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SAFETY PROFILE OF KARISALAI KARPAM BY 28 DAYS REPEATED ORAL TOXICITY STUDY IN ANIMAL MODEL

G. Bharathkumar[1]* and M. Pitchiah Kumar[2]

[1]Asst. Professor, The Tamilnadu Dr. M. G. R. Medical University, Chennai.

[2]State Drug Licensing Authority (IM), Chennai.

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***Corresponding Author**
Dr. G. Bharathkumar
Asst. Professor, The Tamilnadu Dr. M. G. R. Medical University, Chennai.

ABSTRACT

Karisalai Karpam is a hepato protective herb in its combination. In Bogar 7000 the Siddha literature this drug has been mentioned. The composition of *Karisali Karpam* (KK) as per the Siddha traditional preparation are Eclipta alba (*Vellai Karisalai Samoolam*), Wedelia chinensis (*Manjal Karisalai samoolam*), Indigofera tinctoria (*Neeli Samoolam*), Sphaeranthus indicus (*Kottaikarandhai Samoolam*), Centella asiatica (*Vallarai Samoolam*), Acalypha indica (*Kuppeimeni*) and Coldenia procumbens (*Siruserupadai*). KK has various medicinal properties. Herbal remedies are considered safer and less damaging to the human body than synthetic drugs. In India, about 80% of the rural population uses medicinal herbs or indigenous systems of medicine. The toxicity profile of *Karisalai Karpam* has not been addressed, hence it was taken for 28 days repeated oral toxicity study. It was carried out as per OECD guidelines. (Guideline - 407) Animals were divided into three groups of 6 animals each consist of 3 male and 3 female rats. The animals were administrated with the studydrug once daily for 28 days. The animals in group I (control group) received normal saline 5 ml/kg b.w. The animals in group II received low dose of *Karisalai Karpam*200 mg/kg b.w(p.o) and group III receivedhigh dose of *Karisalai Karpam* 400 mg/kg b.w(p.o). The results of Sub-acute oral administration for 28 consecutive days of *Karisalai Karpam* (200 and 400 mg/kg, p.o) have shown no significant changes in the body weight, food intake, water intake general behavior, clinical signs as well as mortality of rats.

KEYWORDS: *Sub acute toxicity, Animal study, Siddha, Poly herbal medicine, Karisalai Karpam.*

1. INTRODUCTION

Medicine is one of the greatest feats of mankind which brings health and happiness. It is very much important to note that the growth of medicine started based on the nature, the customs and the civilization of the respective peoples of the world.[1] *Karisalai Karpam* is a hepato protective herb in its combination. In Bogar 7000 the Siddha literature this drug has been mentioned. The composition of *Karisali Karpam* (KK) as per the Siddha traditional preparation are Eclipta alba (*Vellai Karisalai Samoolam*), Wedelia chinensis (*Manjal Karisalai samoolam*), Indigofera tinctoria (*Neeli Samoolam*), Sphaeranthus indicus (*Kottaikarandhai Samoolam*), Centella asiatica (*Vallarai Samoolam*), Acalypha indica (*Kuppeimeni*) and Coldenia procumbens (*Siruserupadai*). KK has various medicinal properties.

Before starting efficacy profile of any drug, the toxicity profile has to be conducted. Herbal remedies are considered safer and less damaging to the human body than synthetic drugs.[2] In India, about 80% of the rural population uses medicinal herbs or indigenous systems of medicine.[3] During the evaluation of the toxic characteristics of traditional medicines and herbal preparations, the determination of LD_{50} is usually an initial step to be conducted. Data from the acute toxicity study may (a) serve as the basis for classification (b) provide initial information on the mode of toxic action of a substance; (c) help arrive at a dose of a new compound; (d) help in dose determination in animal studies; and (e) help determine LD50 values that provide many indices of potential types of drug activity.[4]

The toxicity profile of *Karisalai Karpam* has not been addressed, hence it was taken for 28 days repeated oral toxicity study. It was carried out as per OECD guidelines. (Guideline - 407)

2. MATERIALS AND METHODS

2.1 Sub-acute toxicity study

Healthy adult Wistar albino rat weighing between 200-220 g were used for the study. The animals were housed in poly propylene cages and were kept in well ventilated with 100% fresh air by air conditioning. A 12 light / dark cycle were maintained. Room temperature was maintained between 22 $\pm$ 2^{o} Cand relative humidity 50–65%. They were provided with food (Sai feeds, Bangalore, India) and water *ad libitum*. All the animals were acclimatized to the laboratory for 7 days prior to the start of the study. The experimental protocol was approved by The Institutional Animal Ethics Committee of Sathyabama University, Chennai, Tamil

Nadu, India. Approvalreference number- SU/CLATR/IAEC/IV/013/2016

Animals were divided into three groups of 6 animals each consist of 3 male and 3 female rats. Group I: Animals received saline 5 ml/kg b.w (p.o) Group II:Animals received low dose of test drug 200 mg/kg (p.o)Group III:Animals received high dose of test drug 400 mg/kg (p.o) The animals were randomly divided into control group and drug treated groups for two different doses viz. low dose (200 mg/kg b.w) and high dose (400 mg/kg b.w). The animals were administrated with the studydrug once daily for 28 days. The animals in group I (control group) received normal saline 5 ml/kg b.w. The animals in group II received low dose of *Karisalai Karpam*200 mg/kg b.w(p.o) and group III receivedhigh dose of *Karisalai Karpam* 400 mg/kg b.w(p.o).

The rats were weighed periodically and observed for signs of toxicity pertains to C.N.S, C.V.S, A.N.S including behavioral changes, food - water intake and morphological changes. At the end of 28th day, the animals were fasted for overnight with free access to water. On 29th day the animals were sacrificed with excess anesthesia. Blood samples were collected from aorta and stored in EDTA (ethylenediamine –tetra actate) for Hematological analysis and for serum generation for biochemical analysis. The vital organs including heart, brain, lungs, spleen, kidneys, liver, stomach, testes, and ovary were harvested and carefully examined for gross lesions. The organs were preserved in 10% formalin for histopathological assessment and interpretation.

2.2 Hematological analysis

Blood samples were analyzed using established procedures and automated Bayer Hematology analyzer. Parameters evaluated include Packed Cell Volume (PCV), Red Blood Cells (RBC) count, Hemoglobin (Hb), Platelet Count, Total and Differential White Blood Cell count (TC & DC), Mean cell Haemoglobin Concentration (MCHC), Mean Red Cell Volume (MCV) and Mean Cell Hemoglobin (MCH).

