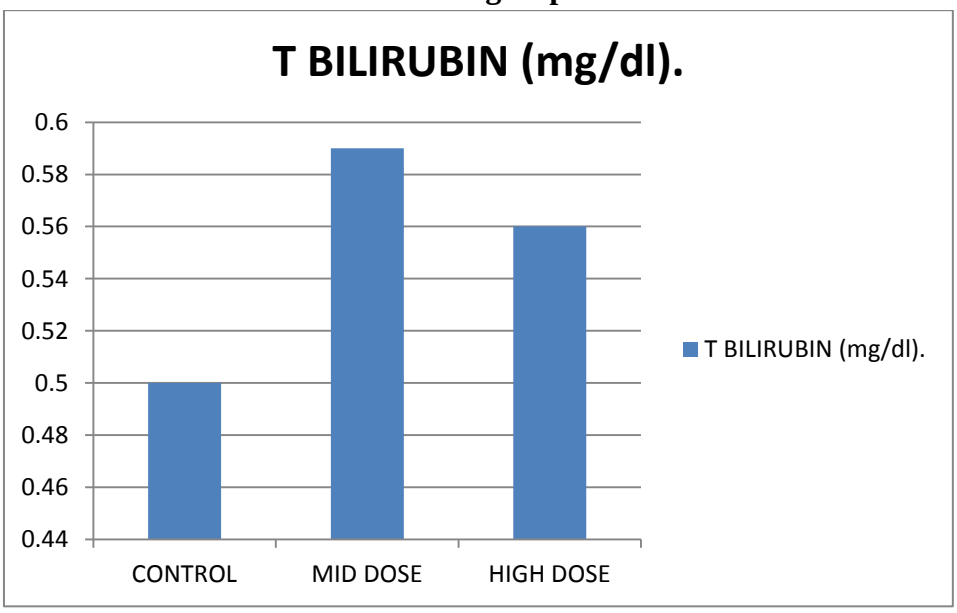


Chart: 18Sub-acute Toxicity – The mean value of SGOT, SGPT, ALP of Control and treated groups

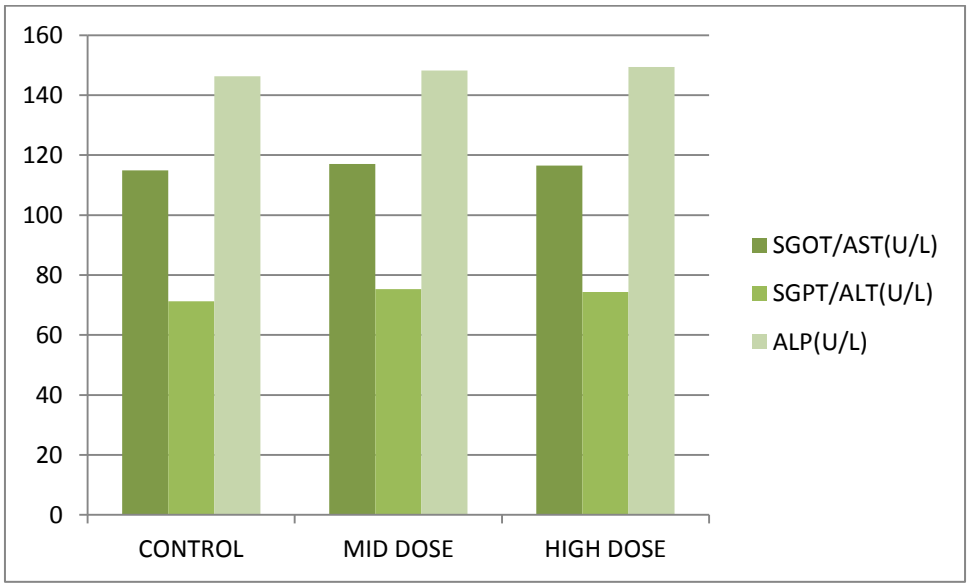
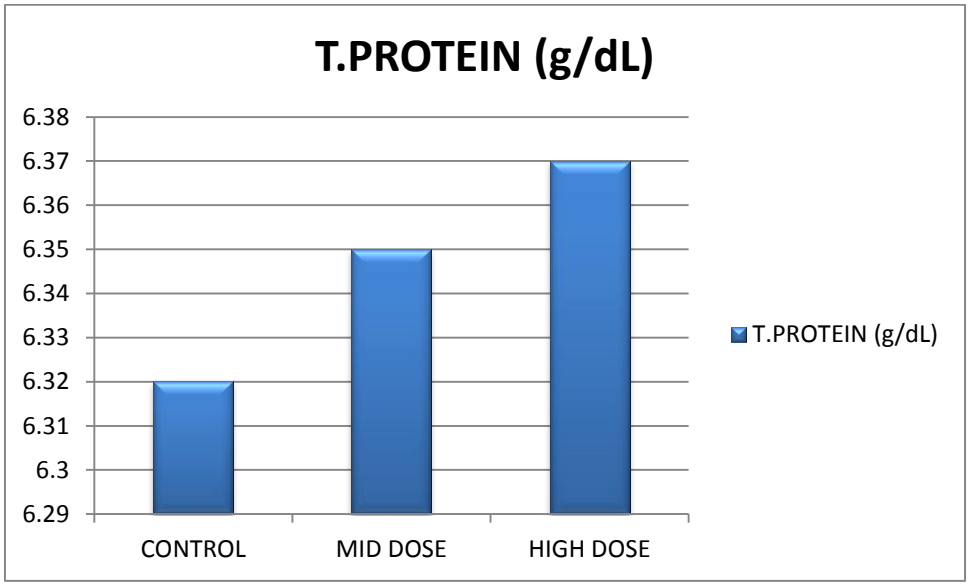


Chart: 19Sub-acute Toxicity – The mean value of T. Protein of Control and treated groups



II.b. Results of Urine parameters

Table: 17 Effect of *Sanjeevi Theeneer* on Urine parameters in rats

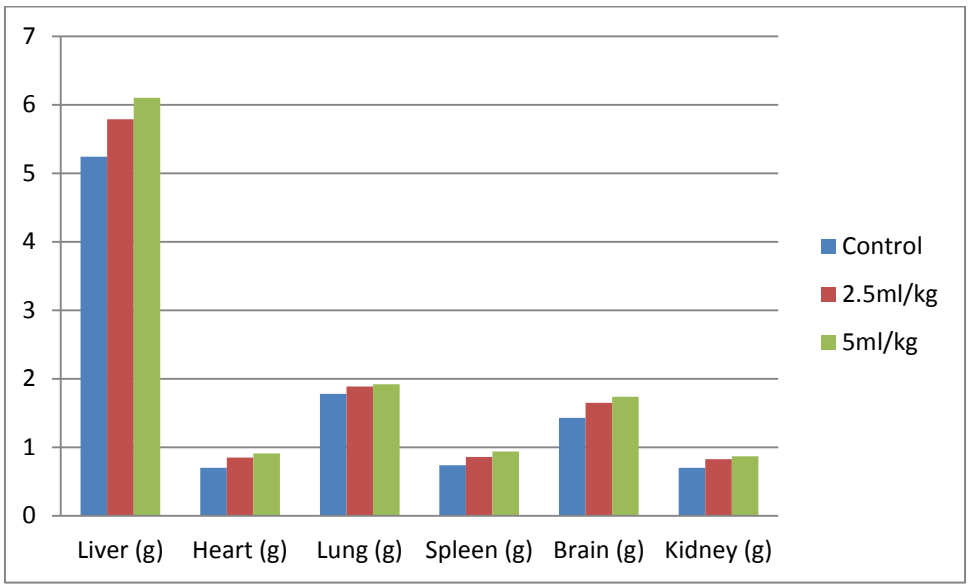
Parameters	Control	2.5ml/kg	5ml/kg
Colour	Yellow	Pale yellow	colourless
Transparency	Clear	Clear	Clear
Specific gravity	1.01	1.02	1.01
pH	7.2	7.3	7.4
Protein	Nil	Nil	Nil
Glucose	Nil	Nil	Nil
Bilirubin	-ve	-ve	-ve
Ketones	-ve	-ve	-ve
Blood	Absent	Absent	Absent
RBCs	Nil	Nil	Nil
Epithelial cells	Nil	Nil	Occasional
Casts	Nil	Nil	Nil

II.c. Results of organ weight in rats

Table: 18 Effect of *Sanjeevi Theeneer* on organ weight in rats

Organ	Control	2.5ml/kg	5ml/kg
Liver (g)	5.24±0.14	5.79±0.3	6.1±0.3
Heart (g)	0.70±0.05	0.85±0.1	0.91±0.06
Lung (g)	1.78±0.25	1.89±0.07	1.92±0.08
Spleen (g)	0.74±0.07	0.86±0.04	0.94±0.02
Brain (g)	1.43±0.18	1.65±0.14	1.74±0.06
Kidney (g)	0.70±0.05	0.83±0.05	0.87±0.02

Chart: 20 Sub-acute Toxicity –Mean value of organ weight in ratsof Control and treated groups



Interpretation of 28-day repeated dose oral toxicity study in rats

- The repeated oral toxicity potentials of *Sanjeevi Theeneer* in Wistar albino rats were studied effectively.
- In the sighting study, the test substance was administered in sequential manner at doses of 2.5ml, 5ml/kg to groups 3, 4 and water to control group respectively. The treated animals were observed for mortality, untoward clinical/toxic signs, and alterations in body weight gain and necropsy findings during the study.
- The treated animals survived throughout the study period of 28 days and did not reveal any treatment related major abnormal clinical signs at the test dose levels.
- Morphological characters of organs (skin, eyes, fur, and nose) appeared normal at the end of the study.
- Food and water intake of the rats did not differ significantly and remained within the normal limits.

RESULTS- REPEATED DOSE 90-DAY ORAL TOXICITY STUDY OF *SANJEEVI THEENEER*

Table: 19 Body weight of wistar albino rats group exposed to *Sanjeevi Theeneer*

DOSE	DAYS						
	1	15	30	45	60	75	90
CONTROL	240.6±33.673	241.4 ± 41.134	241.7 ± 42.661	244.6 ± 36.24	248.4 ± 22.211	250±44.54	256±62.22
MID DOSE	276.4± 12.34	276.3 ± 21.54	276.4 ± 32.32	278.1 ± 32.34	279.7 ± 22.32	282±48.24	286±92.26
HIGH DOSE	287± 36.74	288 ± 32.72	288± 32.46	290 ± 22.78	291.92 ± 26.49	292±36.54	293±66.34
P value (p)*	NS	NS	NS	NS	NS	NS	NS

NS- Not Significant, **(p > 0.01),*(p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test

Chart: 21 Sub Chronic Toxicity - Mean value ofBody weight of Wistar albino rats group

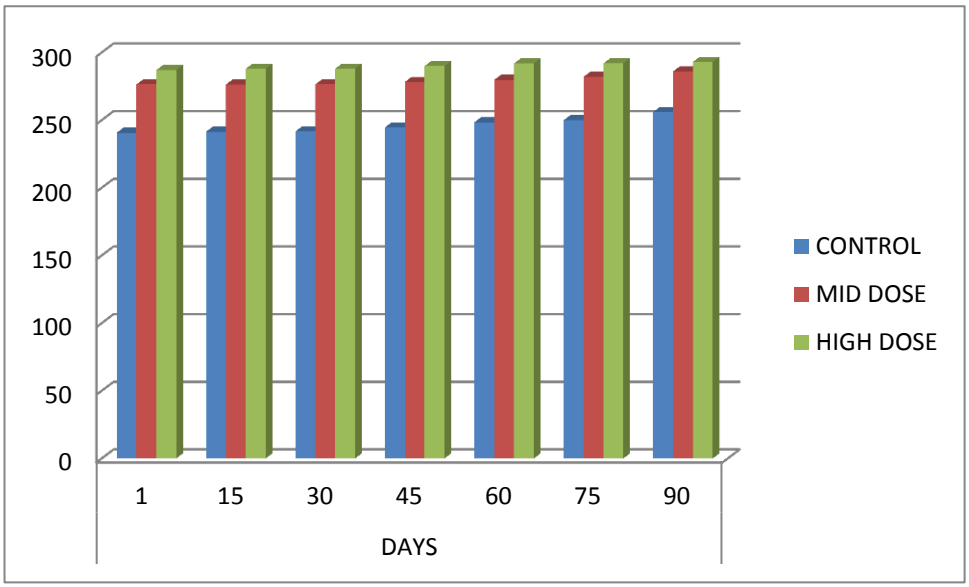


Table: 20Sub Chronic Toxicity - Water intake (ml/day) of Wistar albino rats group exposed to Sanjeevi Theeneer

DOSE	DAYS						
	1	15	30	45	60	75	90
CONTROL	80.5 ± 8.95	81±9.23	88.5±7.42	88±9.96	89.5±2.76	89.65±2.66	90±74.82
MID DOSE	85.7±4.33	86.3±2.11	87.12±3.23	88.4±2.11	89.2±1.24	89±2.21	89±1.12
HIGH DOSE	86.1±1.32	86.2±2.13	87.7±2.25	88.2±1.54	87.4±4.85	88±3.64	89±2.22
P value (p)*	NS	NS	NS	NS	NS	NS	NS

NS- Not Significant, **(p > 0.01),*(p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test.

Chart: 22 Sub Chronic Toxicity - Water intake (ml /day) of Control and treated groups exposed to *Sanjeevi Theeneer*.

