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ACUTE AND SUB-ACUTE TOXICOLOGICAL EVALUATION OF THE SIDDHA SINGLE HERBAL FORMULATION SENGATHAARI ROOT BARK (SRB) DECOCTION IN RATS

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ABSTRACT

Capparis sepiaria known as *Sengathaari* in Tamil is used in Siddha medicine for treating various skin diseases. In the present study acute and sub-acute toxicity studies of *Sengathaari* Root bark (SRB) decoction were carried out in rats using OECD guidelines 423 and 407.In acute toxicity study 2 groups of 3 female Wistar rats were given orally SRB decoction single dose of 10 ml/kg and observed for 14 days. The results indicated there were no clinical signs of toxicity and mortality at the dose level of 10 mg/kg per oral. In sub-acute toxicity study four groups of 10 Wistar rats (5male and 5female) were used. Group I (control) received normal distilled water, while rats in group II, III and IV were given daily oral dose of 1 ml/kg, 2.5 ml/kg and 4 ml/kg respectively for 28 days. The effect of SRB decoction on feed intake, water intake and body weight changes, biochemical, haematological and histological studies of vital organs namely heart, lungs, liver, kidney, testis and uterus were assessed. The results of the sub-acute toxicity study did not show evidence of any changes in body weight, food and water intake, hematological parameters, liver and kidney function tests when compared with the control animals. The vital organs of animals treated with SRB decoction for 28days did not show any histo pathological evidence of pathological lesions. From the results it is concluded that SRB decoction at the dose of 4ml/mg/kg per oral is safe for long-term treatment.

INTRODUCTION

The use of herbal medicines in the treatment of various disease are in practice in traditional medicines by majority of world's population. This is due to its affordability, accessibility and efficacy of herbal remedies. The increasing usage of these herbal formulations paves way to subject to various pharmacological studies mainly but not on toxicological studies. *Capparis sepiaria* known as *Sengathaari* (*Tamil* name) is indicated in Siddha system of medicine for Eczematous disorders, allergic disorders, diseases due to vitiated *Iyyam* and *Vali* diseases.

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In the present study, *Sengathaari* Root bark (SRB) decoction was prepared as per the literature mentioned in the Siddha text Balavagadam [1]. Considering the traditional claim of this formulation, the present study was carried out to evaluate its safety and tolerability in long term treatment.

MATERIALS AND METHODS

Plant Material:

The raw drug *Sengathaari* (*Capparis sepiaria)* root bark was procured from M/s GopalAasan Country drug store, Nagercoil, Tamilnadu, India. It was authenticated by the department of Medicinal Botany, National Institute of Siddha, Chennai, India.

Preparation of the plant material:

The impurities like stone, sand were removed from the root bark and then sauté in an iron vessel and dried in sunlight for a day. The SRB decoction was made by adding 25 gms of *Sengathaari* Root Bark coarse powder with 480 ml of water and was boiled till the water reduced to 1/8 of its original quantity. The SRB decoction powder was kept in a clean, dry, airtight glass container.

Analytical specification of Decoction Choornam:

The analytical specification of decoction choornam was done as per the Protocol for testing Ayurvedic, Siddha and Unani Medicines, *Pharmacopoeial Laboratory for Indian Medicine*, Department of AYUSH, Government of India [2]. The analysis includes loss on drying, total ash value, acid insoluble ash, water soluble extractive, alcohol soluble extractive, heavy metal analysis, microbial contamination, aflatoxin and pesticide content.

Preparation of test samples

25 g of coarse powdered root bark of *Sengathaari* was boiled in 500 ml of water and reduced to 50 ml decoction. 1 ml constitutes the extract of 500 mg *Sengathaari* root bark (SRB). For each time, freshly prepared SRB decoction was made.

EXPERIMENTAL ANIMALS

Acute toxicity studies:

This study was carried out by following the procedure mentioned in OECD 423 guideline [3] with some modification. Six female Wistar rats were randomly selected and acclimatized prior to the study. Each selected animal was kept in separate poly propylene cage and marked with picric acid on the fur for identification. The rats were fasted overnight before the administering of test drug. After the administration of test drug, the rats were deprived of feed for 3 -4 hrs.

