Galore International Journal of Health Sciences and Research
[Indexed, Peer-Reviewed Journal]
Vol. 11; Issue: 3; July-Sept. 2026
Website: www.gijhsr.com
Original Research Article *P-ISSN: 2456-9321*

Evaluation of Acute and Sub Chronic Toxicity Studies of Siddha Polyherbal Formulation, *Thippili chooranam* in Wistar Albino Rats

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DOI: https://doi.org/10.52403/gijhsr.20260326

ABSTRACT

Back ground: Herbal drugs were considered safe for a long time. But as science advances the toxicity of herbal drugs had been pronounced. Toxicity analysis is now mandatory for all herbal drugs even they are considered safe. In this regard, *Thippili Chooranam* a poly herbal formulation indicated for *Iya eraippu noi* – Bronchial Asthma in siddha system is analyzed for its toxicity.
Aim and Objectives: To evaluate the safety of *Thippili Chooranam* in animal model through screening Acute and Sub chronic toxicity as per OECD guidelines. Standard Operating
Procedure: All the ingredients were purified and ground well to prepare Thippili Chooranam. Acute toxicity study was done as per OECD 423 guidelines. Single Oral dose of Thippili Chooranam 2000mg/kg body weight was given to six female Wistar rats and observed for 14 days. On the 15th day they were euthanized and analyzed for toxicity. Sub chronic or 90 days oral toxicity study had been executed as per the OECD 408 guidelines. 20 Wistar rats (10 male and 10 female) of three groups were taken for study. Group I served as control Group II is low dose (200mg/kg b.w.) and Group III is moderate dose (400 mg /kg body weight). On 91st day All were sacrificed and biochemical histopathological examination were done.
Results and Discussion: The histopathological analysis of *Thippili Chooranam* reveals, no remarkable toxicity of the drug and this drug is having a high safety in chronic use.

Key words: *Siddha* poly herbal formulation, *Thippili chooranam*, toxicity studies, *Thippili Iya eraippu noi*

INTRODUCTION

Before the emergence of modern medical system, native systems of medicine saved the people from various ailments. The Asian countries have their own medical system; among them *Siddha* is one of the oldest systems of medicine in the world. It is as old as the Tamil language and practiced endemically at southern parts of India particularly where the *Tamils* dwelled. The *Siddha* philosophy has been compiled with many doctrines of *Siddhars* like *Agasthiyar, Theraiyar* and *Bogar*[1]. The therapeutics of siddha mostly depends on herbals initially. Later when the knowledge of alchemy

emerged many metals, minerals and even the animal products were also used in therapeutics. The pharmacology of siddha clearly explains the symptoms of toxicity of metal and mineral made formulations. It also elaborates the treatment of thier toxicity. Poisonous plants like nux vomica, aconite root can also be used with caution for certain notorious and recalcitrant diseases and if any toxic effects arised, their antidotes were also described in *Siddha* medicine[2]. Nowadays, the modern world does not blindly accept that the herbal drugs are safe. Global acceptance of herbal products will be obtained only when the scientific algorithms are followed. In this study *Thippili Chooranam* a poly herbal formulation that is practiced in siddha for bronchial asthma is taken for acute and sub chronic toxicity studies in Wistar albino rats.

MATERIALS AND METHODS

The drug, *Thippili chooranam*[3] has been selected from the siddha text "*Kannusamy pillai parambarai vaithiyam"*. This poly herbal formulation contains 19 herbals. They were purified as per the description in the text "*sarakkugalin suththi sey muraikal*". The herbal constituents are as follows.

As per the reference, the ingredients of this formulation had been taken with their ratio.

1. Thippili – 10 palam * (350 gms)
2. Chukku – 5 palam (175 gms)
3. Milagu – 1 palam (35 gms)
4. Val milagu - 1 palam (35 gms)
5. Kadukkai - 1 palam (35 gms)
6. Nellikkai (dried) - 1 palam (35 gms)
7. Thandrikkai - 1 palam (35 gms)
8. Araththai - 1 palam (35 gms)
9. Thalisapathri - 1 palam (35 gms)
10. Kodiveli Ver pattai - 1 palam (35 gms)
11. Seeragam - 1 palam (35 gms)
12. Sombu - 1 palam (35 gms)
13. Karunjeerakam - 1 palam (35 gms)
14. Omum - 1 palam (35 gms)
15. Kurosani omum - 1 palam (35 gms)
16. Lavangam - 1 palam (35 gms)
17. Lavanga pattai - 1 palam (35 gms)
18. Lavanga pathri - 1 palam (35 gms)
19. Elam - 1 palam (35 gms)

* *Palam* is the older measurement in siddha that is approximately equal to 35 gms.

Vernacular name in *Tamil*	**English name**	**Botanical Name**	**Family**	**Parts used**	**Actions**
Thippili	Long pepper	*Piper longum*	*Piperaceae*	Fruit	Anti Asthmatic[4]
Chukku	Dry ginger	*Zingiber Officinale*	*Zingiberaceae*	Rhizome	Anti tussive[5]
Milagu	Pepper	*Piper nigrum*	*Piperaceae*	Fruit	Anti asthmatic Anti platelet aggregator[6]
Val Milagu	Tail pepper	*Piper cubeba*	*Piperaceae*	Fruit	Anti allergic[7]
Kadukkai	Chebulic myrobalan	*Terminalia chebula*	*Combretaceae*	Fruit	Immuno modulator, adaptogenic[8]
Nellikkai (dried)	Indian goose berry	*Phyllanthus embellica*	*Euphorbiaceae*	Fruit	Anti tussive, Anti inflammatory [9]
Thandrikkaai	Belleric myrobalan	*Terminalia bellerica*	*Combretaceae*	Fruit	Betalactamase inhi bitor, Antiulcer[10]
Araththai		*Alpinia officinarum*	*Zingiberaceae*	Rhizome	Broncho dilator[11] Anti allergic[12]
Thalisapathri		*Taxus baccata*	*Taxaceae*	Leaves	Anti Asthmatic[13]
Kodiveli Ver pattai	Ceylon lead wort	*Plumbago zeylanica*	*Plumbaginaceae*	Root bark	Anti viral, Anti inflammatory[14]
Seeragam	Cumin seeds	*Cuminum cyminum*	*Apiaceae*	Seeds	Anti inflamma tory[15]
Sombu	Anise seeds	*Pimpinella anisum*	*Apiaceae*	Seeds	Anti viral[16] Anti bacterial[17]
Karunjeeragam	Black cumin	*Nigella sativa*	*Ranunculaceae*	Seeds	Anti histaminic[18] Immune booster.
Omum	Ajwoin	*Carum*	*Apiaceae*	Seeds	Anti inflammato

		copticum			ry[19]anti histaminic
Kurosani omum		*Hyocyamus niger*	*Solanaceae*	Seeds	Analgesic, Spasmolytic [20]
Lavangam	Clove	*Syzygium aromaticum*	*Myrtaceae*	Flower bud	Anti microbial[21] Anti viral [22]
Lavanga Pattai	Cinnamon bark	*Cinnamomum zeylanicum*	*Lauraceae*	Bark	Anti bacterial[23] Im muno modulator[24]
Lavanga pathri	Brinji leaves	*Cinnamomum tamala*	*Lauraceae*	Leaf	Anti inflamm atory [25]
Elam	Cardamom	*Eletaria cardamomum*		Fruit	Anti microbial [26]

The ingredients of this formulation were bought from reputed country drug store at Broadway Chennai. They are authenticated by botanist of D.G.Vaishnav College of Arts and Science, Chennai and Siddha experts in Gunapadam (Siddha Pharmacology) Department, Government Siddha Medical College, Chennai. They were given authentication number as per the records maintained in the department. Specimen samples were preserved in the Gunapadam department for future reference.

