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TOXICITY STUDY OF *VAIVILANGAM CHOORANAM*

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ABSTRACT

The toxicity study of *Vaivilangam Chooranam* was investigated on experimental animals. The experiments were carried out in the premises of the conventional animal facility of the institute. The toxicity study of the drug was carried out to detect possible toxic effects by clinical examination, pre terminal death of animals, body weight, food intake, and histopathology study. The Clinical examinations were measured to find out the alteration in the levels of Glucose, Total Cholesterol, Triglycerides, Urea, Total bilirubin, Creatinine, Albumin, Alanine aminotransferase, Aspartate aminotransferase. The acute and sub-acute toxicity studies were found out by the changes in general behaviour, physical observation and pre terminal deaths. All the studies were carried out separately in both female and male animals. The histopathological analysis of the vital organs brain, heart, lungs, spleen, kidneys, liver, adrenals glands and testes were also examined. The overall result indicates the absence of toxic effects in animals.

KEYWORDS

Vaivilangam Chooranam, Clinical examinations, Histopathology, Toxicity.

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INTRODUCTION

The herbal formulations are very essential in order to assess the quality of drugs, based on the concentration of active principles. Ancient books of Siddha Medicine has mentioned the ingredients of *Vaivilangam chooranam* for anemia, abdominal disorders, obesity, diseases of vatam, snake bite, rat bite, worm infestation, digestive disorders, skin diseases, venereal diseases, *pitha* diseases and *gunma vatham*, flatulence, cough and leucoderma[1]. The components of *Vaivilangam chooranam* are seeds of *Emblica ribes* (Siddha Name: Vaivilangam) (Primulaceae) and seeds of *Vernonia anthelmintica*e (Siddha Name: Kattujeeragam) (Asteraceae) in equal

proportion by weight. The plant constituents have been reported for possessing anthelmintic, alterative, tonic, stomachic properties and also to be effective in dyspepsia, flatulence and skin disease [2].

Raw drug Kattucheeragam (*Vernonia anthelmintica*)

Raw drug Vaivilangam (*Embelica ribes*)

MATERIALS AND METHODS[3-6]

Preparation of Extracts

Both the test formulations were suspended in 0.3% CMC. The suspension was freshly prepared and used. Test formulations were administered orally by using gastric gavage twice daily. Acute toxicity was conducted before initiation of sub-acute toxicity studies. The formulation was non-toxic upto 2g/kg body weight in mice.

Evaluation of Toxicity

The experimental studies on animals were conducted at PSG College of Pharmacy, Coimbatore. This study was approved by the Institutional Animal Ethics Committee of PSG Institute of Medical Science and Research, Coimbatore. The animals were housed individually in polyurethane cages with wire mesh floors in the animal house. All animals were kept in one room and with no other species being housed in the same room. The room was well-ventilated (> 10 air changes per hour) with 100% fresh air (no air circulation). A 12-hour light/dark photoperiod was maintained. Room temperature and relative humidity was set to be maintained between 20 ± 2°C and 30-70 % respectively. The environmental conditions were kept at 21 ± 2°C, with 10-15 air changes per hour and relative humidity was 50-55% with a 12 hour light/dark cycle. The animals had free access to sterile pelleted feed of standard composition containing all macro and micro nutrients. Water which was passed through activated charcoal filter and exposed to UV rays (Aqua guard on-line water filter-cum- purifier) was provided ad libitum. The animals were examined at regular intervals by trained personnel for any behavioral abnormalities. Body weights of these rats were taken after the initial stabilization period. They were ranging between 180-350g. Animals were housed with appropriate identification by colouring the fur in cages with cage cards. After initial weights were taken, the animals were distributed randomly into appropriate groups for conducting the study. The experiments were carried out in the premises of the conventional animal facility of the institute. The protocol was approved by National Toxicology Evaluation Panel appointed by the Indian Council of Medical Research (ICMR) as well as the Institutional Animal Ethical Committee.

Clinical examination was conducted before grouping and at the end of each week of the experimental schedule. All rats were observed twice daily for any pre terminal deaths. Individual body weights were recorded weekly. A measured amount of feed was kept in the cages and then after 24 hrs the left out amount of feed was measured to calculate the

amount of food consumed by the rats. The biochemical parameters such as Glucose, Total Cholesterol, Triglycerides, Urea, Total bilirubin, Creatinine, Albumin, Alanine aminotransferase, Aspartate aminotransferase and haemotological parameters such as RBC, MCV, HCT, Platelet, MPV, WBC, Hb, MCH, MCHC, RDW% were measured after the drug treatment to ascertain the drug induced alteration thereby its toxicity. The tissue samples of Brain, Heart, Lungs, Spleen, Kidney, Liver, Adrenal gland and Testes were collected from all the animals and preserved in 10% buffered neutral formalin. They were sliced adequately wherever necessary. After a minimum of 24 hr fixation, they were sampled and processed by conventional methods, paraffin blocks were made and 6 μm paraffin sections were stained with Hematoxylin and Eosin. They were examined under light microscope. All deviations from normal histology were recorded and compared with corresponding controls.

RESULTS AND DISCUSSION

In the present investigation, the toxicity study of *Vaivilangam chooranam* were found out by undergoing acute toxicity and sub-acute toxicity studies. Acute dose administration of *Vaivilangam chooranam* (2g/kg) in female mice did not produce any mortality and behavioural change up to 7 days and 48 hrs respectively. There is no abnormality in home cage activity and no behaviour changes were noticed in any rat. The faecal/urinary excretions were found to be normal. Hair coat was clean and groomed with no lacrimation, salivation, tremors, and convulsions in all the group of rats were observed. The normal activity of eye lid closure, respiratory rate was observed physically in both control and test compound treated rats. No pre-terminal death was observed during sub-acute toxicity studies. Body weight was monitored weekly. Data indicate that there was no significant loss or gain in the weight of the rats exposed to test compounds at different doses as compared to vehicle treated rats. It indicates normal growth pattern of the rats. Food intake was found to be normal and no significant difference was observed.