2.3 Biochemical analysis

Serum samples were analyzed for High Density Lipoprotein (HDL), Low density Lipoprotein (LDL), Triglycerides (TGL), Blood urea nitrogen, Creatinine, Albumin, Total Protein, Total Cholesterol, Glucose, Uric acid, Aspartate Transaminase (AST), Alanine amino Transaminase (ALT) and Alkaline Phosphatase (ALP) using Mind ray auto analyzer model BS 120.

2.4 Histopathological evaluation

Organs included of heart, brain, lungs, spleen, kidneys, liver, stomach, testes and ovary. Histological slides of organs were made and observed under the microscope. The pathological observations of cross section of these organs were performed on gross and microscopic bases. Histological examinations were performed on the preserved tissues with particular emphasis on those which showed gross pathological changes.

2.5 Statistical analysis

The statistical analysis was carried by one way ANOVA (GRAPH PAD PRISM 5 computer program). Results were expressed as mean ± standard error. A statistical comparison was carried out using the Dunnet's test for the control and treatment group. P-values less than 0.05 were set as the level of significance.

3. RESULTS

Effect of *Karisalai Karpam* on clinical signs of male and female rats in Sub-acute toxicity study

No significant signs of toxicity were observed in male and female rats during the 28 days of consecutive treatment with *Karisalai Karpam* at the dose of 200 and 400 mg/kg b.w. Results were tabulated in Table 1.

Table 1: Clinical Signs of rats treated with *Karisalai Karpam* for 28 days Sub-acute toxicitystudy.

Clinical Signs Parameters for the duration of 28 days	Group IControl	Group IIKK 200mg/ Kg	Group IIIKK 400mg/ Kg
Number of animals observed	5 Male 5 Female	5 Male 5 Female	5 Male 5 Female
Lacrimation	Absence	Absence	Absence
Salivation	Absence	Absence	Absence
Animal appearance	Normal	Normal	Normal
Tonic Movement	Absence	Absence	Absence
Clonic Movement	Absence	Absence	Absence
Laxative action	Absence	Mild	Moderate
Touch Response	Normal	Normal	Normal
Mobility	Normal	Normal	Normal
Respiratory Distress	Nil	Nil	Nil
Skin Color	Normal	Normal	Normal
Stereotype behavior	Absence	Absence	Absence
Piloerection	Absence	Absence	Absence
Limb Paralysis	Absence	Absence	Absence

Posture	Normal	Normal	Normal
Open field behavior	Normal	Normal	Normal
Muscular coordination	Normal	Normal	Normal
Muscle grip	Normal	Normal	Normal
Sedation	Absence	Absence	Absence
Social Behavior	Normal	Normal	Normal
Urine Analysis	No Abnormality	No Abnormality	No Abnormality
Fecal Pellet consistency	Normal	Soft blobs	Fluffy
Mortality	Nil	Nil	Nil

Effect of *Karisalai Karpam* on Body weight, food Intake and Water intake of Male and Female Rats in Sub-acute toxicity study

No significant differences were found between the initial and final body weight of the animals treated with test drug *Karisalai Karpam* and control. A similar absence of significant change was observed in the case of food and water consumption in both male and female rats treated with *Karisalai Karpam* at both the dose level of 200 and 400 mg/kg when compare to control group. Results were tabulated in Table 2, 3, 4, 5, 6 respectively.

Table 2: Effect of *Karisalai Karpam* on Body weight of Male and Female Rats in Sub-acutetoxicity study.

Treatment	Mean body weight in gms			
	Pre-Treatment		Post-Treatment	
	Male	Female	Male	Female
Group I	206.8 ± 6.30	208.2 ± 4.20	213 ± 7.1	219.8 ± 2.72
Group II	208.6 ± 7.26	205.4 ± 4.45	224.2 ± 5.45	229 ± 3.87
Group III	204.8 ± 4.14	205.2 ± 5.49	226 ± 7.8	230.4 ± 4.27

Values are mean ± S.D (n = 5 per group). Control and treatment groups were compared statistically using one way ANOVA followed by Dunnett's test.

Table 3: Quantitative data on the food intake of female rats treated with *Karisalai Karpam* for 28 days in Sub-acute toxicity study.

Treatment	Mean weight of feed (gms)			
	Day 1-7	Day 8 – 14	Day 15- 21	Day 22- 28
Group I	17.14 ± 4.25	21.43 ± 3.45	23 ± 3.36	25.57 ± 3.10
Group II	17.14 ± 2.79	19.29 ± 2.69	22.14 ±2.19	25.43 ± 2.69
Group III	20 ± 2.38	21.57 ± 2.63	24.14 ± 1.95	26.14 ± 2.116

Values are mean ± S.D (n = 5 per group). Control and treatment groups were compared statistically using one way ANOVA followed by Dunnett's test.

Table 4: Quantitative data on the food intake of male rats treated with *KarisalaiKarpam* for 28 days in Sub-acute toxicity study.

Treatment	Mean weight of feed (gms)			
	Day 1-7	Day 8 – 14	Day 15- 21	Day 22- 28
Group I	19.71 ± 1.59	20.43 ±1.17	22.29 ± 1.79	24.43 ± 2.22
Group II	19 ± 3.41	20.86 ± 3.07	23 ± 2.38	26.71 ± 1.60
Group III	21.29 ± 1.38	23.29 ± 1.25	25 ± 0.81	27 ± 2.08

Values are mean ± S.D (n = 5 per group). Control and treatment groups were compared statistically using one way ANOVA followed by Dunnett's test.

Table 5: Quantitative data on the water intake of female rats treated with *Karisalai Karpam* for 28 days in Sub-acute toxicity study.

Treatment	Mean water intake volume in ml			
	Day 1-7	Day 8 – 14	Day 15- 21	Day 22- 28
Group I	20.71 ± 2.69	22.29 ± 2.49	23.57 ± 2.87	33.14 ± 3.02
Group II	21.43 ± 2.63	24.71 ± 2.7	27.14 ± 2.96	30.86 ± 1.86
Group III	19.43 ± 2.22	23.43 ± 2.14	28.41 ± 2.61	31.14 ± 2.73

Values are mean ± S.D (n = 5 per group). Control and treatment groups were compared statistically using one way ANOVA followed by Dunnett's test.

