PRECLINICAL SAFETY EVALUATION OF KOTTAI KARANTHAI CHOORANAM

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"preclinical safety evaluation of *kottai karanthai chooranam"*** is a bonafide and genuine research work carried out by me under the guidance of **Dr. S. MURUGESAN , M.D(S), Lecturer, 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.

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1.INTRODUCTION

A systematic study of the nature and its elements from which is now known as “Siddha system”. The word Siddha means ‘Ever sure’, ‘True’, Ever ready and Everlasting. The Siddha system originated from 18 Siddhars headed by ‘Agathiyar’. Siddha method is well founded under the basic principles of the human system.

The seers of ancient India propounded the Thiri dhathu theory in accordance with which three vital elements namely the Vatham, Pitham, Kabham, in their normal condition regulate all physiological activities and keep the body healthy. When these thiri dhathu become abnormal or when their mutual harmony is disturbed (in which role they are called thiri dosham) they bring about ill health[1].

Siddha have certain diagnostic methodology to evaluate the root cause of disease. The various diagnostic and prognostic tools namely Envaigai thervu, Manikadai nool, Jothidam and Panchapatchi sasthiram.

The treatment in Siddha medicine is aimed at maintaining the three dhosham and seven thathus in equilibrium. Proper diet and a regimen of life including cleansing therapies are advised for a healthy living and to restore equilibrium of dhosham in the early stage of the disease[2] . A substance or agent one that ensures remedy to physiological or pathological illness and one that prevents disease and thereby provides immortality or delay death is called as a Medicine[3]. The siddhars used the medicines prepared from innumerable plants, herbs, metals, poisonous substances, minerals, salts, and other organic substances. Which can render relief to innumerable ailments of mankind suffered and is suffering[4].

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

1. Internal medicine
2. External medicine

In Siddha system of medicine, chooranam based on pure herbals is one of the first line of medicine for the several diseases. Chooranam is one among the 32 internal medicines in Siddha and it has a self-life of three months[5].

Chooranam is fine dry powders of drug. The terms ‘chooranam’ may be applied to the powder of single drug or a mixture of two or more drugs, which are powdered separately prior to their being mixed to homogeneity[4].

The prevalence of skin diseases was 60%. There was female preponderance 55.7% and 44.3% were male participitants[6].

Skin diseases are numerous and a frequently occurring health problem affecting all ages from the neonates to the elderly and cause harm in number of ways. Maintaining healthy skin is important for the healthy life. Herbals have great potential to cure different kinds of skin diseases. More than 80% of people in India depend on traditional health care and use different plant based products for curing skin related problems[7].

Kottai Karnathai Chooranam is a single herbal drug formulation use to treatment of Karappan, Sori, Thozh Pinigal, Maripatta earu kazhium, Vali, Veri, Sinaippu mentioned in Siddha literature[8].

The safety profile of “**Kottai Karnathai Chooranam”** is not proving till now. In present scenario toxicity evidence is need to prove the safety of any drug. So, the author selects “**Kottai Karnathai Chooranam”** to access safety profile through toxicity studies.

2. AIM AND OBJECTIVES

AIM:

To evaluate the safety profile of Kottai Karanthai Chooranam (KKC) on animal models (Wistar albino rats).

OBJECTIVES:

To Study the physicochemical analysis, biochemical analysis and phytochemical analysis of Kottai Karanthai Chooranam.

To study the heavy metal analysis, Pesticide residue, Aflatoxin of Kottai Karanthai Chooranam.

To evaluate the acute toxicity profile of Kottai Karanthai Chooranam as per WHO Guidelines.

To evaluate the long-term toxicity profile of Kottai Karanthai Chooranam as per WHO Guidelines.

3. LITERATURE REVIEW

3.1 SIDDHA ASPECT REVIEW:

கொட்டைக் கரந்தை

Figure 1

பெயர்க் காரணம்:

இதன் மலர்கள் சிறு முட்டை வடிவிலானவும் செம்மை, நீலம், இளம்சிவப்பு, நீலம் கலந்த சிவப்பு நிறங்களை உடையனவாகவும் இருக்கும். இக்கரந்தையில் மலரெனக் கூறப்படுவது தனி மலரன்று. இதனை கரந்தையினர் என்பது பொருந்தும். சற்று நீண்ட சிறு முட்டை வடிவிலான கரந்தையின் இவ்வினரில் நூற்றுக்கணக்கான மலர்கள் இணைந்து கொட்டைபோல இருப்பதால் இது கொட்டைக் கரந்தை என வழங்கப்படுகிறது[9].

இ.ந்து இந்தியாவில் எங்கும் பயிராகும். இப்பூண்டு தென்னிந்தியாவிற்கே உரியது. இது வயல் நிலங்களிலும் தோட்டங்களிலும் வளர்ந்து கிடக்கும்[8].அறுவடை செய்த வயல்களில் விசேஷமாய்க் காணலாம். இது வருடத்திற்கு ஒரு முறை முளைக்கும். இது வெள்ளை, சிவப்பு என இருவகைத்து. வெள்ளைப் பூ அரிது. ஆனால் சிவப்பு எங்கும் கிடைக்கக் கூடியது. இதற்கு மருத்துவ குணம் உண்டு. வாசனை அதிகம்[10].

ஒரு ஆண்டு வாழும்(Annuals) இயல்புடைய சிறுசெடி இனம். இலைகள் காம்பற்றதாய் 2-7 செ.மீ நீளமாக நீண்டு (Obovate -oblong) சிறு மயிரிழை போன்ற வளரிகளுடன் விளிம்பில் பற்களுடையதாய்(Spinous serrate or dentate) அடியில்

குறுகியும் இருக்கும். மலர்கள் நுனியில் தனிமலராக உருண்டையாக இருக்கும் ஹெட் இன்.்பிலாரன்ஸ் (Head inflorescence) வகை. இளமையில் பச்சையாகவும் முதிரிரும்போது சிவந்த நிறத்திலும் இருக்கும்.[11].

கரந்தையில் பல விதமுண்டு அவையாவன:

1.கொட்டைக் கரந்தை - Spheranthus indicus

2.குத்துக்கரந்தை - Erigeron oliquum

3.சிறுகொட்டைக் கரந்தை - Ethulia divaricata

4.சிவ கரந்தை - Sphoe rathus amaranthoides

5.நறுங் கரந்தை - Hianaoser

6.சுனைக்கரந்தை - Ocimum pilosum

7.சுரைக்கரந்தை - Sphoeranthus Neylanicus Nob

8.சூரியக்கரந்தை[8]

சாம்பசிவம் அகராதியில் கூறப்பட்ட வேறு சில வகைகள்:[10]

தமிழ் மருத்துவத்தில் கரந்தையில் 19 வகையான செடிகள் இருப்பதாக கூறப்படுகிறது.

அவையாவன:

1. கொட்டைக் கரந்தை - Indian globe thistle-Sphaeranthus indicus
2. சுரக் கரந்தை - fever toolay or Sphaerantaus amranthoides.
3. சிறுகொட்டைக் கரந்தை - Small seeded basil- Brachycome assamica
4. சிவ கரந்தை - Ceylon toolay-Spheanthus zeylanius.
5. சுனைக்கரந்தை - Sharp basil- Ocimum pilosum
6. சின்ன பூக்கொட்டைக் கரந்தை - Small seeded basil- Brachycome assamica
7. கோடாலிக் கரந்தை - axe basil-Spaeranthus indicus

8. விஷ்ணு கரந்தை - blue. Flowered Vishnu basil-Spaeranthus mollis.
9. வயல் கரந்தை - Indian globe thistle-Sphaeranthus indicus
10. சுரக் கரந்தை - fever toolsy or Sphaeranthaus amaranthoides.
11. குத்துக்கரந்தை - a variety of Sphaeranthus indicus
12. சூரியக்கரந்தை - unidentified plant.
13. நறுங் கரந்தை - Indian globe thistle-Sphaeranthus indicus
14. நாறு கரந்தை - Indian globe thistle-Sphaeranthus indicus
15. வெள்ளை கொட்டைக் கரந்தை அல்லது வெண் கரந்தை - A White species of Sphaeranthus indicus
16. வெள்ளை விஷ்ணு கரந்தை - Flowered Vishnu basil-Spaeranthus mollis. but with white flowers instead of blue. A very rare palnt.
17. சங்க நிறக் கரந்தை - A White species of Sphaeranthus indicus
18. பசுக் கரந்தை - Young plant of Sphaeranthus indicus
19. பிரப்பங் காய்க் கரந்தை - An unknown variety .

கொட்டைக் கரந்தை சற்றேறக் குறைய சிவகரந்தைக்கு ஒப்பெனினும் மணத்தில் சிவகரந்தை சிறப்புடையது.

இவைகளுள் கொட்டைக்கரந்தை சிவக்கரந்தை இவ்விரண்டும் மருந்து வகைகளில் சிறந்தது. இவைகளே பெரும்பாலும் மருந்துகளுக்கு வழங்கப்படும்.

இதிலிருந்து ஒரு வகை எண்ணெய் எடுக்கின்றனர்.

பயன்படும் உறுப்பு : இலை, பூ, விதை, வேர், வேர்ப்பட்டை.

சுவை : கைப்பு

தன்மை : வெப்பம்

பிரிவு : கார்ப்பு[8]

செய்கை:

இலகுமலகாரி

சமனகாரி[12]

இலை, பூ : உடற்தேற்றி

: உள்ளழலாற்றி

விதை, வேர் : பசிதீத்தூண்டி

: புழுக்கொல்லி

சிறப்பு செய்கை

தூய்மையாக்கி

குளிர்ச்சியுண்டாக்கி

உரமாக்கி

குணம்: இதனால் ஒழுக்கு வெள்ளை, கரப்பான், சொறி, சிரங்கு இவை தீருவதல்லாமல், மறிப்பட்ட எருவுங் கழியும். மேலும் வளி, வெறி, சினைப்பு முதலியவையும் ஒழியும். தோற் பிணியும் போம்.

கொட்டைக் கரந்தைதனைக் கூசாம லுண்டவர்க்கு
வெட்டை தணியுமதி மேகம்போந்- துட்டச்
சொறி சிரங்கு வன்கரப்பான் தோற்றாது நாளும்
மிறிமலந் தானிறங்கு மால்.

வழக்கு:

சமூலம்

மேற்கூறிய நோய்களுக்கு இப்பூண்டினை உலர்த்திப் பொடி செய்து வேளைக்கு 4 கிராம் எடை சாப்பிட்டு வரலாம்.

இலை

இலையை நிழலிலுலர்த்திப் பொடி செய்து வேளைக்கு 1 கிராம் எடை வீதம் தினம் இரு வேளை கொடுத்து வர தோலைப் பற்றிய நோய்கள் தீரும். ஆண்மையுண்டாக்கும்.

பூ

மேற்கூறிய இதைப் பொடித்துக் கொடுக்க உரமாக்கியாக வழங்குவதன்றி உடலுக்குக் குளிர்ச்சியையும் உண்டுப் பண்ணும்.

விதை

விதையைப் பொடித்துத் தேனிற் கலந்து கொடுக்க செரியாமை, இருமல் தீருவதுமல்லாமல் வயிற்றுப்புக்களும் வெளிப்படும்.

வேர்

வேர் பொடித்து 2 கிராம் எடை கொடுக்க வயிற்றுப்புழுக்களைக் கொல்லும்.

வேர்ப்பட்டை

இதை அரைத்து வெண்ணெயிற் கலந்துகொடுக்க குருதி எருமுளை போம். இதையே அரைத்து வெளிப் பூச்சாகவும் வழங்கலாம்.

நல்லெண்ணெயுடன் கலந்து 7 மி.லி - 15 மி.லி உச்சிக்கரண்டி வீதம் ஒரு மண்டலம் சாப்பிட்டு வர வன்மை பெறும். ஆண்மை பெறும்[8][13].

இதன் விதை புழுக்களைக் கொல்லும். வேர்த் தூள் சீரணத்திற்கு வலிவைக் கொடுக்கும். இதன் பட்டையை அரைத்து மோரிற் கலந்துட் கொள்ள மூலத்திற்கு நல்லது. இதன் சமூலத்தைச் சூரணித்து வெள்ளை பிரமேகம் , வாதம், கிரந்தி, கரப்பான், மலபந்தம், கிராணி முதலியவைகளைக் குணப்படுத்தக் கொடுப்பதுண்டு.

அன்றியும் இச்சமூலம் தலை, மூளை, இருதயம் , நரம்பு இவைகட்கு பலத்தைக் கொடுக்கும். கஷாயமாகச் சாப்பிட பைத்தியம் கிரந்தி போம். இக்கஷாயத்தோடு சீரகத்தைப் பொடித்துப் போட்டு உட்கொள்ள வயிற்றுக் கோளாறுகள் தீரும். இதன் சமூலத்தின் சாற்றை சூதத்திற்குச் சுருக்குக் கொடுக்க வெள்ளிக் கம்பியைப் போலாகும். இதன் சமூலத்தின் இரசமும் இலை ரசமும் வாத முறைக்கும். கற்ப முறைக்கும் மிக்க உபயோகமானது.[10]

பொதுவாக கரந்தை இனம் சிறு நீரக நோய்களை போக்கவும் பழுக் கொல்லியாகவும் பயன்படும்[14].

கொட்டைக்கரந்தை செடியைப் பூ விடுவதற்கு முன்பாகவே எடுத்து நிழலில் உலர்த்தி இடித்துச் சூரணம் செய்து வைத்துக் கொண்டு வேளைக்கு ¼ தோலா எடை சீனி கூட்டிக் கொடுத்து வர வெள்ளை, உள்ரணம், கரப்பான், கிறாணி இவை போம். தலை, மூளை, நரம்பு, இருதயம் இவைகளுக்கு நல்ல பலத்தைக் கொடுக்கும். இந்த மூலிகையுடன் சமனெனடை தகரையிலையும் கூட்டி இடித்துச் சூரணம் செய்து வைத்துக் கொண்டு வேளைக்கு ¼ தோலா எடை சீனி அல்லது தேன், நெய் கூட்டிக் சில நாள்

கொடுத்து வர வெளுப்பாக உள்ள மயிறும் கருத்து விடும். தேகம் நல்ல ஆரோக்கியம் பெறும்[12].

ஆயுர்வேத மருத்துவத்தில் தோஷங்களை தூண்டிவிடும் வஸ்தியில் பயன்படும் மூலிகைகளில் கொட்டைக் கரந்தை ஒரு மூலிகையாகப் பயன்படுகிறது[15].

கற்ப முறைப்படி கொட்டைக் கரந்தை :

ஆச்சுதே கொட்டையாங் கரந்தைதன்னை
ஆதிகமாய் சமூலந்தான் பிடுங்கிவந்து
வாச்சுதே நிழலுலர்த்தி வைத்துக்கொண்டு
வடிகட்டி நாலிலொன்று கற்கண்டுசேர்த்து
வேச்சுதே வெருகடிதூள் வெந்நீரில்கொள்ள
மிடுக்காடும் கால்கைக்குப் பெலனுண்டாகும்
ஏச்சுதே வாசியென்ற குதிரைதன்னை
யேத்தியே முலைகட்டி யிருக்குந்தானே.

தானான கொட்டையாங் கரந்தைதன்னைச்
சமூலந்தான் பிடுங்கிவந்து சாருவாங்கி
ஆணான அயக்கரண்டி தன்னில்சூதம்
அழுந்தவிட்டு மேலாகச் சுருக்குபபோடு
நானான கால்சாமஞ் சுருக்கிட்டாக்கால்
நல்லவெள்ளிக் கம்பிபோல் ரசமுமாகும்
கானான யிலையரைத்துக் கவசங்கட்டிக்
காரியமாய் வெகுபுடமாய்ப் போட்டிடாயே.

போட்டிடுவாய் புடம்பத்து யிலைகவசங்கட்டிப்
பேரான குகையிலிட்டு உருக்கிக்கொண்டு
நாட்டிடுவாய் தங்கமது நாலுக்கொன்று
நாகந்தான் தங்கத்தி னிடையேகூட்டி

காட்டிடுவாய் கல்வத்தில் நொருக்கிக்கொண்டு

கரந்தையென்ற சாற்றாலே அரைத்து மையோல்

பூட்டுவா யுலரவைத்துப் பொடியாய்ப்பண்ணி

பேரிதுக்குச் சரிகெந்தி கூட்டிக்கொள்ளே.

கொள்ளவே ஓட்டிலிட்டு கெந்தகத்தையுருக்கிக்

குறித்தமுன் பொடிபோட்டு வறுத்துப்போடு

கள்ளவே கல்வத்திற் போட்டுக்கொண்டு

காணிக்கி யெட்டிலொன்று தாளகமுஞ்சிலையும்

வள்ளவே கரந்தையென்ற சாரரைத்து

வாகாக உலரவிட்டுப் பொடியாய்ப்பண்ணி

தெள்ளவே காசியென்ற மேருக்கேற்றிச்

சிலாகையிட்டுச் சிவந்தபதம் தீயையாத்தே[16].

கொட்டைக் கரந்தையை சமூலமாக நிழலில் உலர்த்தி சூரணம் செய்து 1 பங்கு கற்கண்டு பொடி சேர்த்து வெருகடி அளவு தூள் செய்து வெந்நீரில் சாப்பிட்டு வர கை கால் வலுத்து தேகம் பலம் பெறும்.

கரந்தையை கருப்பாய் செய்து நிழலில் உலர்த்தி 4ல் 1 பங்கு கந்தகத்தையும் இரசத்தையும் சேர்த்து அரைத்து பொடியாக்கி தேன் விட்டு அரைத்து சுண்டை அளவு 1 மண்டலம் வரை உண்டு வர தேகம் வலுக்கும்[11].

சங்க இலக்கியமான புறநானூற்றில் கரந்தையின் குறிப்புகள்:

சங்கக்கால நூல்கள் இரு பெரும் பிரிவுகளாக பிரிக்கப்பட்டனர். அவை மக்களின் அகப் பொருள் புறப்பொருள் பற்றி கூறப்படும் நூல்களாக இருந்தது. சங்கக்கால நூல்கள் இரு பெரும் பிரிவுகளுடன் மேலும் 7 பிரிவுகளாக பிரித்தனர். அவை திணை என வழங்கப்பட்டது[17]. புறநானூறு என்னும் தொகை நூல் நானூறு பாடல்களைக் கொண்ட புறத்திணை சார்ந்த ஒரு சங்க தமிழ் நூலாகும். புறநானூற்றின் பாடல்கள் சங்க காலத்தில் ஆண்ட அரசர்களைப் பற்றியும் மக்களின் சமூக வாழ்க்கையையும் எடுத்துறைக்கின்றன.

வெட்சி நிரைக்கவர்தல் மீட்டல் **கரந்தை**யாம்

வட்கார்மேல் செல்வது வஞ்சியாம் - உட்கா

தெதிரூன்றல் காஞ்சி எயில்காத்தல் நொச்சி

அதுவளைத்த லாகு முழிஞை – அதிரப்

பொருவது தும்பையாம் போர்க்களத்து மிக்கோர்

செருவென் றதுவாகை யாம்.[18][19]

இப்புற ஒழுக்கங்களை வெட்சி, **கரந்தை**, வஞ்சி, காஞ்சி, நொச்சி, உழிஞை, தும்பை, வாகை என்ற எட்டுத் திணைகளாகக் குறிப்பபிடுகின்றன[18][19].

பல்ஆ தழீஇய கல்லா வல்வில்

உரைக்குற் கூகை அழைப்ப ஆட்டி

நாகுமுலை அன்ன நறும்பூ **கரந்தை**

விரகுஅறி யாளர் மரபிற் சூட்ட

நிரைஇவண் தந்து நடுகல் ஆகிய

வென்வேல் விடலை இன்மையின் புலம்பிக்

கொய்ம்மழித் தலையொடு கைம்மையுறக் கலங்கிய

கழிகலம் மகடூஉப் போலப்

புல்என் றனையால் பலடஅணி இழந்தே[20].

புறத்திணைகளின் செய்திகளை கூறும் இந்தப் பழம்பாடல் கரந்தைத் திணைக்கான செய்தியை உரைக்கறது. புறத்திணையில் சூறையாடிச் சென்ற தனது ஆநிரைகள் மட்டுமின்றி யானைகளை மீட்டு கொண்டு வர கரந்தைமலர் சூடி போருக்கு செல்லுவது வழக்கமாய் இருந்ததினால் இத்திணைக்கு கரந்தை திணை என பெயர் வந்தது. கரந்தை என்பது கொட்டைக் கரந்தை எனும் ஒரு பூண்டு வகையாகும்[11][18].

சேரும் பிற சரக்குகள் :

கரந்தை சூரணம் :

அளவு : திரிகடிப் பிரமாணம்

தீரும் நோய்கள் : கிரந்திசூலை, அரையாப்பு, பவுத்திரம், மேகவூறல், பற்று, வெள்ளை படறுதல், சொறி, கரப்பான், வெடிக்கரப்பான், கன்னப்புற்று, யோனிப்புற்று, தொடை வாளை, பிளவை, எலிகடி தீரும்[21].

கரந்தை லேகியம் :

அளவு : கொட்டைப் பாக்களவு

தீரும் நோய்கள் : கிரந்தி, அரையாப்பு, குட்டம், புண்கள், சூலை, பவுத்திரம், கண்ணி சூலை, மேகவூறல், சொரி, கரப்பான், மேகப் புற்று, வெடிக்கரப்பான், கன்னப்புற்று, யோனிப்புற்று, தொடை வாளை, பிளவை, புற்று, சர்க்கரை நோய்,விப்புருதி, புண், எலிகடி தீரும்[22].

சோற்றுப்புச் செந்தூரம் :

அளவு : 1 பணவெடை

தீரும் நோய்கள் : சூதக வயிற்றுவலி தீரும்[23].

கர்ப்பமுண்டாக எண்ணெய் :

அளவு : 1 கரண்டி

தீரும் நோய்கள் : கர்ப்பமுண்டாகும்[23]

வாத சுரக் கசாயம் :

அளவு : 3 நாள்

தீரும் நோய்கள் : சுரம், தேகக் கடுப்பு, குடைச்சல், குளிர், கணுக்களின் நோய், பிடிப்பு தீரும்[24].

ஹிங்குவாதி சூரணம்:

தீரும் நோய்கள் : விலா வலி, இதய வலி, சிறுநீர்ப்பையில் வலி, கப வாத குன்மங்களும், பெண் குறிகளிலும், ஆசன வாய்களிலும் உண்டாகும் வலி, மண்ணீரல் நோய், இதய பிடிப்பு, தொண்டை பிடிப்பு, பாண்டு, இழுப்பு தீரும்[15].

கஜ கற்ணி பராக கற்க தைலம் :

வெளி பிரயோகம்

தீரும் நோய்கள் : சந்தசுர முதலிய முறைச் சுரங்களும், பழைமையாக நீடித்த புராண சுரங்களும் தீரும்[25].

சிறிய விடமுட்டி தைலம் :

வெளி பிரயோகம்

தீரும் நோய்கள் : ஊருஸ்தம்ப வாதம், பாதபகேஷபக வாதம், உதாவர்த்த வாதம், 1 பகல் நித்திரையால் வருகின்ற பற்பல வாதரோகங்களும் தீரும்[25].

LITERATURE REVIEW

3.2: MODERN ASPECT

SYNONYMS :

Sphaeranthus hirtus Willd.

Sphaeranthus mollis Roxb.

It grows in rice fields, dry waste places and cultivated lands in tropical parts of India. It is distributed throughout India, Sri Lanka, Africa and Australia from sea level to 1200 m altitude[26]. In hills 50 feet altitude. The size of this herb 1-2 feet in height[27].It is an annual plant consisting of two varieties viz. white and purplish red or rose colored, the former is very rare, but the latter is very common, the whole plant is remarkably fragrant and has medicinal virtues[28].

MORPHOLOGY:

The herb *S. indicus* is much branched, strongly scented, and annual erect with branched tapering roots tap roots. Stems are cylindrical with toothed wings. Leaves are sessile, decurrent, 2–7 cm long, 1–1.5 cm wide, obovate-oblong, rounded or subacute, glandular-hairy, spinous-serrate or dentate, narrowed at the base and greenish-brown in colour. Flowers are borne in terminal, solitary, globose, clusters of heads. Heads of flowers are purple, bracts are short slender and acuminate. In each head, the outer flowers are females, few or many, fertile, the central flowers bisexual, fertile or sterile, involucre narrow, bracts paleaceous, spathulate, acute, ciliate; receptacle small, naked. Corolla of female flowers are purple, slender, tubular, minutely two to three toothed; corolla of hermaphrodite flowers are purplish white, tubular or funnel-shaped, four to five toothed, anther-base sagittate, auricles acute or tailed, style-armed, filiform, sometimes connate. Fruits are oblong and have compressed achenes in which pappus is absent. Odor of herb is slightly aromatic but disappears on long storage.

MICROSCOPIC CHARACTERS

Leaf :

The leaf is dorsiventral and shows abundant trichomes of varying types on both the epidermis. Simple trichomes are three to four celled, thick walled and measure 130.8–145.2 μm in length and 29.0–43.5 μm in width. Trichomes are straight/knee shaped, with a swollen base and with collapsed cell at the middle or at the apex. Midrib shows three to four collateral vascular bundles associated with a group of sclerenchyma cells on either side.

Stem :

The stem shows cork with two to three layers of parenchymatous cells covered with p apillose cuticle having trichomes and can be distinguished by the presence of a discontinuous ring of lignified pericyclic fibers and a well-developed ring of Bicol lateral vascular bundle surrounding the pith. Medullary rays are pitted, lignified and about unitetra seriate.

Root :

The root shows on its outer side mesoderm, a typical brown coloured tissue. It consists of suberized cells, arranged irregularly and forms a protective layer. Radial groups of pericyclic fibers and few stone cells are seen alternating with radially arranged secretory canals in the secondary cortex. Phloem is parenchymatous and radially arranged. Medullary rays are pitted, lignified and about two to five striate.

Parts Used:

Whole Plant

Seeds

Flowers

Roots[26].

REGIONAL NAMES:

Sphaeranthus indicus linn.is known in different names in different Indian languages as mentioned below:

Common name **:** East Indian Globe Thistle[28].

Tamil **:**kottaikaranthai,karanthai,kotook,kottaipalai, kottainalam[29].

Sanskrit **:** Mundi,Sravani Kadamba,Puspika,Alambusta

Hindi **:** Mundi

Telungu **:** Bodasaramu,bodataramu

Kannada **:** Mirnagnee,Atookamanni,Mirangnee

Urdu **:** Mundi

Marathi **:** Mundi, Baras Bondi

Bengalese **:** Surmuriya,Chhagal Nadi,Mudmudiya

Gujarati **:** Gorakhmundi

Punjabi **:**Gorakhmundi

Assamese **:** Kmadarus

Oriya **:** Buikadam[30]

Malayalam **:** Mirangani ,adakkamaniyan[31]

TOXONOMICAL CLASSIFICATION:

Kingdom **:** *Plantae*

Subkingdom **:** *Viridaeplantae*

Phyllum **:** *Tracheophyta*

Subphyllum **:** *Euphyllophytina*

Infraphyllum **:** *Radiatopses*

Class **:** *Magnoliopsida*

Subclass **:** *Asteridae*

Superorder **:** *Asteranae*

Order **:** *Asterales*

Family **:** *Asteraceae*

Genus **:** *Sphaeranthus*

Species **:** *indicus*[26]

SIDDHA PROPERTIES OF SPHAERANTHUS INDICUS:

Suvai (Taste) : Kaippu (Bitter)

Tanmai (Potency) : Veppam (Hot)

Gunam : Ilaku (Soft)

Pirivu (Transformation): Kārppu (Pungent)

Ceikai (Action) : Demulcent

Restorative

Anthelmintic

Tonic[27]

Stomachic

Stimulant

Alternative

Pectoral

Deculcent

Externally : Emollient[33].

PHYTOCHEMISTRY:

Large numbers of phytochemicals were isolated from whole plant, leaves and flowers of S. *indicus.* Aerial parts of this plant showed presence of an essential oil, glycosides, and eudesmanoids, an alkaloid sphaeranthine and an isoflavone 5,4'-dimethoxy-3'prenylbiochanin 7-o-β-galctoside with some interestings esquiterpene. And another one study isolated a new Flavonoid C-glycoside, along with eight known compounds, namely n-pentacosan, hentriacontane, n-triacontanol, β- sitosterol, stigmasterol, β-D-glucoside of β- sitosterol, sphaeranthine and a phenolic glycoside (C_{22} H_{26} O_{12}). The essential oil, obtained by steam distillation of the whole herb, contains ocimene, α-terpinene, methyl-chavicol, α-citral, geraniol, α-ionone, β - ionone, d - cadinene, p -methoxycinnamaldehyde[32].

MEDICINAL USES OF SPHAERANTHUS INDICUS:

It is an important medicinal plant used for the treatment of styptic gastric disorders, skin diseases, anthelmintic, glandular swelling, depression, analgesic, antibiotics, antifungal, laxative and diuretics properties. The decoction of the plant is said to be active against bronchitis, asthma, leukoderma, jaundice and scabies. The powdered bark along with whey is useful in the treatment of piles. Roots and seeds are anthelmintic. Juice of fresh leaves is taken for cough. The plant is also useful in preservation of food grains as it possess insecticidal property[26].

The entire plant when dried and powdered proves useful in cases of gonorrhea and other venereal complaints rheumatic and syphilitic affections, skin eruptions, constipation, chronic diarrhea etc; moreover, it is a good remedy for strengthening the heart, brain and nerves. The decoction of its plant prescribed for lunacy and other mental disorders. The plant mixed with pulverized cumin seeds, it is used in stomach complaints.

