PRECLINICAL SAFETY EVALUATION OF KIRANTHI MEGA CHOORANAM

The Dissertation Submitted by

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"Preclinical safety evaluation of Kiranthi Mega Chooranam''** is a bonafide and genuine research work carried out by me under the guidance of **Dr. S. Murugesan, M.D(S)**., Department of Nanju Maruthuvam, National Institute of Siddha, Chennai -47, and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or another similar title.

Date: **Signature of the candidate**

Place: Chennai - 47. **Dr.G.THANGANILA**

BONAFIDE CERTIFICATE

Certified that I have gone through the dissertation submitted by **Dr .G.THANGANILA (Reg.No: 321716207)** a student of final year M.D(s), Branch-VI, Department of Nanju Maruthuvam, National Institute of Siddha, Tambaram Sanatorium, Chennai - 47, and the dissertation work has been carried out by the individual only. This dissertation does not represent or reproduce the dissertation submitted and approved earlier.

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CONTENTS

1. INTRODUCTION

The Siddha system of medicine is a gift of the mankind by the Siddhars who were the greatest scientists in ancient times. Siddhars who defied death, preached the philosophy of Siddha medicine and theory of immortality. Agathiyar is believed to be the founding father of Siddha medicine. Eighteen Siddhars are considered to be important in Siddha medicine. The word Siddha means "an object to be attained" Siddha system of medicine is an ancient system which provides continuous services to humanity for more than 5000 years in combating diseases and then maintaining its physical, mental and moral health[(1).]

Everything is Pancha boothic in nature. Life and death are Panchaboothic which is inevitable. The same process repeats and it will restore in one place called Parabirammam. Creation, Protection and Destruction are the powers of Parabirammam. The cosmos and the things in cosmos are all Panchaboothic in nature [(2)]

There is an old saying in Tamil.

"நோயற்ற வாழ்வே குறைவற்ற செல்வம்"

Which means Wealthy life is that which is free from diseases (Health is wealth).The human body is not only to enjoy in the pleasure of the world but also to attain the salvation through various means and hence maintaining the body with good health and strength is the must[(3)] .In our AYUSH system in India, Ayurveda, Siddha and Unani medicines are the major system of indigenous medicines. Ayurveda and Siddha are most developed and widely practiced in South India [(4).]The Recent interest on traditional medicine has taken up great dimensions in changing the health care scenario across the globe[(5).] The Siddha system of medicine is practiced mainly in south India which includes drugs of herbal, mineral/metal and animal origin. The drugs used by the Siddhar could be classified into three groups:

- Mooligai/Thavaram (herbal product)
- Thathu (inorganic substances)
- Jeevam or Sangamam (animal products).

According to their mode application the Siddha medicine could be categorized into two classes:

1. Internal medicine
2. External medicine

Siddha, a traditional system of medicine is now attracting people because of their efficacy in curing diseases Chooranam is one among the 32 internal medicines in Siddha and it has a shelf life of three month[6].

Chooranam the dried drugs are finely powdered individually and sieved in a fine cloth and mixed together with other powders to homogeneity. The finely powdered material mixed to homogeneity is then baked in a baking pan using milk or pure water to produce steam. It is then filtered in a cloth and makes it into a fine powder[7].

Globally, there is increasing need of drugs to cure various ailments. Siddha, a traditional system of medicine is now attracting people because of their efficacy in curing diseases. Kiranthi Mega Chooranam is one of the major Siddha medicines which are widely used to cure various ailments. It has specific indication to cure major diseases like Kiranthi, megam, kadividam, kuttam, megavayu, megasoolai. But for the global acceptance of our Siddha medicinal formulation we ensure the safety of the drug through toxicity study in animals. Since, toxicological screening is very important for the extension of the therapeutic potential of Kiranthi Mega Chooranam, I have chosen this drug as my dissertation topic.

2. AIM AND OBJECTIVES

AIM:

To evaluate the safety profile of **Kiranthi Mega Chooranam**(KMC) on animal model Wistar albino rats.

OBJECTIVES:

- To study the physicochemical properties, biochemical analysis and Phytochemical analysis of **Kiranthi Mega Chooranam**.
- To evaluate the acute toxicity profile of **Kiranthi Mega Chooranam** as per WHO Guideline.
- To evaluate the long-term toxicity profile of **Kiranthi Mega Chooranam** as per WHO Guideline.

3. REVIEW LITERATURE

3.1. SIDDHA ASPECT

சிவனார்வேம்பு

வேறு பெயர்கள் [8]

குணபாடம் மூலிகை வகுப்பு :

காந்தாரி, அரனிம்பம்

சித்தர் பரிபாஷை அகராதி :

சிவன் சேவகம்
ஈசன் மூலி
காலனை வென்றோன்.

தட்சநாயனார் வைத்திய அட்டவணை:

சிவா

பசுமூலி அகராதி

சிவன்வேர்

அனுபவ வைத்திய முறைகள்:

முப்புர மெரித்தான் வேர்

வளரியல்பு

சிறுசெடி

பயன்படும் உறுப்பு:

இலை, பூ, தண்டு, வேர்

சுவை - கைப்பு

தன்மை - வெப்பம்

பிரிவு - கார்ப்பு

செய்கைகள்:

உள்ளழலாற்றி

வெப்பமுண்டாக்கி

பொதுகுணம்:

இதனால் தோலை பற்றி வரும் படைகள் நாட்பட்ட புண், சொறி சிரங்கு, பிளவை, கொடுமை செய்யும் வளிநோய்கள், மந்தம், குறைநோய், பாம்பு நஞ்சு நீங்கும், அழகு உண்டாகும்.

"குட்டஞ் சிரங்கு குறைப்புப்பச மாந்தை
கட்டப் பிணிகள் கழலுமே - திட்டம்
உரனிம்பங் காயத்துக் குண்டாகு மேலை
அரனிம்ப மென்னு மருந்தால்"

- தேரன் வெண்பா

பயன்கள்:

- இலை, பூ இவற்றை குடிநீரிட்டு குடிக்க அது தோலை பற்றிய படை முதலிய நோய்களை விலக்கும்.
- வேரை எண்ணெயிலிட்டு காய்ச்சி சொறி, சிரங்கு முதலியவைகளுக்கு பூச போகும்.
- சிவனார் வேம்பு தைலத்தை உள்ளுக்கு காசெடை கொடுத்து மேலுக்கு பூச பெருநோய், படை முதலியன போகும்.
- சிவனார் வேம்பு தைலத்துடன் சிவனார் வேம்பு பொடி சேர்த்துக் கொடுக்க, பெருநோய், கரப்பான், வெடிசூலை, நச்சுப்புண் தீரும்.
- இதன் வேரை இடித்து வெண்ணெய் குழைத்து வீக்கங்களுக்கும் பூசலாம்.

சிவனார்வேம்பு சேரும் பிற மருந்துகள்

1.சிவனார்வேம்பு
2.வெள்ளறுகு
3.சங்கம் வேர்பட்டை
4.பிரப்பங்கிழங்கு முற்றின வேப்பம்பட்டை

} வகைக்கு 10 பலம்

இவைகளை இடித்து தூள் செய்து கொள்ளவும்.

அளவு : திரிகடி அளவு 3 நாள் உட்கொள்ள வெள்ளை, வெட்டை தீரும்.

2. பறங்கிப்பட்டை பதங்கம்

பறங்கிப்பட்டை 6பலம் எடுத்து பாக்களவு வெட்டி சங்கங் குப்பி சாற்றில் பிரட்டிஎடுத்து காய வைக்கவும். இவ்வாறு மூன்று முறைசெய்து சிவனார்வேம்பு, சங்கம் வேர்,அமுக்கராவேர்,சிறுபிரப்பங்கிழங்கு, கொடிவேலி மூலம், கொன்றைபட்டை, எருக்கம்வேர், சாரணைவேர், கருஞ்சூரைவேர், நாய்க் கொட்டான்பட்டை, வெள்ளருகு இவை வகைக்கு 5பலம் எடுத்து தூளாக்கி சேர்க்கவும். இதை ஓர் பாண்டத்தில் இட்டு சீலைமண் செய்து வைக்கவும். வேறொரு பாண்டத்தில் புளியம் பொருக்குகளை வைத்து அதன் மீது மருந்துள்ள பாண்டத்தை வைத்து மேலே பொருக்குகளை கொட்டி மேல் மூடியிட்டு சீலை மண் செய்யவும். இதனை தீயிட்டு 9 நாள் எரிக்கவும். உள்ளே மருந்து யாவும் பதங்கமாக மேலேறி நிற்கும். அப்பதங்க தூளுடன் ஓரளவாக சீனியும் வெங்கார பொடியும் கலந்து கொள்ளவும்.

அளவு : வெருகடியளவு இருவேளை ஒரு மண்டலம் (48 நாட்கள்).

தீரும் நோய்கள் : கரப்பான், கிரந்தி, வெட்டை, குட்டம், மேகம், கிராணி, காந்தல்

(அகத்தியர் வைத்திய காவியம் 1500 ப.எண். 78)

3. மகா வல்லாதி இளகம்:

அளவு : 3கிராம் தினம் இருவேளை, 45 நாட்கள்

தீரும் நோய்கள் : 21 வகை மேகம், வெள்ளை, வெட்டை, புற்று, மூலவாயு, தோல் நோய்கள்.

(போகர் வைத்தியம் 750 பாடல் எண் 175 - 187)

4. வெள்ளைதீர சூரணம்:

சிவனார் வேம்பு வேர்பட்டை

செய்முறை :

சிவனார் வேம்பு வேரின் பட்டையை கொண்டு வந்து பசுவின் நெய்யுடன் உண்டாலோ கருங்குருவை அரிசி, நெருஞ்சிக்காய் (சரி அளவு) சேர்த்து கல்லுரலிடித்து புட்டாக செய்து சாப்பிட்டாலோ கற்றாழை சோற்றையெடுத்து நன்றாக அலம்பிவிட்டு கடுக்காயுடன் கூட்டி உண்டாலோ வெள்ளைத் தீரும்

(சரபேந்திரர் வைத்திய முறைகள் ப.எண்: 105)

5.சிவனார் வேம்பு குழித் தைலம்:

சிவனார் வேம்பு வேர் - 340 கிராம்

வாலுளுவை அரிசி - 340 கிராம்

இவற்றை ஆகாசகருடன் கிழங்குச்சாற்றால் அரைத்து வில்லை செய்துலர்த்திக் குழித்தைலம் இறக்கவும். இதில் கற்பூரம் சிறிது கூட்டி கொடுக்க வெடி சூலை, நாட்பட்டபுண், அடிப்பட்டபுண் இவற்றிற்கு பூச தீரும்.

(குணபாடம் மூலிகை வகுப்பு)

6. சிவனார் வேம்பு சூரணம்:

சிவனார் வேம்பு சூரணம் 10 பலம், குழி தைலம் 10 படி வாலுளுவை, தாளிசபத்திரி, கிராம்பு, ஏலம், அதிமதுரம், கோஷ்டம், சாதிக்காய், சாதிப்பத்திரி, கருஞ்சீரகம், மிளகு, கடுக்காய், திப்பிலி, அதிவிடயம், பறங்கிபட்டை, சிறுநாகப்பூ, வால்மிளகு, கர்கடக சிங்கி வகைக்கு 9 கிராம் எடுத்து இடித்து சூரணித்து சிவனார் வேம்பு சூரணத்துடன் சேர்த்து பாதியளவு வெள்ளை சர்க்கரை சேர்த்து தினம் ஒருவேளை வெருகடி அளவு சிவனார் வேம்பு தைலத்துடன் சேர்த்து உண்ணவும்.

தீரும் நோய்கள்:

வெட்டை, மேகசூலை, மேகவாயு, கரப்பான், 18 வகை குட்டம், சொறிசிரங்கு, விஷக்கடி, சில்விஷங்கள், விரணம், எரிச்சல் தீரும்.

- பிரம்மமுனி வைத்திய சூத்திரம்

SCIENTIFIC VALIDATION:

Indigofera aspalathoides- சிவனார் வேம்பு

Classification [9]:

Kingdom : Plantae

Order : Fabales

Family : Fabaceae

Sub family : Faboideae

Tribe : Indigofereae

Genus : Indigofera. L

Species : Indigofera aspalathoides Vahl

Anti-diabetic activity:

Preliminary investigation was carried out to evaluate the anti diabetic effect of the alcoholic extract by oral glucose tolerance test (OGTT), normoglycaemic and anti hyperglycaemic activity in streptozotocin (STZ) – nicotinamide induced non-insulin dependent diabetes mellitus rats. Graded dose (250 and 500mg/kg) of the alcoholic extract suspended in gum acacia were administered to normal and experimental diabetic rats. Effect on glucose tolerance and hyperglycemic studies showed only less remarkable decrease in blood glucose level at both dose levels as compared to glibenclimide. Normoglycaemic study revealed significant percentage decrease in blood glucose level from the initial value in normal rats 21.20% and 25.20% (250 and 500mg/kg respectively) as compared to the control group 1.85%. Result showed that alcoholic extract of Indigofera aspalathoides is a source of compounds with antidiabetic activity [10] .

Nephro protective activity:

The methanol extract of Indigofera aspalathoides was studied against gentamicin induced nephrotoxicity in wistar male albino rats. Various biochemical markers such as blood urea, serum Creatinine, serum uric acid, serum electrolytes and antioxidant parameters such as Renal SOD, catalase, LPO and GPx were analysed.The result showed significant reduction in elevated serum marker levels and significant increase in renal SOD, catalyse level. Histopathological study revealed the protective effect at the dose level of 500mg/kg while the 250mg/kg showed only partial protection [(11)].

Wound healing activity:

The wound healing property of chloroform extract of Indigofera asphalathoides vahl. Ex DC.was evaluated in two different dose levels employing excision wound model. The wound treated with plant drug showed higher rate of wound contraction, increased level of Hydroxy proline, Hexosamine, SOD, Ascorbic acid and decreased levels of Lipid peroxides as well as histopathological studies also showed progressive collagenation and few macrophages compared to the control rats [(12)].

நிலாவாரை- Cassieae senna

வேறுபெயர்கள்

நிலவாகை

நாட்டு நிலாவிரை

ஆலகாலம்

கமதாயம்

தாளினி

ஆவாகை

குயத்தினலகை.

-தேரன் வெண்பா

நிலாவாரை பூக்கும் தாவர வகையைச் சேர்ந்த இத்தாவரம் அலங்காரத் தாவரமாகவும் மூலிகை மருத்துவத்திற்கும் பயன்படுகிறது.

வளரியல்பு : செடி

பயன்படும் உறுப்பு: இலை

சுவை : கைப்பு (வெகுட்டல்)

தன்மை : வெப்பம்

பிரிவு : கார்ப்பு

செய்கை : நீர்மலம்போக்கி

மலமிளக்கி

பொதுகுணம்:

"நிலாவாரை யின்குணந்தான் நீகேள் மயிலே!

பலமூல வாயுவெப்பு பாவைச்- சிலகிரந்தி

பொல்லாத குன்மம் பொருமுமலக் கட்டுமுதல்

எல்லா மகற்றுமென எண்"

(அகத்தியர் குணவாகடம்)

- இவற்றின் கல், மண், காம்பு, காய் நீக்கி இலையை மட்டும் எடுத்து பிட்டவியல் செய்து உலர்ந்தபின் எடுத்து வைத்து பயன்படுத்திக் கொள்ளலாம்
.
- நிலாவிரையின் வேரோடு பிரப்பங்கிழங்கு, மிளகு, சுக்கு இவற்றை சேர்த்து அரைத்து உட்கொள்ள வளிக்குற்றத்தால் பிறந்த நோய்கள் நீங்கும்

- மெலிந்தவர், முதிர்ந்தவர்கள், சிறுகுழந்தைகளுக்கு சிறந்த கழிச்சல் மருந்தாகும்.

- அளவு: 650 மி.கி- 1950 மி.கி.

- கடுக்காய்பிஞ்சு, நெல்லி வற்றல், சுக்கு, நிலாவரை, சோம்பு, உப்பு, தனியா இவற்றைத் தனித்தனியாக இடித்து தலா 50 கிராம் அளவில் எடுத்து வாதுமை எண்ணெய் தேவையான அளவு சேர்த்து அடுப்பில் வைத்து 225 கிராம் தேன், 50 மில்லி எலுமிச்சம்பழச்சாறு சேர்த்து லேகியபதம் வரும்வரை சிறு தீயாக எரிக்க வேண்டும். பதத்தில் எடுத்து பத்திரப்படுத்திக் கொண்டு இரவு படுக்கும்போது ஒரு நெல்லிக்காய் அளவு சாப்பிட்டு வந்தால், சுலபமாக மலமிளகும். வாத, பித்த, கபம் என்னும் மூன்று தோஷங்களால் ஏற்படும் அனைத்து வகை தோஷங்களும் நிவர்த்தியாகும்.

- சுகபேதிக்கு : ரோஜா மொக்கு 25 கிராம், நிலாவரை 15 கிராம், சுக்கு 10 கிராம், கிராம்பு 5 கிராம் இவைகளைச் சிதைத்து 500 மில்லி தண்ணீரில் போட்டு நன்றாகக் கொதிக்க வைத்து வடிகட்டி, பெரியவர்களுக்கு 120 மில்லி சிறுவர்களுக்கு 60 மில்லி ஒரு வயதுக் குழந்தைக்கு 15 மில்லி வீதம் கொடுத்தால் சுகபேதியாகும்.

Scientific validation- Cassia senna L - நிலாவாரை

Kingdom: Plantae
Clade: Angiosperms
Order: Fabales
Family: Fabaceae
Tribe: Cassieae
Genus: Senna

Pharmacology of senna

Senna leaves and pods show laxative activity. Leaves contain glycosides, sennoside A, B, C and D. Two naphthalene glycosides have been isolated from leaves and pods. Anthraquinone gives the medicinal action of senna. It appears that the aglycone portion is responsible for its action. The breakdown of the anthraquinone glycosides in the digestive tract can occur in one of two ways. The bowel flora can directly hydrolyze them in a similar way to that of free active aglycone. Alternatively, in the presence of bile and the sugar moiety, the free aglycone can be absorbed into the blood stream and secreted later into the colon[13] .

Antimicrobial activity of senna

The extracts of Cassia angustifolia showed anti-microbial activity. Different extracts (ethanol, methanol, petroleum ether and aqueous solutions) of Cassia angustifolia plant are extracting out. Antimicrobial efficacy of various extracts was assessed by disc diffusion method against Gram positive bacteria-Staphylococcus aureus, Gram negative-Escherichia coli and Pseudomonas aeruginosa and fungi-Aspergillus niger, Aspergillus flavus, Fusarium oxisporum and Rhizopus stolonifer. Phytochemical screening of the extract showed the presence of alkaloids, flavonoids, carbohydrates, proteins, tannins and triterpenoids in cassia angustifolia[13].

Anti-fungal activity of Senna

Senna act as anti-fungal agent and act against D.N.A of E. Coli bacteria. Sennosides affect the intestinal tract and induce diarrhoea. It has shown that senna produces DNA lesions in Escherichia coli cultures and can act as an antifungal agent [13] .

Most valuable medicinal plant

India has rich dietary resources and a combination of different foodstuffs can provide adequate quantity of nutrients and medicinal values in sustainable manner. In this context, less familiar crops like Senna have a vital role to play as their economic value is beyond dispute. These crops generally are rich source of sennosides, glycosides and other nutrients and can provide a solution to the problem of malnutrition and other diseases to a great extent. It is considered one of the world's most useful crop as almost every part of the senna can be used [14].

தவசு முருங்கை- Justicia tranquebariensis

வேறு பெயர்கள்

பிண்ணாக்கு பூண்டு,
நரி முருங்கை

வளரியல்பு : குறுஞ்செடி
பயன்படும் உறுப்பு: இலை
சுவை : துவர்ப்பு
தன்மை : வெப்பம்
பிரிவு : கார்ப்பு

செய்கை :கோழையகற்றி

தவசுமுருங் கைத்தழைக்குத் தையலே கேளாய்!
அவசியம் பீநசம்உண் ணாக்கும்- உவசர்க்க
ஐயஞ்சு வாசகபம் அண்டாது குத்திருமல்
வையம் விடுத்தேகும் வழுத்து.

-அகத்தியர் குணவாகடம்

- இதனால் மூக்குநீர் பாய்தல்,உண்ணாக்கு வளர்ச்சி,பொடியிருமல்,ஐயம்,இரைப்பு ஆகிய நோய்கள் தீரும்.
- இதன் இலைச்சாற்றினை 30 மி.லி அளவில் கொடுக்க பிள்ளை பெற்ற அழுக்கு வெளிப்படும்.
- இதன் சாற்றில் வெள்ளியை பழுக்க காய்ச்சி தோய்த்து எடுக்க வெட்டையாகும்.
- இதன் இலைச்சாற்றினை பலவகை நஞ்சு கடிகளுக்கு மிளகு சேர்த்து கொடுக்க தீரும்.

தவசு முருங்கை சேரும் பிற மருந்துகள்

1.நாகமல்லிகை வேர் மருந்து :

நாகமல்லிகை வேர்

தவசு முருங்கை வேர்

அபின்

சாரணைவேர்

கொடிவேலி வேர்

செய்முறை:

மருந்துச்சரக்குகளை எலுமிச்சம் பழச்சாறுவிட்டு நன்றாக அரைத்து எடுத்துக் கொள்ளவும்.

தீரும்நோய்:

வெளிமூலம்

பிரயோகம்:

வெளிப்பிரயோகம்

SCIENTIFIC VALIDATION - தவசி முருங்கை- Justicia tranquebariensis

Kingdom : Plantae
Phylum : Tracheophyta
Class : Magnoliopsida
Order : Lamiales
Family : Acanthaceae
Genus : Justicia L.
Species : Justicia tranquebariensis L.f.

Anti-inflammatory activity

Anti-inflammatory activity of leaf extracts of Justicia tranquibariensis. Ethanolic extract of the plant showed significant anti-inflammatory activity when compared to the standard drug sample [(15)].

Anthelminthic activity :

Ethanolic extract of justicia tranquibariensis was taken for anthelmintic activity against indian earthworm (pheretima posthuma),roundworm (ascaridia galli) and tapeworms (raillietina spiralis). Four different concentrations (20, 40, 80 and 100 mg/ml) of ethanolic extracts were tested and results were expressed in terms of time for paralysis and time for death of worms. Piperazine citrate (10 mg/ml) was used as reference standard and double distilled water as a control group. The anthelmintic activity of justicia tranquibariensis root extract has therefore been demonstrated for the first time [(16)].

Antibacterial activity:

The antibacterial activity of leaves of justicia tranquebariensis linn., against 10 pathogenic bacteria strains. 25mg/ml showed more level of activity than 5mg/ml against all the tested micro organisms in a dose dependent manner. Both chloroform as well as ethanol extract were found to possess antibacterial activity.

But chloroform extract showed better activity than ethanolic extract against a range of bacteria, as revealed by in vitro agar well diffusion method. The inhibitory effect of the extract was compared with standard antibiotic amoxicillin[17].

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Anti Oxidant activity

The study suggests that the Justicia tranquebariensis have the maximum number of bioactive components and higher amount of antioxidant potential in the ethyl acetate extract, therefore the ethyl acetate extract may act as a significant activity and can be further analyzed for many pathogenic disorders as well as may be helpful in the future for preventing or slowing the progress of diseases involved [18].

தூதுவளை- Solanum trilobatum

வேறுபெயர்கள் :

தமிழ் : அலர்க்கம், சிங்கவல்லி

சமஸ்கிருதம்: அளர்க்கம்

தெலுங்கு : முள்ளமுஸ்தே

வளரியல்பு : கொடி (முட்கொடி)

பயன்படும் உறுப்பு : இலை, பூ, காய்

சுவை : சிறு கைப்பு, கார்ப்பு

தன்மை : வெப்பம்

பிரிவு : கார்ப்பு

செய்கை : வெப்பமுண்டாக்கி
கோழையகற்றி
உரமாக்கி

சமூலம் குணம்

தூது பத்திரி யூண்சுவை யாக்கும்பூ
தாது வைத்தழைப் பித்திடும் காயது
வாத பித்தக பத்தையு மாற்றுவேர்
ஓதும் வல்லிபன் நோயுமொ ழிக்குமே.

(தேரன் குணவாகடம்)

காய்:

காயை வற்றலிட்டு பாகம் செய்து உண்டு வர, ஐயநோய், அழல்நோய், வளிநோய் முதலியன அடங்காது. குடல்வாதம் போம்.

பழம்

இறுகிய மார்புச்சளி, இருமல், முக்குற்றங்கள், நீரேற்றம் இவை போம். இருமுறை வெளிக்குப் போம். பாம்பு நஞ்சு தீரும்.

இலை

இலைக் குடிநீரை, இருமல்,இரைப்பு இவைகளுக்குக் கொடுக்கலாம்.

இலையைப் பிழிந்து காதில்விட காதடைப்பு, காதெழுச்சி போகும்.

இலையை துவையல், குழம்பு முதலியன செய்து உண்ண கோழைக்கட்டு அறும்.

கற்பம்

தூதுவளைக் கீரை, வேர், காய், வத்தல், ஊறுகாய் இவற்றை நாற்பது நாட்கள் புசித்துவரின், கண்ணில் ஏற்பட்ட தீக்குற்ற மிகுதி, மற்ற கண்ணோய்கள் யாவும் நீங்கும்.

நெய்

இதனை நெய் காய்ச்சி கொடுத்து வர ஐய நோய், இருமல்நோய் போகும்.

