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ACUTE AND SUB ACUTE TOXICITY STUDY ON SIDDHA DRUG *VELVANGA PARPAM*

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ABSTRACT: Benign Prostatic Hyperplasia (BPH) is a common progressive disease among men, with an incidence that is high among elderly. *Velvanga parpam* (VP) has been employed as traditional remedy for Benign Prostatic Hyperplasia (BPH) which is a herbo mineral formulation. As a mandate, steps were taken to evaluate safety profile of VP in rats and mice following OECD guidelines. Acute toxicity studies, different doses of VP were administered orally to rats once daily for one week. Sub-acute toxicity studies were carried in four different groups in which VP was administrated orally to rats once daily for 28 days in various doses ranging from 2.5, 5, 10 Mg/kg for mice respectively. Detailed hematological, biochemical, necropsy and histopathological evaluation of organs was performed for all animals. The VP was well tolerated and no toxic manifestations were seen in any animal. Histopathological analysis revealed that Spleen, Testes, Pancreas, Lung, Liver, Brain, Heart, Stomach, Intestine, Bone, Ovary, and Kidney tissues of treated groups did not show any signs of toxicity. Mortality observed in highdose. The VP was found to be safe in animals. No toxic effect was observed up to 5mg/kg of *Velvanga parpam* in acute and sub-acute toxicity studies.

INTRODUCTION: Benign Prostatic Hyperplasia (BPH) is a common progressive disease among men, with an incidence that is high among elderly. Histological BPH, which typically develops after the age of 40 years, ranges in prevalence from 50% at 60 years to as high as 90% by 85 years of age [1-3].

BPH may also result from the proliferation of epithelial and stromal cells, and may further contribute to constriction of the urethra, leading to bladder outlet obstruction [4, 5].

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Approximately 50% of patients with histological BPH report moderate to severe LUTS, consisting of storage and voiding symptoms [6].

In the recent years, complementary and alternative medicine (CAM) has upsurge globally for the treatment and prevention of many aliments which are non-communicable and chronic in nature [7].The interventional drug *Velvanga Parpam* (VP) has been quoted by Veeramamunivar Vagada Thirattu for the treatment of Ukkara soolai (BPH) [8, 9.]

The pre-clinical toxicity studies are essential for determining a safe dose for humantrials [10]. Consequently an effort was made to evaluate acute and sub-acute toxicity of herbo-mineral siddha formulation of *Velvanga Parpam* in laboratory animals.

MATERIALS & METHODS:

Preparation of the *Velvanga Parpam*:

a) **Ingredients:** The siddha medicine VP has the ingredients of velvangam (Tin), kattrazhai (*Alove vera*) and muttaiodu (The egg shell) [8].

b) **Procedure:** Velvangam is taken in an iron bowl and powder of egg shell is poured on the melted velvangam and stirs it thoroughly until the velvangam completely merge. Add juice of aloe vera and grind it for 12 hours (4 samam) and keep it in calcinations process [8].

Aim: Aim of the study is to evaluate the acute and sub-acute toxicity of the siddha drug '*Velvanga Parpam*'.

Animals: Mice of either sex weighing 25-30g and rats weighing 210-240g were obtained from the animal house of Vel's University. The animals were used with the approval of the Institute animal ethics committee (IAEC) and obtained from Vel's University, Chennai-117 on 11.08.2012 bearing no. (VP) (XIII/VELS/PCOL/22/2000/CPCSEA/IAEC). They were fed with a balanced standard pellet diet and maintained under standard laboratory conditions, providing 24-280C temperature, standard light cycle (12 h light, 12 h dark) and water ad libitum.

Animals were kept in cages with raised floors of wide mesh to prevent coprophagy. Animal welfare guidelines were observed during the maintenance period and experimentation. The rats were randomly assigned to control and different treatment groups, six animals per group. The animals were acclimatized for one week under laboratory conditions.

Acute Toxicity Study-OECD 425 guidelines [11]: Acute oral toxicity test for the *Velvanga Parpam* was carried out as per OECD Guidelines 425. As with other sequential test designs, care was taken to ensure that animals are available in the appropriate size and age range for the entire study. The test substance is administered in a single dose by gavage using a stomach tube or a suitable intubation cannula.