The juice of the whole plant is used for converting mercury into a silver like rod by alchemical process of absorption . According to Siddhar's science, the juice of the entire plant and that of the leaves are found to be of great use in Alchemy and rejuvenation.The whole plant pulverized with cumin seeds and mixed with honey is used in coughs. The plant is also used for local application[28].

Plant leave's juice used in hepatic and gastric disorders. Decoction used in cough and other chest troubles; credited with antitubercular properties. Leaves eaten as a pot-herb. Herbs used as a fish poison, also stuffed into holes of crabs to kill them. Aqueous extract poisonous to American cockroaches. Herb yields an essential oil and fatty oil[31].

Distilled water prepared like rose-water from the herb is recommended by Hakims for bilious affections for the dispersion of various kinds of tumors. Roots is used as a stomachic and anthelmintic in doses of about 40 grains daily in the form of powder; also the seeds have the same properties. They are useful in worms and indigestion, and given with honey, in cases of cough. Flowers (flower head) are highly esteemed as alternatives, depurative, refrigerants and tonics, useful as blood-purifiers in skin diseases. Oil prepared from the root by steeping it in water and then boiling.it in sesamum oil until all the water is expelled, taken on empty stomach every morning for 41 days in doses of 2 dirhams is a valuable aphrodisiac. It is used in glandular swelling in the neck with benefit and also a good remedy in jaundice. Leaves dried in the shade and powdered are used in doses of 20 grains twice a day in chronic skin disease as anti-syphilitic and nervine tonic. The drug is also useful in urethral discharges and jaundice[33].

Methanolic extract of the plant showed repellent and feeding deterrent activity against Thibolium castaneum. A decoction of the plant is diuretic and used in urethral discharge. Fruits are reported to posses digestive properties. Roots are anthelmintic[34] .

OTHER SYSTEM OF MEDICINE USES:

In ***Ayurvedic*** **system of medicine**, the whole herb is used in insanity, tuberculous glands, indigestion, bronchitis, spleen diseases, elephantiasis, anaemia, pain in the uterus and vagina, piles, biliousness, epileptic convulsions, asthma, leukoderma, dysentery, vomiting, urinary discharges, pain in the rectum, looseness of the breasts, hemicrania. The whole herb is used in *Ayurvedic* preparations to treat epilepsy and mental disorders. Leaves dried in the shade and powdered are used in doses of 20 grains twice a day in chronic skin diseases as an anti-syphilitic and a nervine tonic. Hot water extract of the herb is used as an anthelminitic, as a diuretic, as a fish poison and as an aphrodisiac. Flowers are tonic, cooling, alterative and used in conjunctivitis and give

strength to weak eyes. The oil prepared using the plant root is reportedly useful in treating scrofula and as an aphrodisiac. The external application of a paste of this herb is beneficial in treating pruritus and edema, arthritis, filariasis, gout and cervical adenopathy. Pulverized seeds have antimicrobial property. It is also stuffed into holes of crabs to kill them. Aqueous extract is poisonous to American cockroaches.

In ***unani,*** the herb is used as a tonic, laxative, emmenagogue, and also it increases the appetite, enriches the blood, lessens inflammation, cools the brain and gives luster to the eye, is good for sore eyes, jaundice, scalding of urine, gleet, biliousness, boils, scabies, ringworm in the waist, diseases of the chest. The plant is traditionally used for diarrhoea. The entire plant is used as an emmenogogue. Hot water extract of the entire plant is used for glandular swelling of the neck and for jaundice [27].

PHARMACOLOGICAL STUDIES

Anti-diabetic activity:

This study was conducted by Prabhu KS, Lobo R, Shirwaikar A. they have evaluated the anti-hyperglycemic effect of *S.indicus* extract was carried out in diabetic rats induced by nicotinamide(120mg/kg i.p)and streptozotocin(STZ)(60 mg/kg i.p).Oral administration of alcholic extract of *S.indus* for 15 days exhibited in significant reduction in blood glucose levels and increases in hepatic glycogen and plasma insulin levels and significant improvement in oral glucose tolerance test. Glibenclamide was used as a reference standard [35].

Anti-hyperlipidemic activity

This study was conducted by Pande VV, Dubey S. they have evaluated the anti-hyperlipidemic activity on *S.indicus*. In this study, the alcoholic extract of flower heads in atherogenic diet induced hyperlipidemia in rats was investigated for the dose of 500 mg/kg/day,p.o. for 8 days. The extract effectively suppressed the hyperlipidemia by decreasing total cholesterol, triglyceride, LDL and very low density lipoprotein(VLDL); increasing the HDL[36].

Antioxidant activity

This study was conducted by Shirwaikar A, Prabhu KS, Punithathey have evaluated the anti-oxidant activity on *S.indicus*. In this study, the free radical scavenging potential of the plant was studied by using different antioxidant models of screening. The ethanolic extract at 1,000 μg/ml showed maximum scavenging of the radical cation, 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonate) observed up to 41.99% followed by the scavenging of the stable radical 1,1-diphenyl, 2-picryl hydrazyl (33.27%), SOD (25.14%) and nitric oxide radical (22.36%) at the same concentration. However, the extract showed only moderate scavenging activity of iron chelation (14.2%). Total antioxidant capacity of the extract was found to be 160.85 nmol/g ascorbic acid. The results justify the therapeutic applications of the plant in the indigenous system of medicine, augmenting its therapeutic value [37].

Anti-arthritic activity:

This study was conducted by Badgujar LB, Ghosh P, Gaur V, Bodhankar SL they have evaluated the anti-arthritic activity of the petroleum ether extract of the flowers in the doses 10,30 and 100 mg/kg/day p.o. was investigated against complete Freund's adjuvant induced arthritis in laboratory rats. Indomethacin (2 mg/kg/day p.o) was the standard drug. The dose of 100 mg/kg/day p.o. showed significant anti-arthritic activity [38].

Anti-inflammatory activity:

This study was conducted by Meher BR, Rath BG, Biswal S. they have evaluated the anti-inflammatory effect of ethanolic extract was evaluated. The extract in different doses (100,200 and 400 mg/kg,p.o.) exhibited dose dependent and significant anti-inflammatory activity in acute (carrageenan induced hind paw edema, $P<0.05$) and chronic (cotton pellet granuloma formation, $P<0.05$) model of inflammation [39].

Immunomodulatory activity

This study was conducted by Bafna AR, Mishra SH they have evaluated the immune modulatory activity on *S.indicus*. In this study, the petroleum ether extract from the flower heads of *S. indicus* Linn. was found to be effective in increasing phagocytic activity, hemagglutination antibody titer and delayed type hypersensitivity. The extract

acts by stimulating both humoral and cellular immunity as well as phagocytic function [40].

Nephroproductive effect:

This study was conducted by Srinivasan VM, Jessy KK, Alex AE they have evaluated the nephroproductive effect on *S.indicus* . In this study, the ethanolic extract was screened for nephroprotective effect in gentamicin induced acute renal injury in rats. The extract in the dose of 300 mg/kg was found to increase blood urea, serum creatinine and decrease the total protein and serum albumin of the treated group compared to normal group [41].

Broncho dilatory effect:

This study was conducted by by Sarpate RV, Deore TK, Tupkari SV they have evaluated the bronchodilatory effect on *S..indicus*. In this study, methanolic extract and its fraction viz, petroleum ether, benzene, chloroform and ethyl acetate exhibited significant protection against bronchospasm, induced by histamine in guinea pigs. Significant protection exhibited by methanolic extract was comparable with the standard chlorophenarmine maleate(2mg/kg)[42].

Analgesic and antipyretic activity:

This study was conducted Nanda BK, Jena J, Rath B, Behera BR they have evaluated the analgesic and antipyretic activity of the successive taking petroleum ether, benzene, chloroform, ethanol and triple distilled water extracts (200 mg/kg and 400 mg/kg b.w) of whole plant was screened for analgesic and antipyretic activities on Albino rats by Eddy's hot plate, Tail immersion and Brewer's yeast induced pyrexia method. The petroleum ether, chloroform and ethanol extracts showed significant analgesic activity in both doses as compared to the standard drug diclofenac sodium. The chloroform and ethanol extracts showed potential significant antipyretic activity from 1 h onward whereas aqueous extracts exhibited activity from 2 h onward as compared to the standard drug paracetamol amongst various extracts [43].

Anthelmintic activity:

This study was conducted by Malairajan P, Babu GV, Saral A, Mahesh S, Gitanjali have evaluated the anthelmintic activities of ethanolic and aqueous extracts

(10,50,100 mg/ml concentration levels) of the whole plant were tested against *Pheretima posthuman* and *Ascardiagalli*. Both extract exhibited anthelmintic activity in a dose-dependent manner. The most significant activity was observed at the highest concentration of 100 mg/ml against both types of worms[44].

Antimicrobial activity

This study was conducted by Singh SK, Saroj KM, Tripathi VJ, Singh AK, Singh RH they have evaluated the anti-microbial activity on *S.indicus.*. In this study, the bicyclic sesquiterpene lactone isolated from the petroleum ether extract of the aerial part has been found to be potent against *staphylococcus aureus, Escherichia coli,fusarium sp., Helminthosporium sp.,*and other microorganism[45] .

Attaurrahman, Shekhani MS, Perveen S, Habiburrehman, Yasmin A, Ziaulhaque A, et al. they have evaluated the anti-microbial activity on *S.indicus.*. In this study,7Hydroxyfrullanolide,a sesquiterpene lactone showed antimicrobial activity[46].

Dubey KS, Ansari AH, Hardaha M.they have evaluated the anti-microbial activity on *S.indicus*. In this study, alcoholic and aqueous extracts of the plant were highly effective against *Alternaria solani, fusarium oxysporum* and *penicillium pinophilum* by preventing their growth to a greater extent[47].

Dubey LN, Sahu B. they have evaluated the antimicrobial activity of terphenoidal compound isolated from *S.indus* showed activity against *Basillus subtilis*[48].

Upadhyay R, Mishra N. they have evaluated the in-*vitro* antimicrobial activity of aqueous extract of flower was evaluated against coliforms *E.coli*(10,536)and total coliforms by using disc diffusion method. The extracts showed significant inhibition against coliform strains[49].

Antiviral activity

This study was conducted by Vimalanathan S, Ignacimuthu S, Hudson JB. they have evaluated the anti-viral activity on *S..indicus*. In this study, the methanol extract was found to exhibit inhibitory activity against *Mouse corona* virus *Herpes simplex* virus at a concentration of 0.4 µg/ml[50].

Dhar ML, Dhar MM, Dhawan BN, Mehrotra BN, Ray C. they evaluated the screening of Indian plants for biological activity on *S..indicus*. The plant also showed antiviral activity against vaccina and viruses[51].

Anxiolytic activity

This study was conducted by Ambavade SD, Mhetre NA, Tate VD, Bodhankar SL they have evaluated the anxiolytic activity on *S..indicus*. In this study, the petroleum ether (10 mg/kg), alcohol (10 mg/kg) and water extracts (30 mg/kg) of flowers were tested to assess the anxiolytic activity in mice. Petroleum ether extract of *S. indicus* flowers produced prominent anxiolytic activity[52].

Ovicidal activity

This study was conducted by Sharma MC they have evaluated ovicidal activity on *S.inducus* . In this study, Sesquiterpene lactone, isolated from a petroleum ether extract of *S. indicus*, was screened for its effects on the hatching of eggs and the metamorphosis of larvae of *Culex quinquefasciatus* at concentration of 50-250 ppm. Rates of fecundity and fertility were found to be affected in the larval-treated adult females. Egg hatching was also significantly lowered. Mortality in the larvae, pupae and adults produced a marked decrease in mosquito populations in laboratory experiments[53].

Macrofilaricidal activity

This study was conducted by Tiwari A, Saxena RC. they have evaluated macrofilaricidal activity on *S.inducus* . In this study,the methonolic extract showed macrofilaricidal activity(4 mg/ml) against adult *setaria digitate,*the cattle filarial worm when tested by worm motility assay method[54].

Attenuation effect on prostatic hypertrophy

This study was conducted by Nahata A, Dixit VK they have evaluated effect of prostatic hypertrophy on *S.inducus* . In this study,the attenuating effect of petroleum ether, ethanoic, aqueous extracts and β-sitosterol on prostatic hyperplasia induced by testosterone in Albino rats. Finasteride was used as a positive control(1mg/kg p.o.). The petroleum ether extract exhibited the best activity, although the ethanol and aqueous

extracts also exhibited significant activity thereby indicating the potential use of *S.indicus* in the treatment of prostatic hyperplasia[55].

Mast cell stabilizing activity

This study was conducted by Mathew JE, Srinivasan KK, Dinakaran V, Joseph A. they have evaluated mast cell stabilizing activity on *S.inducus* . In this study,the protective effect of different extract of whole plant against the compound 48/80 and sheep serum induced mast cell degranulation was evaluated. The ethanol extract at the dose levels of 150 mg/kg and 300 mg/kg and ethyl acetate extract at the dose levels of 100 mg/kg,150 mg/kg and 300 mg/kg showed slightly better protection of mast cell degranulation (77-86%) than ketotifen (75%) in the sheep serum model. These extract also showed better mast cell stabilizing activity (77-88 %) than the standard drug (69%) when peritoneal mast cells are treated with compound 48/80.These result suggest that *S.indicus* has potent mast cell stabilizing effects thereby inhibiting mediator release from mast cells[56].

Effect on psoriasis

This study was conducted by Sharma they evaluated the effect of *S.indicus* on psoriasis was studied and found to exhibit the potent activity[57].

Wound healing activity

This study was conducted by Sadaf F, Saleem R, Ahmed M, Ahmad SI, Navaid-ul-Zafar they evaluated the wound healinf activity on *S.indicus.* In this study ,A cream containing ethanolic extract of aerial parts of *S. indicus*, L. (Asteraceae) was evaluated for wound healing activity in guinea pigs. The cream was applied *in-vivo* on the paravertebral area of six excised wounded models once a day for 15 days. The cream significantly enhanced the rate of wound contraction and the period of epithelialization comparable to neomycin[58].

4.MATERIALS AND METHODS

4.1.PREPARATION OF TEST DRUG:

SELECTION OF TEST DRUG:

The test drug Kottai Karanthai Chooranam was selected for the evaluation of the toxicity studies in Wister albino rats.

COLLECTION:

The Kottai Karanthai Samoolam were collected from Annavasal,

Pudukkottai Dt.

AUTHENTICATION:

The Herbal drug Kottai Karanthai Samoolam is identified and authenticated by Assistant Professor, Department of Medicinal Botany, National Institute of Siddha, Chennai-47.

INGREDIENTS:

Kottaikaranthai Samoolam

METHOD OF PURIFICATION:

The decomposed leaves, insects infected leaves should be eliminated. Mud, sand sticking to the Kottaikaranthai Samoolam are removed by water wash and dried in shade[59].

METHOD OF PREPARATION:

Then it was grounded well to obtain fine powder. After this process, the dried powder was sieved through white cloth. Then the chooranam was stored in an air tight container and it was labelled as Kottai Karanthai chooranam.

THERAPEUTIC DETAILS OF KOTTAI KARANTHAI CHOORANAM (KKC):

Form of Medicine : Chooranam (Powder)

Route of administration : Internal

Clinical dose : 4 gram

Adjuvant : Water

Indication : Karappan - Eczema

Sori - Pruritis

Sirangu - Scabies

Thozh Pinigal - Skin disorders

Maripatta earu kazhiyum - Constipation

Vali - Arthritis

Veri - Psychiatric disorder

Sinaippu - Erythema

CHOORANAM:

Definition:

Chooranam are fine dry powders of drug. The terms ‘chooranam’ may be applied to the powder of single drug or a mixture of two or more drugs,which are powdered separately prior to their being mixed to homogeneity[4].

Self life of Chooranam:

Chooranam retain their potency for three months[60].

Figure 2: kottai Karanthai Chooranam

STANDARDIZATION OF KOTTAIKARANTHAI CHOORANAM

After preparation of Kottai Karanthai Chooranam I have analyse for standardization and safety evaluation. Here is given details of procedure of standardization method and Safety evaluation of toxicity study.

4.2. QUALITATIVE ANALYSIS OF KOTTAIKARANTHAI CHOORANAM

ORGANOLEPTIC CHARACTER ANALYSIS:

Organoleptic Character of Kottai Karanthai chooranam was analyzed as per standard procedure.

PHYSICO-CHEMICAL ANALYSIS

The physico-chemical properties of kottai Karanthai chooranam was carried as per standard procedure at Noble Research Solutions, Thanigai nagar, Kolathur, Chennai -99.

PROCEDURE:

Determination of Moisture Content (Loss on Drying):

Test drug was accurately weighed in evaporating dish. The sample was dried at 105^{o}C for 5 hours and then 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%. when the constant weight was obtained the percentage of moisture content were calculated with reference to the air -dried drug.

Determination of Total Ash:

Test drug was accurately weighed in silica dish and incinerated at the furnace a temperature 400 ^{o}C until it turns white in color which indicates absence of carbon. Percentage of total ash will be calculated with reference to the weight of air-dried drug.

Determination of Acid Insoluble Ash:

The ash obtained by total ash test will be boiled with 25 ml of dilute hydrochloric acid for 6mins. Then the insoluble matter is collected in crucible and will be washed with hot water and ignited to constant weight. Percentage of acid insoluble ash will be calculated with reference to the weight of air-dried ash.

Determination of Alcohol Soluble Extractive:

Test sample was macerated with 100 ml of Alcohol in a closed flask for twenty-four hours, shaking frequently during six hours and allowing it to stand for eighteen

hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105ºC, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

Determination of Water- Soluble Extractive:

Test sample was macerated with 100 ml of chloroform water in a closed flask for twenty-four hours, shaking frequently during six hours and allowing it to stand and for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105ºC, to constant weight and weigh. Calculate the percentage of water-soluble extractive with reference to the air-dried drug.

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 100 micron- 1mm. later the particle size was analysed 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[61][62].

BIOCHEMICAL ANALYSIS:

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

Table 1:

S.No	EXPERIMENT	OBSERVATION	INFERENCE
1.	Appearance of sample	Brown	
2.	**Test for silicate:** a. A little (500mg) of the sample is shaken well with distilled water. b. A little (500mg) of the sample is shaken well	Not soluble	Absence of silicate.

	with con.HCL/Con.H2So4.		
3.	**Action of heat:** A small amount (500mg) of the sample is taken in a dry test tube and heated gently at first and then strong.	White fumes evolved.	Presence of carbonate.
4.	**Flame test:** A small amount (500mg) 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.	No bluish green flame appeared.	Absence of Copper
5.	**Ash test:** A filter paper is soaked into a mixture of sample and dil.cobalt nitrate solution and introduced into the Bunsen flame and ignited.	Yellow color flame appeared.	presence of Sodium.

Preparation of Extract:

5g of Kottai Karanthai Chooranam was taken in a 250 ml of clean beaker and 50ml of distilled water was added to it. Then it was boiled well for about 10 min. Then it is allowed to cool and filtered into a 100 ml volumetric flask and made up to 100 ml with distilled water.

Table No:2. Test for Acid Radicals:

S.NO	EXPERIMENT	OBSERVATION	INFERENCE
1.	**Test For Sulphate:** 2ml of the above prepared extract was taken in a test tube and 2ml of 4% dil. Ammonium oxalate solution was added.	Presence of Cloudy appearance.	Sulphate present.
2.	**Test For phosphate:** 2ml of the extract was treated with 2ml of con.HNo3 and 2ml of dil.ammonium molybdate solution.	Presence of Yellow precipitate.	Phosphate present.
3.	**Test For Carbonate:** 2ml of the extract was treated with 2ml dil. magnesium sulphate solution	Presence of Cloudy appearance.	Carbonate Present.
4.	**Test for Chloride:** 2ml of the above prepared extracts is added with 2ml of dil-HCL is added until the effervescence ceases off.	Presence of Cloudy appearance.	Chloride Present.
5.	**Test for Sulphide:** 1gm of the substance is treated with 2ml of con. HCL.	Presence of Cloudy appearance.	Sulphide Present.

6.	**Test for Fluoride & Oxalate:** 2 ml of the extract was added with 2ml of dil.acetic acid and 2 ml dil.calcium chloride solution and heated.	absence of Cloudy appearance.	Absence of fluoride & oxalate.

Table no 3: Test for Basic Radicals:

S.NO	EXPERIMENT	OBSERVATION	INFERENCE
1.	**Test For Lead:** 2ml of the extract was added with 2ml of dil. potassium iodine solution.	Yellow Precipitate was not obtained.	Lead absent.
2.	**Test For Aluminium:** In the 2ml of extract dil. sodium hydroxide was added in 5 drops to excess.	Yellow colour was formed.	Aluminium present.
3.	**Test For Iron:** To the 2ml of extract add 2ml ammonium thiocyanate solution and 2ml of con HNo3 is added.	Red colour formed.	Iron present.
4.	**Test For Zinc:** In 2ml of the extract dil. sodium hydroxide solution was added in 5 drops to excess and dil. Ammonium chloride was added.	White precipitate was formed.	Zinc present.

5.	**Test For Calcium:** 2ml of the extract was added with 2ml of 4% dil. ammonium oxalate solution .	Cloudy appearance was formed.	Calcium present.
6.	**Test For Ammonium:** In 2ml of extract 1 ml of Nessler's reagent and excess of dil. sodium hydroxide solution was added.	Brown colour formed.	Ammonium present.
7.	**Test For Mercury:** 2ml of the extract was treated with 2ml of diluted sodium hydroxide solution.	Yellow precipitate not formed	Mercury absent.
8.	**Test For Arsenic:** 2ml of the extract was treated with 2ml of dil. sodium hydroxide solution.	Brownish red precipitate not formed.	Arsenic absent.
9.	**Test for Copper:** a. One pinch (50mg) of substance is made into paste with con.HCL in a watch glass and introduced into the nonluminous part pf the flame.	No blue color precipitate is formed.	Absence of copper.
10.	**Test for Magnesium:** To 2ml of extract dil.sodium hydroxide solution is added in drops to excess.	No white precipitate is obtained.	Absence of magnesium.

11.	**Test for potassium:** A pinch (25mg) of substance is treated off with 2 ml of dil. Sodium nitrite solution and then treated with 2ml of dil.Cobalt nitrate in 30% dil. Glacial acetic acid.	yellowish precipitate is obtained.	Presence of potassium.
12.	**Test for Sodium:** 2 pinches (50mg) of the substance is made into paste by using HCl and introduced into the blue flame of Bunsen burner.	No yellow color flame appeared.	Presence of Sodium.

Table no : 4 Other constituents :

S.NO	EXPERIMENT	OBSERVATION	INFERENCE
1.	**Test for Starch:** 2ml of extract is treated with weak dil.iodine solution.	Blue color formation is present.	Presence of starch.
2.	**Test For Reducing Sugar:** 5ml of Benedict's qualitative solution was taken in a test tube and allowed to boil for 2 minutes and added 8 to 10 drops of the extract and again boil it for 2 minutes.	Red colour developed.	Reducing color present.

3.	**Test For The Alkaloids:** 2ml of the extract is treated with 2ml of dil. Potassium iodide solution. 2ml of the extract is treated with 2ml of dil. picric acid.	Yellow precipitation formed.	Alkaloid present.
4.	**Test For Tannic Acid:** 2ml of the extract was treated with 2ml of dil. ferric chloride solution	Black precipitate formed.	Tannic acid present.

PHYTOCHEMICAL SCREENING OF KOTTAIKARANTHAI CHOORANAM

The preliminary phytochemical screening test was carried out for extract of Kottai Karanthai Chooranam as per the standard procedure at Noble Research Solutions, Thanigai nagar, Kolathur, Chennai -99.

Test for alkaloids:

Mayer's Test:

To the test sample, 2ml of mayer's reagent was added, a dull white precipitate revealed the presence of alkaloids.

Test for coumarins:

To the test sample, 1 ml of 10% sodium hydroxide was added. The presence of coumarins is indicated by the formation of yellow color.

Test for saponins:

To the test sample, 5 ml of water was added and the tube was shaken vigorously. Copious lather formation indicates the presence of Saponins.

Test for tannins:

To the test sample, ferric chloride was added, formation of a dark blue or greenish black color showed the presence of tannins.

Test for glycosides:

Borntrager's Test:

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

Test for flavonoids:

To the test sample about 5 ml of dilute ammonia solution were been added followed by addition of few drops of conc. Sulfuric acid. Appearance of yellow color indicates the presence of Flavonoids.

Test for phenols:

Lead acetate test:

To the test sample; 3 ml of 10% lead acetate solution was added. A bulky white precipitate indicated the presence of phenolic compounds.

Test for steroids:

To the test sample, 2ml of chloroform was added with few drops of conc. Sulphuric acid (3ml), and shaken well. The upper layer in the test tube was turns into red and sulphuric acid layer showed yellow with green fluorescence. It showed the presence of steroids.

Triterpenoids:

Liebermann–Burchard test: To the chloroform solution, few drops of acetic anhydride was added then mixed well. 1 ml concentrated sulphuric acid was added from the sides of the test tube, appearance of red ring indicates the presence of triterpenoids.

Test for Cyanins:

Aanthocyanin:

To the test sample, 1 ml of 2N sodium hydroxide was added and heated for 5 min at 100^{o}C. Formation of bluish green colour indicates the presence of anthocyanin.

Test for Carbohydrates:

Benedict's test:

To the test sample about 0.5 ml of Benedic's reagent is added. The mixture is heated on a boiling water bath for 2 minutes. A characteristic coloured precipitate indicates the presence of sugar.

Test for Proteins:

Biuret Test:

To extracts 1% solution of copper sulphate was added followed by 5% solution of sodium hydroxide, formation of violet purple colour indicates the presence of proteins[63].

4.3.QUANTITATIVE ANALYSIS:

The High Performance Thin Layer Chromotography Analysis was carried out as per the standard procedure at Noble Research Solutions, Thanigai nagar, Kolathur, Chennai -99.

Thin layer Chromatography Analysis:

Test sample was subjected to thin layer chromatography (TLC) as per conventional one dimensional ascending method using silica gel 60F254, 7X6 cm (Merck) were cut with ordinary household scissors. Plate markings were made with soft pencil. Micro pipette were used to spot the sample for TLC applied sample volume 10-micro liter by using pipette at distance of 1 cm at 5 tracks. In the twin trough chamber with the specified solvent system After the run plates are dried and was observed using visible light Short-wave UV light 254nm and light long-wave UV light 365 nm[64].

High Performance Thin Layer Chromatography Analysis:

HPTLC method is a modern sophisticated and automated separation technique derived from TLC. Pre-coated HPTLC graded plates and auto sampler was used to achieve precision, sensitive, significant separation both qualitatively and quantitatively. High performance thin layer chromatography (HPTLC) is a valuable quality assessment tool for the evaluation of botanical materials efficiently and cost effectively. HPTLC method offers high degree of selectivity, sensitivity and rapidity combined with single-step sample preparation. This method can be conveniently adopted for routine quality

control analysis. It provides chromatographic fingerprint of phytochemicals which is suitable for confirming the identity and purity of Phyto therapeutics.

Chromatogram Development:

It was carried out in CAMAG Twin Trough chambers. Sample elution was carried out according to the adsorption capability of the component to be analyzed. After elution, plates were taken out of the chamber and dried.

Scanning:

Plates were scanned under UV at 366nm. The data obtained from scanning were brought into integration through CAMAG software. Chromatographic finger print was developed for the detection of phytoconstituents present in each sample and their respective Rf values were tabulated[65].

PESTICIDE RESIDUE:

The pesticide residueanalysis was carried out for extract of Kottaikaranthai Chooranam as per the standard procedure at Noble Research Solutions, Thanigai nagar, Kolathur, Chennai -99.

Procedure:

Test sample were extracted with 100 ml of acetone and followed by homogenization for brief period. Further filtration was allowed and subsequent addition of acetone to the test mixture. Heating of test sample was performed using a rotary evaporator at a temperature not exceeding 40ºC until the solvent has almost completely evaporated. To the residue add a few milli litters toluene and heat again until the acetone is completely removed. Resultant residue will be dissolved using toluene and filtered through membrane filter[66][67].

AFLATOXIN:

The Aflatoxin analysis was carried out for extract of Kottaikaranthai Chooranam as per the standard procedure at Noble Research Solutions, Thanigai nagar, Kolathur, Chennai -99.

Standard:

Aflatoxin B1

Aflatoxin B2

Standard:

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, 5 µL, 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 front and allow the plate to air-dry. Locate the spots on the plate by examination under UV light at 365 nm[68].

HEAVY METAL ANALYSIS:

The analysis of heavy metals and trace elements were estimated by using Inductively Coupled Plasma Optical Emission Spectrometry (ICP- OES). The Experimental Procedure was done at SAIF, IIT Madras, Chennai-32.

ICP-OES:

Perkin Elmer Optima 5300DV was used for standard ICP-OES analysis. The optimized operating conditions are given in (table 5), the wave length of analytical lines is given in table (5) and the test drug *Kottai Karanthai Chooranam* underwent microwave digestion for sample preparation. With respect to other kind of analysis where chemical speciation was relevant. Only total essential concentration was analysed by ICP-OES[69].

Table 5 : ICP- OES Operating Conditions:

Rf frequency	40 M Hz
Range	165 – 782 nm
Detection limit	Up to ppm level using SCD detector

TEST FOR SPECIFIC PATHOGEN:

Test for specific pathogen Analysis was carried out as per the standard procedure at Noble Research Solutions, Thanigai nagar, Kolathur, Chennai -99.