தூதுவளைக் குடிநீர்

தூதுவளை

ஆடாதோடை

இம்பூறல்

சங்கம்வேர்

சுக்கு

வீழி

திப்பிலி

பற்படாகம்

வழுதுணைவேர்

கண்டக்காலி

செய்முறை

மேற்கண்ட சரக்குகளை சம அளவு எடுத்து, 4பங்கு நீர் விட்டு எட்டில் ஒன்றாக வற்ற வைத்து வடித்துக் கொள்ளவும்.

அனுபானம்

தேன்

தீரும்நோய்கள்

சுரம், ஐயசுரம், வளிசுரம்

தூதுவளை சேரும் பிற மருந்துகள்

1.சர்வவாயு சூரணம்

தூதுவளைச் சமூலம்
மிளகு
இந்துப்பு
இலவங்கம்
நற்சீரகம்
கருஞ்சீரகம்
ஏலரிசி
திப்பிலி
பெருங்காயம்
வெள்ளைப்பூண்டு
அதிமதுரம்
சுக்கு
சிவதை வேர்
சாரணைவேர்
சீமை கடுக்காய்

செய்முறை

சூரணித்து துணியில் வடிகட்டி வைத்துக்கொள்ளவும்.

அளவு

1 ½ வராகன்

அனுபானம்

நெய், தேன், சுக்குக் குடிநீர், சோம்புத் திராவகம்

உபயோகம்

மூலரோகங்கள், குன்மரோகங்கள், கிராணிகள், வயிற்றிரைச்சல், யோனிப்புற்று,யோனிச்சூலை, அழிரணங்கள், துர்மாமிசம், வீக்கம், குஷ்டம்.

(ஆதாரம்- மூலரோக சிகிச்சா போதினி பக்க எண்-121)

2.தூதுவளைக்கிருதம்

தூதுவளைச்சமூலம்
ஆடாதோடைவேர்
கண்டங்கத்திரி வேர்
கடுக்காய்
நெல்லிவற்றல்
தான்றிக்காய்
மிளகு
கோஷ்டம்
தாளிசபத்திரி
திப்பிலி
முசுமுசுக்கை வேர்
இஞ்சிச்சாறு
ஆவின்நெய்
ஆவின்பால்

பிரயோகம்:

உள்பிரயோகம்

அளவு:

1-2 தேக்கரண்டி, இருவேளை

தீரும்நோய்கள் :

ஈளை, இருமல், காசம், சுவாசம், உப்பிசம், இரத்தகாசம்.

(ஆதாரம் : கண்ணுசாமி பரம்பரைவைத்தியம் பக்க எண்-239-241)

3.கருணை சூரணம்

திரிகடுகு
ஓமம்
தூதுவளை வேர்
கண்டங்கத்திரி வேர்
சித்திரமூல வேர்
பெருங்காயம்
வெங்காரம்
அப்பளக்காரம்
இந்துப்பு
சோத்துப்பு
திரிபலா
சீரகம்
கருஞ்சீரகம்
சங்கம்வேர்
சிவதை வேர்
கருணைக்கிழங்கு

செய்முறை

வகைக்கு அரைப்பங்கு எடுத்து சூரணித்துக் கொண்டு, அதனுடன் கருணைக்கிழங்கு சூரணம் ஒரு பங்கு சேர்த்து கலந்து கொள்ளவும்.

அளவு

1 ¼ வராகன்

அனுபானம்

தேன்

தீரும்நோய்கள்

பித்தம், வாயு தீரும்

ஆதாரம்: சரபேந்திர வைத்திய முறைகள் பக்க எண் 184-185

Scientific validation- Solanum trilobatum- தூதுவளை

Kingdom:	Plantae
Clade:	Tracheophytes
Clade:	Angiosperms
Order:	Solanales
Family:	Solanaceae
Genus:	Solanum
Species:	trilobatum

Anti-oxidant activity of Solanum trilobatum:

The chloroform extract of Solanum trilobatum shows an Antioxidative effect in certain concentration by some level of extent in further increased concentration, when compared with anti-oxidative compounds such as Butylated Hydroxy Toluene (BHT), Superoxides, and other compounds, the extract shows more effective anti-oxidant property than the compounds used to test [19]

Chemotherapeutic Efficacy of Solanum trilobatum:

The results obtained in the present study with reference to Lipid peroxidation, antioxidant enzymes and histopathological analysis suggest that Solanum trilobatum and paclitaxel possesses significant chemotherapeutic activity in experimental lung cancer. The antioxidant and chemotherapeutic properties of Solanum trilobatum and paclitaxel may be responsible for the observed chemotherapeutic action. Hence these results suggested that combined chemotherapy of Solanum trilobatum along with paclitaxel could be more advantageous in cancer[20].

Hepatoprotective activity of Solanum trilobatum:

The Hepatoprotective experimental toxicity was induced by known or unknown drug and also using transition metals or heavy metals to induce hepatotoxicity in test animal.which influence the antioxidant enzymes and provides protection against free radical-induced hepatic damage, also authors showed that the Solanum trilobatum was extracted using chloroform and orally administrated to Swiss albino mice.Finally, they found it furnished protection against toxicity induced by the mercury in a dose-dependent manner.The extract obtained from the Solanum trilobatum also experimented that it reduces the lipid content by lowering the cholesterol level in test animals[21] .

Anti-inflammatory effect of Solanum trilobatum:

The antiinflammatory effect of solasodine (50 mg/kg p.o.), of a purified component named sobatum (50 mg/kg p.o.) and of methanol extract of Solanum trilobatum (100 mg/kg p.o.) was evaluated. All the tested articles showed significant antiinflammatory activity[22].

Anti diabetic activity of Solanum trailobatum:

The study was conducted to evaluate the antihyperglycemic activity of the ethanolic extracts of S. trilobatum (STEt) leaves on blood glucose of albino rats. Diabetic rats had much reduced body weight than normal rats. Administration of the extracts at the dose of 400 mg/kg body wt./day resulted in a marked decrease in the levels of fasting blood glucose with a concomitant increase in\body weight. S. trilobatum extract at 400 mg/kg was found to be comparable to glibenclamide. STZdiabetic rats\treated with STEt (400 mg/kg) significantly reversed all these changes to near normal. These results suggest that STEt induce antihyperglycemic as well as antihyperlipidemic activities in STZ-diabetic rats [23].

வெள்ளறுகு- Enicostemma littorale

வேறுபெயர்

தமிழ் பெயர் வெள்ளறுகு, வல்லாரி

வளரியல்பு : பூண்டு

பயன்படும் உறுப்பு: பூண்டு

சுவை : கைப்பு

தன்மை : வெப்பம்

பிரிவு : கார்ப்பு

செய்கை

பசித்தீத்தூண்டி

உரமாக்கி

உடற்றேற்றி

மலமிளக்கி

வெப்பகற்றி

பொதுகுணம்

"குன்மமொடு வாய்வு குடல்வாதம் சூலையிவை

சென்மம்விட் டோடிச் சிதையுங்காண் – வன்முலையாய்

உள்ளுறுகி ரந்திசொறி யொட்டிய சிரங்குமறும்

வெள்ளறுகு தன்மை விரும்பு."

(அகத்தியர் குணவாகடம்)

இதனால் குன்மம், வளிநோய்கள், குடல்வாயு, கீல்பிடிப்பு, நரம்புகளை பற்றிய கழலைகள் , தினவு, சிறு சிரங்கு ஆகிய நோய்கள் குணமாகும்.

இதனுடன் வெண்மிளகு, பூண்டு சேர்த்து கொடுக்க சீழ் ஒழுகும் வெள்ளை குணமாகும்.

அளவு: 2-4 கிராம், குடிநீர் 35-70 மி.லி

வெள்ளறுகு சேரும் பிற மருந்துகள்

1.**வெள்ளறுகாதிக் கியாழம்**

வெள்ளறுகு
கோரைக்கிழங்கு
நாணல் } வகைக்கு 1 பிடி

செய்முறை

மேற்கண்ட சரக்குகளை இடித்து ஒரு படி, நீரில் போட்டு 1/2 படியாக வற்ற வைத்து வடித்துக் கொள்ளவும்.

அனுபானம்

சர்க்கரை

தீரும்நோய்

நீரடைப்புகள்

(ஆதாரம் :சரபேந்திர வைத்திய முறைகள் பக்க எண் 289-290)

2. குஷ்டாமிர்தம்

வெள்ளறுகுச்சாறு
ஆடுதின்னாப்பாளைச்சாறு
வல்லாரைச்சாறு
தேங்காய்ப்பால் — வகைக்கு 1 படி

தாளகம்
மயில்துத்தம்
கருஞ்சீரகம்
கார்போக அரிசி — வகைக்கு 2 ½ பலம்

நெல்லிக்காய் கந்தகம்
நீரடிமுத்து பருப்பு — வகைக்கு 5 பலம்

பிரயோகம் / அளவு

5-6 துளி, **5** நாள் காலை வெறும்வயிற்றில்

அனுபானம்

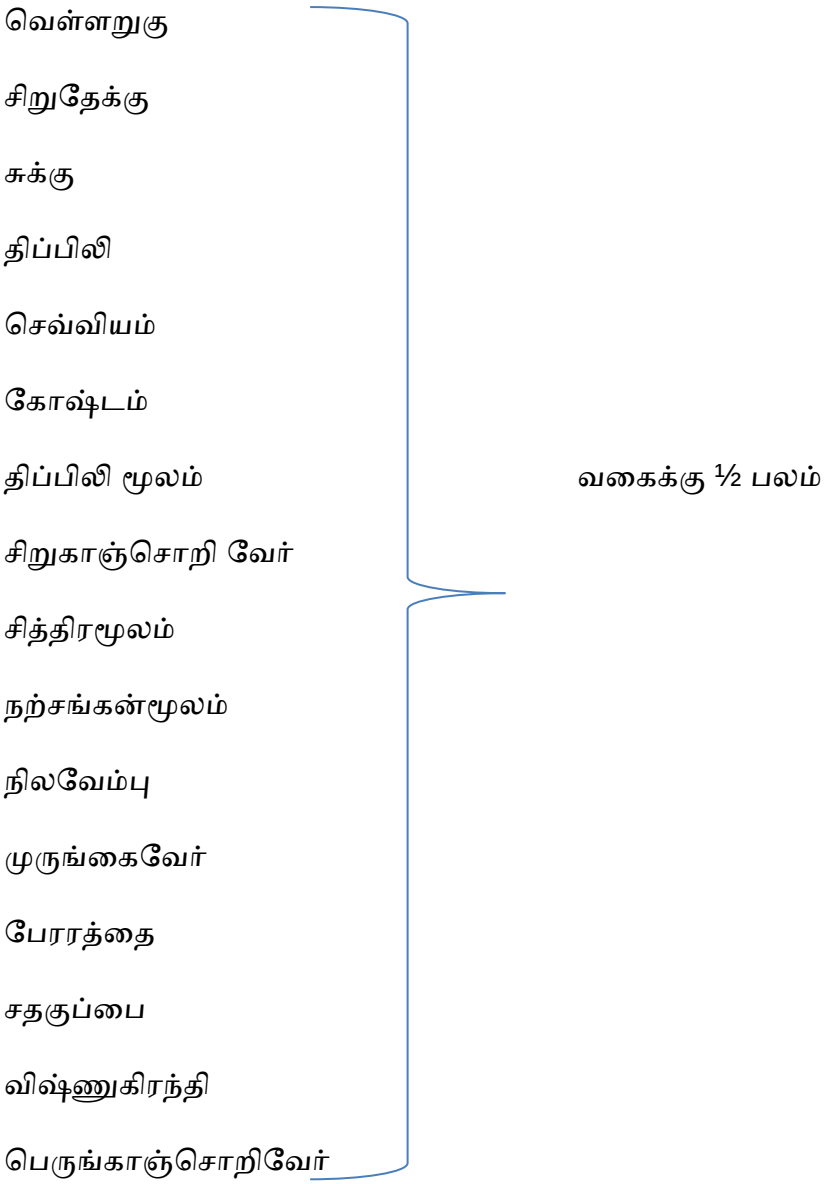
கற்கண்டு சூரணம்

தீரும்நோய்கள்

குஷ்டம், தடிப்பு, சொறி, சிரங்கு, தவளைச்சொறி, மரு, மேக விரணம்.

(ஆதாரம் கண்ணுசாமி பரம்பரை வைத்தியம் பக்க எண்- 453-455)

3.வாத சுரக் கியாழம்



செய்முறை

மேற்கண்ட சரக்குகளை நன்றாக இடித்து எட்டுப் பாகமாகப் பங்கிட்டு வைத்துக் கொள்ளவும். தேவைப்படும் போது, ஒரு பாகத்தை 1/2 படி நீர்விட்டு 30 நாழிகை ஊறிய பின் அடுப்பிலேற்றி வாய்மூடி சிறுதீயாக எரித்து ஆழாக்காகச் சுண்டக் காய்ச்சி வடித்துக் கொள்ள வேண்டும்.

அளவு

இந்த கியாழத்தை மூன்றாகப் பங்கிட்டு 4மணி நேரத்திற்கு ஒருமுறை கொடுக்கவும். மூன்று நாட்கள் ஒன்பது வேளை இவ்வாறு கொடுப்பது அவசியம்.

தீரும்நோய்

வாத சுரம்

(ஆதாரம் கண்ணுசாமி பரம்பரை வைத்தியம் பக்க எண் 50-51)

SCIENTIFIC VALIDATION- Enicostemma Littorale-வெள்ளறுகு

Kingdom: Plantae

Subdivision: Angiospermae

Class: Dicotyledonae

Subclass: Gamopetalae

Order: Gentianales

Family: Gentianaceae

Genus: Enicostemma

Species: Littorale

Nomenclature:

The word Enicostemma is probably formed from the three words, “en” means inside, “icos” means 20 and “stemma” means wreath or circle due to the many flowers arranged in circles in the leaf axils along the stem [(24)].

Antitumour activity of E. littorale:

The antitumour activity of methanolic extract of E. littorale has been evaluated against Dalton's ascitic lymphoma (DAL) in Swiss albino mice by Kavimani et al. A significant enhancement of mean survival time of methanolic extract of E. littorale treated tumour bearing mice was found with respect to control group. Treating with methanolic extract of E. littorale enhances peritoneal cell counts. When these methanolic extract of E. littorale treated animals underwent intraperitoneal inoculation with DAL cells, tumour cell growth was found to be inhibited. After 14 days of inoculation, methanolic extract of E. littorale is able to reverse the changes in the haematological parameters, protein and PCV consequent to tumour inoculation.

Diabetic neuropathy activity of E. littorale

Poor glycemic control and oxidative stress is one of the main reasons for the development of diabetic neuropathy. The protective effects of E. littorale Blume was investigated for hypoglycemic and antioxidant effect in alloxan induced diabetic neuropathy in male Charles foster rats by Bhatt et al [36]. Nociceptive responses were compared by formalin and tail flick in hot immersion test in both diabetic and non diabetic rats. Treating with E. littorale extract for 45 days significantly improved nociception in diabetic rats. The changes in lipid peroxidation and antioxidant enzymes like SOD, GPx and CAT levels, decrease in Na-K+ ATPase activity were also restored by E. littorale treatment. This study provides an experimental evidence for the preventive effect of E. littorale on nerve function and oxidative stress in animal model of diabetic neuropathy. Hence, E. littorale may be clinically tried for treating diabetic neuropathy since it was used as a folklore medicine in diabetic patients[(25)].

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Antibacterial effect of Enicostemma littoreale:

The study systematically evaluated the antibacterial effect of Enicostemma littoreale nanoparticles against a Gram Positive and Gram negative bacteria. The Ag NPs and ZnO NPs effectively inhibited the growth of both Gram Negative and Gram Positive bacteria. Our results show that Ag NPs and ZnO NPs synthesized using an aqueous extract of Enicostemma littorale have potential antibacterial activity against both Gram Negative and Gram Positive bacteria[(26)].

Phytochemical value of Enicostemma littoreale:

The crude extracts of E.littorale was used to assess its anti-microbial activity against Gram positive ,Gram negative bacteria and some fungal strains through the above mentioned assays. All the phytochemical tests showed the presence of tannins, flavanoids, alkaloids, betacyanin, quinone, glycosides, and phenols in methanolic crude extract. Interesting we observed that all the four extracts exhibited significant antimicrobial activity against all microorganisms used in the study[(27)].

கொட்டைக்கரந்தை - (Sphaeranthus indicus)

வளரியல்பு : பூண்டு

பயன்படும் உறுப்பு: இலை, பூ, விதை,
வேர்ப்பட்டை.

சுவை : கைப்பு

தன்மை : வெப்பம்

பிரிவு :கார்ப்பு

செய்கை

இலை, பூ :உடற்றேற்றி, உள்ளழலாற்றி

விதை, வேர் : பசித்தீத்தூண்டி, புழுக்கொல்லி

சிறப்புச்செய்கை:

தூய்மையாக்கி

குளிர்ச்சியுண்டாக்கி

உரமாக்கி

குணம் :

கொட்டைக் கரந்தைதனைக் கூசாம லுண்டவர்க்கு

வெட்டை தணியுமதி மேகம்போந் – துட்டச்

சொறி சிரங்கு வன்கரப்பான் றோற்றாது நாளும்

மறிமலமுந் தானிறங்கு மால்.

(அகத்தியர் குணவாகடம்)

இதனால் ஒழுக்கு வெள்ளை, கரப்பான், சொறி, சிரங்கு இவை தீருவது மட்டுமல்லாமல் மறிபட்ட எருவும் கழியும். மேலும் வளி, வெறி, சினைப்பு முதலியவையும் அழியும். தோற்பிணியும் போம்.

சமூலம்

இப்பூண்டினை உலர்த்திப் பொடி செய்து வேளைக்கு 4 கிராம் எடை சாப்பிட்டு வரலாம் அல்லது இதன் கிழங்கைக் குடிநீரிட்டுச் சாப்பிட்டு வந்தால் குணத்தில் கூறப்பட்டுள்ள நோய்கள் நீங்கும்.

இலை

இலையை நிழலிலுலர்த்திப் பொடி செய்து, வேளைக்கு 1கிராம் எடை வீதம், தினம் இருவேளை கொடுத்து வர, தோலைப் பற்றிய நோய்கள் தீரும். ஆண்மை உண்டாகும்.

பூ

இதைப் பொடி செய்து உண்டுவரின் உரமாக்கியாக செயல்படுவதுடன், உடலுக்கு குளிர்ச்சியையும் உண்டு பண்ணும்.

விதை

விதையைப் பொடித்துத் தேனில் கலந்து கொடுக்க செரியாமை, இருமல் தீரும். வயிற்றுப்புழு வெளிப்படும்.

வேர்

வேர் பொடித்ததை 2கிராம் எடை கொடுக்க, வயிற்றுப் புழுக்களைக் கொல்லும்.

வேர்ப்பட்டை

இதை அரைத்து வெண்ணெய்யில் கலந்து கொடுக்க, குருதி எருமுளை போகும். இதையே வெளிப்பூச்சாகவும் பயன்படுத்தலாம்.

நல்லெண்ணெய் உடன் 7-15 மிலி வேர்ப்பட்டைச் சாறு கலந்து, உச்சிக் கரண்டி வீதம் ஒரு மண்டலம் சாப்பிட்டு வர உடல்வன்மை பெறும். ஆண்மை பெருகும்.

கொட்டைக் கரந்தை சேரும் பிற மருந்துகள்

இலவணச் செந்தூரம்

சோற்றுப்பு	5 பலம்
கொட்டைக் கரந்தைச் சாறு	

செய்முறை :

சோற்றுப்பை கல்வத்திலிட்டு கொட்டைக் கரந்தைச் சாறுவிட்டு இரண்டு சாமம் அரைத்து வில்லை தட்டி கடும் இரவியில் உலர்த்தி, அகலில் வைத்து மேல் அகல் மூடி, சீலை செய்து 10-12 வறட்டியில் புடமிடவும்.இவ்வாறுசெய்து, 10தடவை புடமிட ரோஜாப்பூ வர்ணத்தில் மருந்து கிடைக்கும். இதனை அரைத்து குப்பியில் அடைக்கவும்.

அளவு

குன்றி எடை, இருவேளை

அனுபானம்

தேன்

தீரும்நோய்கள்

குன்மம், செரியாமை, பேதி, வயிற்றுவலி

(ஆதாரம் கண்ணுசாமி பரம்பரை வைத்தியம். பக்க எண் 364-365)

SCIENTIFIC VALIDATION- Sphaeranthus indicus- கொட்டைக்கரந்தை

Kingdom: Plantae
Order: Asterales
Family: Asteraceae
Genus: Sphaeranthus
Species: S. indicus

Antihyperlipidemic activity of *Sphaeranthus indicus*:

Hyperlipidemia is the most prevalent indicator for susceptibility to atherosclerotic heart disease. The antihyperlipidemic activity of the alcoholic flower head extract of *S. indicus* was investigated in atherogenic diet induced hyperlipidemia in rats. The alcoholic extract when administered at a dose of 500 mg kg^{-1} effectively suppressed the atherogenic diet induced hyperlipidemia. The extract also caused considerable decrease in body weight, total cholesterol, triglyceride, Low-Density Lipoprotein (LDL) and Very Low Density Lipoprotein (VLDL) whereas significant increase was observed in the level of High-Density Lipoprotein (HDL) as compared to control. The extract showed a marked reduction in LDL: HDL-c ratio and atherogenic index. It was concluded that effects of *S. indicus* extract may be due to an increase in the activity of Lecithin: Cholesterol acetyl transferase, which incorporates free cholesterol free LDL into HDL and transfers it back to VLDL and intermediate density lipoprotein. The reduction in the triglyceride level might be due to increase in activity of the endothelium bound lipoprotein lipase. *S. indicus* may, therefore, be capable of exerting a potential protective role in atherosclerosis[(28)].

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Antimicrobial Activity of *Sphaeranthus indicus*

The *S. indicus* hexane extracts of flower and aerial parts showed good antibacterial activity against gram positive organisms. Flower extracts were more active than the aerial parts. It also possessed strong antifungal activity against *Candida* and other tested fungi. The findings of the present research may lead to the development of natural antimicrobial agents[29].

Antidiabetic property of *Sphaeranthus indicus*

The anti hyperglycaemic effects of Sphaeranthus indicus extract in rats rendered diabetic by nicotinamide (120 mgkg−1 i.p.) and streptozotocin (STZ) (60 mgkg−1 i.p). Fasting plasma glucose levels, serum insulin levels, serum lipid profiles, magnesium levels, glycosylated haemoglobin, changes in body weight and liver glycogen levels were evaluated in normal and diabetic rats. Oral administration of S. indicus for 15 days resulted in significant decrease in blood glucose levels and increases in hepatic glycogen and plasma insulin levels. Fasting normal rats treated with the alcoholic extract of S. indicus showed significant improvement in oral glucose tolerance test. Glibenclamide was used as a reference standard[30] .

4. MATERIALS AND METHODS

4.1 PREPARATION OF THE TEST DRUG:

Collection of raw drugs:

The herbal drugs such as:

1. Nilavaarai ilai (Cassia senna)

2. Thavasi murungai samoolam (Justicia tranquebariensis)

3. Sivanaar vembu samoolam (Indigofera aspalathoides)

4. Vellarugu samoolam (Enicostemma littorale)

5. Thoothuvalai samoolam (Solanum trilobatum)

6. Kottai karanthai samoolam (Sphaeranthus indicus)

Some of the drugs were collected from the Kolli hills and other drugs were purchased from the authenticated raw drug store *K Ramaswamy Chetty, a Siddha and Ayurveda Raw Material Store*, Chennai-001.

4.1.1 Authentication:

The herbal drugs were identified and authenticated by Assistant Professor of Medicinal Botany, National Institute of Siddha, Chennai-47.

4.1.2 Method of purification[31] :

The impurities and nerve roots from the Nilavaarai leaves were removed then wash with water and dried well, and collect the other whole plants (Samoolam) remove the impurities like sand, dirt wash with water and dried well in the sun light. The impurities of other raw drug were removed and dried in the sun light for two days then the all raw drugs were kept and stored in water resistant bag.

Preparations of Kiranthi Mega Chooranam [32] :

Ingredients: -

Nilavaarai ilai

Thavasi murungai samoolam

Sivanaar vembu samoolam — Equal quantity

Vellarugu samoolam

Thoothuvalai samoolam

Kottai karanthai samoolam

Method of preparation:

After purification of all the above ingredients are dried well and equal quantity the plant leaves and Whole plants were taken make as fine powder and filtered well(Vasthira kayam) mix chooranam and stored in the air tight container

Dosage: 6-8 gram

Indication: Kiranthi (Syphilis), Kadividam (Poisonous bites), Kuttam (Skin diseases), Thadippu (Urticaria), Megasoolai (Syphilitic arthritis).

INCREDIENTS OF KIRANTHI MEGA CHOORANAM

Sivanaar vembu (Indigofera aspalathoides)

Nilavaarai (Cassieae senna)

Vellarugu (E.littorale)

Thavasi murungai (Justicia traiquibensis)

Thuthuvelai (Solanum trilobatum)

Kottai karanthai (Sphaeranthus indicus)

STANDARDIZATION OF KIRANTHI MEGA CHOORANAM

4.2. Qualitative analysis:

This covers identification and characterization of crude drug with respect to phytochemical constituent. It employs different analytical technique to detect and isolate the active constituents. Phytochemical screening techniques involve botanical identification, extraction with suitable solvents, purification, and characterization of the active constituents of pharmaceutical importance[33].

a)Physico-Chemical analysis:

The Physico- chemical properties of Kiranthi Mega Chooranam was carried as per standard procedure at The Tamil Nadu Dr. M.G.R. Medical University No.69, Anna Salai, Guindy, and Chennai-600032.

b) Organoleptic characterization:

The Organoleptic characterization of the polyherbal formulation Kiranthi Mega Chooranam refers to its color, taste, odour and texture. The taste was distinctively classified as bitter, salty, sour, astringent, and sweet.

