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Sub-Acute Toxicity Study of Hydro-Alcoholic Extracts of *Sansevieria cylindrica* and *Plumeria obtusa* in Wistar Albino Rats

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Abstract

Sansevieria cylindrica Bojer ex. Hook (Asparagaceae) and *Plumeria obtusa* L. (Apocynaceae) are useful traditional medicines. The present study assessed the toxicity of a combination of hydro-alcoholic extract of aerial parts of both these plants through repeat administration in Wistar albino rats. This study is an extension of an acute toxicity study, wherein female rats were orally administered with a single dose of 2000mg/kg of both plant extracts. Sub-acute toxicity of both plants (200 and 400 mg/kg p.o.) was studied by daily dosing of Wistar rats of both sexes for 28 days. Toxicity, mortality, body weight changes, food and water intake, and neurological assessments were recorded. Hematological, biochemical, and urine analysis was done before and after dosing on day 29. Absolute and relative organ weight and histological evaluation were performed on day 29. The acute toxicity study revealed no lethal effects indicating that LD_{50} is greater than 2000mg/kg. The sub-acute study observed a significant increase ($p < 0.05$) in body weight, feed, and water intake. The body weight gain in rats from the treatment group was non-significant ($p > 0.05$) compared to the control group. A significant ($p < 0.05$) increase in lymphocytes, granulocytes, RBC, platelet, creatinine, albumin, triglycerides, and alkaline phosphatase were also observed. The evaluation of different neurobehavior parameters showed a significant increase ($p < 0.05$). Histopathological analysis showed mild liver distortion in male rats in both treatment groups. Most of the significant observations were considered incidental findings indicative of low toxicity of plant extract during long-term use.

Keywords: *Plumeria obtusa*, Safety, *Sansevieria cylindrica*, Sub-Acute Toxicity, Toxicity

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1. Introduction

Sansevieria cylindrica (SC) plant belongs to the Asparagaceae family. It is commonly found in the African continents, whereas it is also grown for ornate reasons in Egypt, India, and other countries[1,2]. They grow up to 2 m (7 ft) above the soil and have a diameter of around 3 cm (1 inch). Their leaves are long and thin, sub-cylindrical in shape with a smooth-striped surface and greenish-grey in colour[3]. *Sansevieria* species exert a wide range of biological activities like antitumor, anti-bacterial, hunting free radicals, antidiabetic effects, etc. They have also demonstrated suppression of capillary permeability, which might help in anti-inflammatory activity[4].

The *Plumeria obtusa* (PO) plant is geographically distributed in Greater Antilles, Florida, Northern Central America, and Southern Mexico[5,6]. It is known by different names in different countries like Rote Frangipani (Germany), Frangipanier (France), Araliya (Sri Lanka), Temple tree (United Kingdom), White frangipani (Australia), Champa or Chafa (India), Melia (Hawaii) belonging to the Apocynaceae family[7]. *Plumeria* has many medicinal uses such as emmenagogue, febrifuge, hemostatic, laxative, purgative, rubefacient, stimulant, and vermifuge[8,9].

The scientific reports on phytochemical or plant extracts in experimental animals at high doses showed their toxic effects such as carcinogenic (capsaicin, chili powder, safrole), neurotoxic (*Papaver somniferum*, *Erytroxylum* sp., and *Cannabis sativa*), genotoxic (thymol and carvacrol), teratogenic (pyrrolizidine alkaloid monster, heliotrope), cytotoxic (*Allium sativum*, *Lagerstroemia speciosa*, *Calotropis procera, Chenopodium murale, Pulicaria orientalis, Tribulus Terrestris, Withania somniferum, Blumea lacera* and safrole-20, 30-oxide), nephrotoxic (aristolochic acid, turmeric, Juniper berries), etc[10]. These reports are alarming and indicate the need for evaluation of the safety of phytochemicals for their repeated use. Traditional knowledge and controlled clinical trial data are necessary to demonstrate plant extract's pharmacokinetics, efficacy, and safety or its bioactive compounds[11]. However, research in the toxicological domain (such as acute, sub-acute, sub-chronic, and chronic studies) not only adds value to the ethnopharmacology of phytochemicals but also will ensure its safe use and may avoid the occurrence of any untoward effects which were considered to be a significant obstacle with the synthetic drugs[12]. Sub-acute toxicity studies are usually carried out after the availability of initial information on acute toxicity studies, as they provide information on possible health hazards likely to arise from repeated exposure to drugs/chemicals over a limited period of time[13]. In addition, sub-acute toxicology studies in rodents are considered to be an obligatory step to support the progression to clinical trials and the eventual marketing of drug molecules[14].