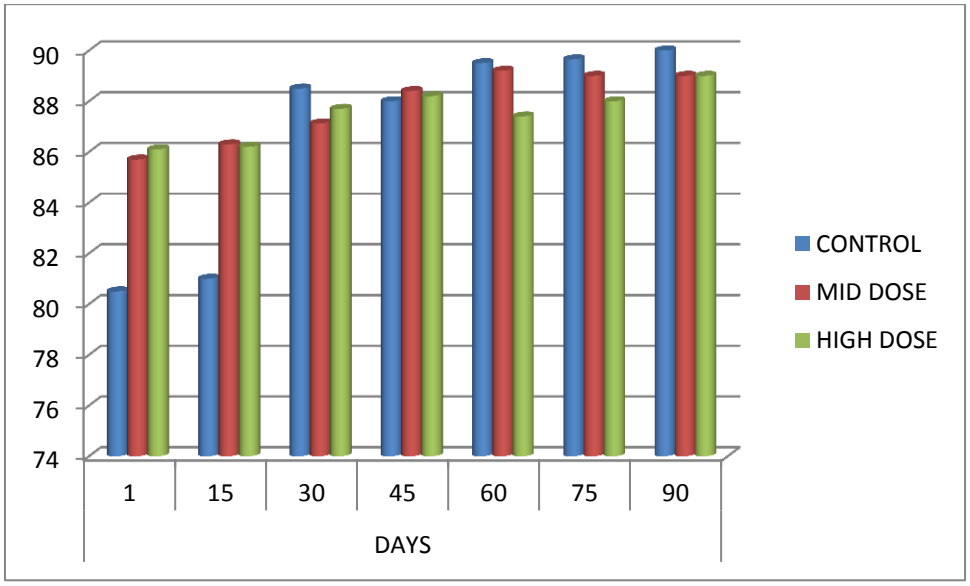


Table: 21 Food intake (gm/day) of Wistar albino rats group exposed to *Sanjeevi Theeneer*

DOSE	DAYS						
	1	15	30	45	60	75	90
CONTROL	180.5 ± 6.35	181±7.14	188.5±6.22	182±4.954	189.5±4.16	189.65±1.26	190±44.12
MID DOSE	182.7±4.33	182.3±3.21	187.12±3.23	188.4±6.12	189.2±2.22	189±2.44	194±4.22
HIGH DOSE	186.1±1.22	186.2±2.12	187.7±2.22	188.2±1.24	189.4±4.85	190±8.84	196±6.82
P value (p)*	NS	NS	NS	NS	NS	NS	NS

NS- Not Significant, **(p > 0.01),*(p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test

Chart: 23 Sub Chronic Toxicity - Food intake (gms /day) of Control and treated groups exposed to *Sanjeevi Theeneer*

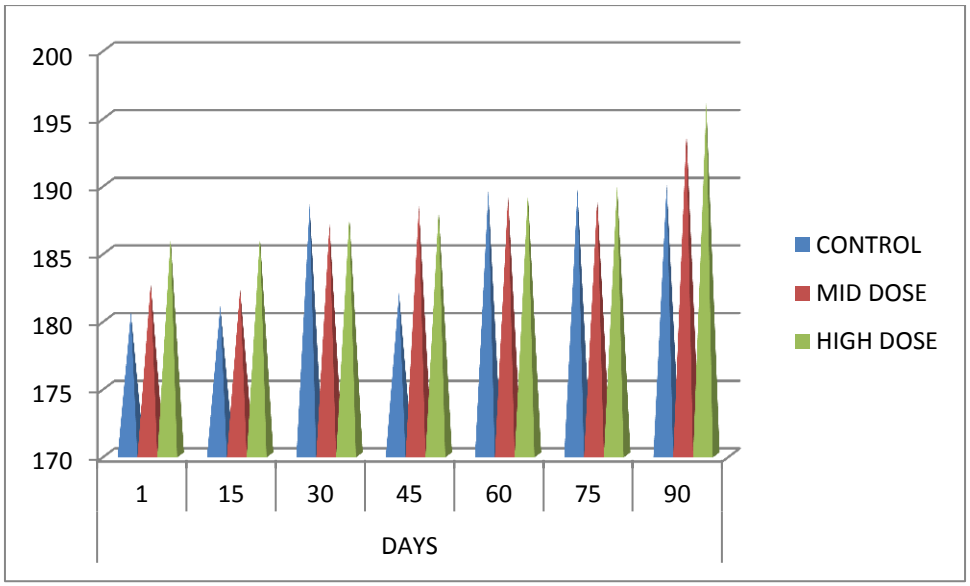


Table: 22 Haematological parameters of Wistar albino rats group exposed to *Sanjeevi Theeneer*

Category	Control	Mid dose	High dose	P value (p)*
Haemoglobin(g/dl)	16.4±0.48	16.15±0.26	16.28±0.56	N.S
Total WBC ($\times 10^3$ l)	12.91±0.25	12.24±0.31	12.60±2.14	N.S
Neutrophils(%)	32.45±0.03	34.31±1.26	34.50±4.10	N.S
lymphocyte (%)	80.14±2.18	82.30±1.46	85.18±6.16	N.S
Monocyte (%)	0.96±0.02	0.92±0.06	0.91±0.04	N.S
Eosinohil(%)	0.74±0.04	0.76±0.04	0.87±0.02	N.S
Basophil (%)	0.2±0.07	0.19±0.06	0.22±0.07	N.s
Platelets cells10^3/µl	824.16±4.46	863.12±4.0	867.06±7.64	N.S
Total RBC 10^6/µl	8.69±0.11	8.62±0.47	8.04±0.42	N.S
PCV%	36.79±0.6	53±1.34	55.61±1.14	N.S
MCHC g/dL	34.4±1.22	39.67±2.12	40.30±2.14	N.S
MCV fL(µm^3)	51.04±7.24	52.50±2.14	54.64±2.24	N.S

N.S- Not Significant, **($p > 0.01$), *($p > 0.05$), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

Chart: 24 Sub Chronic Toxicity - Mean value of HB, T.RBC of Control and treated groups exposed to *Sanjeevi Theeneer*.

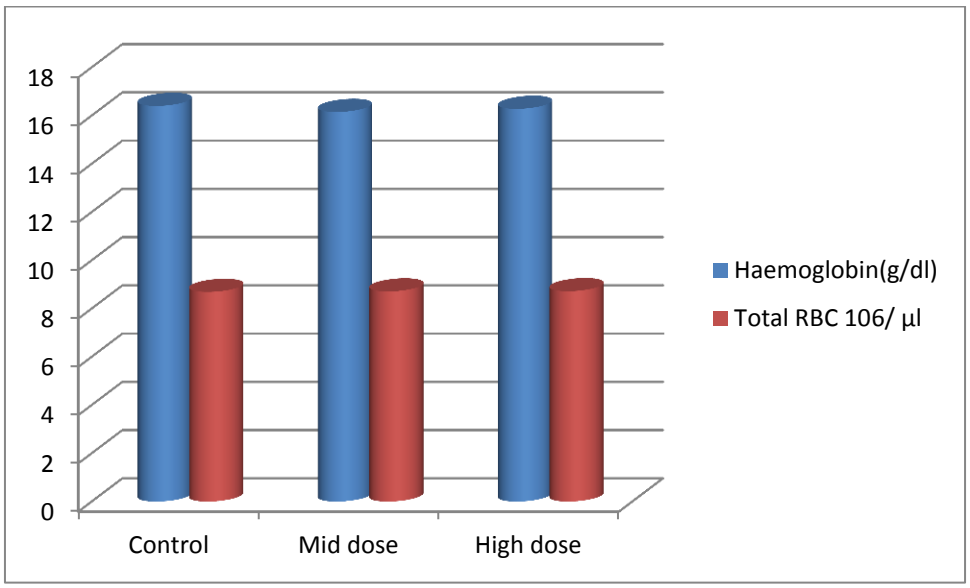


Chart: 25 Sub Chronic Toxicity - Mean value of T.WBC of Control and treated groups exposed to *Sanjeevi Theeneer*.

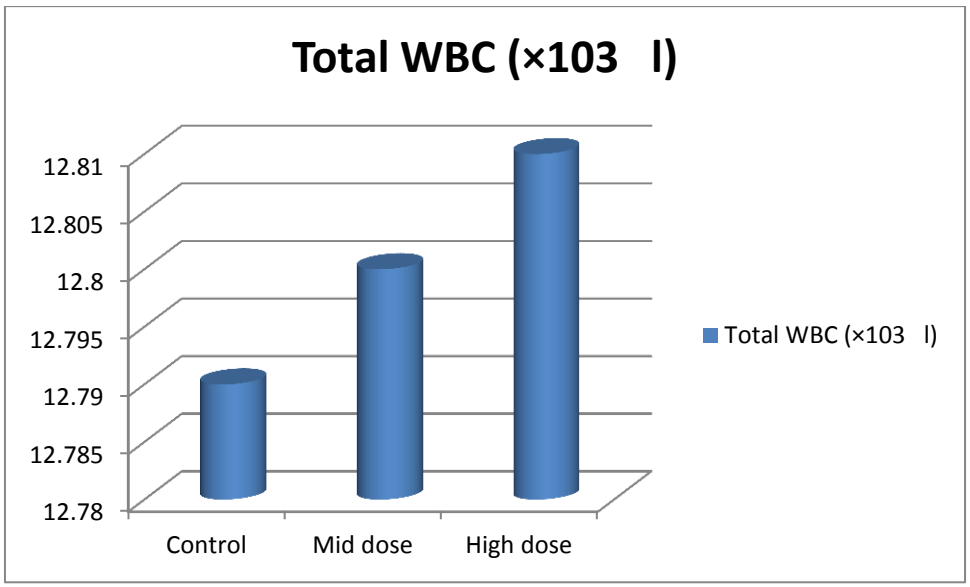


Chart: 26 Sub Chronic Toxicity - Mean value of Neutrophil, Lymphocyte of Control and treated groups exposed to *Sanjeevi Theeneer*.

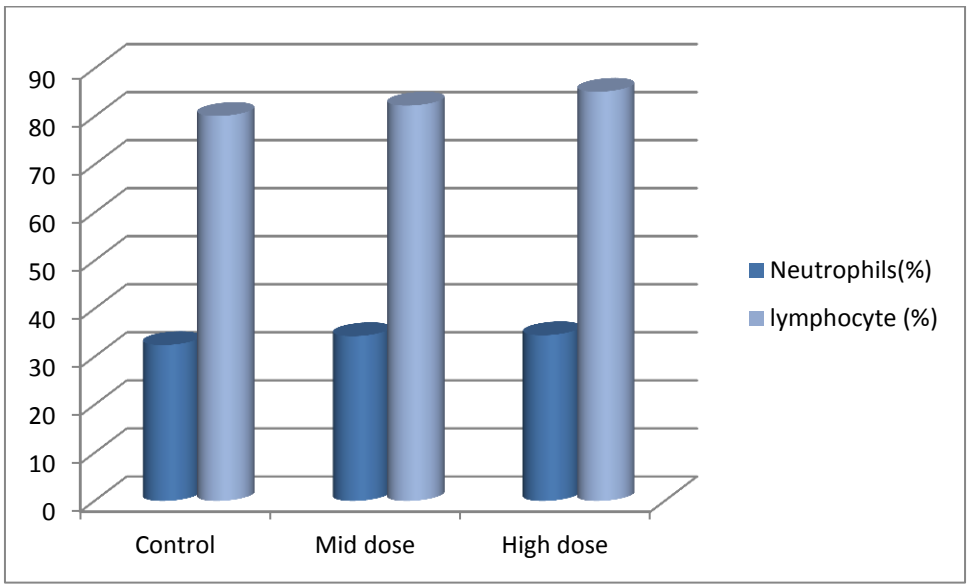


Chart: 27 Sub Chronic Toxicity - Mean value of Monocyte, Eosinophil, Basophil of Control and treated groups exposed to *Sanjeevi Theeneer*.

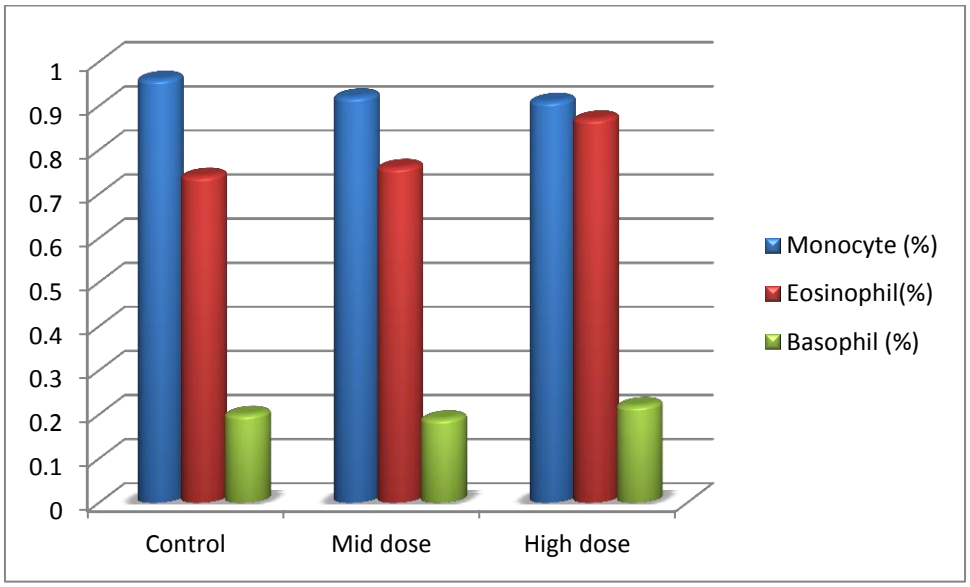


Chart: 28 Sub Chronic Toxicity - Mean value of PCV, MCHC, MCV of Control and treated groups exposed to *Sanjeevi Theeneer*.

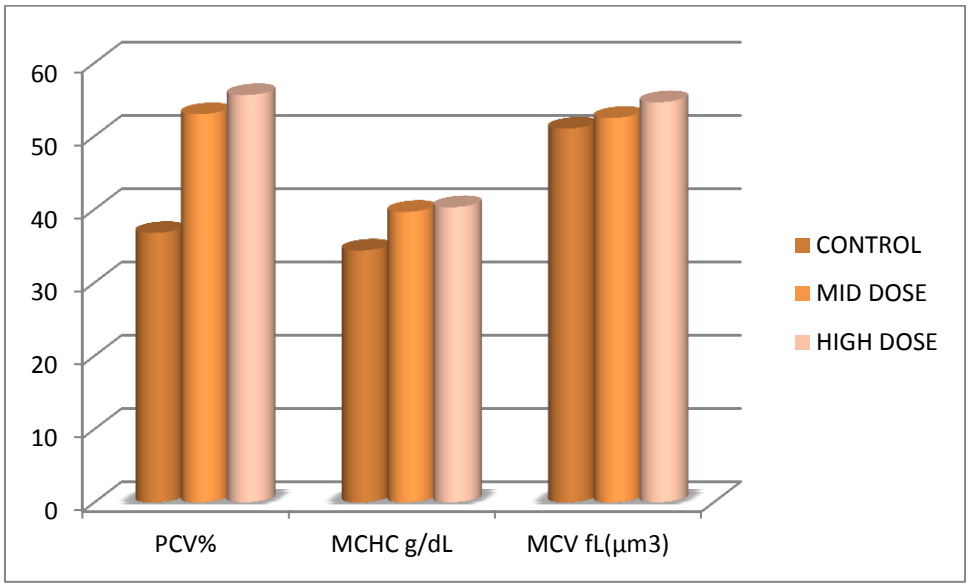


Chart: 29 Sub Chronic Toxicity - Mean value of Platelets of Control and treated groups exposed to *Sanjeevi Theeneer*.