Procedure:

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

5 g of the drug without preliminary drying was weighed accurately in a tarred evaporating dish, dried at 105ºC for 5 hours, cooled in desiccator and weighed. Later the drying and weighing process was continued at one-hour interval until difference between two successive weighing's of sample corresponds to not more than 0.25 percent. When the constant weight was obtained the percentage of moisture content were calculated with reference to the air-dried drug.

2. Determination of Total Ash:

2 to 3 g of drug was weighed in the pre weighed and tarred Gooch crucible, kept in the muffle furnace at a temperature not exceeding 450ºC until the ash free from carbon was obtained, then cooled in desiccator, weighed and the percentage of the total ash content were calculated with reference to the air-dried drug.

3. Determination of Acid Insoluble Ash:

The ash obtained from total ash was boiled with 25ml of dilute hydrochloric acid for 5-minute sand the insoluble matter was collected in an ash less filter paper, washed with hot water and ignited to constant weight. Later the percentage of the acid insoluble ash content was calculated with reference to the air-dried drug.

4. Determination of Water-soluble extractive:

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

5. Determination of Alcohol soluble extractive:

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

6. Particle size for the powdered drug formulation:

The drug to be tested was accurately measured and evenly distributed over the mesh in sieve apparatus of mesh size 100micron- 1mm. Later the particle size was analyzed by sieving it at uniform speed. As the particles passes through the sieves it shows that drug particle size is less than that of the sieve size.

b) Biochemical analysis

The bio-chemical analysis of Kiranthi Mega Chooranam as done at Biochemistry lab National Institute of Siddha, Chennai-47.

S.NO	EXPERIMENT	OBSERVATION	INFERENCE
1.	**Appearance of the sample**	Dark brown in color	
2.	**Solubility:** A little of the sample is shaken well with distilled water	Sparingly soluble	Presence of silicate
3.	**Action of heat:** A small amount of the sample is taken in a dry test tube and heated gartly at first and then strong.	White fumes evolved	Presence of carbonate
4.	**Flame test:** A small amount of the sample is made into a paste with con.HCL in a watch glass and introduced into non luminous part of the Bunsen flame.	Bluish green flame not appeared.	Absence of copper
5.	**Ash test:** A filter paper is soaked into a mixture of sample and cobalt nitrate solution and introduced into the Bunsen flame and ignited	No yellow colour flame	Absence of sodium

Preparation of Extract:

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

S.NO	EXPERIMENT	OBSERVATION	INFERENCE
	TEST FOR ACID RADICALS		
1.	**Test for sulphate:** 2ml of the above prepared extract is taken in a test tube to this added 2ml of 4% ammonium oxalate solution.	No cloudy appearance present.	Absence of sulphate.
2.	**Test for chloride:** 2ml of the above prepared extract is added with diluted HNo3 till the effervescence ceases. Then 2 ml of silver nitrate solution is added.	No cloudy appearance present.	Absence of chloride.
3.	**Test for phosphate:** 2ml of the extract is treated with 2ml of ammonium molybdate solution and 2ml of con. Hno3.	No cloudy yellow appearance present.	Absence of phosphate.
4.	**Test for carbonate:** 2ml of the extract is treated with 2ml magnesium sulphate solution.	Cloudy appearance present.	Presence of carbonate.

5.	**Test for sulphide:** 1gm of the substance is treated with 2ml of con HCL.	No rotten egg smelling gas evolved.	Absence of sulphide.
6.	**Test for fluoride and oxalate:** 2ml of extract is added with 2ml of dil.Acetic acid and 2ml calcium chloride solution and heated.	No cloudy appearance.	Absence of fluoride and oxalate.
7.	**Test for nitrite:** 3 drops of the extract is placed on a filter paper, on that 2drops of acetic acid and 2 drops of benzidine solution is placed.	No characteristic changes.	Absence of nitrite.
8.	**Test for borate:** 2 pinches of the substance is made into paste by using sulphuric acid and alcohol (95%) and introduced into the blue flame.	Bluish green colour flame not appeared.	Absence of borate.
II. Test for basic radicals			
1.	**Test for lead:** 2ml of the extract is added with 2ml of potassium iodide solution.	No yellow precipitate is obtained.	Absence of lead.
2.	**Test for copper:** One pinch of substance is made into paste with con Hcl in a watch glass and introduced into the non luminuous part of flame.	Blue colour flame not appeared.	Absence of copper.

3.	**Test for aluminium:** 2ml of the extract sodium hydroxide is added in drops to excess	Yellow colour appeared	Presence of aluminium
4.	**Test for iron:** 2ml of extract add 2ml of ammonium thiocyanate solution and 2ml of con HNO3 is added	Mild red colour appeared	Presence of iron
5.	**Test for zinc:** 2ml of the extract sodium hydroxide solution is added in drops to excess.	White precipitate is formed.	Presence of zinc
6.	**Test for calcium:** 2ml of the extract is added with 2ml of 4% ammonium oxalate solution.	Cloudy appearance and white precipitate obtained	Presence of calcium
7.	**Test for magnesium:** 2ml of extract sodium hydroxide solution is added in drops to excess.	White precipitate is obtained	Presence of magnesium
8.	**Test for ammonium:** 2ml of extract few ml of nessler's reagent and excess of sodium hydroxide solution are added.	No brown colour appeared	Absence of ammonium
9.	**Test for potassium:** A pinch of substance is treated with 2ml of sodium nitrite solution and then treated with 2ml of cobalt nitrate in 30% glacial acetic acid.	Yellowish precipitate is obtained	Presence of potassium

10.	**Test for sodium:** 2 pinches of the substance is made into paste by using HCL and introduced into the blue flame of Bunsen burner.	No yellow colour flame appeared	Absence of sodium
11	**Test for mercury**: 2ml of the extract is treated with 2ml of sodium hydroxide solution	No yellow precipitate is obtained	Absence of mercury
12.	**Test for arsenic:** 2ml of the extract is treated with 2ml of sodium hydroxide solution.	No brownish red precipitate is obtained	Absence of arsenic

III. Miscellaneous			
1.	**Test for starch:** 2ml of extract is treated with weak iodine solultion.	Sky blue colour developed	Presence of starch
2.	**Test for reducing sugar:** 5ml of benedict's qualitative solution is taken in a test tube and allowed to boil for 2 minutes and added 8 to 10 drops of the extract and again boil it for 2 minutes. The colour changes are noted.	No brick red colour developed	Absence of reducing sugar
3.	**Test for the alkaloids:** a. 2ml of the extract is treated with 2ml of potassium iodide solution. b. 2ml of extract is treated with 2ml of picric acid. c. 2ml of the extract is treated with 2ml of phosphor tungstic acid.	Yellow colour developed	Presence of alkaloids

4.	**Test for tannic acid:** 2ml of extract is treated with 2ml of ferric chloride solution.	No black precipitate is obtained	Absence of tannic acid
5.	**Test for unsaturated compound:** 2ml of extract 2ml of potassium permanganate solution is added.	Potassium permanganate is not decolourised.	Absence of unsaturated compound.
6.	**Test for amino acid:** 2 drops of the extract is placed on a filter paper and dried well.	Not violet colour developed.	Absence of amino acids.
7.	**Test for type of compound:** 2ml of the extract is treated with 2ml of ferric chloride solution.	Red colour developed.	Anti pyrine, aliphatic amino acids and meconic acid are present.

c) Phytochemical screening of Kiranthi Mega Chooranam

The preliminary phytochemical screening test was carried out for extract of **Kiranthi Mega Chooranam** as per the standard procedure at The Tamilnadu Dr.M.G.R. Medical University, Guindy, Chennai 32.

Detection of alkaloids:

Extracts were dissolved individually in diluted hydrochloric acid and filtered.

Mayer's test:

2 ml of extract was treated with few drops of Mayers‟ reagent, formation of yellow coloured precipitate indicates the presence of alkaloids.

Detection of carbohydrate:

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

Molisch's test:

2 ml of filtrate was treated with few drops of alcoholic Alpha naphthol solution in test tubes. Formation of the violet ring at the junction indicates presence of carbohydrates.

Detection of glycosides:

Extract were hydrolyzed with dil.HCL and then subjected to test for glycosides.

a) Modified Borntrager's test:

Extract were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonium solution. Formation of rose- pink color in the ammonical layer indicates the presence of anthranol glycosides.

b) Cardiac glycoside (Keller- Killiani test):

Extract was shaken with distilled water (5ml). To this, glacial acetic acid(ml) containing a few drops of ferric chloride was added, followed by H_2SO_4 (1ml) along the side of the test tube. The formation of brown ring at the interface gives positive indication for cardiac glycoside and a violet ring may appear below the brown ring.

Detection of saponins:

Foam test:

0.5-gram extract was shaken with 2 ml of water. If foam produced persists for 10minutes, it indicates the presence of saponins.

Detection of phenols:

Ferric Chloride test

2 ml of extracts was treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Detection of tannins:

Gelatin test:

To the extracts, 1% of gelatin solution containing sodium chloride was added, formation of white precipitate indicates the presence of tannins.

Detection of flavonoids:

Alkaline reagent test:

Extract was treated with few drops of 10% sodium hydroxide, formation of intense yellow colour then on addition of diluted hydrochloric acid it becomes colourless, it indicates the presents of flavonoids.

Lead acetate test:

Extract was treated with few drops of lead acetate solution, yellow colour precipitate indicates presence of flavonoids.

Detection of proteins:

a) Xanthoprotein test:

The extract was treated with few drops of conc. Nitric acid. Formation of yellow color indicates the presence of proteins.

Detection of amino acids:

a) Ninhydrin test:

To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue color indicates the presence of amino acid.

Detection of diterpenes:

Copper Acetate test:

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

Test for gum and mucilage:

The extract was dissolved in 10 ml of distilled water and to this 2ml of absolute alcohol with the constant stirring white cloudy precipitate indicates the presence of gum and mucilage.

Test for Quinones:

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

4.3. Quantitative analysis.

Standardization of herbal medicines is the process of prescribing a set of standards or inherent characteristics, constant parameters, definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety and reproducibility. It is the process of developing and agreeing upon technical standards[(34)].

The High Performance Thin Layer Chromatography Analysis and Heavy metal Analysis was carried out as per standard procedure at Noble Research Solutions, Chennai 99.

a) High Performance Thin Layer Chromatography:

HPTLC method is the modern sophisticated and automated separation technique derived from TLC. Precoated HPTLC graded plates and auto sampler was used to achieve precision, sensitive, significant separation both qualitatively and quantitatively. HPTLC is a valuable quality assessment tool for the evaluation of botanical material efficiently and cost effectively. HPTLC method offers high degree of selectivity, sensitivity and rapidity combined with single step sample preparation. Thus, this method can be conveniently adopted routine quality control analysis. It provides chromatographic finger print of phytochemicals which is suitable for conforming the identity and purity of medicinal plant raw material.

Chromatogram development:

It was carried out in CAMAG TLC SCANNER III sample elution was carried out according to the adsorption capability of the component to be analysed. After elution plates were taken out of the chamber and dried [35].

b) Heavy metal analysis:

Environment conditions in developing countries, pollution from irrigation water, the atmosphere and soil, sterilization methods and in adequate storage conditions all play an important role in contamination of medicinal plants by heavy metals [36].

Standard: Hg, As, Pb and Cd - Sigma

Methodology:

Atomic Absorption Spectrometry (AAS) is a very common and reliable technique for detecting metals and metalloids in environmental samples. The total heavy metal content of the sample was performed by Atomic Absorption Spectrometry (AAS) Model AA 240 Series. In order to determination the heavy metals such as mercury, arsenic, lead and cadmium concentrations in the test item.

Sample Digestion:

Test sample was digested with 1mol/L HCL for determination of arsenic and mercury. Similarly, for the determination of lead and cadmium the sample were digested with 1mol/L of HNO3.

Standard reparation:

As & Hg- 100 ppm sample in 1mol/L HCl

Cd & Pb- 100 ppm sample in 1mol/L HNO3

c)Microbial contamination:

The microbial load and the test for specific pathogen and pesticide residue of Kiranthi Mega Chooranam was carried as per standard procedure at V.S Clinical research and hospital Pvt. Ltd, Chennai-13.

Determination of microbial contamination:

10 g of the preparation being examined was dissolved properly in a buffered sodium chloride peptone solution, pH 7.0 with no antimicrobial activity. Later the volume was made up to 100ml using the same media chosen, maintaining the ph. For determining bacterial and fungal count petri dishes 9 to 10 cm in diameter was plated with15 ml of liquefied casein soyabean digest agar and Sabouraud dextrose agar with antibiotics at not more than 45º along with 1 ml of the pre-treated preparation. Alternatively, it was also spread plated with the pre-treated preparation on the surface of solidified medium in a Petri dish of the same diameter. The drug of preparation was diluted so that a colony count of not more than 300could be expected. Two petri plates were plated at same dilution and incubated at 30º to 35ºC for 5 days for bacteria and 20º to 25ºC for 5 days for fungus until a more reliable count was obtained in a shorter time. After incubation the results were chosen from the plate with greatest number of colonies but not more than 300 colonies.

Test for specific pathogen:

10 g of the preparation being examined was dissolved properly in a buffered sodium chloride peptone solution, pH 7.0 with no antimicrobial activity. Later the volume was made up to 100mlusing the same media chosen, maintaining the pH. Escherichia coli10 ml of pretreated drug preparation mixed with 50ml of nutrient broth, placed in a screw capped container shaken properly and allowed to stand for 1 hour. Later the cap was loosened and incubated at 37^{O}C for 18-24hrs. As a primary test for E. coli 5ml of Mac Conkey broth mixed well with 1ml of enrichment culture in a centrifuge tube, incubated in water bath at 36-38^{O}C for about 2 days. As the acid and gas formation was not found it indicates that the drug to be tested was free from E. coli. Salmonella spp.10 ml of pre-treated drug preparation along with 1 g of the product was added with 100 ml of nutrient broth in a sterile screw capped jar, shaken properly and, allowed to stand for 4 hours. Later the cap was loosened and incubated at 35º to 37ºC for 24 hours. As a primary test 1.0 ml of enrichment culture was added to 2 tubes containing 10ml of selenite F broth and tetrathionatebile-brilliant green broth, incubated at 36º to 38ºC for 48 hours. From each of these two cultures sub culturing was done by two of the following four agar media like bismuth sulphate agar, brilliant green agar, desoxycholatecitrate agar and xylose-lysine-desoxycholate agar then Incubated at 36º to 38º C for 18 to 24 hours. As the colonies were not found it indicates that the drug to be tested was free from Salmonella spp.

Pseudomonas aeruginosa:

The pre-treated preparation was inoculated in100 ml of fluid soyabean-casein digest medium along with 1g of preparation being examined, mixed well and incubated at 35º to 37ºC for 24 to48 hours. On examination of media if growth was found streaked aportion of the medium on the surface of cetrimide agar medium, each plated on Petridishes. Covered and incubated at 35º to37º for 18 to 24 hours. Streaked the colonies from the agar surface of cetrimide agar on the surfaces of pseudomonas agar medium for detection of fluorescein and pseudomonas agar Medium for detection of pyocyanin contained in Petri dishes, covered, inverted and incubated at 33º to 37ºC for not less than 3 days. The streaked surfaces were examined under ultra-violet light, placed 2 to 3 drops of a freshly prepared 1% w/v solution of N, N, N1, N1-tetramethyl-4-phenylenediamine dihydrochloride on filter paper and prepared the smear. As pink color formation was not found it indicates that the drug to be tested was free from Pseudo monasaeruginosa.

Staphylococcus aureus:

The pre-treated preparation was inoculated in 100 ml of fluid soyabean-casein digest medium along with 1g of preparation being examined, mixed well and incubated at 35º to 37ºC for 24 to48 hours. If growth occurs, coagulase test was conducted by transferring the colonies from the agar surface of media to individual tubes each containing 0.5 ml of mammalian preferably rabbit plasma with or without additives incubated in water-bath at 37ºC and examined at 3 hours and Subsequently at suitable intervals up to 24 hours. As coagulation was not found it indicates that the drug to be tested was free from Staphylococcus aureus.

Determination of pesticide residues:

There are a number of factors which contribute to heavy metal contamination on agricultural soils including fertilizers, pesticides, atmospheric deposition from town wastes, industrial emissions, and metal production. For analysis of the test drug reagents without any external components were chosen and the samples were analyzed using Gas chromatographic methods. Later the amount of different components such as organophosphorus, organochlorine and pyrethroid contents was recorded.

d) Aflatoxin:

The presence of fungi should be carefully investigated and/or monitored, since some common species produce toxins, especially aflatoxins. Aflatoxins in herbal drugs can be dangerous to health even if they are absorbed in minute amounts. Aflatoxin-producing fungi sometimes build up during storage. Procedures for the determination of aflatoxin contamination in herbal drugs are published by the WHO . After a thorough clean-up procedure, TLC is used for confirmation. In addition to the risk of bacterial and viral contamination, herbal remedies may also be contaminated with microbial toxins, and as such, bacterial endotoxins and mycotoxins, at times may also be an issue[37].

The Aflatoxins analysis of the test sample was carried out as per standard procedure at Noble Research Solutions, Chennai-99.

Standard

Aflatoxin B1

Aflatoxin B2

Aflatoxin G1

Aflatoxin G2

Solvent:

Standard samples were dissolved in a mixture of chloroform and acetonitrile (9.8:0.2) to obtain a solution having concentrations of 0.5 µg per ml each of aflatoxin B1 and aflatoxin G1 and 0.1 µg per ml each of aflatoxin B2 and aflatoxin G2.

Test solution: Concentration 1 µg per ml

Procedure:

Standard Aflatoxin was applied on to the surface to pre coated TLC plate in the volume of 2.5 µL, 5µL, 7.5 µL and 10 µL. Similarly, the test sample was placed and Allow the spots to dry and develop the chromatogram in an unsaturated chamber containing a solvent system consisting of a mixture of chloroform, acetone and isopropyl alcohol (85:10: 5) until the solvent front has moved not less than 15 cm from the origin. Remove the plate from the developing chamber, mark the solvent from and allow the plate to air-dry. Locate the spots on the plate by examination under UV light at 365 nm.

4.4.Toxicological evaluation of Kiranthi Mega Chooranam

The following in vivo toxicity studies were carried out on Kiranthi Mega Chooranam (KMC) by World Health Organization (WHO) guideline for testing traditional medicines[38] .

Acute Oral Toxicity study (WHO Guideline)

Long term toxicity study (WHO Guideline)

The toxicity studies were carried out at National Institute of Siddha. The study was done after getting permission from the Institutional Animal Ethics Committee.

IAEC Approved No**: NIS/IAEC-II /28082018/19**

For acute and long-term toxicity study -

For Acute and Long-term toxicity studies test animals were obtained from Tamil Nadu Veterinary and Animal Sciences University, Madhavaram. Animals were kept in animal house, National Institute of Siddha, Chennai.

Description of the method:

Selection of the animals:

Animals were selected as per WHO guideline. Healthy adult animals of Wistar albino rat, both male and female rats were used for acute oral toxicity study and Long term toxicity study. The female animals used in the studies were nulliparous and non-pregnant.

Housing and feeding conditions:

Temperature: In the experimental animal room: 22ºC (± 3ºC)

Humidity: 60 ± 10 %

Lighting: Artificial, the sequence being 12 hours light, 12 hours dark.

The animals were housed in polypropylene cages provided with bedding of husk. The animals had free access to RO water.

For feeding, Standard pellet diet (bought from SaiMeera foods pvt. Ltd, Bangalore) was used.

Preparation of animals:

The animals were randomly selected, to permit individual identification by cage number and individual marking on the fur of each animal was made with picric acid. The animals were kept in their cages for 7 days prior to dosing to allow for acclimatization to the laboratory conditions. The principles of laboratory animal care were followed.

Test Substance:

Kiranthi Mega Chooranam (KMC) was brown in color, without taste and odour. The drug was dissolved in ghee to obtain and ensure the uniformity in drug distribution.

Route of administration:

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

Preparation of doses:

The stock solution was prepared freshly as dose per animal suspended in 1ml hot water**.**

TOXICOLOGICAL PROFILE:

4.4.1. Acute oral toxicity study (WHO guidelines)

- Species and strain : Wistar Albino rat
- Sex : Male and Female
- Age/Weight : 6 weeks/150-175gms
- Test guideline : WHO guidelines
- Groups : Grouped by randomization
- Duration of exposure : Single dose
- Study duration : 14 days
- Number of animals : 10 / group,
- Route of administration : Oral

Number of animals and dose level:

Acute oral toxicity of the test drug will be evaluated in rats following WHO guideline. Animals will be divided into 2 groups by randomization method, each group containing 10 animals (5 females and 5 males). One group as control and the other as test group. Control group is treated with hot water and test group were treated with the test drug Kiranthi Mega Chooranam ten times more than the therapeutic dose (2000mg per kg b.wt)

GROUP	NO OF RATS
Group-1 Control (Hot water)	5 Male, 5 Female
Group-2 Test drug (2000 mg /kg b.wt)	5 Male, 5 Female

Administration of doses:

The test drug was administered in a single dose by using oral gavage. Animals were fasted prior to drug administration. Following the period of fasting, the animals were weighed and test drug was administered. The control groups received equal volume hot water. The test drug was administered at 10 times the therapeutic dose (2000 mg / kg b.wt). The food was withheld for 3-4 hours after dosing the animal.

Observations:

Observations were made and recorded systematically and continuously observed after the drug administration as per the guideline.

- ½ hour, 1 hour, 2 hours, 4 hours and up to 24 hours observation.
- All rats were observed twice daily for further 14 days.
- Body weight were Calculated weekly once.
- Feed and water intake were Calculated daily.

Cage side observations:

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

Gross necropsy:

At the end of 14^{Th} day animals will be sacrificed for gross necropsy. It includes examination of the external surface of the body, all orifices, and organs like brain, thymus, lungs, heart, spleen, liver, kidneys, adrenals and sex organs of all animals. If there will any occurrence of mortality during the trial period, the vital organs will be subjected to Necropsy.

4.4.2. Long term toxicity study : (WHO guidelines)

- Species and strain : Wistar albino rats
- Sex : Male and Female
- Age/Weight : 6 weeks/150-175mg
- Test guideline : WHO guidelines
- Groups : Grouped by randomization
- Duration : 90 days
- Number of animals : 20/group (10/sex)
- Route of administration : Oral

Grouping of animals:

Long term toxicity study was carried out at different dose levels. The animals in both sex was divided in four groups (group I, II, III & IV). Each group consists of 20 animals (10 males and 10 females). Group-I served as control and the other three groups II, III and IV for test drug of Low dose (108 mg/kg/b.wt), Mid dose (540 mg/kg/b.wt) and High dose (1080 mg/kg b.wt) respectively The low dose was calculated from the therapeutic dose (6g) and body surface area of rats.

No of animals used for long term toxicity study:

GROUPS	NO OF RATS
Control (Hot water)	(10 Male, 10 Female)
Low Dose	(10 Male, 10 Female)
Mid Dose	(10 Male, 10 Female)
High Dose	(10 Male, 10 Female)

Total 80 (40 Female + 40 Male)

Administration of doses:

The animals were dosed with the test drug daily for a period of 90 days. The test drug mixed with ghee and was administered by oral gavage, and this was done in a single dose to the animals once in daily for 90 days.

Observations:

During the study, body weight of the animals, water and food consumption were evaluated weekly; mortality events were evaluated daily. By the end of 90 days, on 91st day animals were sacrificed by excessive aesthesia. Blood were collected in all overnight (12 hours) fasted rats through abdominal aorta and it were processed for below mentioned investigations. Vital organs were collected from the animals and subjected to histopathology.

Laboratory test:

- Complete heamogram
- Renal function test
- Liver function test
- Lipid profile

Gross necropsy:

It includes examination of the external surface of the body, all orifices, and organs like brain, thymus, lungs, heart, spleen, liver, kidneys, adrenals and sex organs of all animals.

Histopathology:

Vital organs were collected from all animals and preserved in 10% buffered neutral formalin for preparation of sections using microtome. Tissues samples of liver, kidney, spleen, brain, heart, testis, ovary and lungs from control and highest dose group animals were subjected to Histopathological investigations. The organs were sliced into 3-5µm sections. The organ pieces were fixed in 10% formalin for 24 hours. Samples were dehydrated in an auto technique and the cleaned in benzene to remove absolute alcohol. Embedding was done by passing cleared samples through three cups containing molten paraffin at $50^{O}C$ and then in a cubical block of paraffin made by the L molds. It was followed by microtome and the slides were prepared then stained with Haematoxylin- eosin.