Initially, a single dose of 5000 mg/kg of test drug SRB (10 ml/kg of SRB decoction) was chosen and administered to three rats and observed for mortality and clinical signs of toxicity (General behavior, respiratory pattern, cardiovascular signs, motor activities, reflexes and changes in skin and fur texture) at 30 min, 1, 2 and 4 hours and thereafter once a day for the next 13 consecutive days. Since there was no mortality and abnormal signs, further three animals were administered with SRB decoction at the same dosage and observed for mortality and clinical signs of toxicity. Body weight was recorded once in a week, at the end of 14th day animals were sacrificed and organs were observed for gross pathological changes. The experimental protocol was approved by Institutional Animal Ethics Committee of KMCH college of Pharmacy, Coimbatore, India (KMCRET/MD(S)/08/2014-15).

Sub-Acute Toxicity Study (28 days repeated oral toxicity):

Dose administration

This study was carried out by following OECD 407 guidelines [4] (3rd October, 2008) adopted for the testing of chemicals and was modified according to the experimental need. In acute toxicity study, LD50 value was found to be more than 5000 mg/kg. For sub-acute toxicity study, low dose was fixed as one tenth of LD50 value i.e.500 mg/kg (1 ml/kg of SRB decoction), high dose was fixed as four folds of low dose i.e. 2000 mg/kg (4 ml/kg of SRB decoction) and intermittent dose was fixed as 1250 mg/kg (2.5 ml/kg of SRB decoction) for administration.

Randomization

Both sexes of Wistar Albino rats were randomized into four groups of ten animals each (5 males, 5 females). Group I served as control group. Group II, III and IV served as low, middle and high doses of SRB decoction respectively. All the test substances were administered once daily via oral route through gastric gavage for 28 days.

Housing

The Wistar rats were housed in standard polypropylene cages with stainless steel top grill. Paddy husk was used as bedding. The paddy husk was changed at least twice in a week.

Environmental conditions

The animals were kept in a clean environment with 12 hour light and 12 hour dark cycles. The air was conditioned at 22±3^{0}C and the relative humidity was maintained between 30-70% with 100% exhaust facility. The cages corresponding to each experimental group were distributed on racks in such a manner that external factors, such as environmental conditions, were balanced as far as possible.

Feed schedule

Feed was provided *adlibitum* throughout the study period, except overnight fasting (18-20 hours) prior to dose administration. After the test substance administration, food was withheld for further 3-4 hours. Prior to the beginning of treatment, the food intake of each cage was recorded for period of 28 days and the mean weekly intake per rats was calculated.

Water

The water was offered *adlibitum* in bottles and was periodically analyzed to detect the presence of possible contaminants. The water consumption in each cage was measured daily for a period of 28 days.

Clinical signs of toxicity

All the experimental animals were observed for mortality and morbidity twice a day, till the completion of treatment. Clinical observations were made once daily to detect signs of toxicity, preferably at the same time in each day (1hour after vehicle or SRB decoction administration).

Body weight

The body weight of each rat was recorded one week before the start of treatment and during the course of the treatment on the day of initial, 3rd, 7th, 10th, 14th, 17th, 20th, 24th and 28th days (day of sacrifice). The mean weights for the different groups and sexes were calculated from the individual weights.

Blood Collection

Blood was collected in heparin/EDTA tube through retro-orbital sinus from all the animals of different groups on 28th day under light ether anesthesia after fasting for 16 hours. The samples were used to evaluate hematological parameters like RBC, WBC, and Platelets. The collected blood samples were also centrifuged 10000 rpm in 10 minutes to separate the serum. The separated serum was used to evaluate biochemical parameters like SGOT, SGPT, Alkaline phosphatase and Bilirubin.

Haematological and biochemical parameters

At the end of 28thday treatment, live rats were fasted over night and on 29th day under CO2 inhalation, immediately 5 ml of blood was collected through cardiac puncture in a EDTA and a tube without anti-coagulant. The haematological parameters such as Haemoglobin (Hb), Red Blood Cell count (RBC), White Blood Cell count (WBC), Differential count - Lymphocyte, Monocyte and Granulocyte, Red Cell Distribution Width (RDW), Haematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Platelet (Plt), Platelet Crit (PCT), Platelet Distribution Width (PDW) and Mean Platelet Volume (MPV) were done in the EDTA mixed blood samples using ErbaMannhein® haematologyanalyser.