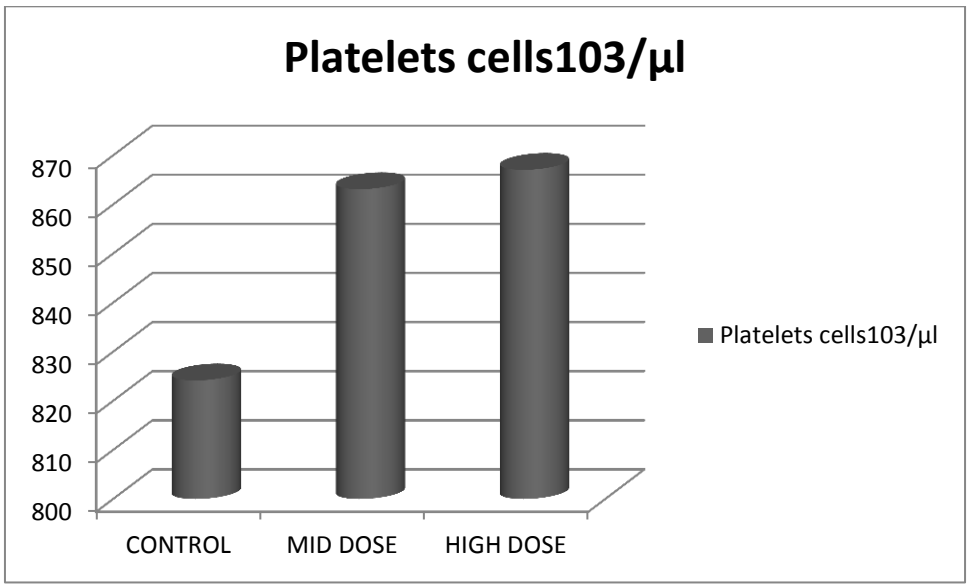


Table: 23 Biochemical Parameters of of Wistar albino rats group exposed to *Sanjeevi Theeneer*

BIOCHEMICAL PARAMETERS	CONTROL	MID DOSE	HIGH DOSE	P Value (p)*
GLUCOSE (R) (mg/dl)	73.45±13.4	74.26±11.20	76.42±11.6	N.S
T. CHOLESTEROL(mg/dl)	110.26±1.83	105.42±1.78	100.22±1.73	N.S
TRIGLY(mg/dl)	46.35±1.48	43.58±1.30	44.66±1.33*	N.S
LDL	73.8±2.43	72±2.44	73.64±24.32	NS
VLDL	14.2±2.44	15.40±6.64	15±34.36	NS
HDL	25.66±6.88	24.68±4.66	24.78±21.22	NS
Albumin (g/dL)	3.1±0.17	3.30±22.02	3.52±6.86	NS

NS- Not Significant, **(p > 0.01), * (p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test

Chart: 30 Sub Chronic Toxicity - Mean value of Blood Glucose of Control and treated groups exposed to *Sanjeevi Theeneer*.

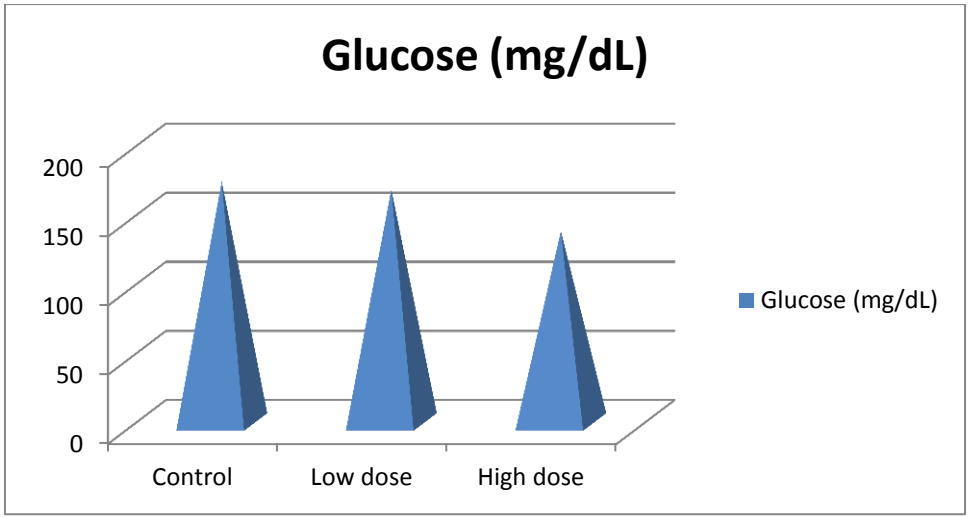


Chart: 31 Sub Chronic Toxicity - Mean value of T. Cholestrol, Triglycerides of Control and treated groups exposed to *Sanjeevi Theeneer*.

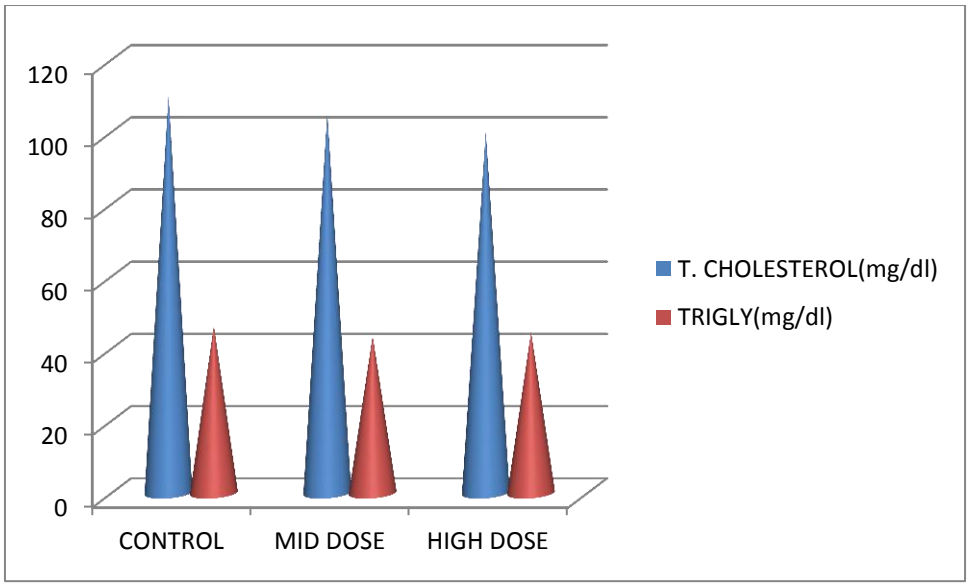
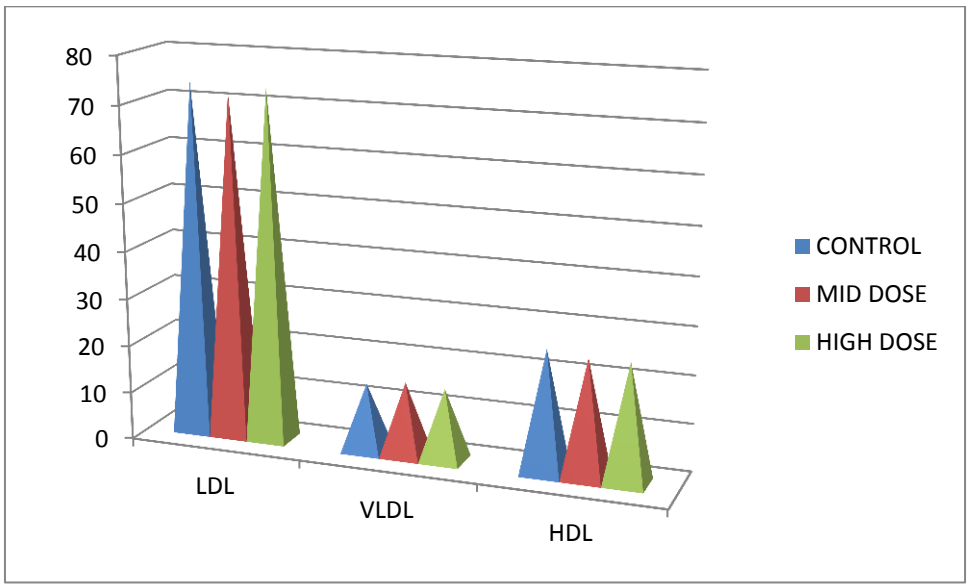


Chart: 32 Sub Chronic Toxicity - Mean value of LDL, VLDL, HDL of Control and treated groups exposed to *Sanjeevi Theeneer*.



Treatment	T BILIRUBIN (mg/dl).	SGPT/ALT (U/L)	SGOT/AST (U/L)	ALP (U/L)	T.PROTEIN (g/dL)
Control	0.55±0.07	31.24±2.37	115±1.39	150.04±04.28	6.5±0.38
Mid dose	0.55±0.08	24.22±1.19	117±1.53	140.20±01.48	6.73 ± 0.23
High dose	0.54±0.05	28.08±2.14	116.55±1.03	130.14±10.64	6.60 ±0.46

Chart: 33 Sub Chronic Toxicity - Mean value of Serum Albumin of Control and treated groups exposed to *Sanjeevi Theeneer*

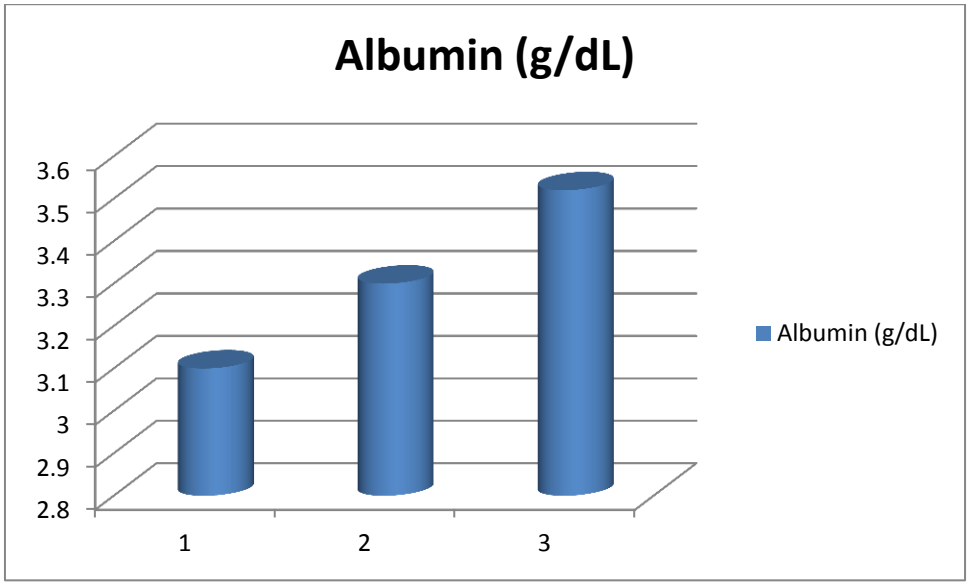


Table: 25 Liver Function Test of Wistar albino rats group exposed to *Sanjeevi Theeneer*

Chart: 34 Sub Chronic Toxicity - Mean value of T. Bilirubin of Control and treated groups exposed to *Sanjeevi Theeneer*.

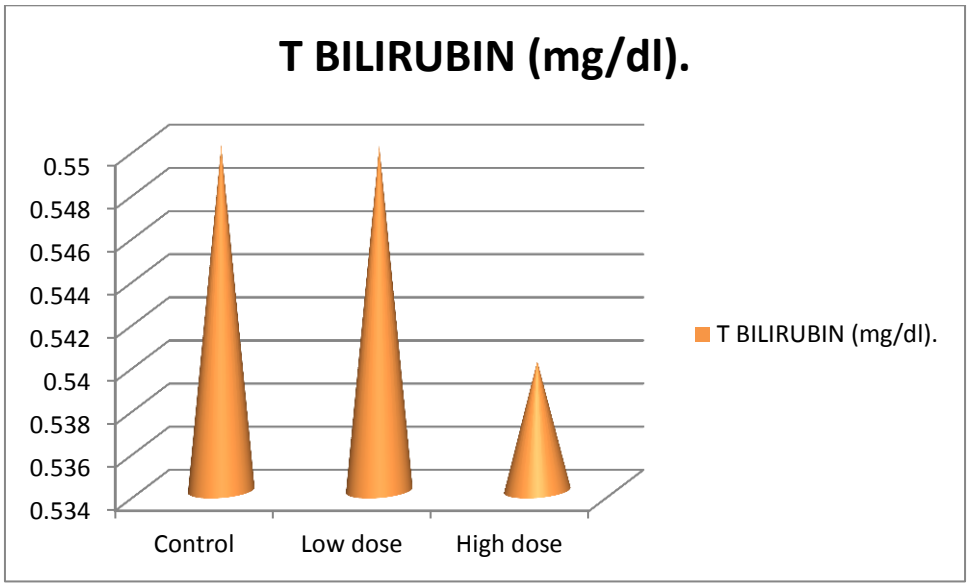


Chart: 35 Sub Chronic Toxicity - Mean value of SGPT, SGOT, ALP of Control and treated groups exposed to *Sanjeevi Theeneer*.