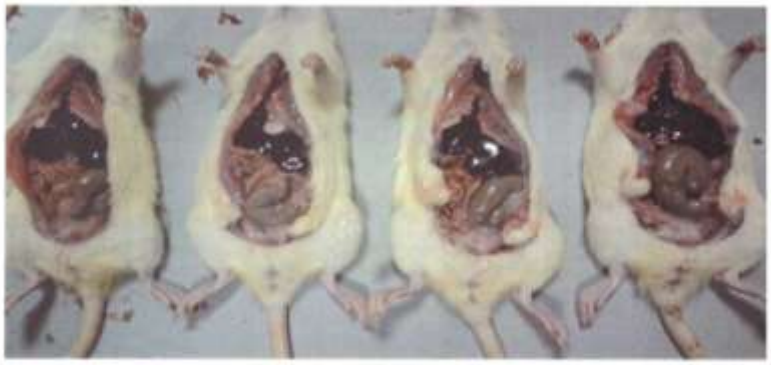
All the herbal ingredients were dried well after purification and pounded into an iron mortar with pestle to make a fine powder. The powder is sieved and packed tightly into a food grade plastic container and taken for the study.

The proposal for the study had been submitted before the Institutional Animal Ethical Committee, Department of Veterinary Pharmacology and toxicology, Madras Veterinary College, Vepery Chennai under the control of The Tamilnadu Veterinary and Animal Sciences University, Chennai and got approval for the proposal. The IAEC No is **IAEC NO: 40/SA/IAEC/2024.**

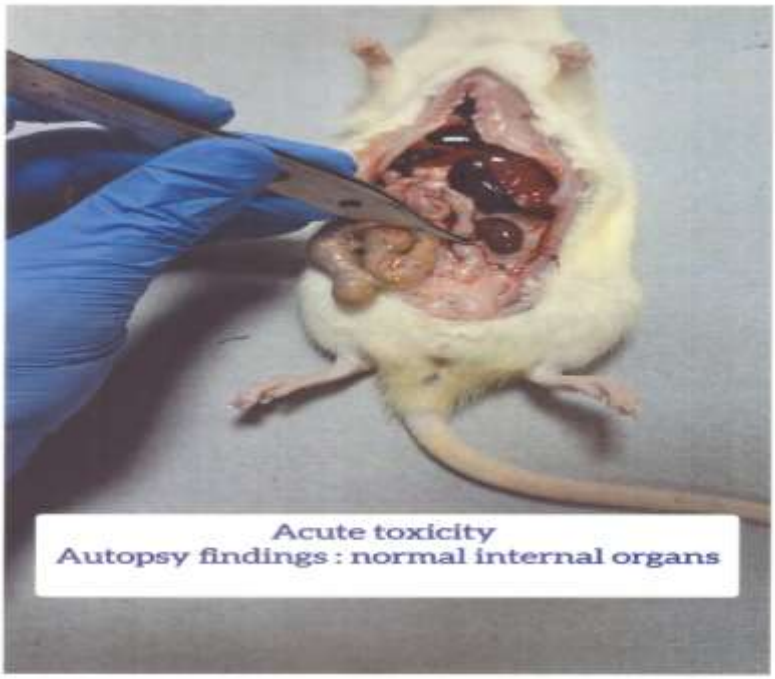
Wistar albino rats were purchased from TANUAS, Madhavaram, Chennai. All the animals were housed in poly propylene cages. They were maintained 12 hours dark and light cycle in 37 C with required humidity and ventilation. They were fed with rat pellets and water *ad libitum*. After 7 days of acclimatization, rats were taken for the Acute and Sub chronic (90 days) toxicity studies.

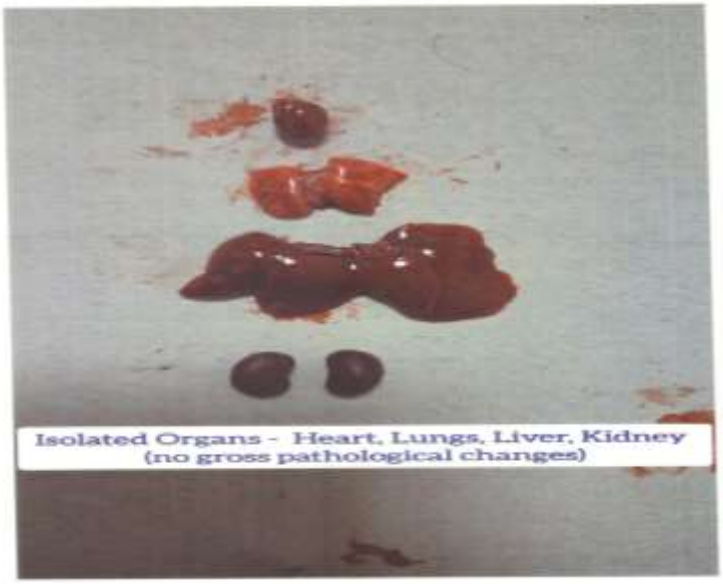
Acute oral toxicity test was conducted as per the OECD 423 guidelines. 6 female Wistar rats weighing average 150 gms were taken for the study. The animals were kept overnight fast for food with free access to water. The weight of all animals was noted initially and *Thippili chooranam* was administered orally in the dose of 2000 mg/ kg body weight. They were observed for 72 hours and then 14 days for behavioral changes, body weight, and for mortality. Any symptoms of toxicity was observed continuously for the first 2-4 hours and later for the next 14 days. Body weight will be recorded periodically. Observation includes changes occurring in skin, fur and mucous membranes. Tremors, convulsions, sedation, respiratory distress, cardio vascular collapse, diarrhoea, lethargy and mortality were observed with special attention. At the end of the study all the animals were sacrificed and observed for gross pathological changes.

Acute oral Toxicity study of Thippili Chooranam in female Wistar Rats.



Acute toxicity
Autopsy findings: no gross pathological changes





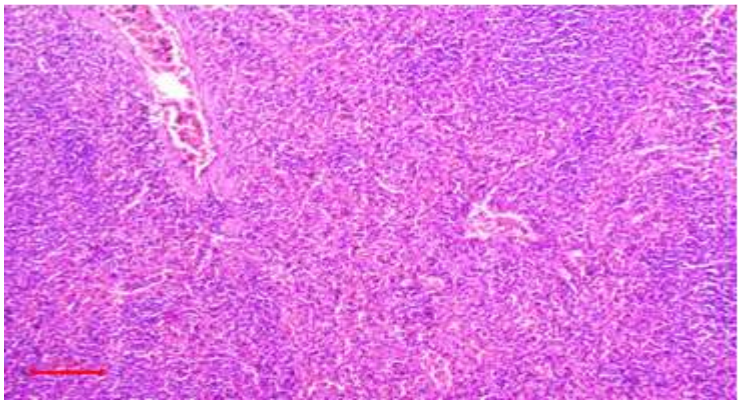
Sub Chronic Toxicity study was conducted in Wistar albino rats with the study drug Thippili Chooranam as per OECD guidelines 408

For sub chronic toxicity of *Thippili Chooranam,* animals were divided into 3 groups. Each group contains 10 female and 10 male rats. First group of animals were considered as control where as other 2 groups were treated groups with low (200 mg/kg body weight) and high dose (400 mg/kg body weight) of study drug. The animals were treated orally with *Thippili Chooranam* once daily for 90 days. The animals in Group I received normal saline 5ml/kg body weight. The animals in Group II received 200mg/kg body weight and Group III received 400mg/kg body weight of *Thippili Chooranam*. The rats weighed periodically and observed signs for toxicity indicating cardiovascular, neuro muscular and autonomic nervous systems. Changes in the intake of food and water will be observed. At the 90th day, the animals were kept fasted overnight with free water intake. All the groups on 91st day they were subjected to necropsy. Blood samples will be collected for biochemical and hematological Para meters. All the vital organs will be examined for gross lesions and sent for histo pathological analysis.