Methodology

One part of the test sample was dissolved in 9 mL of sterile distilled water and the test sample was directly inoculated in to the specific pathogen medium (EMB, DCC, Mannitol, Cetrimide) by pour plate method. The plates were incubated at 37oC for 24 - 72h for observation. Presence of specific pathogen identified by their characteristic colour with respect to pattern of colony formation in each differential media.

Table no: 6-Specific Pathogen

Detail of Specific Medium and their abbreviation

Organism	Abbreviation	Medium
E-coli	EC	EMB Agar
Salmonella	SA	Deoxycholate agar
Staphylococcus Aureus	ST	Mannitol salt agar
Pseudomonas Aeruginosa	PS	Cetrimide agar

Observation

No growth was observed after incubation period. Reveals the absence of specific pathogen.

STERILITY TEST BY POUR PLATE METHOD:

The sterility test by pour plate method was carried out as per the standard procedure at Noble Research Solutions, Thanigai nagar, Kolathur, Chennai -99.

Objective:

The pour plate techniques were adopted to determine the sterility of the product. Contaminated / un sterile sample (formulation) when come in contact with the nutrition rich medium it promotes the growth of the organism and after stipulated period of incubation the growth of the organism was identified by characteristic pattern of colonies. The colonies are referred to as Colony Forming Units (CFUs).

Methodology:

Test sample was inoculated in sterile petri dish to which about 15 mL of molten agar 45^{o}C were added. Agar and sample were mixed thoroughly by tilting and swirling the dish. Agar was allowed to completely gel without disturbing it. (about 10 minutes). Plates were then inverted and incubated at 37^{o} C for 24-48 hours and further extended for 72 hrs for fungal growth observation. Grown colonies of organism was then counted and calculated for CFU.

TOXICOLOGICAL EVALUATION OF KOTTAIKARANTHAI CHOORANAM

4.4.TOXICITY STUDIES:

The following in vivo toxicity studies were carried out on kottai Karanthai Chooranam (KKC) by World Health Organization (WHO) guideline for testing traditional medicines.

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 Ethical Committee.

IAEC Approved No:

For Acute and Long-term study- NIS/IAEC-VII /28082018/17

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:

Animal were selected as per guideline. Healthy adult animals of Wistar albino rats, 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 romm:22◦C(±3◦C)

Humidity : 60 ±10%

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

The animal were housed in polypropylene cages provided with bedding of husk.

The animal had free access to RO water.

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

Preparation of animals:

The animal 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 condition. The principles of laboratory animal care were followed.

Test substances:

Kottai Karanthai Chooranam(KKC) was brown in colour, strong characteristic odour, moderately fine in nature. The drug was dissolved in water 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 dose:

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

TOXICOLOGICAL PROFILE:

Acute oral toxicity study (WHO guidelines)

Species and strain	: Wistar Albino rat
Sex	: Male and female
Age /weight	: 6 weeks/ 150-200 gm
Test guideline	: WHO guideline
Groups	: grouped by randomization
Duration of exposure	: single dose

Study duration : 14 days

Number of animals : 10/ groups

Route of administration : Oral

Number of animal and dose level:

Acute oral toxicity of the drug will be evaluated in rats following WHO guideline. Animals will be divided into 2 groups by randomization method, each group containing 20 animals (5 females and 5 males). One group as control and the other as test group. Control group is treated with water and test group were treated with the test drug Kottai Karanthai Chooranam ten times more than the therapeutic dose (2000 mg per kg b.wt).

Table no 7: Number of animal used for Acute oral toxicity study

S.NO	Group	Treatment	No of rats
1.	Group I	Control (water)	10(5M+5 F)
2.	Group II	Kottai Karanthai Chooranam (KKC) - 2000mg/kg per oral	10(5M+5 F)

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 weighted and test drug was administered. The control groups received equal volume 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 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 14th 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, kidney, 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.

Long term toxicity study (WHO guidelines)

Species and strain	:	Wistar Albino rat
Sex	:	Male and female
Age /weight	:	6 weeks/ 150-200 gms
Test guideline	:	WHO guideline
Groups	:	grouped by randomization
Duration of exposure	:	single dose
Study duration	:	90 days
Number of animals	:	20/ groups(5/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 (groups 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, IV for test drug of low dose (360 mg/kg/b.wt), Mid dose (760 mg/kg/b.wt), High dose (1440 mg/kg/b.wt), respectively. The low dose was calculated from the therapeutic dose (4g) and body surface area of rats.

Table 8: Number of animals used for long term toxicity study

S.NO	GROUP	TREATMENT	NUMBER. OF ANIMALS
1.	GROUP I	Control (Water)	20(10M + 10F)
2.	GROUP II	Low dose - Kottai Karanthai Chooranam- 360mg/kg Per oral	20(10M + 10F)
3.	GROUP III	Mid dose - Kottai Karanthai Chooranam 720mg/kg per oral	20(10M + 10F)
4.	GROUP IV	High dose - Kottai Karanthai Chooranam 1440mg/kg per oral	20(10M + 10F)

Total 80 (40 Female + 40 Male)

Total no. of animals 100 (50 Male and 50 Female)

Administration of dose:

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

Observation:

During the study, body weight of the animals, water and food consumption were evaluated weekly: mortality events were evaluated daily.

Laboratory Investigations:

On the 90th day, the animals were fasted overnight, then anesthetized to collect blood samples from the abdominal aorta in two tubes: one with EDTA for haematological parameters, another one without any anticoagulant and was centrifuged at 4000 rpm at 4°C for 10 minutes to obtain the serum for biochemical parameters. Vital organs were collected from the animals and subjected to histopathology.

Haematological Investigations:

Blood samples of control and Test drug treated rats were analysed for haemoglobin (Hb), total red blood corpuscles (RBC), white blood corpuscles (WBC) count, Platelet, Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), by auto analyser.

Biochemical Investigations:

Serum samples of control and Test drug treated animals were analysed for, Bilirubin, BUN, Creatinine, Triglyceride, Total Cholesterol, HDL, LDL, VLDL, SGOT/AST, SGPT/ALT using standard methods.

Gross necropsy:

It includes examination of the external surface of the body, all orifices, and organs like brain, thymus, lungs, heart, spleen, liver, kidney, adrenals and sex organs of all animals were preserved, and they were subjected to histopathology.

Histopathology:

Vital organs were collected from all animals and preserved in 10 % buffered neutral formalin for preparation of sections using microtone. Tissue samples of liver, kidneys, spleen, brain, heart, lungs stomach and gonads from control and highest dose group animals were subjected to histopathological investigations. The organ pieces (3-5µm thick) of all the animals. The organ pieces were fixed in 10% formalin for 24 hrs. Samples were dehydrated in an auto technic and then cleaned in benzene to remove

absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50ºC 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. RESULT

After preparation of Kottai Karanthai Chooranam I have analyse for standardization and safety evaluation. Here is given details about result of standardization method and Safety evaluation of toxicity study.

5.1.QUALITATIVE ANALYSIS

Table 9: Organoleptic Character of Kottai Karanthai Chooranam(KKC)

State	Solid
Nature	Moderately fine
Odor	Strong characteristic
Touch	Dry
Flow Property	Non- Free flowing
Appearance	Greenish brown

Figure no :3- Organoleptic Character

Table 10: Physico-chemical analysis:

S.NO	PARAMETERS	PERCENTAGE
1.	Loss on Drying at 105 °C (%)	4.033 ± 2.801
2.	Total Ash (%)	2.867 ± 0.4509
3.	Acid insoluble Ash (%)	0.3933 ± 0.2501
4.	Water soluble Extractive (%)	9.33 ± 0.1153
5.	Alcohol Soluble Extractive (%)	20.33 ± 4.869
6.	Particle Size	Completely passes through sieve size of 1 mm(90 % passes through 400 micro meter sieve and 10% passes through 1mm sieve)

Biochemical analysis of Kottai Karanthai Chooranam(KKC)

Table 11: Acid Radicals :

S.NO	PARAMETERS	RESULTS
1.	Test for Sulphate	+
2.	Test for Phosphate	+
3.	Test for Carbonate	+
4.	Test for Chloride	+
5.	Test for Sulphide	+
6.	Test for Fluoride & Oxalate	-

+ -> Indicates Positive and - -> Indicates Negative

From table 11, The Biochemical analysis for acid radical reveals that ***kottai Karanthai Chooranam*** contains Sulphate, Phosphate , Carbonate, Chloride, Sulphide.

Table 12: Basic Radicals:

S.NO	PARAMETERS	RESULTS
1.	Test for Lead	-
2.	Test for Aluminium	+
3.	Test for Iron	+
4.	Test for Zinc	+
5.	Test for Calcium	+
6.	Test for Ammonium	+
7.	Test for Mercury	-
8.	Test for Arsenic	-
9.	Test for Copper	-
10.	Test for Magnesium	-
11.	Test for Potassium	+
12.	Test for Sodium	+

From Table 12, The Biochemical analysis for basic radical reveals that ***kottai Karanthai Chooranam*** contains Aluminium, Calcium, Iron, zinc, ammonium, Sodium, Potassium.

Table:13 Other constituents:

S.NO	PARAMETERS	RESULTS
1.	Test for Starch	+
2.	Test for Reducing sugar	+
3.	Test for Alkaloids	+
4.	Test for Tannic acid	+

From Table 13, The Biochemical analysis for Other constituents reveals that ***kottai Karanthai Chooranam*** contains Starch, Reducing sugar, Alkaloid, Tannic acid.

5.2.QUALITATIVE ANALYSIS

Table 14: Phytochemical Investigation:

S.NO	TEST	OBSERVATION
1.	Alkaloids	-
2.	Flavonoids	-
3.	Glycosides	-
4	Steroids	-
5.	Triterpenoids	-
6.	Coumarin	-
7.	Phenol	+
8.	Tannin	+
9.	Protein	-
10.	Saponins	+
11.	Sugar	-
12.	Anthocyanin	-
13.	Betacyanin	-

From Table 14, The Qualitative analysis for Phytochemical analysis reveals that ***kottai Karanthai Chooranam*** contains Phenol,Tannin, Saponin.

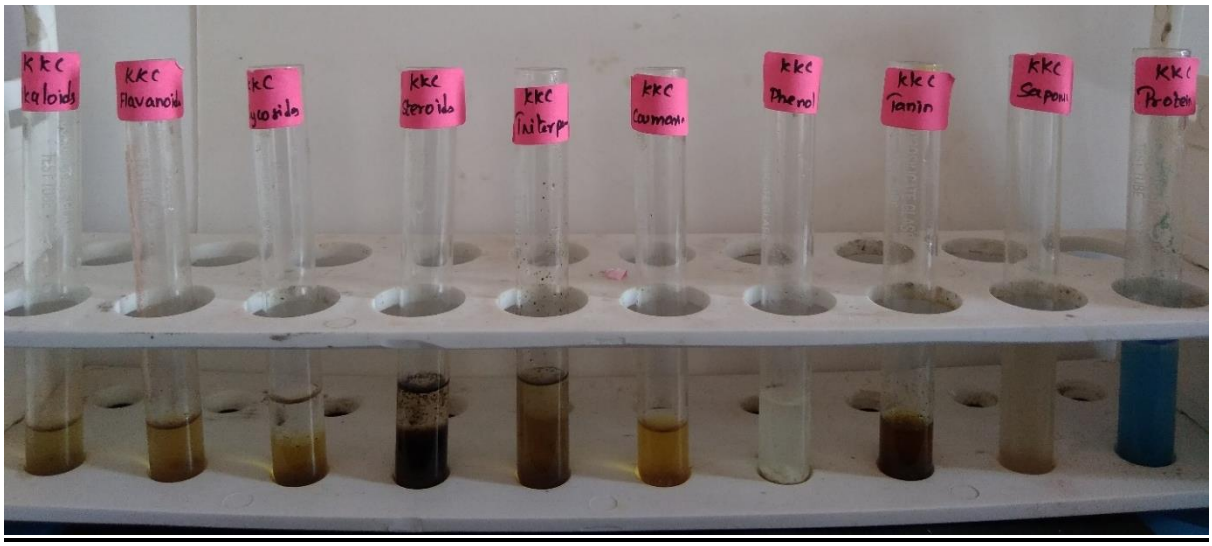


Figure 4: Qualitative Phytochemical Investigation

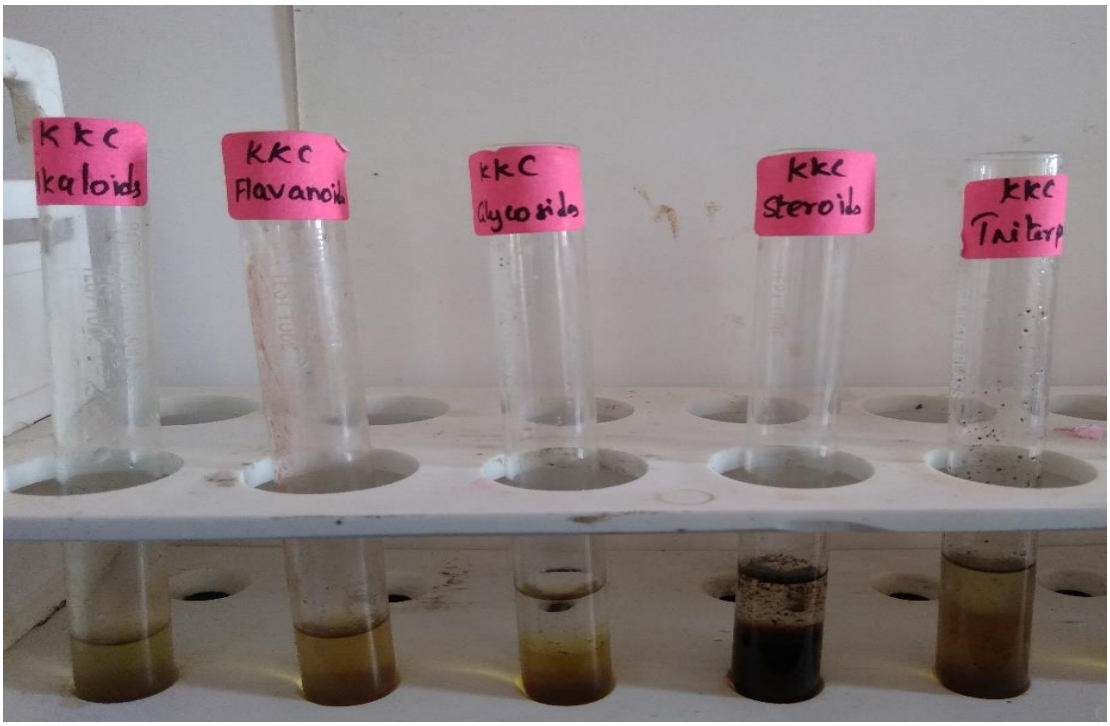


Figure 5:Test for Alkaloids, Flavonoids, Glycosides, Steroids and Triterpenoids

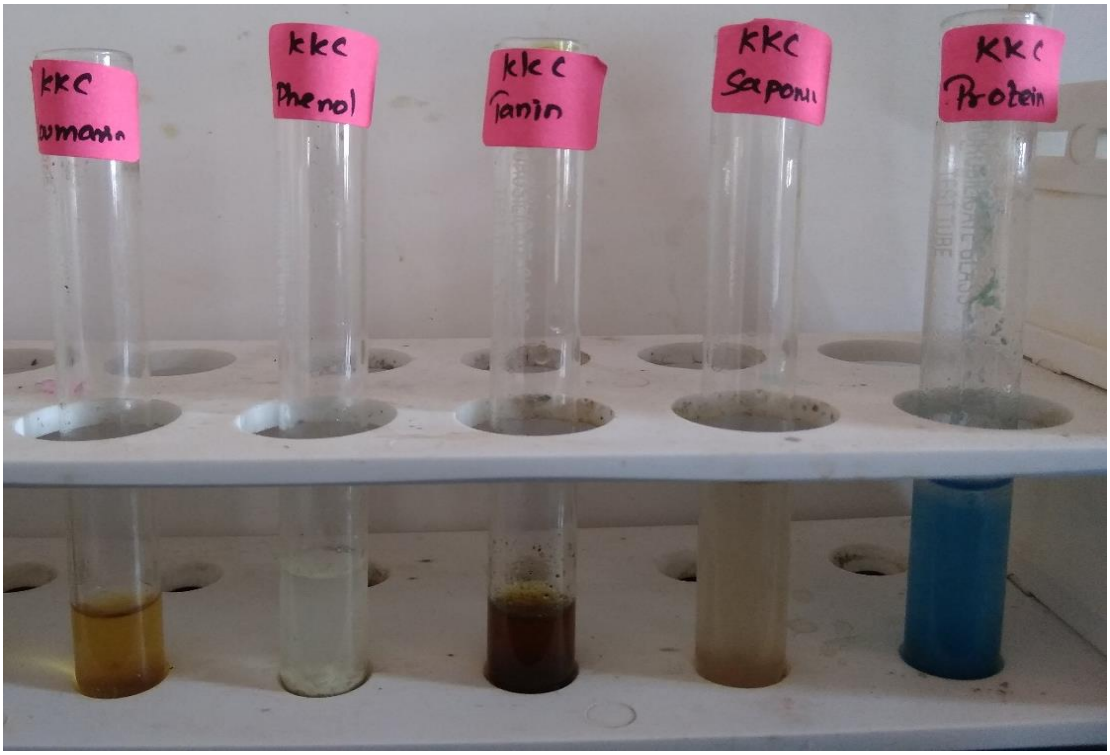


Figure 6: Test for Coumarin, Phenol, Tannins, Saponin, Proteins

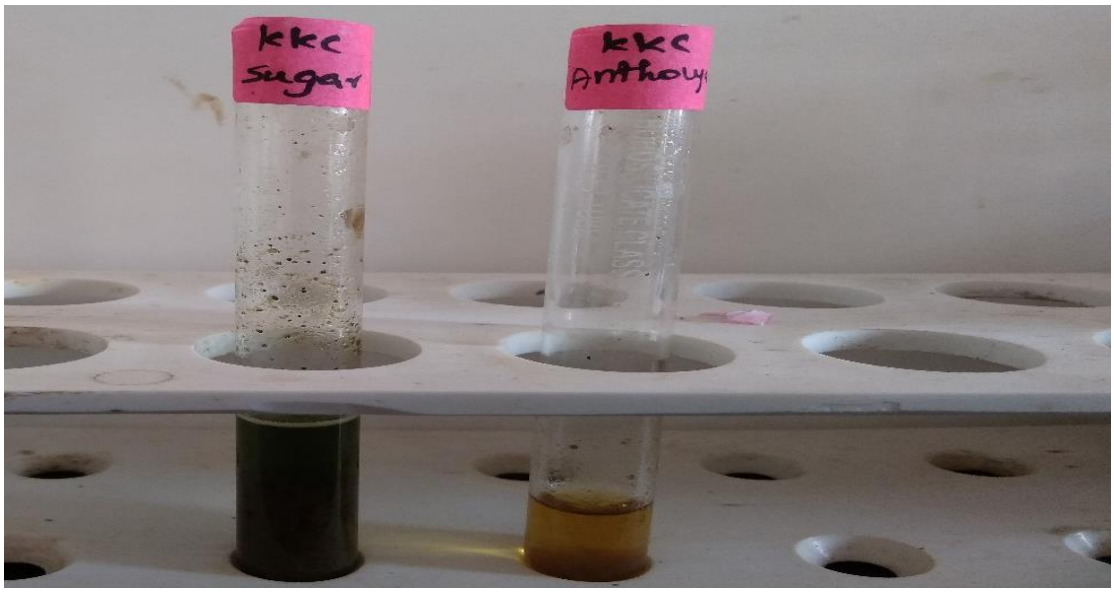
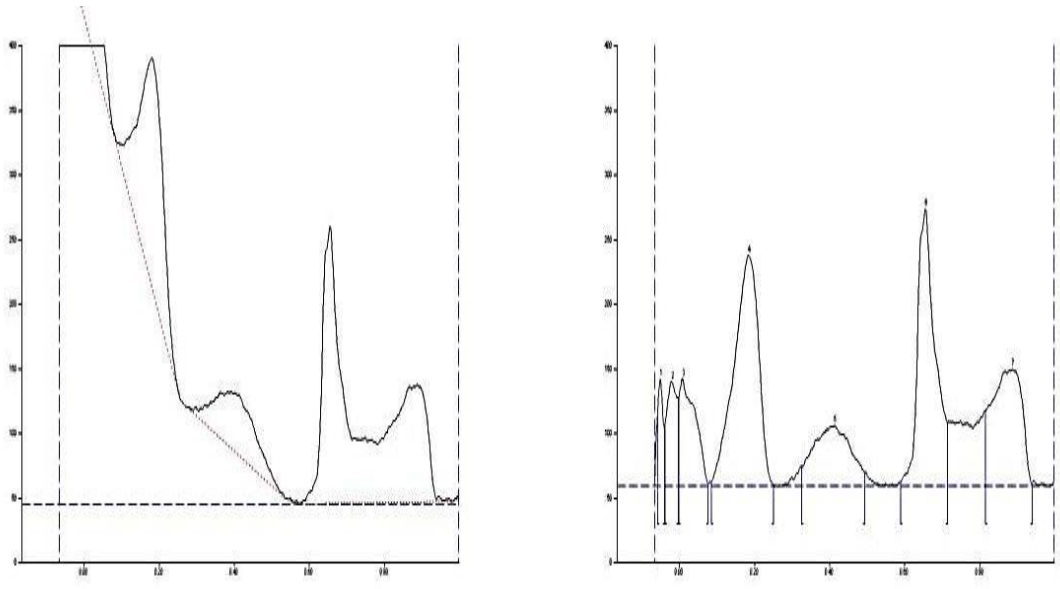


Figure 7: AnthoCyanin and carbohydrates

Table 15: HIGH CERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC) ANALYSIS OF KOTTAIKARANTHAI CHOORANAM.

PEAK	Start Rf	Start height	Max Rx	Max height	Max %	End Rf	End height	Area	Area %
1.	-0.06	57.7	-0.05	83.1	10.69	-0.04	44.8	784.4	2.78
2.	-0.04	47.3	-0.02	80.9	10.40	-0.00	68.6	1539.6	5.46
3.	-0.00	69.0	0.01	83.0	10.68	0.08	2.1	2484.9	8.81
4.	0.09	3.0	0.18	178.4	22.94	0.25	1.2	8134.3	28.83
5.	0.33	15.0	0.41	47.0	6.04	0.50	10.9	3265.6	11.58
6.	0.59	2.9	0.66	214.9	27.63	0.71	49.5	6912.6	24.50
7.	0.82	58.7	0.89	90.5	11.63	0.94	1.8	5090.3	18.04

Figure:8 High Performance Thin Layer Chromatography (HPTLC) Analysis Of Kottaikaranthai Chooranam



HPTLC finger printing of Sample KKC

Figure 9: TLC Visualization of KKC - TLC plate visualization at 366 nm

Report:

HPTLC finger printing analysis of the sample reveals the presence of seven prominent peaks corresponds to presence of seven versatile phytocomponents present with in it. Rf value of the peaks ranges from 0.09 to 0.82. Further the peak 4 occupies the major percentage of area of 28.83 which denotes the abundant existence of such compound.

Table 16: PESTICIDE RESIDUE:

Pesticide Residue **I.Organo Chlorine Pesticides**	**Sample KKC**	**AYUSH Limit (mg/kg)**
Alpha BHC	BQL	0.1mg/kg
Beta BHC	BQL	0.1mg/kg
Gamma BHC	BQL	0.1mg/kg
Delta BHC	BQL	0.1mg/kg
DDT	BQL	1mg/kg
Endosulphan	BQL	3mg/kg
II.Organo Phosphorus Pesticides		
Malathion	BQL	1mg/kg
Chlorpyriphos	BQL	0.2 mg/kg
Dichlorovos	BQL	1mg/kg
III. Organo carbamates		
Carbofuran	BQL	0.1mg/kg
III.Pyrethroid		
Cypermethrin	BQL	1mg/kg

BQL- Below Quantification Limit

Result:

The results showed that there were no traces of pesticides residues such as Organo chlorine, Organo phosphorus, Organo carbamates and pyrethroids in the sample provided for analysis.

TABLE 17: AFLOTOXIN ANALYSIS OF KOTTAI KARANTHAI CHOORANAM

Aflatoxin	Kottai Karanthai Chooranam	AYUSH Specification Limit
B1	Not Detected – Absent	0.5 ppm
B2	Not Detected – Absent	0.1 ppm
G1	Not Detected – Absent	0.5 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 TLC plates when compare to the standard, which indicates that he sample were free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2.

HEAVY METAL ANALYSIS:

ICP-OES Analysis

Table 18: Inductively Coupled Plasma Optical Emission Spectrometry Analysis of Kottai Karanthai Chooranam (KKC)

S.NO	ELEMENT NAME	STANDARD VALUE	OBTAINED VALUE
1.	As	188.979	BDL
2.	Ca	315.807	24.150mg/L
3.	Cd	228.802	BDL
4.	Cu	327.393	BDL
5.	Fe	238.204	2.340mg/L
6.	Hg	253.652	BDL

7.	K	766.491	120.821mg/L
8.	Mg	285.213	01.020mg/L
9.	Na	589.592	13.110mg/L
10.	Ni	231.604	BDL
11.	Pb	220.353	BDL
12.	P	213.617	58.541 mg/L
13.	Zn	213.856	01.587 mg/L

(BDL-Below Detection Limit)

Results And Interpretation Of Icp – Oes Analysis:

The presence of some metals such as Calcium, Iron, Mercury, Potassium, Manganese, Sodium, Phosphorus and Zinc were detected in the sample of *Kottai Karanthai Chooranam*. The most important heavy metals such as lead, mercury, nickel, copper, arsenic and cadmium are present in BDL as per the WHO permissible levels in this purified sample.

TEST FOR SPECIFIC PATHOGEN

Table no 19:

Organism	**Specification**	**Result**	**Method**
E-coli	Absent	Absent	As per AYUSH Specification
Salmonella	Absent	Absent	
Staphylococcus Aureus	Absent	Absent	
Pseudomonas Aeruginosa	Absent	Absent	

Result:

No growth / colonies were observed in any of the plates inoculated with the Kottai Karanthai Chooranam.

STERILITY TEST BY POUR PLATE METOD:

Observation:

No growth was observed after incubation period. Reveals the absence of specific pathogen.

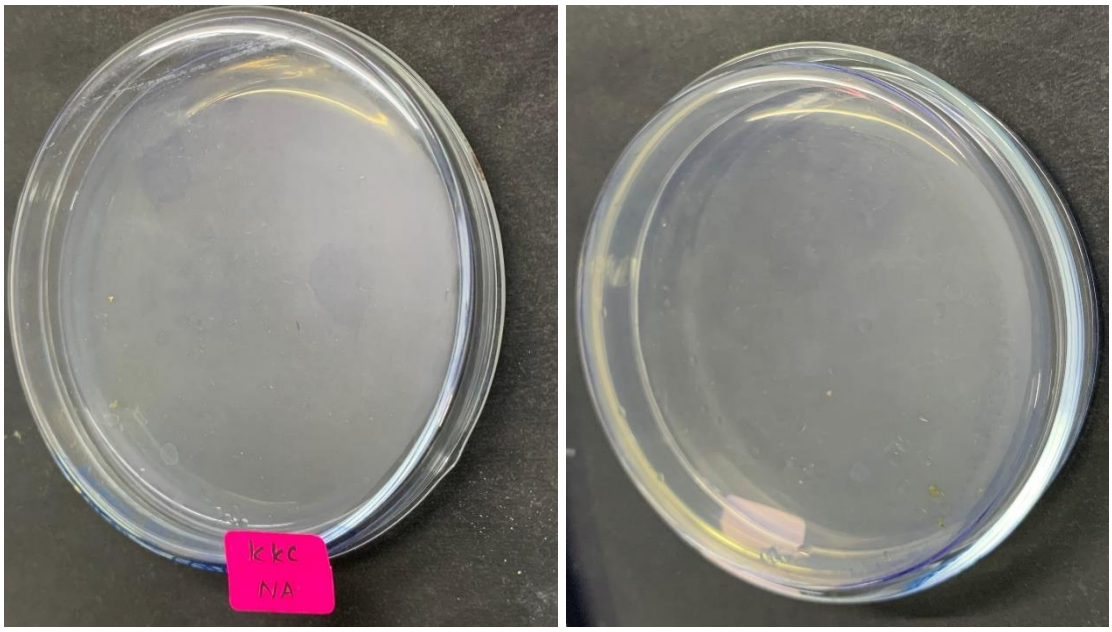


Figure no :10 Sterility test for Pour Plate Method

Result:

No growth / colonies were observed in any of the plates inoculates with the test sample.

Table no: 20 Sterility Test

Test	**Result**	**Specification**	**As per AYUSH/WHO**
Total Bacterial Count	Absent	NMT 10^5CFU/g	As per AYUSH specification
Total Fungal Count	Absent	NMT 10^3CFU/g	

TOXICOLOGICAL EVALUATION OF KOTTAIKARANTHAI CHOORANAM

5.3.TOXICITY STUDIES:

ACUTE ORAL TOXICITY STUDY OF KOTTAI KARANTHAI 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 21: Effect of Kottai Karanthai Chooranam on behavioural signs of acute toxicity study.