The fasted body weight of each animal is determined and the dose is calculated according to the body weight. After the substance has been administered, food was withheld for a further 2 hours in mice.

The animals were observed continuously for the first 4 h and then each hour for the next 24 h and at 6 hourly intervals for the following 48 h after administering of the test drug, to observe any death or changes in general behavior and other physiological activities. Single animals are dosed in sequence usually at 48 h intervals. However, the time interval between dosing is determined by the onset, duration, and severity of toxic signs.

Treatment of an animal at the next dose was delayed until one is confident of survival of the previously dosed animal. General behavior, respiratory pattern, cardiovascular signs, motor activities, reflexes, change in skin and fur, mortality and the body weight changes were monitored daily. The time of onset, intensity and duration of these signs, if any, were recorded [12, 13].

Sub-Acute Toxicity: In a 28-days, sub-acute toxicity study, twenty four either sex (3+3) rats were divided into four groups of 6 rats each. Group I that served as normal control was administered with distilled water (p.o.) while groups II, III and IV were administered daily with the Velvanga Parpam (p.o.) for 28 days at a dose of 2.5, 5.0 and 10mg/kg respectively.

The animals were then observed daily for gross behavioral changes and any other signs of sub-acute toxicity [**Table 1]**. The weight of each rat was recorded on day 0 and weekly throughout the course of the study [**Table 2**], food and water consumption per rat was calculated [**Table 3 & 4**].

At the end of the 28 days they were fasted overnight, each animal was anaesthetized with diethyl ether, following which they were then dissected and blood samples were obtained by cardiac puncture into heparinized tubes. The blood sample collected from each rat was centrifuged with 3000 X g at 4^{o}C for 10 min to separate the serum and used for the biochemical assays.

TABLE 1: DOSE FINDING EXPERIMENT AND ITS BEHAVIORAL SIGNS OF TOXICITY

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.	50	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-
2	300	+	+	-	+	-	+	+	+	+	-	-	-	-	-	-	+	-	+	+	+
3	2000	+	+	-	+	-	+	+	+	+	+	-	-	+	-	-	+	-	+	+	+

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

TABLE 2: BODY WT (G) OF ALBINO RATS EXPOSED TO *VELVANGA PARPAM* FOR 28DAYS

Dose (mg/kg/day)	Days				
	1	7	14	21	28
Control	210.11±5.00	212.15±4.05	214.25±5.20	218.22±6.12	221.00±5.00*
2.5	215.00±4.31	217.12±4.52	220.31±4.16*	222.64±8.21**	224.62±6.10*
5.0	214.13±5.00	214.10±4.82	211.00±3.18	210.10±5.00	207.72±4.21*
10	212.32±5.20	210.20±5.40	210.12±5.24	205.02±4.38	204.12±3.12*

Values are mean of 6 animals ± S.E.M. *P<0.05; **P<0.01.

TABLE 3: FOOD (G/DAY) INTAKE OF ALBINO RATS EXPOSED TO *VELVANGA PARPAM* FOR 28 DAYS

Dose (mg/kg/day)	Days (gms/rats)				
	1	7	14	21	28
Control	45.00±2.75	44.54±2.18	46.45±2.19	45.00±2.58	47.50±3.42
2.5	44.21±2.48	45.43±2.42	46.24±2.46	49.12±2.49	48.18±3.00
5.0	42.35±2.10	42.00±2.54	44.20±2.42	45.81±3.56	46.40±3.00
10	43.43±2.61	45.24±2.80	44.11±2.80	45.19±2.02	45.06±3.11

Values are mean of 6 animals ± S.E.M. ^{ns}P>0.05Vs control.

TABLE 4: WATER (ML/DAY) INTAKE OF MALE AND FEMALE ALBINO RATS EXPOSED TO *VELVANGA PARPAM* FOR 28 DAYS

Dose (mg/kg/day)	Days (ml/rat)				
	1	7	14	21	28
Control	52.04±2.80	52.02±3.43	55.21±3.15	50.13±3.14	50.24±3.20
2.5	52.28±2.42	51.20±3.04	48.20±4.02	46.18±3.00	42.52±2.48*
5.0	48.14±2.18	50.14±3.72	44.28±3.34	42.12±2.92	44.14±3.37
10	50.43±3.52	52.72±3.00	50.22±3.80	46.42±3.12	44.32±3.15

Values are mean of 6 animals ± S.E.M. *P<0.05; vs control.