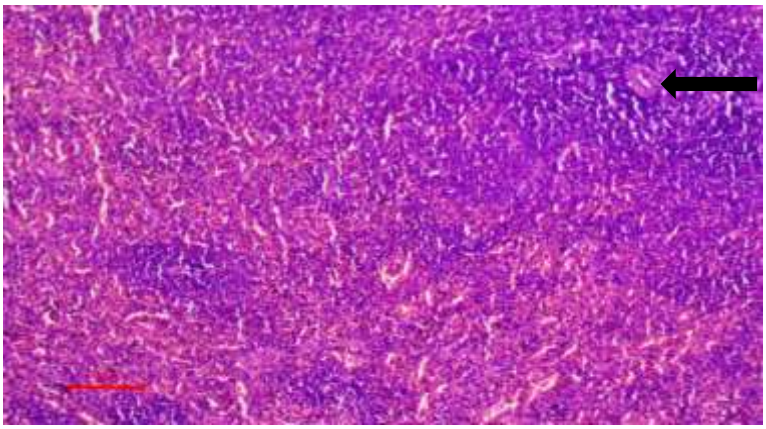
Biochemical parameters

Parameters	Control group	Low dose group	High dose
BUN	13.53 mg/dl	15.65 mg/dl	18.25 mg/dl
Creatinine	0.26 mg/dl	0.30 mg/dl	0.33 mg/dl
ALT	49.0 IU/L	32.0 IU/L	45.0 IU/L
AST	77.0 IU/L	70.0 IU/L	93.0 IU/L

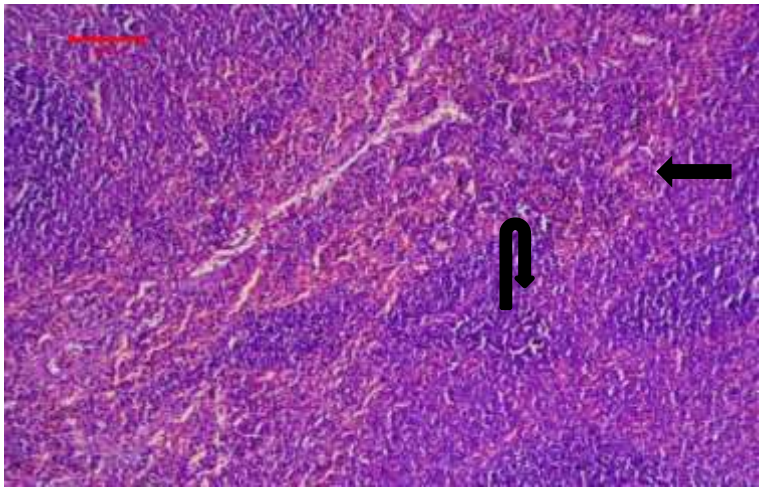
Female Rat - Spleen



Control Female rat – spleen

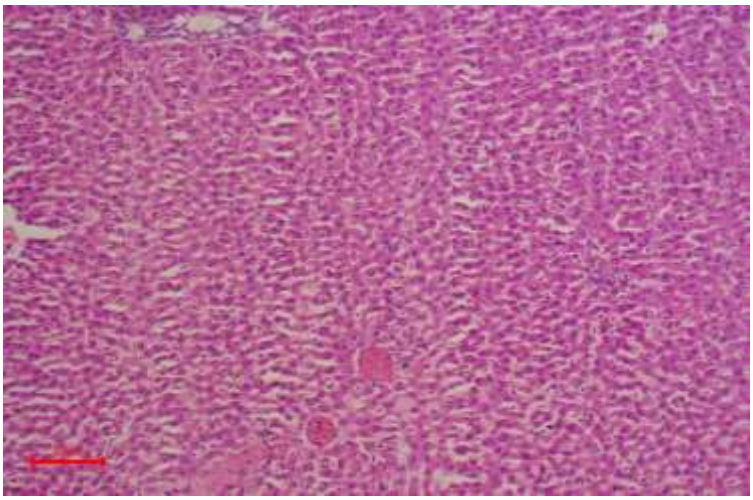


Low Dose Female – Spleen Mild dilatation of splenic sinus congestion in red pulp

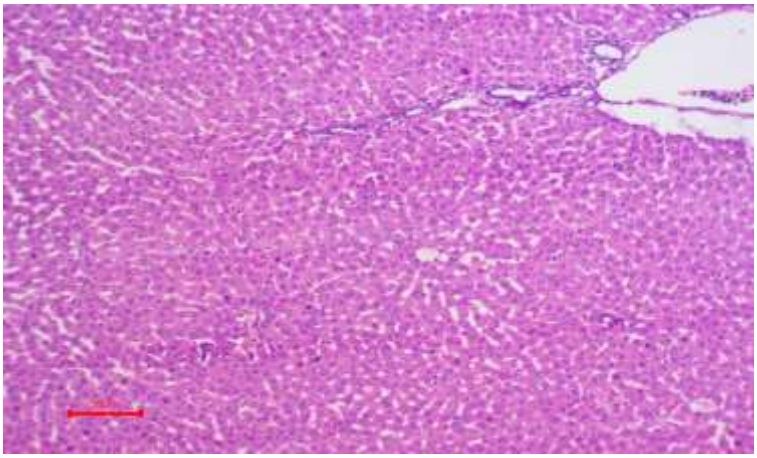


High dose Spleen of Female. Arrow Dilatation of splenic sinus. Congestion in red pulp curved arrow.