S.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.	Test group 10 times therapeutic dose (2000 mg/b.wt)	+	-	-	+	+	+	--	-	-	-	-	-	-	-	-	-	-	-	-	-

1.Alertness 2. Aggressiveness 3. Pile erection 4. Grooming 5.Gripping 6.Touch Response 7. Motor Activity 8. Tremors 9. Convulsions 10. Muscle Spasm 11. Catatonia 12.Muscle relaxant 13.Hypnosis 14.Analgesic 15.Lacrimation 16.Exophthalmos 17.Diarrhea 18.Writhing 19. Respiration 20. Mortality.

+ Presence of Activity

- Absence of Activity

1.All the data were summarized in the form of table revealed that there was no abnormal signs and behavioural changes in all animals at the dose level of 2000 mg/kg body weight administered orally, during the study period.

2.There was no morbidity and mortality rate were observed in all test animals. Body weight of all test drug treated animals were gradually increased.

3. There was no necropsy findings seen of all the orifices and vital organs of 2000mg/kg.b.wt of Kottai Karanthai Chooranam treated animals in acute toxicity study.

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

LONG TERM ORAL TOXICITY STUDY OF KOTTAIKARANTHAI CHOORANAM

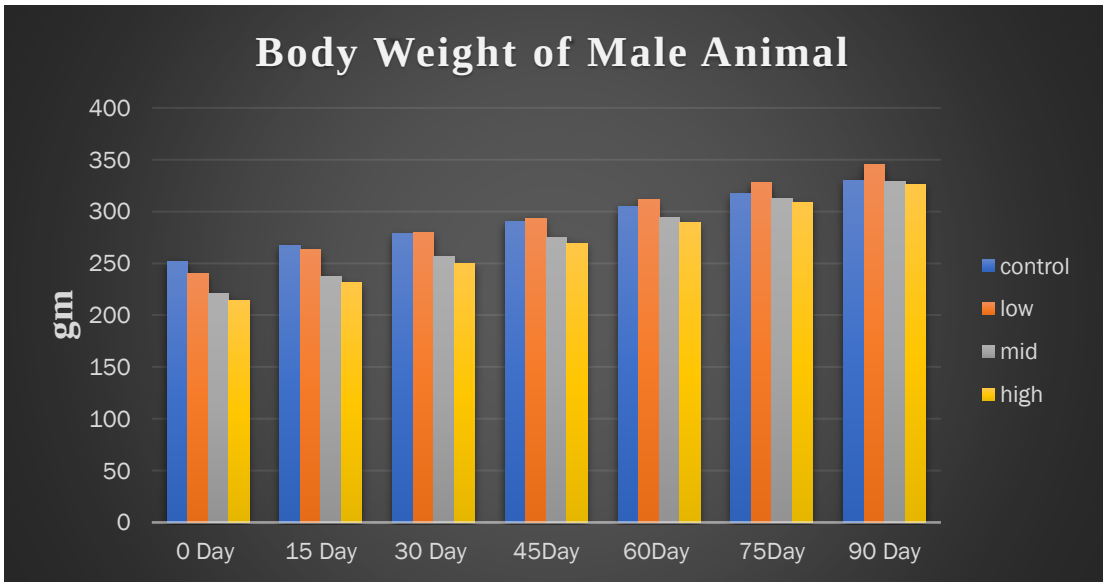
Long term oral toxicity study carried out as per WHO guideline for a period of 90 days. The changes observed in the feed intake, water intake, body weight changes, haematological and biochemical parameters were mentioned in below table.

Table 22: Effect of Kottai Karanthai Chooranam on Body Weight changes of Male wistar albino rats in long term toxicity study.

Groups	Control	Low dose	Mid dose	High dose
0th day	252±19.23	240 ±19.29	221.2±21.83	214.5±13.83
15th day	266.8±13.8	263.1 ± 18.33	237.5±20.23	231.3±13.66
30th day	279±11.57	279.6 ± 19.46	256.4±20.35	250.3±14.56
45th day	290±10.17	293.6 ± 18.79	275.3±20.8	269.3±14.23
60th day	304.8±8.52	311.5 ± 18.34	294.7±18.9	289.5±13.87
75th day	317.4±8.73	327.9 ±17.36	312.2±18.22	308.4±13.37
90th day	330±10	345 ±21.85	329.2±19.7	326.4±13.67

Values were expressed as mean± S.D. for rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates *P<0.05, **P<0.01.

Figure 11: Effect of Kottai Karanthai Chooranam on Body Weight changes of Male wistar albino rats in long term toxicity study.



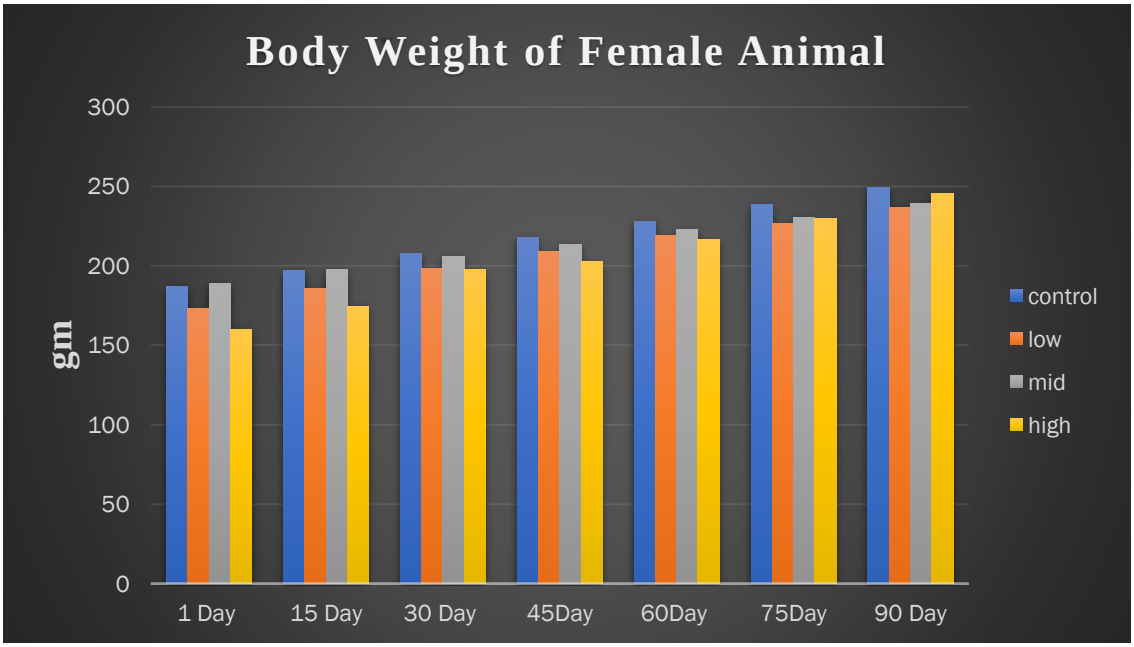
Male body weight of both control and test dose treat group revealed normal body weight throughout the study. There is no significant change occur in body weight of low dose, mid and high dose compared with control group (Table 22).

Table 23: Effect of Kottai Karanthai 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	187±12.04	173±8.23	188.6±11.03	160.2±12.3
15th day	196.8±13.1	185.8±8.62	197.4±10.12	174.6±12.79
30th day	207.6±16.65	198.1±12.95	206.1±10.21	197.5±34.92
45th day	217.8±17.06	208.8±16.92	213.5±9.61	203±13.69
60th day	228±19.45	218±19.11	222.8±9.19	216.5±14.3
75th day	238.8±20.57	226.9±22.39	230.5±8.11	229.5±14.56
90th day	249.6±21.75	237±25.4	239.2±8.01	245.5±12.42

Values were expressed as mean± S.D. for rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates *P<0.05, **P<0.01.

Figure 12: Effect of Kottai Karanthai Chooranam on Body Weight changes of Female wistar albino rats in long term toxicity study.



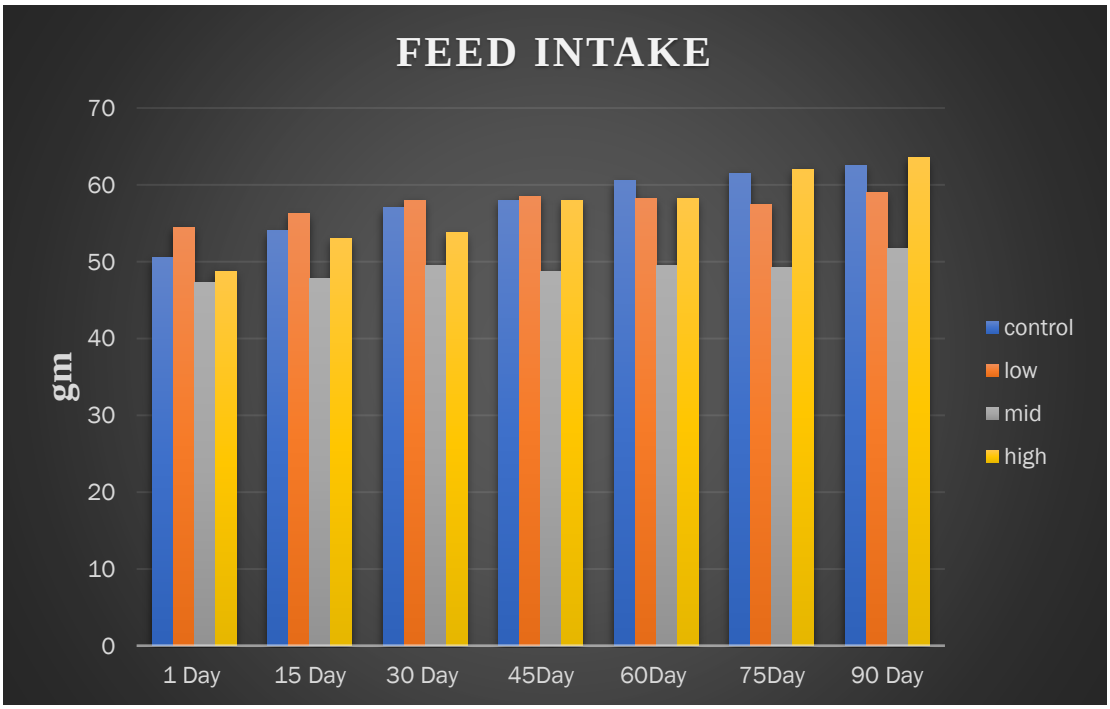
Female body weight of both control and test dose treat group revealed normal body weight throughout the study. There is no significant change occur in body weight of low dose, mid and high dose compared with control group (Table 23).

Table 24: Effect of Kottai Karanthai Chooranam on feed intake changes of wistar albino rats in long term toxicity study.

Groups	Control	Low dose	Mid dose	High dose
0th day	50.5±13.43	54.5±15.02	47.25±15.3	48.75±18.2
15th day	54±16.97	56.25±13.72	47.75±17.03	53±19.91
30th day	57±18.38	58±15.72	49.5±15.58	53.75±19.93
45th day	58±16.97	58.25±14.64	48.75±16.47	58±19.06
60th day	60.5±16.26	58.25±11.89	49.5±17.91	58.25±18.83
75th day	61.5±16.26	57.5±13.86	49.25±15.96	62±17.9
90th day	62.5±14.84	59±13.9	51.75±15.3	63.5±17.33

Values were expressed as mean± S.D. for rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates *P<0.05, **P<0.01.

Figure 13: Effect of Kottai Karanthai Chooranam on feed intake changes of wistar albino rats in long term toxicity study.



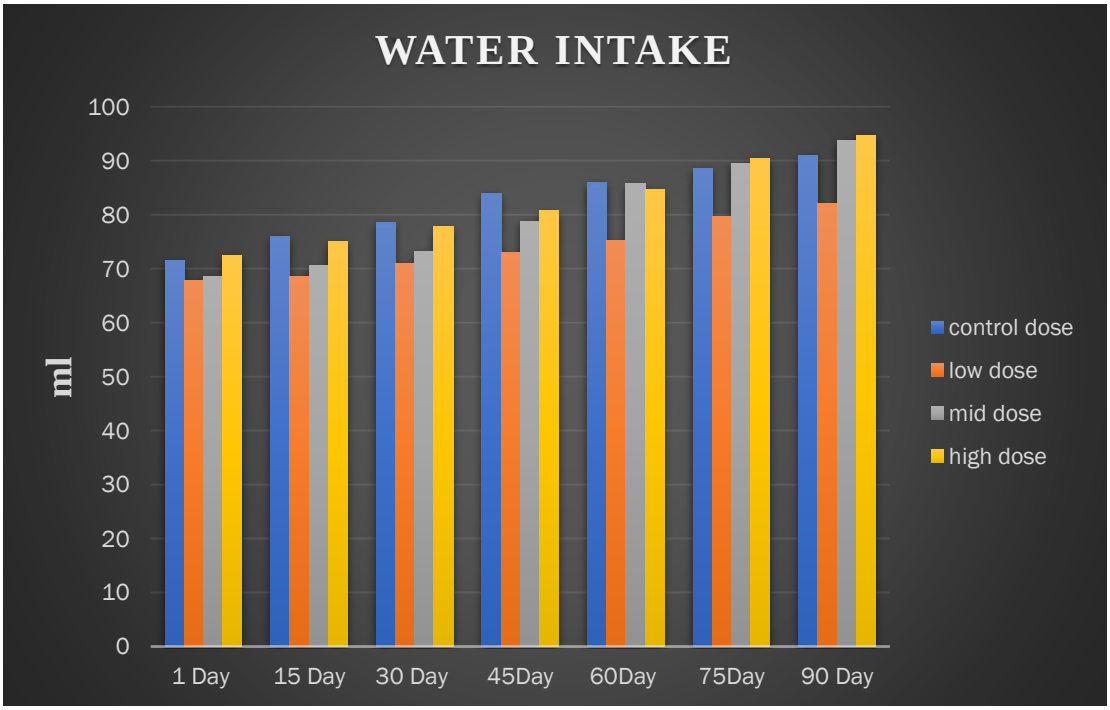
Feed consumption of the animals showed no significant difference in Food intake the test group animals were observed when compared with control group during the study period. (Table 24).

Table 25: Effect of Kottai Karanthai Chooranam on Water intake changes of wistar albino rats in long term toxicity study.

Groups	Control	Low dose	Mid dose	High dose
0th day	71.5±2.12	67.75±6.23	68.5±5.32	72.5±11.56
15th day	76±1.41	68.5±5.19	70.5±5.25	75±11.69
30th day	78.5±0.7	71±5.22	73.25±5.56	77.75±11.38
45th day	84±1.41	73±3.91	78.75±7.41	80.75±11.29
60th day	86±1.41	75.25±1.7	85.75±2.21	84.75±10.68
75th day	88.5±2.12	79.75±1.25	89.5±1.29	90.5±13.02
90th day	91±1.41	82±2	93.75±1.25	94.75±13.45

Values were expressed as mean± S.D. for rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates *P<0.05, **P<0.01.

Figure 14: Effect of Kottai Karanthai Chooranam on water intake changes of wistar albino rats in long term toxicity study.



Water consumption the difference in Water intake of control and test group of animal were observed during the study period. There was no significant difference occurs in the test group compared with control group (Table 25).

Table 26: Effect of Kottai Karanthai Chooranam on Haematological parameters of wistar albino rats in long term toxicity study.

Parameters	Control	Low dose	Mid dose	High dose
RCB (X 10^6µl)	6.77±1.09	7.12±1.25	8.15±0.66	7.55±1.08
WBC (X 10^3/µl)	7.53±1.91	8.18±2.3	8.55±1.38	7.94±1.5
Platelet (X 10^3/µl)	787.2±100.6	706.25±147.16	797.15±92.2	766.7±97.28
Haemoglobin (g/dl)	13.24±1.33	13.2± 1.78	14.12±1.84	13.05±1.15
MCH (pg)	19.26±1.78	18.79±1.75	19.53±1.1	19.67±1.66
MCV (fl)	58.66±5.84	61.21±5.84	60.58±4.3	58.99±5.07
Neutrophils (10^3/mm^3)	2.37±0.73	2.54±0.71	2.62±0.58	2.58±0.67
Eosinophils (%)	1.41±0.21	1.45±0.61	1.37±0.98	1.67±0.96
Basophils (%)	0.2±0.42	0.43±0.58	0.74± 0.58	0.38±0.52
Lymphocytes (%)	78.37±9.37	74.71±6.93	74.18±6.94	76.91±8.35
Monocytes (%)	3.61±1.23	3.22±1.16	3.33±1.29	4.02±1.09

Values were expressed as mean± S.D. for rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates *P<0.05, **P<0.01.

Figure 15: Effect of Kottai Karanthai Chooranam on Haematological parameters – RBC of wistar albino rats in long term toxicity study.

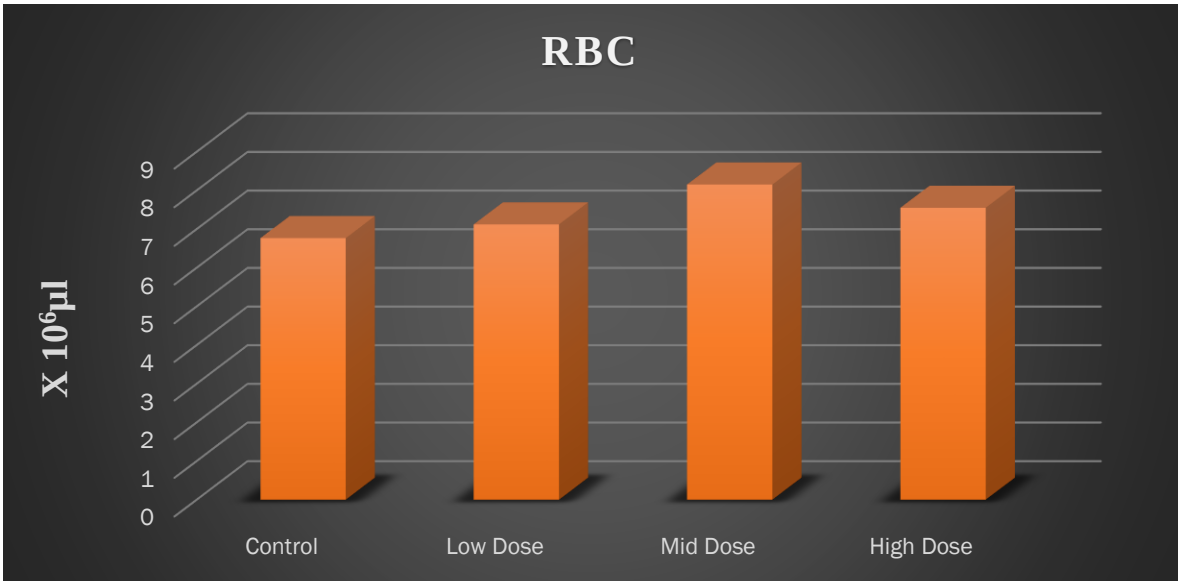


Figure 16: Effect of Kottai Karanthai Chooranam on Haematological parameters – WBC of wistar albino rats in long term toxicity study

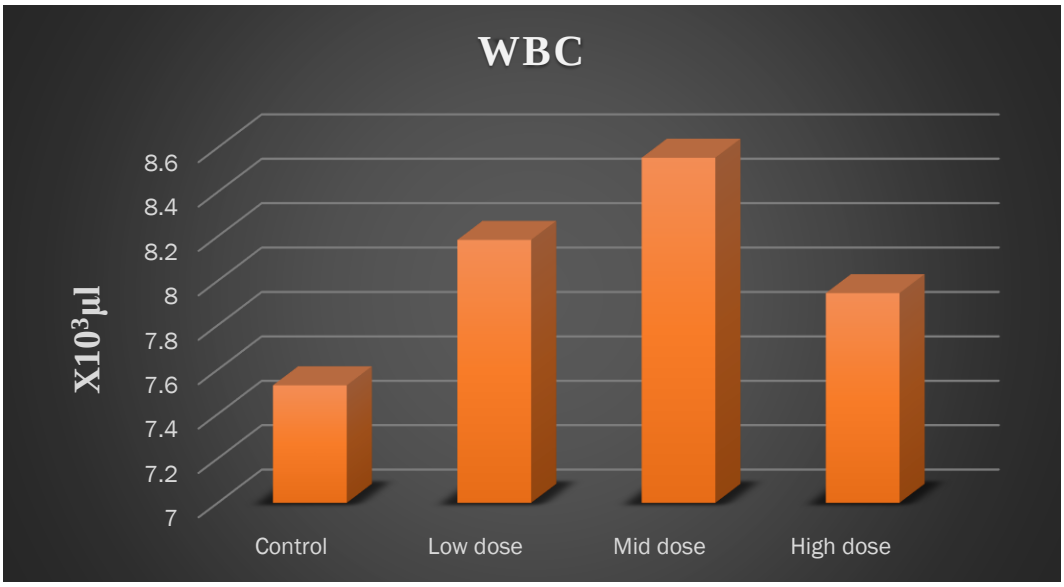


Figure17: Effect of Kottai Karanthai Chooranam on Haematological parameters – Platelet of wistar albino rats in long term toxicity study.

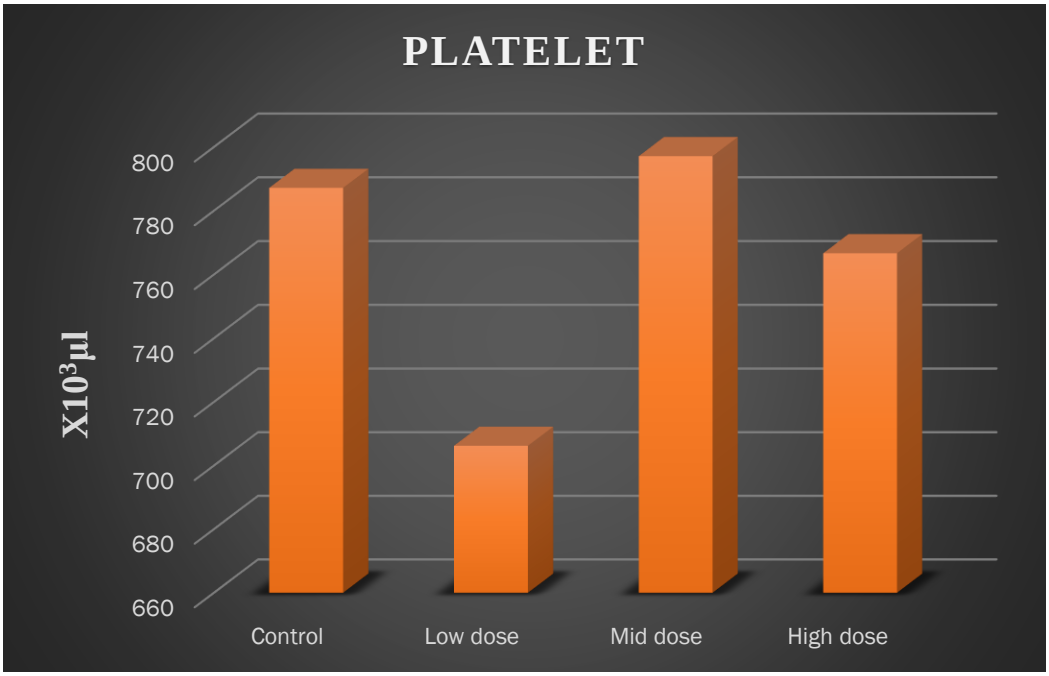


Figure 18: Effect of Kottai Karanthai Chooranam on Haematological parameters - Haemoglobin of wistar albino rats in long term toxicity study.

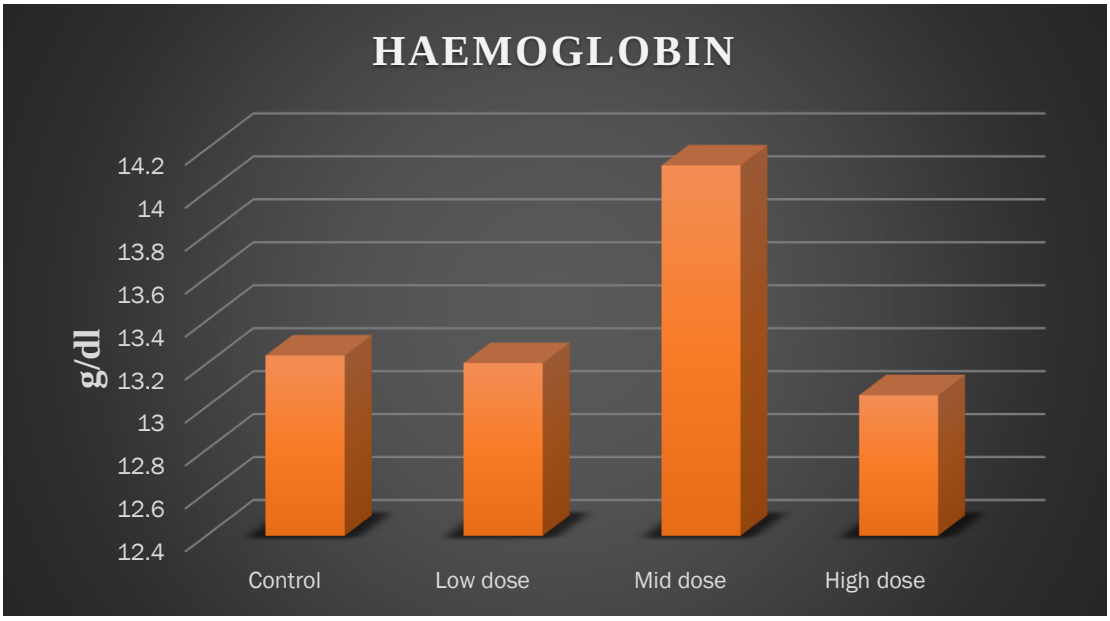


Figure 19: Effect of Kottai Karanthai Chooranam on Haematological parameters – MCV of wistar albino rats in long term toxicity study.

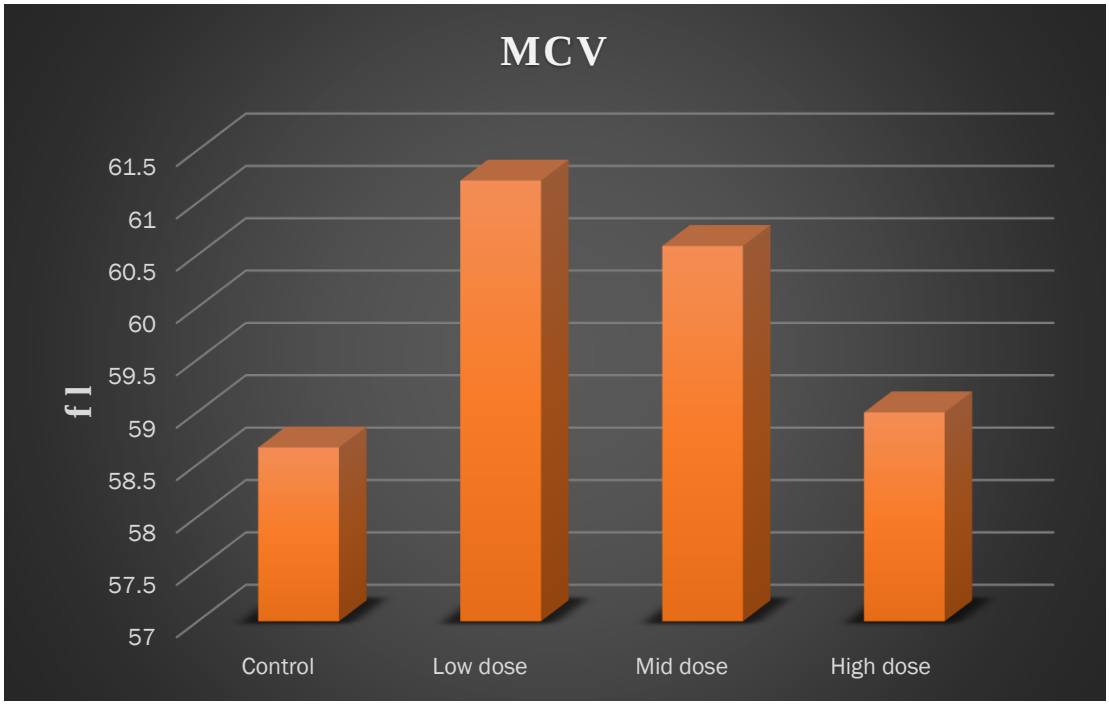


Figure 20: Effect of Kottai Karanthai Chooranam on Haematological parameters MCH of wistar albino rats in long term toxicity study.