The biochemical parameters such as glucose, cholesterol, triglycerides, albumin, alanine transaminase (ALAT) have not shown any significant alterations upon 28 days administration in both male and female rats. The chooranam was (upto 600 mg / kg) found to be safe in sub-acute toxicity studies. The *Vaivilangam chooranam* at higher dosage (600 mg / kg) in female rats has shown increase of serum creatinine, total bilurubin, direct bilurubin and aspartate transaminase (ASAT) levels but without any statistical significance. Serum parameters mentioned above are found to be normal at other two dosages in female rats. In male rats, all the above parameters have not been altered at all three different dosages of chooranam taken for this study. The haematological parameters such as RBC, WBC, platelet counts, Hb measurement were not altered with *Vaivilangam chooranam* treatment for 28 days. The chooranam was found to be safe upto 600 mg/kg with respect to haematological functions. All values in figures expressed as Mean±SEM. One way ANONA followed by post hoc Tukey test. *,**,*** denotes $p<0.05$,$p<0.01$,$p<0.001$ as compared to control respectively. The histopathological analysis of the vital organs brain, heart, lungs, spleen, kidneys, liver, adrenals glands and testes have given below. The tissues have shown normal cellular organization without any lesions or necrosis with high dose administration of the Siddha formulations this observation indicates that the *Vaivilangam chooranam* is not having any toxicity in animals. The results are depicted in Table No.1 and Figure No.1-21.

Table No.1: Haematological parameters (After drug treatment in animal model)

Group		RBC	MCV	HCT	PLATELET	MPV	WBC	HGB	MCH	MCHC	RDW%
Control M1	Mean	8.3	44.8	33.3	443.3	6.4	17.9	7.6	12.0	25.1	15.9
	SD	1.7	2.9	2.1	48.0	0.4	1.7	1.2	1.4	1.1	1.2
Control F1	Mean	7.8	47.5	30.1	394.7	6.4	18.3	6.9	10.9	24.9	15.8
	SD	1.8	4.5	4.5	162.0	0.2	0.8	1.2	1.4	1.4	0.6
150mg/kg M2	Mean	7.8	44.3	33.9	450.4	6.5	17.9	7.4	11.1	24.3	15.9
	SD	0.7	1.8	3.4	33.7	0.2	1.0	1.4	0.9	1.4	1.9
150mg/kg F2	Mean	7.1	47.7	30.7	423.0	6.5	18.0	7.2	11.3	24.7	15.6
	SD	1.2	3.3	4.4	124.1	0.2	0.7	1.3	1.4	0.7	0.9
300mg/kg M3	Mean	7.5	45.1	33.5	451.2	6.5	17.6	7.7	12.2	25.0	15.8
	SD	0.9	1.0	3.3	36.6	0.2	1.6	0.5	0.9	1.2	1.5
300mg/kg F3	Mean	7.4	48.0	31.2	422.4	6.5	17.5	6.9	11.6	24.0	15.9
	SD	1.8	5.0	5.9	124.2	0.3	1.5	1.3	1.2	1.0	1.5
600mg/kg M4	Mean	7.7	43.9	33.9	450.4	6.2	18.6	8.3	10.8	24.7	17.7
	SD	0.5	1.7	3.4	33.7	0.1	1.0	0.8	0.3	0.4	0.5
600mg/kg F4	Mean	6.3	48.7	30.7	421.8	6.0	17.4	7.7	12.1	25.1	17.0
	SD	0.8	3.6	4.5	121.9	0.1	1.2	0.9	0.7	0.9	3.7