The blood samples without anticoagulant were used for estimating biochemical parameters such as Glucose, Cholesterol, Triglyceride (TG), Protein, Urea, Creatinine, Bilirubin, Serum glutamic-oxaloacetic transaminase (SGOT), Serum glutamic pyruvic transaminase (SGPT) and Alkaline Phosphatase (ALP) using Erba system Pack kits in Fully Automated Biochemistry analyzer. Sodium, Potassium and Chloride content were estimated by using electrolyte analyser from Roche®.

Necropsy and Histo-pathological study

After withdrawal of blood, all the rats were sacrificed for gross necropsy and histopathological study. Organs including brain, lungs, heart, liver, kidney, stomach, spleen, testis and uterus and were studied for gross necropsy and weighed for calculating relative organ weight. Histopathological studies on liver, kidney, lungs, heart, testis and uterus were carried out for control and intervention three group. The tissues of collected organs were fixed in 10% Neutral buffered formalin for 24 h. The tissues were trimmed, embedded in molten paraffin wax and sectioned (4-5 microns thickness) using rotary microtome. The sections were floated in hot water and placed in the glass slide. The slides were stained with Haematoxylin and Eosin (H&E), mounted in DPX and examined under light microscope.

TERMINAL STUDIES

Sacrifice and macroscopic examination

On completion of 4 weeks of treatment, 18 Wistar rats were sacrificed by ether inhalation. A full autopsy was performed on all animals which included examination of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents both *in situ* and after evisceration. As the number of animals exceeded the number that could be sacrificed in one day, the autopsies were carried out over three consecutive days at the end of the treatment period.

Organ weights

After the macroscopic examination the following organs were weighed after separating the superficial fat: Brain, Heart, Spleen Kidneys, Testes, Liver, Lungs, pancreas and stomach.

Histopathology

The target organs were collected and preserved in 10 % formalin for the histopathological evaluation. The organs from control and drug treated animals were preserved in 10 % neutral formalin for histopathological examination.

Statistical analysis

All data were expressed as mean ± standard deviation (SD). The test groups were compared with control group for testing significance and done by One-way Analysis of Variance (ANOVA) followed by Dunnett Multiple Comparisons Test using GRAPH PAD INSTAT version 3 software programs. Values of $p<0.05$ were considered significant.

RESULTS

Physico-chemical Parameters

The Physico-chemical parameters of SRB decoction powder performed using the PLIM guideline was reported in Table1.

Acute toxicity study

From acute toxicity study it was observed that the

administration of SRB Decoction at a dose of 10 ml/kg to the Female Wistar rats do not produce drug-related toxicity and mortality. So No-Observed-Adverse-Effect- Level (NOAEL) of SRB Decoction is 10 ml/kg.

Sub-Acute Toxicity study

Clinical signs:

All animals in this study were free of toxic clinical signs throughout the dosing period of 28 days.

Mortality:

All animals in control and in all the treated dose groups survived throughout the dosing period of 28 days.

Body weight:

Results of body weight determination of animals from control and different dose groups exhibited comparable body weight gain throughout the dosing period of 28 days.

Food consumption:

During dosing and the post-dosing recovery period, the quantity of food consumed by animals from different dose groups was found to be comparable with that by control animals.

Organ Weight:

Group Mean Relative Organ Weights (% of body weight) were calculated by comparison of organ weights of treated animals with respective control animals on day 29 was found to be comparable similarly.

Responses to sensory reactivity:

The responses of sensory reactivity to auditory, visual and proprioceptive stimuli were normal in animals belonging to both the controls as well as drug treatment groups.

Hematological investigations:

The results of hematological investigations (Table 4) conducted on day 29 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 or the effect was not dose dependent.

Biochemical Investigations:

Results of biochemical investigations (Table 3) conducted on day 29 and recorded in Table 3, revealed the following significant changes in the values of hepatic serum enzymes studied. When compared with those of respective control. The values were within normal biological and laboratory limits.