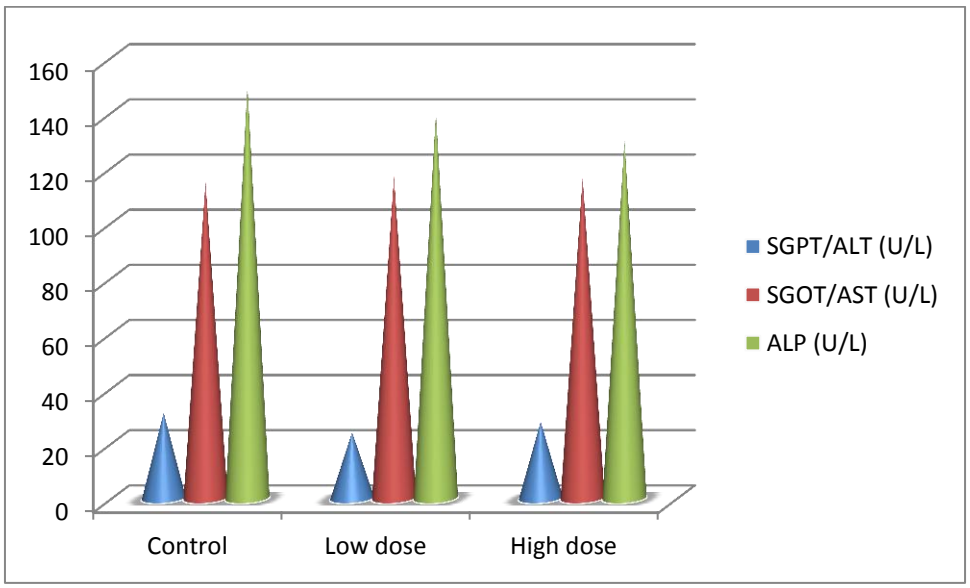


Table: 26 Renal function test of of Wistar albino rats group exposed to *Sanjeevi Theeneer*

PARAMETERS	CONTROL	MID DOSE	HIGH DOSE	P Value (p)*
UREA (mg/dl)	13.35±0.99	14.06±1.38	14.48±1.42	N.S
CREATININE(mg/dl)	0.58±0.08	0.62±0.04	0.66±0.02	N.S
BUN(mg/dL)	15.12±0.10	16±0.44	16.10±2.12	NS
URIC ACID(mg/dl)	5.37±0.35	5.7±1.25*	5.48±0.23	N.S

NS- Not Significant, **(p > 0.01), * (p >0.05) , n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

Chart: 36 Sub Chronic Toxicity - Mean value of Urea, BUN of Control and treated groups exposed to *Sanjeevi Theeneer*.

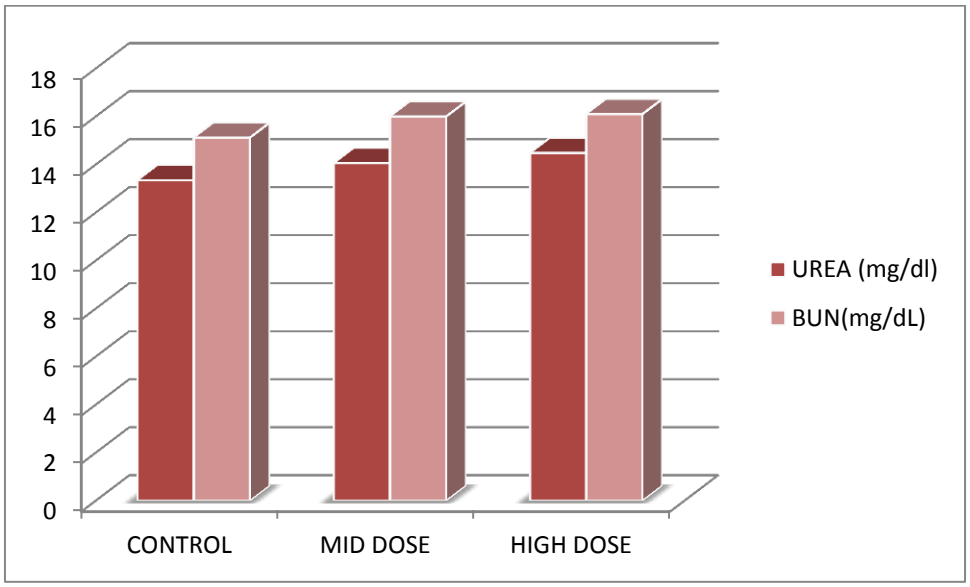


Chart: 37 Sub Chronic Toxicity - Mean value of Uric acid of Control and treated groups exposed to *Sanjeevi Theeneer*.

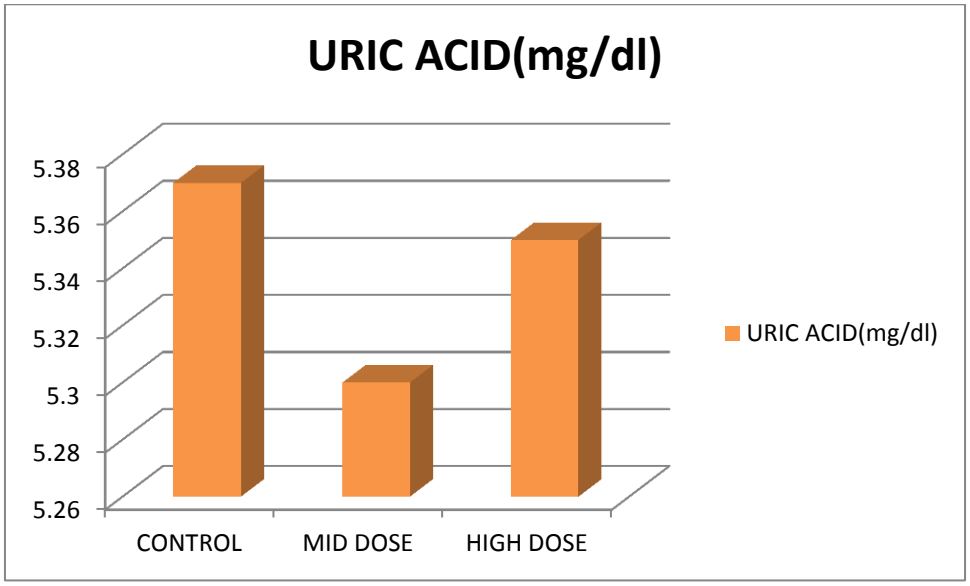
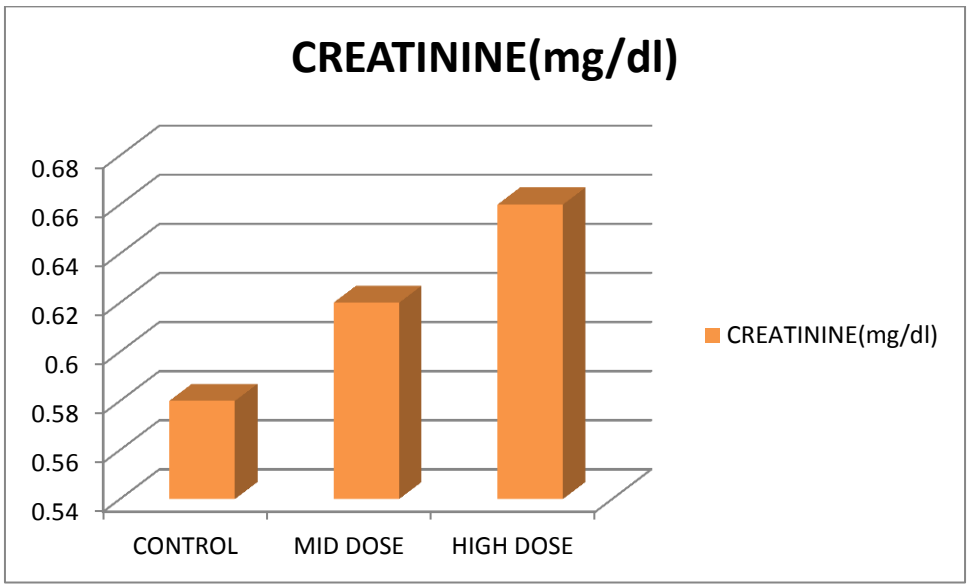


Chart: 38 Sub Chronic Toxicity - Mean value of Creatinine of Control and treated groups exposed to *Sanjeevi Theeneer*.



Interpretation of 90-day repeated dose oral toxicity study in rats

- The repeated oral toxicity potentials of *Sanjeevi Theeneer* in Wistar albino rats were studied effectively.
- In the sighting study, the test substance was administered in sequential manner at doses of 2.5ml, 5ml/kg to groups 2, 3, 4 and water to control group respectively. The treated animals were observed for mortality, untoward clinical/toxic signs, alterations in body weight gain and necropsy findings during the study.
- The treated animals survived throughout the study period of 90 days and did not reveal any treatment related major abnormal clinical signs at the test dose levels.
- Morphological characters of organs (skin, eyes, fur, and nose) appeared normal at the end of the study.
- Food and water intake of the rats did not differ significantly and remained within the normal limits.

Results - DPPH radical scavenging assay of *Sanjeevi Theeneer*

Table: 27 Percentage inhibition of test drug *Sanjeevi Theeeneer* (ST) on DPPH radical scavenging assay

Concentration (µl)	% Inhibition of Ascorbic Acid	% Inhibition of ST
10 µg/ml	23.7 ± 8.34	6.881 ± 1.79
20 µg/ml	35.93 ± 4.57	15.77 ± 2.42
40 µg/ml	45.56 ± 9.71	24.29 ± 2.49
60 µg/ml	57.41 ± 3.32	33.55 ± 6.20
80 µg/ml	68.89 ± 7.74	45.4 ± 5.04
100 µg/ml	85.19 ± 2.37	55.4 ± 3.58

Data are given as Mean ± SD (n=3)

Chart: 39 - Percentage inhibition of test drug *Sanjeevi Theeeneer* (ST) on DPPH radical scavenging assay

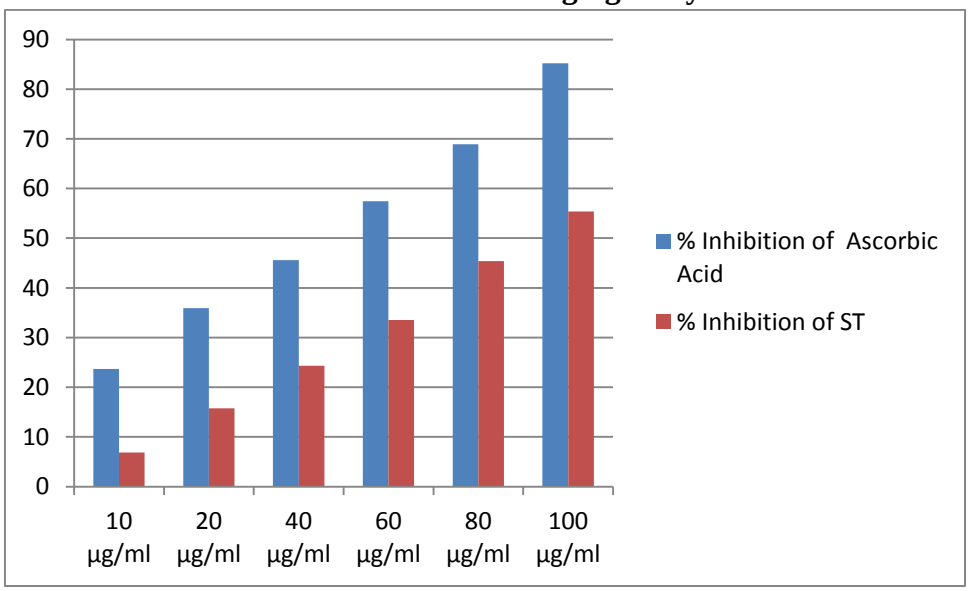


Table: 28 IC50 Values for DPPH radical scavenging Assay by Sanjeevi Theeneer (ST) and Standard.

Test Drug / Standard	IC50 Value DPPH Assay ± SD (µg /ml)
ASCORBIC ACID	46.91 ± 9.93
ST	90.19 ± 8.57

Data are given as Mean ± SD (n=3)

Interpretation: *Sanjeevi Theeneer* was studied for its Anti-oxidant potential on DPPH radical scavenging assay by comparing with standard Ascorbic acid. Sanjeevi Theeneer was able to reduce the stable DPPH radical to 50 % reduction with IC_{50} of 90.19 ± 8.57)as compared with Ascorbic acid (46.91± 9.93).

Chart: 40 - IC50 Values for DPPH radical scavenging Assay by Sanjeevi Theeneer (ST) and Standard.

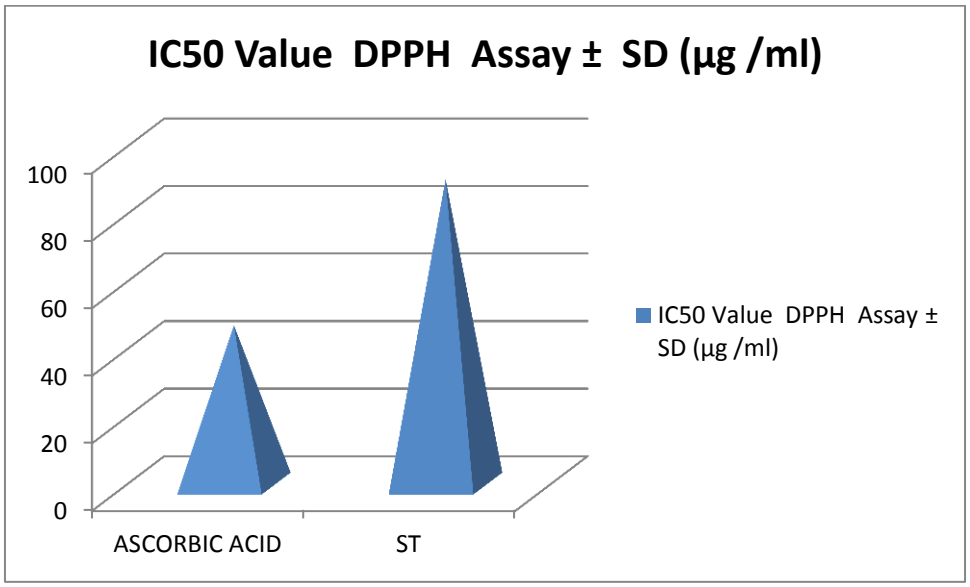
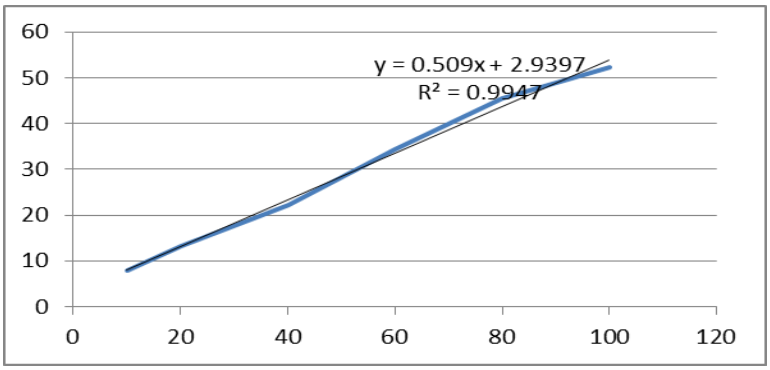
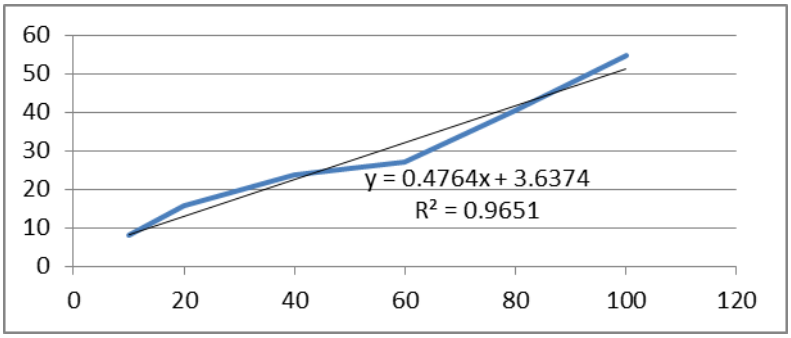