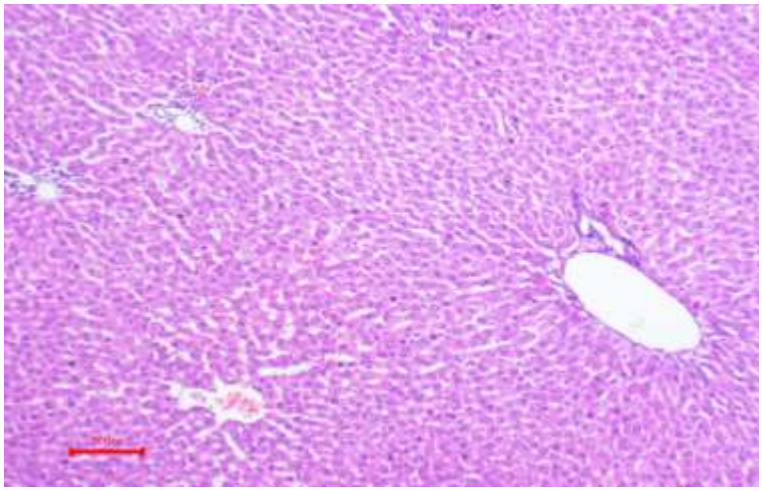
Female Rat – Liver



Control Female Rat - Liver Normal study

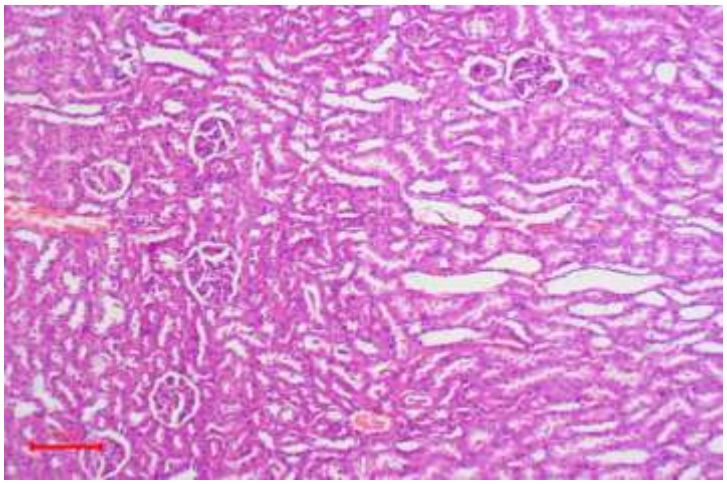


Low dose Female Rat – Liver Normal study

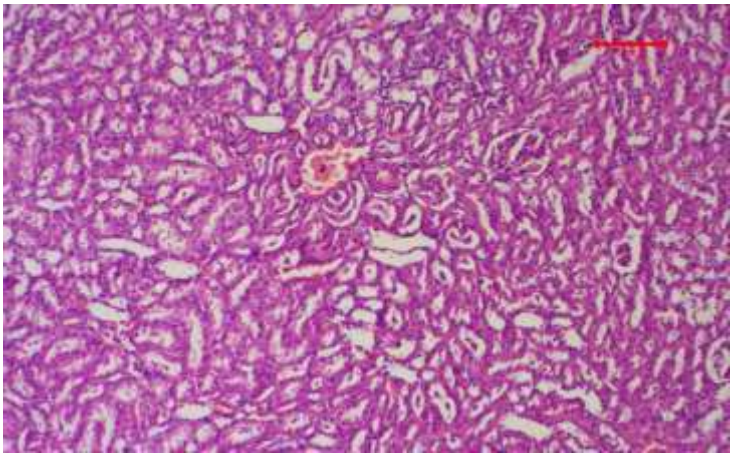


High dose Female – Liver (Normal study)