Statistical analysis:

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

5. RESULTS

5.1. Qualitative analysis

TABLE 1: Physico-Chemical Properties of Kiranthi Mega Chooranam

S.NO	PARAMETERS	PERCENTAGE
1	Moisture Content (Loss On Drying)	9.6 %
2	Total Ash	8.5%
3	Acid Insoluble Ash	4.3%
4	Water Soluble Extractive	12%
5	Alcohol Soluble Extractive	21%
6	Particle Size	Completely passes through sleeve size of 1 mm(90% passes through 400 micrometer sleeve and 10% Passes through 1 mm sleeve)

TABLE 2: Organoleptic characterization of Kiranthi Mega Chooranam

S.NO	PARAMETERS	RESULTS
1	Appearance	Powder
1	Colour	Brown
2	Odour	Odourless
3	Taste	Slightly bitter

TABLE 3: Biochemical Analysis of Kiranthi Mega Chooranam

S.NO	PRAMETERES	RESULTS
1.	Test for ammonium	-
2.	Test for sodium	-
3.	Test for magnesium	-
4.	Test for aluminium	-
5.	Test for potassium	-
6.	Test for calcium	+
7	Test for ferrous iron	+
8	Test for zinc	+
9	Test for arsenic	-
10	Test for mercury	-
11	Test for lead	-
12	Test for sulphate	-
13	Test for chloride	-
14	Test for fluoride and oxalate	+
15	Test for starch	+
16	Test for phosphate	+
17	Test for carbonate	+
18	Test for reducing sugar	-
19	Test for alkaloids	+

(+) - Present; (-) - Absent

TABLE 4: Phytochemical Analysis of Kiranthi Mega Chooranam

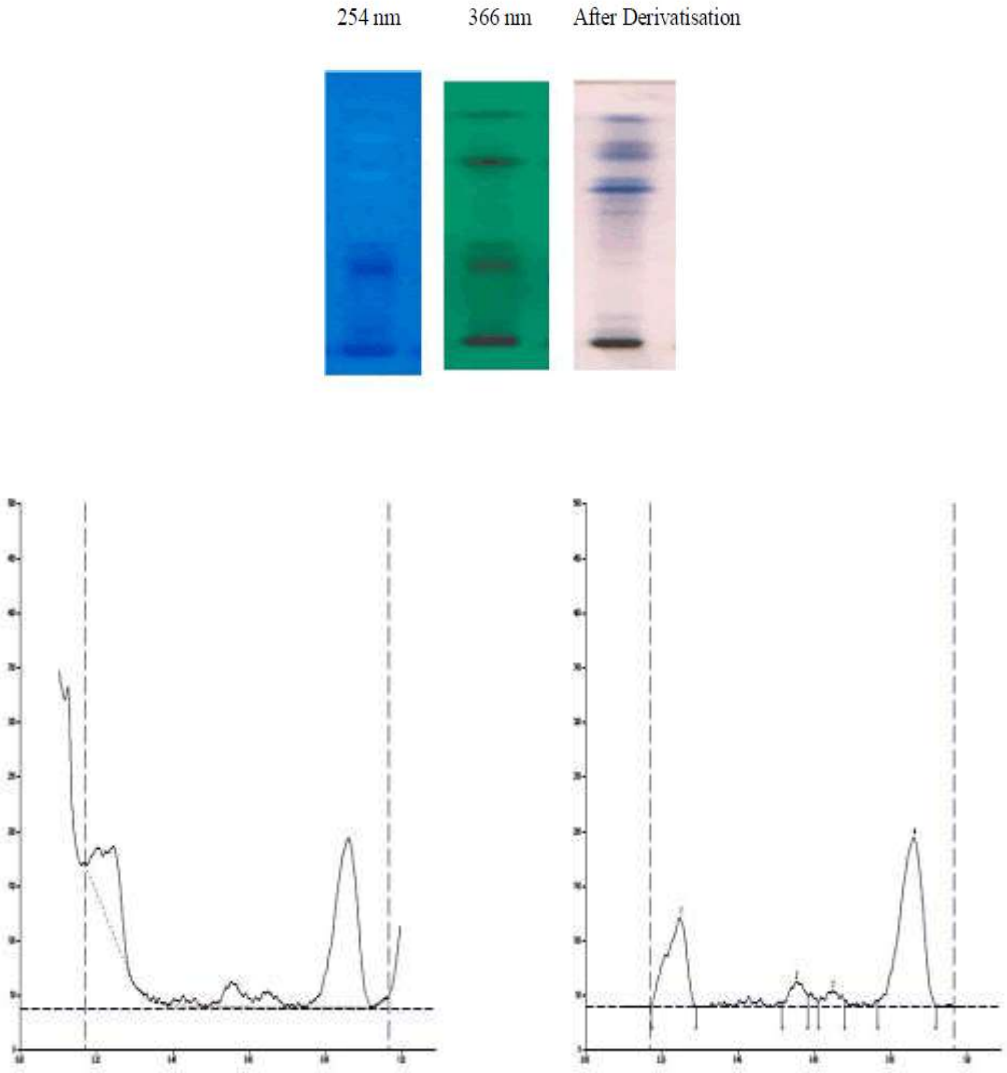
S.NO	PHYTOCHEMICALS	TEST NAME	H2O EXTRACT
1.	Alkaloids	Mayer‟s test	+ ve
2.	Carbohydrates	Molisch‟s	+ve
3.	Glycosides	Modified borntrager‟s test Cardiac glycosides	+ve -ve
4.	Saponin	Foam test	+ve
5.	Phenols	Ferric Chloride test	+ve
6.	Tannins	Gelatin test	+ve
7.	Flavonoids	Alkaline reagent test Lead acetate	+ve +ve
8.	Proteins	Xanthoprotein test	+ve
9.	Diterpenes	Copper acetate test	+ve
10.	Gum & mucilage	Extract +Alcohol	+ve
11.	Quinones	NAOH+Extract	-ve
12.	Fat &Fixed oil	Spot test	-ve

5.2 Quantitative analysis

High Performance Thin Layer Chromatography(HPTLC) Analysis of Kiranthi Mega Chooranam

PEAK	START Rf	START HEIGHT	MAX Rf	MAX HEIGHT	MAX %	END Rf	END HEIGHT	AREA	AREA %
1	0.16	1.8	0.25	81.8	29.50	0.29	0.2	2745.5	28.69
2	0.52	3.4	0.55	22.5	8.99	0.58	10.8	577.6	6.09
3	0.62	5.2	0.65	14.4	5.70	0.63	5.3	406.9	4.24
4	0.77	5.6	0.86	153.1	56.43	0.91	0.6	5840.2	62.02

Figure 1: High Performance Thin Layer Chromatography (HPTLC) Analysis of Kiranthi Mega Chooranam



REPORT:

HPTLC finger printing analysis of the sample reveals the presence of four prominent peaks corresponds to presence of four versatile phytocomponents present with in it. Rf value of the peaks ranges from 0.16 to 0.64. Further the peak 4 and 1 occupies the major percentage of area of 56.20 and 29.67 % which denotes the abundant existence of such compounds.

TABLE 6: Heavy Metal Analysis of Kiranthi Mega Chooranam

Name of the heavy metal	Absorption maximum	Result	Maximum limit
Mercury	253.7nm	0.12	1ppm
Lead	217.0nm	BDL	10ppm
Arsenic	193.7nm	0.29	3ppm
Cadmium	228.8nm	BDL	0.3ppm

BDL-Below Detection Limit

Report:

The results of the present investigations have clearly shows that the sample has no traces of heavy metals such as cadmium and lead. Further the results show the presence of arsenic and mercury.

TABLE 7: Microbial Load Test of Kiranthi Mega Chooranam

S.NO	PARAMETERS	RESULT
1	Total Bacterial Content	640cfu/g
2	Total Fungal Content	65cfu/g
3	E.Coli	Absent
4	Salmonella.Spp	Absent
5	S.Aureus	Absent
6	Pseudomonas Aeruginosa	Absent

TABLE 8: Aflatoxin Analysis of Kiranthi Mega Chooranam

AFLATOXIN	SAMPLE	AYUSH SPECIFICATION LIMIT
B1	Not detected-absent	0.002 ppm
B2	Not detected- absent	0.01 ppm
G1	Not detected- absent	0.03 ppm
G2	Not detected- absent	0.1 ppm

Result:

The results shown that there was no spots were been identified in the test sample loaded on TLC plates when compare to the standard , which indicates that the sample were free from Aflatoxin B1,Aflatoxin B2, Aflatoxin G1, Aflatoxin G2

TABLE 9: Pesticide Residue Analysis of Kiranthi Mega Chooranam

S.NO	PARAMETERS	UNITS	RESULTS
1	Aldrin	mg/kg	BDL(DL:0.1)
2	Dicofol	mg/kg	BDL(DL:0.1)
3	Dieldrin	mg/kg	BDL(DL:0.1)
4	Endosulan1	mg/kg	BDL(DL:0.1)
5	Endosulfan2	mg/kg	BDL(DL:0.1)
6	Endosulfan sulphate	mg/kg	BDL(DL:0.1)
7	Heptachlor(sum,ofitsepoxides)	mg/kg	BDL(DL:0.1)
8	Hexacholrobenzene(HCB)	mg/kg	BDL(DL:0.1)
9	Methoxychlor	mg/kg	BDL(DL:0.1)
10	o,p-DDT	mg/kg	BDL(DL:0.1)
11	P,p-DDD	mg/kg	BDL(DL:0.1)
12	P,p-DDE	mg/kg	BDL(DL:0.1)
13	P,p-DDT	mg/kg	BDL(DL:0.1)
14	AlphaHCH	mg/kg	BDL(DL:0.1)
15	BetaHCH	mg/kg	BDL(DL:0.1)
16	GammaHCH	mg/kg	BDL(DL:0.1)
17	DeltaHCH	mg/kg	BDL(DL:0.1)
18	Endrin(sum,ofitsaldehyde and ketone)	mg/kg	BDL(DL:0.1)
19	Chlordane(cisandgamma)	mg/kg	BDL(DL:0.1)

20	Butachlor	mg/kg	BDL(DL:0.1)
21	Captan	mg/kg	BDL(DL:0.1)
22	Chlorpyriphos	mg/kg	BDL(DL:0.1)
23	Monocrotophos	mg/kg	BDL(DL:0.1)
24	Propetamphos	mg/kg	BDL(DL:0.1)
25	Chlorfenvinphos	mg/kg	BDL(DL:0.1)
26	Dimethoate	mg/kg	BDL(DL:0.1)
27	Fenitrothion	mg/kg	BDL(DL:0.1)
28	Ethion	mg/kg	BDL(DL:0.1)
29	Malathion	mg/kg	BDL(DL:0.1)
30	Monocrotophos	mg/kg	BDL(DL:0.1)
31	Parathion-methyl	mg/kg	BDL(DL:0.1)
32	Parathion-ethyl	mg/kg	BDL(DL:0.1)
33	Phorate	mg/kg	BDL(DL:0.1)
34	Phospphomidon	mg/kg	BDL(DL:0.1)
35	Profenophos	mg/kg	BDL(DL:0.1)
36	Quinalphos	mg/kg	BDL(DL:0.1)
37	Triazophos	mg/kg	BDL(DL:0.1)
38	Disulfoton	mg/kg	BDL(DL:0.1)
39	Deltamethrin	mg/kg	BDL(DL:0.1)
40	Fenpropethrin	mg/kg	BDL(DL:0.1)
41	Fenvalerate	mg/kg	BDL(DL:0.1)
42	Alpha cypermethrin	mg/kg	BDL(DL:0.1)
43	Beta cyfluthrin	mg/kg	BDL(DL:0.1

S.NO	PARAMETERS	UNITS	RESULTS
44	Thiodicarb	mg/kg	BDL(DL:0.1)
45	Thiobencarb	mg/kg	BDL(DL:0.1)
46	Propoxur	mg/kg	BDL(DL:0.1)
47	Mehomyl and Thiodicarb (sum of methymoyl and thiodicarb expressed as methomyl)	mg/kg	BDL(DL:0.1)
48	Iprovalicard	mg/kg	BDL(DL:0.1)
49	Indoxacarb (sum of R and S isomers)	mg/kg	BDL(DL:0.1)
50	Fenobucarb	mg/kg	BDL(DL:0.1)
51	Dazomet (Methylisothicyanate resulting from the use of dazomet metam)	mg/kg	BDL(DL:0.1)
52	Carbosulfan	mg/kg	BDL(DL:0.1)
53	Benfuracard	mg/kg	BDL(DL:0.1)
54	Tau – Fluvalinate	mg/kg	BDL(DL:0.1)
55	Permethrin (sum of isomers)	mg/kg	BDL(DL:0.1)

5.3. TOXICITY STUDIES

5.3.1. Acute oral toxicity study of Kiranthi Mega Chooranam

Acute toxicity study carried out as per WHO guidelines, there were no treatment related death or signs of toxicity developed in Wistar albino rats at dosage of 10 times the therapeutic dose **(**2000 mg/kg b.wt) throughout the study period. Further, no gross pathological changes have been seen in the internal organs of both control and treated groups.

TABLE 10: Effect of **Kiranthi Mega Chooranam** on behavioural signs of acute toxicity study

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

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. Catatonia 12. Muscle relaxant 13. Hypnosis 14.Analgesia 15.Lacrimation 16. Exophthalmos 17.Diarrhoea 18.Writhing 19.Dyspnoea 20. Mortality

Note:

+ Presence of activity

- Absence of activity

5.3.2 Long term toxicity study of Kiranthi Mega Chooranam

Long term toxicity study carried out as per WHO guideline for a period of 90 days. The changes observed in the food intake, water intake, body weight changes, haematological and biochemical parameters were mentioned in below tables.

TABLE 11: Effect of **Kiranthi Mega Chooranam** on Body weight changes of Male Wistar albino rats in long term toxicity study.

GROUPS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
0^{th} day	183.7±11.4	181.5±8.6	192.5±8.6	194.4±6.1
15^{th} day	198.7±10.1	196.5±12.5	209.2±4.1	209.2±5.1
30^{th} day	218.2±13.6	221.8±17.6	231.6±5.3	229.2±6.4
45^{th} day	239.6±17.7	241.6±16.5	253.8±5.9	256 ± 8.6 *
60^{th} day	264.2±15.8	265.6±17.6	285.2± 9.2	280.2±8.6*
75^{th} day	288.2±16.0	287.6±12.9	308±9.2**	312±8.8**
90^{th} day	316.2±18.2	308±15.5	335±12.9*	340 ±6.5**

Values are mean± S.D. (Dunnett's test).*P<0.05,**P<0.01,N=10

Graph 1: Effect of **Kiranthi Mega Chooranam** on Body weight changes of male Wistar albino rats in long term toxicity study

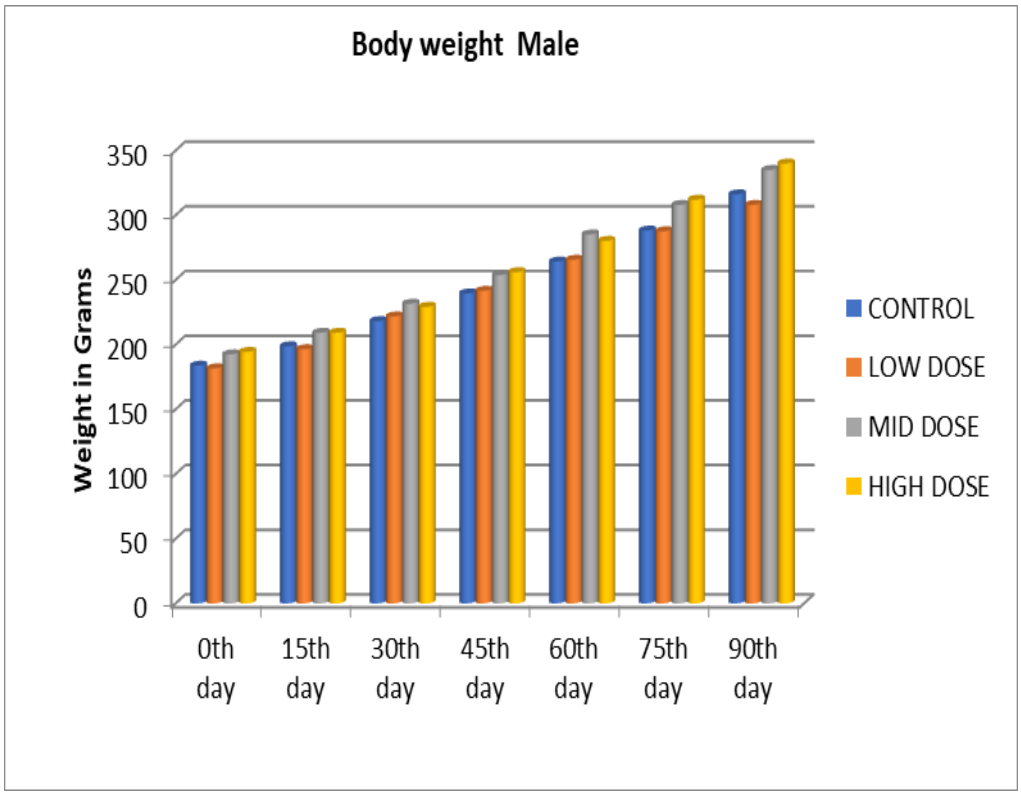


TABLE 12: Effect of **Kiranthi Mega Chooranam** on Body weight changes of Female Wistar albino rats in long term toxicity study

GROUPS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
0th day	151.3±12	157.2±15.7	167.4±16.4	170±16.2
15th day	164±11.6	172.6±16.2	182.2±22.1	178.3±16.0
30th day	173.4±12.8	186.6±17.1	193±26.5	195.2±19.0
45th day	193.6±6.8	194.6±15.2	199.4±18.6	205.2±20.0
60th day	214.4±6.0	215.6±11.5	214.6±20.8	219.8±18.1
75th day	241.6±10.1	240.4±15.0	247.2±25.0	262.8±26.1
90th day	261.2±12.6	272.8±19.6	270.6±24.5	284.8±26.9*

Values are mean± S.D. (Dunnett‟s test).*P<0.05,**P<0.01,N=10

Graph 2: Effect of **Kiranthi Mega Chooranam** on Body weight changes of female Wistar albino rats in long-term toxicity study.

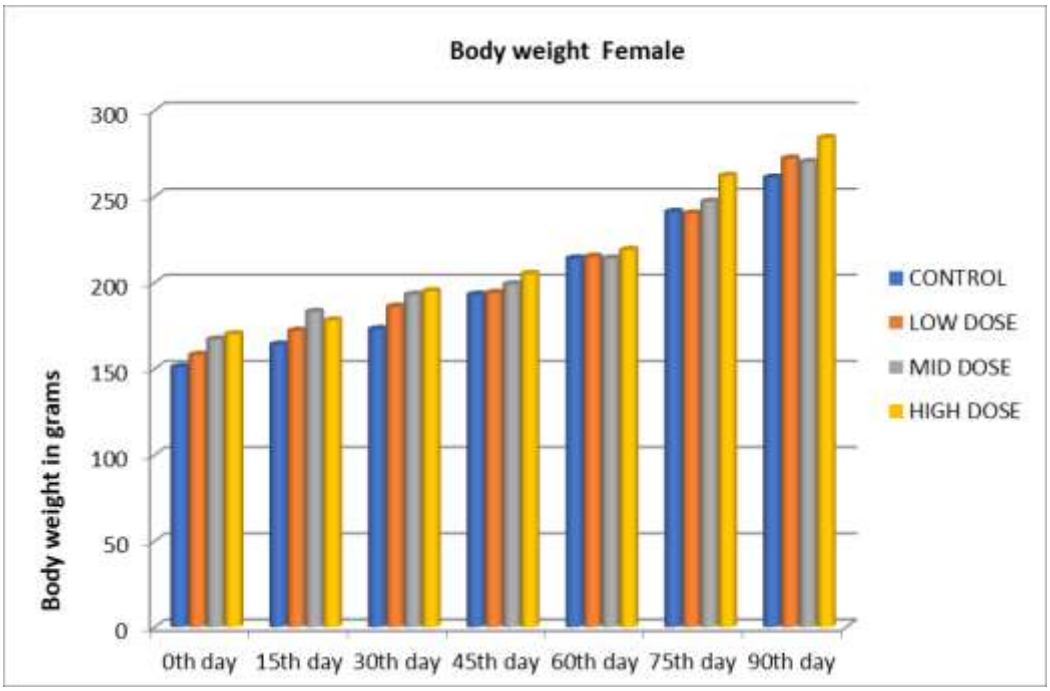


TABLE 13: Effect of **Kiranthi Mega Chooranam** on feed intake of Wistar albino rats in long term toxicity study

GROUPS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
1st day	41.5±2.53	31±6.07	35±14.1	56±14.6
15th day	40.2±12.8	36.8±5.87	42.3±9.44	45.4±13.2
30th day	35.2±8.5	39.5±7.01	43.5±10.6	45.8±12.1
45th day	36.6±7.9	39.1±10.5	43.9±12.5	51.5±14.1*
60th day	39.5±9.12	42.5±10.0	45.5±17.3	50.5±12.0
75th day	43.4±12.6	47.9±13.4	47.8±13.6	48.8±15.5
90th day	44.5±11.5	46.4±18.5	55.5±13.4*	57.7±15.5

Values are mean± S.D. (Dunnett's test).*P<0.05,**P<0.01,N=10

Graph 3: Effect of **Kiranthi Mega Chooranam** on feed intake changes of Wistar albino rats in long term toxicity study.

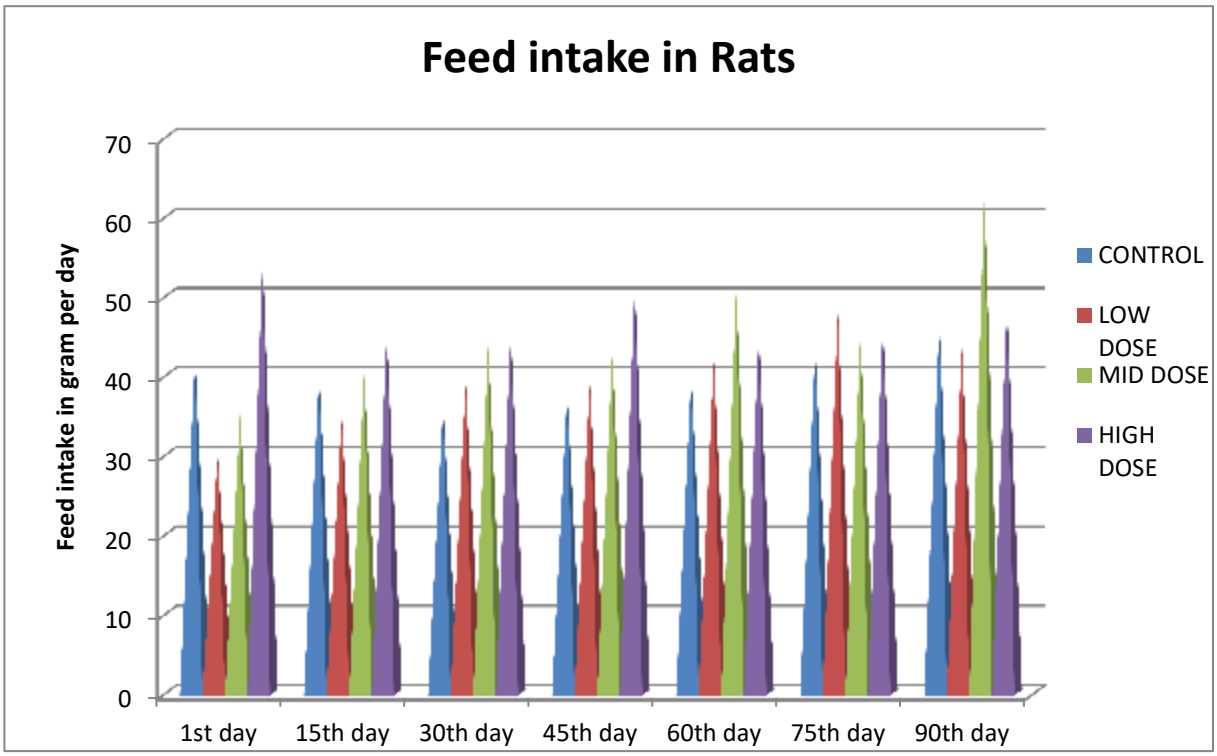


TABLE 14: Effect of **Kiranthi Mega Chooranam** on water intake of Wistar albino rats in long term toxicity study

GROUPS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
1st day	86±7.07	62±14.1	71±28.2	89±20.2
15th day	60±17.4	55.4±16	73.3±21.8	80.9±25.1
30th day	66±18.7	74.1±22.1	75.6±25.2	81±27.3
45th day	68±22.5	79.5±23.1	82.1±21.6	87.4±28.8
60th day	70±20.8	81.2±24.9	87.6±32.9	84.3±25.2
75th day	71.3±22.5	84.2±28.1	88.2±32.5	88.8±31.1
90th day	82.3±23.5	89.3±32.1	91.3±33.8	93±29.8

Values are mean± S.D. (Dunnett's test).*P<0.05,**P<0.01,N=10

Graph 4: Effect of **Kiranthi Mega Chooranam** on water intake changes of Wistar albino rats in long term toxicity study.