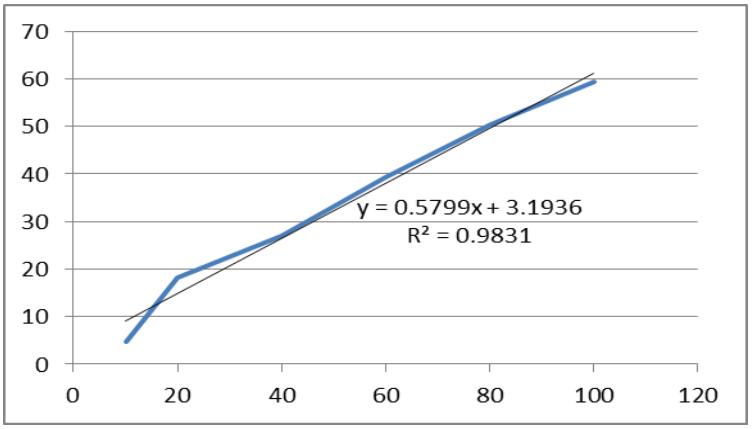
Chart: 41 Percentage inhibition of ST on DPPH radical scavenging assay



Triplicate 1

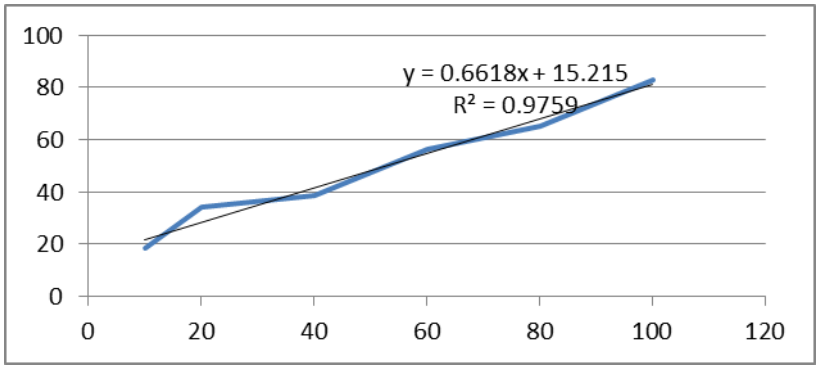


Triplicate 2



Triplicate 3

Chart: 42 Percentage inhibition of Standardon DPPH radical scavenging assay



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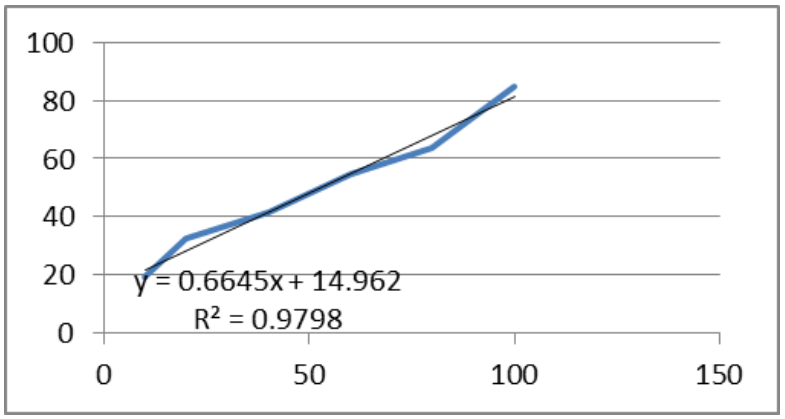

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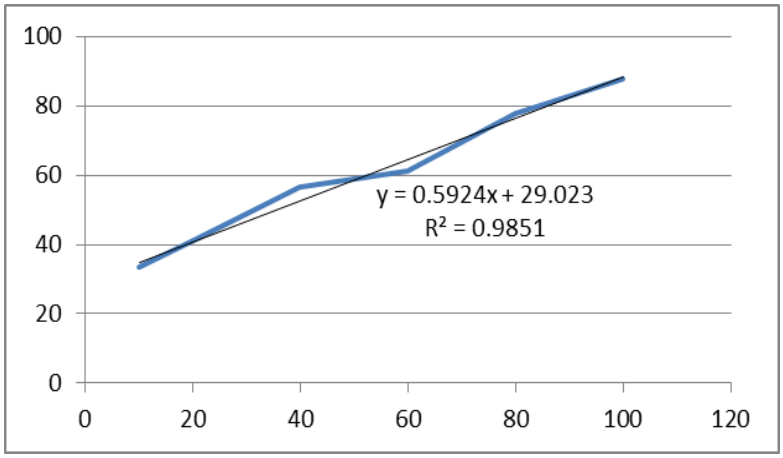
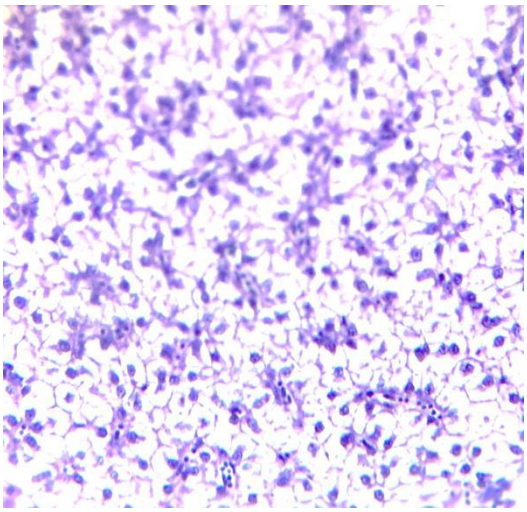


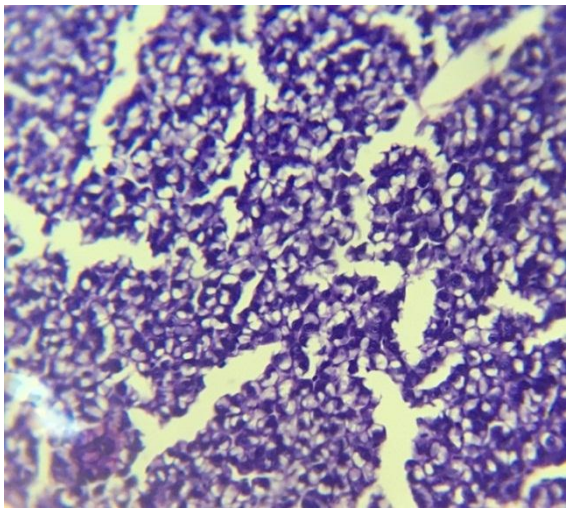
Triplicate 3

RESULTS - **Histo Pathology of liver section of zebra fish Danio rerio**

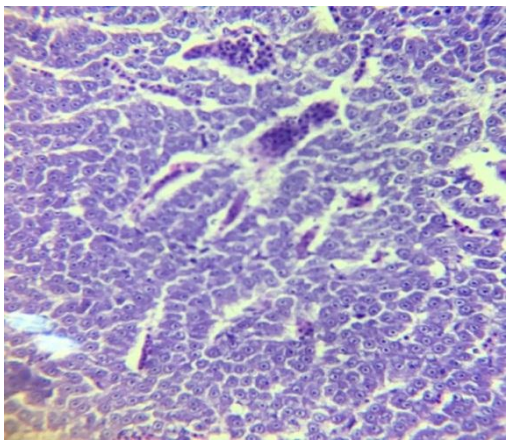
Control – Group I



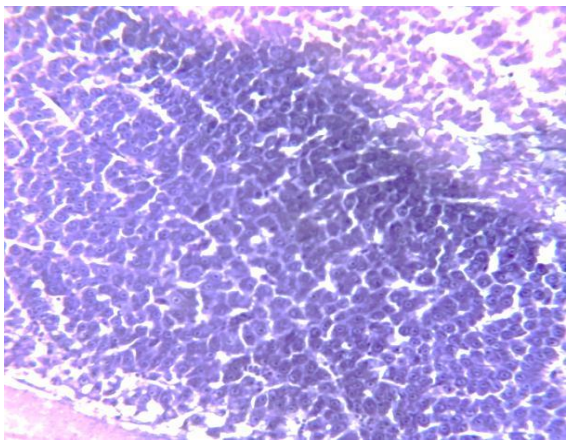
Negative Control – Group II



ST- Low Dose – Group III



ST-High Dose – Group IV



Interpretation of Histopathology study:

- Histopathology liver samples belongs to control groups reveals the presence of perfect polygonal shape of hepatic parenchyma cells with prominent nucleus further sinusoids appears with regular interval.
- Photomicrograph of liver section belongs to group II (Disease control) reveals the presence of frequent cytoplasmic vacuolation and with abnormally dilated sinusoids.
- Sample belongs to group III retains the basic structure polygonal shaped hepatic parenchyma with occasional indication of karyorrhexis.
- Appearance of liver parenchyma was almost normal with prominent large nucleus with mild inflammatory changes were observed in sample belongs to Group IV.

- The results of the present investigation indicates that paracetamol treated groups shows sever liver degeneration whereas treatment with test drug ST at both the dose level significantly attenuated the paracetamol induced damage in group III and IV. Hence from the study it was concluded that the drug ST has possess promising hepato protective activity in dose dependent manners and restores the basic liver architecture by means of its rejuvenating potential against paracetamol induced toxicity in zebra fish model.

RESULTS – HEPATO PROTECTIVE STUDIES OF SANJEEVI THEENEER

Table: 29 Haematological parameters of Wistar albino rats group exposed to *Sanjeevi Theeneer*

Parametres	Control	Toxicant Control	ST 0.5ml/kg.+ Paracetamol	ST 1ml/kg+ Paracetamol	Sillymarin 25 mg/kg, + Paracetamol
Haemoglobin(g/ dl)	16.2 ±0.48	9.20±0.26	12.15±0.26	15.28±0.56	14.7±0.56
Total WBC (×10^3 l)	7.91±0.25	9.22±0.73	8.9 ±0.31	8.30±2.14	7.50 ± 0.30
Platelets cells10^3/µl	160.16±4.4	90.42±8.16	110.12±4.0	121.06±7.64	130 ± 4
Total RBC 10^6/µl	8.69±0.11	6.3 ±0.57	7.2±0.47	7.7±0.42	7.3±0.42
PCV%	43.79±0.5	27.24±1.12	33 ±1.34	37.61±1.14	35±1
MCHC g/dL	34.4±1.22	45.06±0.69	41.67±2.12	40.30±2.14	36.67±1
MCV fL(µm^3)	51.04±7.24	37.10±2.23	40.50±2.14	46.64±2.24	44.5±2.24

Chart: 43 Hepato Protectivity - Mean value of HB in Control and treated groups.

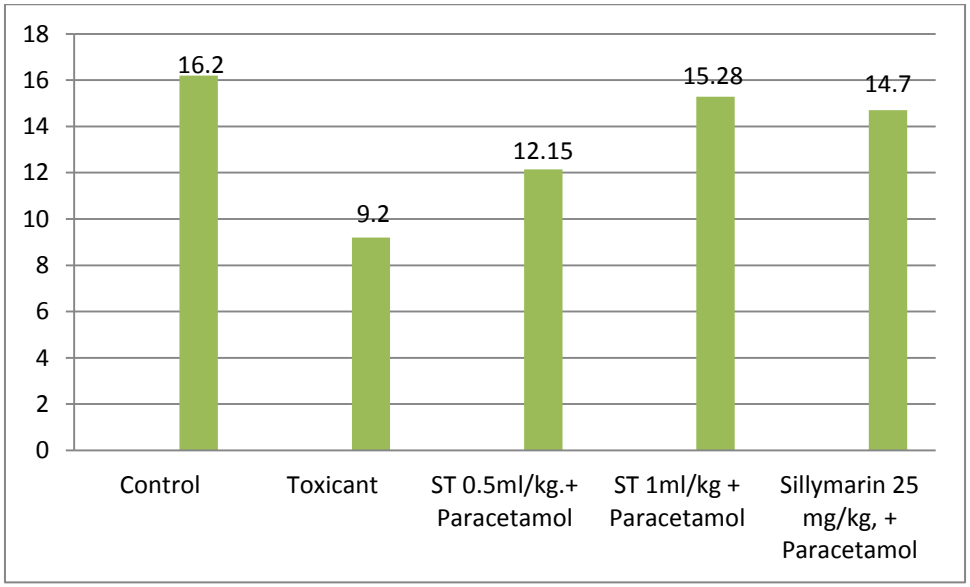


Chart: 44 Hepato Protectivity - Mean value of WBCin Control and treated groups.

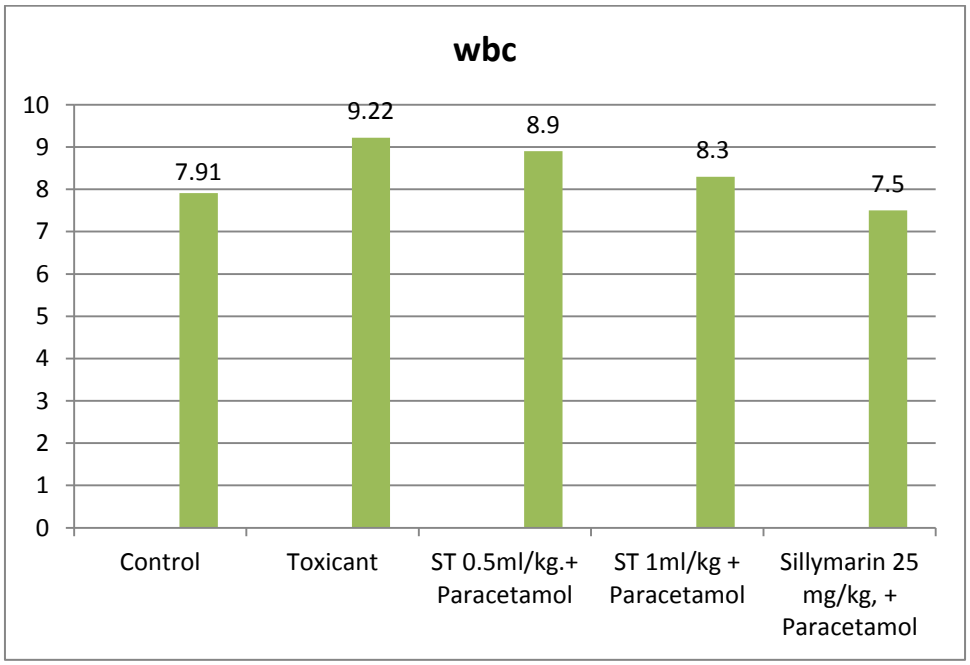


Chart:45 Hepato Protectivity - Mean value of Plateletin Control and treated groups.