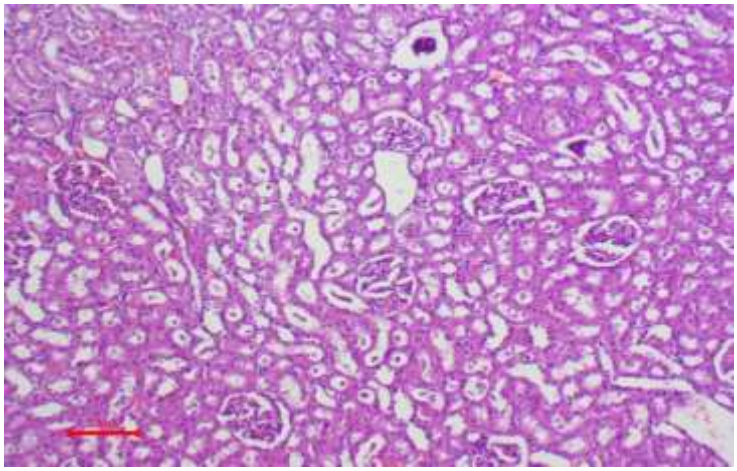
Female Rat – Kidney



Control Female Rat - Kidney Normal study

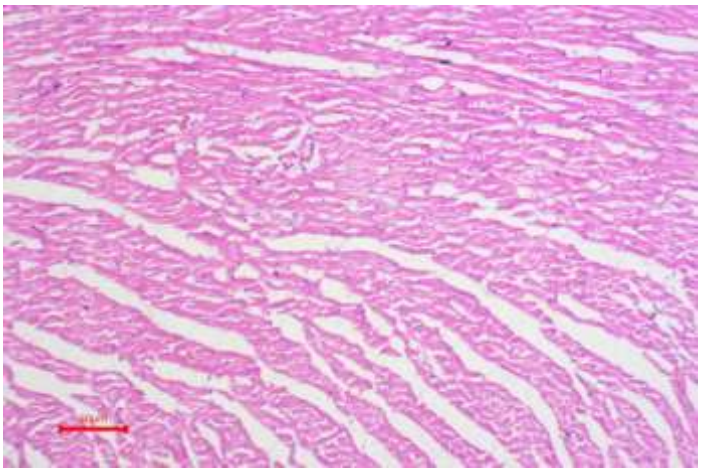


Low dose Female – Kidney Normal study

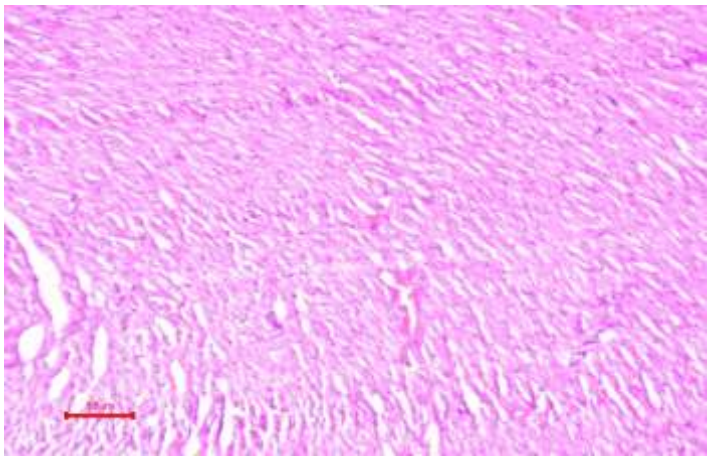


High Dose Female – Kidney Normal study

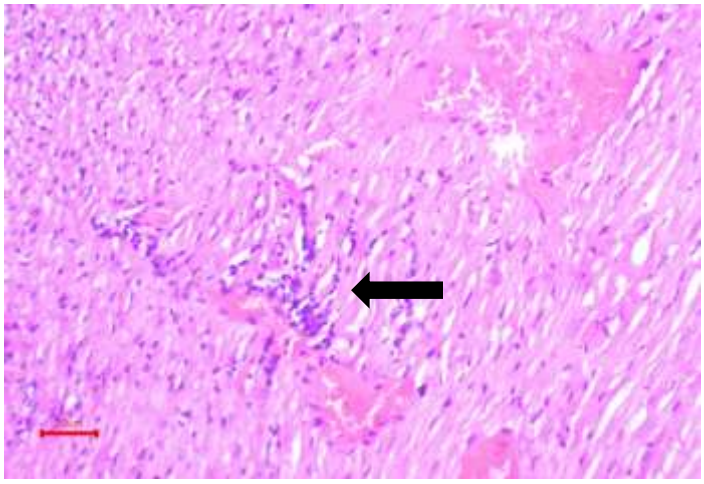
Female Rat – Heart



Control Female – Heart Normal study

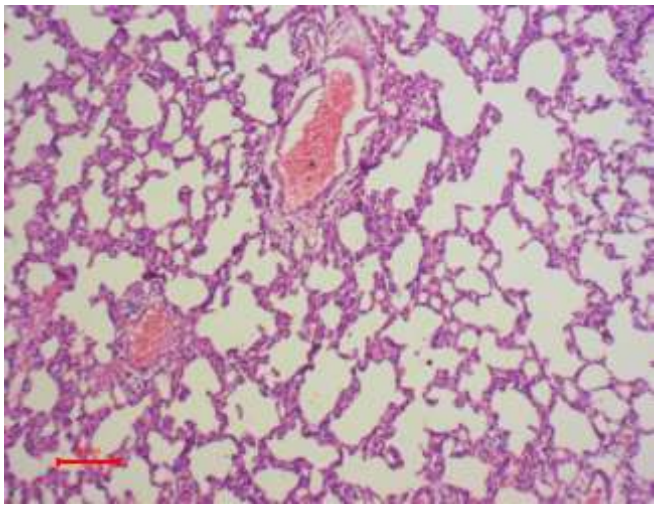


Low dose Female – Heart Normal study

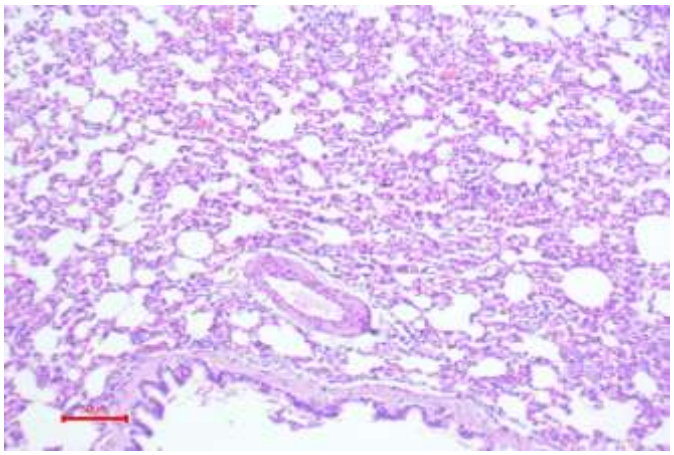


High dose Female – Heart focal lymphocytic infiltration – myocarditis.