Histopathology:

In histopathological examination, revealed normal architecture in comparison with control and treated animal.

Table 1. Physico-chemical Parameters of Sengathaari root bark decoction powder

SI.No	Test	SRB Powder
1	Organoleptic characters: a. Colour	Light Brown
2	Loss on drying	11.52%
3	Total – ash value	6.51%
4	Acid insoluble ash in diluted Hcl	2.08%
5	Water soluble extract	30.12%
6	Alcohol soluble extract	4.77%
7	Microbial contamination a. Total Bacterial Count b. Total Fungal Count c. *Escherichia coli* d. *Staphylococcus aureus* e. Salmonella Spp f. Pseudomoasaeruginosa	 $19x10^2$ cfu/g Less than 10 Absent Absent Absent Absent
8	Test for aflatoxins (B1, B2, G1, G2)	Not detected
9	Pesticide residue	Not detected

Table 2. 28 Days Repeated Oral Toxicity Effect of SRB Decoction on Organ Weight of Wistar Rats

Organ weight (in grams)	Control	Low dose (1ml/kg)	Middle dose (2.5 ml/kg)	High dose(4 ml/kg)
Brain	1.394±0.085	1.253±0.053ns	1.385±0.066ns	1.251±0.112ns
Heart	0.911±0.007	0.896±0.005ns	0.802±0.018**	1.021±0.036**
Lungs	2.067±0.024	1.168±0.164***	1.179±0.071***	2.108±0.041ns
Liver	8.476±0.925	6.097±0.268*	5.540±0.328**	6.192±0.338*
Kidney (L)	1.003±0.030	0.733±0.0185ns	0.692±0.006***	0.924±0.0434**
Kidney (R)	0.999±0.004	0.739±0.0182***	0.701±0.007***	0.918±0.012**
Testis	3.048± 0.050	2.200±0.0866***	2.215±0.123***	2.733±0.194ns
Uterus	0.412±0.018	0.604±0.020ns	0.526±0.015ns	0.701±0.011ns

Values are expressed as the mean ± S.D; Statistical significance (p)calculated by one way ANOVA followed by dunnett's ns- no significant *P< 0.001, **P < 0.01, ***P < 0.05 calculated by comparing treated group with CONTROL group.

Table 3. 28 Days Repeated Oral Toxicity Effect of SRB Decoction on Biochemical Parameters of Wistar Rats

Variable	Control	Low dose(1 ml/kg)	Middle dose(2.5 ml/kg)	High dose (4 ml/kg)
Blood glucose (Male) (mg/dl)	77.00±4.211	87.33±6.637	78.33±4.356	80.67±9.969
Blood glucose(Female) (mg/dl)	92.00±2.191	93.67±5.846	87.33±6.381	96.33±4.958
Urea(mg/dl)	41.07± 2.122	34.03±0.66ns	32.10±2.309*	32.57±2.88*
Uric acid (mg/dl)	2.030±0.136	2.727±0.215ns	1.810±0.322ns	1.523±0.308ns
Creatinine (mg/dl)	0.596±0.037	0.716±0.0426*	0.536±0.0152ns	0.486±0.0269ns
Total cholesterol (mg/dl)	58.33±6.245	47.50±2.245ns	51.77±3.274ns	46.20±1.220ns
HDL-Cholesterol (mg/dl)	25.47±0.941	16.67±1.884**	25.20±0.545ns	24.37±2.726ns
Triglycerides (mg/dl)	126.1±8.664	73.01±3.476***	90.10±8.417**	75.83±3.928***
Total bilirubin (mg/dl)	0.856±0.033	1.233±0.084**	0.816±0.025ns	0.740±0.090ns
SGOT (AST) (u/l)	87.07±7.93	103.5±10.34**	118.8±5.78ns	105.4±4.321***
SGPT (ALT) (u/l)	54.00±3.395	40.13±0.706***	0.536±0.0152*	50.50±2.726ns
Alkaline phosphatase (u/l)	315.6±22.11	175.3±17.88***	141.8±24.53***	145.5±9.314***