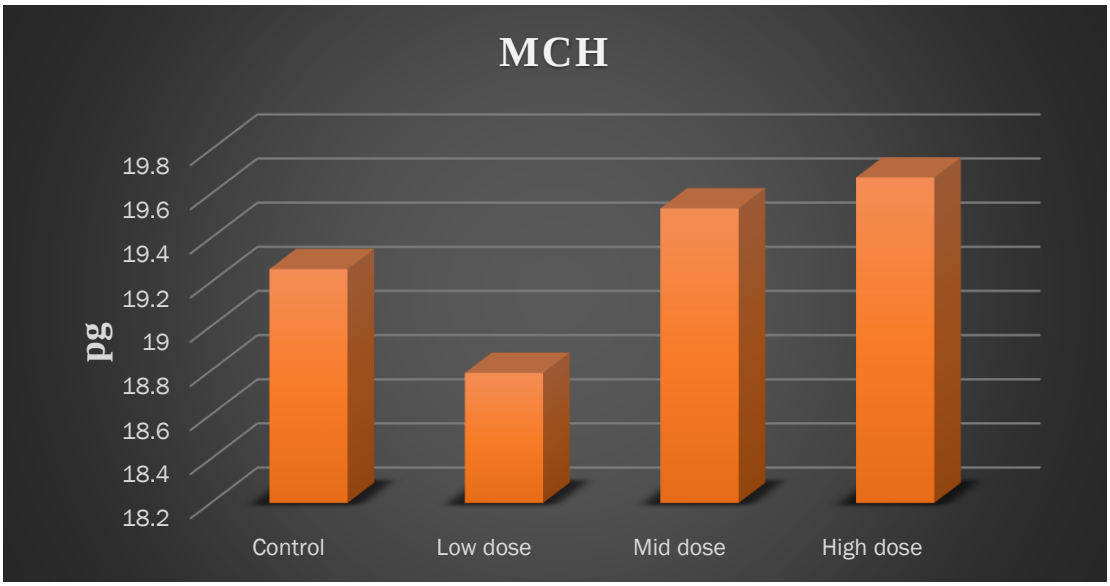
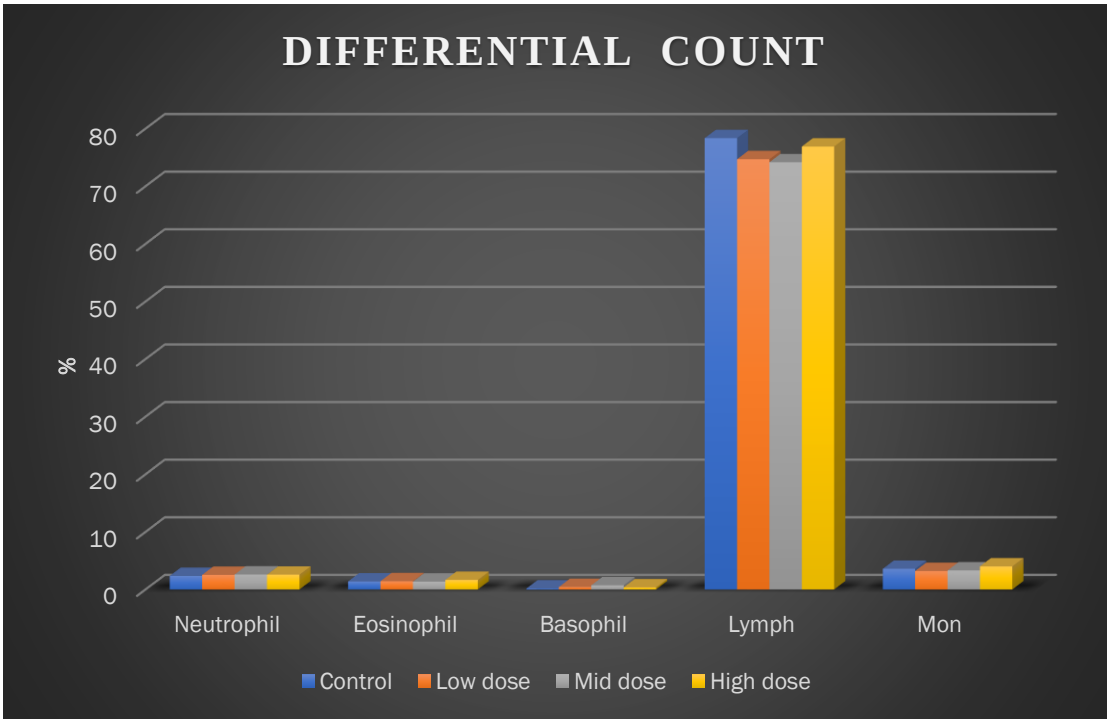


Figure 21: Effect of Kottai Karanthai Chooranam on Haematological parameters – Differential counts of wistar albino rats in long term toxicity study.



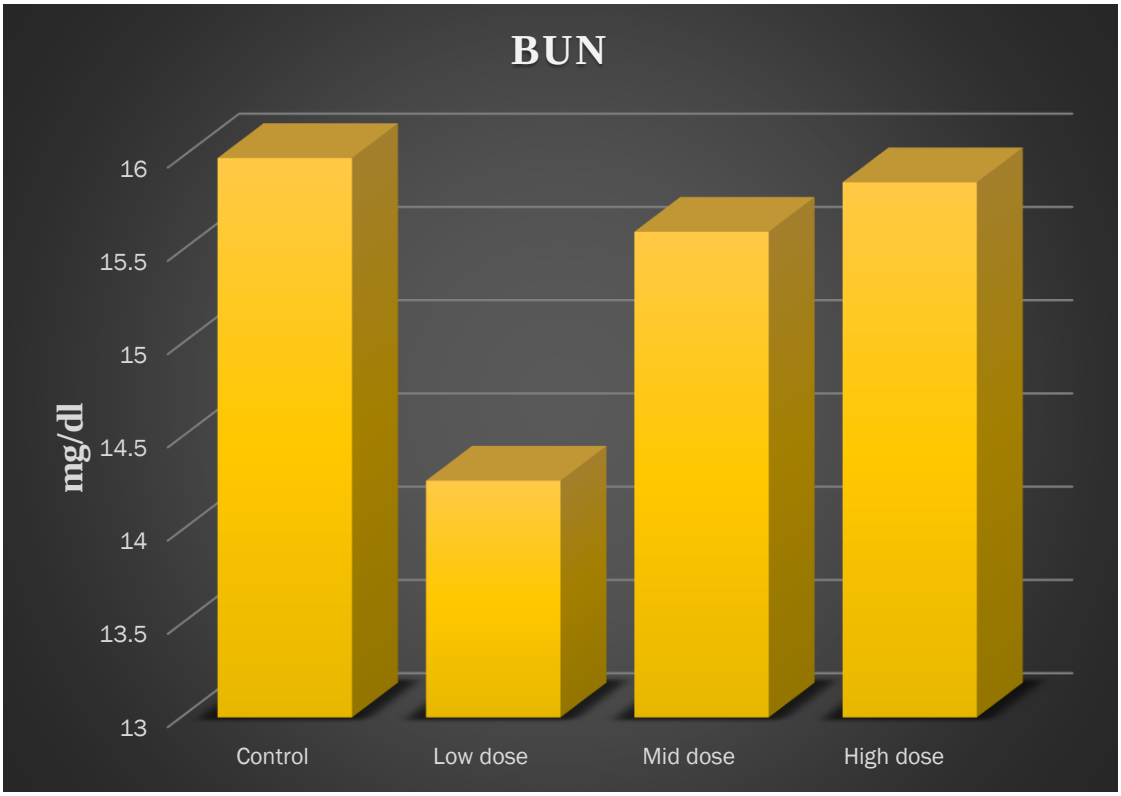
The haematological analysis revealed no significant changes in as all test drug treated groups when compared with control group (Table. No 26).

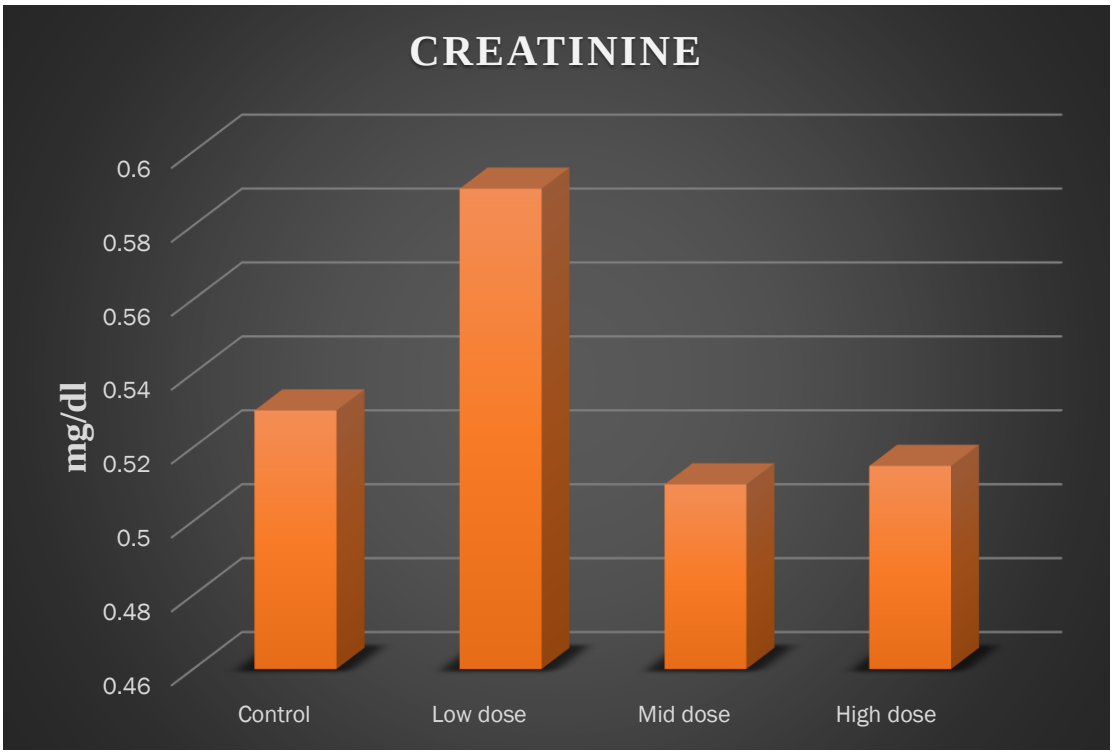
Table 27 : Effect of Kottai Karanthai Chooranam on Biochemical parameters – Renal function test of wistar albino rats in long term toxicity study.

Parameters	Control	Low dose	Mid dose	High dose
BUN(mg/dl)	16±2.44	14.27±2.7	15.6±1.35	15.87±2.1
Serum Creatinine(mg/dl)	0.53±0.13	0.59±0.17	0.51±0.15	0.51±0.12

Values were expressed as mean± S.D. for rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates *P<0.05, **P<0.01.

Figure 22 &23: Effect of Kottai Karanthai Chooranam on Biochemical parameters – BUN & creatinine levels of wistar albino rats in long term toxicity study.





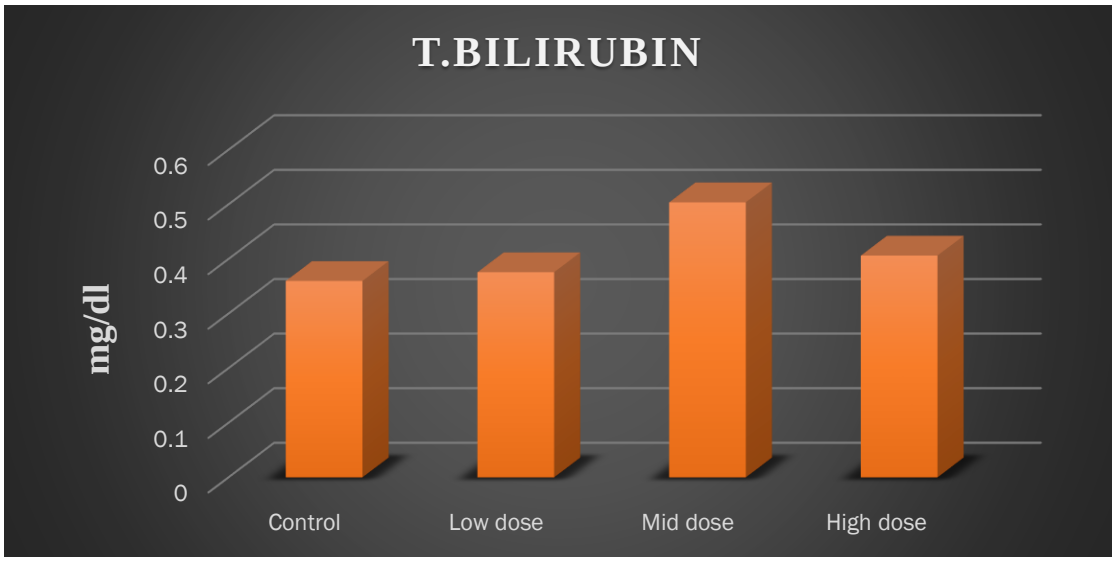
Biochemical investigation of Renal function test results suggest there was no significant changes observed in all test drug animals when compared with control group (Table no:27).

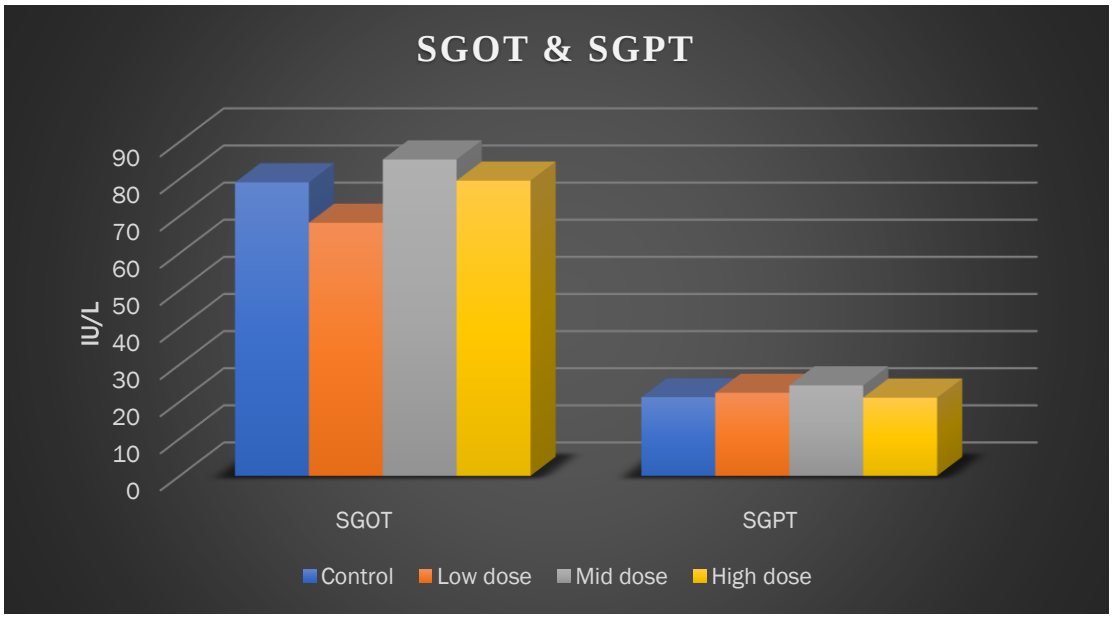
Table 28: Effect of Kottai Karanthai Chooranam on Biochemical parameters –Liver function test of wistar albino rats in long term toxicity study.

Parameters	Control	Low dose	Mid dose	High dose
Total bilirubin(mg/dl)	0.36±0.14	0.37±0.18	0.5±0.15	0.4±0.13
SGOT (U/dl)	79.1±18.82	68.2±18.85	85.25±19.78	79.55±15.36
SGPT (U/dl)	21.2±8.4	22.4±8.75	24.41±5.03	21.1±6.19

Values were expressed as mean± S.D. for rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates *P<0.05, **P<0.01.

Figure 24 &25: Effect of Kottai Karanthai Chooranam on Biochemical parameters – Total Bilirubin and SGOT & SGPT of wistar albino rats in long term toxicity study.





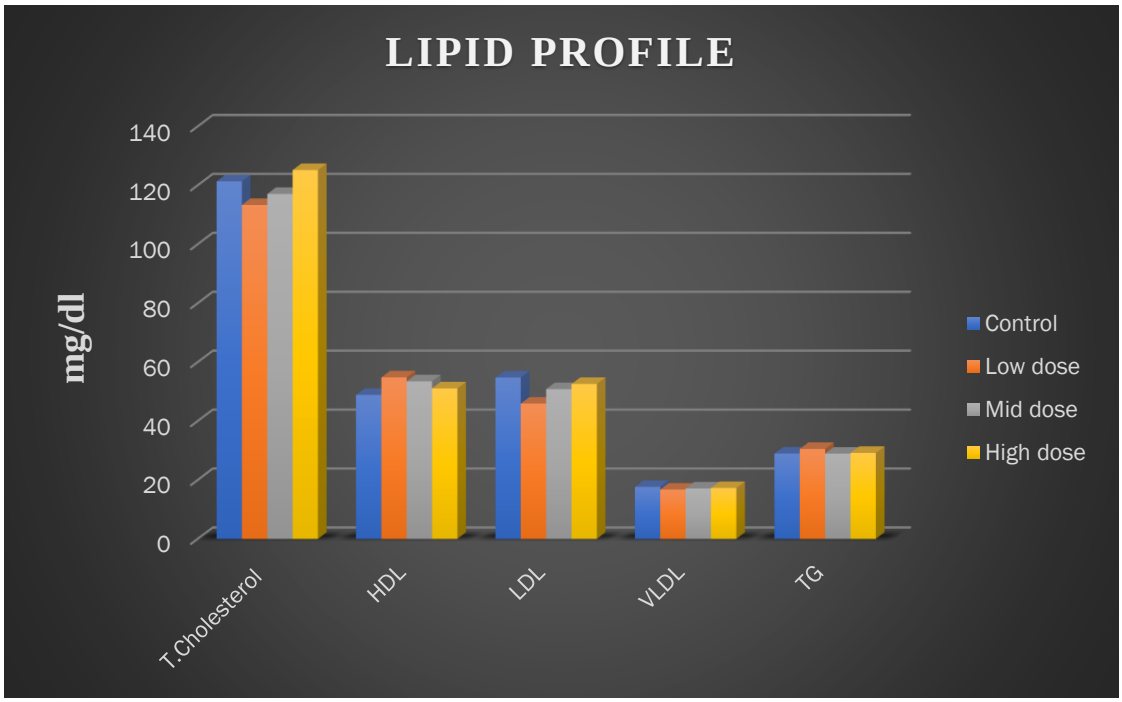
The result of the liver function test revealed, test drug treated groups shows no significant changes in liver parameters when compared to control group (Table No:28).

Table 29 : Effect of Kottai Karanthai Chooranam on Biochemical parameters-Lipid profile of wistar albino rats in long term toxicity study.

Parameters	Control	Low dose	Mid dose	High dose
Total cholesterol (mg/dl)	121.39±17.7	113.36±15.46	117.1±8.62	125.2±16.03
HDL (mg/dl)	48.9±5.74	54.85±10.17	53.5±5.79	51.1±5.75
LDL (mg/dl)	54.8±11.98	45.95±10.89	50.85±3.91	52.6±8.94
VLDL (mg/dl)	17.69±1.33	16.76±1.76	17.15±0.97	17.29±1.25
Triglyceride	29±6.32	30.55±7.48	28.95±4.12	29.2±5.11

Values were expressed as mean± S.D. for rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates *P<0.05, **P<0.01.

Figure 26: Effect of Kottai Karanthai Chooranam on Biochemical parameters – Lipid profile of wistar albino rats in long term toxicity study



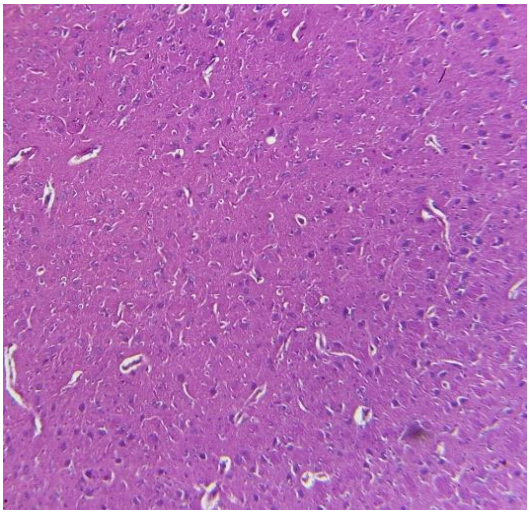
The result of the lipid profile revealed, test drug treated groups shows no significant changes in lipid profile parameters when compared to control group (Table No:29).

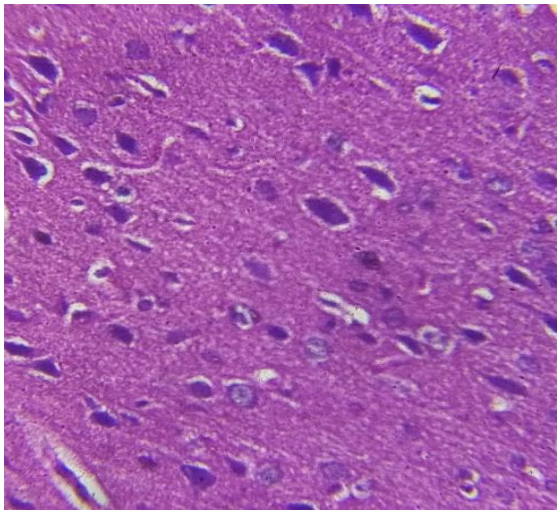
Figure :27 **HISTOPATHOLOGICAL CHANGES OF CONTROL AND HIGH DOSE GROUP OF KOTTAI KARANTHAI CHOORANAM TREATED ANIMALS (MALE AND FEMALE WISTAR ALBINO RATS)**

HISTOPATHOLOGY OF BRAIN

CONTROL - MALE

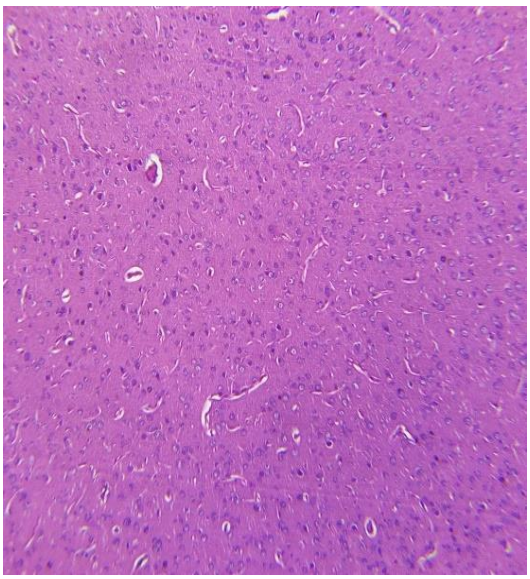
Low Power Magnification 10X High Power Magnification 40X