Hematological and blood biochemical analyses: At the end of the study, all animals were kept fasted for 16-18 h and then anesthetized with anesthetic ether on the 28th day. Blood samples for hematological and blood chemical analyses were taken from retro orbital vein. Heparinized blood samples were taken for determining complete blood count [**Table 5**] (white blood cell count, differential white blood cell count, platelet count, red blood cell count, hematocrit, and hemoglobin) by semi-automated hematology analyzer.

TABLE 5: HEMATOLOGICAL PARAMETERS AFTER 28DAYS TREATMENT WITH *VELVANGA PARPAM* IN RATS

Parameter	Control	2.5 mg/kg	5 mg/kg	10 mg/kg
Red blood cell (mm^3)	8.18±0.72	7.59±0.44	7.25±0.52	7.13±0.50
HB (%)	14.42±0.30	12.10±0.35	12.51±0.40	11.02±0.44*
Leukocyte ($x10^6$/mL)	10215±112.55	10415±215.14	10206±224.11	10346±212.00
Platelets/ul	1440±34.10	1392±32.12	1172±30.22**	998±23.16**
MCV (gl)	55.62±5.42	55.10±5.72	55.45±5.22	56.18±4.62
Neutrophil	5.54±1.43	5.22±1.20	4.84±0.92*	5.14±3.27
Lymphocyte	92.32±2.90	91.48±3.12	93.20±3.24	94.30±3.86
Monocyte	2.15±0.30	2.31±0.34	2.25±0.22	2.28±0.31
Eosinophil	1.00±0.00	1.0±0.22	1.0±0.11	1.00±0.12
Basophil	0	0	0	0
ESR (mm)	1±00	1±00	1±00	1±00
PCV	42.30±2.52	45.11±2.18	45.52±3.04	45.42±3.00

Values are mean of 6 animals ± S.E.M. *P<0.05; **P<0.01. Vs control.

The serum from non-heparinized blood was carefully collected for blood chemistry and enzyme analysis, glucose, Creatinine, total protein, albumin, total and direct bilirubins, serum glutamate-oxaloacetate trans-aminase (SGOT), serum glutamate pyruvate transaminase (SGPT), and alkaline phosphatase (ALP) were automatically determined using auto analyzer [**Table 6, 7, 8 & 9**] [14].

TABLE 6: EFFECT OF TREATMENT WITH *VELVANGA PARPAM* BIOCHEMICAL PARAMETERS

Dose (mg/kg)	Control	2.5 mg/kg	5 mg/kg	10 mg/kg
Total Bilirubin (mg/dL)	0.200±0.05	0.220±0.06**	0.225±0.05**	0.218±0.04**
Bilirubin direct (mg/dL)	0.1±0.04	0.1±0.05	0.1±0.04	0.1±0.05
Bilirubin indirect (mg/dL)	0.1±00	0.1±00	0.1±00	0.1±00
ALP (U/L)	380.32±10.10	414.20±12.13**	456.82±10.02	494.21±12.22
SGOT (U/L)	176.21±5.18	160.26±6.52**	156.23±5.10*	154.12±5.50*
SGPT(U/L)	45.2±2.32	44.18±3.22	45.83±2.52	44.60±4.17
Total Protein(g/dl)	9.02±1.22	8.07±0.30*	8.15±0.27*	8.10±0.42*
Albumin (g/dl)	3.17±0.25	3.10±0.24	3.16±0.23	3.10±0.22
Globulin (g/dl)	5.00±0.18	4.18±0.22*	4.28±0.24*	4.28±0.23*

Values are mean of 6 animals ± S.E.M. *P<0.05; **P<0.01.Vs control.