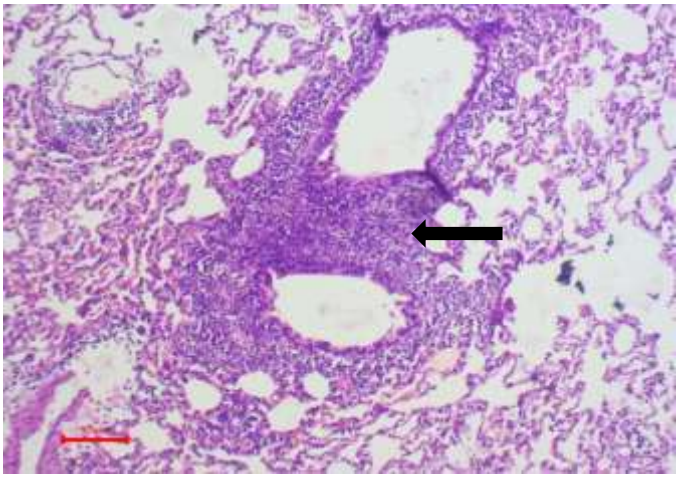
Female Rat – Lungs



Control Female – Lungs Normal study

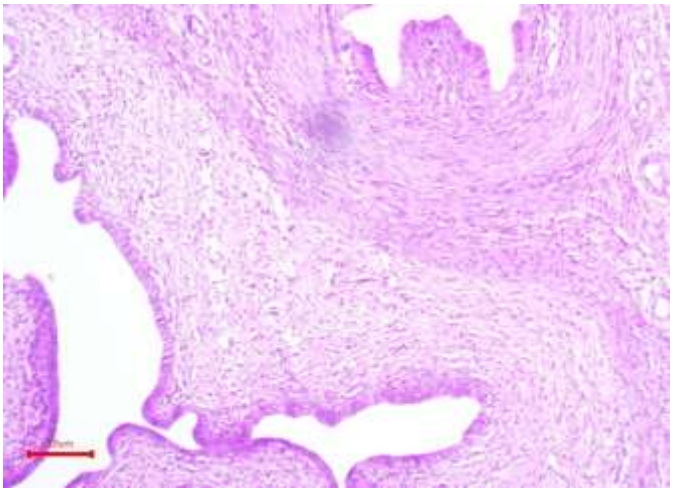


Low dose Female – Lungs Normal study

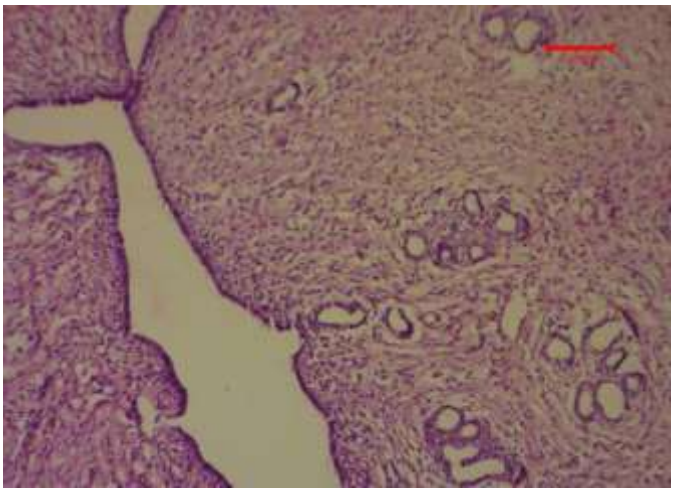


High dose Female – Lungs Peri bronchiolar lymphoid Hyperplasia

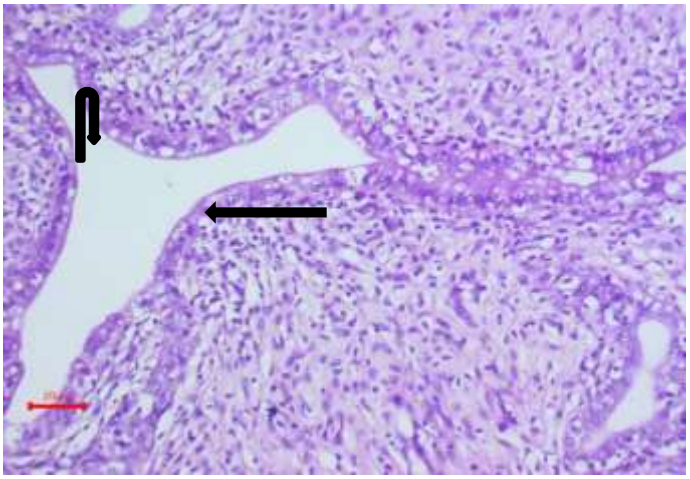
Female Rat – Uterus



Control Female - Uterus Normal study

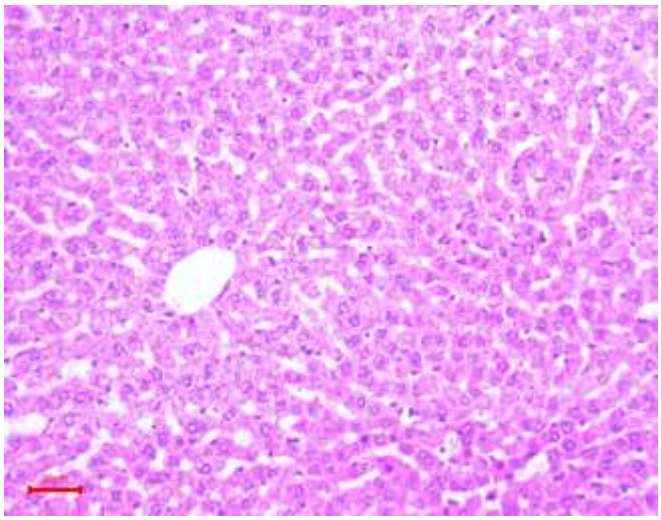


Low dose Female – Uterus Normal study

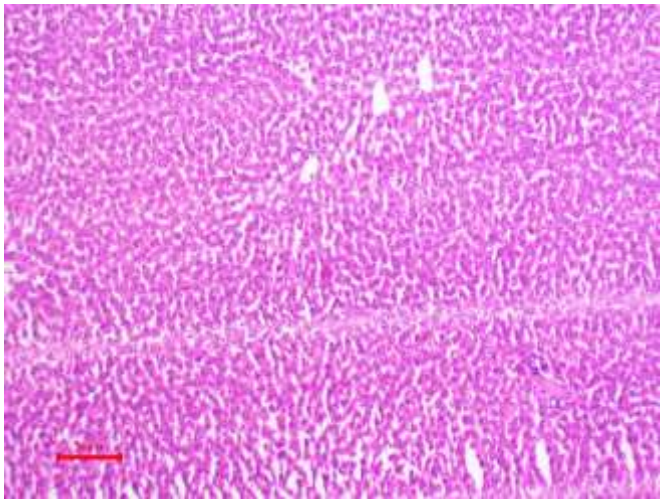


High dose Female -Uterus mild vacuolar degeneration of surface epithelium. Curved arrow Lumen