The aforementioned facts necessitate the need to assess the toxicological profile of SC and PO plants. We have already performed the 14-day single oral acute dose toxicity (2000mg/Kg; body weight (b.wt.) study of these plant extracts and found them safe[15]. Currently, a study on the sub-acute safety profile of the plant extract needs to be completed. Therefore, in the present study, we have evaluated the sub-acute toxicological effects of these plants in Wistar rats of both sexes. This study will add value to its ethnopharmacological profile. Further, it may provide a platform to search for ways of decreasing its toxic effects (if any).

2. Materials and Methods

2.1 Chemicals and Reagents

All the chemicals and solvents of analytical grade were purchased from S. D. Fine Chemicals Mumbai, India.

2.2 Experimental Animals

The sub-acute toxicity investigation was conducted based on the study conducted by Agarwal *et al.*, with modification on healthy adult female Wistar albino rats (age 8-12 weeks, body weight 180-250 g)[16]. The rats were provided by Crystal Biological Solutions, Pune (Reg. No. 2030/PO/RcBiBt/S/18/CPCSEA). The female rats chosen were nulliparous and were not pregnant. Before testing, the animals were acclimatized to the laboratory environment for seven days. Each polypropylene cage held three rats. The cages were housed under standard settings (22 ± 1 °C temperature, 60-70 % relative humidity, and a 12-hour light/12-hour dark cycle). Animal care and handling were done per the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines. The bedding was made from fresh paddy husk. The rats had free access to normal rat pellet food (M/s, Nutrivet Life Sciences) and drinking water during the study.

2.3 Ethical Approval

The experimental design of the present study was reviewed and approved with reference no. DYPIPSR/IAEC/21-22/P-03 by the Institutional Animal Ethics Committee (IAEC) of Dr. D. Y. Patil Unitech Society's

Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune-411018, India (Regd. no.: 198/PO/Re/S/2000/CPCSEA) and animals were maintained as per the guidelines of the CPCSEA India.

2.4 Limit Test (Acute Toxicity Study)

As per OECD TG 423, rats in Group I and Group II were assigned to SC and PO plant extract, respectively, at the dose of 2000mg/kg, b.wt. The Group III animals received extract in a 1:1 ratio (at the dose of 1000 mg/kg, b.wt. for each plant extract). All the rats were observed for any mortality and toxicities for 14 days. The safety bleed and urine collection were performed at the baseline and end of the study.

2.5 Sub-Acute Toxicity Study Design

The sub-acute toxicity study was carried out on Wistar albino rats in accordance with OECD standards TG 407 with a few minor adjustments and modifications[17]. The study included 18 rats (9 male and 9 female) which were randomly divided into three groups (n = 3M/3F) maintaining mean body weight variation less than 25%. Rats groups were as follows:

a. The control group received distilled water for 28 days.
b. SC and PO hydro-alcoholic extracts (SCPOHAE) [SC+PO (1:1 ratio)] received 200 mg/kg, b.wt. (100 mg/kg of each plant extract) for 28 days.
c. SCPOHAE [SC+PO (1:1 ratio)] received 400 mg/kg, b.wt. (200 mg/kg of each plant extract) for 28 days.

Each rat was marked with an identification marker. The test substances (extracts) were suspended in carboxymethyl cellulose (CMC) (1%, v/v) and orally administered to rats through oral gavage for 28 days.

2.6 Experimental Assessment

2.6.1 In-Life Clinical Observations

Animals were observed twice a day for mortality. Following dosing with test extracts, each rat of the control and treatment group was observed closely for changes in skin color, hair, and eye, lethargy, tremors, diarrhea, salivation, respiration, behavior, sleep, and motor activity on days 1, 7, 14, 21, and 28[18].

2.6.2 Body Weight and Body Weight Gain

Each rat's body weight and food intake were recorded prior to dosing (Day 1) and thereafter on each alternate day and day 29 (fasting body weight prior to sacrifice) in control and treatment groups. The mean body weight gains were determined at the end of the treatment period using the relation[19];

Weight gain (%) = $(W_f - W_i) \times 100$; where, W_f = final weight; W_i = initial weight.

2.6.3 Food and Water Consumption

Animals were allowed ad libitum access to food and water throughout the study. Food and water consumption of treatment and control groups were measured on Day 1 (prior to dosing) and after that on each alternate day of the in-life experiment. Animals were fasted overnight prior to termination at the end of the study.