Table 6: Quantitative data on the water intake of male rats treated with *Karisalai Karpam* for 28 days in Sub-acute toxicity study.

Treatment	Mean water intake volume in ml			
	Day 1-7	Day 8 – 14	Day 15- 21	Day 22- 28
Group I	18.67 ± 3.67	23.17 ± 3.65	28.17 ± 2.63	33.17 ± 1.72
Group II	20.71 ± 2.69	22.29 ± 2.49	23.57 ± 2.87	33.14 ± 3.02
Group III	20.86 ± 1.06	21.86 ± 1.09	23.57 ± 0.97	32.43 ± 2.50

Values are mean ± S.D (n = 5 per group). Control and treatment groups were compared statistically using one way ANOVA followed by Dunnett's test.

Effect of *Karisalai Karpam* on Hematological and Biochemical parameters of male and female rats in Sub-acute toxicity study

No statistically significant differences with respect to complete blood cell count, liver function, kidney function, lipid profile, glucose and protein analysis were observed in hematological and biochemical parameters of rats treated with *Karisalai Karpam* at both the dose level of 200 and 400 mg/kg when compare to control group. Results were tabulated in Table 7, 8, 9, 10 respectively.

Table 7: Effect of *Karisalai Karpam* on Haematology profile of female rats in sub-acute study.

Parameters	Group I	Group II	Group III
WBC count (×103µl)	9.82±2.22	10.42±3.31	10.94±2.89
RBC (×10 6 µl)	7.02±1.29	7.384±0.81	7.16±1.76
PLT (×10 3 µl)	444.2±141	448.6±121.2	545.6±139.5
MCV (fl)	58±4.7	60.4±4.93	63.2±6.7
MCH (pg)	18.86±1.34	19.2±2.93	19±2.55
MCHC (g/dl)	32.14±3.60	33.88±6.45	33±4.18
HGB (g/dl)	12.4±1.14	13.8±1.70	14.2±2.66
Lymphocyte (%)	72.6±2.30	72.16±6.61	69.2±10.64
Monocyte (%)	3.98±1.05	2.2±1.14	3.54±0.94
Granulocyte (%)	23.64±5.96	20.16±5.83	25±7.17
MPV (fl)	4.2±0.83	5.56±1.12	6±0.70
RDW (%)	12.72±1.67	15.06±2.94	13.82±2.08
HCT (%)	36.4±6.42	44.6±3.64	36.4±5.98
PDW	14.74±2.61	15.94±3.09	16±3.31
PCV (%)	44±5.47	40.8±12.91	41.4±13.48
Neutrophils (%)	68.8±3.34	68.4±7.05	55.6±11.76

Values are mean ± S.D (n = 5 per group). Control and treatment groups were compared statistically using one way ANOVA followed by Dunnett's test.

Table 8: Effect of *Karisalai Karpam* on Haematology profile of male rats in sub-acute study.

Parameters	Group I	Group II	Group III
WBC count (×103µl)	9.68±2.93	16.32±1.35	15.24±1.09
RBC (×10 6 µl)	7.5±0.26	8.504±0.36	8.174±0.47
PLT (×10 3 µl)	240.8±15.45	400.8±67.81	601±71.32
MCV (fl)	55.8±2.36	57.96±4.68	55.16±3.05
MCH (pg)	17.34±0.72	18.06±0.73	18.3±0.81
MCHC (g/dl)	31.96±1.34	34.66±3.91	33.94±2.72
HGB (g/dl)	13.8±0.78	15.56±0.56	15.32±0.76
Lymphocyte (%)	76.74±6.11	79.82±5.16	81.56±6.09
Monocyte (%)	4.88±0.70	3.34±0.75	3.36±0.74
Granulocyte (%)	18.7±4.50	18.38±4.16	17.96±4.18
MPV (fl)	5.04±0.63	4.96±0.75	6.38±1.32
RDW (%)	11.92±0.83	12.64±1.32	13.16±0.65
HCT (%)	39.76±4.29	46.42±2.84	46.4±1.24
PDW	16.74±1.05	15.1±1.05	17.28±0.99
PCV (%)	48.6±4.72	46±4.72	49.4±5.59
Neutrophils (%)	71.8±4.26	72.6±4.26	68.2±7.19

Values are mean ± S.D (n = 5 per group). Control and treatment groups were compared statistically using one way ANOVA followed by Dunnett's test.

Table 9: Effect of *Karisalai Karpam* on Bio-chemistry profile of female rats in sub-acute study.

Parameters	Group I	Group II	Group III
Blood sugar (mg/dl)	100.8±22.64	92.6±18.23	104±16.63
BUN (mg/dl)	16.6±3.64	13.8±4.76	14.8±3.56
Serum creatinine (mg/dl)	0.86±0.30	0.9±0.33	0.98±0.17
Serum total cholesterol (mg/dl)	119.4±12.9	111.6±13.48	129.8±28.29
Serum triglycerides level (mg/dl)	89±9.40	78.2±7.79	88.6±15.01
Serum HDL cholesterol (mg/dl)	47.2±10.23	63.2±6.05	67.6±.93
Serum LDL	40.6±7.23	51.2±e11.03	58.2±20.24
cholesterol (mg/dl)			
Serum VLDL cholesterol (mg/dl)	18.4±2.07	16±3.74	18.4±2.40
Serum total protein(g/dl)	5.4±1.14	5.56±1.05	6.5±0.72
Serum albumin(g/dl)	3.7±0.80	4.34±0.51	3.42±0.65
(AST) (IU/ml)	80.4±11.28	88±15.41	113.2±27.6
(ALT) (IU/L)	33±9.11	30.8±7.32	38.2±6.49
(ALP) (IU/L)	140.8±15.12	111±14.5	219.6±30.36

Values are mean ± S.D (n = 5 per group). Control and treatment groups were compared statistically using one way ANOVA followed by Dunnett's test.

Table 10: Effect of *Karisalai Karpam* on Bio-chemistry profile of male rats in sub-acute study.