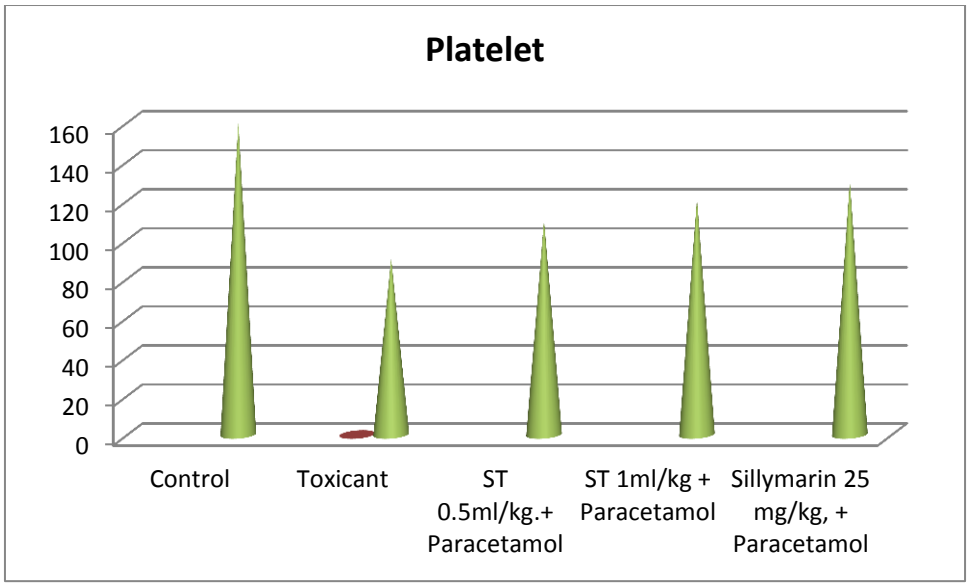


Chart: 46 Hepato Protectivity - Mean value of T. RBCin Control and treated groups.

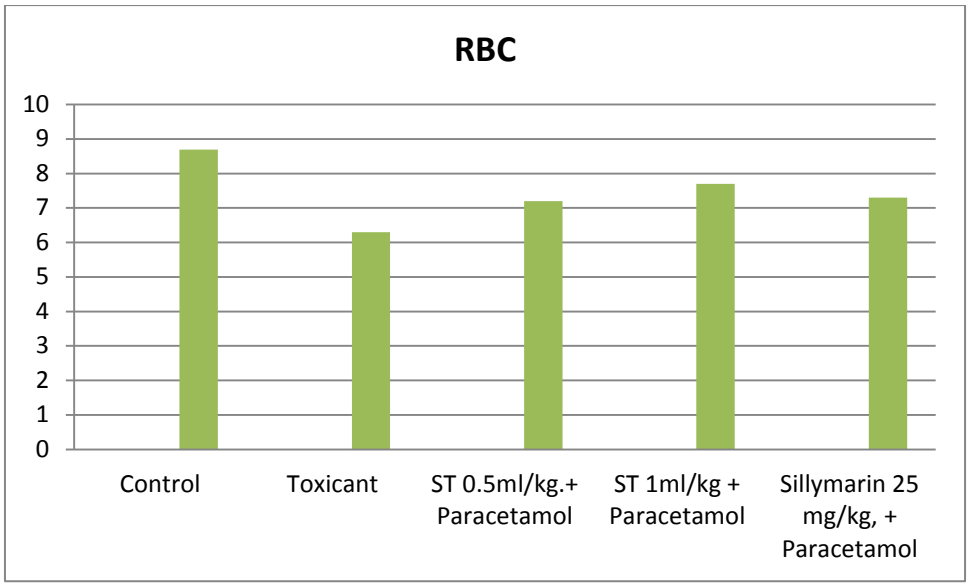


Chart: 47 Hepato Protectivity - Mean value of PCV%in Control and treated groups

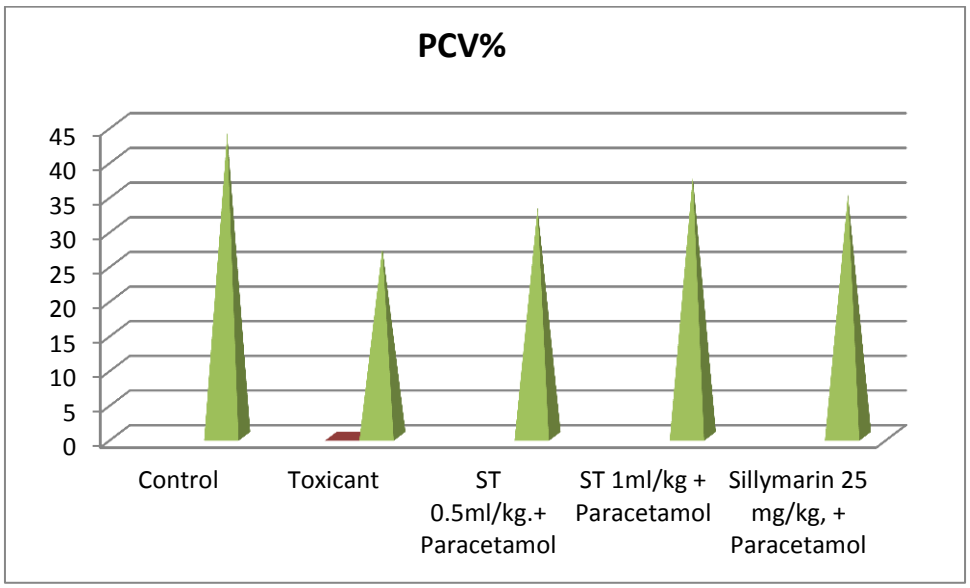


Chart: 48 Hepato Protectivity - Mean value of MCHCin Control and treated groups

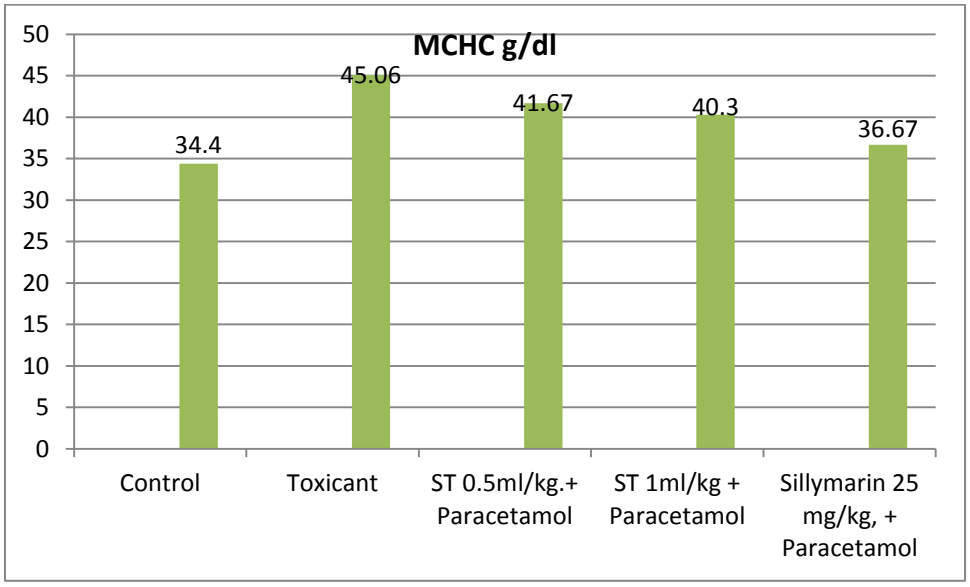


Chart: 49 Hepato Protectivity - Mean value of MCVin Control and treated groups

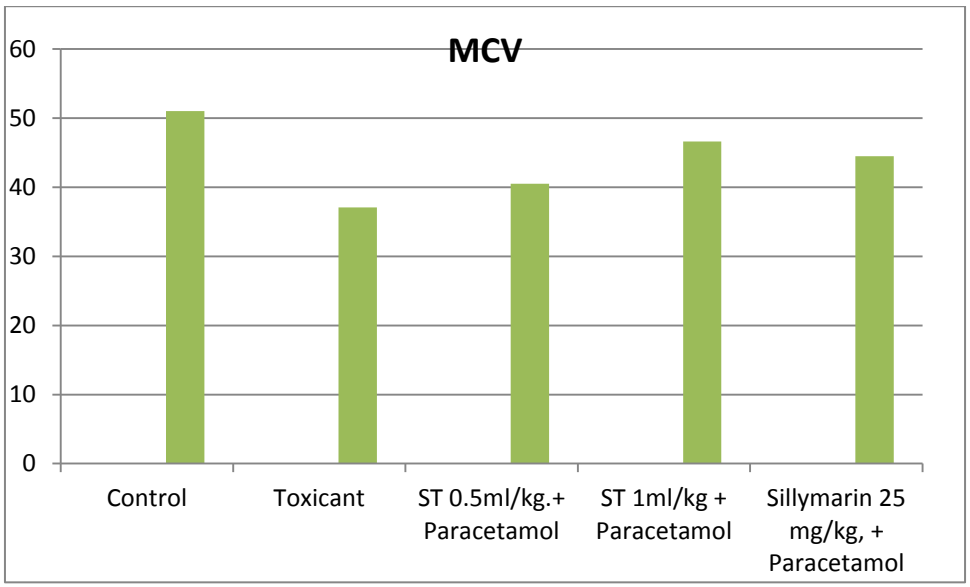


Table : 29 Effect of ST on direct bilirubin, total bilirubin levels in Paracetamolinduced hepatotoxic rats.

Group	Treatment	Dose (p.o.)	Direct bilirubin (mg/dl)	Total bilirubin (mg/dl)
A	Normal control	Distilled water	0.168 ± 0.60	0.293±0.029
B	Toxicant Control	Paracetamol-1 ml/kg,	1.579 ±0.099	1.6±0.005
C	ST - 1	0.5ml/kg, *p.o.*+paracetamol	1.22 ±0.032**	1.43±0.059**
D	ST - 2	1ml/kg, *p.o.*+ paracetamol	0.56 ±0.040**	0.50 ±0.1105**
E	Standard (Silymarin)	25 mg/kg, + Paracetamol	0.286 ±0.012**	0.433±0.049**

Values are mean ± SEM (n=6) one way ANOVA followed by Dunnet's test.

Chart: 50 Hepato Protectivity - Mean value of Direct and Total Bilirubinin Control and treated groups

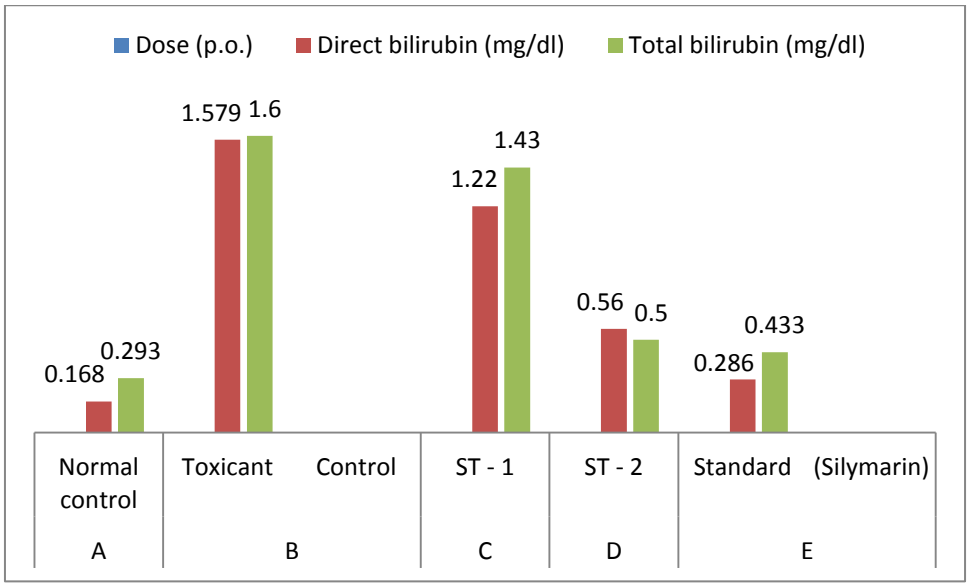


Table: 30 Effect of ST on ALT, AST, ALP levels in Paracetamol induced hepatotoxic rats.

Group	Treatment	Dose (p.o)	ALT (U/L)	AST (U/L)	ALP (mg/dl)
A	Normal control	Distilled water	27.145±0.325	33.05±4.5	32.50±0.50
B	Toxicant Control	Paracetamol-1 ml/kg,	102 ±2.0	152.05 ±2.50	130.95±0.550
	ST - 1	0.5ml/kg, *p.o.*+paracetamol	86.5±2.0**	110.75±8.750**	81.6±0.30**
C	ST - 2	1ml/kg, *p.o.*+ paracetamol	53.6±1.30**	77.5±3.0**	60.35±0.250**
D	Standard (Silymarin)	25 mg/kg, + Paracetamol	35.5±1.0**	60.5±1.0**	40.05±0.55**

Values are mean ± SEM (n=6) one way ANOVA followed by Dunnet's test. Where, ** represents significant at P<0.01.