2.6.4 Clinical Pathology

The rats were fasted overnight before blood sample collection. The blood samples were collected on day 1 and day 29 for hematological analysis through retro-orbital puncture using capillary in pre-calibrated tubes containing anticoagulant (EDTA) for assessment of hematology and without anticoagulant for assessment of biochemistry parameters. All animals were anesthetized using isoflurane before the collection of blood.

2.6.4.1 Hematological Analysis

The BD 21 Auto Hematology analyzer was used to analyze blood samples (HASTECH technology-Veterinary Model). Total White Blood Cell (WBC) count, Lymphocyte (LYM), Lymphocyte Percentage (LYM%), Middle Cell Number (MID), Middle Cell Number percentage (MID%), granulocyte number (GRAN), granulocyte percentage (GRAN%), total Red Blood Cell (RBC) count, hemoglobin, Hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Platelet Count (PLT), Mean Platelet Volume (MPV), Platelet Distribution Width (PDW), Platelet Crit (PCT), Platelet Large Cell Ratio (PLCR), and Platelet Large Cell Count (PLCC) were evaluated.

2.6.4.2 Biochemical Analysis

The blood sample was centrifuged at 1000 rpm for 20 minutes, and the serum was separated. The separated serum was stored at -20°C and later used for evaluation of Blood Glucose (BG), urea, Total Protein (TP), Creatinine (CR), Albumin (ALB), Total Bilirubin (TB),

Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT), Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST), Triglyceride (TG), Total Cholesterol (TC), etc. An automated biochemistry analyzer (Epoch Microplate Spectrometer, SN/Model-1505231C, Biotech Instruments Inc.) and standard diagnostic test kits (Pathozyme Diagnostics, Maharashtra, India) were used for analyses of these parameters.

2.6.4.3 Urinalysis

The qualitative analysis of urine samples was carried out before dosing and on the last day after the dosing. Metabolic cages (Ajinkya Enterprises, Maharashtra, India) were used for the overnight collection of urine from all the animals. Urine samples were analyzed utilizing a Uriscan Pro Optima (BioSys Laboratories, Inc., CA) automated urine analyzer and urine test strips for color, appearance, pH, specific gravity, proteins, blood, ketones, glucose, urobilinogen, and bilirubin. The urine was also assessed for microscopic examination under 10 X magnification using 250µL of a urine sample.

2.6.5 Gross Necropsy and Organ Weight

All the animals fasted overnight were euthanized with ketamine hydrochloride (100mg/kg, i.m.). The external surfaces, all orifices, and the thoracic, abdominal, cranial, and pelvic cavities were all examined macroscopically[20]. The liver, kidney, brain, spleen, testis (male rats), ovaries, and uterus (female rats) of rats were removed during necropsy. These vital organs were thoroughly cleaned using water and kept in a 10% neutral buffered formalin solution. Then the absolute organ weights were recorded in grams using a calibrated balance, and Relative Organ Weight (ROW) was determined using the formula given below:

ROW = [Absolute organ weight (g)/Body weight of rat on sacrifice day (g)] x 100

These organs were also examined under the microscope for the development of lesions or other remarkable changes. For histopathological assessment, the vital organ samples were dehydrated in a graded sequence of ethanol (70-99.9 %), rinsed with toluene, and wrapped in paraffin after fixation. On a rotating microtome, thin tissue sections of 3-5 µm were produced, and the material was subsequently stained with Hematoxylin and Eosin (HE)[21]. Microscopically, the parts were examined for pathological tests, and photomicrographs were taken.

2.6.6 Neurotoxicological Evaluation

2.6.6.1 Functional Observational Battery (FOB)

FOB was performed according to reported standard procedures with few minor modifications[22,23]. The scoring criteria given in the reported literature were used for neurotoxicological assessment[24]. The FOB was recorded during an initial week (Day 1), during mid-week (Day 14), and during the final week (Day 28) of the dosing period. After dosing, the rats were kept in home cages for 10 minutes and observed for posture, presence or absence of tremors and convulsions, spontaneous vocalization, and biting. Handheld observations such as ease of removal and handling, lacrimation, salivation, and piloerection were conducted. Following these observations, sensorial responses were also monitored, such as response to various stimuli (click stimulus using a metal clicker, pinch on the tail using the metal tweezers, constriction of the pupil to a penlight stimulus, touch in the corner of the eye using fine cotton thread). Then, the animals were placed in an open field covered with clean absorbent paper on the surface and allowed to explore for 2 min freely. The parameters investigated during that period were arousal activity, gait, mobility, unusual movements, and stereotype activities. After the allotted time period, the presence/absence of diarrhea on the absorbent paper was recorded. The rats were transferred to a sound-attenuated observation room and allowed to acclimate for 30 to 40 minutes.