Parameters	Group I	Group II	Group III
Blood sugar (mg/dl)	86.2±25.4	106.4±3.28	138±21.91
BUN (mg/dl)	20.6±3.2	19.4±2.88	18.4±4.72
Serum creatinine (mg/dl)	0.596±0.11	0.86±0.23	0.84±0.38
Serum total cholesterol (mg/dl)	98.4±28.7	80.4±44.47	136±9.56
Serum triglycerideslevel (mg/dl)	94.4±8.8	109.8±7.91	86.4±13.32
Serum HDL cholesterol (mg/dl)	70±7.5	64±7.17	59.8±11.48
Serum LDL cholesterol (mg/dl)	20±4.8	32.2±12.07	33.2±24.41
Serum VLDL cholesterol (mg/dl)	16.16±5.57	22±3.80	18.78±2.83
Serum total protein(g/dl)	5.6±0.84	5.56±1.29	5.42±0.61
Serum albumin(g/dl)	3.62±0.59	3.7±0.91	3.52±0.77
(AST) (IU/ml)	106.6±20.48	114.2±17.64	131.8±5.11
(ALT) (IU/L)	35.2±7.53	29.6±8.90	38.2±7.85

(ALP) (IU/L)	128.2±37.12	176.4±55.67	194.6±22.41

Values are mean ± S.D (n = 5 per group). Control and treatment groups were compared statistically using one way ANOVA followed by Dunnett's test.

Effect of *Karisalai Karpam* on organ weight of male and female rats in Sub-acutetoxicity study

No significant change were recorded in organ weight of male and female rats treated with *KarisalaiKarpam* at the dose of 200 and 400 mg/kg when compare to control group animals. Macroscopic examination of the vital organs of treatment group animals revealed no abnormalities in the colour or texture when compared with the organs of the control group. Results were tabulated in Table 11, 12 respectively.

Table 11: Quantitative data on absolute organ weight of female rats treated with *Karisalai Karpam* for 28 days in Sub-acute toxicity study.

Treatment	Heart (gms)	Liver (gms)	Kidneys (gms)	Spleen (gms)	Brain (gms)	Lung (gms)	Stomach (gms)	Ovary (gms)
Group I	0.78± 0.06	5.0± 0.52	1.18± 0.04	0.4± 0.06	1.87± 0.19	1.07± 0.13	0.77± 0.07	0.036± 0.006
Group II	0.76± 0.07	5.22± 0.64	1.18± 0.04	0.43± 0.25	1.76± 0.40	1.02± 0.07	0.85± 0.10	0.035± 0.003
Group III	0.74± 0.06	5.44± 0.51	1.23± 0.03	0.39± 0.04	1.93± 0.39	1.04± 0.12	0.82± 0.13	0.038± 0.002

Values are mean ± S.D (n = 5 per group). Control and treatment groups were compared statistically using one way ANOVA followed by Dunnett's test.

Table 12: Quantitative data on absolute organ weight of male rats treated with *Karisalai Karpam* for 28 days in Sub-acute toxicity study.

Treatment	Heart (gms)	Liver (gms)	Kidneys (gms)	Spleen (gms)	Brain (gms)	Lung (gms)	Stomach (gms)	Testes (gms)
Group I	0.758 ±0.10	5.08 ±0.63	1.21 ± 0.03	0.39 ±0.07	1.87 ±0.10	1.13 ±0.06	0.78 ±0.05	2.20 ±0.09
Group II	0.65 ± 0.05	4.74± 0.35	1.22 ± 0.07	0.40 ±0.06	1.75± 0.09	1.05± 0.05	0.84± 0.08	2.03± 0.17
Group III	0.71 ± 0.07	5.14± 0.25	1.152± 0.08	0.37± 0.04	1.82± 0.16	1.10± 0.08	0.81± 0.09	1.90± 0.18

Values are mean ± S.D (n = 5 per group). Control and treatment groups were compared statistically using one way ANOVA followed by Dunnett's test.

Effect of *Karisalai Karpam* on Histopathological changes of male and female rats in Sub-acute toxicity study

The histological section of the kidney belongs to group II and III treated with 200 and 400 mg/kg of test drug revealed normal morphology of glomeruli and tubules with no signs of inflammation when compare to that of the group I saline treated rats.

Histopathological observation of heart belongs to group II and III treated with 200 and 400 mg/kg of test drug revealed the presence of bundles of cardiac fiber with normal myofibrillar striations when compare to that of the group I saline treated rats.

Microscopic section of liver samples belongs to group II and III treated with 200 and 400 mg/kg of test drug revealed the presence of normal hepatocytes with no signs of degeneration and edema when compare to that of the group I saline treated rats.

Histology of brain belongs to group II and III treated with 200 and 400 mg/kg of test drug revealed the presence of normal cortex showing neurons, glial cells and capillaries. Section ofcerebellum shows distinct molecular and granular layer. Neuronal architecture appears normal with sufficient numbers. Similar morphology was appeared in sample belongs to group I rats.

Microscopic examination of lung belongs to group II and III treated with 200 and 400 mg/kg of test drug revealed normal alveoli and alveolar sac with no signs of infiltration when compare to that of the group I saline treated rats.

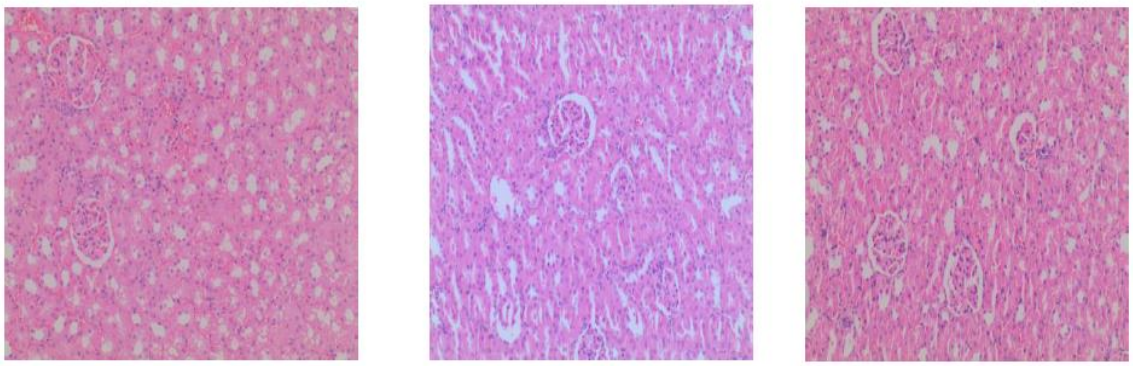
Cross section of spleen samples belongs to group II and III treated with 200 and 400 mg/kg of test drug revealed the presence of typical red pulp and white pulp and central arteriole with few macrophages when compare to that of the group I saline treated rats.