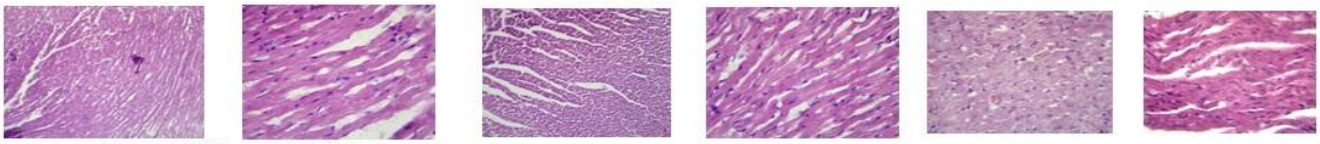
Values are expressed as the mean ± S.D; Statistical significance (p)calculated by one way ANOVA followed by dunnett's ns- no significant *P< 0.001, **P < 0.01, ***P < 0.05 calculated by comparing treated group with CONTROL group.

Table 4. 28 Days Repeated Oral Toxicity Effect of SRB Decoction on Haematological Parameters of Wistar Rats

Variable	Control	Low dose (1 ml/kg)	Middle dose (2.5 ml/kg)	High dose(4 ml/kg)
RBC (cell/cumm)	6.073±0.103	6.197±0.074ns	5.260±0.056***	6.137±0.101ns
WBC (cell/cumm)	12.70±0.131	10.47±0.331*	13.57±0.625ns	11.70±0.904ns
HB (mg/dl)	15.10±0.292	15.60±0.193ns	12.67±0.220***	15.40±0.318ns
PCV (%)	46.90±0.955	47.80±0.579ns	39.00±0.660***	47.23±0.967ns
POLYMORPHS (%)	8.667±1.116	10.33±1.647ns	9.33±3.106ns	4.00±0.365ns
LYMPHOCYTES (%)	83.67±0.918	82.00±0.966ns	81.67±2.140ns	89.67±0.557**
MONOCYTES (%)	3.00±0.365	3.333±0.210ns	4.333±0.421*	2.667±0.421ns
EOSINOPHILS (%)	4.667±0.421	4.333±0.557ns	4.667±0.557ns	3.667±0.421ns
MCH (%)	25.43±0.283	25.60±0.348ns	24.07±0.164**	25.07±0.117ns

Values are expressed as the mean ± S.D; Statistical significance (p)calculated by one way ANOVA followed by dunnett's ns- no significant, *P< 0.001,**P < 0.01, ***P < 0.05 calculated by comparing treated group with CONTROL group.

Figure 1. HISTOPATHOLOGICAL REPORT OF SUB ACUTE(28 DAYS REPEATED ORAL) TOXICITY EFFECT OF SRB DECOCTION ON OF WISTAR RATS

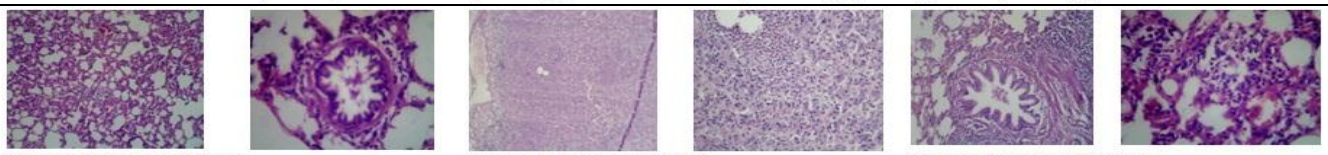


FigureA2(a) and A2(b) Focal loss of architecture and mild inflammation

FigureA3(a) and A3(b) myocardium and myocytes

FigureA4(a) and A4(b) Myocytes and myocardial fibres

Note: a-10x; **b**-40x; **2**- low dose(1 ml/kg) ; **3**- middle dose(2.5 ml/kg) ; **4**- high dose(4 ml/kg); **A-Heart**