2.6.6.2 Open Field Arena

In this experiment, rats were tested in the open field to determine whether plant extracts modulate gross locomotor activity, exploration, and anxiety-like behaviors during the dosing period's final week (Day 28)[25,26]. The open field apparatus consisted of a metal square box of 72 x 72 x 36 cm (L x W x H) size having a white Formica floor. The wall surfaces were smooth to avoid any injury to rats. Using a blue marker, the floor was further divided into 16 equal square (18 x 18 cm) blocks. A central square was drawn using a red marker, an intersection of four neighboring squares. The apparatus was kept in a sound-attenuated room, and lighting was provided using a 60-watt white bulb which was fixed above the box. The floor was cleaned using 70% ethyl alcohol after each animal test. A video camera connected to a computer with MazeMaster 2.0 software was attached 2 cm above the apparatus to capture the animal behavior in the arena. The rats were allowed to acclimatize in the room for 5-10

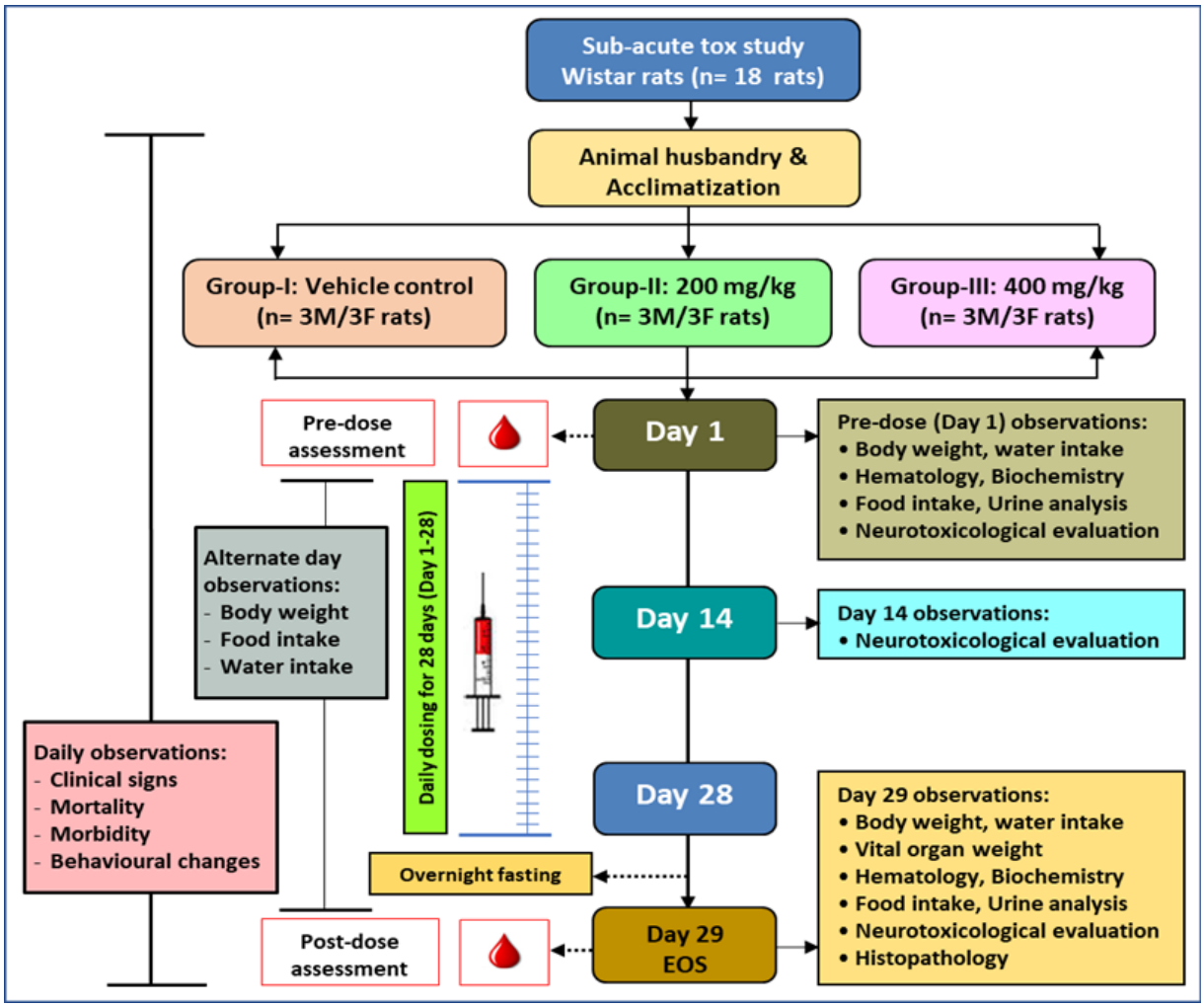


Figure 1. The overall study schema of the sub-acute toxicity study, which was carried out in both male and female Wistar rats by daily dosing through oral administration for 28 days. EOS: End of study.

minutes before initiating the test. Each rat was then gently placed in the box and allowed free movement in the arena for 5 minutes. Observations such as center square entries, time in center, stay in the center, latency period, velocity, and distance traveled were captured through software and video recording. In addition, other parameters like grooming, freezing, defecation, and urination were also observed and recorded.

2.6.6.3 Locomotor Activity

The locomotor activity was performed using an actophotometer on Wistar albino rats according to the standard procedures[27]. The spontaneous locomotor activity was recorded on Day 1, Day 14, and Day 28 of the dosing period. Actophotometer consists of a series of photocells. When the beam of light from these cells is interrupted by rats, reading is observed on a digital counter in the form of counts. Each animal was observed for 10 min after placing it in the cage. The counts were recorded when rats cut off the beam of light falling on the photocell of an actophotometer. The number of obstructions recorded for extracts was compared with the control group. The overall study schema of the sub-acute toxicity study is displayed in Figure 1.

2.7 Statistical Analysis