H&E staining of stomach samples belongs to group II and III treated with 200 and 400 mg/kg of test drug revealed normal anatomy of muscular stomach – Epithelial layer keratinized stratified squamous epithelium, Lamina propria and Submucosa when compare to that of the group I saline treated rats.

Histopathological analysis of ovary samples belongs to group II and III treated with 200 and 400 mg/kg of test drug revealed normal ovary showing many corpus luteum (CL) and few mature ovarian follicles with no signs of abnormality when compare to that of the group I

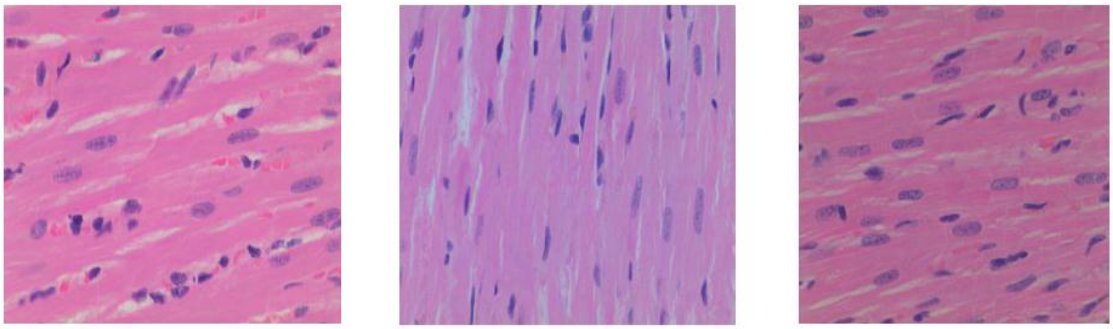
saline treated rats.

Microscopic section of testes belongs to group II and III treated with 200 and 400 mg/kg of test drug revealed the presence of normal seminiferous tubules with various stages germ cells when compare to that of the group I saline treated rats.



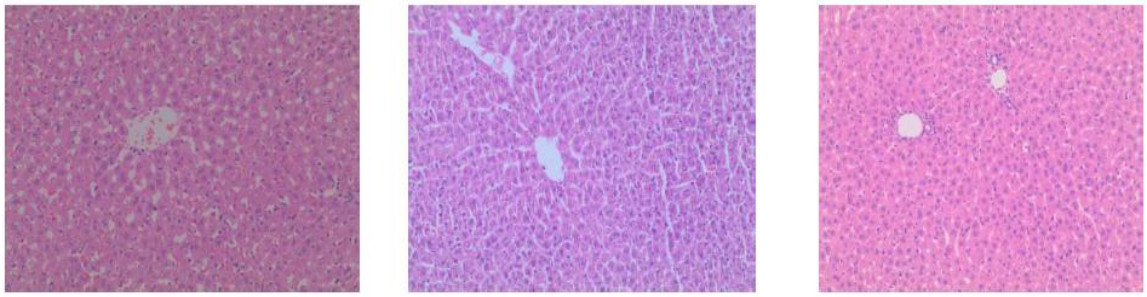
Group I **Group II** **Group III**

Fig. 1: Histopathology of Kidney (Sub-Acute toxicity) Female Rat High Power Magnification 40X.



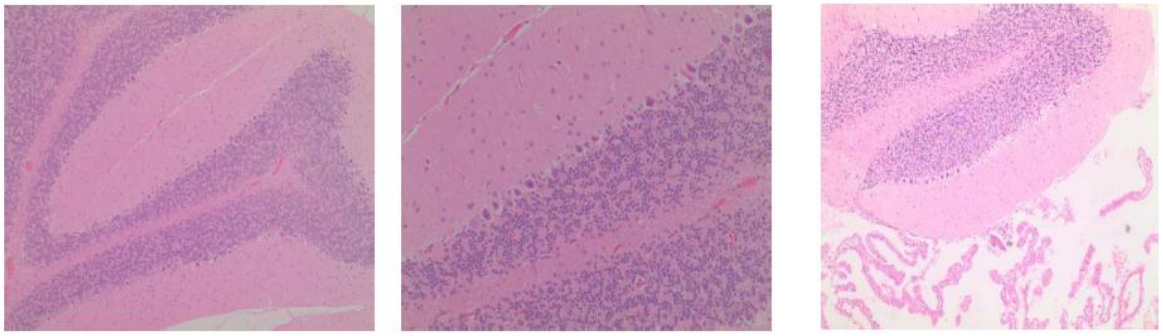
Group I **Group II** **Group III**

Fig. 2: Histopathology of Heart (Female Rat) High Power Magnification 40X.



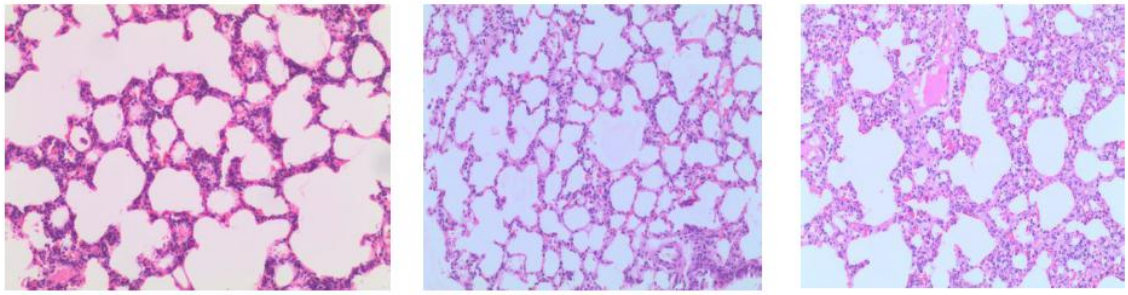
Group I **Group II** **Group III**

Fig. 3: Histopathology of Liver (Female Rat) High Power Magnification 40X.