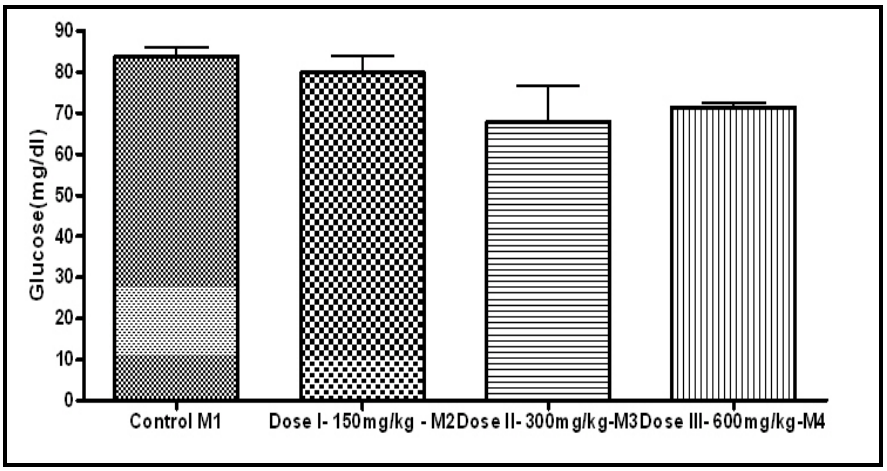


Figure No.1: Effect of different treatments on Glucose levels in male rat

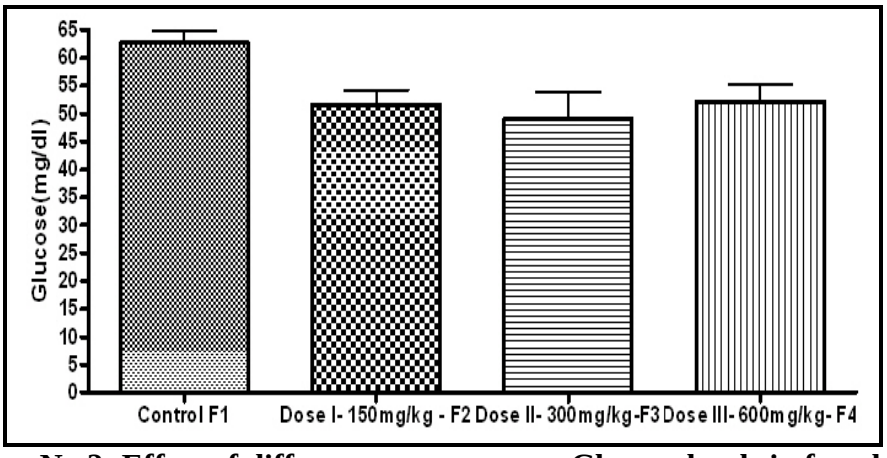


Figure No.2: Effect of different treatments on Glucose levels in female rat

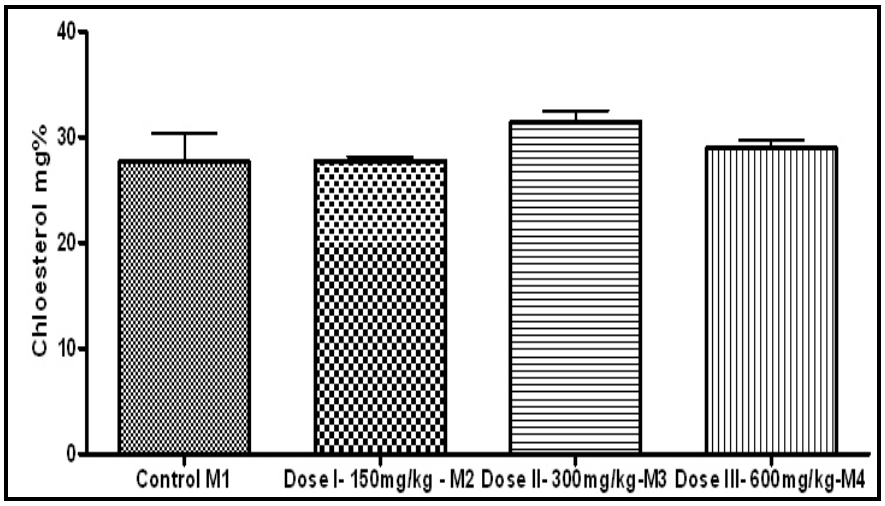


Figure No.3: Effect of different treatments on Cholesterol levels in male rat

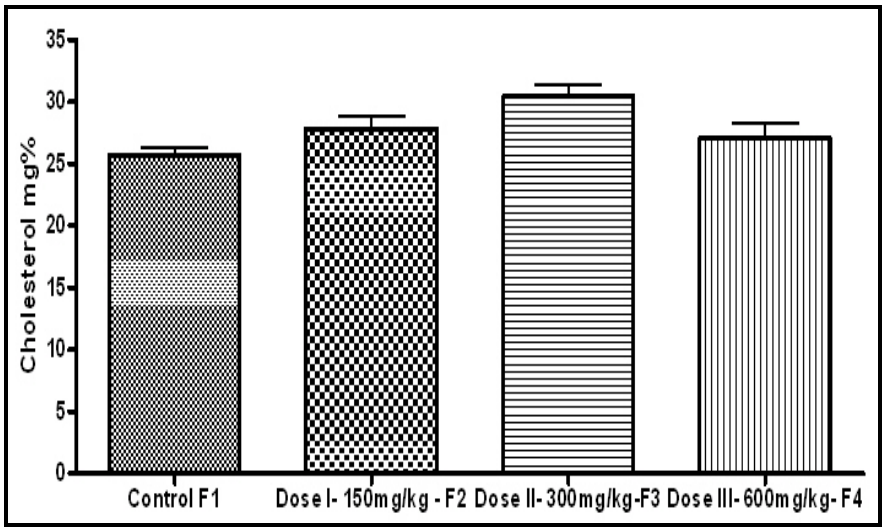


Figure No.4: Effect of different treatments on Cholesterol levels in female rat

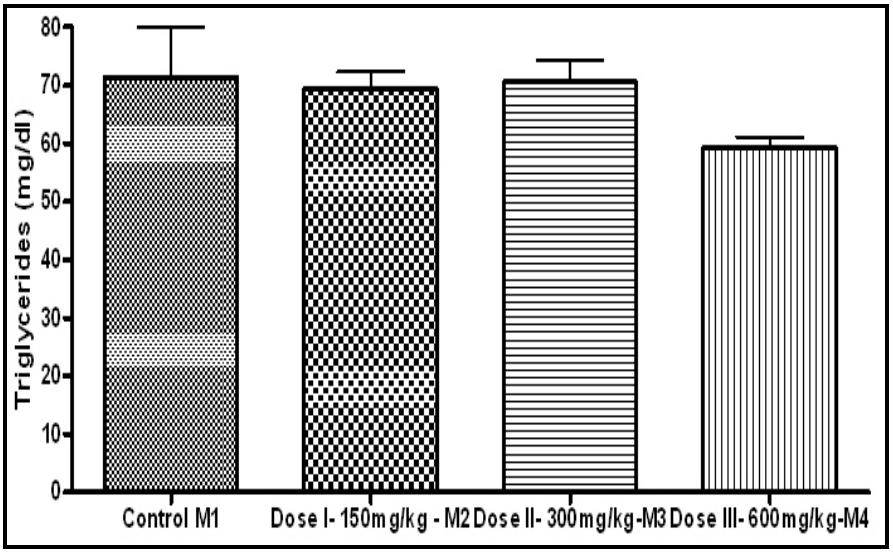


Figure No.5: Effect of different treatments on Triglycerides levels in male rat

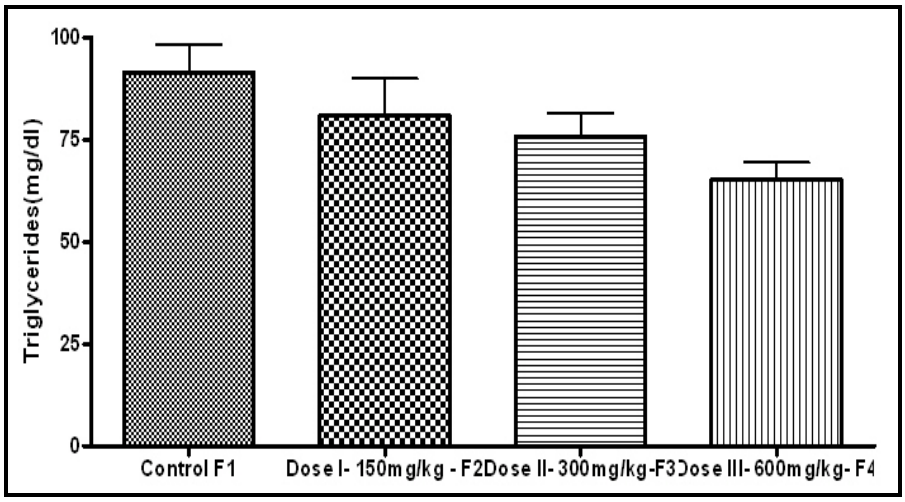


Figure No.6: Effect of different treatments on Triglycerides levels in female rat