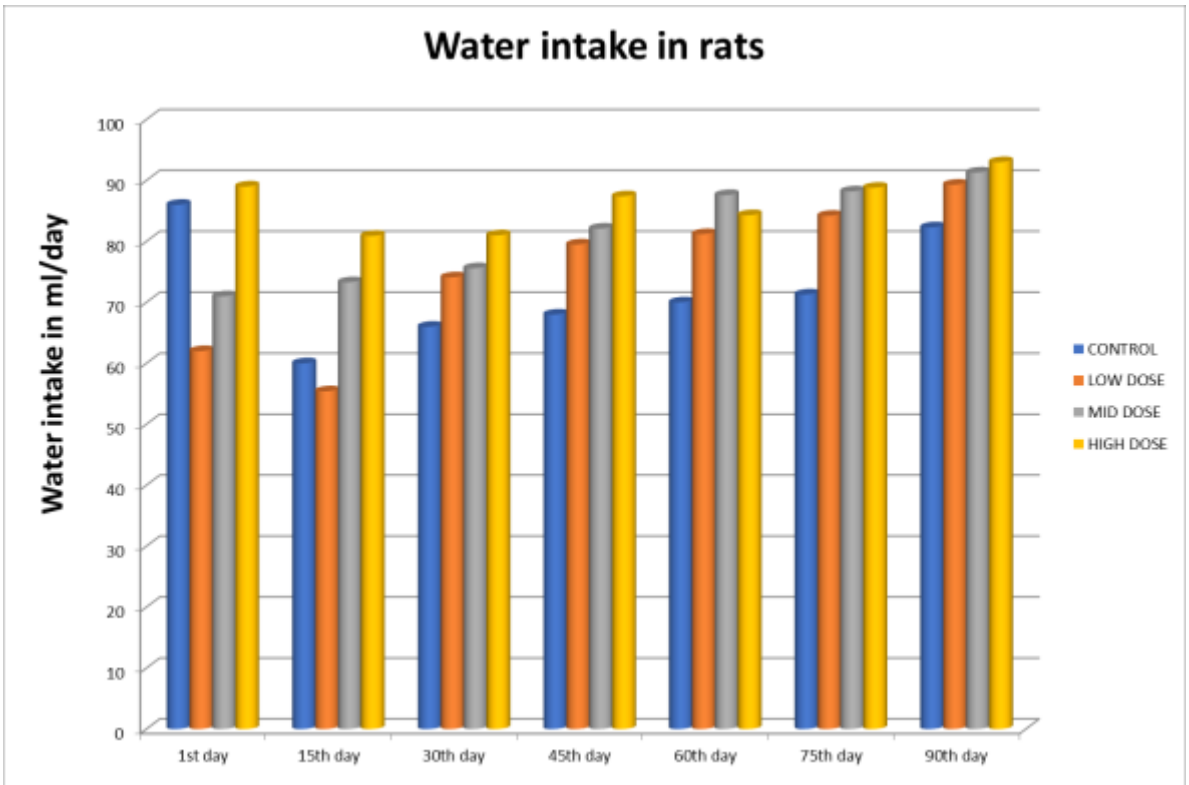
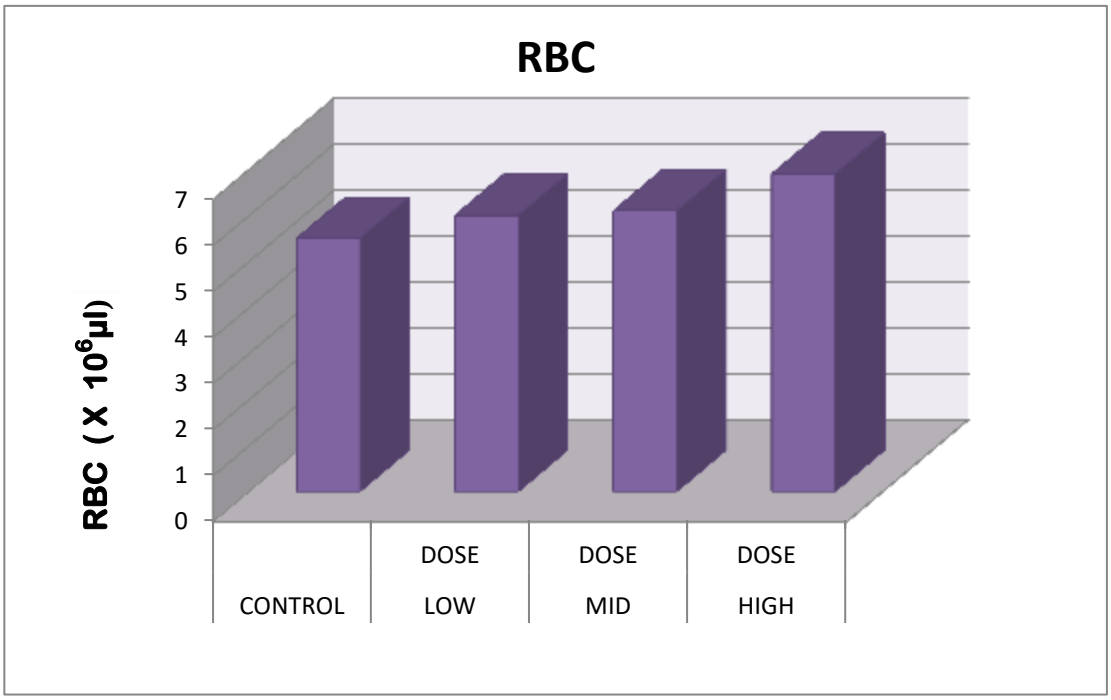


TABLE 15: Effect of **Kiranthi Mega Chooranam** Haematological parameters of Wistar albino rat in long term toxicity study

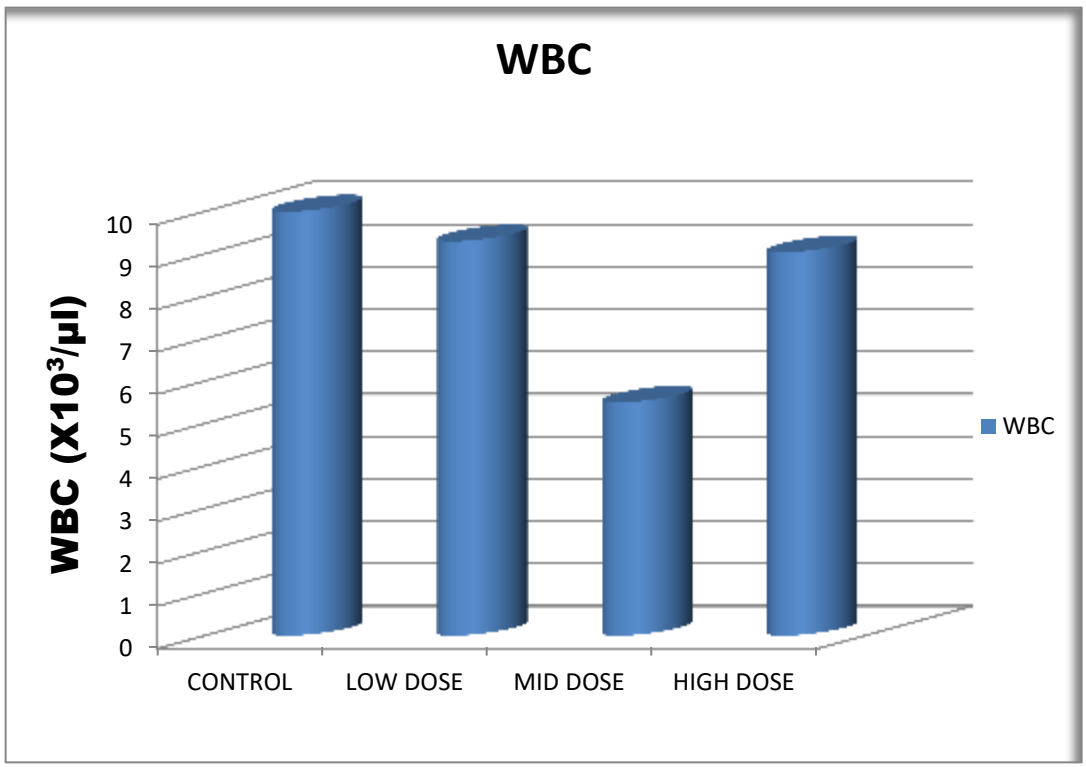
PARAMETERS	**CONTROL**	**LOW DOSE**	**MID DOSE**	**HIGH DOSE**
RBC (X 10^6µl)	5.5±1.32	6±1.07	6.1±0.9	6.9±1.53
WBC (X10^3/µl)	10±2.08	9.3±2.48	5.4±0.96**	9.04±1.35
Platelet(X10^3/µl)	752.8±172.6	642.9±164.5	762.6±113.7	723±138.4
Haemoglobin(g/dl)	12±2.03	13.2±1.43	12.1±2.38	13.5±2
MCH(pg)	18.2±1.57	17.5±1.64	19.10	17.3±1.14
MCV(fl)	58±5.10	58.1±4.24	58.5±6.4	62.1±6.11
Neutrophils 10^3/mm3	22.3±0.72	22.6±0.78	22..1±0.75	21.6±0.43
Eosinophils (%)	1.3±0.27	1.3±0.29	1.4±0.30	1.4±0.32
Basophils (%)	0.3±0.38	0.1±0.32	0.3±0.40	0.3±0.38
Lymphocytes (%)	73.7±12.2	74.2±12.1	75.8±10.66	75.1±8.89
Monocytes (%)	4.2±0.74	3.1±0.81	3.2±1.50	3.9±0.96

Values are mean± S.D. (Dunnett's test).*P<0.05,**P<0.01,N=10

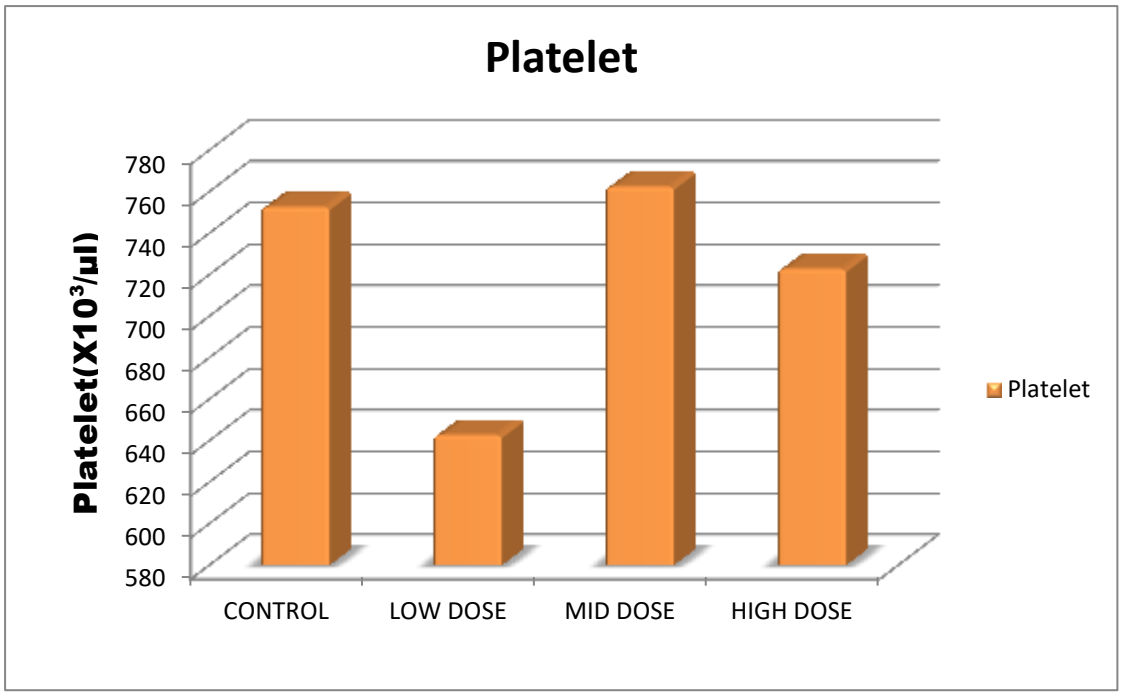
Graph 5: Effect of **Kiranthi Mega Chooranam** on haematological parameters- RBC of Wistar albino rat in long term toxicity studies.



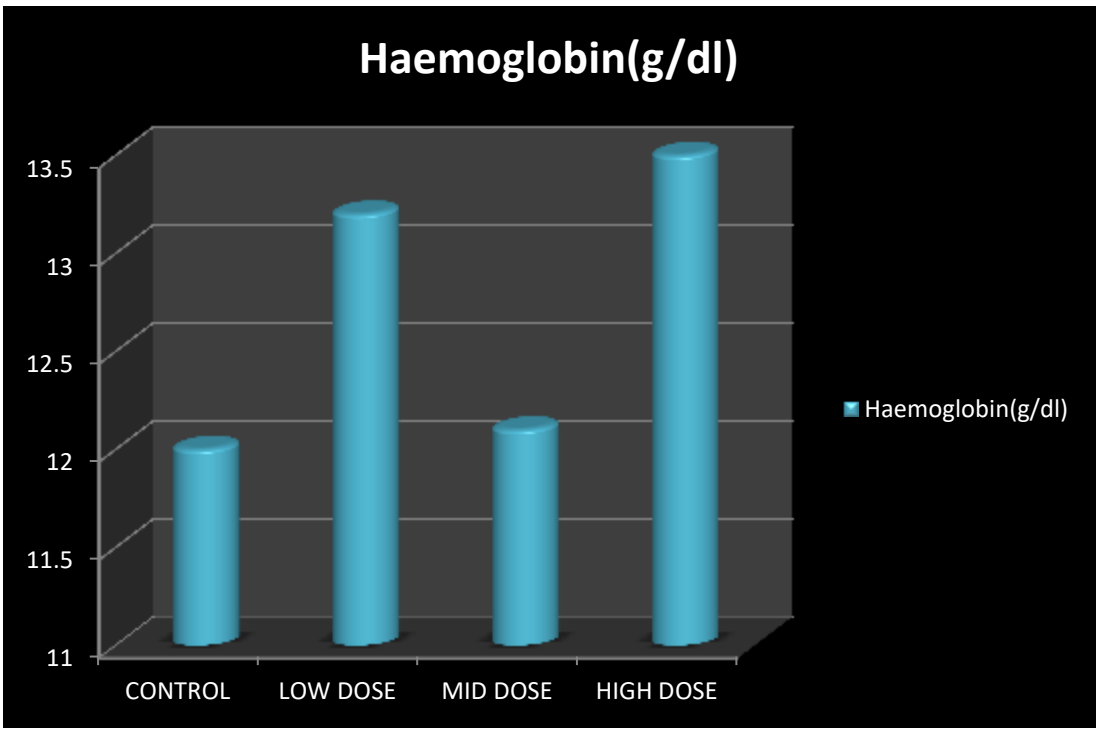
Graph 6: Effect of **Kiranthi Mega Chooranam** on haematological parameters- WBC of Wistar albino rat in long term toxicity study.



Graph 7: Effect of **Kiranthi Mega Chooranam** on haematological parameters- Platelets of Wistar albino rat in long term toxicity study



Graph 8: Effect of **Kiranthi Mega Chooranam** on haematological parameters- HB of Wistar albino rat in long term toxicity study.



Graph 9: Effect of **Kiranthi Mega Chooranam** on haematological parameters –Differential counts of Wistar albino rat in long term toxicity study

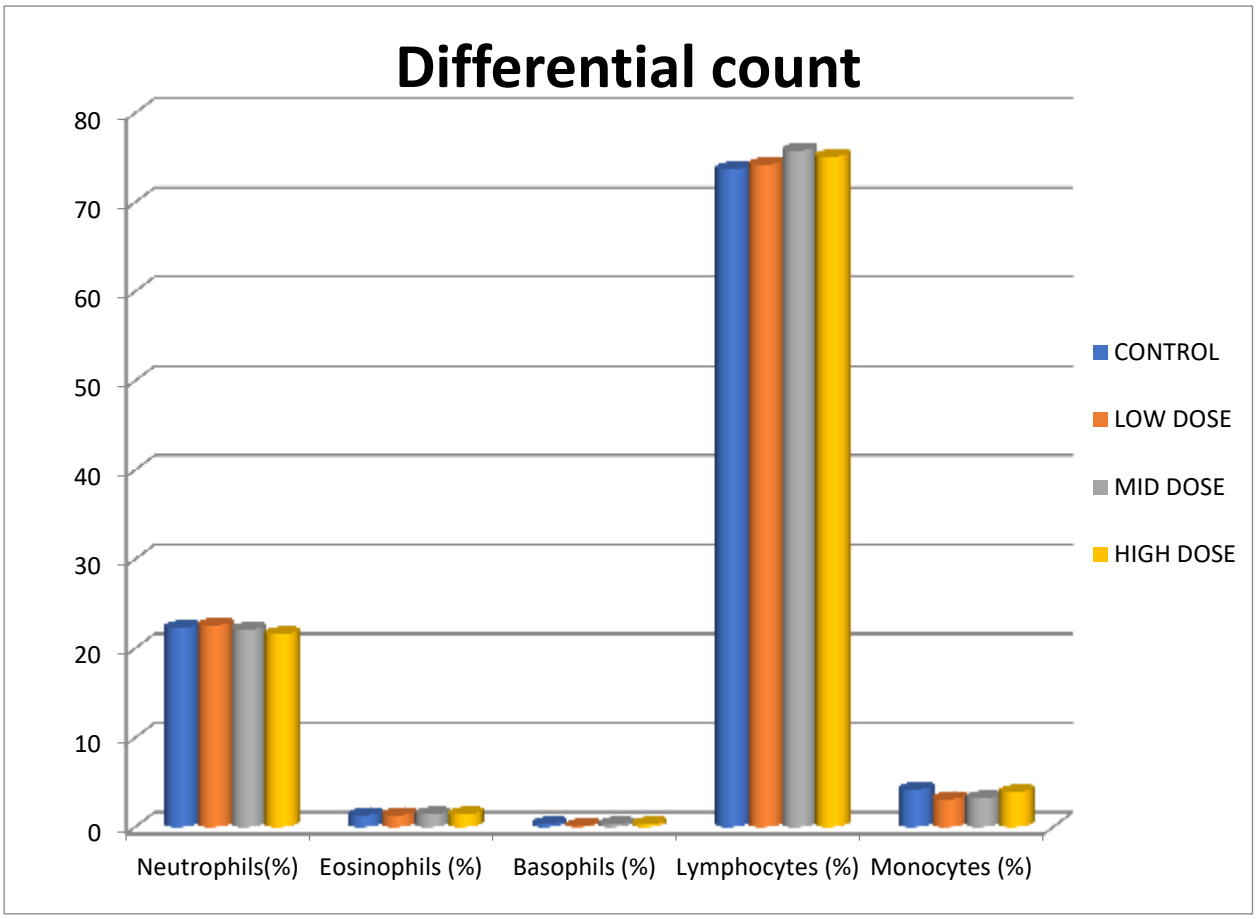


TABLE 16: Effect **of Kiranthi mega Chooranam** on biochemical parameters- Renal function test of Wistar albino rat in long term toxicity study

Parameters	Control	Low dose	Mid dose	High dose
BUN (mg/dl)	15.8±3.32	16.1±2.54	15.1±3.15	14.9±3.27
Serum Creatinine(mg/dl)	0.7±0.12	0.69±0.17	0.71±0.22	0.73±0.16

Values are mean± S.D. (Dunnett‟s test).*P<0.05,**P<0.01,N=10

Graph 10: Effect of **Kiranthi Mega Chooranam** on biochemical parameters- BUN of wistar albino rat in long term toxicity study

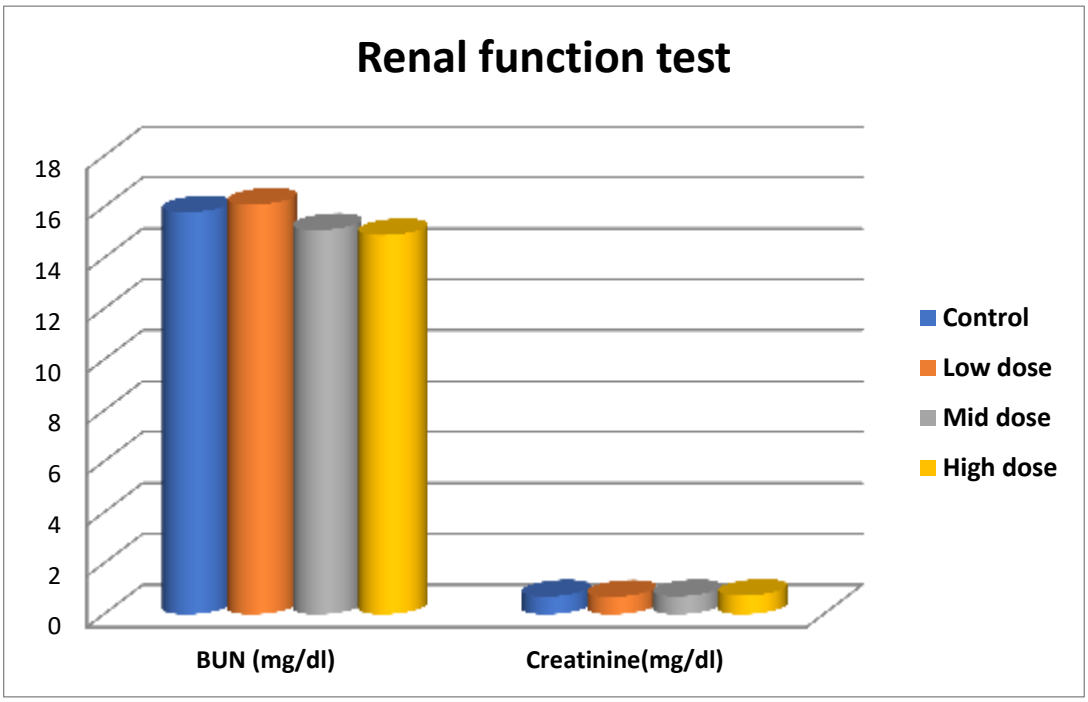
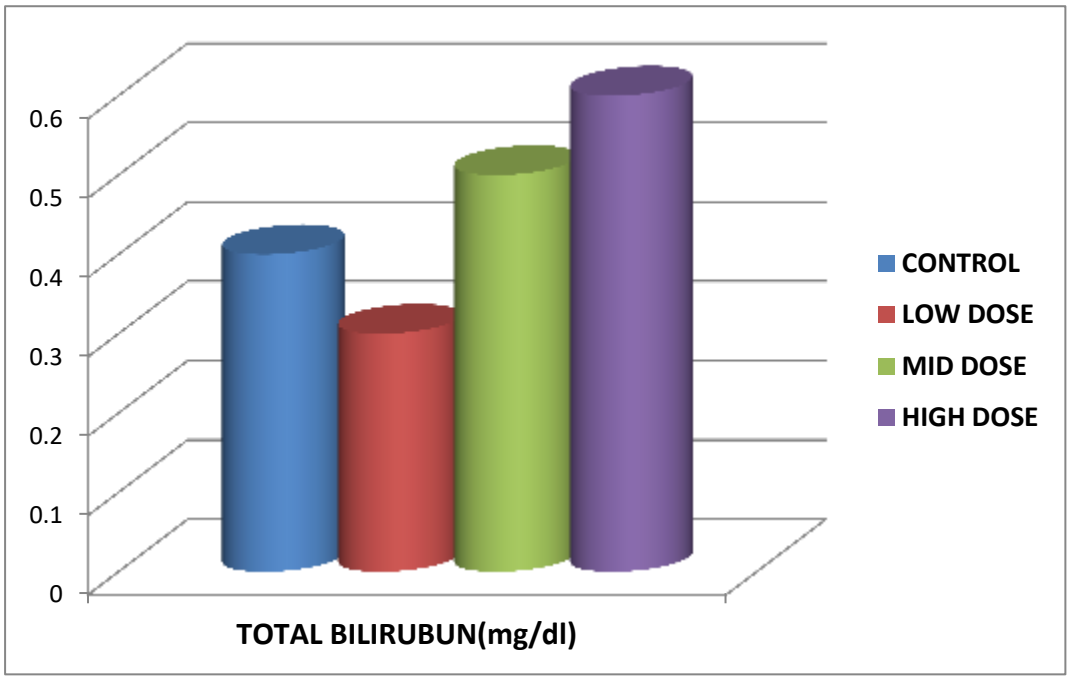


TABLE 17: Effect of **Kiranthi Mega Chooranam** biochemical parameters-Liver function test of wistar albino rat in long term toxicity study

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
TOTAL BILIRUBIN (mg/dl)	0.4±0.13	0.3±0.14	0.5±0.13	0.6±0.14
SGOT(U/dl)	99±16.4	89±16.9	98±16.4	101±16.9
SGPT(U/dl)	26±12.20	28±1.50	32±0,12	35 ±12.0

Values are mean± S.D. (Dunnett's test).*P<0.05,**P<0.01,N=10

Graph 11: Effect of **Kiranthi Mega Chooranam** on biochemical parameters-Total Bilirubin of Wistar albino rat in long term toxicity study



Graph 12: Effect of **Kiranthi Mega Chooranam** on biochemical parameters SGOT-SGPT - of wistar albino rat in long term toxicity study