TABLE 7: RFT

Dose (mg/kg)	Control	2.5 mg/kg	5 mg/kg	10 mg/kg
Urea (mg/dL)	55.40±2.35	54.32±3.62	55.42±2.18	55.82±2.30
Creatinine (mg/dL)	0.77±0.05	0.76±0.05	0.78±0.06	0.76±0.05
Uric acid (mg/dL)	1.62±0.12	1.16±0.18**	1.26±0.16*	1.06±0.12**
Nam.mol	142.80±5.22	144.5±5.00	142.12±5.22	140.28±5.10
Km.mol	20.45±2.48	19.40±2.60	20.05±2.42	20.18±2.02
Clm.mol	100.25±4.46	100.20±5.22	99.78±4.72	100.02±4.10

Values are mean of 6 animals ± S.E.M. *P<0.05; **P<0.01.Vs control.

TABLE 8: LIPID PROFILE

Dose (mg/kg)	Control	2.5 mg/kg	5 mg/kg	10 mg/kg
Total cholesterol (mg/dL)	40.82±2.52	41.10±2.42	40.28±3.24	41.00±3.01
HDL (mg/dL)	13.02±1.42	13.20±1.47	13.20±2.42	13.24±2.23
LDL (mg/dL)	44.00±2.80	44.05±3.60	43.38±3.20	44.22±3.20
VLDL (mg/dl)	16.32±2.60	15.22±2.42	16.10±1.42	15.00±1.14
Triglycerides (mg/dl)	86.04±3.02	85.18±2.22	86.32±3.40	85.14±2.72
TC/HDL ratio (g/dl)	3.66±0.25	3.70±0.28	3.70±0.30	3.52±0.28
Blood glucose (mg/dl)	125.30±6.47	126.05±5.20	126.15±5.62	125.21±2.57

Values are mean of 6 animals ± S.E.M. ^{ns}P>0.05;Vs control.

TABLE 9: URINE ANALYSIS

Parameters	Control	2.5 mg/kg	5 mg/kg	10 mg/kg
Colour	Yellow	Yellow	Yellow	Yellow
Transparency	Clear	Slightly turbid	Slightly cloudy	Slightly turbid
Specific gravity	1.010	1.010	1.010	1.010
pH	>7.2	>8.0	>8.0	>9.0
Protein	Nil	3+	3+	3+
Glucose	Nil	Nil	Nil	Nil
Bilirubin	-ve	-ve	-ve	-ve
Ketones	-ve	+ve	+ve	+ve
Blood	Absent	Absent	Absent	Absent
Urobilinogen	Normal	Abnormal	Abnormal	Abnormal
Pus cells	0-cells/HPF	1-cell/HPF	2-cells/HPF	1-cell/HPF
RBCs	Nil	Nil	0-1cells/HPF	Nil
Epithelial cells	Nil	1-cell/HPF	Nil	1-cell/HPF
Crystals	Nil	Nil	Nil	Nil
Casts	Nil	Nil	Nil	Nil
Others	Bacteria seen	Bacteria seen	Bacteria seen	Bacteria seen

Necropsy: All rats were sacrificed after the blood collection. The positions, shapes, sizes and colors of internal organs were evaluated. The spleen, testes, pancreas, lung, liver, brain, heart, stomach, intestine, bone, ovary and kidney tissues were excised from all rats to visually detect gross lesions, and weighed to determine relative organs' weights and preserved in 10% neutral formalin for Histopathological assessment [**Table 10**]. The tissues were embedded in paraffin, and then sectioned, stained with haematoxylin and eosin and were examined microscopically [**Figure-1, 2, 3 & 4**].

TABLE 10: EFFECT OF ORAL ADMINISTRATION OF A *VELVANGA PARPAM* ON ORGAN WEIGHT

Dose (mg/kg)	Control	2.5 mg/kg	5 mg/kg	10 mg/kg
Liver (g)	5.27±0.17	5.00±0.15	4.82±0.12	4.71±0.18*
Heart (g)	0.62±0.04	0.60±0.05	0.58±0.04	0.58±0.04
Lung (g)	1.45±0.06	1.44±0.14	1.46±0.24	1.50±0.15**
Spleen (g)	0.65±0.05	0.65±0.04	0.66±0.04	0.65±0.05
Ovary (g)	1.71±0.14	1.73±0.15	1.70±0.18	1.72±0.15
Testes (g)	1.48±0.10	1.45±0.12	1.46±0.15	1.46±0.15
Brain (g)	1.56±0.15	1.58±0.13	1.56±0.14	1.53±0.14
Kidney (g)	0.73±0.04	0.71±0.04	0.70±0.04	0.72±0.05
Stomach (g)	1.36±0.14	1.35±0.12	1.36±0.11	1.35±0.15

Values are mean of 6 animals ± S.E.M. *P<0.05; **P<0.01Vs control.