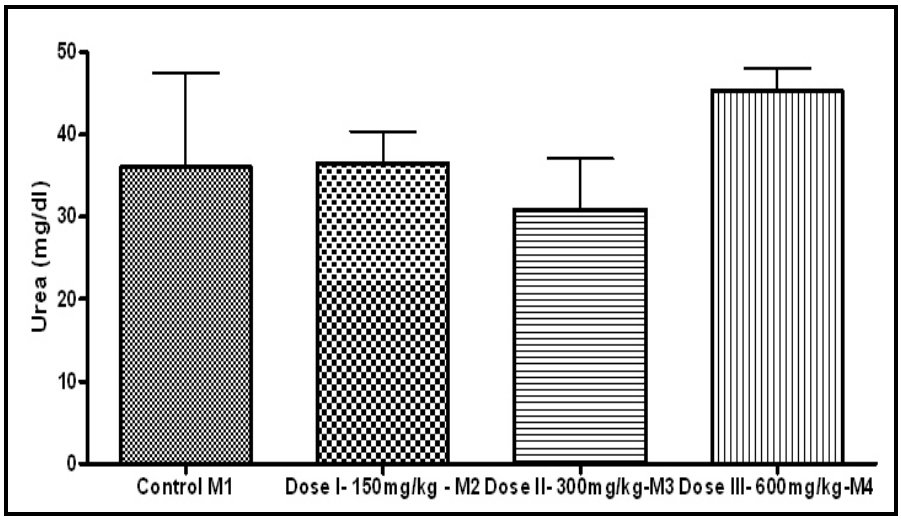


Figure No.7: Effect of different treatments on Urea levels in male rat

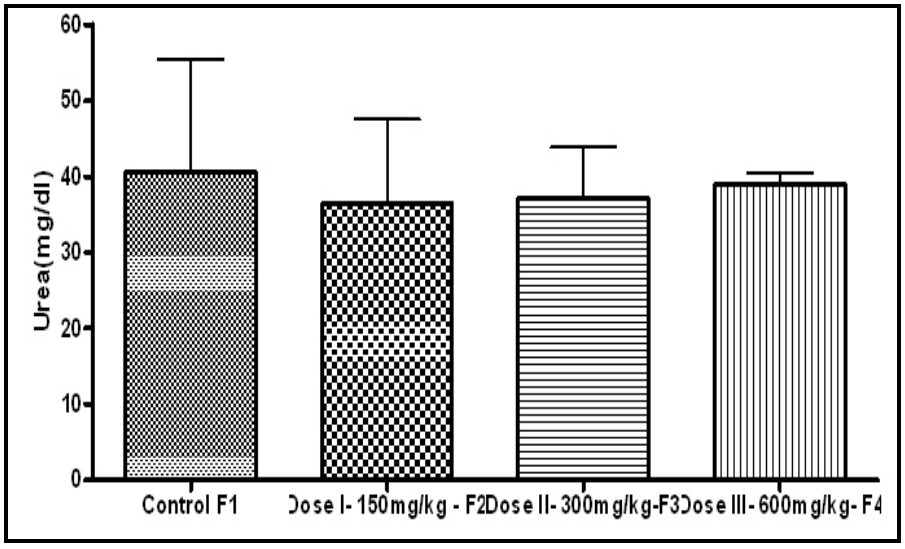


Figure No.8: Effect of different treatments on Urea levels in female rat

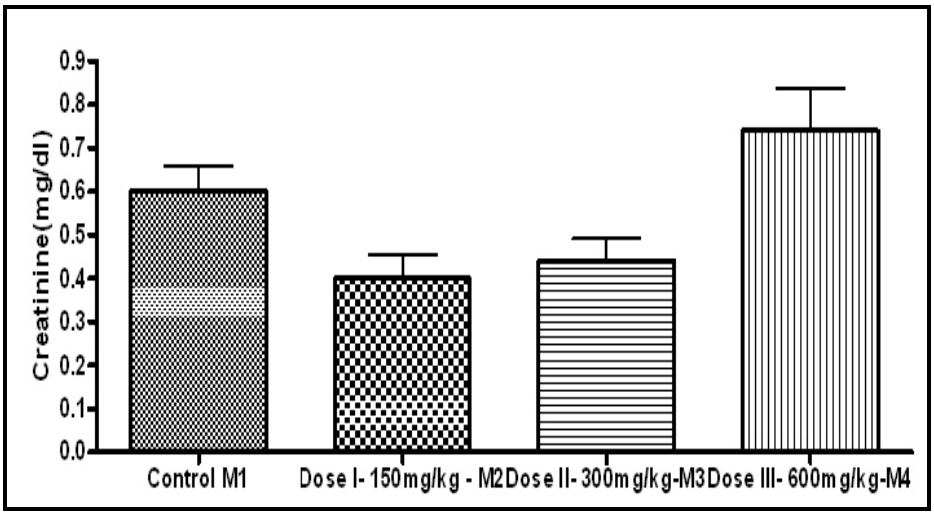


Figure No.9: Effect of different treatments on creatinine levels in male rat

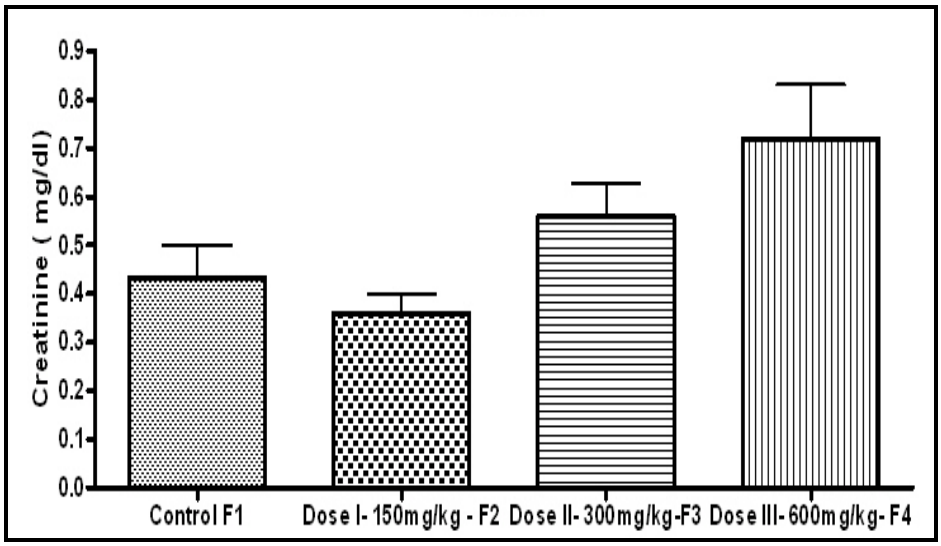


Figure No.10: Effect of different treatments on creatinine levels in female rat