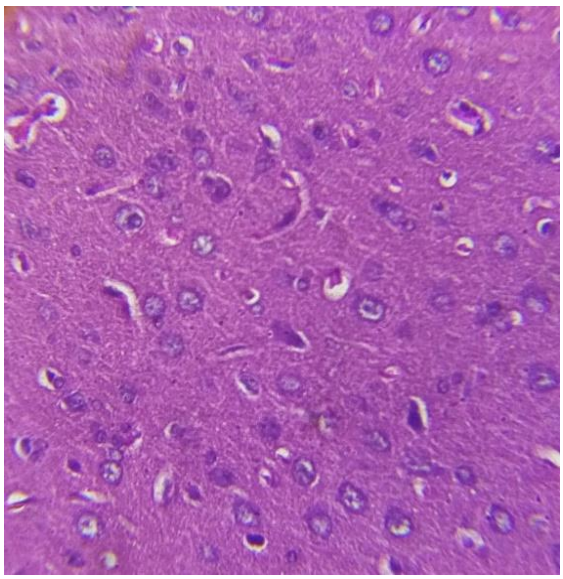




CONTROL – FEMALE

Low Power Magnification 10X High Power Magnification 40X

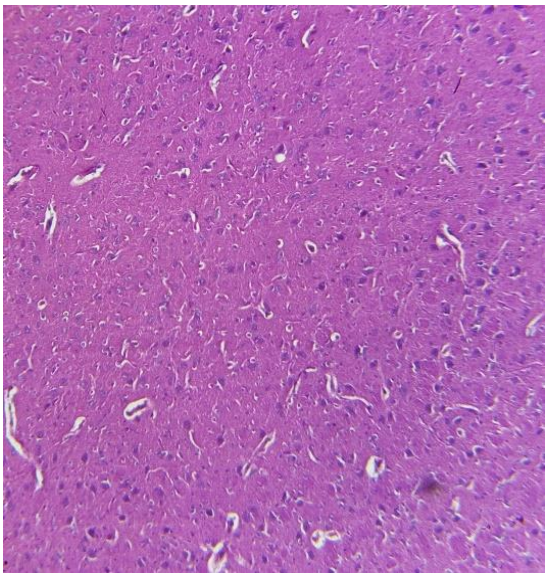




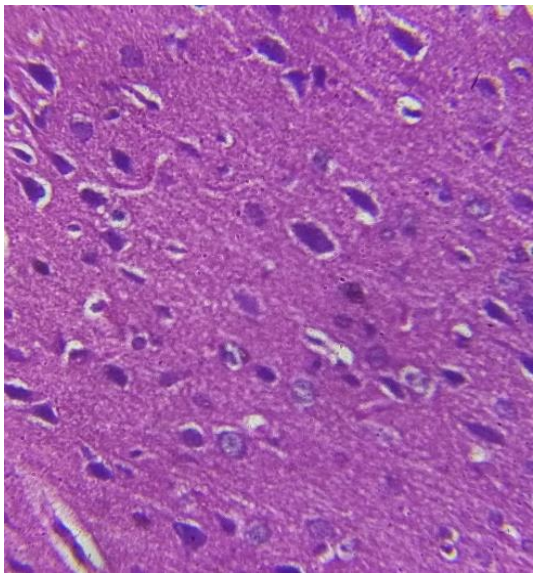
HISTOPATHOLOGY OF BRAIN

HIGH DOSE- MALE

Low Power Magnification 10X

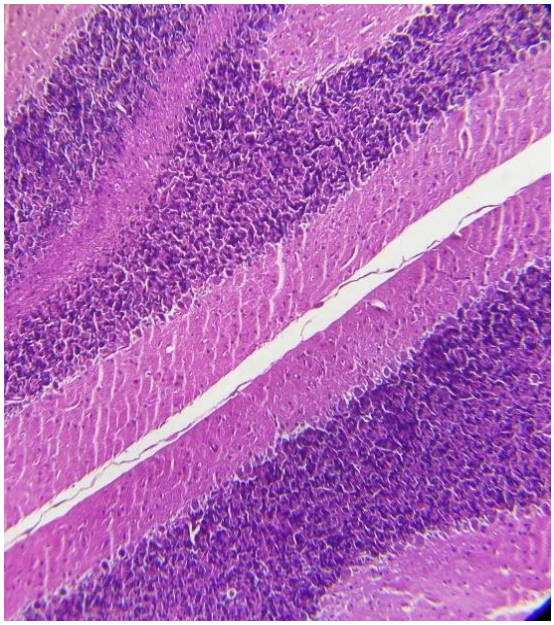


High Power Magnification 40X

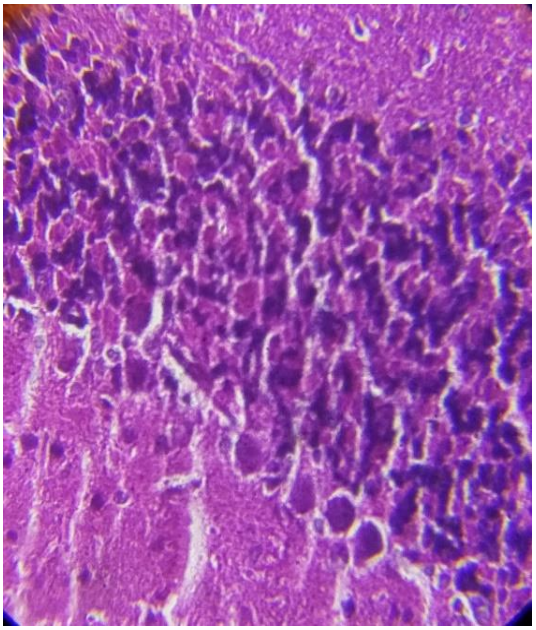


HIGH DOSE- FEMALE

Low Power Magnification 10X



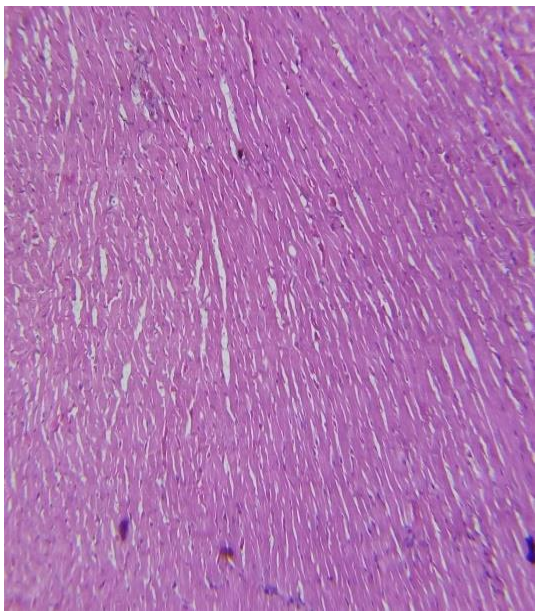
High Power Magnification 40X



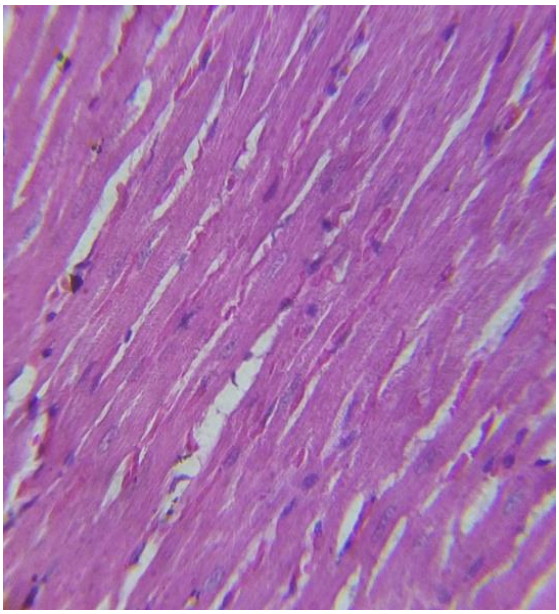
HISTOPATHOLOGY OF HEART

CONTROL - MALE

Low Power Magnification 10X

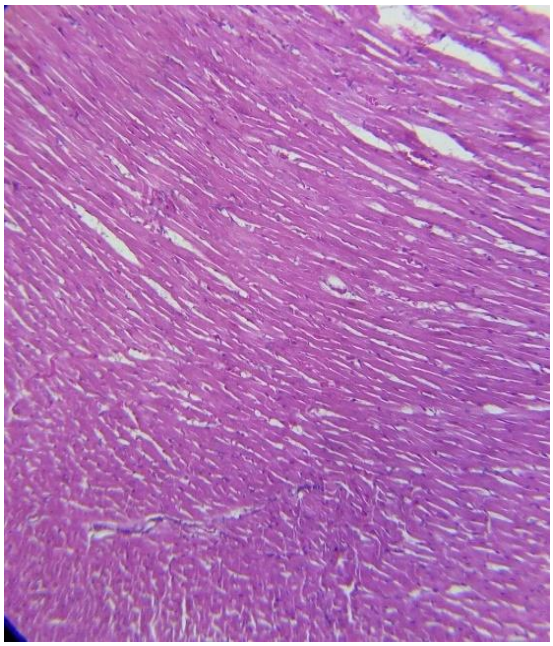


High Power Magnification 40X

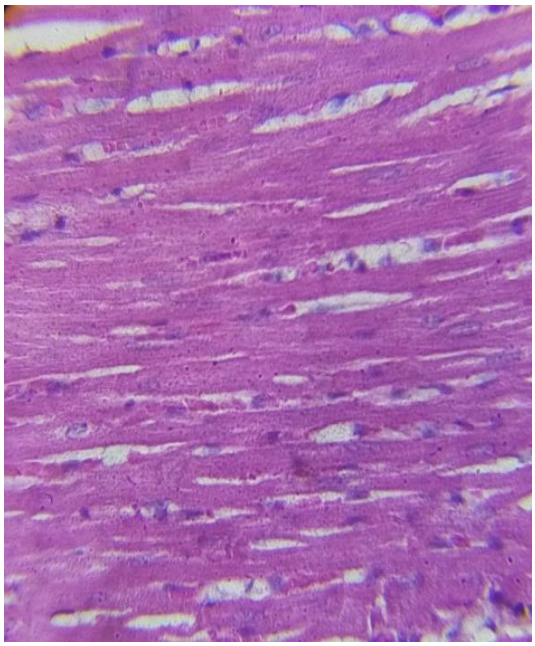


CONTROL – FEMALE

Low Power Magnification 10X



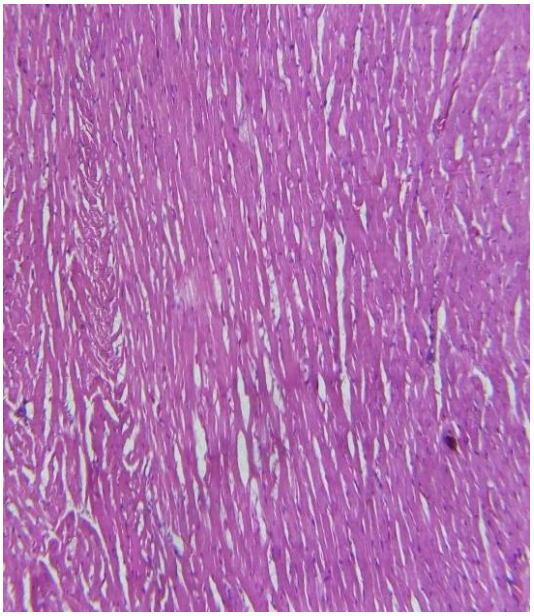
High Power Magnification 40X



HISTOPATHOLOGY OF HEART

HIGH DOSE – MALE

Low Power Magnification 10X



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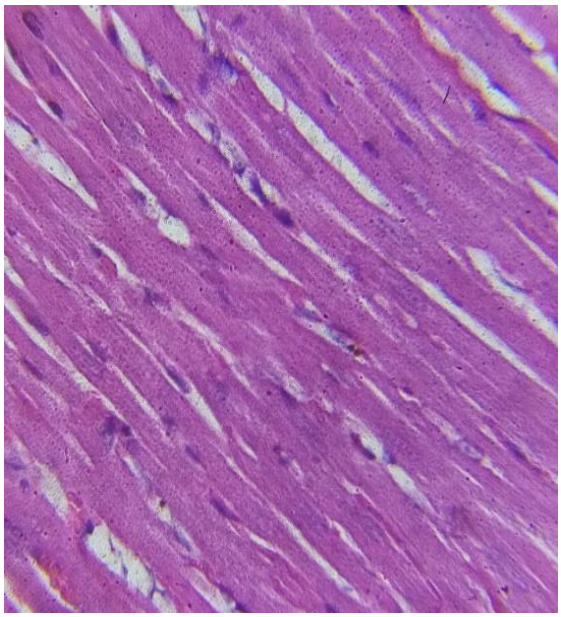
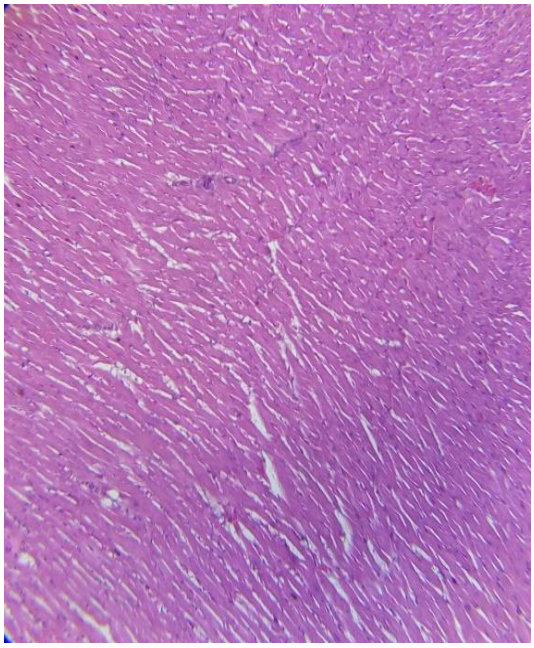


HIGH DOSE – FEMALE

Low Power Magnification 10X



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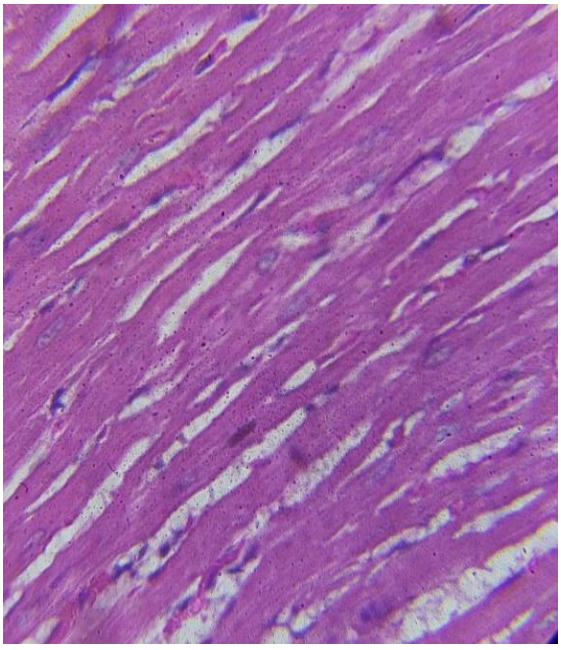
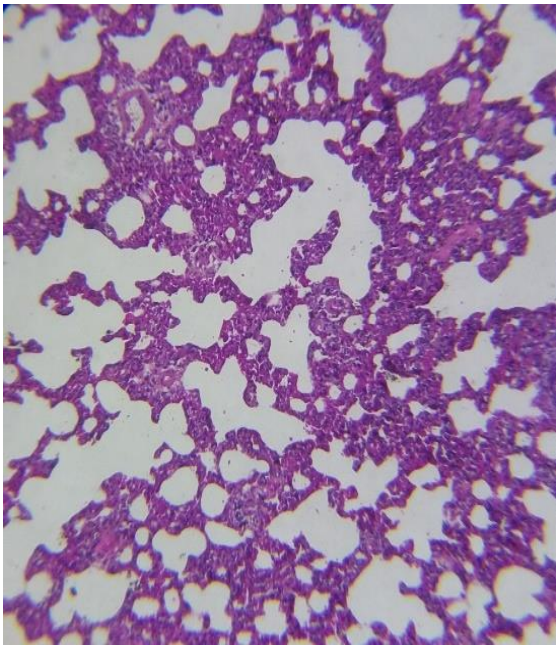


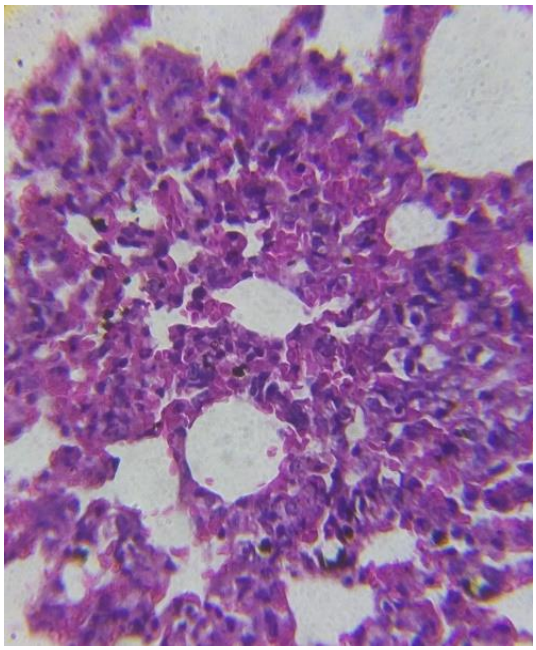
HISTOPATHOLOGY OF LUNG

CONTROL - MALE

Low Power Magnification 10X

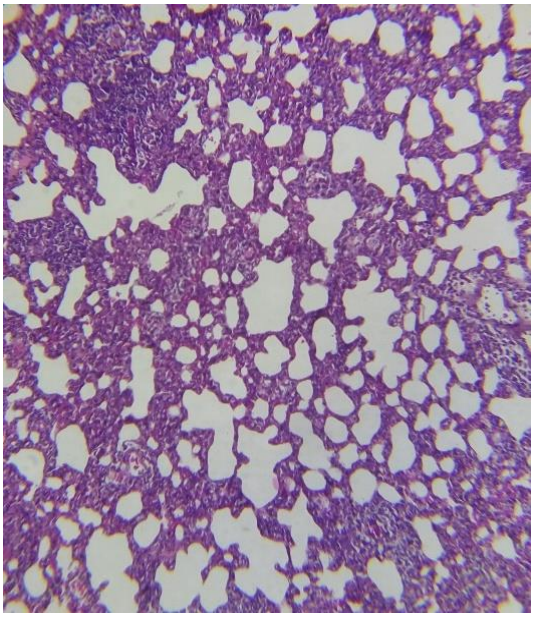


High Power Magnification 40X

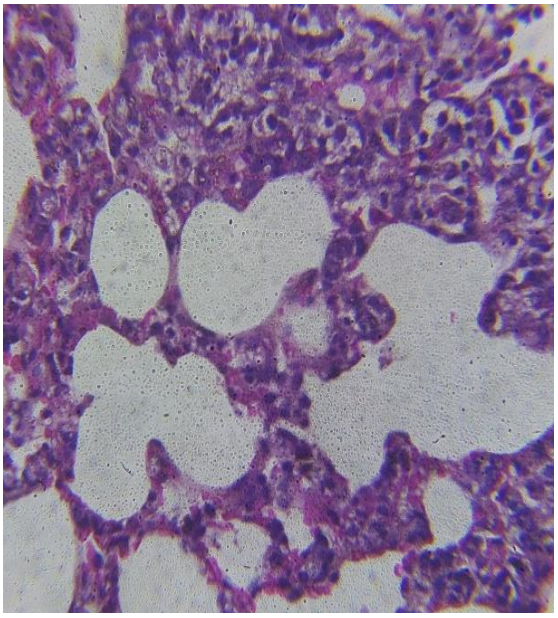


CONTROL - FEMALE

Low Power Magnification 10X



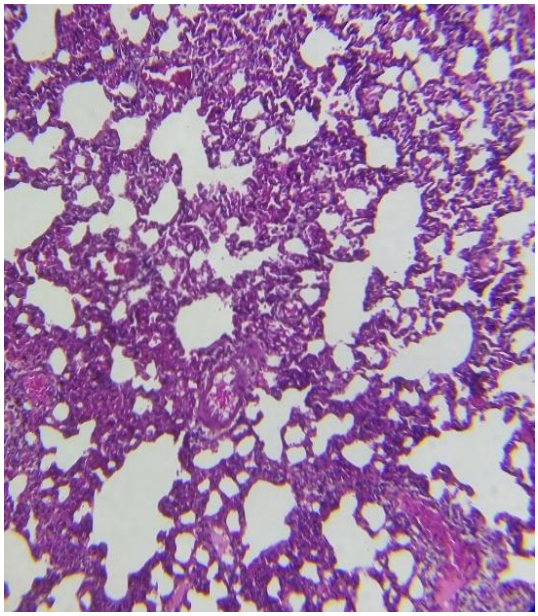
High Power Magnification 40X



HISTOPATHOLOGY OF LUNG

HIGH DOSE - MALE

Low Power Magnification 10X



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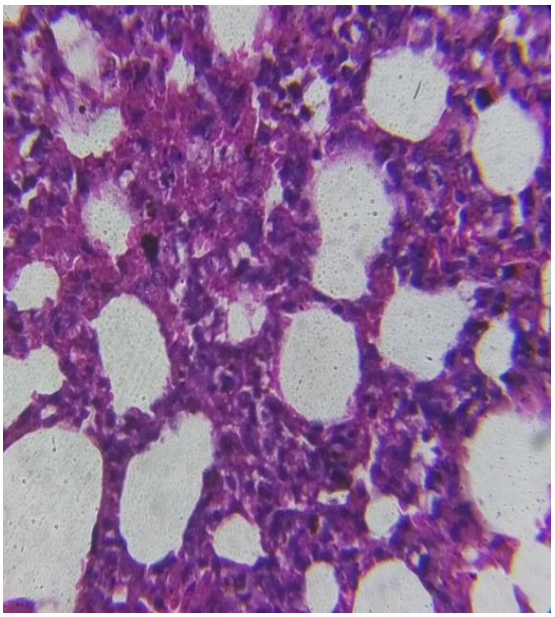
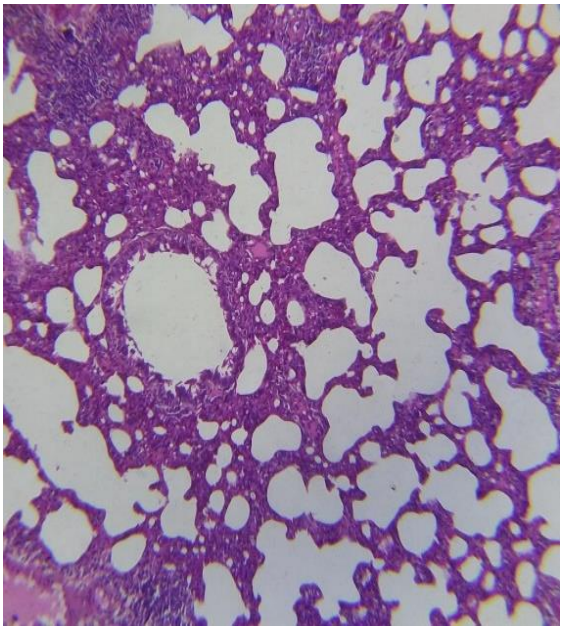
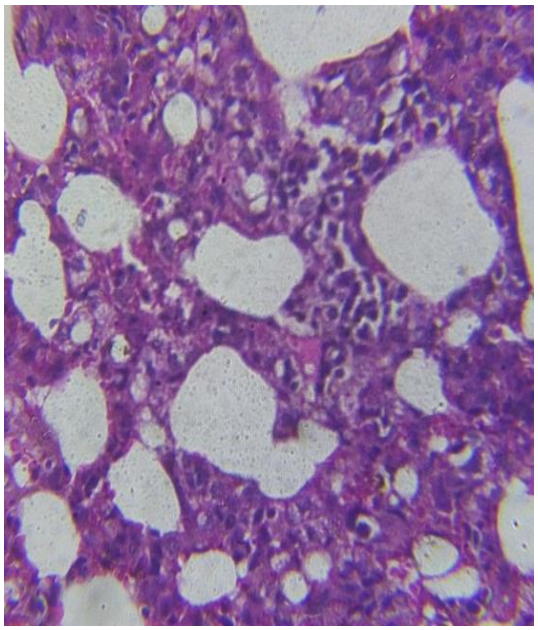


HIGH DOSE -FEMALE

Low Power Magnification 10X



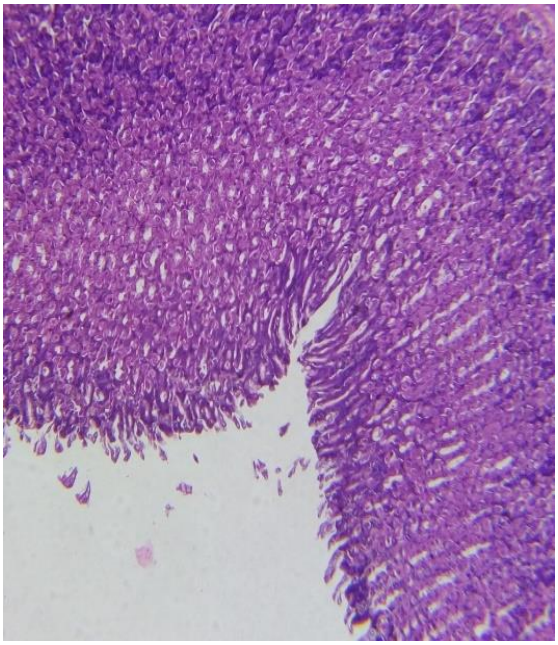
High Power Magnification 40X



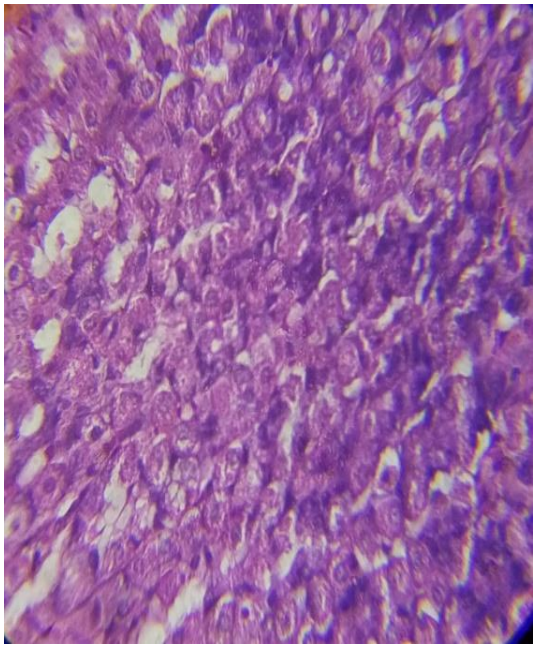
HISTOPATHOLOGY OF STOMACH

CONTROL - MALE

Low Power Magnification 10X

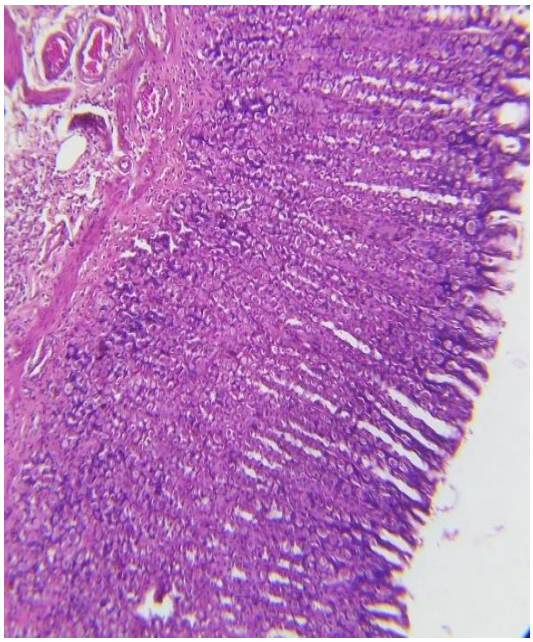


High Power Magnification 40X



CONTROL -FEMALE

Low Power Magnification 10X



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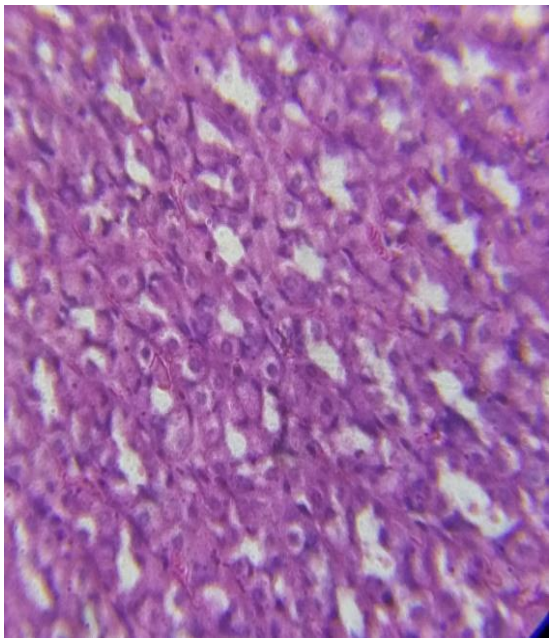
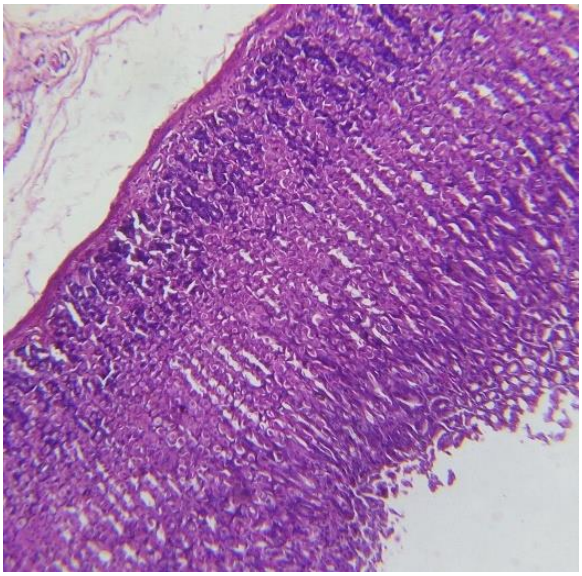


HISTOPATHOLOGY OF STOMACH

HIGH DOSE – MALE

Low Power Magnification 10X



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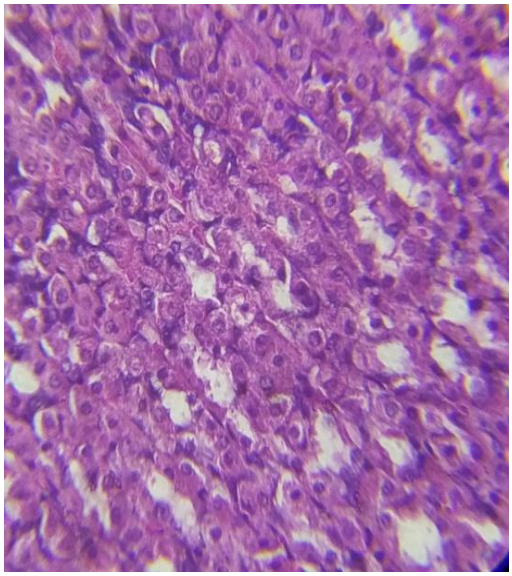
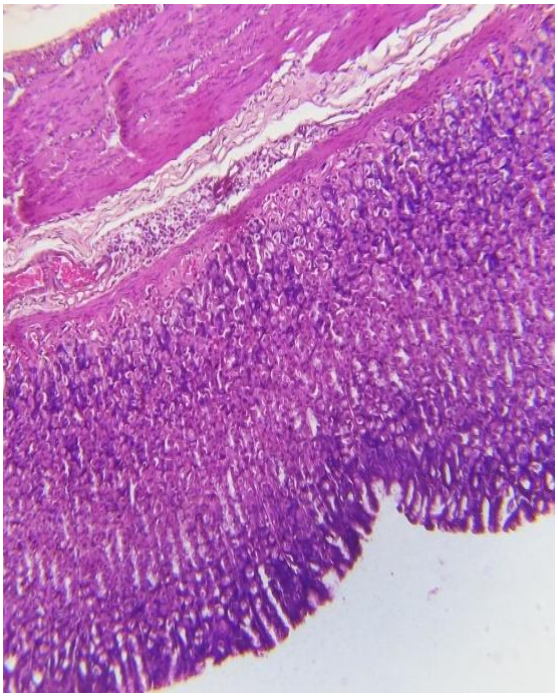


HIGH DOSE – FEMALE

Low Power Magnification 10X



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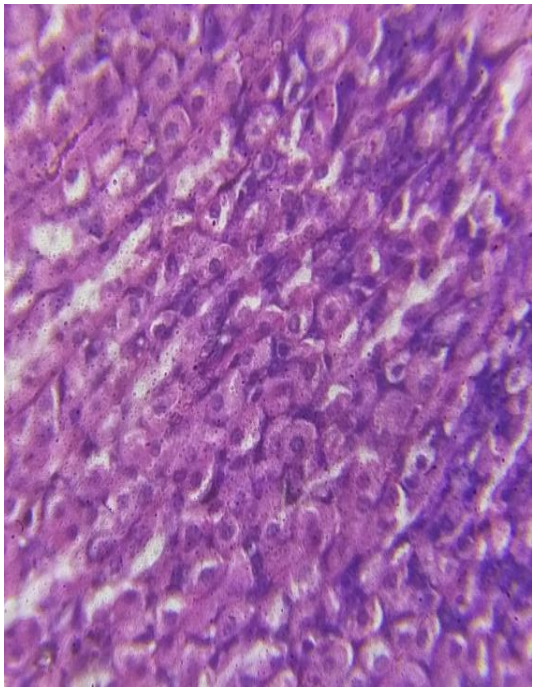
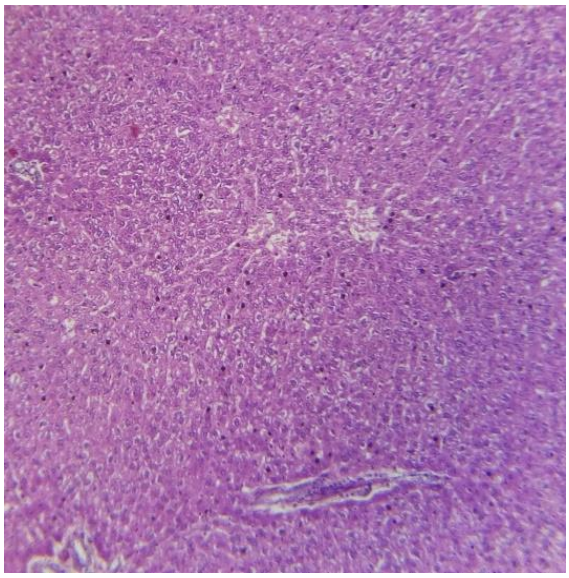