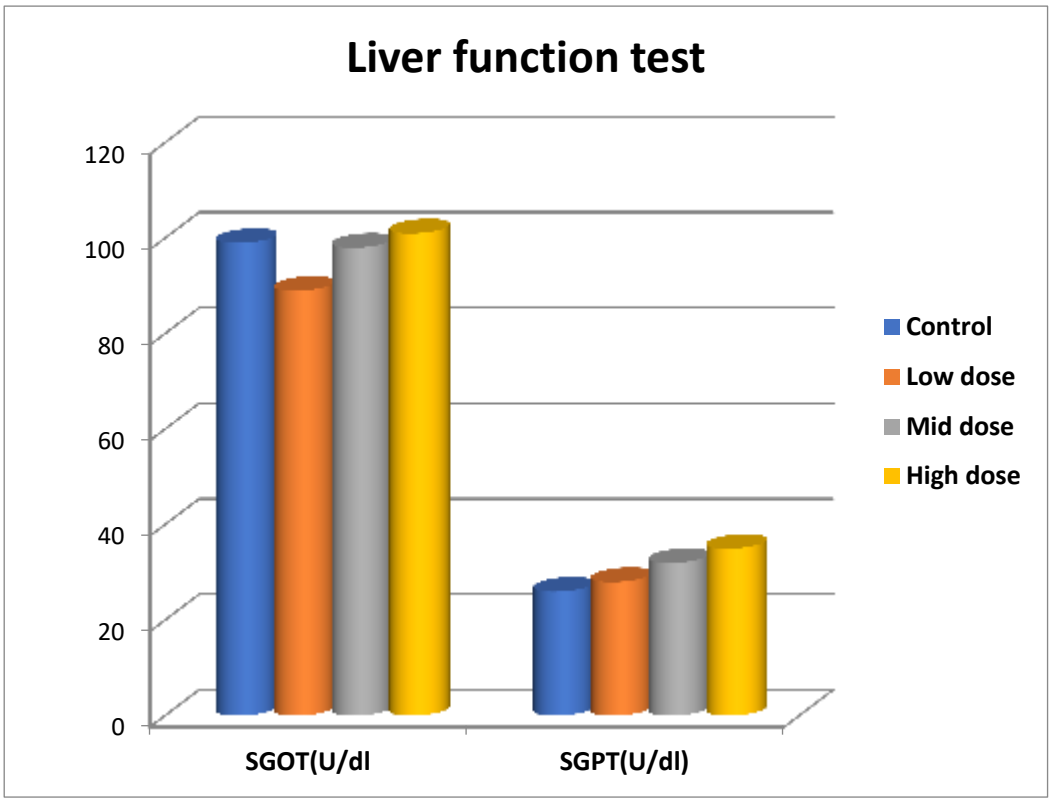
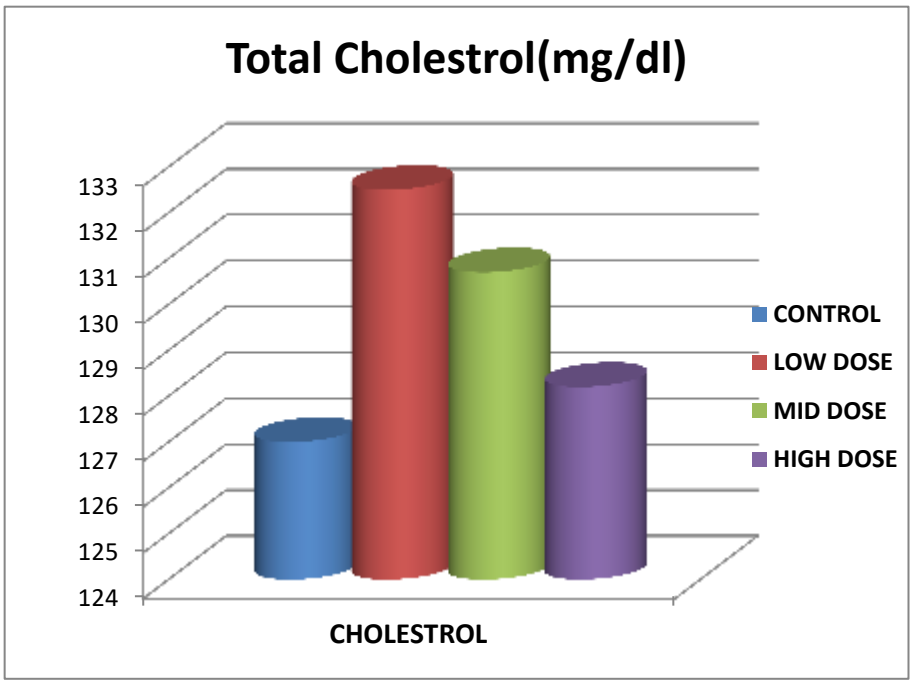


TABLE 18: Effect of **Kiranthi Mega Chooranam** on biochemical parameters-Lipid profile of wistar albino rat in long term toxicity study

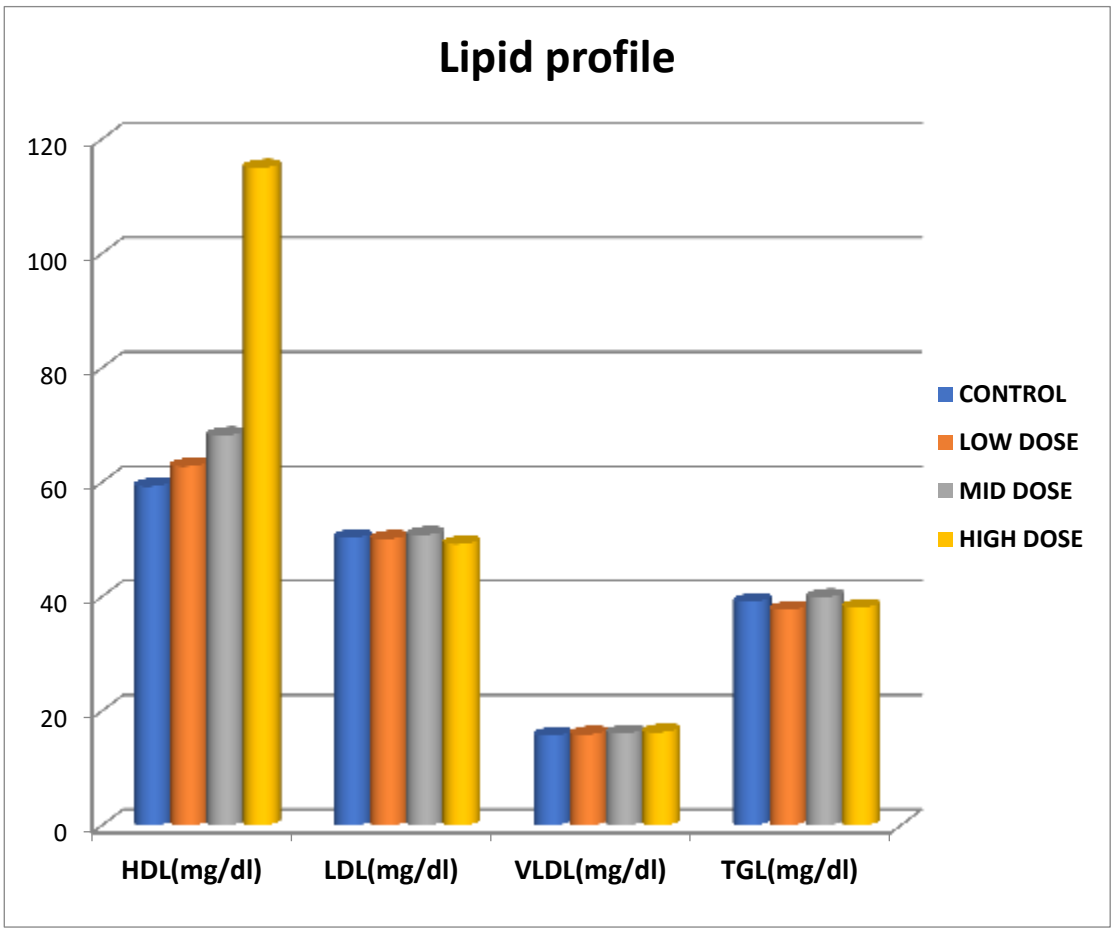
PARAMETERS (mg/dl)	**CONTROL**	**LOW DOSE**	**MID DOSE**	**HIGH DOSE**
TOTAL CHOLESTROL (mg/dl)	125±18.13	132.6±15.8	137±27.15	139.8±25.3
HDL(mg/dl)	59.3±5.86	62.8±10.8	68.2±6.05	115±27**
LDL(mg/dl)	50.3±21.0	50.1±20.8	50.8±21.4	49.2±20.3
VLDL(mg/dl)	15.7±2.25	15.9±2.15	16.1±4.02	16.3±4.12
TGL(mg/dl)	39.1±8.21	37.7±10.6	40.1±7.88	38.1±11.6

Values are mean± S.D. (Dunnett"s test).*P<0.05, **P<0.01, N=10

Graph 13: Effect of **Kiranthi mega Chooranam** on biochemical parameters-Total Cholesterol of wistar albino rat in long term toxicity



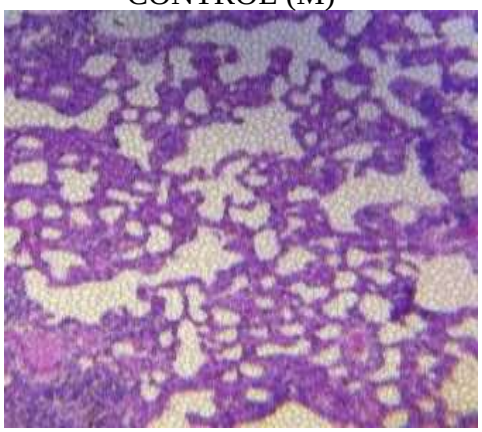
Graph 14: Effect of **Kiranthi Mega Chooranam** on biochemical parameters-Lipid profile of wistar albino rat in long term toxicity study



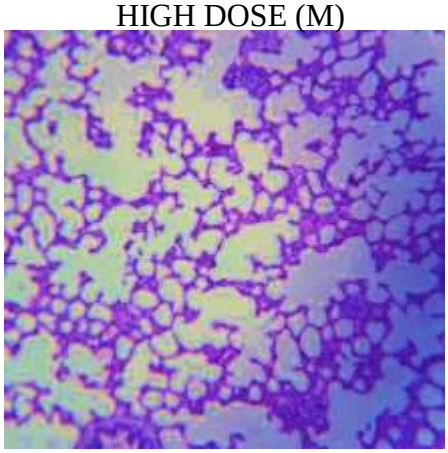
Histopathological changes of control and high dose group of Kiranthi Mega Chooranam treated animals (male and female wistar albino rat) under magnification power 10x & 40x for 90 days long term toxicity studies:

Low Power Magnification (10X) of albino rat - Lung

CONTROL (M)

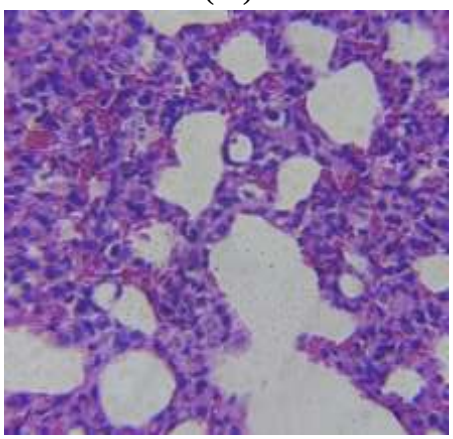


HIGH DOSE (M)

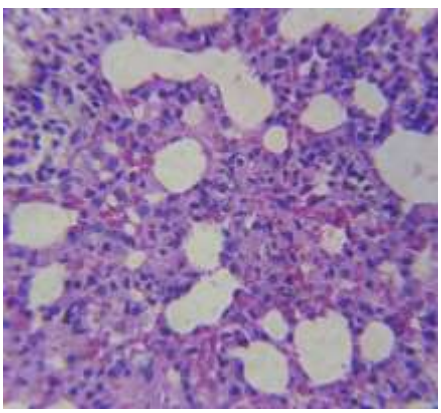


High Power Magnification (40X) of albino rat - Lung

CONTROL (M)



HIGH DOSE (M)

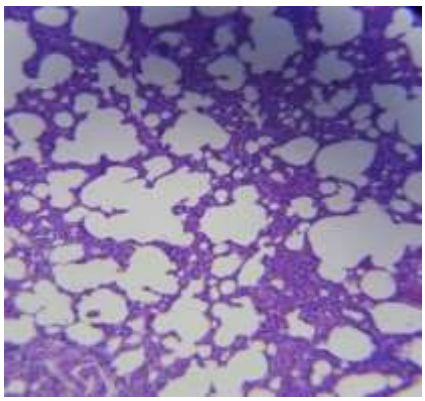


CONTROL : Bronchial opening appears regular with no signs of infiltration

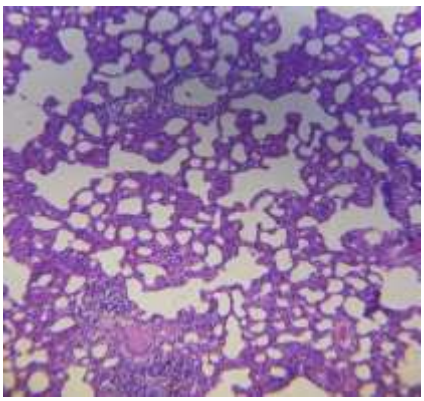
HIGH DOSE : Pulmonary vessels and bronchioles appear normal.

Low Power Magnification (10X) of Female albino rat - Lung

CONTROL (F)

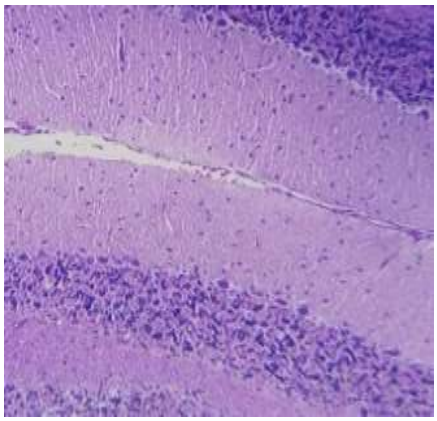


HIGH DOSE (F)

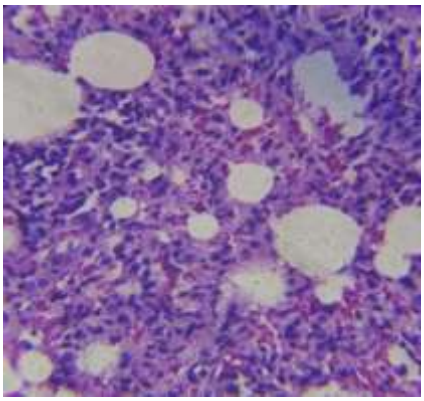


High Power Magnification (40X) of Female albino rat - Lung

CONTROL (F)



HIGH DOSE (F)

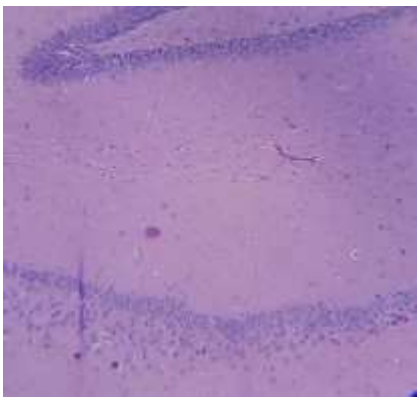


CONTROL : Perfect network of simple squamous epithelium were observed

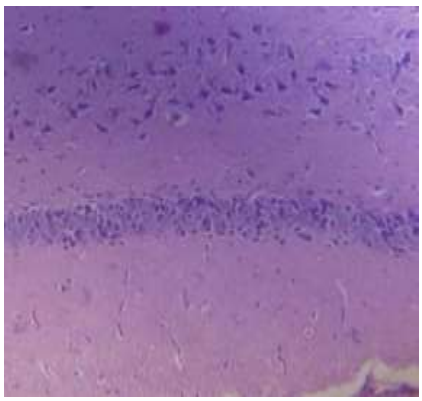
HIGH DOSE : Normal lung parenchyma with regular airway histology was observed

Low Power Magnification (10X) of Male albino rat - Brain

CONTROL (M)

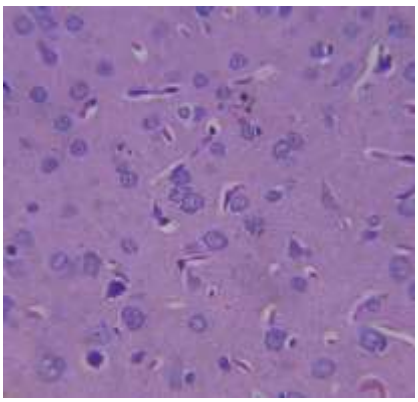


HIGH DOSE (M)

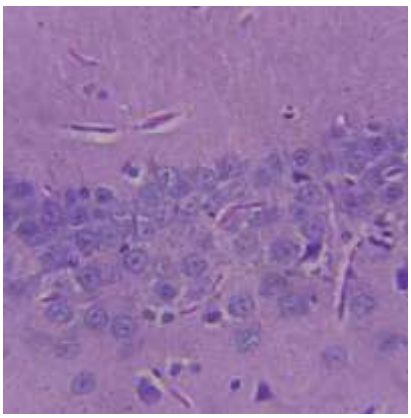


High Power Magnification (40X) of Male albino rat - Brain

CONTROL (M)



HIGH DOSE (M)

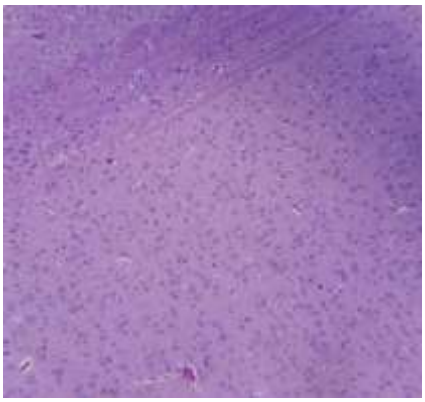


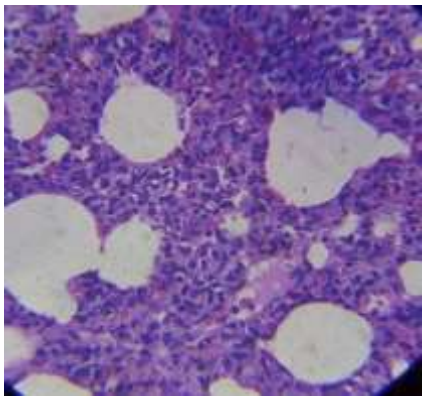
CONTROL : The arrangement of the neurons appears intact with no signs of degeneration or apoptotic changes

HIGH DOSE : Appearance of Hippocampus neurons was normal with dense network.

Low Power Magnification (10X) of Female albino rat - Brain

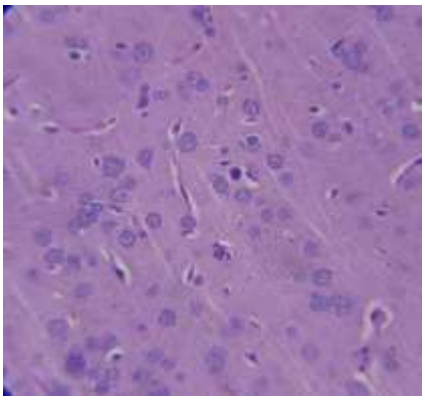
CONTROL (F) HIGH DOSE (F)

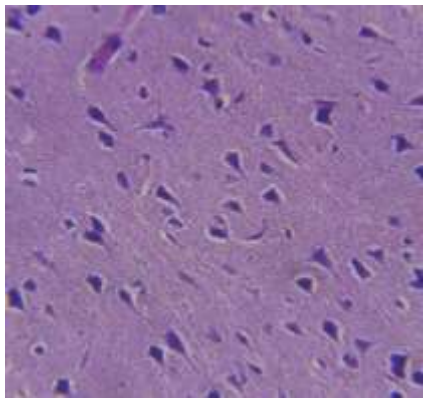




High Power Magnification (40X) of Female albino rat - Brain

CONTROL (F) HIGH DOSE (F)





CONTROL:

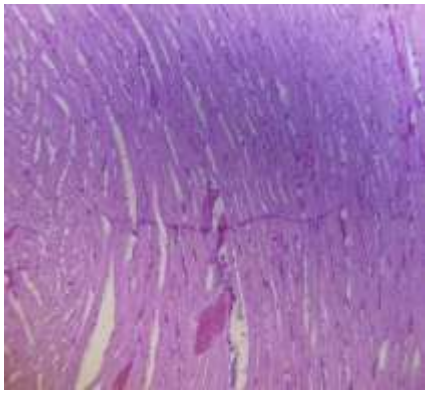
The cerebral sections have showed normal architecture in both cortex and medulla without any changes in their cells without any inflammatory cells or gliosis.

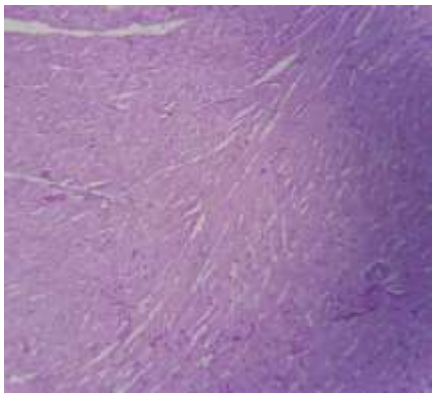
HIGH DOSE:

Normal appearance of cerebral cortex and medulla with intact parenchyma .

Low Power Magnification (10X) of Male albino rat - Heart

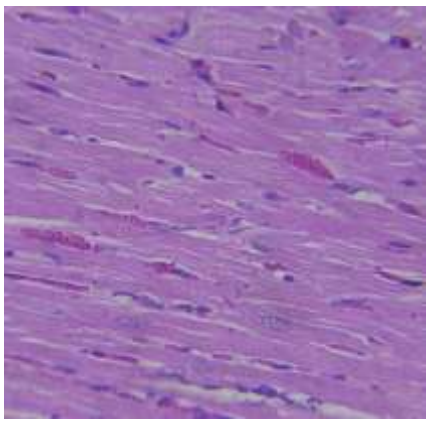
CONTROL (M) HIGH DOSE (M)

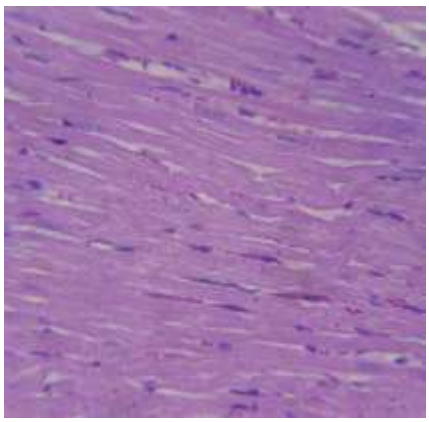




High Power Magnification (40X) of Male albino rat - Heart

CONTROL (M) HIGH DOSE (M)





CONTROL:

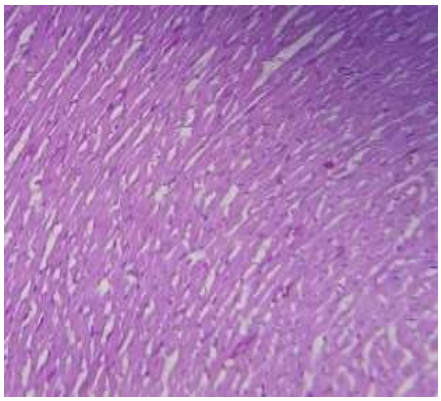
Myocardial tissue appears normal with orderly striated heart muscle fibres and a clear nuclear and muscle bands

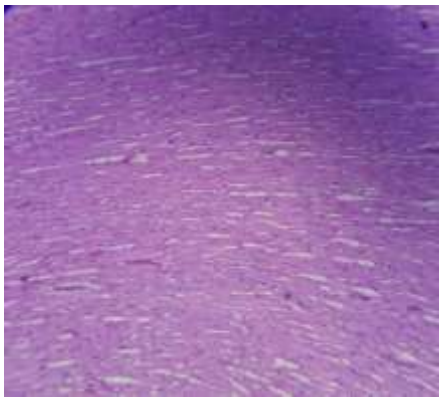
HIGH DOSE:

Cardiac fibres appears normal with regular striations

Low Power Magnification (10X) of Female albino rat - Heart

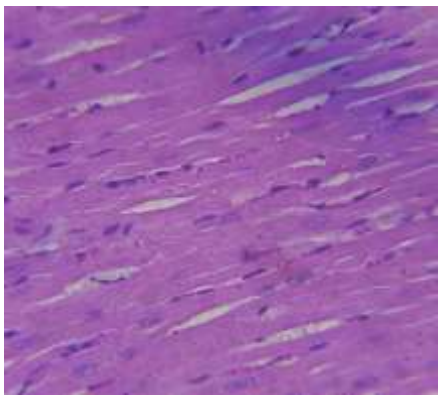
CONTROL (F) HIGH DOSE (F)

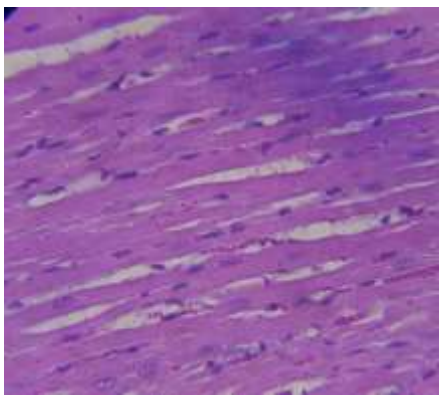




High Power Magnification (40X) of Female albino rat - Heart

CONTROL (F) HIGH DOSE (F)





CONTROL:

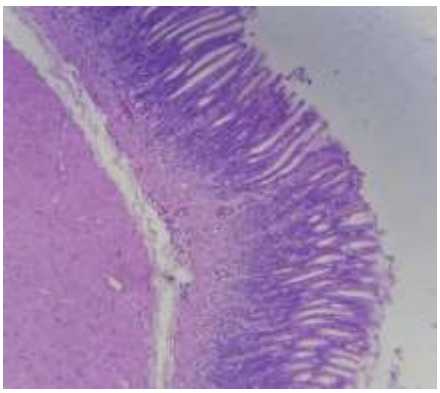
The appearance of cardiomyocyte was normal with the dark nuclear region. The nuclei of muscle fibers appear central arrangement.

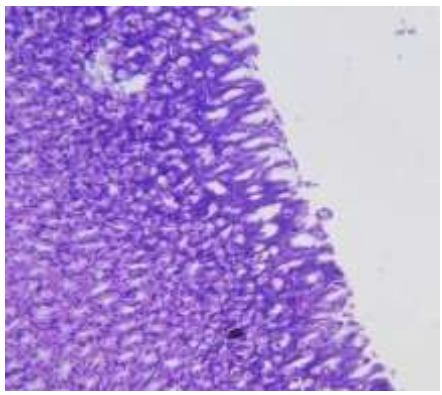
HIGH DOSE:

Myocardial cells appears normal with well-defined mycoplasma.