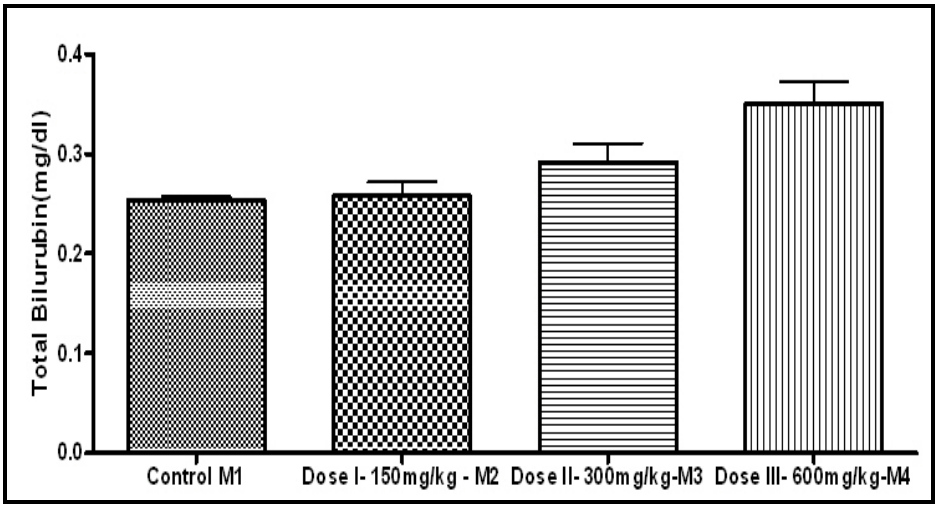


Figure No.11: Effect of different treatments on total bilirubin levels in male rat

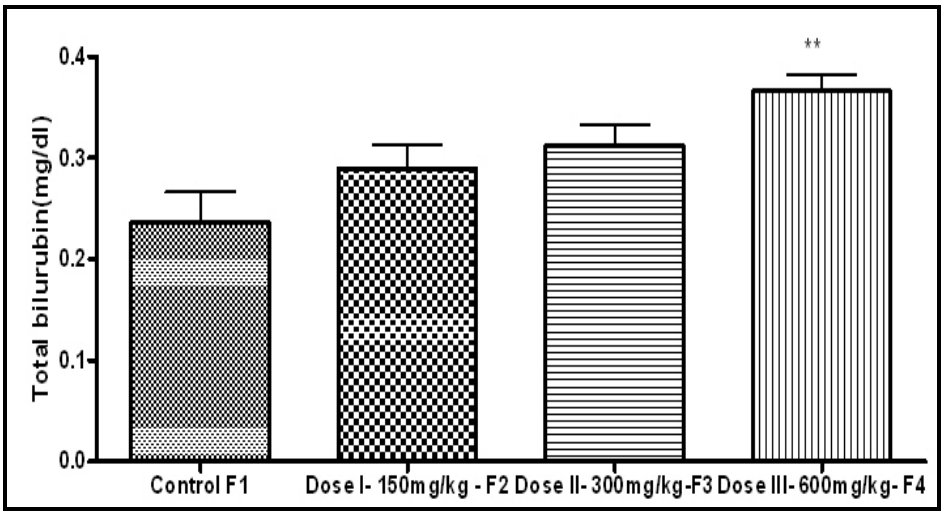


Figure No.12: Effect of different treatments on total bilirubin levels in female rat

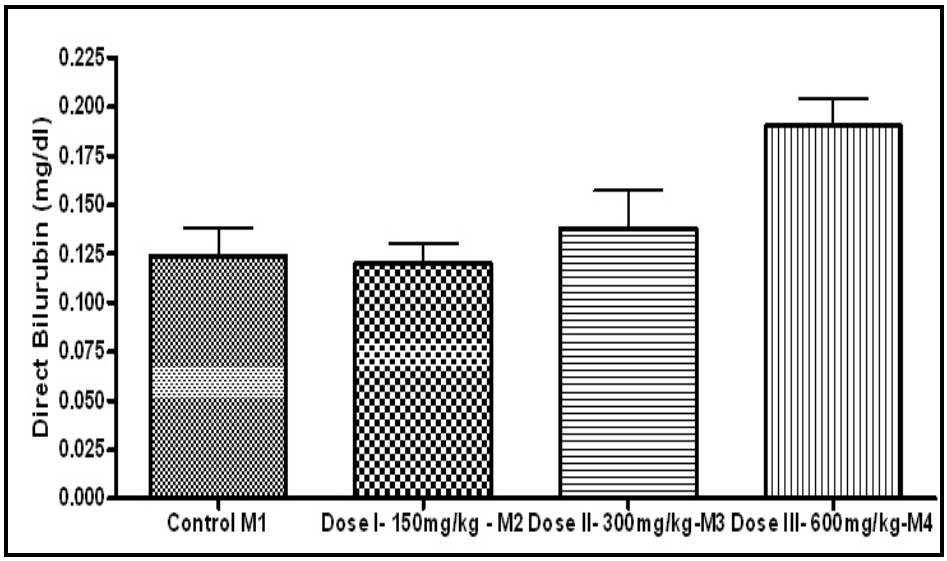


Figure No.13: Effect of different treatments on direct bilirubin levels in male rat

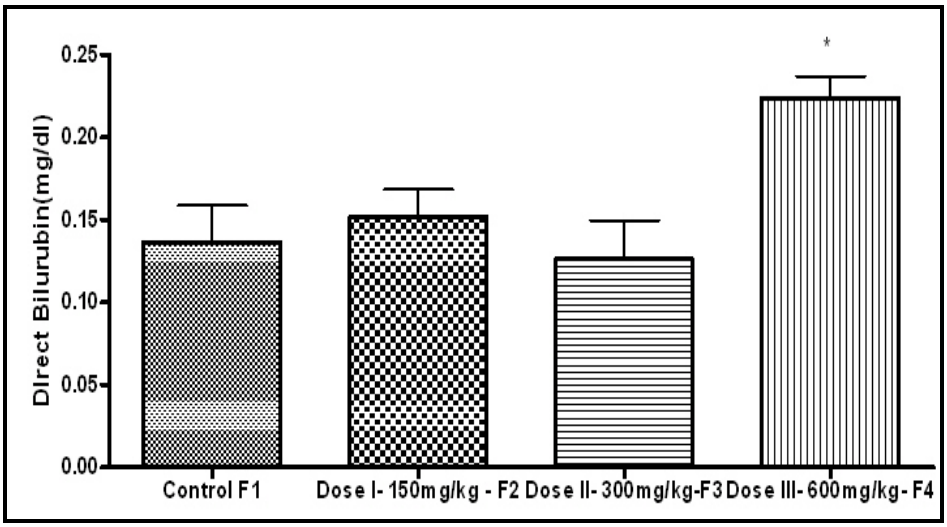


Figure No.14: Effect of different treatments on direct bilirubin levels in female rat