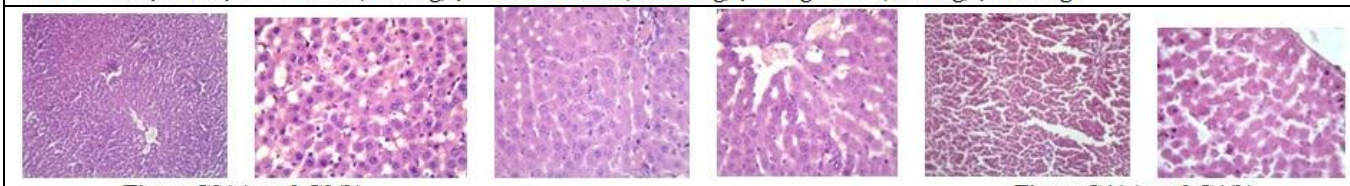


FigureB2(a) and B2(b) interstitium with inflammation and normal bronchi

FigureB3(a) and B3(b) consolidation with inflammation with lymphocytes and polymorphs

FigureB4(a) and B4(b) lung parenchyma with normal bronchi and brochiole with peribronchial inflammation and interstitial inflammation

Note: a-10x; **b**-40x; **2**- low dose(1 ml/kg) ; **3**- middle dose(2.5 ml/kg) ; **4**- high dose(4 ml/kg) ; **B-Lungs**

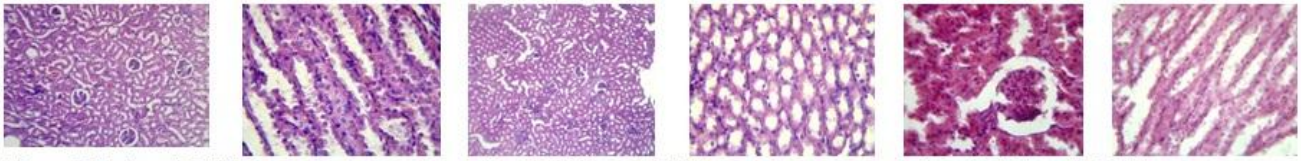


FigureC2(a) and C2(b) lobular architecture and cytoplasmic vacuolation

FigureC3(a) and C3(b) normal hepatocytes

FigureC4(a) and C4(b) lobular architecture with mild inflammation and sinusoidal dilatation

Note: a-10x; **b**-40x; **2**- low dose(1 ml/kg) ; **3**- middle dose(2.5 ml/kg) ; **4**- high dose(4 ml/kg) ; **C-Liver**

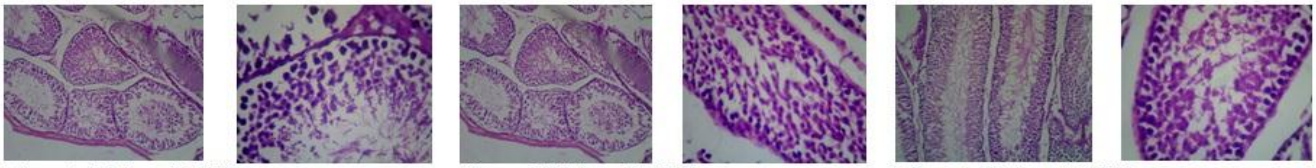


FigureD2(a) and D2(b) normal cortex medulla and interstitium

FigureD3(a) and D3(b) normal cortex, medulla and tubules

FigureD4(a) and D4(b) normal glomeruli and tubules

Note: a-10x; **b**-40x; **2**- low dose(1 ml/kg) ; **3**- middle dose(2.5 ml/kg) ; **4**- high dose(4 ml/kg) ; **D-Kidney**

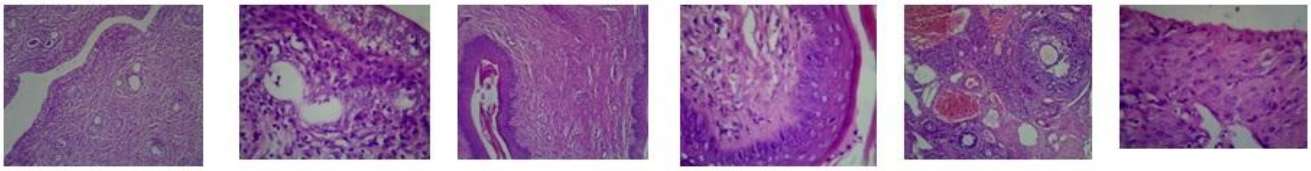


FigureE2(a) and E2(b) normal maturation

FigureE3(a) and E3(b) maturation in varying stages(primary and secondary stages)