The data was computed and reported as mean and standard deviation. Graph Pad Prism, 6.0 software, and Microsoft Excel 2019 (Microsoft Corp., Redmond, WA, USA) were used for statistical analysis and presentation of the data. Dunnett's t-test was used to compare the means before and after the dose of the plant extract. A highly

significant ($p < 0.01$) and significant ($p < 0.05$) p-values were determined for comparative analysis.

3. Results

In the acute dose toxicity study, the SCPOHAE individually and also as a 1:1 blend at the limited dosage (2000mg/kg, b.wt.) did not cause death and did not induce any remarkable and abnormal clinical signs, indicative of systemic toxicity, in rats during the treatment period of 14-days. The No-Observed-Adverse-Effect Level (NOAEL) of these extracts in rats was greater than 2000mg/kg.

Several parameters were assessed in the current sub-acute toxicity study. Over the course of 29 days after the repeated oral doses, no death was reported in any of the treatment groups. None of the rats developed any remarkable morbidity or clinical toxicities. Figure 2 represents the changes in body weight and body weight gain. There was a non-significant ($p > 0.05$) increase in body weight of male rats from treatment groups, except on days 19 and 29, wherein a significant increase in body weight ($p < 0.05$) was observed at 200 and 400mg/kg doses of plant extract, respectively. In female rats, the increase in body weight was significant ($p < 0.05$) in group 2 on days 5, 7, 11, 13, 17, 23, 27, and 29 in comparison to the control group. A similar trend was observed in group 3 on days 13, 15, and 17. The percentage weight gain in rats from treatment groups was found to be comparable in a non-significant ($p > 0.05$) manner.

Statistically higher ($p < 0.05$) food consumption was observed in female rats during the first week on Day 5 and Day 3 at 200 and 400 mg/kg doses of plant extract, respectively. Similarly, male rats also reported significantly ($p < 0.05$) higher food consumption on Day 5 and Day 21 in Group 2 and Group 3, respectively, compared to the control group. All male and female rats' drinking habits were statistically unaffected ($p > 0.01$), with a minor non-treatment-related decline or increase in water intake. (Figure 3).

The effect of plant extracts on hematological parameters on Day 29 is tabulated in Table 1. The percent lymphocytes and platelet count were significantly increased ($p < 0.05$) in male rats at 400 mg/kg of extract doses. In the case of female rats, the percent granulocytes were significantly decreased ($p < 0.05$) in both treatment groups. The HCB and HCT were also significantly affected than the control group in female rats at 400mg/kg doses. Other hematological