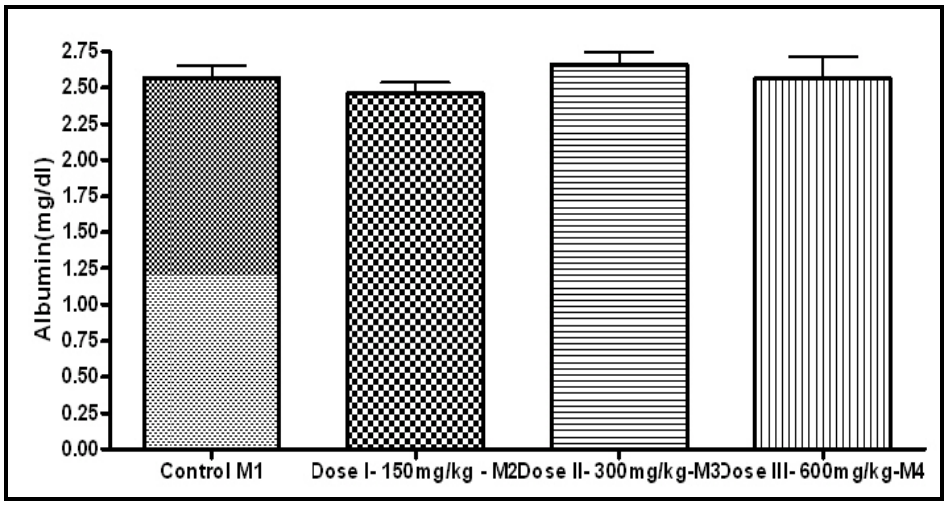


Figure No.15: Effect of different treatments on Albumin levels in male rat

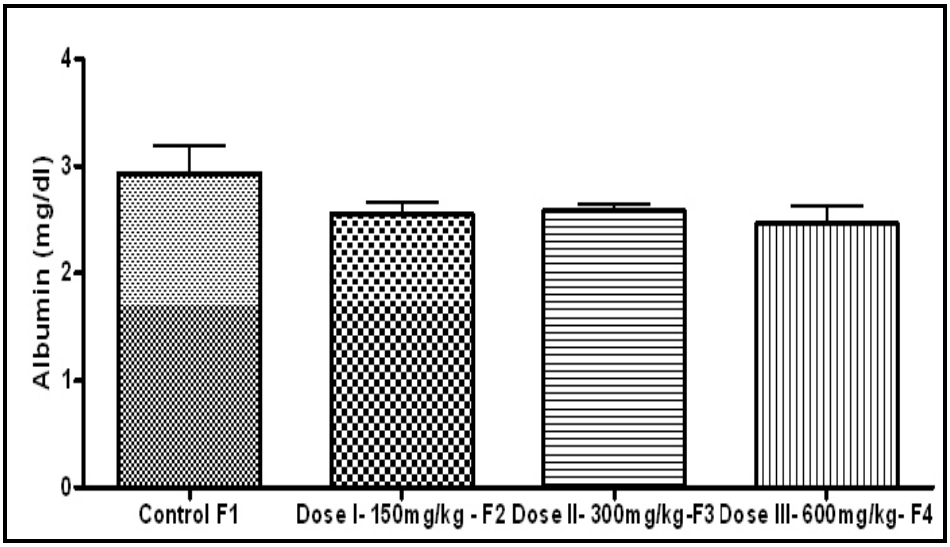


Figure No.16: Effect of different treatments on Albumin levels in female rat

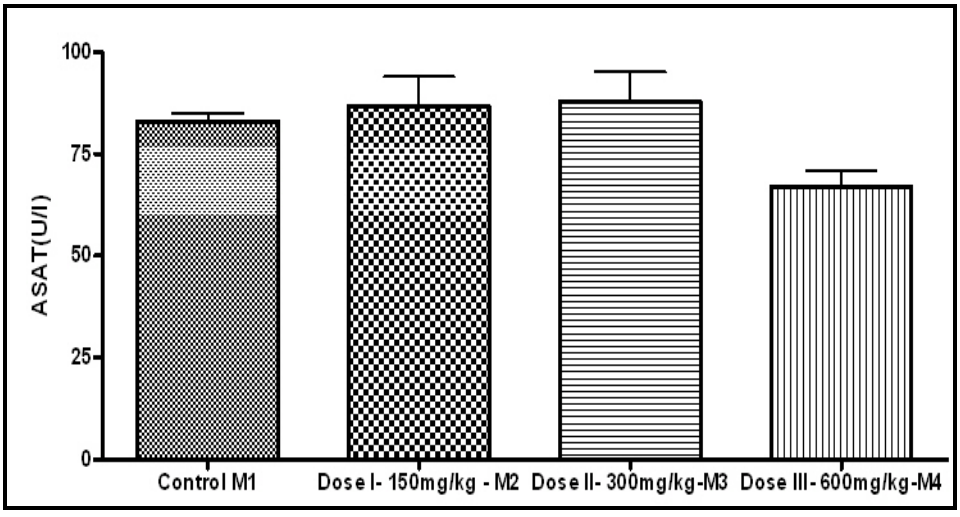


Figure No.17: Effect of different treatments on ASAT levels in male rat

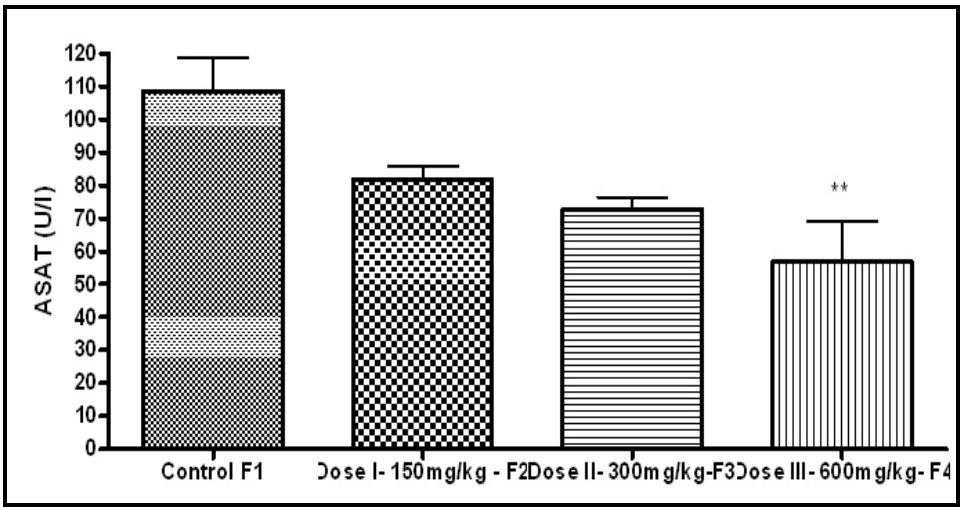


Figure No.18: Effect of different treatments on ASAT levels in female rat

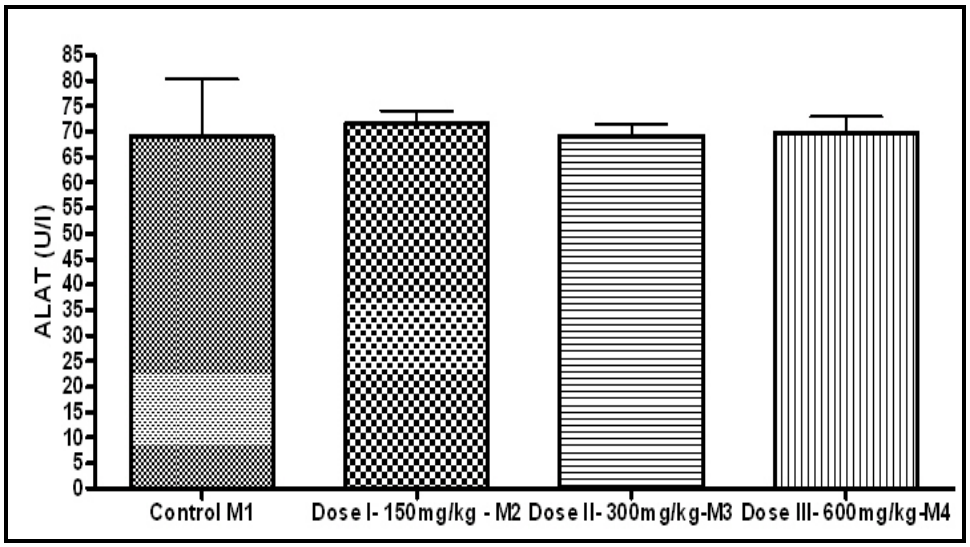