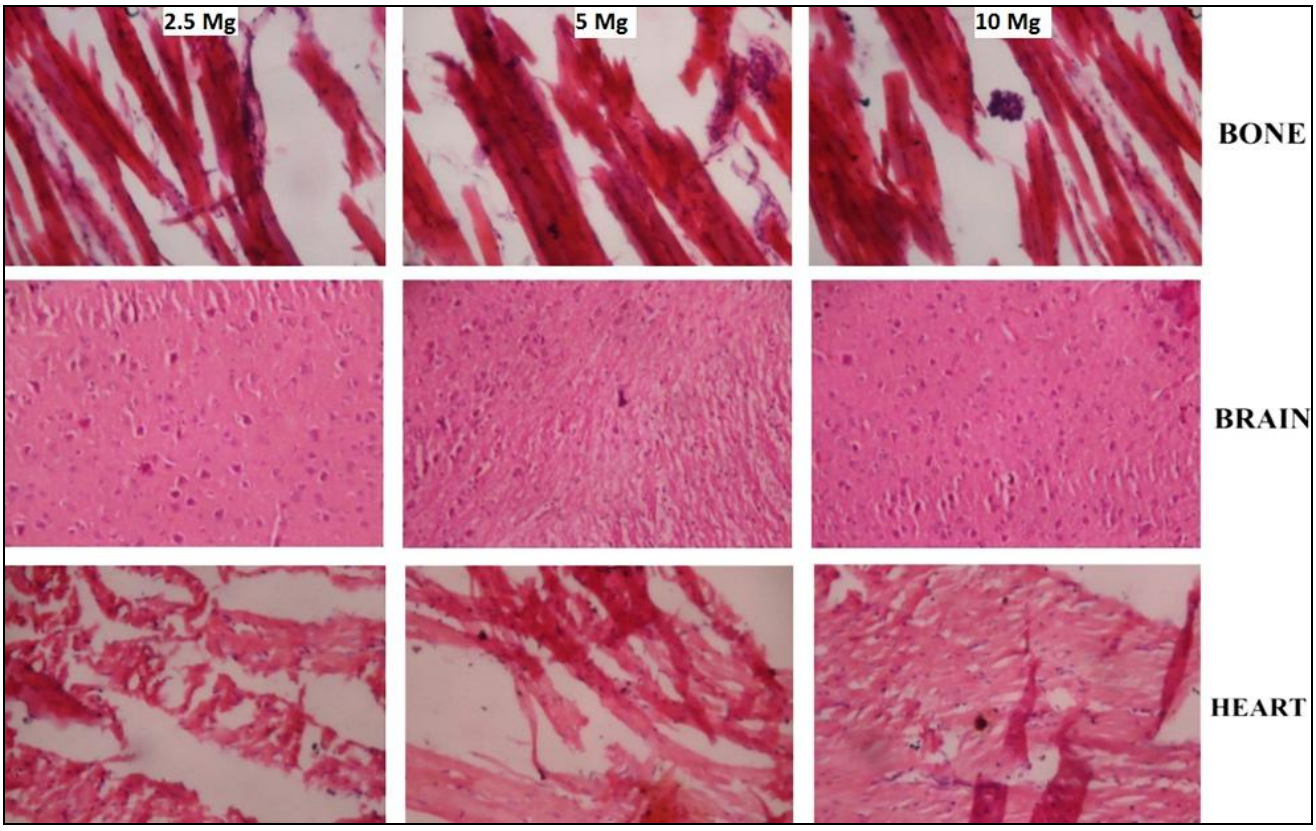

FIGURE 1

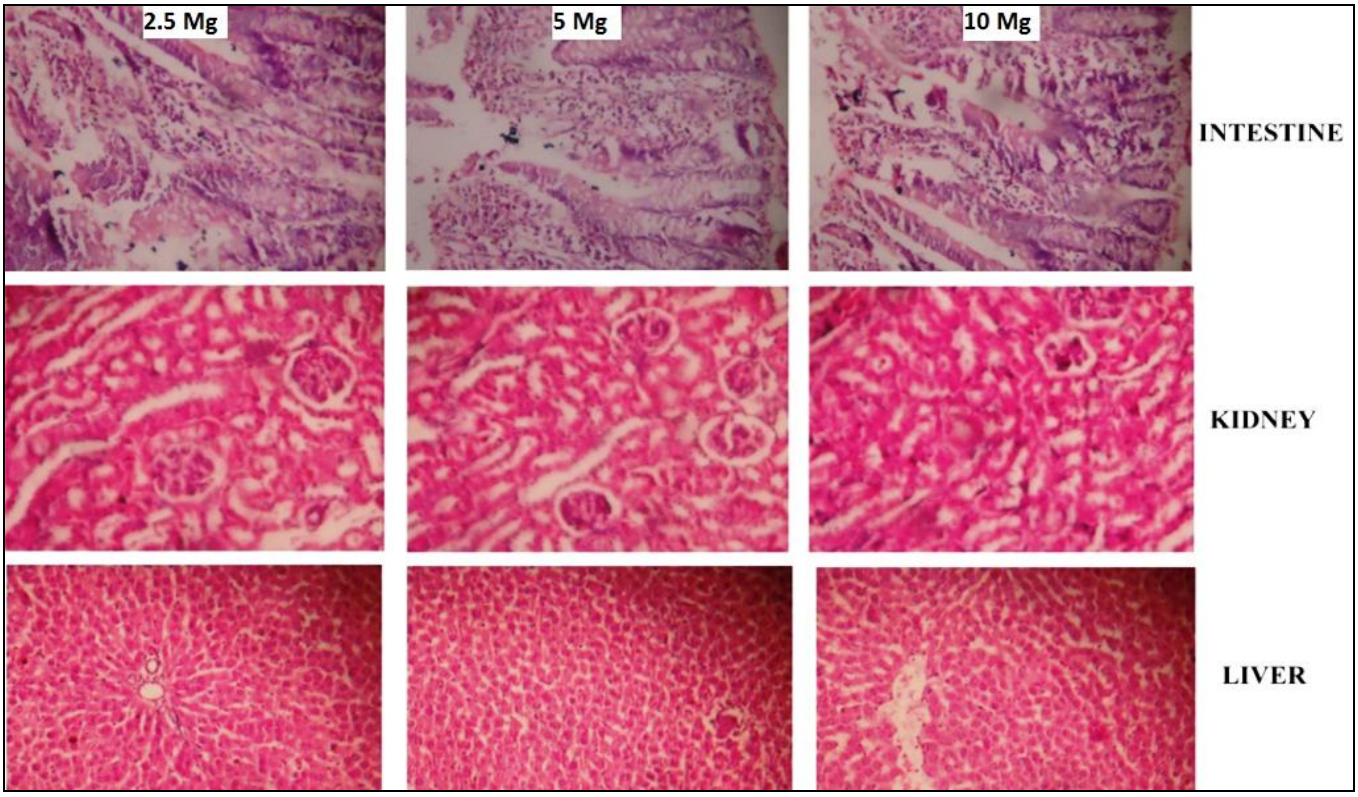

FIGURE 2

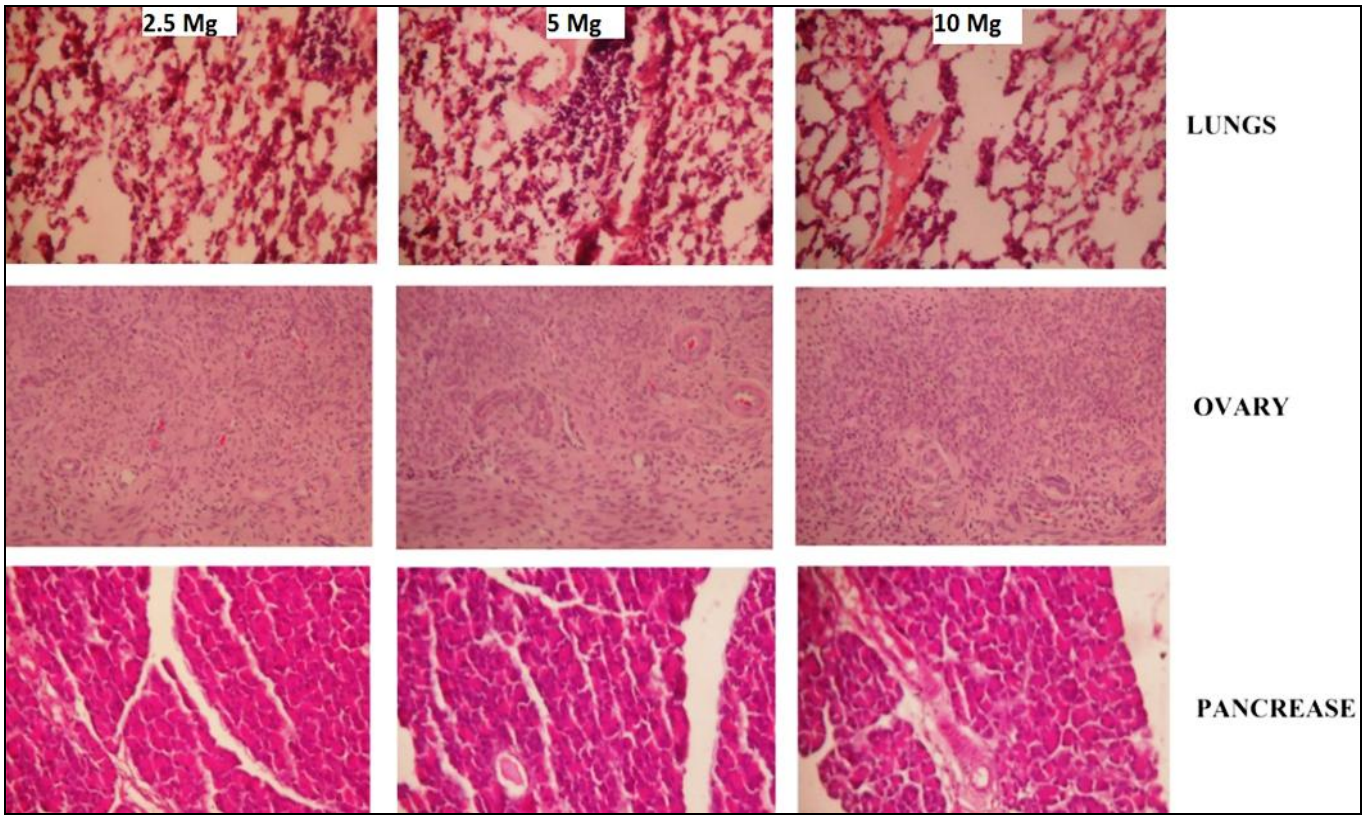

FIGURE 3

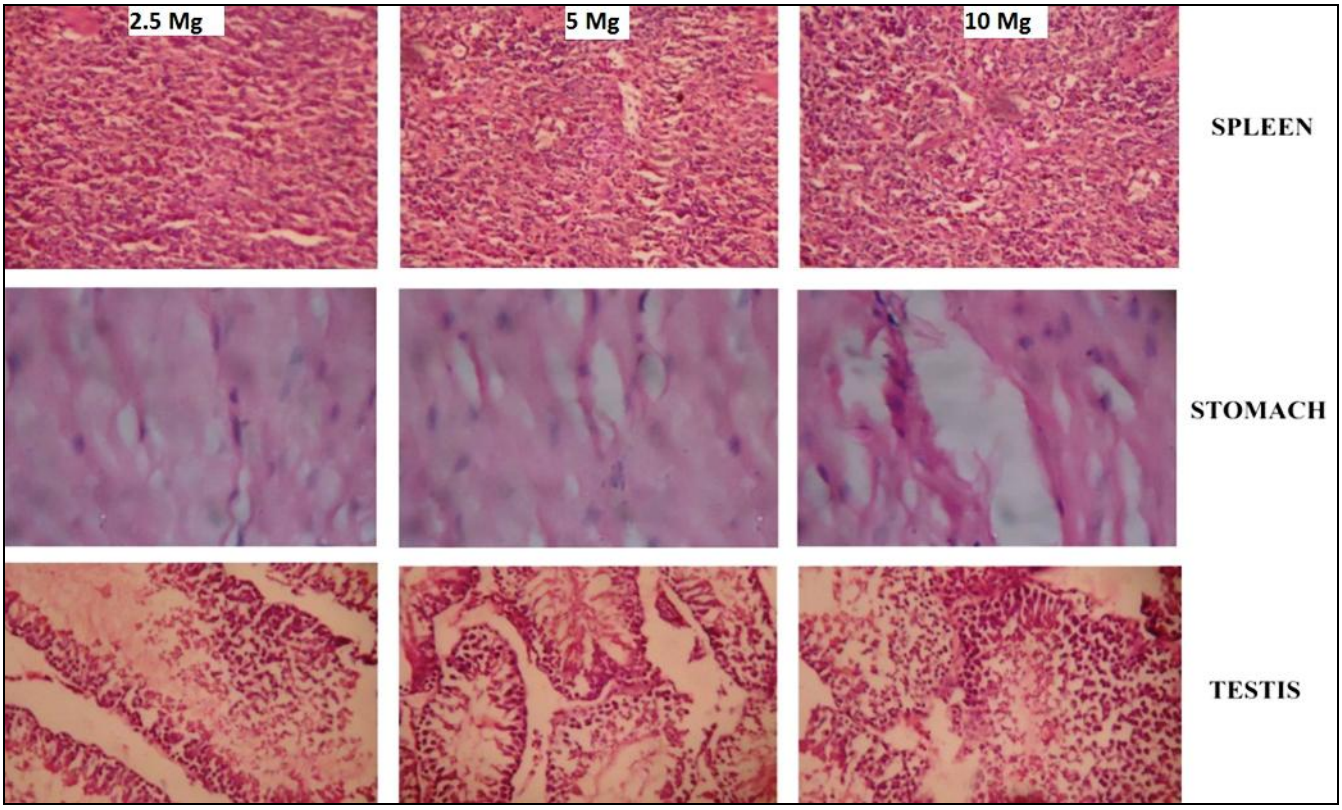

FIGURE 4

Statistical analysis: Values were represented as mean ± SEM. Data were analyzed using one-way analysis of variance (ANOVA) using Graph Pad Instat-V3 software. P values < 0.05 were considered significant.

RESULTS: All the animals from control and all the treated dose groups up to 5mg/kg survived throughout the dosing period of 28 days. No signs of major or significant intoxication were observed in animals from lower to higher dose groups during the dosing period of 28 days. Animals from all the treated dose groups exhibited comparable body weight gain with that of controls throughout the dosing period of 28 days.