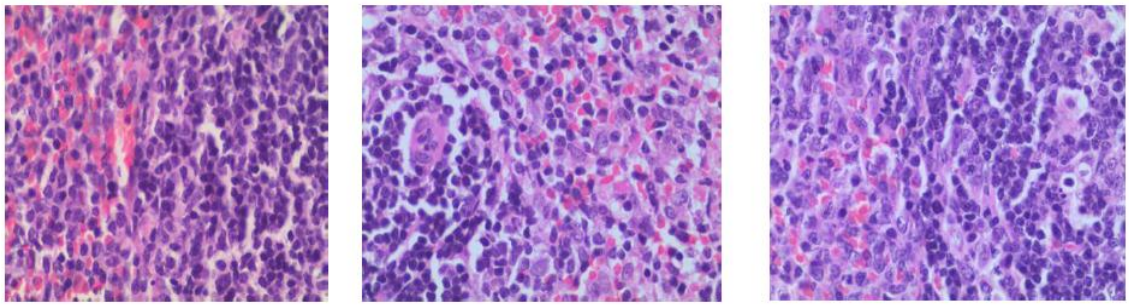
Group I **Group II** **Group III**

Fig. 4: Histopathology of Brain (Female Rat) High Power Magnification 40X.



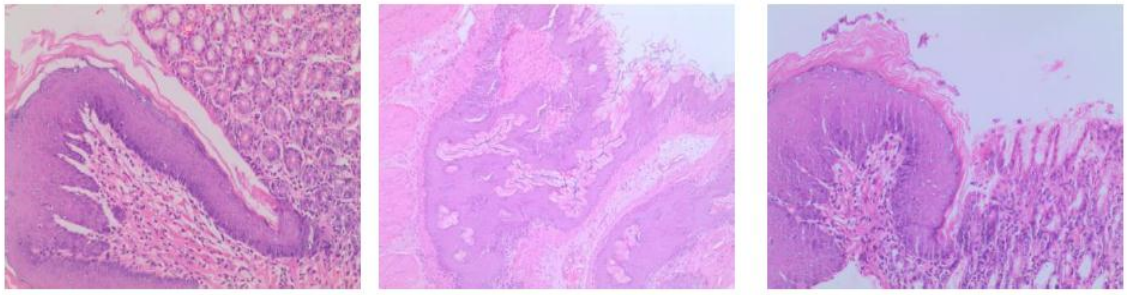
Group I **Group II** **Group III**

Fig. 5: Histopathology of Lung (Female Rat) High Power Magnification 40X.



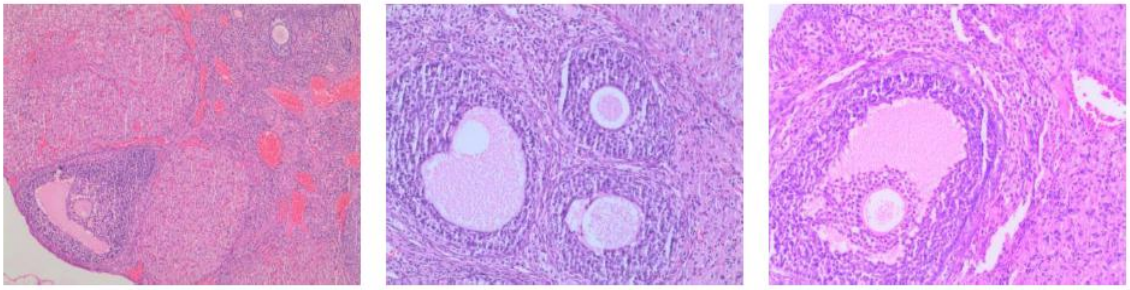
Group I **Group II** **Group III**

Fig. 6: Histopathology of Spleen (Female Rat) High Power Magnification 40X



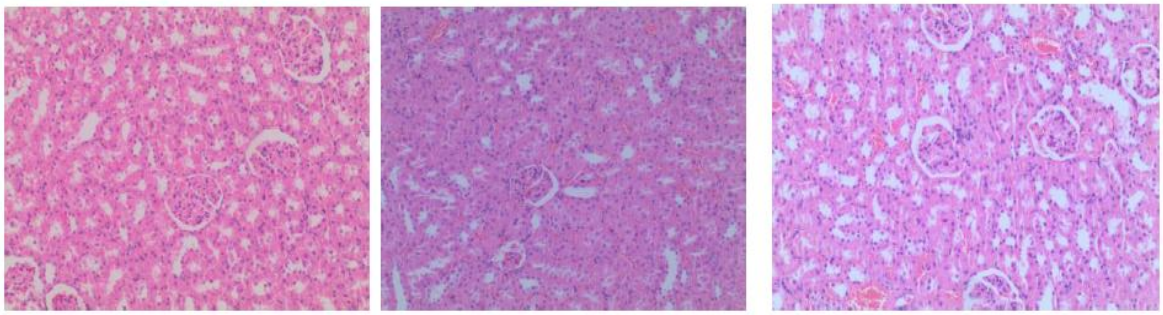
Group I **Group II** **Group III**

Fig. 7: Histopathology of Stomach (Female Rat) High Power Magnification 40X.



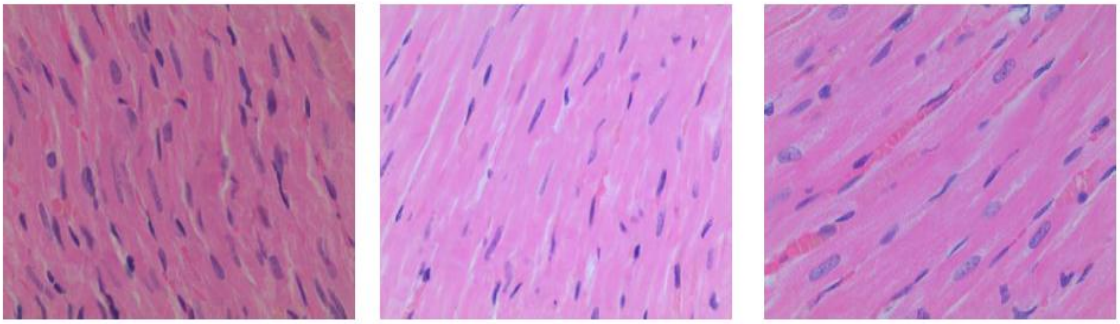
Group I **Group II** **Group III**

Fig. 8: Histopathology of Ovary (Female Rat) High Power Magnification 40X.