Figure No.19: Effect of different treatments on ALAT levels in male rat

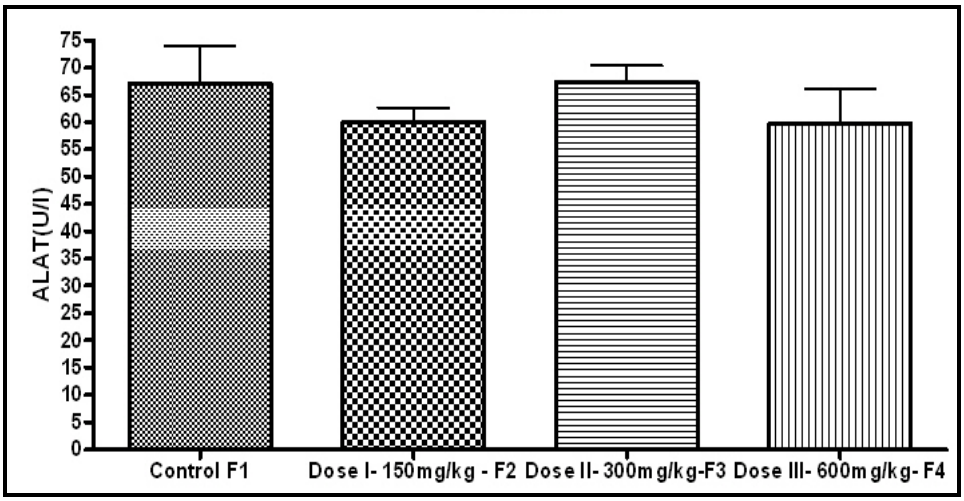
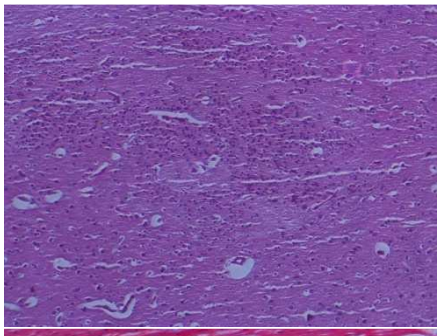
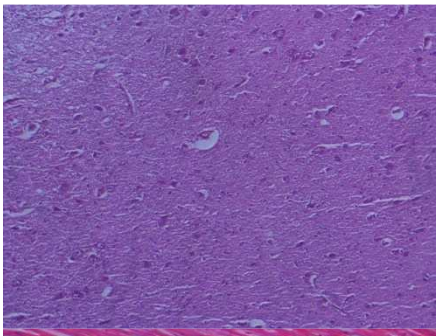
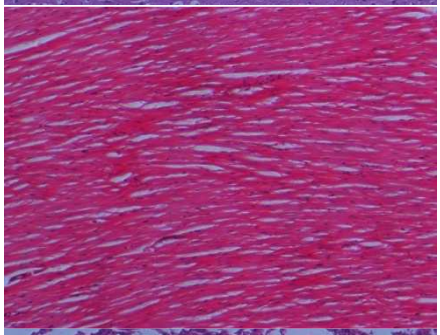
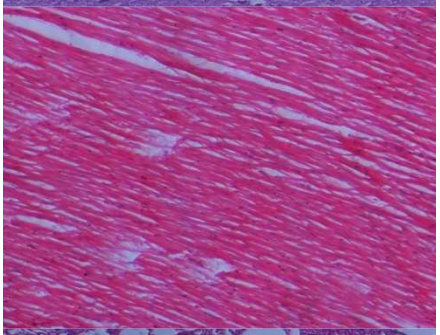
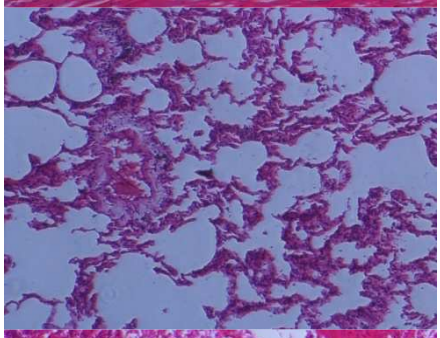
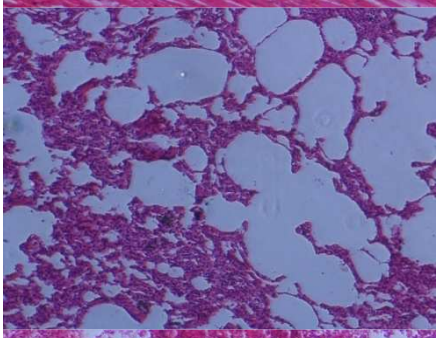
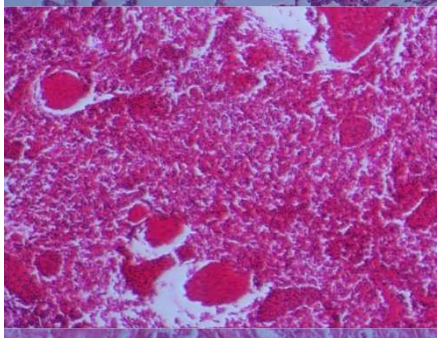
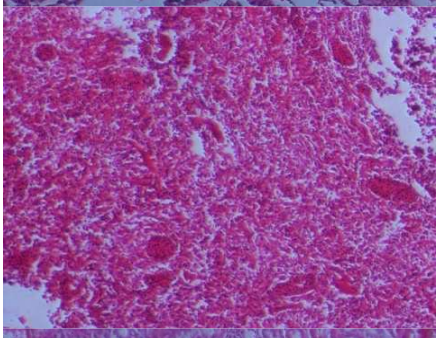
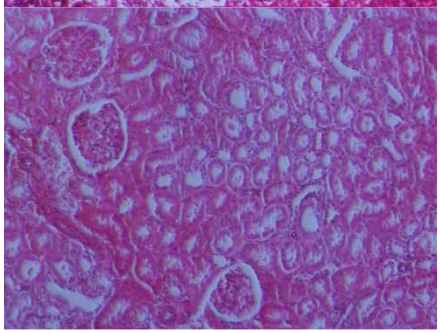
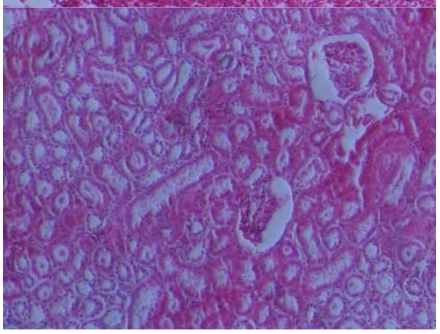