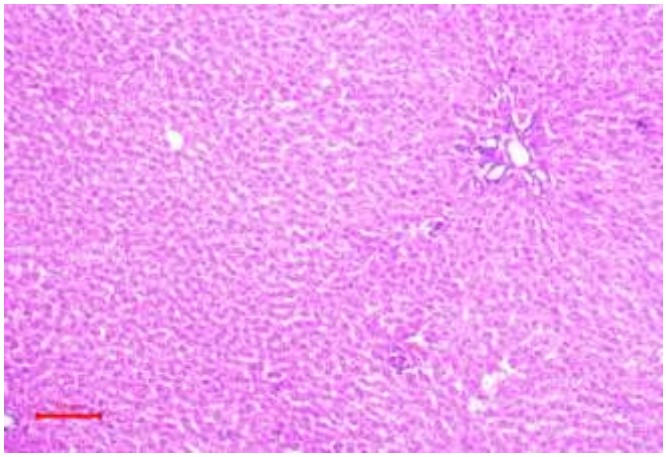
Male Rat – Liver



Control Male Rat – Liver Normal study

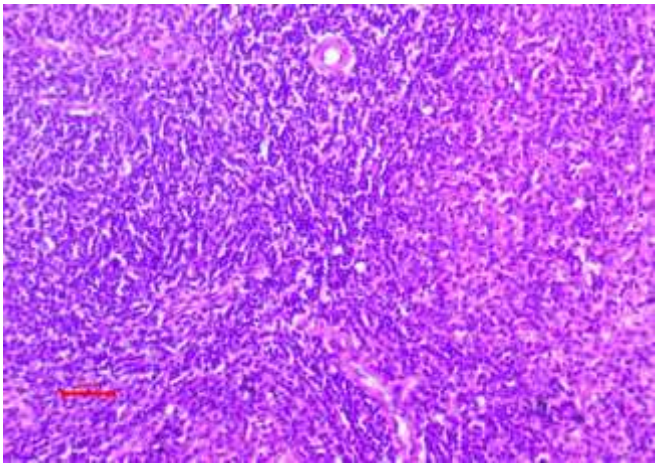


Low dose Male – Liver Normal study

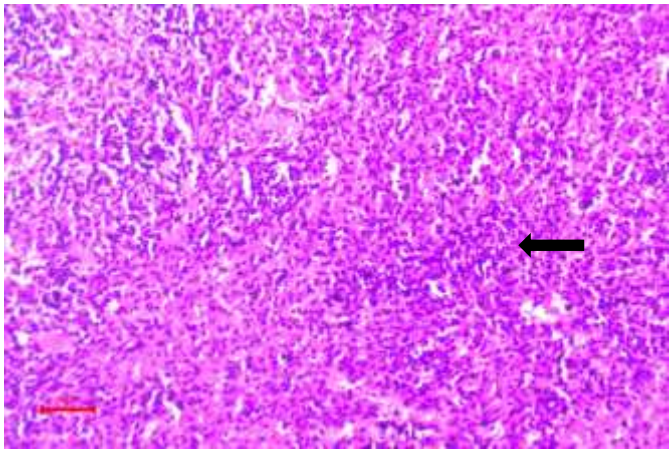


High dose Male - Liver Normal study

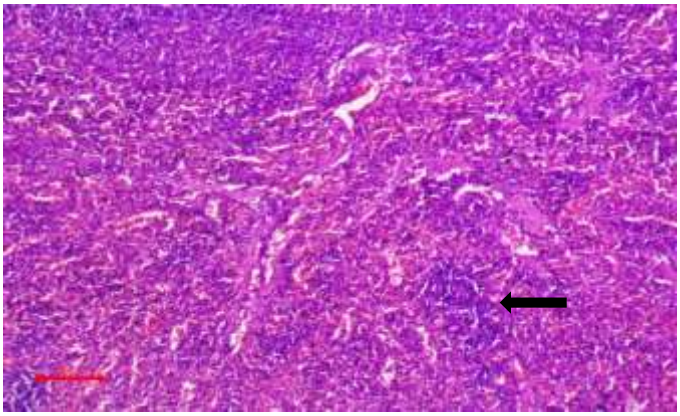
Male Rat – Spleen



Control Male - Spleen Normal study

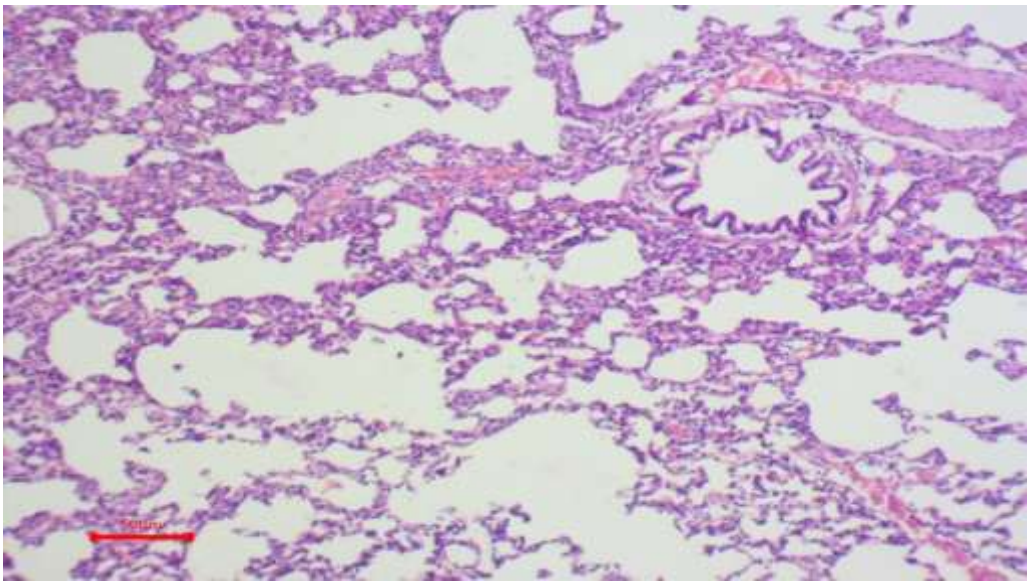


Low dose Male – Spleen Congestion of red pulp

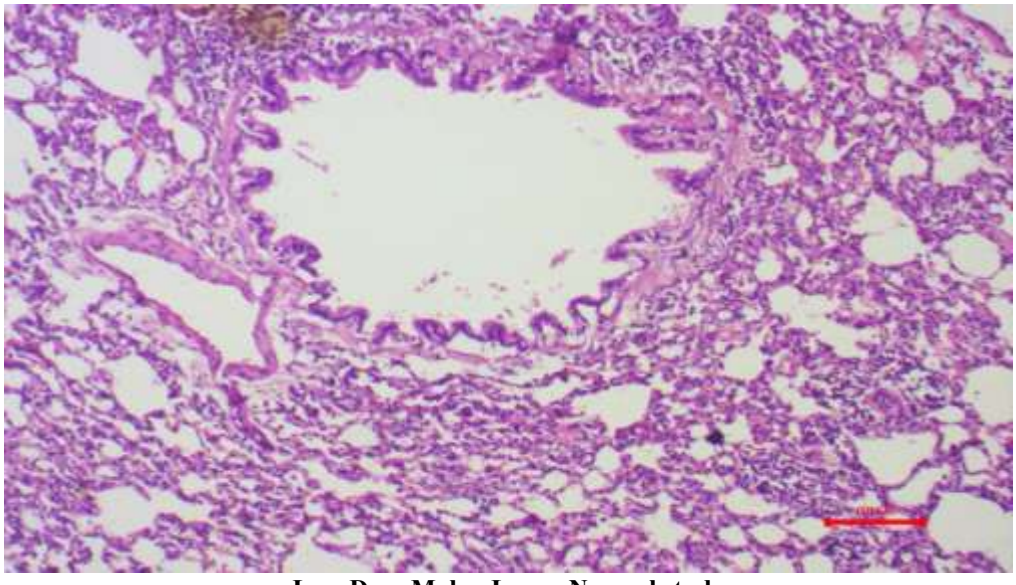


High dose Male – Spleen Congestion of red pulp

Male Rat – Lungs

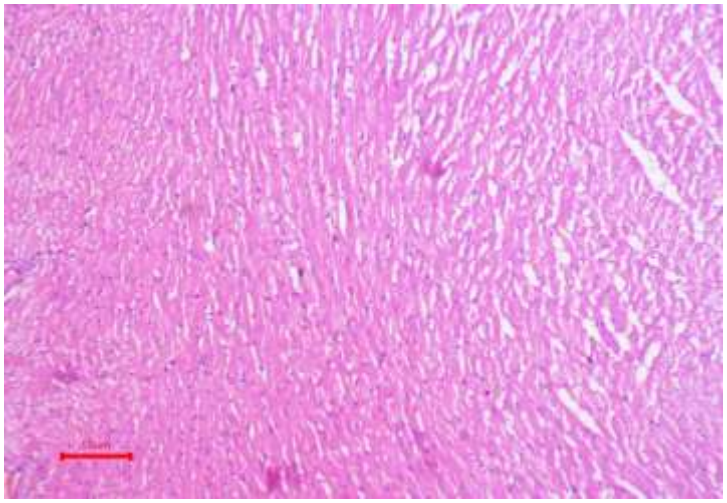


Control Male – Lungs Normal study

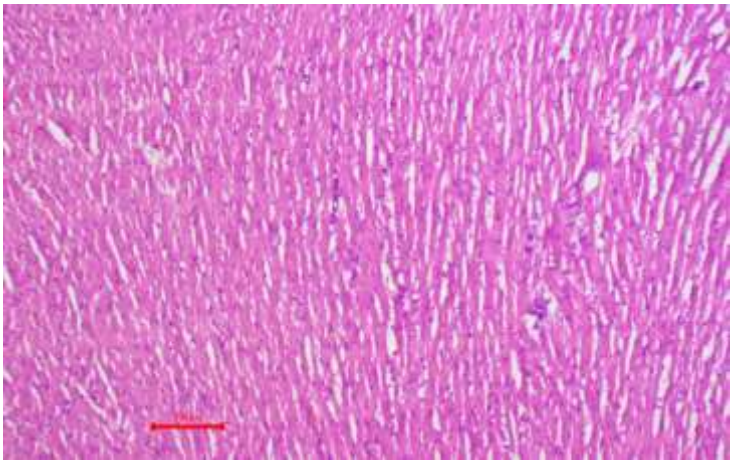


Low Dose Male - Lungs Normal study

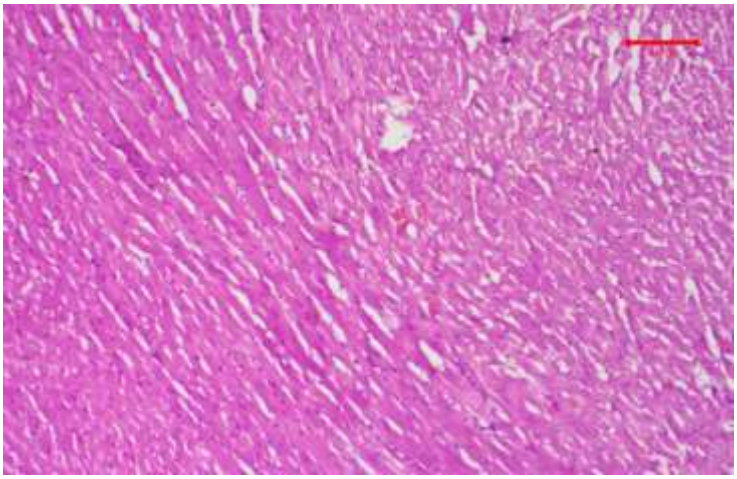
Male Rat - Heart



Control Male – Heart Normal study

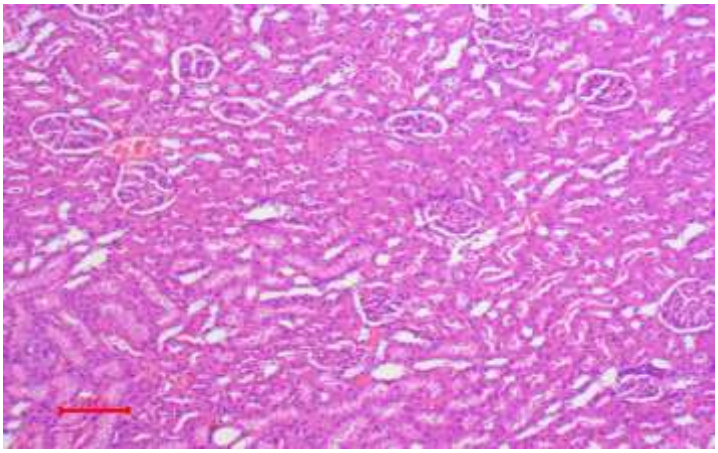


Low dose Male – Heart Normal study

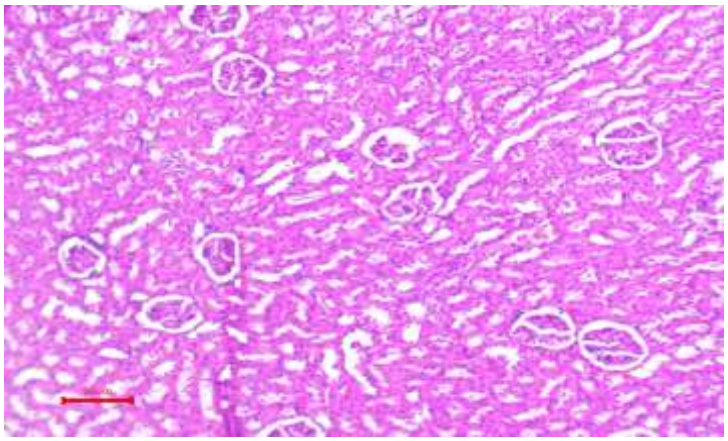


High dose Male – Heart Normal study

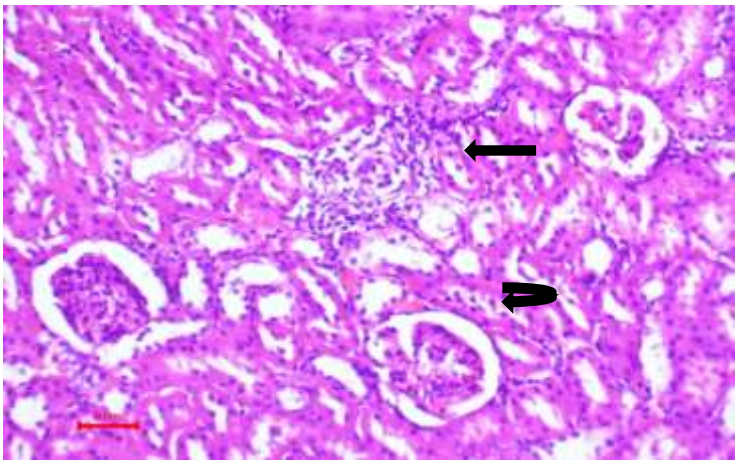
Male Rat - Kidney



Control Male -Kidney Normal study

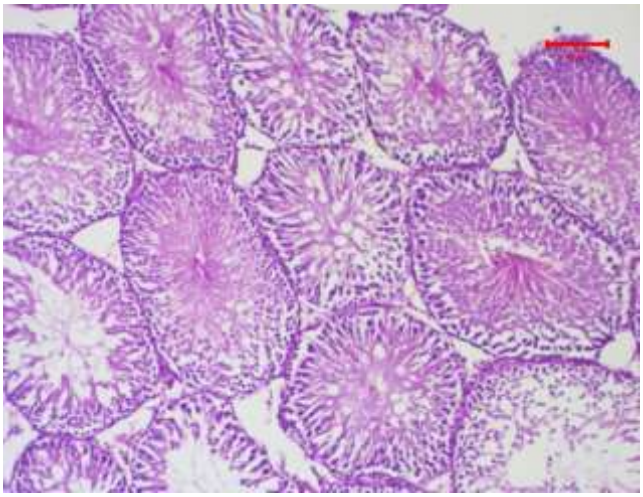


Low dose Male - Kidney Normal study

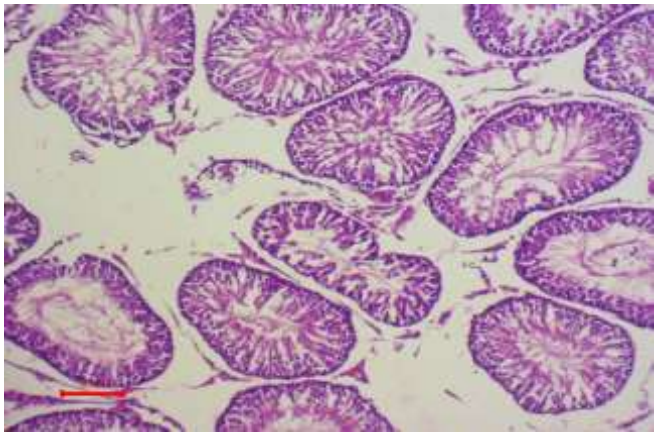


High dose Male – Kidney. focal Lymphocytic infiltration. Curved arrow vacuolar changes in tubular epithelium

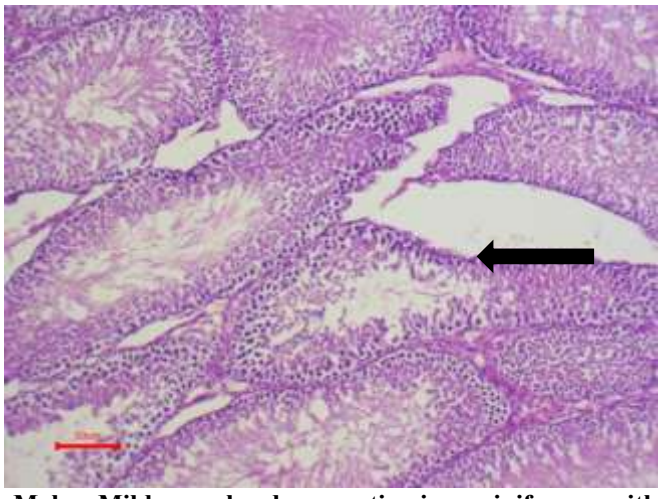
Male Rat – Testis



Control Male – Testis Normal study



Low dose Male – Testis Normal study



High dose Male – Mild vacuolar degeneration in seminiferous epithelial cells.