Chart: 51 Hepato Protectivity - Mean value of ALT, AST, ALPin Control and treated groups

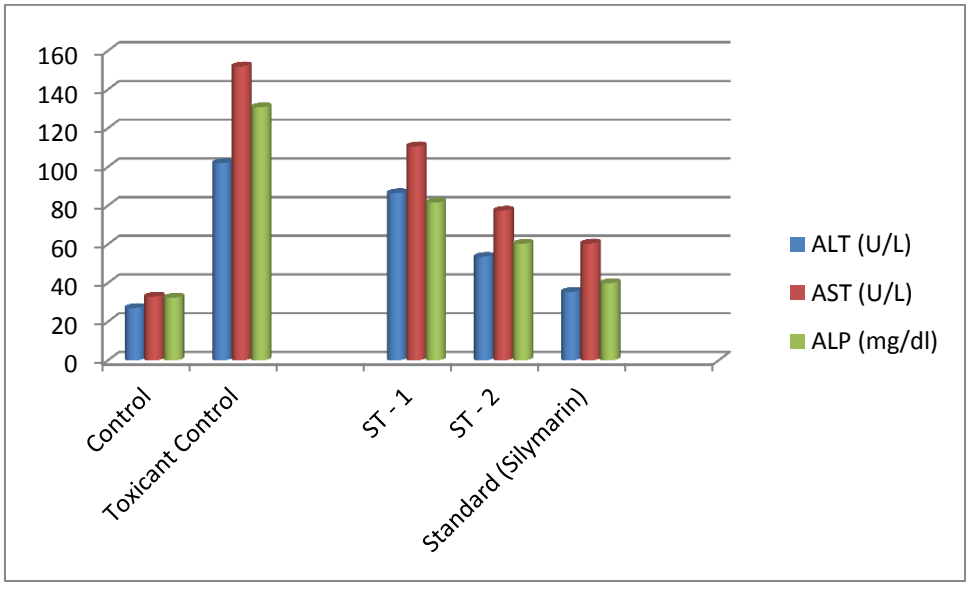
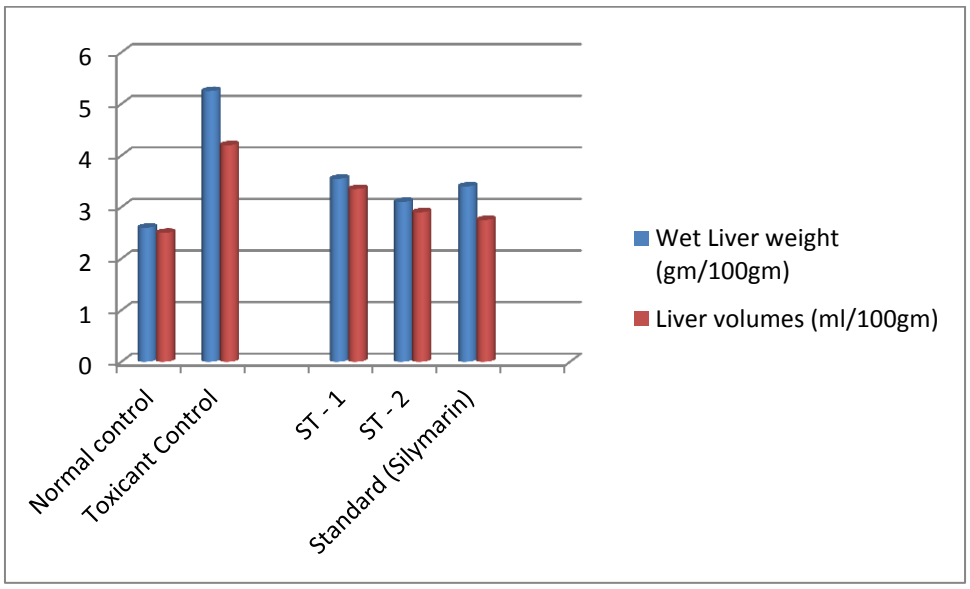


Table: 31 Effect of ST on Wet liver weight & Wet liver volumes in Paracetamol induced hepatotoxic rats.

Group	Treatment	Dose	Wet Liver weight (gm/100gm)	Liver volumes (ml/100gm)
A	Normal control	Distilled water	2.60± 0.370	2.50 ±0.10
B	Toxicant Control	Paracetamol-1 ml/kg, *p.o.*	5.25 ± 0.150	4.20 ±0.15
C	ST - 1	0.5ml/kg, *p.o.*+paracetamol	3.55± 0.10**	3.35 ±0.015**
D	ST - 2	1ml/kg, *p.o.*+ paracetamol	3.10± 0.125**	2.90 ±0.150**
E	Standard (Silymarin)	25mg/kg, *p.o.*+ paracetamol	3.40± 0.05**	2.75 ±0.05**

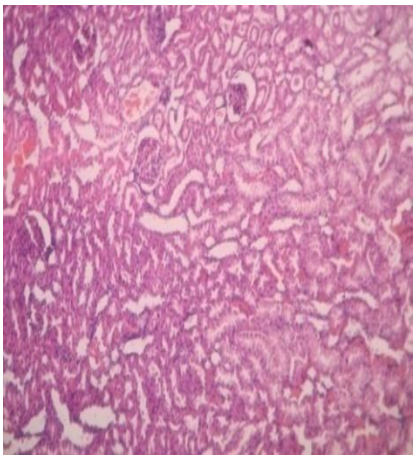
Values are mean ± SEM (n=6) one way ANOVA followed by Dunnet's test. Where, ** represents significant at P<0.01.

Chart: 52 Hepato Protectivity - Mean value of Wet liver weight and Liver volume in Control and treated groups

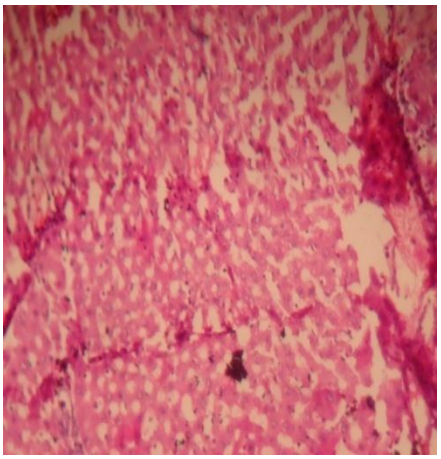


HISTOPATHOLOGY REPORTS OF LIVER

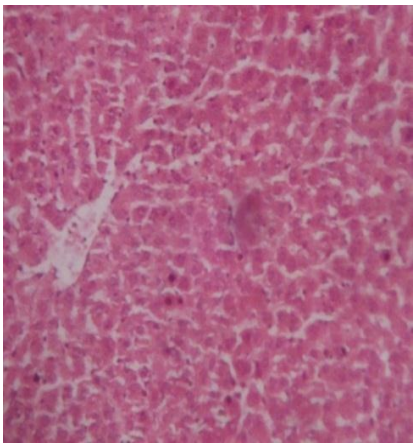
NORMAL CONTROL



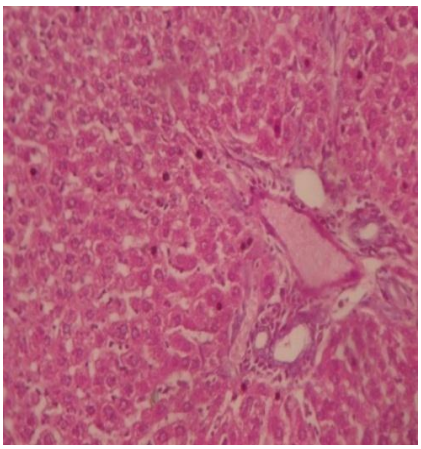
TOXIC CONTROL



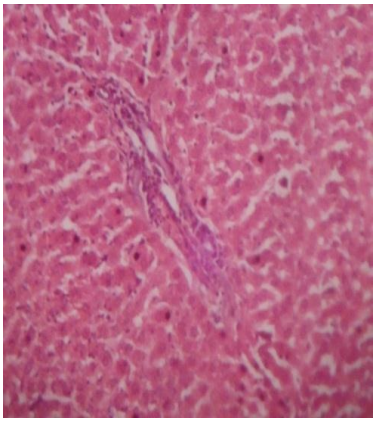
ST - 1



ST - 2



STANDARD



Interpretation : The present studies were performed to assess the hepatoprotective activity in rats against Paracetamol as hepatotoxin.

Biochemical Parametres

- The administration of the toxicant PCM showed a distinct rise in the levels of serum marker enzymes namely ALT, AST, ALP and Total Bilirubin as shown in Table. no 29, 30.
- The activities of serum SGPT, SGOT and ALP and the concentration of total bilirubin in the ST treated groups (Tables. 29, 30) and the Silymarin group, were significantly decreased when compared to PCM treated group and almost near the normal value when compared to the negative control.
- The drug treatment was carried out at two dose levels 0.5ml and 1ml /kg, both of which along with the standard treated group showed a significant reduction in the elevated enzyme levels.
- These data suggests a dose dependent Hepato protective activity of ST. The present studies were performed to assess the hepatoprotective activity in rats against PCM as hepatotoxin to prove its claims in clinical practice against liver disorders.

Histopathological Analysis

- Group A: Photomicrograph of liver tissue of control group showing normal hepatic cells with central Vein (CV) and sinusoidal dilation (S).
- Group B: showed fatty changes and slight increase in liver weights compared to the control groups. Photomicrograph of liver tissue of Toxicant group showing severe centri lobular necrosis (N) with disappearance of nuclei.
- Group C: Photomicrograph of liver tissue of ST 1 group treated with Sanjeevi Theeneer at 0.5ml/kg showing mild degree of necrosis (N) with mild inflammatory cells.
- Group D: Photomicrograph of liver tissue of ST 2 group treated with Sanjeevi Theeneer at 1ml/kg showing normal hepatocytes with regenerating hepatocytes and mild inflammation in the portal area (M).
- Group E: Photomicrograph of liver tissue of Silymarin treated group 25mg/kg showing normal hepatocytes, portal vein and portal artery.

- Estimating the activities of serum marker enzymes, like ALT, AST, ALP can make the assessment of liver function when liver cell plasma membrane is damaged, a variety of enzyme normally located in the cytosol are released into the blood stream. Their estimation in the serum is a useful quantitative marker of the extent and type of hepato cellular damage.
- Reduction in the levels of ALP, ALT and AST towards the normal value is an indication of regeneration process.
- Reduction in ALP levels with concurrent depletion of raised bilirubin levels suggests the stability of the biliary function during injury with PCM.
- These findings suggested the ST administered has significantly neutralized the toxic effects of PCM and helped in regeneration of hepatocytes. The tendency of these enzymes to return to near normally in ST administered group is a clear manifestation of anti-hepatotoxic effects.
- The measurement of liver body weight ratio is a more accurate approach to determine the changes in liver size compared to the measurement of liver weight alone as the liver weight largely depends on the size of the rat.
- The enlargement of livers in PCM-treated rats suggested hepatic lesions and liver injury associated with the toxic effects of PCM. These significant changes in the liver weights may be attributed to the accumulation of extracellular matrix protein and collagen in liver tissue.
- From the Table 31 it was evident that ST was able to reduce or normalize the wet liver weight and wet liver volumes and all the elevated biochemical parameters due to the PCM intoxication.
- The ST thus has a potential application in the condition of jaundice. A possible mechanism of the ST on bilirubin levels may be interference with cytochrome P450, resulting in the hindrance of the formation of hepatotoxic free radicals, thereby protecting the integrity of the membrane. Hence, it may be possible that the mechanism of hepatoprotection by ST is due to its antioxidant effect.

RESULTS – BRONCHODILATOR ACTIVITY (isolated tracheal chain Preparation)

Figure: 5 Dose response Curve with Trial drug *Sanjeevi Theeneer* in isolated Guinea Pig Tracheal Chain Preparation

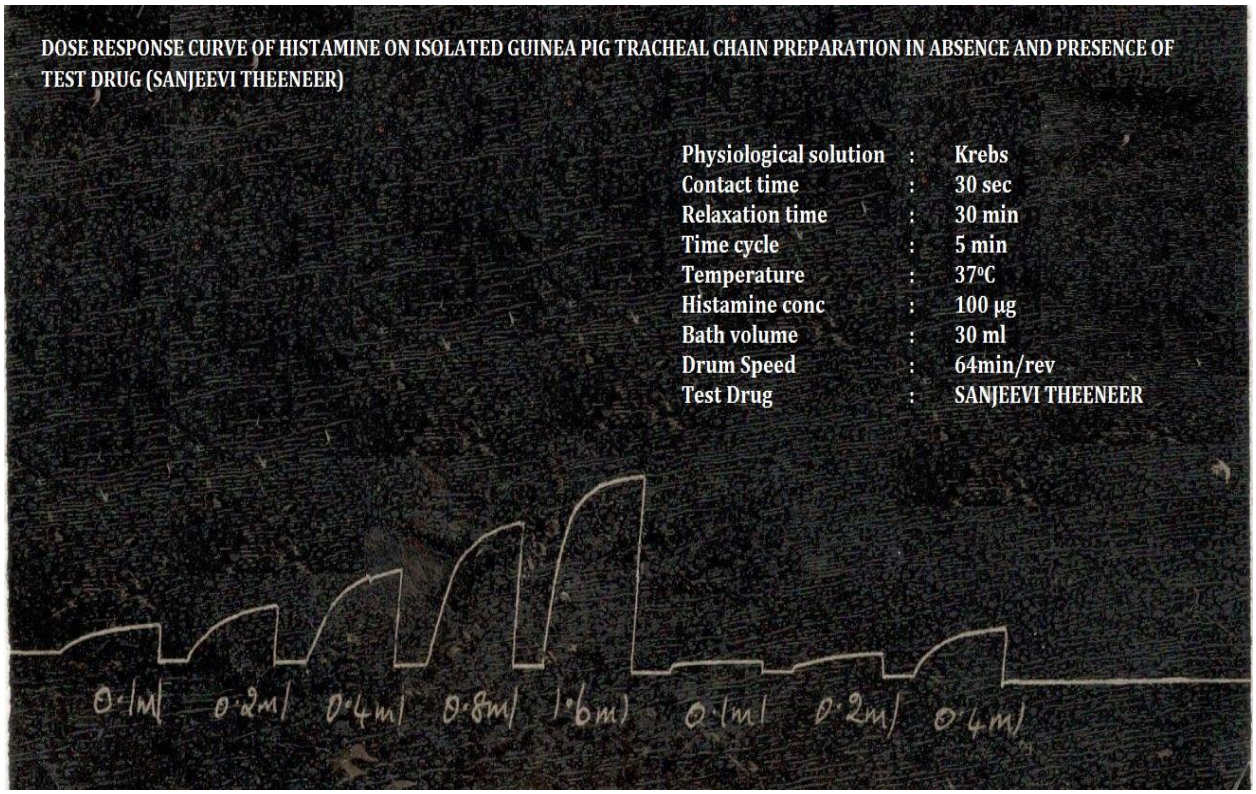


Table: 32 Pharmacological analysis- Broncho dilator activity of Sanjeevi Theeneer in Guinea pig Tracheal chain preparation

Treatment	Conc.in µg/ml	Height of response in mm	Percentage inhibition
Histamine	10 20 40 80 160 320	08 09 10 12 14 19	-
Sanjeevi Theeneer 15 ml +Histamine	10 160	12 9	14 % 35 %
Sanjeevi Theeneer 30 ml +Histamine	20 320	11 08	42% 57%

% inhibition = [(normal activity - inhibited activity) / (normal activity)] x 100%

Results

Histamine causes contraction of the guinea pig tracheal chain. At the test Sanjeevi Theeneer showed a dose dependent inhibition of contractions induced by histamine on guinea pig at 5 ml and 10 ml which is plotted in the graph above in terms of concentration in µg/ml versus height in mm . 10 µg and 160 µg/of histamine produces 12, 9 mm height of response in the presence of *Sanjeevi Theeneer* at 5 ml with inhibitory efficacy of 14% and 35 %. 20 µg and 320 µg of histamine produces 11, 8 mm height of response in the presence of *Sanjeevi Theeneer* at 10 ml with inhibitory efficacy of 42% and 57 %. This proves its dose dependent inhibition of Histamine induced contractions.