Low Power Magnification (10X) of Male albino rat - Stomach

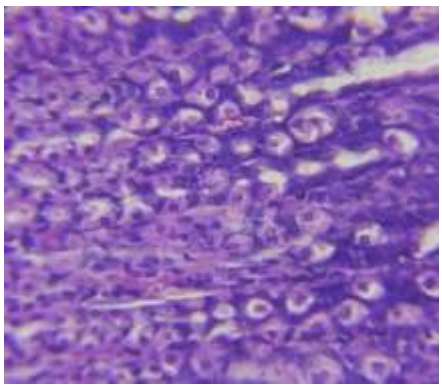
CONTROL (M) HIGH DOSE (M)

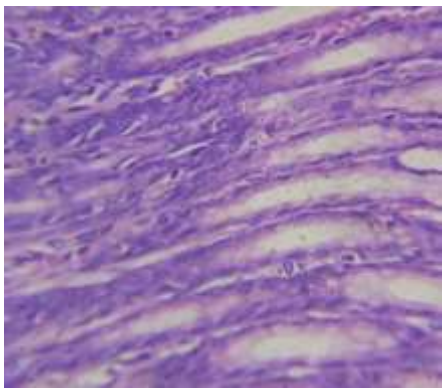




High Power Magnification (40X) of Male albino rat - Stomach

CONTROL (M) HIGH DOSE (M)





CONTROL:

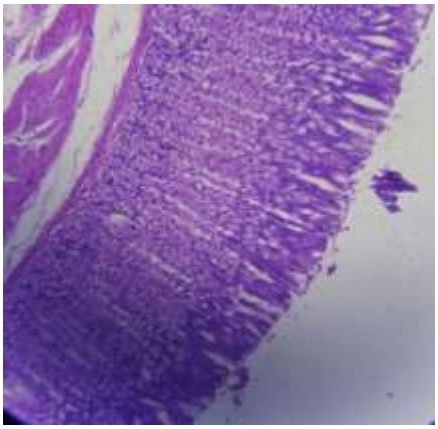
Histology of gastric wall composed of normal mucosa, muscular mucosa, sub mucosa, muscularis propiria and adventitia

HIGH DOSE:

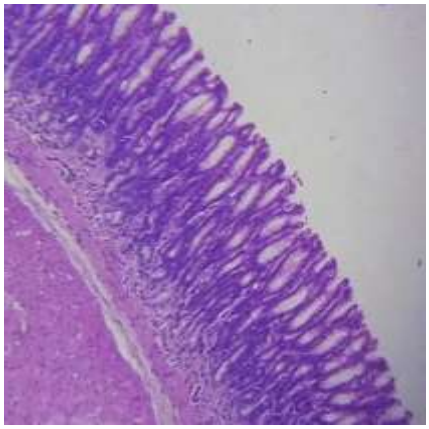
Mucosal wall appears normal with no evidence of infiltration and inflammation.

Low Power Magnification (10X) of Female albino rat - Stomach

CONTROL (F)

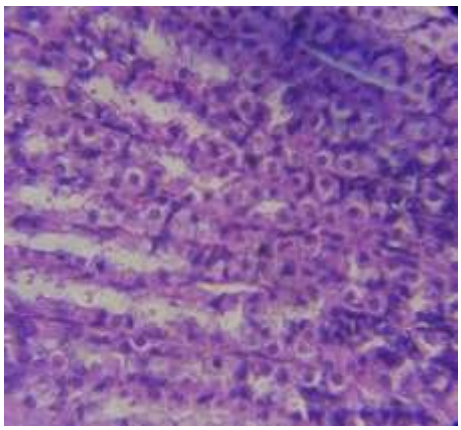


HIGH DOSE (F)

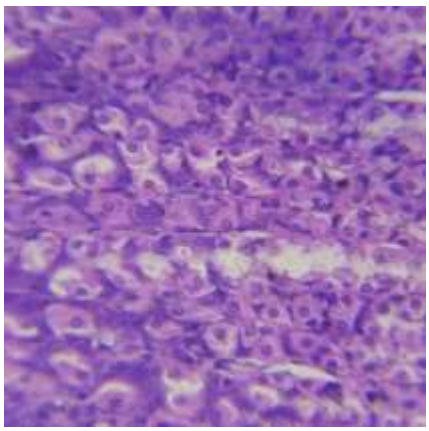


High Power Magnification (40X) of Female albino rat - Stomach

CONTROL (F)



HIGH DOSE (F)



CONTROL :

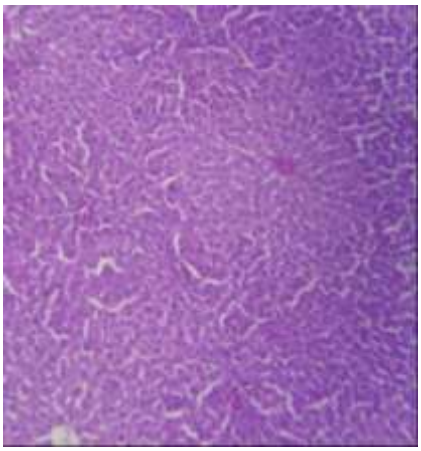
Mucosal wall appears normal with regular arrangement of connective tissue

HIGH DOSE:

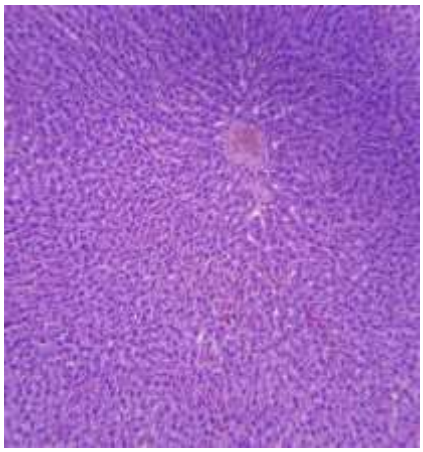
The continuity of mucosa was normal with no evidence of ulceration.

Low Power Magnification (10X) of Male albino rat - Liver

CONTROL (M)

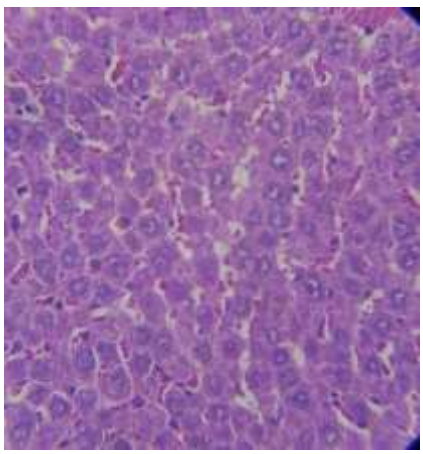


HIGH DOSE (M)

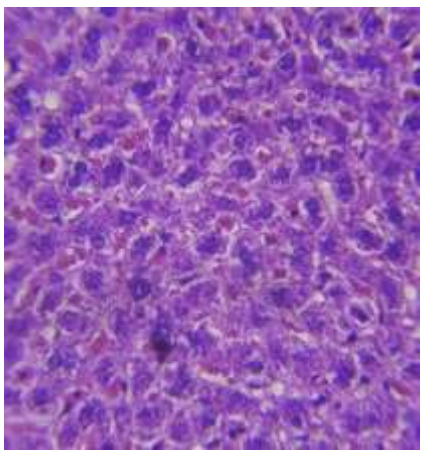


High Power Magnification (40X) of Male albino rat - Liver

CONTROL (M)



HIGH DOSE (M)



CONTROL:

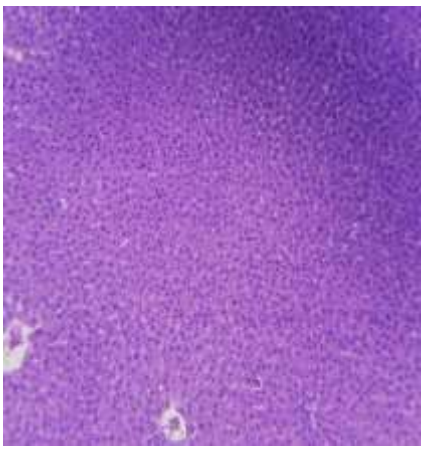
Liver parenchyma appears normal with no evidence of necrosis.

HIGH DOSE:

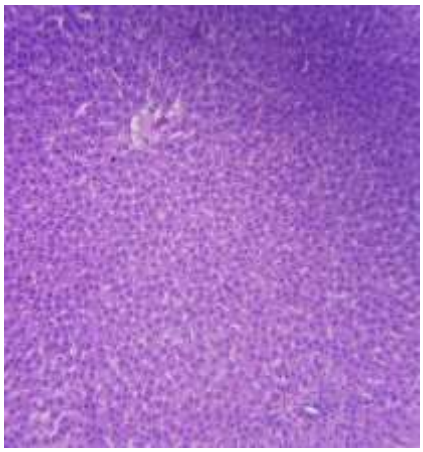
No evidence of inflammation and collagen (fibrosis).

Low Power Magnification (10X) of Female albino rat - Liver

CONTROL (F)

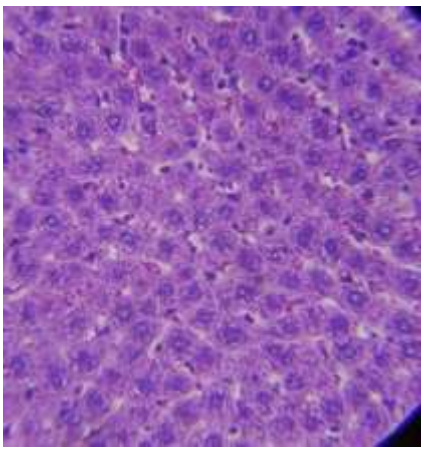


HIGH DOSE (F)

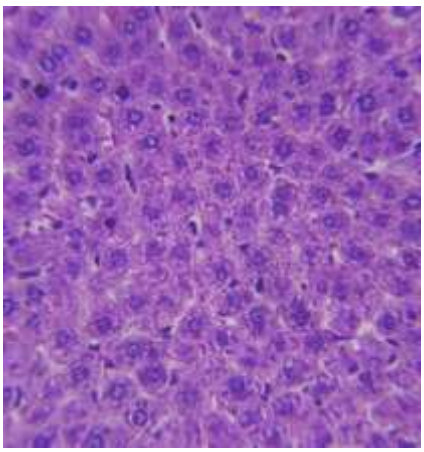


High Power Magnification (40X) of Female albino rat - Liver

CONTROL (F)



HIGH DOSE (F)



CONTROL:

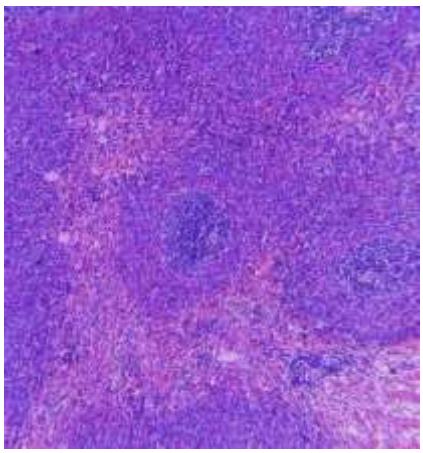
Numerous hepatocytes appears with shrunken nucleus.

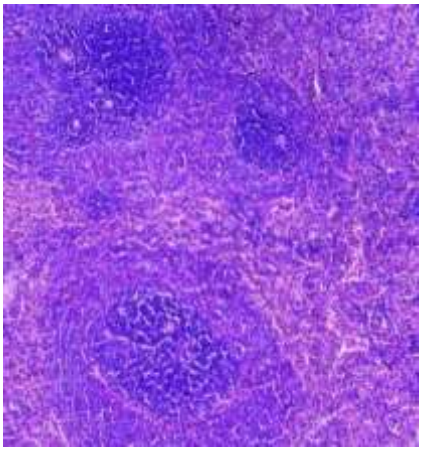
HIGH DOSE:

Diffused vascular changes were observed in the mid-zonal region

Low Power Magnification (10X) of Male albino rat - Spleen

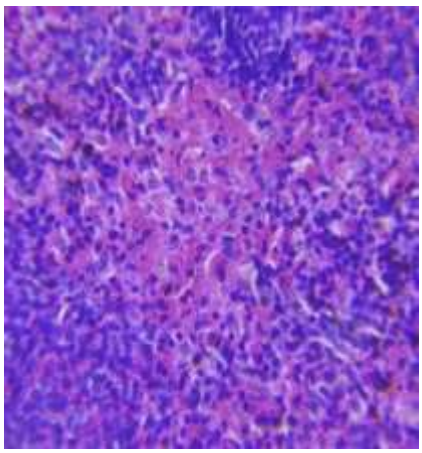
CONTROL (M) HIGH DOSE (M)

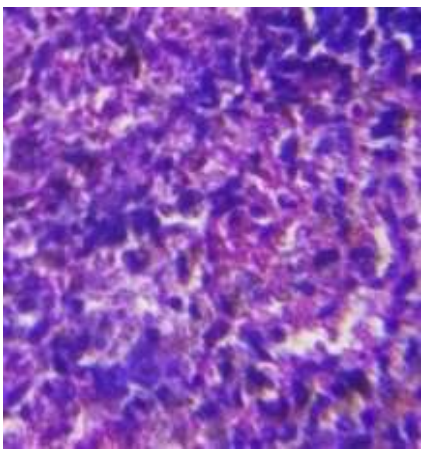




High Power Magnification (40X) of Male albino rat -Spleen

CONTROL (M) HIGH DOSE (M)





CONTROL:

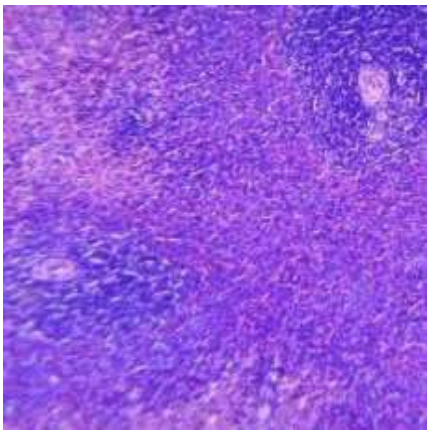
Appearance of splenic sinuses, Splenic cord and endothelial orientation was normal

HIGH DOSE:

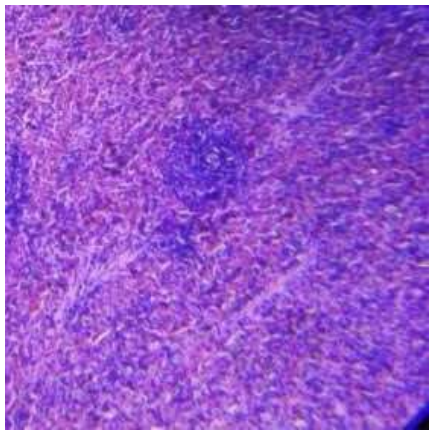
No signs of immunological reaction.

Low Power Magnification (10X) of Female albino rat - Spleen

CONTROL (F)

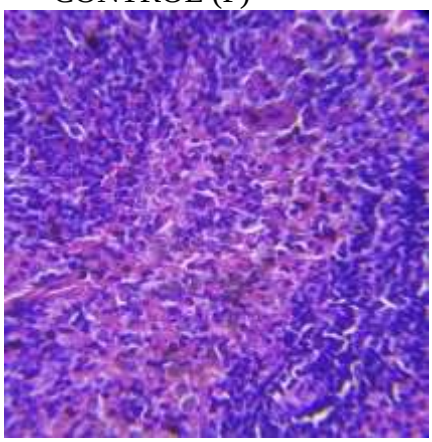


HIGH DOSE (F)

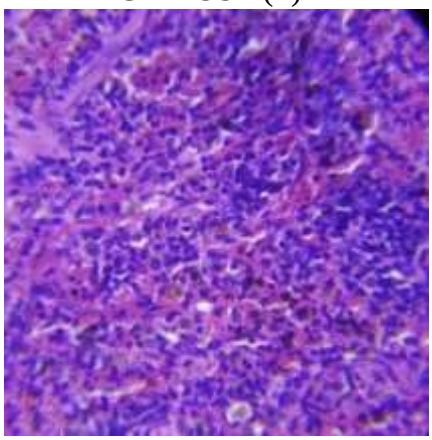


High Power Magnification (40X) of Female albino rat - Spleen

CONTROL (F)



HIGH DOSE (F)



CONTROL:

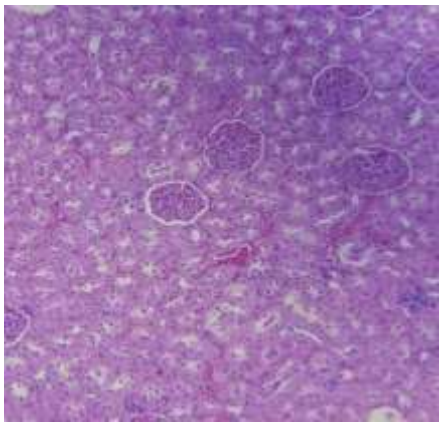
Appearance of splenic red pulp was normal.

HIGH DOSE:

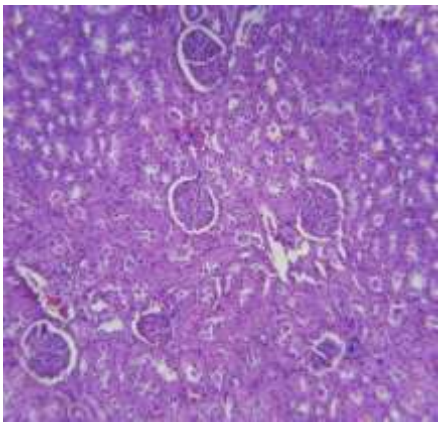
Marginal vascular zone radiated in between red and white pulp.

Low Power Magnification (10X) of Male albino rat- Kidney

CONTROL (M)

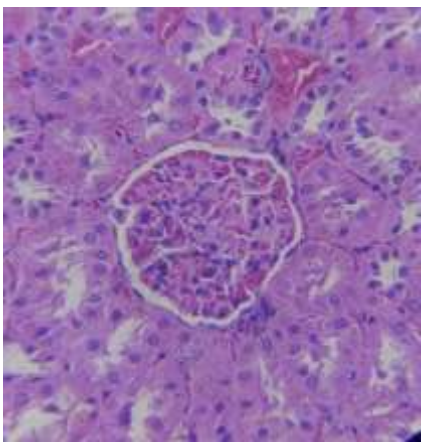


HIGH DOSE (M)

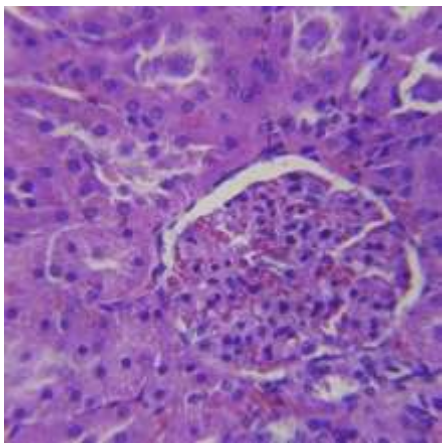


High Power Magnification (40X) of Male albino rat - Kidney

CONTROL (M)



HIGH DOSE (M)



CONTROL:

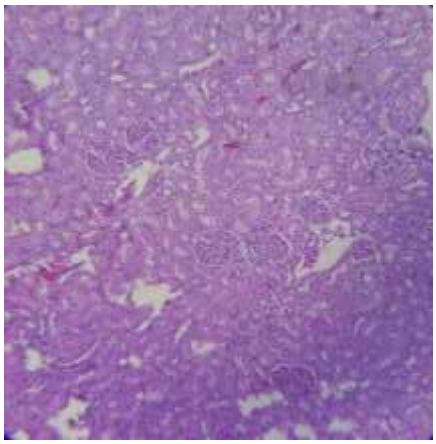
Appearance of Podocytes and parietal epithelium in the glomeruli appears normal

HIGH DOSE:

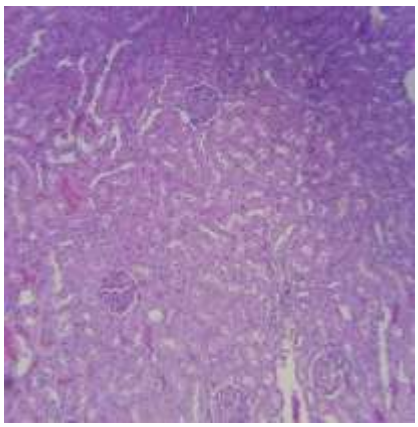
Glomerular cell integrity appears mild derangement

Low Power Magnification (10X) of Female albino rat - Kidney

CONTROL (F)

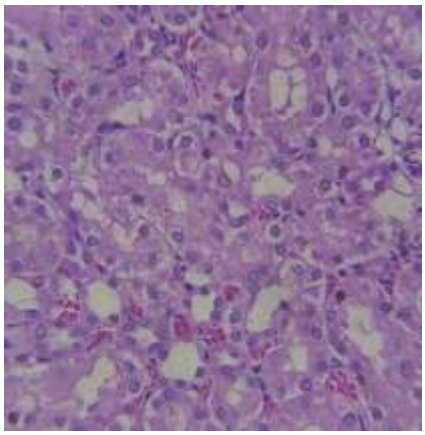


HIGH DOSE (F)

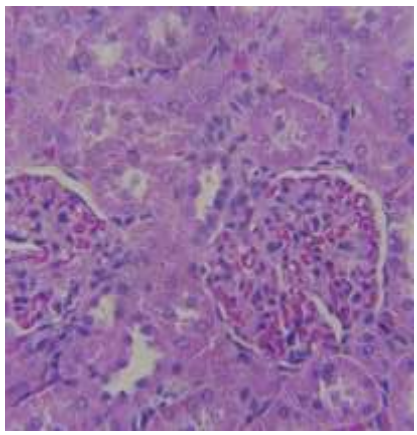


High Power Magnification (40X) of Female albino rat - Kidney

CONTROL (F)



HIGH DOSE (F)



CONTROL:

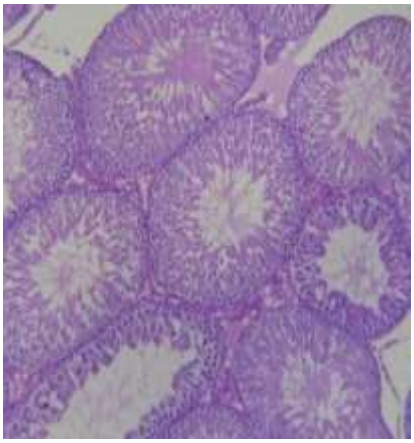
Kidney section showing normal, intact renal tubules as well as renal glomeruli.

HIGH DOSE:

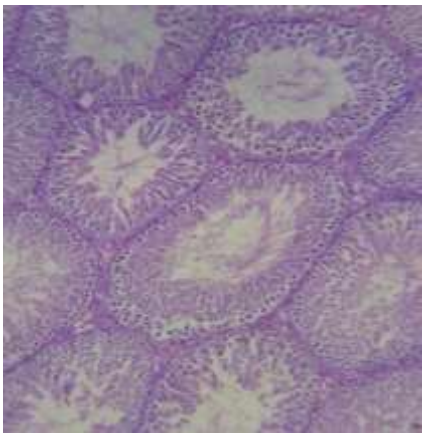
Proximal and distal convoluted tubule appears normal

Low Power Magnification (10X) of Male albino rat - Testes

CONTROL (M)

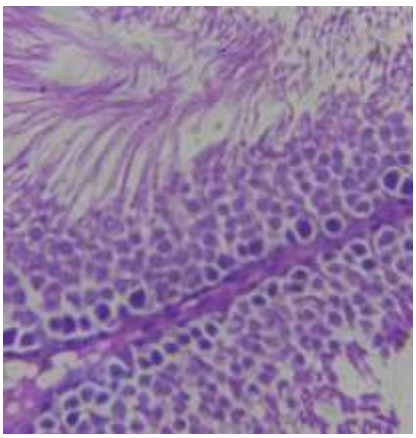


HIGH DOSE (M)

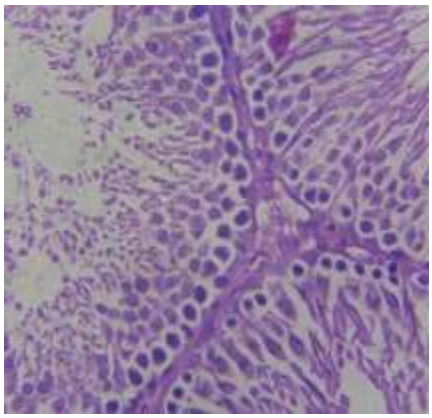


High Power Magnification (40X) of Male albino rat- Testes

CONTROL (M)



HIGH DOSE (M)



CONTROL:

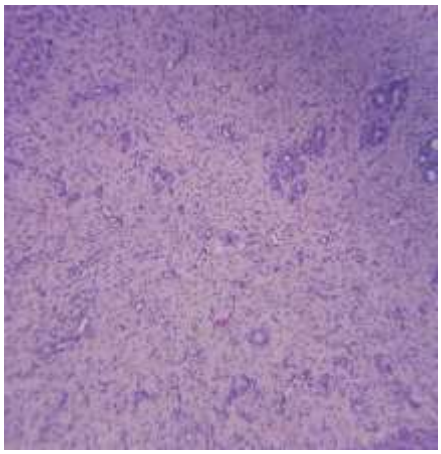
Appearance of leydig cells, interstitial tissue , seminiferous tubule, Sertoli cells and spermatogonia were normal

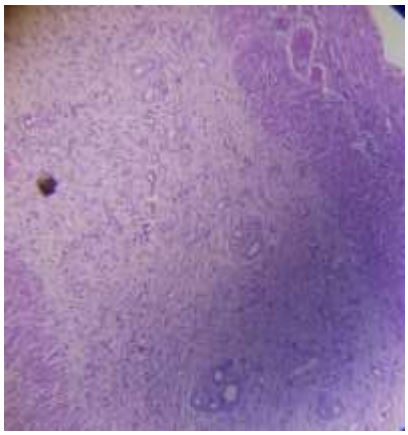
HIGH DOSE:

Sperm oriented towards the center of Sertoli cells with cluster of tail projected outside was observed.