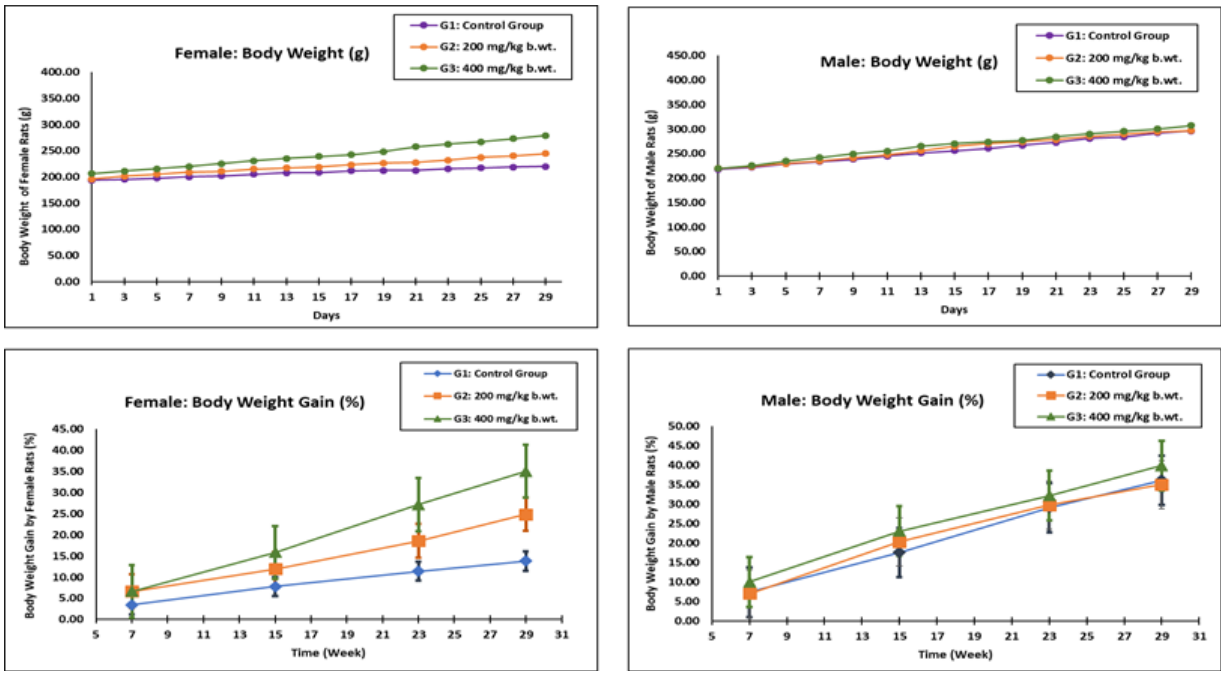


Figure 2. Changes in body weight and body weight gain. Body weight (g) by days and body weight gain (%) by week of male and female control group rats and treated with SCPOHAE at 200 and 400 mg/kg doses in the sub-acute toxicity study. Values are presented as Mean ± SD, n = 3; Significant in relation to the control group at $p < 0.05$, $p < 0.01$ using one-way ANOVA followed by Dunnett's test.