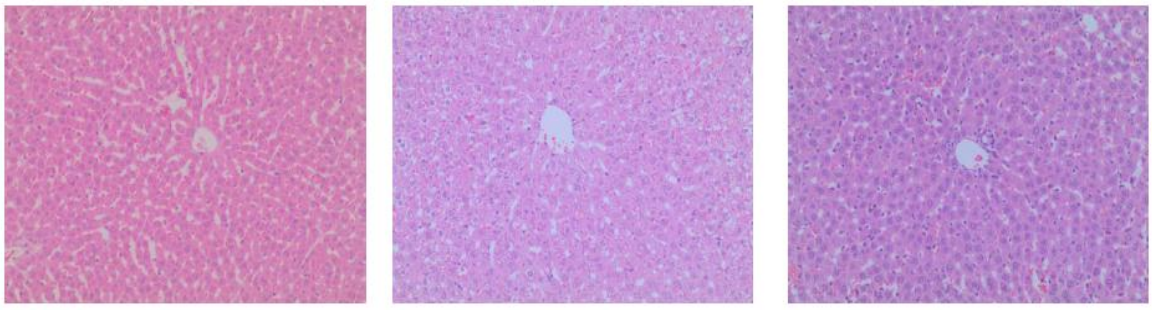
Group I **Group II** **Group III**

Fig. 9: Histopathology of Kidney (Male Rat) High Power Magnification 40X.



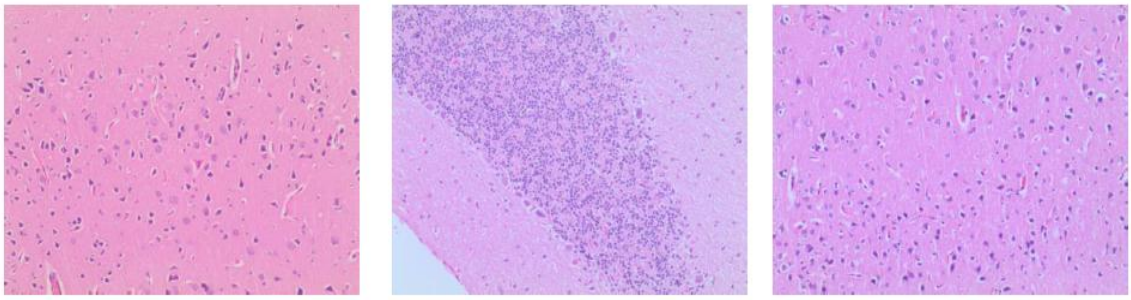
Group I **Group II** **Group III**

Fig. 10: Histopathology of Heart (Male Rat) High Power Magnification 40X.



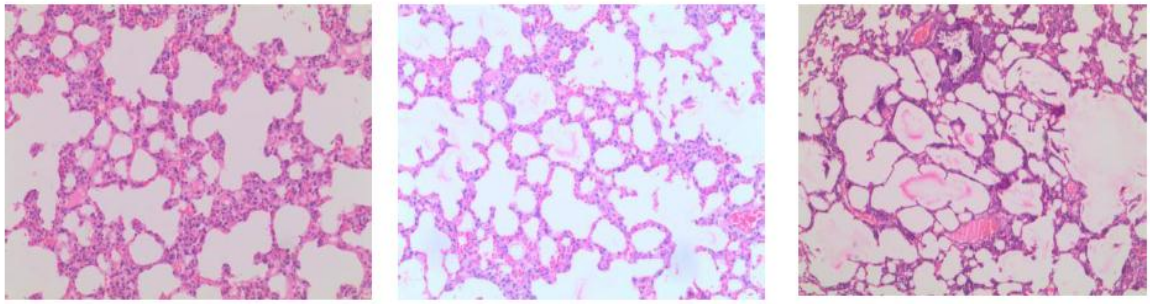
Group I **Group II** **Group III**

Fig. 11: Histopathology of Liver (Male Rat) HighPower Magnification 40X.



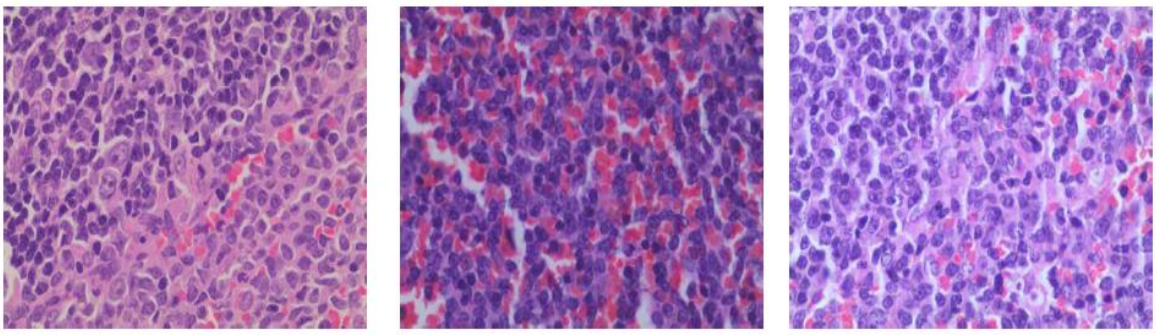
Group I **Group II** **Group III**

Fig. 12: Histopathology of Brain (Male Rat) High Power Magnification 40X.



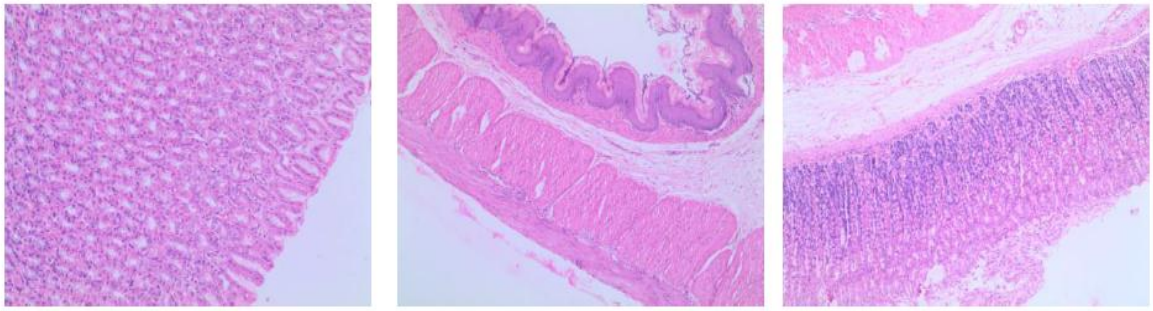
Group I **Group II** **Group III**

Fig. 13: Histopathology of Lung (Male Rat) High Power Magnification 40X.