Figure : 6 Dose response Curve with Trial drug *Sanjeevi Theeneer*in isolated Guinea Pig Ileum Preparation

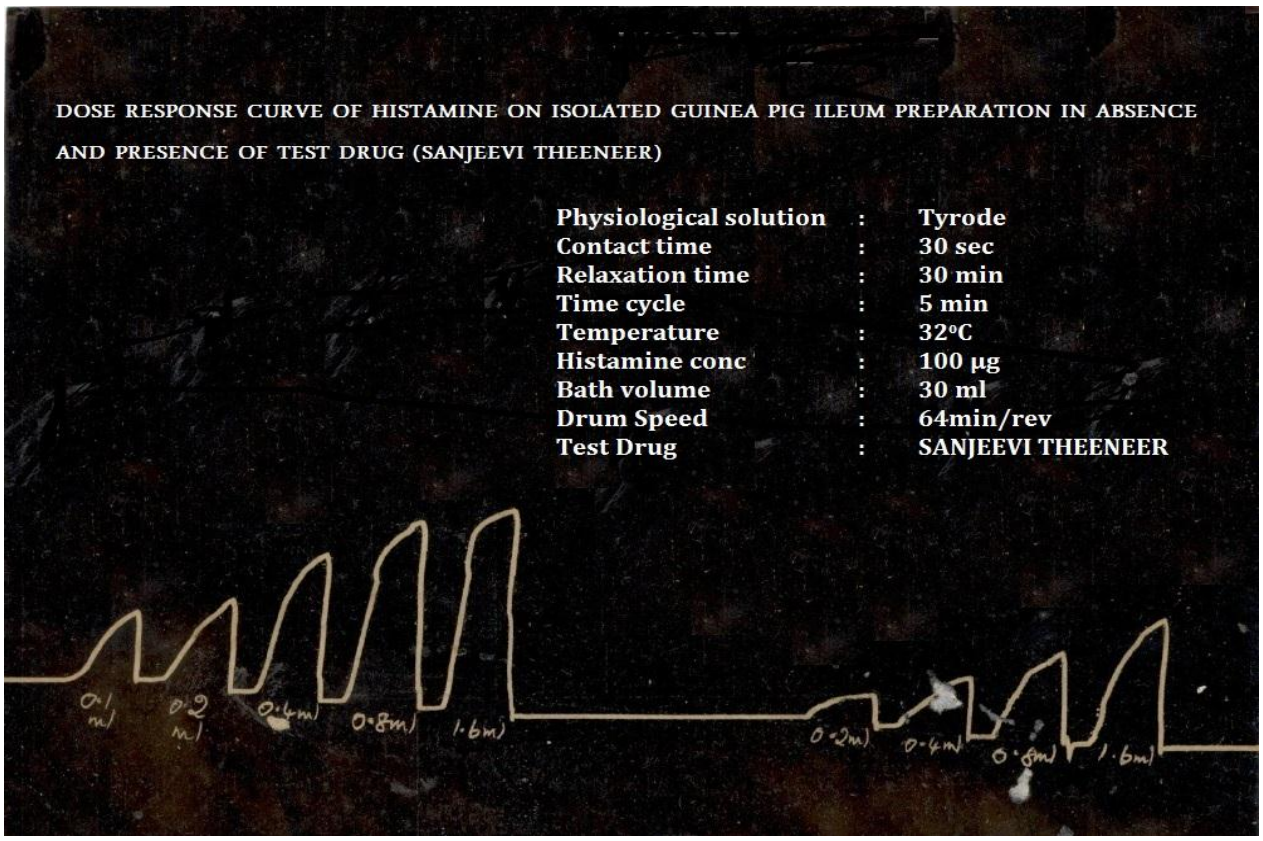


Table: 33 Pharmacological analysis- Broncho dilator activity of Sanjeevi Theeneer in Guinea pig Ileum preparation

Treatment	Conc.in µg/ml	Height of response in mm	Percentage inhibition
Histamine	10 20 40 80 160 320	06 07 9 12 14 19	-
Sanjeevi Theeneer 15 ml+Histamine	10 160	10 9	28% 35 %
Sanjeevi Theeneer 30 ml+Histamine	20 320	12 08	37% 57%

% inhibition = [(normal activity - inhibited activity) / (normal activity)] x 100%

Results

Histamine causes contraction of the guinea pig ileum, the test Sanjeevi Theeneer showed a dose dependent inhibition of contractions induced by histamine on guinea pig at doses of 5 ml and 10 ml which is plotted in the graph above in terms of concentration in µg/ml versus height in mm . 10 µg and 160 µg/of histamine produces 10, 9 mm height of response in the presence of *Sanjeevi Theeneer* at 5 ml with inhibitory efficacy of 28 % and 35 %. 20 µg and 320 µg of histamine produces 12, 8 mm height of response in the presence of *Sanjeevi Theeneer* at 10 ml with inhibitory efficacy of 37 % and 57 %. This proves its dose dependent inhibition of Histamine induced contractions.

In isolated guinea pig ileum preparation, there is a right side shift of dose response curve of histamine in the presence of Sanjeevi Theeneer. This proves its Anti- Histamine activity.

9. DISCUSSIONS

The Classical distillate Sanjeevi theeneer had been subjected to various studies and it confirms the literature evidences. Literary collections, Physicochemical Studies, , Bio-chemical analysis, Preliminary Phyto chemical analysis, Toxicity studies, Pharmacological studies were done to prove the Anti-Oxidant, Hepato protective, Bronchodilator and Anti-Histamine activities of.*Sanjeevi Theeneer*. Literary review about the ingredients of this trial drug from various *Siddha* texts supports the activity.

- ✓ **Literary collections:**

  Literary collections include drug review, which consist both Botanical aspect and *Gunapadam* aspect, pharmaceutical and scientific reviews in support of the study.

- ✓ **Drug review:**
  - The Trial drug is a rich formulation with Anti- oxidant herbs, most are described as *kalpa mooligai* (Herbal Elixirs).
  - *Siddha* Literatures are supporting the usage of Ingredients of *Sanjeevi Theeneer* in conditions of Liver diseases and Respiratory ailments like Bronchial Asthma.
  - All the Ingredients of the trial drug were used in Traditional *Siddha* practices for diseases pertaining to Liver and Respiratory system.
  - Most of the Ingredients of Sanjeevi Theeneer has been scientifically validated for its Anti- oxidant, Hepato protective, Bronchodilator and Anti-Histamine activities.

- ✓ **Drug Analysis:**
  - **Organo leptic characters:** *Sanjeevi Theeneer* is a clear aqueous distillate, light lemon yellow coloured with pleasant aroma and mild pungent taste.
  - The analytical part reports the percentage of volatile content and the pH specifying alkaline nature of the distillate .The standards are promising as per the traditional quality parameters

- **Heavy metal and Microbial Load Analysis:** The heavy metal and microbial load presence were within the permissible limits. Both the results indicate the nontoxicity and purity of the distillate- *Sanjeevi theeneer*.

- **Biochemical analysis:** Shown the presence of Alkaloids which are important bio active constituents possessing antispasmodic, analgesic, bactericidal effects. A synergistic effect of all these flavonoids, alkaloids, glycosides, tannins, phenols, saponins, increases the potency of the drug against Hepatic damages.

- **Preliminary Phyto Chemical Analysis:** Qualitative analysis of phytochemical variables in *Sanjeevi theeneer* reports the presence of Phenolic compounds. Phenols exhibit wide range of pharmacological and biological functions especially considering the cardiovascular health. Their role in prevention of atherosclerosis (Anti-atherosclerotic) and inflammation (Anti-inflammatory) and also in improving endothelial function has been well established [23, 24] various studies prove it as an Anti-carcinogenic, Ant apoptosis and Anti-ageing compound.

- **GC-MS analysis**: Reported the presence of major compound Oleic acid (% Peak area: 40.85), Dasycarpidan (% Peak area: 1.98), Thymol (% Peak area: 34.32 and other secondary metabolites which may have significant biological properties contributing to the Therapeutic potential of *Sanjeevi Theeneer* as an Anti-oxidant, Hepato protective, Broncho dilator and Anti-histamine.

✓ **Toxicity studies:**

- **Acute Toxicity Studies:** The treated rats with *Sanjeevi Theeneer* 5 ml/kg in the acute toxicity study did not show any mortality, any untoward clinical sign, any behavioral signs, alterations in body weight and necropsy findings at the end of the study. This indicates that the dosages administered were below toxic level and proves the safety of the drug.The acute toxicity helped to fix the doses for pharmacological activities.

- **The Repeated dose 28 day oral toxicity and Repeated dose 90-day oral toxicity study:** The trial drug Sanjeevi Theeneer were administered in Wistar rats. The treated animals survived throughout the study period of 28 days and 90 days did not reveal any treatment related major abnormal clinical signs at the test dose levels. The overall percentage of body weight gain in rats treated with the drug was found to be normal indicating that the test animals were in a healthy condition during the 90 days of observation period.
- In histo pathological studies both mid dose and high dose, treated rats shown no significant abnormalities. The necropsy studies showed no remarkable changes.
- This strongly stress the fact of the drug having no toxic effect on the body metabolism.The necropsy studies showed no remarkable changes.So the trial drug Sanjeevi Theeneer may be further established under human trials.

✓ **Pharmacological studies:**

The pharmacological activities of Sanjeevi Theeneer like Anti oxidant, Hepato protective, Broncho dilator and Anti Histamine have shown significant outcome and results.

- **Anti oxidant Activity** (Invitro - DPPH radical scavenging assay of *Sanjeevi Theeneer)*

  Sanjeevi Theeneer was studied for its Anti-oxidant potential on DPPH radical scavenging assay by comparing with standard Ascorbic acid. Sanjeevi Theeneer was able to reduce the stable DPPH radical to 50 % reduction with IC_{50} of 90.19 ± 8.57)as compared with Ascorbic acid (46.91± 9.93). So results confirmative as a resultant Anti-oxidant.

- **Hepato protective Activity** in Wistar Albino rats**:**The activities of serum SGPT, SGOT and ALP and the concentration of total bilirubin in the ST treated groups and the Silymarin group, were significantly decreased when compared to PCM treated group and almost near the normal value when compared to the negative control.

- The drug treatment was carried out at two dose levels 0.5ml and 1ml /kg, both of which along with the standard treated group showed a significant reduction in the elevated enzyme levels.
- In histo pathological studies *Sanjeevi Theeneer* administered has significantly neutralized the toxic effects of PCM and helped in regeneration of hepatocytes.
- These data suggests a dose dependent Hepato protective activity of ST. The tendency of these enzymes to return to near normally in ST administered group is a clear manifestation of anti-hepatotoxic effects.
- The present studies were performed to assess the hepatoprotective activity in rats against PCM as hepatotoxic to prove its claims in clinical practice against liver disorders.
- **Broncho dilator and Anti-Histamine Activity:** In isolated tracheal chain and isolated guinea pig ileum preparation, there is a right side shift of dose response curve of histamine in the presence of Sanjeevi Theeneer. This proves its anti-asthmatic and Anti- Histamine activity.
- **Hepato protective Activity in Zebra fish (Danio rerio) models:** The results of the Histopathological investigation indicates that paracetamol treated groups shows sever liver degeneration whereas treatment with test drug ST at both the dose level significantly attenuated the paracetamol induced damage in group III and IV. Hence from the study it was concluded that the drug *Sanjeevi Theeneer* possess promising hepato protective activity in dose dependent manners and restores the basic liver architecture by means of its rejuvenating potential against paracetamol induced toxicity in zebra fish model.

From the discussion, this preclinical study of *Sanjeevi Theeneer* proves the safety of the drug and its efficacy as a Hepato protective, Broncho dilator and Anti-Histamine in animal model. Its Anti-oxidant potential has been validated in In vitro studies. The drug may be further assessed and established for Clinical trial studies.

10. SUMMARY

The test drug *Sanjeevi Theeneer*was selected from the *Classical* literature Siddha Formulary of Indiafor its Hepato protective, Broncho dilator and Anti Histamine studies.

Review of literature in various categories was carried out. *Siddha* aspect, botanical aspect, scientific aspects and Pharmaceutical review disclosed about the drug and the disease. Pharmacological review was done to establish the methodologies.The drug was subjected to analysis such as physicochemical, Preliminary phyto chemical analysis, Gas chromatography analysis and Anti-oxidant Assays.

Toxicological study was made according to OECD guidelines comprising the acute, repeated dose 28-days oral toxicity and repeated dose 90-days oral toxicity study.It showed the safety of the drug which proved its utility in long time administration without any harm to the human being.

Pharmacological studywas done. It revealed the Hepato protective, Broncho dilator and Anti Histamine activities in animal model. This present study suggests *Sanjeevi theeneer* has remarkable medicinal value in the treatment of Liver diseases. Thus, the herbal formulation *Sanjeevi Theeneer is* validated for its safety and efficacy for treating liver diseases, Asthma and it would be a great drug of choice and as a Health supplement.

11. CONCLUSION

Sanjeevi Theeneer, the Herbo mineral Classical distillate was prepared as per the Siddha literature. The prepared drug meets all the Traditional quality parameters andalso fulfills the Standardization parameters of Theeneer mentioned in AYUSH guidelines. Based on OECD 423,407, 408 the trail drug *Sanjeevi Theeneer* is considered as nontoxic when studied on Animal Models. The **Anti-oxidant Studies, Hepato protective studies, Bronchodilator and Anti Histamine activities** was scientifically validated. Hence, it can be concluded as a safe and therapeutically effective drug in Liver diseases and Bronchial Asthma and moreover can be used a rejuvinative Health supplement.

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