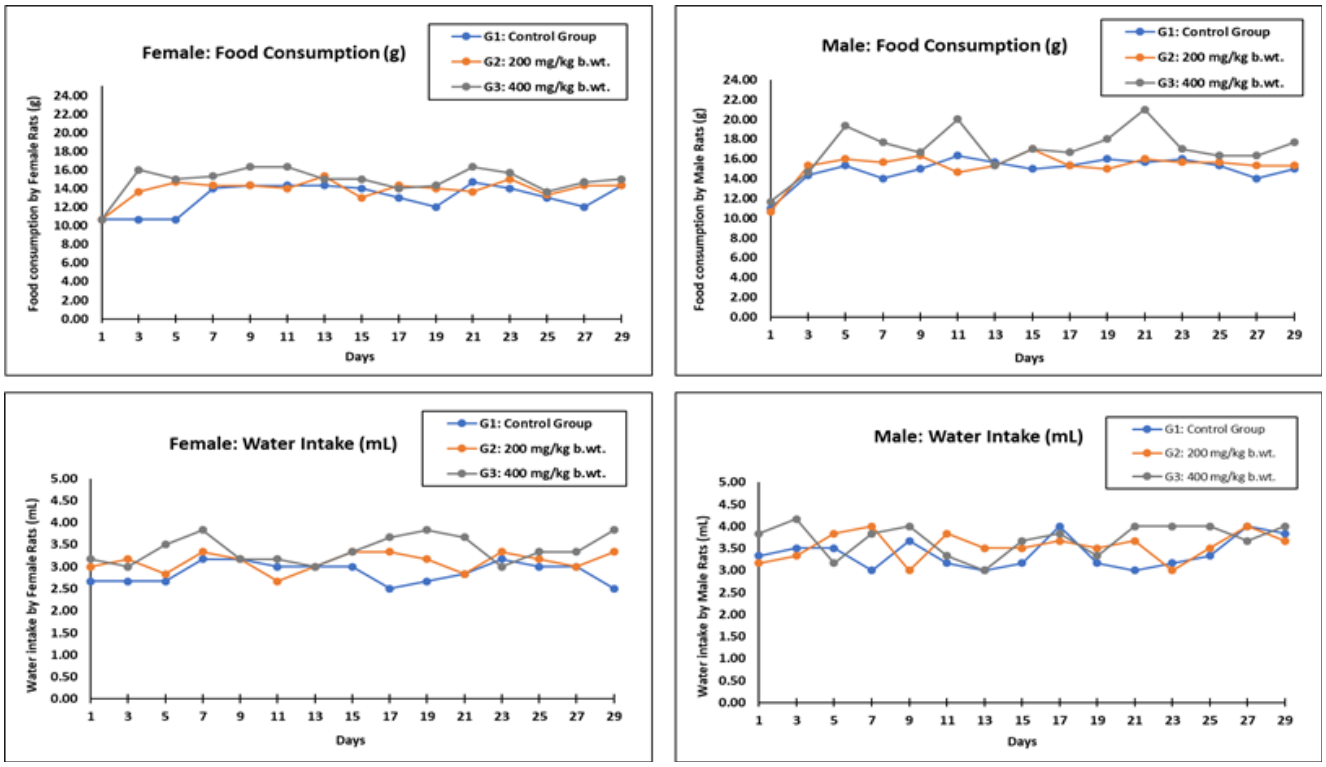


Figure 3. Changes in food consumption and water intake. Feed (g/) and water (ml) consumption by days of male and female rats of the control group and treated with SCPOHAE at the doses of 200 and 400 mg/kg in the sub-acute toxicity study. Values are presented as Mean ± SD, n = 3; Significant in relation to the control group at $p < 0.05$, $p < 0.01$ using one-way ANOVA followed by Dunnett's test.

parameters did not significantly differ from the control group. Assessment of baseline hematological parameters on Day 1 is presented in Table S1.

Table 2 portrays the biochemical parameters assessed on Day 29 of the study. The assessment of baseline biochemical parameters on Day 1 is also presented in Table S2. A significant increase ($p < 0.05$) in ALP and potassium levels was observed in male rats at 200mg/kg doses of extract. The glucose and triglyceride levels decreased ($p < 0.05$) in male rats in group 3 than in the

Table 1. Effect of plant extracts on hematological parameters on Day 29

Parameters	Groups (Male)			Groups (Female)		
	Control	200 (mg/kg)	400 (mg/kg)	Control	200 (mg/kg)	400 (mg/kg)
WBC (10^9/L)	9.10 ± 1.47	7.20 ^{b*} ± 0.56	8.47 ± 1.52	8.17 ± 1.78	9.17 ± 3.75	8.20 ± 1.54
LYM (#)	6.43 ± 1.25	4.27 ± 1.10	6.90 ± 2.42	3.47 ^{b*} ± 0.67	5.60 ± 1.57	6.30 ± 1.64
LYM (%)	70.47 ± 6.38	77.87 ± 6.06	88.00 ^{c*} ± 2.42	79.23 ± 1.19	63.10 ^{c*} ± 7.79	76.13 ± 6.10
MID (#)	0.73 ± 0.21	0.40 ± 0.10	0.43 ± 0.06	0.27 ± 0.06	0.67 ± 0.38	0.60 ± 0.10
MID (%)	7.83 ± 1.33	7.03 ± 1.40	5.50 ± 1.32	6.03 ± 0.68	7.07 ± 1.81	7.37 ± 0.38
GRAN (#)	5.39 ± 0.94	4.40 ± 1.11	3.19 ± 0.62	6.29 ± 0.55	3.92 ^{c*} ± 1.03	2.99 ^{c*} ± 1.51
GRAN (%)	21.70 ^{b*} ± 6.56	15.10 ± 4.67	13.17 ± 12.52	14.73 ± 0.80	29.83 $^{b*, c*}$ ± 6.36	16.50 ± 5.76
NEUT (#)	1.83 ^{b*} ± 0.06	1.50 ± 0.36	1.73 ± 0.57	1.33 ± 0.21	2.13 ± 0.72	2.57 ± 0.91
NEUT (%)	20.31 ± 1.94	18.10 ± 4.43	19.90 ± 3.00	14.11 ^{b*} ± 4.33	18.59 ± 3.35	19.12 $^{b*, c*}$ ± 2.18