Figure No.20: Effect of different treatments on ALAT levels in female rat

Histology	Control	High Dose (600mg/kg)
• Brain		
• Heart		
• Lungs		
• Spleen		
• Kidneys		

- **Liver**
- **Adrenal glands**
- **Testes**
- **Ovary**

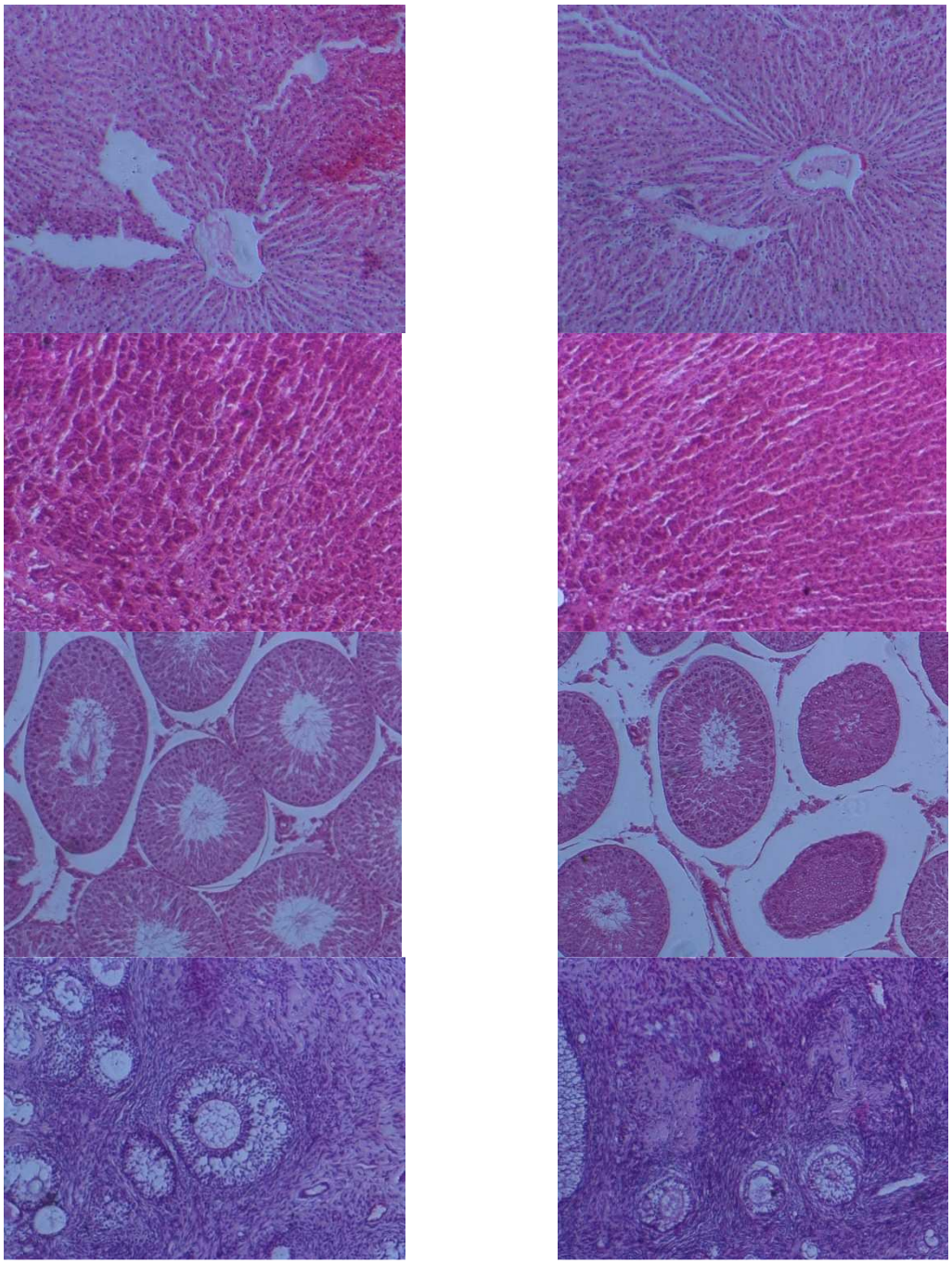


Figure No.21: Histopathological analysis of vital organs

CONCLUSION

The acute and sub-acute toxicity studies of *Vaivilangam chooranam* do not exhibit any toxic effect on animals. The drug was found to be safe on biological and haematological parameters. According to the statistical analysis, all values are expressed as Mean±SEM. One way ANONA followed by post hoc Tukey test. *,**,*** denotes $p<0.05$,$p<0.01$,$p<0.001$ as compared to control respectively. Thus, some useful therapeutic importance may develop out of the research work.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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