Food consumption of control and treated animals was found to be comparable throughout the dosing period of 28days. Ophthalmoscopic examination, conducted prior to and at the end of dosing period on animals from control and all the treated dose groups did not reveal any abnormality. The results of haematological investigations revealed following significant changes in the values of different parameters investigated when compared with those of respective controls; however, the increase or decrease in the values obtained was within normal biological and laboratory limits.

A slight decrease in total RBC count values were obtained for animals in the dose group of 2.5 and 5mg/kg ($P<0.05$). Decreased values of platelets ($P<0.05$) were observed for animals in dose groups administered 5-10mg/ kg body weight of *Velvanga Parpam* sacrificed on day 28. Results of Biochemical investigations conducted on days 28 and revealed the following significant changes in the values of different parameters studied when compared with those of respective controls; however, the values obtained were within normal biological and laboratory limits.

Protein level is elevated in animals of 2.5 and 5mg/kg dose group ($P<0.05$). Aspartate Amino transferase levels slightly decreased in animals of 5 and 10mg/kg group ($P<0.01$). 8) Functional observation tests conducted at termination revealed no abnormalities. Urine analysis, conducted at the end of the dosing period in week 4 revealed no abnormality attributable to the treatment.

Organ weight data of animals sacrificed at the end of the dosing period was found to be comparable with that of respective controls. Gross pathological examination did not reveal any abnormality. Histo-pathological examination did not reveal any abnormality.

CONCLUSION: Based on these findings, no toxic effect was observed upto 5mg/kg of *Velvanga Parpam* on oral route over a period of 28 days. So, it can be concluded that the *Velvanga Parpam* can be prescribed for therapeutic use in human with the dosage recommendations of upto 5mg/kg body weight p.o.

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