RESULTS AND DISCUSSION

Acute Single oral dose toxicity study as per OECD guidelines 423 was carried out for Thippili chooranam. It revealed that the poly herbal formulation was safe in high dose of 2000mg/ kg body weight. 90 days Sub chronic study was carried out as per the OECD guidelines 408. All the vital organs of the animals had been sent for histo pathological investigations. They revealed

no gross changes in cell structure, necrosis or tissue destruction. Mild congestion is pronounced in spleen. Biochemical parameters and hematological parameters were nearly normal to the control group of rats. A mild increase in BUN and ALT levels noted in high dose rats. This reveals the study drug *Thippili chooranam* is safe drug for chronic use in humans.

Declaration by Authors

Ethical Approval: Approved
Acknowledgement: None
Source of Funding: Self-funding. No Funding has been obtained from any one/ any organization for this study
Conflict of Interest: The authors declare no conflict of interest.

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How to cite this article: S. Shankar, K. S. Uma., M. Ravichandiran, N. Kabilan, P. Amutha. Evaluation of acute and sub chronic toxicity studies of Siddha polyherbal formulation, *Thippili chooranam* in Wistar albino rats. *Gal Int J Health Sci Res*. 2026; 11(3): 225-242. *DOI: https://doi.org/10.52403/gijhsr.20260326*

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