FigureE4(a) and E4(b) varying stages of maturation and testicular parenchyma

Note: a-10x; **b**-40x; **2**- low dose(1 ml/kg) ; **3**- middle dose(2.5 ml/kg) ; **4**- high dose(4 ml/kg) ; **E-Testis**



FigureF2(a) and F2(b) normal uterus and subepithelium

FigureF3(a) and F3(b) normal endocervix and entocervix

FigureF4(a) and F4(b) varying stages of ovarian follicles and stroma

Note: a-10x; **b**-40x; **2**- low dose(1 ml/kg) ; **3**- middle dose(2.5 ml/kg) ; **4**- high dose(4 ml/kg) ; **F-Uterus**

DISCUSSION

Acute oral toxicity:

SRB Decoction was administered single time at the dose of 10 ml/kg and observed for consecutive 14 days. Doses were selected based on the pilot study and literature review. All animals were observed daily once for any abnormal clinical signs. No mortality was observed during the entire period of the study. Data obtained in this study indicated no significance physical and behavioral signs of any toxicity due to administration of SRB decoction at the dose of 10 ml/kg. At the 14th day, all animals were observed for functional and behavioral examination. In functional and behavioral examination, home cage activity, hand held activity were observed. Home cage activities like Body position, Respiration, Clonic involuntary movement, Tonic involuntary movement, Palpebral closure, Approach response, Touch response, Pinna reflex, Sound responses, Tail pinch response were observed. Handheld activities like Reactivity, Handling, Palpebral closure, Lacrimation, Salivation, Piloercetion, Papillary reflex, abdominal tone, Limb tone were observed. Functional and behavioral examination was normal in all treated groups. The acute toxicity study showed no mortality of rats up to the dosage of 5000mg/kg. No behavioral changes or abnormal clinical signs of toxicity were observed up to the above dosage throughout the end of 14 day study period. No gross pathological abnormality in the organs was found even at this high dose. LD50 value was found to be more than 5000mg/kg body weight and therefore this test drug SRB decoction falls under (Unclassified) category V with reference to Globally Harmonized classification System (GHS) [5].

28 days repeated oral toxicity:

All the animals from control and all the treated dose groups up to 500 mg/kg survived throughout the dosing period of 28 days. No signs of toxicity were observed in animals from different 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 28 days. Haematological analysis conducted at the end of the dosing period on day 29, revealed no abnormalities attributable to the treatment. Biochemical analysis conducted at the end of the dosing period on day 29 no abnormalities 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.

Histopathology examination:

Histopathological examination revealed normal architecture in comparison with control and treated animal. The heart showed normal myocardial tissue in all three groups, blood vessels showed congestion with mild loss of architecture with no evidence of inflammation, necrosis or ischemia. The lung showed normal bronchi, bronchioles and alveoli in low dose while there was inflammation in the interstitium in the middle dose and peri bronchial inflammation in the high dose group with no evidence of granuloma in all three groups. The liver showed normal lobular architecture in all three groups while there was mild congestion of the central vein and portal tract showed bile duct hyperplasia in all three groups. The kidney showed normal cortex, medulla, glomeruli and tubules in all three groups and there is no evidence of inflammation or tubular necrosis. The testis showed normal testicular parenchyma, seminiferous tubules and spermatocytes and there was no evidence of maturation arrest in all three groups. The uterus showed normal epithelium and normal development of oocyte.

CONCLUSION

The acute oral toxicity study on Wistar rats showed that SRB Decoction did not produce any toxic effect at dose of 10 ml/kg. In 28 days repeated oral toxicity study it did not cause either any lethality or adverse changes with general behavior of rats and also there were no observable detrimental effects (1 ml/kg, 2.5 ml/kg, 4 ml/kg body weight) over a period of 28 days when administered orally in rats.

CONFLICT OF INTEREST STATEMENT

The authors do not have conflict of interest over this research work.

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