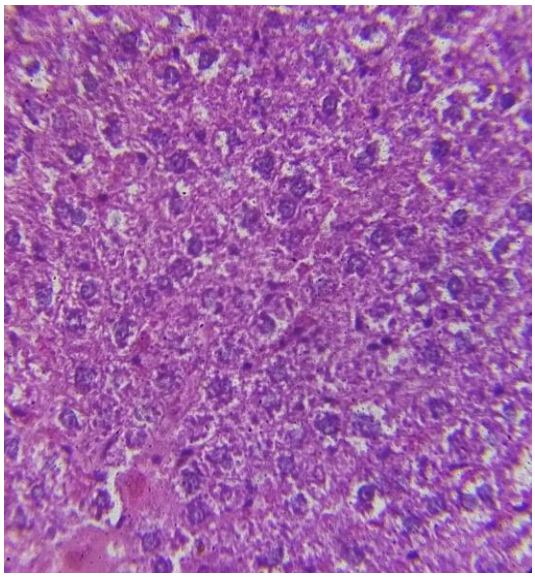
HISTOPATHOLOGY OF LIVER

CONTROL - MALE

Low Power Magnification 10X

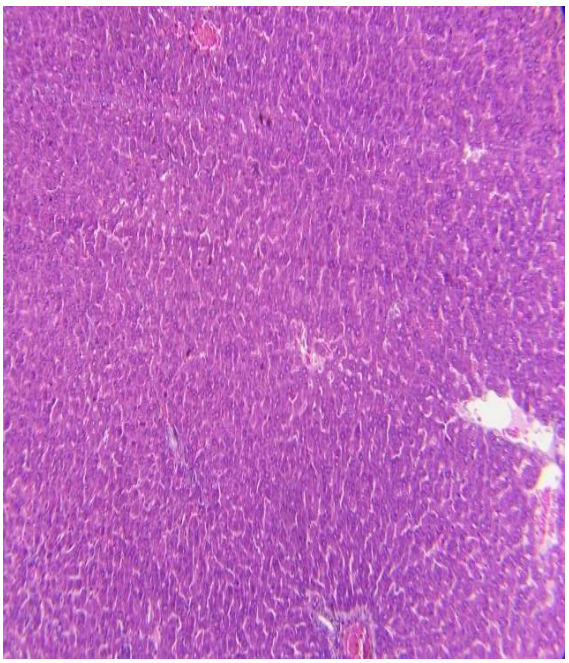


High Power Magnification 40X



CONTROL - FEMALE

Low Power Magnification 10X



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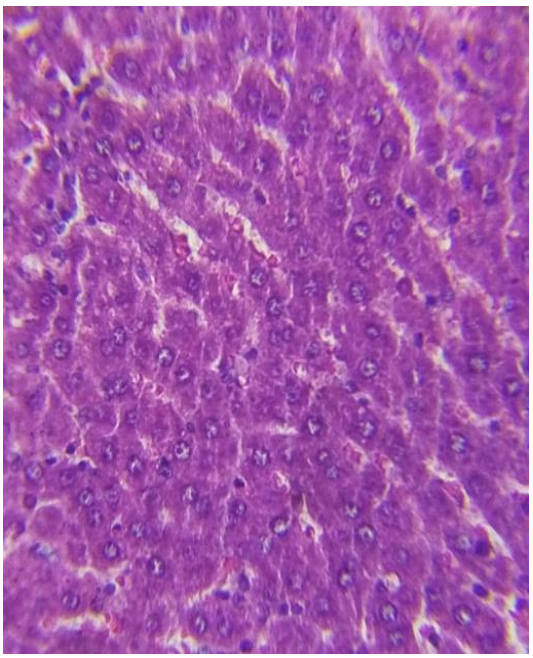
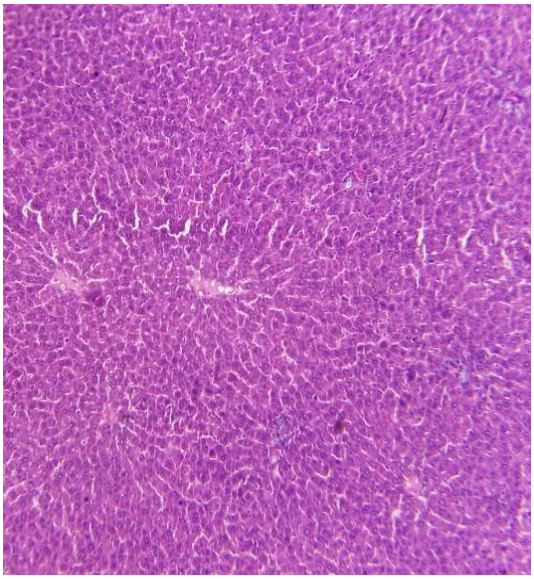


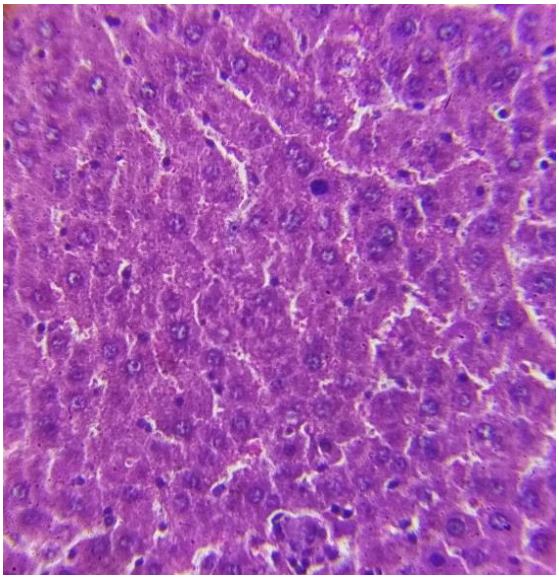
HISTOPATHOLOGY OF LIVER

HIGH DOSE - MALE

Low Power Magnification 10X

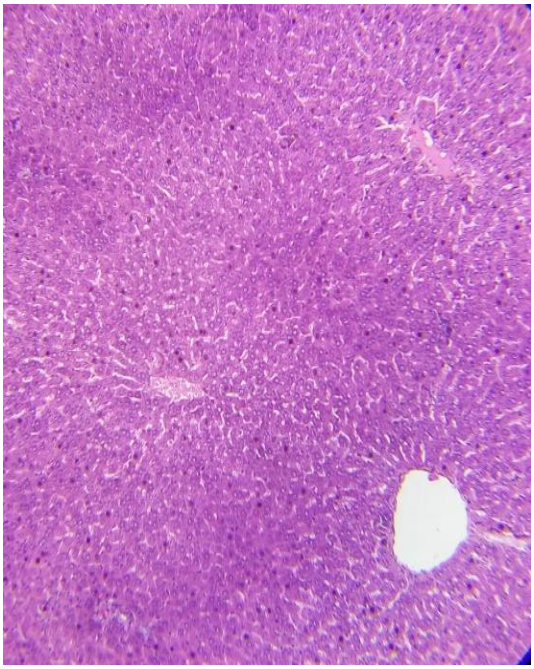


High Power Magnification 40X



HIGH DOSE - FEMALE

Low Power Magnification 10X



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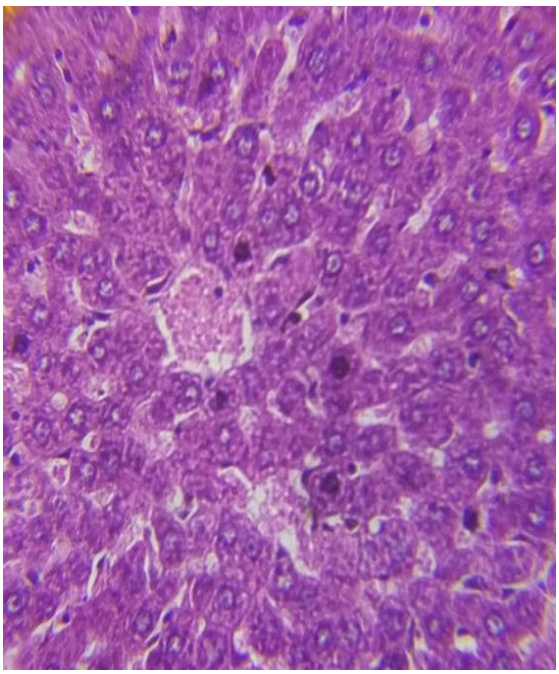
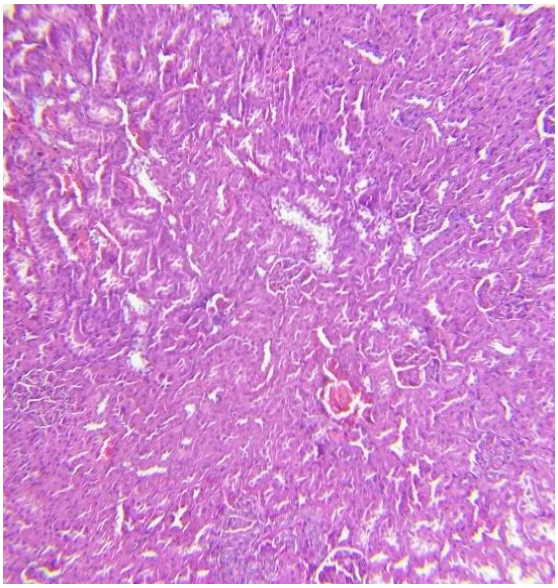


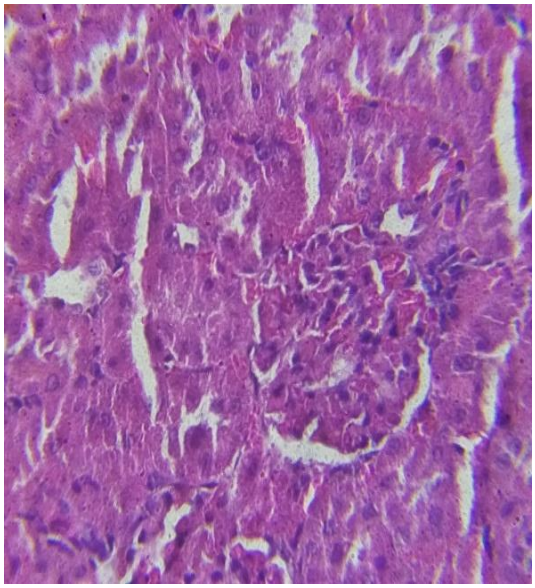
HISTOPATHOLOGY OF KIDNEY

CONTROL - MALE

Low Power Magnification 10X

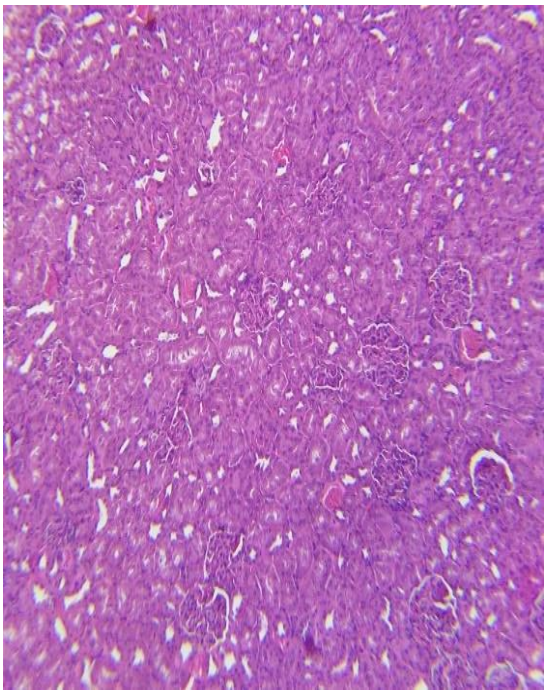


High Power Magnification 40X



CONTROL - FEMALE

Low Power Magnification 10X



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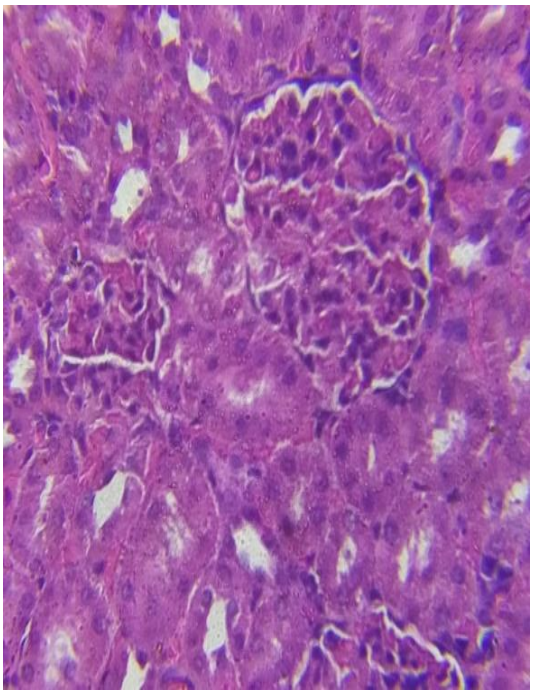
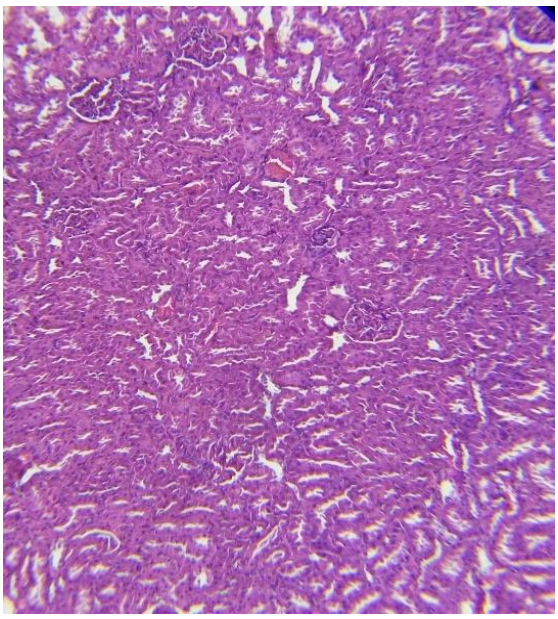


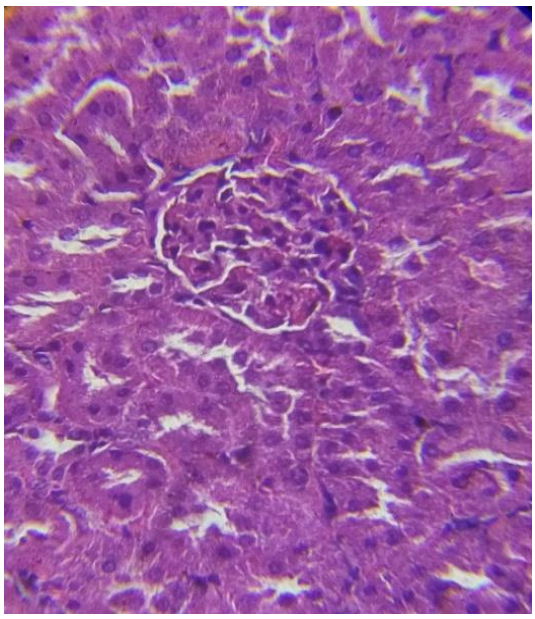
HISTOPATHOLOGY OF KIDNEY

HIGH DOSE - MALE

Low Power Magnification 10X

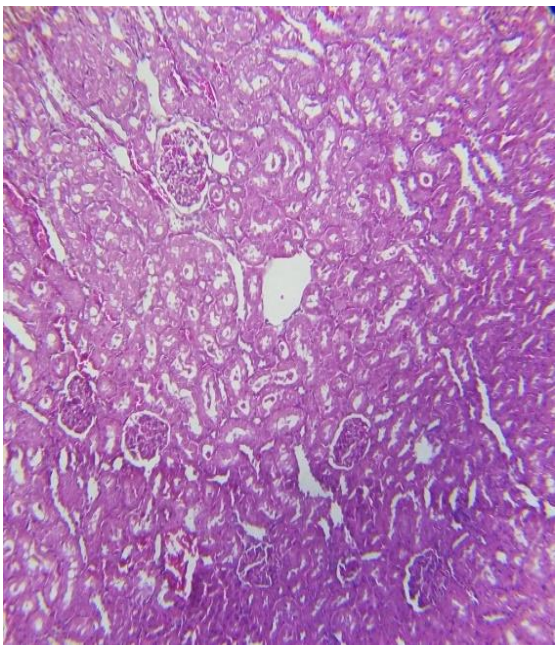


High Power Magnification 40X

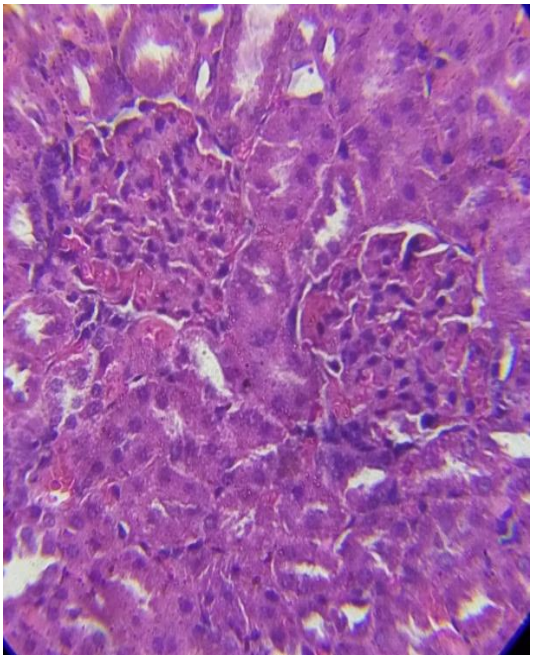


HIGH DOSE - FEMALE

Low Power Magnification 10X



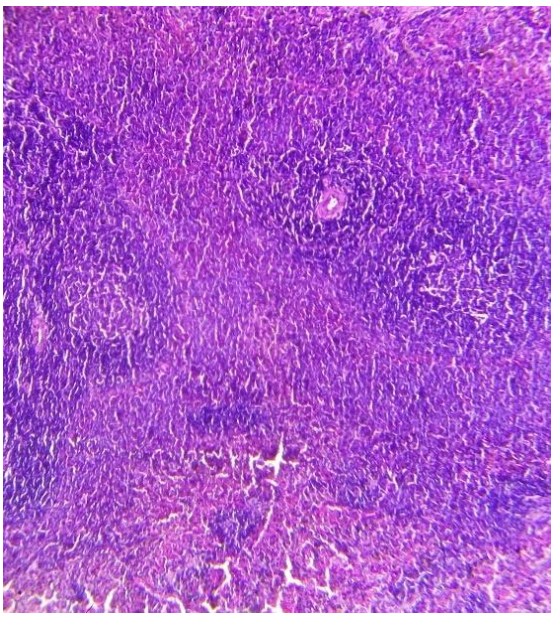
High Power Magnification 40X



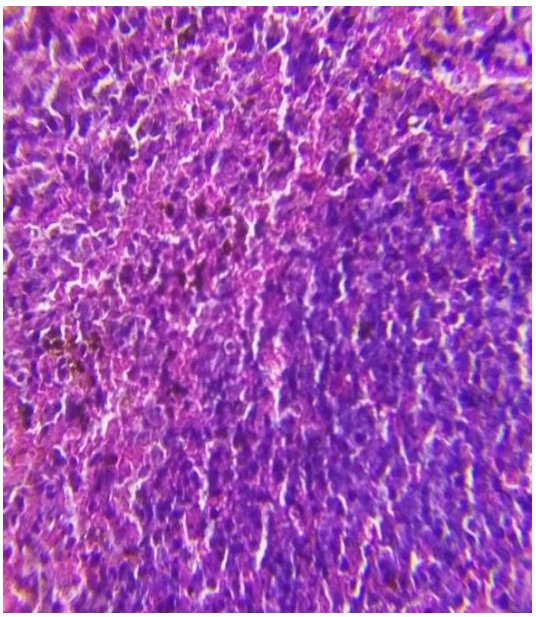
HISTOPATHOLOGY OF SPLEEN

CONTROL - MALE

Low Power Magnification 10X

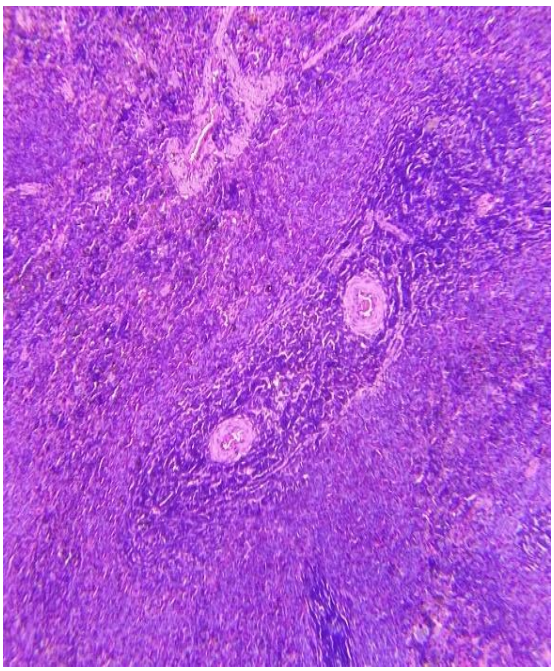


High Power Magnification 40X



CONTROL - FEMALE

Low Power Magnification 10X



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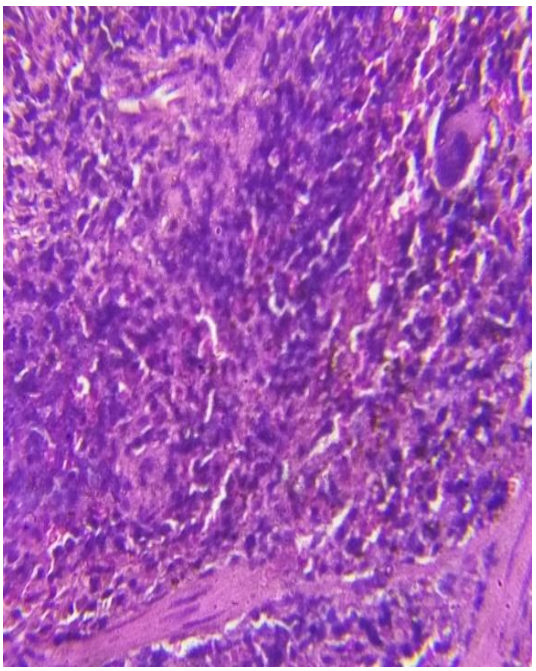
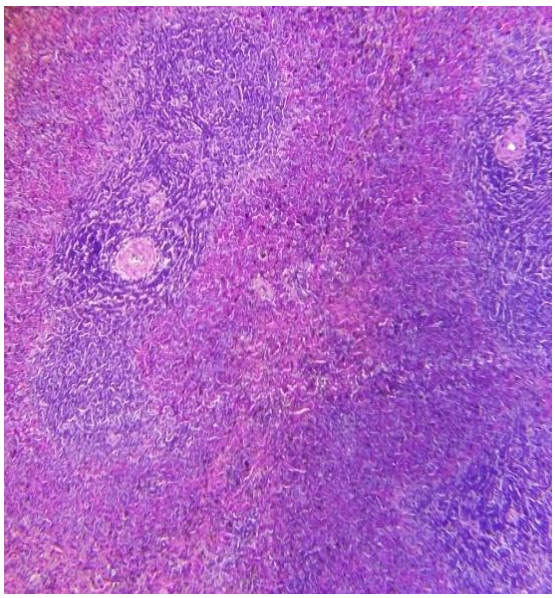