Low Power Magnification (10X) of Female albino rat - Uterus

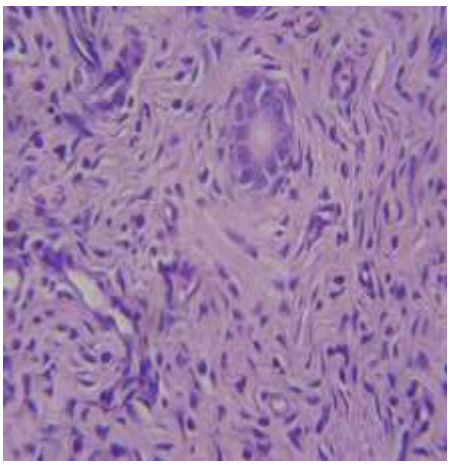
CONTROL (F) HIGH DOSE (F)

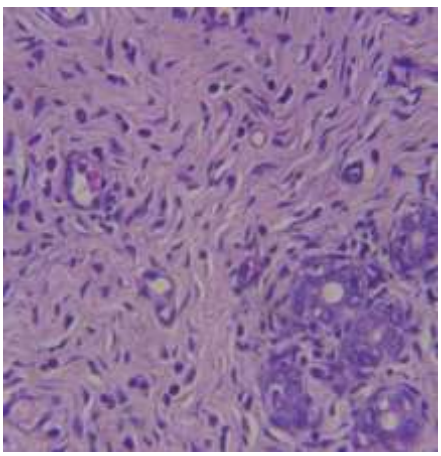




High Power Magnification (40X) of Female albino rat - Uterus

CONTROL (F) HIGH DOSE (F)





CONTROL:

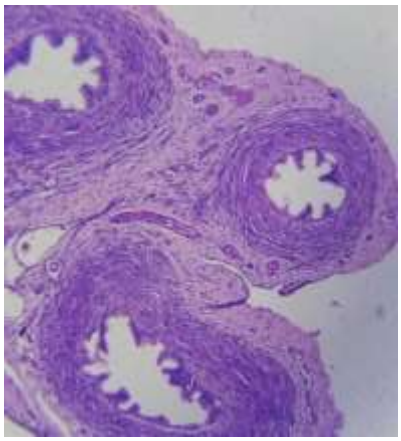
Appearance of antral follicle, primary oocyte and secondary follicles are normal

HIGH DOSE:

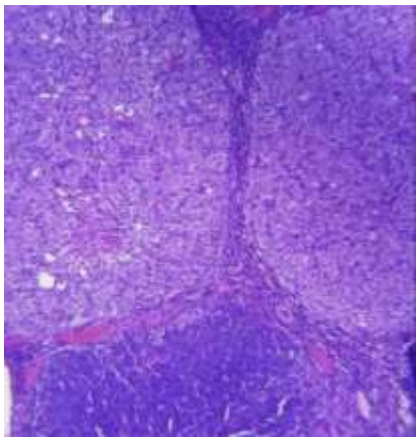
Primordial follicles with few mature ovarian follicles with no signs of abnormality.

Low Power Magnification (10X) of Female albino rat - Ovary

CONTROL (F)

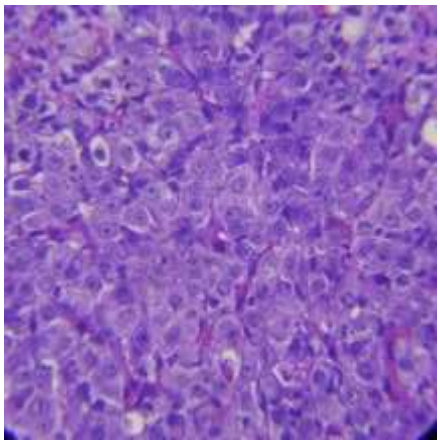


HIGH DOSE (F)

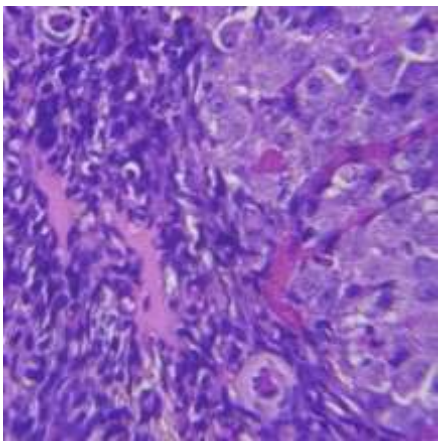


High Power Magnification (40X) of Female albino rat- Ovary

CONTROL (F)



HIGH DOSE (F)



CONTROL:

Histopathological analysis of ovary showing normal corpus luteum (CL) and Primordial follicles with few mature ovarian follicles with no signs of abnormality

HIGH DOSE:

Sequential arrangement of granulosa cells around oocyte was normal and regular.

6. DISCUSSION

Siddha Formulations containing two or more than two herbs are called poly herbal formulation. Drug formulation in Siddha is based on two principles: Use as a single drug and use of more than one drug. The latter is known as poly herbal formulation. The concept of poly herbalism is peculiar to Siddha system of medicine although it is difficult to explain in term of modern parameters. Historically, the Siddha literatures highlights the concept of synergism behind poly herbal formulations. Even though the single herb formulation have been well established due to their active Phytoconstituents, they usually present in minute amount and sometimes they are insufficient to achieve the desirable therapeutic effects. Scientific studies have revealed that these plants of varying potency when combined may theoretically produce a greater result, as compared to individual use of the plant and also the sum of their individual effect, thus positive herb-herb interaction produce synergism, which could be pharmacokinetic synergism or pharmacodynamic synergism. Popularity of polyherbal formulation is due to their high effectiveness in a vast number of diseases. They have wide therapeutic range (effective at low dose and safe at high dose), fewer side effects, eco-friendly, cheaper and readily available polyherbal formulations are always safe.

Kiranthi Mega Chooranam is one of the Siddha poly herbal formulation which treat the various diseases. The major ingredients were collected directly from their habitation and purification done as per literature. The chooranam contain abundant level of bio active agents and pharmacological studies were evaluated the ingredients of the KMC have been reported for antimicrobial, antioxidant, antiulcer, anti-inflammatory, anti-histamine, hepatoprotective properties. The primary objective of this drug has got any adverse effect in long term administration or not. First the test drug going to the process of standardization for quantitative and qualitative analysis. The following analyses were performed.

- Physico- chemical analysis
- Bio Chemical analysis
- Microbial load analysis
- Aflatoxin test
- Heavy metal analysis
- HPTLC analysis
- Pesticide residue test
- Phytochemical analysis

The safety profile was evaluated by acute and long term toxicity study on wistar albino rats as per WHO guidelines. Standardization of the drugs means confirmation of its identity and determination of its quality and purity[(39)]. By the way of standardization, metal-based drugs can be evaluated for their performances, limitations, optimal dosage, contraindications, and applications [(40)].

The percentage of loss on drying of Kiranthi Mega Chooranam was 9.6% (normal range 1-20%) since loss on drying of the test drug is low, the stability of the drug is higher. The ash limit test is used to determine the quality and purity of crude drugs. The total ash value of Kiranthi Mega Chooranam was 8.5% (normal range 1-25%). The acid insoluble ash as limit test is designed to measure the amount of ash insoluble to diluted hydrochloric acid. The acid insoluble ash value of Kiranthi Mega Chooranam were 4.3 % and it shows that a very small of inorganic constituents is insoluble in acid.

Extractive values are primarily useful for the determination of exhausted or adulterated drugs. The extractive value of the crude drug determines the quality as well as purity of the drug. Thus, alcohol and water soluble extractive values were determined[(41)]. The extract value of water in Kiranthi Mega Chooranam is 12% and alcohol is 21% . From the above result we concluded that water is little better solvent of extraction than alcohol.

The Biochemical analysis of the test drug indicates Acid radicals, Basic radicals, and other constituents demonstrates the presence of Silicate, Phosphate, Zinc, Iron, Calcium, starch, carbonate, alkaloids, fluoride and oxalate. Phytochemical investigation to detect the presence of various Phytoconstituents in formulation Kiranthi Mega Chooranam showed the presence of carbohydrates, glycosides, saponin, phenols, flavonoids, proteins, diterpenes, gum& mucilage. Presences of the above components in biochemical and phytochemical analysis increases the therapeutic value of Kiranthi Mega Chooranam.

HPTLC finger printing analysis of the sample reveals the presence of four prominent peaks corresponds to presence of four versatile phytocomponents present with in it. The plate was scanned at 254nm, 366nm and 620nm after post derivatisation. Rf , color of the spots and densitometric scan were recorded using CAMAG Scanner 4[(42)]Rf value of the peaks ranges from 0.16 to 0.64. Further the peak 4 and 1 occupies the major percentage of area of 56.20 and 29.67 % which denotes the abundant existence of such compounds.

WHO has need for quality assurance of herbal products including testing of microbial contamination, aflatoxin, pesticide residue, were studied in mega Chooranam showed below detection limit of aflatoxin and all pesticide. The results concluded that the below detection limit of microbial contamination, aflatoxin, pesticide residue indicated the quality of the ingredients of Kiranthi Mega Chooranam. The drug was quantitatively analysis for heavy metal content by AAS. This analysis showed the below detection limit of mercury and arsenic and absence of cadmium and lead.

Acute toxicity study there was no abnormal signs reported at the dose level of (2000 mg/kg b.wt) within 24 hours in wistar albino rats. No mortality and no pathological changes have been seen in internal organs of both control and treated groups in the 14 days study period. And the body weight, food intake and water intake of the animal is normal.

Long term toxicity study was conducted for about 90 days as per WHO guideline in 3 doses Low dose (270mg/kg b.wt), Mid dose (540mg/kg b.wt), High dose (1080mg/kg b.wt). Animals were observed throughout the period. In wistar albino rats there was significant increase in body weight in mid dose and high dose from 45^{th} day to 90^{th} day (table11).

When compared to control group and there was slight significant increase in feed intake at 45^{th} day in high dose (table13). At the end of 90 days study, on 91^{st} day animals were sacrificed and blood samples were collected and investigated. The result shown that there was substantial increase in RBC and WBC (Table15) when compared to control group. It altered the blood components but within the physiological limit. In biochemical parameters there was significant (**$P<0.01$) changes in lipid profile when compared to control group. It altered the lipid metabolism but within the physiological limit. Renal parameters and hepatic parameters are normal compared to the control group. The histopathological study on the organs such as lung, kidney, spleen, liver, brain, heart, stomach, uterus ,ovary and testes was normal in high dose groups when compared to control.

7. SUMMARY

Chooranam is poly herbal formulation one among the 32 internal medicines in Siddha and it has a shelf life of three months. In present scenario toxicological evidence is need to prove the safety of drugs. Globally, there is increasing need of drugs to cure various ailments. Siddha, a traditional system of medicine is now attracting people because of their efficacy in curing diseases. Kiranthi Mega Chooranam is one of the major Siddha medicines which are widely used to cure various ailments. It has specific indication to cure major diseases like Kiranthi (Syphilis), Kadividam (Poisonous bites), Kuttam (Skin diseases), Thadippu (Urticaria), Megasoolai (Syphilitic arthritis).

The aim of the research work was to study the safety of the experimental formulation by acute and long term toxicity study in the animal models as per WHO Guideline. The ingredients of the test drugs were collected from the hill station and purchased from standard raw drug markets. The drugs were identified and authenticated by Assistant Professor of Medicinal Botany, National Institute of Siddha, Chennai-47. All the distinct components have been purified as per Siddha literature and a formulation was prepared in Gunapadam lab NIS.

Kiranthi Mega Chooranam was analyzed quantitatively and qualitatively with, Physico- chemical, Biochemical, Microbial Load, Pesticide Residue, Aflatoxin, Heavy metal analysis and to evaluate safety by acute and long-term toxicity studies.

Initially the drugs were subjected to physico-chemical analysis. It reveals the increase in bioavailability and purity of the drug. Then the sample was analyzed for bio chemical constituents.

Phytochemical investigation to detect the presence of various phyto constituents in formulation Kiranthi mega Chooranam showed the presence of carbohydrates, glycosides, saponin, phenols, flavonoids, proteins, gum& mucilage, quinones. Presences of the above components in biochemical and phytochemical analysis increases the therapeutic value of Kiranthi Mega Chooranam.

Microbial load, pesticide residue, aflatoxin level was quantitatively measured in the test drug the result specifies the below detectable limit of them indicates the the quality of the ingredients of Kiranthi Mega Chooranam.

Heavy metal analysis was carried out in the test drug to ensure the absence of mercury, lead, arsenic, cadmium. The HPTLC analysis of the sample was done which denotes the abundant existence of such major compounds.

Acute toxicity study there was no abnormal signs reported at the dose level of (2000 mg/kg b.wt) within 24 hours in wistar albino rats. No mortality and no pathological changes have been seen in internal organs of both control and treated groups in the 14 days study period. And the body weight, food intake and water intake of the animal is normal.

Long term toxicity study was conducted for about 90 days as per WHO guideline in 3 doses Low dose (270mg/kg b.wt), Mid dose (540mg/kg b.wt), High dose (1080mg/kg b.wt). Animals were observed throughout the period. There was some significant changes in body weight and feed and water intake after the 45^{th} day, when test group was compared to control group but within the biological limit

After completion of the 90 days study, on 91^{st} day animals were sacrificed and blood samples were collected and investigated. The result revealed that there was significant present in RBC and WBC (Table15) when compared to control group. The renal and hepatic parameter were denotes slight significant changes (**$P<0.01$) but within the physiological limit.

The histopathological study on the organs such as lung, brain, kidney, liver, spleen, heart , stomach, ovary, testis, uterus was normal in Control and High dose group of the animals.

8. CONCLUSION

The Siddha polyherbal formulation Kiranthi Mega Chooranam is one of the best drug widely used to cure major ailments the results reveals purity and bioavailability of the drug. As per the result of heavy metal analysis of this drug the major heavy metals were found to absent, so this drug is safe enough for oral consumption. In vivo toxicity study reveals that there was no mortality and signs of toxicity observed for acute oral administration of Kiranthi Mega Chooranam till the dose of ten times the therapeutic dose of 2000mg/kg b.wt in prescribed manner. In long term toxicity study there was significant changes in haematological and biochemical parameters in test drug treated groups when compare to control group. The histopathological report are also confirms that there was no cellular changes at all doses level. It clearly demonstrate that No Observed Adverse Effect Level (NOAEL) is up to the high dose level (1080mg/kg b.wt), which is ten times of that therapeutic dose. Based on these results it can be concluded that the dose level of Kiranthi Mega Chooranam 6-8gm for a duration of one mandalam (48 days) (bd/day) was mentioned in Siddha literature **Anuboga vaithiya Navaneetham** is safe dosage for consumption.

In future it is to be carried out to study the pharmacological activity and clinical trials to prove the efficacy of the drug.

9. BIBLIOGRAPHY

1. Kandasamy Pillai- History of Siddha Medicine by Department of Indian Medicine& Homeopathy Chennai. Second edition 1998, pg.no-1,852,853.
2. Principals of Diagnosis in Siddha, Department of Indian Medicine and Homeopathy, First edition 2009, Page no: 11 -12.
3. Dr.R.Thiyagarajan, Siddha Maruthuvam Sirappu, Department of Indian Medicine and Homeopathy, Chennai , 2nd d edition1995, Page no: 3.
4. The wealth of India, National Institute of Science Communication and Information Resources, New Delhi, Vol -1, 2004, Page no: 1,121.
5. Mugamathu Icbal, Sattam Saarntha Maruthuvam, Indian Medicine and Homeopathy Department, Chennai -106, 2002, Page no: 564
6. Shukla SS, Saraf S, Saraf S Fundamental Aspect and basic concept of Siddha medicine. Vol - 2. Systemic reviews in pharmacy/Jan - June 2011/issue.
7. Anaivaari R.Anandan., M.Thulasimani, Siddha Materia Medica by Department of Indian Medicine & Homeopathy, 2008, pg.no 58.
8. K.S.Murugesa muthaliyar, Gunapadam(Mooligai) Indian medicine and Homeopathy department, 9th edition pg.no 134,576, 499,488.
9. Pullaiah, Encyclopedia of world medicinal plants, Regency Publications, New Delhi, Vol III, 2006, 1143-44.
10. Rajendran K, Anie shirwaikarb, Srinivasan KK, Short term hypoglycemic and anti-hyperglycaemic studies on alcoholic extract of Indigofera aspalathoides, Journal of Natural medicines 2007; 4(2): 80-83.
11. Saritha Band Brindha P, Evaluation of wound healing activity of Indigofera aspalathoides Vahl Ex Dc- a traditional Siddha drug, International Journal of Pharmacy and Pharmaceutical Sciences 2012; 4(2), 2012.
12. Parmar Rajesh Sarman-, Nephroprotective activity of Indigofera aspalathoides Vahl on Gentamicin induced nephrotoxicity in rats 2010 (Un published data)

13. Ramchander, Pawan Jalwal and Anil Middha, Recent advances on senna as a laxative: A comprehensive review, Journal of Pharmacognosy and Phytochemistry 2017; 6(2): 349-353.

14. D. Balasankar , K. Vanilarasu , P. Selva Preetha, S.Rajeswari, M.Umadevi , Debjit Bhowmik Senna – A Medical Miracle Plant, Journal of Medicinal Plants Studies, Vol. 1 Issue. 3 2013.

15. Akilandeswari S., Kumarasundari S.K., Valarmath R., Manimaran S., Sivakumar M., Studies On AntiInflammatory Activity Of Leaf Extract Of Justicia Tranquebariensis L., Indian Journal of Natural Products, 2001, 17(1), 14-16.

16. Jiju V., Investigation of Invitro Antihelmenthic Activity of Justicia Tranquebariensis, World Journal of Pharmaceutical Research, Vol 4, Issue 06, 2015.

17. Shabana Begum M., Muhammad Ilyas M.H., Burkanudeen A., Evaluation of Antibacterial Activity of Leaves of Justicia Tranquebariensis L., The Pharmacist, 2008, 3(1), 35-37.

18. Krishnamoorthi R and Bai VR: Phytochemical analysis and antioxidant property of *Justicia tranquebariensis*. Int J Pharmacognosy 2015; 2(5): 254-58.

19. Purushothaman Balakrishnan, Thameem Ansari Musafar Gani, A perspective on bioactive compounds from Solanum trilobatum, Journal of Chemical and Pharmaceutical Research, 2015, 7(8):507-512.

20. Ramachandran Venugopal, Palaniyandi Senthilnathan, Chemotherapeutic Efficacy of Solanum trilobatum along with Paclitaxel in Lung Cancer Bearing Swiss Albino Mice International Journal of Cancer Research, 2007, Volume: 3, Issue: 2 , Page No.: 119-126

21. Kumaranshanmugam, A perspective on bioactive compounds from Solanum trilobatum et al J Chem.Pharm.Res.,2015,7(8):507-512.

22. S.Emmanuel,S.Ignacimuthu,R.Perumalsamy,T.Amalraj,AntiInflammatory activity of Solanumtrilobatum,Elsvier,Fitoterapia,Volume-77,Iss ues7-8,December 2006,Pages 611-612.

23. Anthoni SA, John J, Geegi PG, Prabhash T. Int. J. Inst. Pharma. Life Sci. 2011; 1(3):112–119.

24.Nadkarni AK, Dr. KM. Nadkarni's Indian Materia Medica, Bombay: Popular Prakashan Private Ltd. 1976, 1.

25. R.Kowsalya, A Vinoth and S Ramya,, Antibacterial activity of silver and zinc nanoparticles loaded with Enicostemma Littorale, Journal of Pharmacognosy and Phytochemistry 2019; SP2: 335-340

26. Nidhi Mishra, Kamini Kaushal, An ayurvedic herb: Enicostemma littorale blume, Journal of Medicinal Plants Studies 2017; 5(1): 78-82.

27. P.Praveena , D.Sudarsanam, Phytochemical and Pharmacognostic Value of Enicostemma littorale, International Journal of Pharmacognosy and Phytochemical Research 2014-15; 6(4); 795-800.

28. Pande, V.V. and S. Dubey, 2009. Antihyperlipidemic activity of Sphaeranthus indicus on atherogenic diet induced hyperlipidemia in rats. Int. J. Green Pharm., 3: 159-161.

29. Duraipandiyan, V., P. Kannan and S. Ignacimuthu, 2009. Antimicrobial Activity of *Sphaeranthus indicus* L. Ethnobot. Leaflets, 13: 320-325.

30. Prabhu, K.S., R. Lobo and A. Shirwaikar, 2008. Antidiabetic properties of the alcoholic extract of Sphaeranthus indicus in streptozotocin-nicotinamide diabetic rats. J. Pharm. Pharmacol., 60: 909-916.

31. Sarakku suddi seimuraikal (200), Department Indian Medicine and Homeopathy Chennai-106.

32. Hakkim P. Mohammed Abdulla Sahib, Anuboga vaithiya navaneetham, part-10, Thamarai Noolagam, 2nd edition, 2002, Pg 83.

33. Kunle,Oluyemisi Folashade, Standardization of herbal medicines - A review International Journal of Biodiversity and Conservation Vol. 4(3), pp. 101-112, March 2012.

34. Mukherjee, P. K. & Wahile, A. (2006). Integrated approaches towards drug development from Ayurveda and other Indian system of medicines. Journal of Ethnopharmacology, 103, 25–35.

35. Wagner H. Plant Drug Analysis, A thin layer chromatography Atlas 2nd edition. Heidelberg: Springer-Verlag Belgium; 2002: 305,227.

36. Grant, C. A., Buckley, W. T., Bailey, L. D., & Selles,F. (1998). Cadmium accumulation in crops. Canadian Journal of Plant Science, 78, 1–17.

37. De Smet PAGM (1999). Overview of herbal quality control. Drug Inform. J., 33: 717-724.

38. World Health Organization. WHO guidelines for assessing quality of herbal medicine with reference to contaminants and residue, Geneva, WHO Press; 2007.

39. Achana A. Bele et al., Standardization of herbal drugs: An overview; International Research Journal of Pharmacy, 2011, 2(12), 56-60.

40. Vandana Garg et al., Facts about standardization of herbal medicine: a review; Journal of Chinese Integrative Medicine, Oct. 2012, Vol. 10, No. 10 .

41. R. K. Chaudhari, N. O. Girase, Determination of soluble extractives and Physico-chemical studies of bark of Sesbania sesban (L) Merr. Journal of Chemical and Pharmaceutical Research, 2015, 7(8):657-660.

42. Sethi PD. High Performance Thin Layer Chromatography, 1st ed. New Delhi, CBS Publishers and Distributors; 1996.

ANNEXURE

(19)

CERTIFICATE

This is certify that the project title KIRANTHI MEGA CHOORANAM has been approved by the IAEC. Total No. of animal approved: 90 Rats

Approval No: NLS/IAEC/TU/28082018/19 (45M+45F)

V. Banumathi

Prof. Dr. V. BANUMATHI
Chairman IAEC

Prof. Dr. K.NACHIMUTHU
CPCSEA nominee 28/8/2018

Chairman/Member Secretary of IAEC: CPCSEA nominee:

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

Name of the investigator : Dr. G.THANGANILA
Department : Nanju maruthuvam

Received the original Certificate

20/11/18.

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NATIONAL INSTITUTE OF SIDDHA, CHENNAI – 600047

BOTANICAL CERTIFICATE

Certified that the following plant drugs used in the Siddha formulation **"Kiranthimega Chooranam"** taken up for Post Graduation Dissertation studies by **Dr.G.Thanganila** M.D.(S), II year, Department of Nanju Maruthuvam, 2019, are identified through Visual inspection, Experience, Education & Training, Organoleptic characters, Morphology and Taxonomical methods as

Cassia senna Linn. (Caesalpiniaceae), Leaf
Justicia tranquebariensis Roxb. (Acanthaceae), Whole plant
Indigofera aspalathoides Vahl ex DC. (Fabaceae), Whole plant
Enicostemma littorale Blume. (Gentianaceae), Whole plant
Solanum trilobatum Linn. (Solanaceae), Whole plant
Sphaeranthus indicus Linn. (Asteraceae), Whole plant

MEDICINAL BOTANY * National Institute of Siddha CHENNAI 600 047

Certificate No: NISMB3792019

Date: 08-04-2019

Authorized Signatory

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

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CERTIFICATE

Ministry of AYUSH

NATIONAL INSTITUTE OF SIDDHA

Ministry of AYUSH, Government of India

Tambaram Sanatorium, Chennai - 600 047.

WORKSHOP ON
RESEARCH METHODOLOGY & BIOSTATISTICS

This is to certify that

Dr. G. THANGANILA

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

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

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

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NATIONAL INSTITUTE OF SIDDHA

An Autonomous Body under Ministry of AYUSH
Govt. of India

Workshop on

Laboratory Animal Care and Basic Research Techniques

(11-15 February, 2019)

CERTIFICATE

This is to certify that

Dr.G.Thanganila

has participated as Trainee in the workshop on

"Laboratory Animal Care and Basic Research Techniques"

held between 11.02.2019 & 15.02.2019 at National Institute of Siddha, Tambaram Sanatorium, Chennai.

Dr.V.Suba
Organising Secretary

Dr.B.R.Senthilkumar
Coordinator

Prof.Dr.V.Banumathi
Chairperson / Director, NIS

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