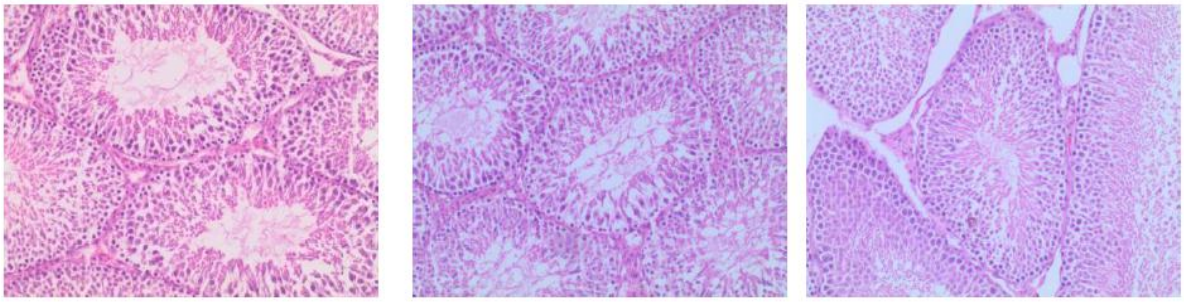
Group I **Group II** **Group III**

Fig. 14: Histopathology of Spleen (Male Rat) HighPower Magnification 40X.



Group I **Group II** **Group III**

Fig. 15: Histopathology of Stomach (Male Rat) High Power Magnification 40X.



Group I **Group II** **Group III**

Fig. 16: Histopathology of Testes (Male Rat) High Power Magnification 40X.

4. DISCUSSION

The results on the sub acute toxicity study has proven that *Karisalai Karpam* tolerance were observed at the dose of 200 and 400 mg/kg. Body weight changes are the key marker of adverse effects of siddha drugs and chemicals. Sub-acute oral administration for 28 consecutive days of *Karisalai Karpam* (200 and 400 mg/kg, p.o) have shown no significant changes in the body weight, food intake, water intake general behavior, clinical signs as well as mortality of rats. This implicate that long term administration of *Karisalai Karpam* could be safe and it shall be used for chronic ailments. Blood is a sensitive index of the physiological changes in response to any environmental pollutant in an animal. It has been documented that toxicant or potentially toxic substances induce conspicuous and significant changes in the haematological parameters.[5]

The hematopoietic system is one of the most sensitive targets for toxic substances and it is also an important marker of physiological and pathological status in human and animal studies.[6,7] The transaminases (AST, ALT, APT) are well known enzymes used as biomarkers predicting possible toxicity.[8] Generally, damage to the parenchymal liver cells will result in elevations of both these transaminases.

Karisalai Karpam at dose level of 200 and 400 mg/kg doesn't causes any alteration in RBC, WBC, PCV, Hb, Platelet Count, Total and Differential White Blood Cell count, MCHC, MCV and MCH. No significant alteration was observed in HDL, LDL, TGL, Blood urea nitrogen, Creatinine, Albumin, Total Protein, Total Cholesterol, Glucose, Uric acid, AST, ALT and ALP level of rats treated with 200 and 400 mg/kg of *Karisalai Karpam*.

Histopathological biomarkers are indices of induced organ toxicity or injury.[9] Macroscopic examination of the vital organs of *Karisalai Karpam* treated animals revealed no

abnormalities in the colour or texture when compared with the organs of the control group. No significant change were recorded in organ weight of male and female rats treated with *Karisalai Karpam* at the dose of 200 and 400 mg/kg when compare to control group animals. The histological section of the kidney. heart, liver, brain, lung, spleen, stomach, testes and ovary of experimental rats treated with 200 and 400 mg/kg of test drug *Karisalai Karpam* shown no significant changes when compare to control group animals.

5. CONCLUSION

The results from the 28 days repeated oral toxicity study reveals that sub acute oral administration of *Karisalai Karpam* is safe and collectively the findings of the present study would suggest a long term usage of this drug has no adverse effect in study animals.

6. ACKNOWLEDGEMENT

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7. REFERENCE

1. Dr. G. Ganapathy, Siddha Medicine History, Published by: Tamil Valarchi Kazhagam, University building, Chepauk, Chennai, 2010; 05: 25.
2. Alam MB, Hossain MS, Chowdhury NS, Mazumder MEH, Haque ME. In vitro and in vivo antioxidant and toxicity evaluation of different fractions of Oxalis corniculata linn. J Pharmacol Toxicol, 2011; 6: 337–348.
3. Mukherjee PK, Wahile A. Integrated approaches towards drug development from Ayurveda and other Indian system of medicines. J Ethnopharmacol, 2006; 103: 25–35.
4. Ukwuani AN, Abubakar MG, Hassan SW, Agaie BM. Toxicological studies of hydromethanolic leaves extract of Grewia crenata. International Journal of Pharmaceutical Science and Drug Research, 2012; 4: 245–249.
5. Jain N, Sharma P, Sharma N, Joshi S C. Haemato-biochemical profile following sub acute toxicity of malathion in male albino rats. Pharmacologyonline, 2009; 2: 500–506.
6. Ferrario D, Croera C, Brustio R, Collotta A, Bowe G, Vahter M. Toxicity of inorganic arsenic and its metabolites on haematopoietic progenitors "*in-vitro*" comparison between species and sexes. *Toxicol*, 2008; 249: 102-108.

7. Ahmad M, Elnakady Y, Farooq M, Wadaan M. Lithium induced toxicity in rats: blood serum chemistry, antioxidative enzymes in red blood cells and histopathological studies. *Biol Pharm Bull*, 2011; 34: 272-277.
8. Rahman MF, Siddiqui MK, Jamil K. Effects of Vepacide (*Azadirachtaindica*) on aspartate and alanine aminotransferase profiles in a sub chronic study with rats. *J Hum Exp Toxicol*, 2001; 20: 243–249.
9. Kumar RS, Gupta M, Mazumdar UK, Rajeshwar Y, Kumar TS, Gomathi P, Roy R.Effects of methanol extracts of Caesalpiniabonducella and Bauhinia racemosa on hematology and hepatorenal function in mice. J Toxicol Sci, 2005; 30(4): 265-74.