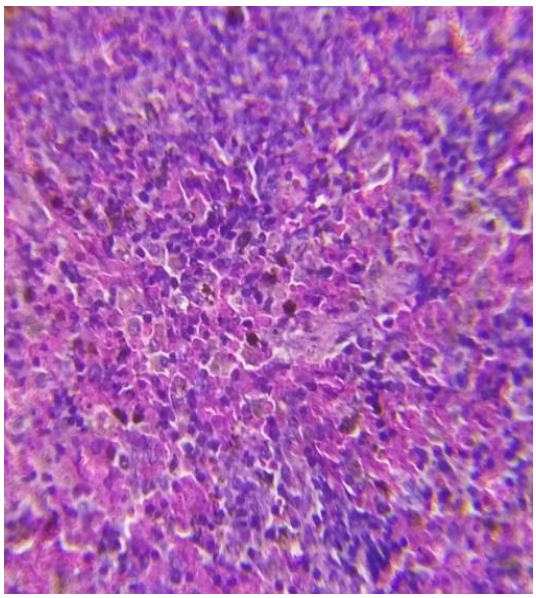
HISTOPATHOLOGY OF SPLEEN

HIGH DOSE - MALE

Low Power Magnification 10X

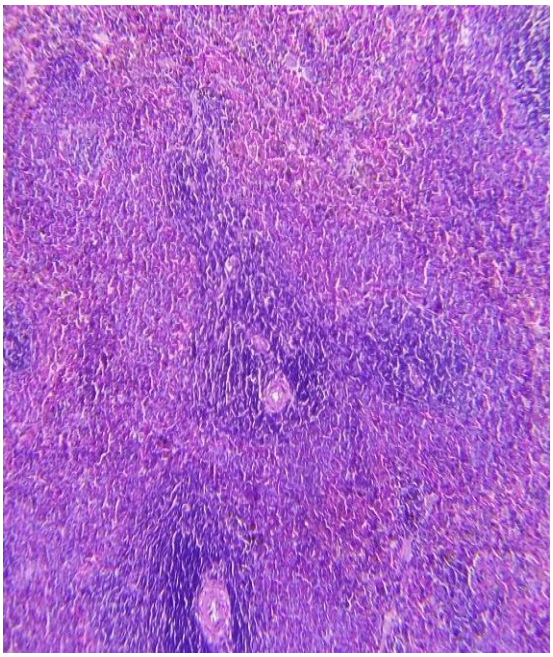


High Power Magnification 40X

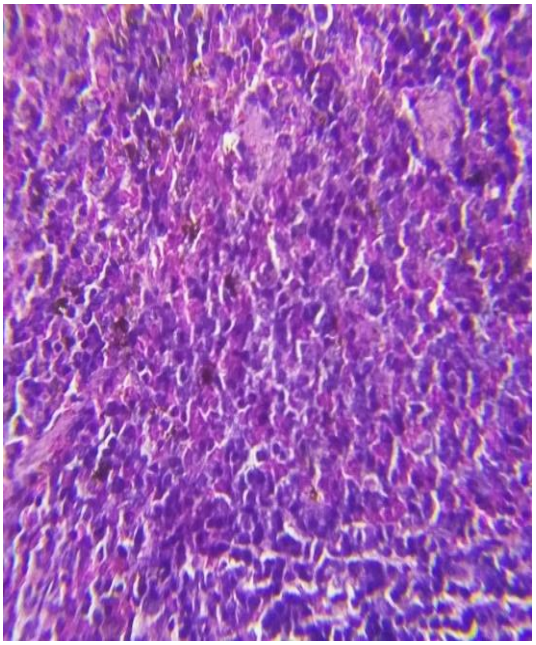


HIGH DOSE - FEMALE

Low Power Magnification 10X



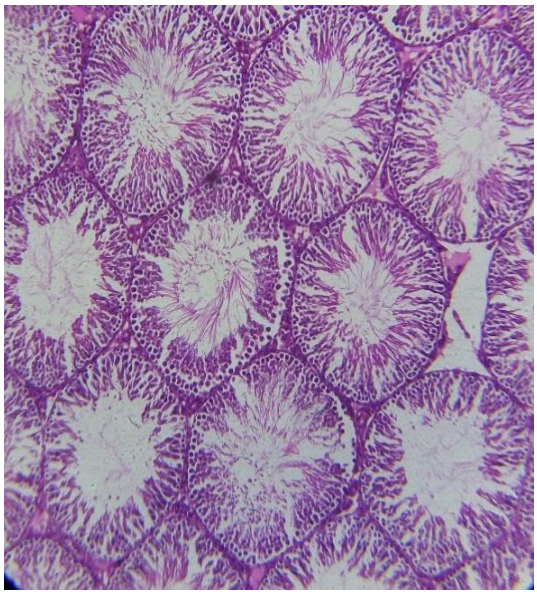
High Power Magnification 40X



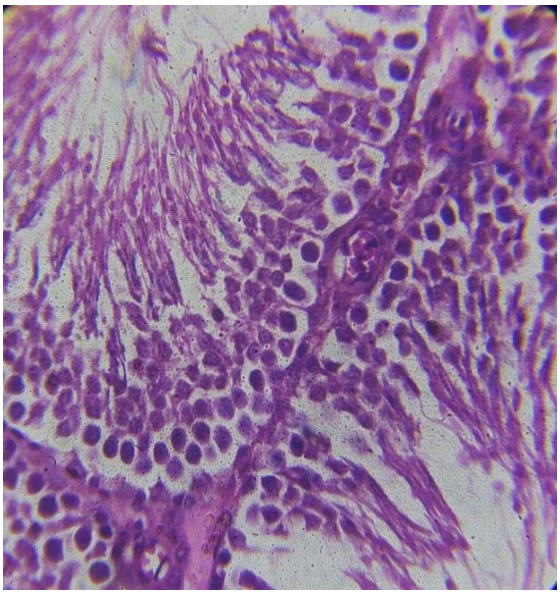
HISTOPATHOLOGY OF TESTIS

CONTROL - MALE

Low Power Magnification 10X

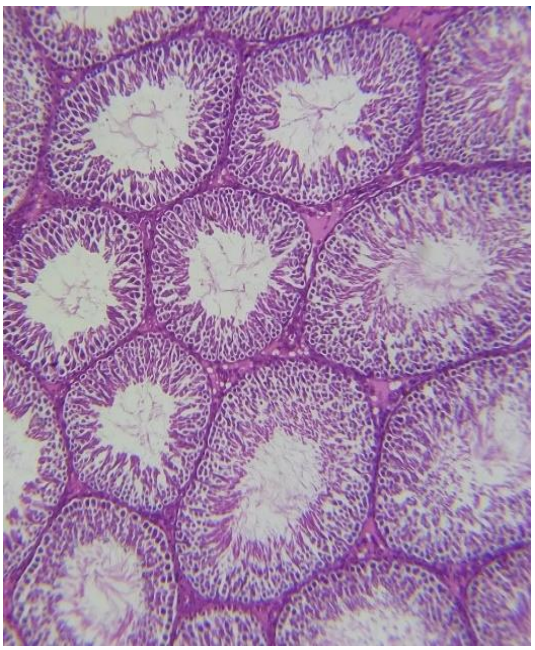


High Power Magnification 40X

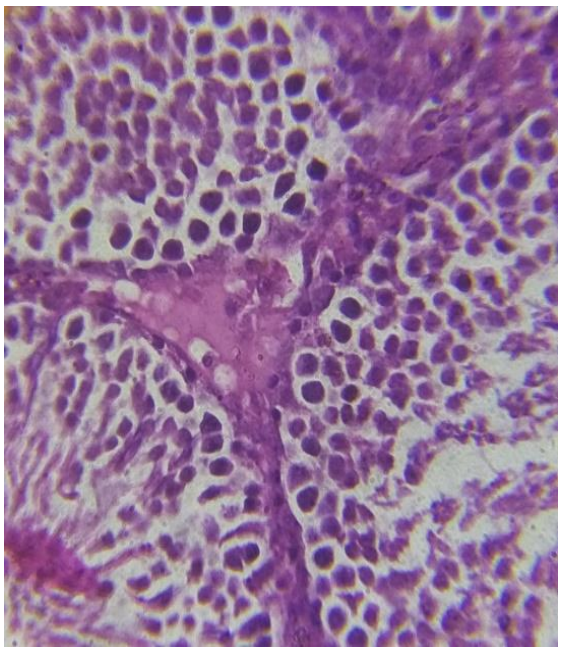


HIGH DOSE -MALE

Low Power Magnification 10X



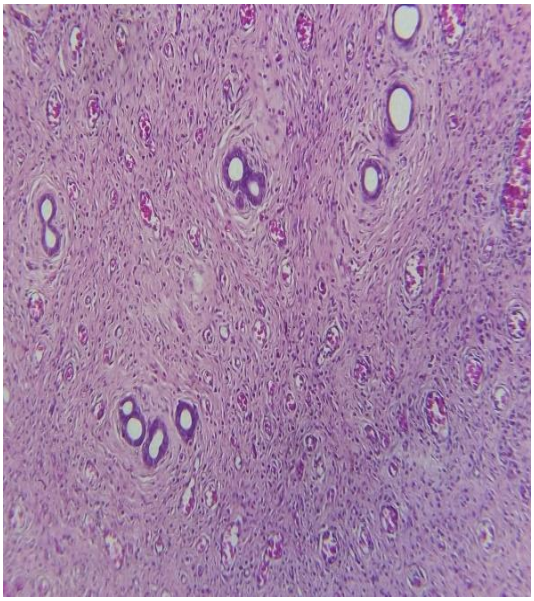
High Power Magnification 40X



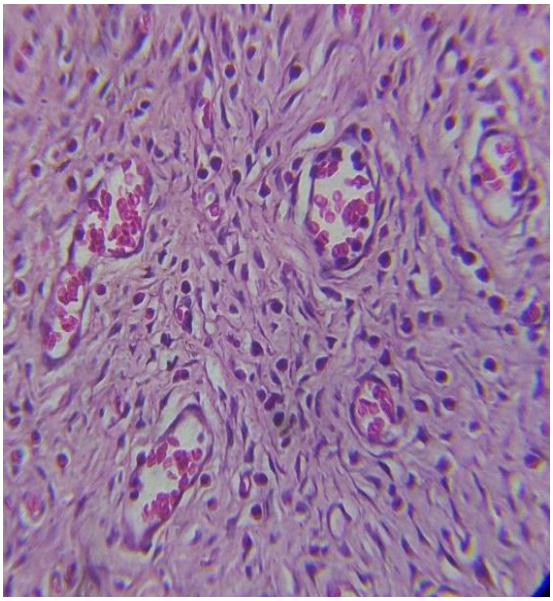
HISTOPATHOLOGY OF UTERUS

CONTROL - FEMALE

Low Power Magnification 10X

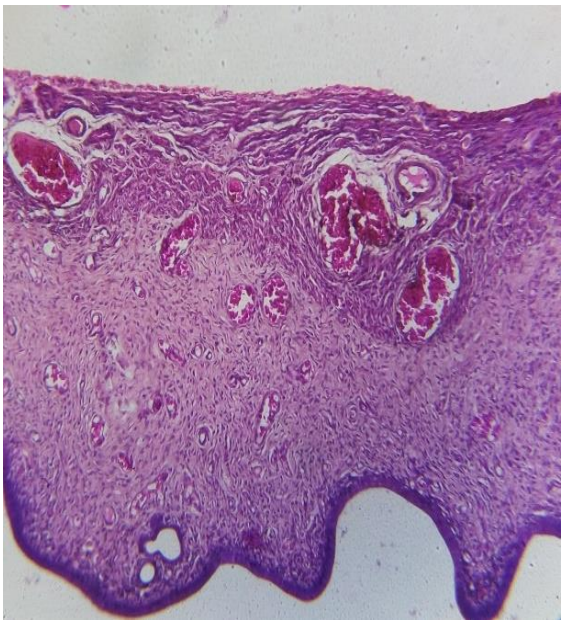


High Power Magnification 40X

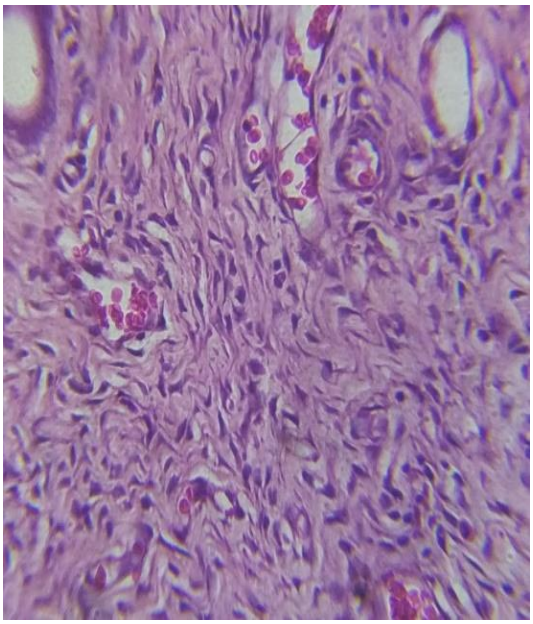


HIGH DOSE - FEMALE

Low Power Magnification 10X



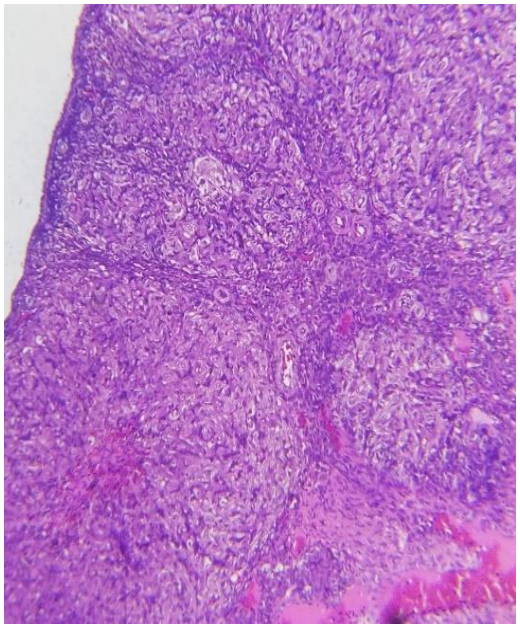
High Power Magnification 40X



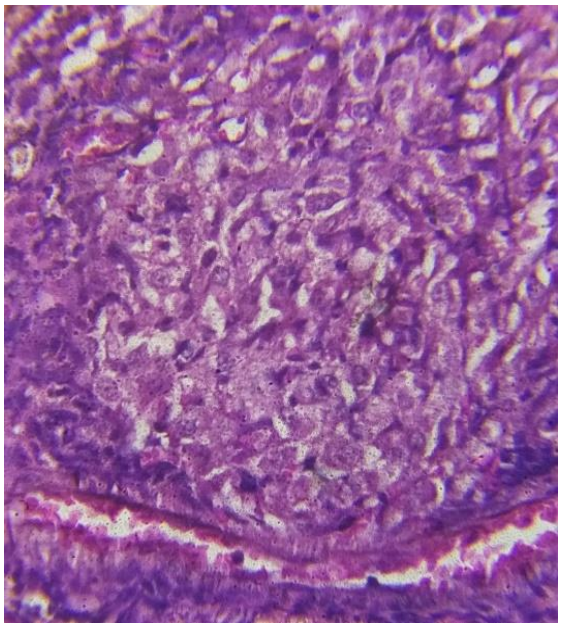
HISTOPATHOLOGY OF OVARY

CONTROL - FEMALE

Low Power Magnification 10X

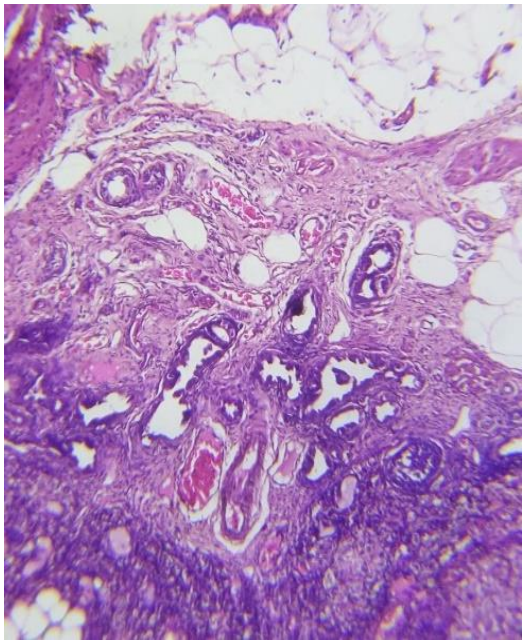


High Power Magnification 40X



HIGH DOSE - FEMALE

Low Power Magnification 10X



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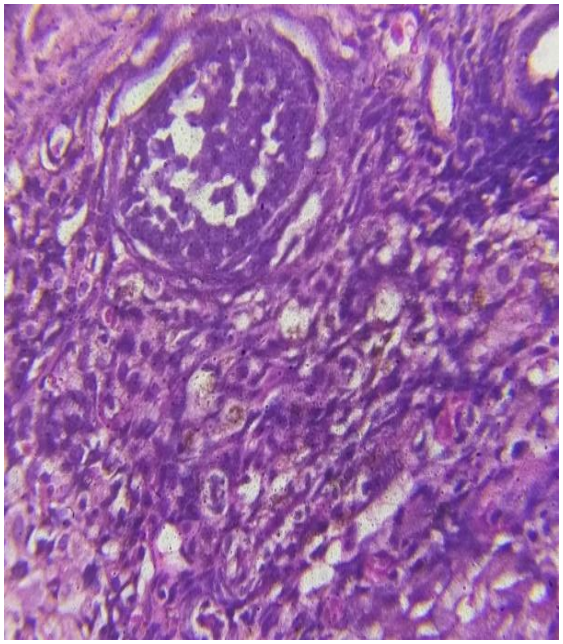

Table 30: **Histopathology Results of long term oral toxicity study of *Kottai Karanthai Chooranam***

MALE:

ORGAN	GROUP	RESULT
BRAIN	Control	Shows normal histology of striatum.
	High dose	Cerebral region shows regular and promising histology of neuronal populations
HEART	Control	Normal appearance of myocytes
	High dose	Showed normal appearance of heart fibres without any histological alterations.
LUNGS	Control	Inter alveoli septum and bronchioles appears normal
	High dose	Showing normal alveoli and collagen fibres
STOMACH	Control	Normal surface epithelium, mucosa and sub-mucosa
	High dose	Normal gastric glands and gastric pits
LIVER	Control	Normal liver histology with No signs of nodular degeneration and cirrhosis
	High dose	Liver parenchyma appears normal with no evidence of necrosis
KIDNEY	Control	Proximal and distal convolutes tubules was normal with no evidence of atrophy
	High dose	Normal glomeruli with no evidence of lymphocytic infiltrate and inflammation
SPLEEN	Control	Erythropoietic cells (EP) are scattered throughout the red pulp
	High dose	Morphology of capsule, nodes, red and white pulp appears normal
TESTIS	Control	Histo cytology of testicular tissue shows well differentiated germ cells
	High dose	Normal, intact rounded or oval seminiferous tubules.

Table 31: **Histopathology Results of long term oral toxicity study of *Kottai Karanthai Chooranam***
FEMALE:

ORGAN	GROUP	RESULT
BRAIN	Control	The cerebral sections showed normal architecture in both cortex and medulla.
	High dose	Cerebral region shows regular and promising histology of neuronal populations.
HEART	Control	Fibres appears normal elongated and rod shaped.
	High dose	Showed normal appearance of heart fibres without any histological alterations.
LUNG	Control	Alveolar epithelium and capillaries appears normal
	High dose	Showing normal alveoli and collagen fibres
STOMACH	Control	Gastric epithelium and mucosa appears normal
	High dose	Normal gastric glands and gastric pits
LIVER	Control	Normal, homogenous, intact hepatic parenchyma; hepatic lobules, with normal central vein
	High dose	Liver parenchyma appears normal with no evidence of necrosis
KIDNEY	Control	Appearance of proximal and distal convolutes tubules was normal
	High dose	Normal appearance of proximal Convoluted Tubule (PCT), Distal Convoluted Tubule (DCT) and Collecting Duct.
SPLEEN	Control	Regular appearance of red pulp
	High dose	Morphology of capsule, nodes, red and white pulp appears normal.
Ovary	Control	Follicular cells, cytoplasm and nucleus appears normal
	High dose	Appearance of antral follicle, primary oocyte and secondary follicles are normal
Uterus	Control	Regular histology of uterine epithelium and endometrial glands.
	High dose	Arrangement of stratum basal, functional and surface epithelium seems normal

6.DISCUSSION

Kottai Karanthai Chooranam is used for Karappan (Eczema), Sori (Pruritis), Sirangu (Scabies), Thozh pinigal (Skin disease), Maripatta earu Kazhiyum (Constipation), Vali (Arthritis), Veri (Psychiatric disorder), Sinaippu (Erythema). First to evaluate the safety profile of the test drug Kottai Karanthai Chooranam. The present study with an objective of finding whether this drug has got any adverse effect in long administration or not. Kottai Karanthai Chooranam is a single drug preparation medicine. Literature review of Kootai Karanthai was collected in Siddha aspects and evaluated pharmacological actions. This literature review support uses of Kottai Karanthai Chooranam as per siddha literature. There was no quality and safety assessment of Kottai Karanthai Chooranam studied. In this present study, quality and safety of Kottai Karanthai Chooranam has been confirmed through necessary quality parameters analysis and Acute & long- term Toxicity studies as per WHO guidelines.

Qualitative assessment and safety profile of Kottai Karanthai Chooranam has been confirmed through Organoleptic Characters, Physico-chemical analysis, Phyto-chemical analysis, Bio-chemical analysis, Instrumental analysis -HPTLC, Pesticide residue, Aflotoxin, Heavy Metal Analysis, Specific pathogen, Acute and Long-term toxicity studies.

Organoleptic characters of on Kottai Karanthai Chooranam revealed that the drug appears solid state, greenish brown in colour, nature of the powder is moderately fine and strong characteristic odour (Table no:9) (Fig no: 3).

Physicochemical analysis of the Kottai Karanthai Chooranam was done as per standard procedure (Table No: 10).

The loss on drying test is used to determine the amount of volatile matter (i.e water drying off from the drug). Moisture is one of the major factors responsible for deterioration of the drugs.The percentage of loss on drying of Kottai Karanthai Chooranam was 4.033 ±2.801 % (Normal range 1 - 20 %). Low moisture content is always desirable for higher stability of drugs. As per the results the loss on drying of Kottai Karanthai Chooranam is low, so the stability of the Kottai Karanthai Chooranam is higher.

The Ash limit Tests are designed to measure the amount of the residual. A high ash value is indicative of contamination, substitution, adulteration or carelessness in preparing the drug[70].Acid insoluble ash use to detect the contamination from sand or soil[70]**.** The total Ash content and Acid Insoluble Ash values of Kottai Karanthai Chooranam were 2.867 ± 0.4509 % (Normal range: 1-25%) and 0.3933 ± 0.2501 (Normal range: 0.1 – 10%). The total ash value of Kottai Karanthai Chooranam is low, it implies that the inorganic constituents are low and indicates the purity of the drug.

Extractive value determined the amount of active constituents in a given amount of medicinal plant material when extracted with solvent. In that particular solvent depends upon the nature of the drug and solvent used. The determination of water soluble and alcohol soluble extractives is used as a means of evaluating crude drug[70]. The extract values of Alcohol in Kottai Karanthai Chooranam is 20.33 ± 4.869 % and water is 9.33 ± 0.1153%.

The Biochemical analysis reveals that kottai Karanthai Chooranam contains Sulphate, Phosphate, Carbonate, Chloride, Sulphide, Aluminium, Calcium, Iron, Sodium, Potaasium, Zinc, Ammonium, Starch, Reducing sugar, Alkaloid, Tannic acid showed in Table no: 11, 12, 13.

Sulphate, comprised of the elements sulphur and oxygen is the fourth most abundant anion in our blood. A critical component of extracellular matrix proteins, it aids in the detoxification of drugs, food additives, and toxic metals. It also prevents blood from coagulating during transit through capillaries[71].

Phosphate is one of the body's electrolytes, which are minerals that carry an electric charge when dissolved in body fluids such as blood. Bone contains about 85 % of the body's phosphate[72].

Chloride is an essential electrolyte located in all body fluids responsible for maintaining acid/base balance, transmitting nerve impulse and regulating fluid in and out of cell [73].

Zinc is an essential trace element for plant growth and also plays an important role in various cell processes and also zinc is important for the production of insulin hormone and carbonic anhydrase in the body. Zinc helps in regulating immune function, sperm production, and fetus development. [74].

Ammonia plays an important role in protein synthesis in the human body and also maintain the body's PH balance[75].Aluminium providing a safe barrier to bacteria and contamination in food preservation. Aluminium compound increases the effects in vaccines and medicine [76].

The calcium maintain strong bones and decrese the risk of cardiovascular disease and stroke. It helps during pregnancy reduce the risk of pre eclampsia[77]. Iron preserve many vital function in the body, including general energy, focus, immune system [78].

Phytochemical investigation (Table no:14) (Figure no:4,5,6,7) to detect the presence of various phyto constituents in formulation Kottai Karanthai Chooranam showed the presence of Phenol, Tanin, Saponin. Presence of the above components in biochemical and phytochemical analysis increases the therapeutic value of Kottai Karanthai Chooranam.

saponins act as adjuvant and it can be added to vaccines (e.g. foot-and-mouth disease vaccines) and help to improve immune response. Among the different biological effects of saponins are antibacterial and antiprotozoal and anticancer activities[74].

Tannins antimicrobial activities of tannins are well documented. The growth of many fungi, yeasts, bacteria, and viruses was inhibited by tannins. Tannins have also been reported to exert other physiological effects, such as to accelerate blood clotting, reduce blood pressure, decrease the serum lipid level, produce liver necrosis, and modulate immunoresponses.

The HPTLC analysis (Table no: 15) (Figure no:8,9) of the sample showed that presence of presence of seven prominent peaks corresponds to presence of seven versatile phytocomponents present in which peak 4 occupies the major percentage of area of 28.83 which denotes the abundant existence of such compound.

Pesticide analysis of Kottai Karanthai chooranam (Table no:16) showed there was no presence of pesticides residues such as Organo chlorine, Organophosporus and pyrethroids in Kottaikaranthai chooranam. Aflatoxin analysis of Kottai Karanthai chooranam (Table no: 15) reveals, Kottai Karanthai Chooranam were free from Aflatoxin B1, B2 and Aflatoxin G1, G2.

Heavy metal analysis through ICP-OES results (Table-18) showed that the Heavy metals like lead, mercury, nickel, copper, arsenic and cadmium were found in below detection level. Hence it may be safe for human consumption. It also shows the presence of physiologically important minerals like Calcium, Copper, Iron, Potassium, Manganese, Sodium, Phosphorus and Zinc which are within ppm limit.

In specific pathogen of Kottai Karanthai chooranam (Table no:19) showed there was no growth / colonies such as E-coli, Salmonella, Staphylococcus Aureus, Pseudomonas Aeruginosa were observed in any of the plates. It denotes quality and purity of Kottai Karanthai Chooranam.

In microbial contamination test of Kottai Karanthai chooranam (Table no:20) showed there was no bacterial & fungal growth / colonies were observed in any of the plates inoculates with the test sample.

WHO has need for quality assurance of herbal products including testing of microbial contamination, aflatoxin, pesticide residue, were studied in Kottai Karanthai Chooranam showed below detection limit of aflatoxin and all pesticide. From the results we concluded that the below detection limit of microbial contamination, aflatoxin, pesticide residue indicated the quality of the ingredients of Kottai Karanthai Chooranam.

In Acute toxicity study (Table no: 21) carried out as per WHO guideline. The study result showed there was no treatment- related behavioural changes and death or signs of toxicity developed in test drug treated rats at dosage levels of 2000mg/kg body weight throughout the study period. And the body weight, feed intake and water intake of the animal is normal. Further, at the end of the study no gross pathological changes were seen in all the internal organs of both control and test drug treated groups.

To ensure the safety of ***Kottai Karanthai Chooranam***, Long term toxicity Study was also carried out as per WHO guideline. Totally 4 groups used in this study. Each group contain 10 Male and 10 Female. Group I set as control received water. 3 groups of II, III, IV Group received 3 doses Low dose (360 mg/kg b.wt) ,mid dose 720 mg/kg b.wt, high dose (1440 mg/kg b.wt) of ***Kottai Karanthai Chooranam*** . Animal were observed through out the period. During study period there was no behavioural changes and no signs of toxicity were observed.

Body weight of male animal of test drug treated group & control group was gradually increased and there was no significant changes observed in body weight between test drug treated group and control group (Table no: 22 & Figure no: 11). Body weight of Female animal of test drug treated group & control group was gradually increased and there was no significant changes observed in body weight between test drug treated group and control group (Table no: 23& Figure no:12). The feed and water intake of all treated animals were gradually increased during the study period and not significant to compared with control group (Table no:24,25 & Figure no:13,14).

Haematological parameters results suggest, that there was no significant changes in complete blood count. (Table No: 26) when compared with control group. Bio - chemical parameters also no significant changes when compared with control group. The results of bio chemical parameters suggest there is no alteration in the serum components (Table no: 27,28,29).

At the end of the study period all the animals sacrificed and all the vital organs and cavities were observed. There were no gross pathological changes noted. The histopathology of Brain, Heart, Lungs, Liver, Kidney, Stomach, Testis, Uterus and Ovary were done in control, High dose treatment group of Kottai Karanthai Chooranam. The histopathology results(Table no:30,31 & figure no: 27) revealed all the studied organs are normal. Finally the acute and long-term toxicity study results suggest the No Observed Adverse Effect Level (NOAEL) is up to the high dose level(1440 mh/kg b.wt), which is ten times of that therapeutic dose.

7.SUMMARY

The experimental formulation Kottai Karanthai Chooranam has been choosen for my dissertation work based on Gunapaadam muthalpaagam porut panbunool. Kottai Karanthai Chooranam prepared with the purified ingredient. It has been mentioned for Karappan (Eczema), Sori (Pruritis), Sirangu (Scabies), Thozh pinigal (Skin disease), Maripatta earu Kazhiyum (Constipation), Vali (Arthritis), Veri (Psychiatric disorder), Sinaippu (Erythema).

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 guidelines. The ingredient was collected from Annavasal, Pudukkottai Dt. The drug were identified and authenticated by Assistant Professor of Medicinal Botany, National Institute of Siddha, Chennai-47. The ingredient have been purified as per Siddha literature and formulation was prepared in Gunapadam Lab of National Institute of siddha, Chennai -47.

Kottai Karanthai Chooranam was analysed quantitively and qualitatively with Organoleptic character, Physico-chemical, Biochemical, Pesticide Residue, Aflatoxin to evaluate safety by acute and long term toxicity studies.

Initially the drug were subjected to physico-chemical analysis. It reveals the increase in bioavailability and purity of the drug. Then the sample was analysed for chemical constituents. It showed the presence of sulphate, phosphate, carbonate, Chloride, Sulphide, Zinc, Sodium, Potassium, Aluminium, ammonium, iron, reducing sugar, alkaloid, tannic acid.

Phyto-chemical investigation to detect the presence of various phyto constituents in formulation Kottai Karanthai Chooranam showed the presence of Phenol, Tanin, Saponin. Presence of the above components in biochemical and phytochemical analysis increases the therapeutic value of Kottai Karanthai Chooranam.

The HPTLC analysis of the sample reveals that presence of seven prominent peaks corresponds to presence of seven versatile phytocomponents present with in it. Rf

value of the peaks ranges from 0.09 to 0.82. Further the peak 4 occupies the major percentage of area of 28.83 which denotes the abundant existence of such compound.

Pesticide analysis of Kottai Karanthai Chooranam showed there was no presence of pesticides residues such as Organo chlorine, Organophosporus and pyrethroids in Kottaikaranthai chooranam. Aflatoxin analysis of Kottai Karanthai Chooranam reveals, Kottai Karanthai Chooranam were free from Aflatoxin B1, B2 and Aflatoxin G1, G2.

Heavy metal analysis through ICP-OES results showed that the Heavy metals like lead, mercury, nickel, copper, arsenic and cadmium were found in below detection level. Hence it may be safe for human consumption. It also shows the presence of physiologically important minerals like Calcium, Copper, Iron, Potassium, Manganese, Sodium, Phosphorus and Zinc which are within ppm limit.

In specific pathogen Kottai Karanthai chooranam showed there was no growth / colonies such as E-coli, Salmonella, Staphylococcus Aureus, Pseudomonas Aeruginosa were observed in any of the plates. It denotes quality and purity of Kottai Karanthai Chooranam. In microbial contamination test of Kottai Karanthai chooranam (Table no:20) showed there was no bacterial & fungal growth / colonies were observed in any of the plates inoculates with the test sample

In Acute toxicity study (Table no:16) carried out as per WHO guideline. The study result showed there was no treatment- related behavioural changes and death or signs of toxicity developed in test drug treated rats at dosage levels of 2000mg/kg body weight throughout the study period. And the body weight, feed intake and water intake of the animal is normal. Further, at the end of the study no gross pathological changes were seen in all the internal organs of both control and test drug treated groups.

Long term toxicity study of kottai Karanthai Chooranam showed did not produced any behavioural changes, mortality and morbidity with in the study period. The body weight of the all the animals were gradually increased during the study period and not significant to compared with control group. The feed and water intake of all treated animals were gradually increased during the study period and not significant to compared with control group.

Haematological parameters of all the test animals were normal when compared with control group. Serum analysis also with in normal level when compared with control group.

Histopathology examination of test drug treated animals results revealed Kottai Karanthai Chooranam did not induce any lesions of toxicological significance in all vital organs examined under the experimental conditions.

This acute and long-term toxicity study results suggest the No Observed Adverse Effect Level (NOAEL) is up to the high dose level (1440 mh/kg b.wt), which is ten times of that therapeutic dose.

8.CONCLUSION

Qualitative analysis and Safety profile of Kottai Karanthai Chooranam was studied. The results of qualitative parameter analysis revealed quality and purity of drug. The results obtained through qualitative analysis of Kottai Karanthai Chooranam used as the standard for future research. Safety of Kottai Karanthai Chooranam was studied as per WHO guideline. In acute toxicity study results reveals that there was no mortality and signs of toxicity observed for acute oral administration of Kottai Karanthai Chooranam till the dose of ten times the therapeutic dose of 2000mg/kg b.wt in mentioned manner. In Long-term toxicity of Kottai Karanthai Chooranam was studied at 3 different dose levels such as, Low dose (360 mg/kg b.wt) ,mid dose 720 mg/kg b.wt, high dose (1440 mg/kg b.wt). This study results showed there was no behavioural changes, mortality and morbidity, no toxicity findings in haematological, biochemical, LFT and RFT in all test animals and no histopathological changes was observed in all the vital organs of control, high dose treated group. As per this study results No Observed Adverse Effect Level (NOAEL) is up to the high dose level (1440 mh/kg b.wt), which is ten times of that therapeutic dose.

Based on these results it can be concluded that the dose level of Kottai Karanthai Chooranam 4 gm for a duration of oru mandalam (48 days) (bd/day) was mentioned in Siddha literature Gunapaadam muthal paagam (Porut panbu nool) is safe dosage for consumption.

In future, efficacy of Kottai Karanthai Chooranam will be carried out to strengthen the clinical use of Kottai Karanthai Chooranam**.**

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10.ANNEXURE

The scanned copy of following certificates to be enclosed in annexure.

1. IAEC approval certificate.

2. Authentication and identification certificate of Ingredients of ***Kottai Karanthai Chooranam.***.

3. Research methodology and biostatistics workshop participant certificate.

4. Laboratory and Animal care and basic research technique workshop participant certificate.

CERTIFICATE

This is certify that the project title Preclinical Safety Evaluation of **"Kottaikaranthai chooranam"** A Siddha formulation **has been approved by the IAEC.** Total No. of animal approved: 90 Rats (M45 + F45). Approval No: NIS/IAEC-VII/2808 2018/17

V. Banumathi

Prof. Dr. V. Banumathi MD(S),
Chairman IAEC,

Prof. Dr. K. Nachimuthu
CPCSEA Nominee

Chairman/Member Secretary of IAEC:

CPCSEA Nominee:

Name of the principle investigator: Dr. S. Sujitha,
Department of Nanjumaruthuvam

Name of the Guide : Dr. S. Murugesan,
Lecturer ,

NATIONAL INSTITUTE OF SIDDHA, CHENNAI – 600047

BOTANICAL CERTIFICATE

Certified that the following plant drug used in the Siddha formulation **"Kottaikaranthai Chooranam"** taken up for Post Graduation Dissertation studies by **Dr.M.Sujitha** 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

Sphaeranthus indicus Linn. (Asteraceae), Whole plant

MEDICINAL BOTANY ★ CHENNAI 600 047 ★ National Institute of Siddha

Certificate No: NISMB3802019

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, INDIA

CERTIFICATE

सत्यमेव जयते
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. M. SUJITHA

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.

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.M.Sujitha

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