(*Continued*)

Table 1. *Continued*

Parameters	Groups (Male)			Groups (Female)		
	Control	200 (mg/kg)	400 (mg/kg)	Control	200 (mg/kg)	400 (mg/kg)
RBC (10^12/L)	6.27 ± 0.36	6.97 ± 1.87	7.88 ± 1.15	6.23 ^{b*} ± 0.93	8.68 ± 2.03	9.03 ^{c*} ± 0.61
HGB (g/dL)	14.33 ± 1.10	16.33 ± 4.04	18.23 ± 2.90	13.87 ± 1.91	19.47 ^{c*} ± 4.26	19.87 ^{c*} ± 2.25
HCT (%)	59.40 ± 7.19	37.90 ± 9.85	42.77 ± 6.11	58.70 ± 1.35	51.43 ± 12.11	48.23 $^{b*, c*}$ ± 5.40
MCV (fL)	54.33 ^{b*} ± 2.06	54.53 ± 0.95	54.40 ± 0.69	52.90 ^{b*}± 1.37	59.70 ^{b*} ± 7.30	53.33 ^{b*} ± 2.54
MCH (pg)	22.80 ^{b*} ± 0.72	23.50 ^{b*} ± 0.56	23.07 ± 0.46	22.20 ± 0.53	22.43 ± 0.99	21.90 ^{b*} ± 1.06
MCHC (g/dL)	42.07 ± 0.40	43.20 ± 0.79	42.50 ± 0.75	42.20 ± 0.66	37.93 ^{b*} ± 3.21	41.13 ^{c*} ± 0.31
RDW-CV (%)	11.63 ± 0.25	11.57 ± 0.76	11.63 ± 0.46	12.63 ± 0.93	20.10 ± 6.58	13.60 ^{b*} ± 1.54
RDW-SD (fL)	26.87 ± 1.50	26.87 ^{b*} ± 1.50	26.00 ± 1.52	26.22 ± 1.59	39.83 ^{b*} ± 9.82	31.20 ^{b*} ± 6.88
PLT (10^9/L)	271.33 ± 17.62	298.67 ± 72.86	385.33 ^{c*} ± 42.52	255.67 ± 22.37	246.17 ^{b*} ± 68.12	287.67 ± 41.62
MPV (fL)	4.50 ± 0.10	4.93 $^{b*, c*}$ ± 0.12	4.93 ^{b*} ± 0.21	4.33 ± 0.21	5.00 ^{c*} ± 0.36	4.57 ^{b*} ± 0.15
PDW (fL)	6.10 ^{b*} ± 0.36	6.97 $^{b*, c*}$ ± 0.12	7.33 ^{b*} ± 0.81	5.70 ± 0.20	7.77 ^{b*} ± 1.46	6.93 ^{b*} ± 1.25
PCT (%)	0.12 ± 0.01	0.14 ^{b*} ± 0.04	0.19 ± 0.02	0.11 ^{b*} ± 0.01	0.16 ^{b*} ± 0.04	0.13 ± 0.02
P-LCR (%)	8.10 ± 1.57	10.83 $^{b*, c*}$ ± 1.17	11.77 $^{b*, c*}$ ± 2.57	9.67 ^{b*} ± 1.10	10.03 ^{b*} ± 2.27	9.43 ^{b*} ± 1.06
P-LCC (10^9/L)	20.33 ± 3.21	36.67 ± 3.21	45.00 ± 4.36	27.00 ^{b*} ± 5.00	41.00 ^{c*} ± 6.08	37.67 $^{b*, c*}$ ± 9.61

Values are expressed as Mean ± S.D. (n = 3). b = measurement in comparison with baseline value on Day 1, c= measurement in comparison with control group on Day 29, **p < 0.01 (highly significant) and *p < 0.05 (significant).

Table 2. Effect of plant extracts on biochemical parameters on Day 29

Parameters	Groups (Male)			Groups (Female)		
	Control	200 (mg/kg)	400 (mg/kg)	Control	200 (mg/kg)	400 (mg/kg)
Glucose (mg/dL)	94.88 ^{b*} ± 1.80	118.45 ± 22.28	69.34 ^{c*} ± 12.11	94.30 ± 16.65	112.75 ± 8.88	89.35 ± 18.90
Creatinine (mg/dL)	0.56 ± 0.12	0.66 ± 0.60	0.64 ± 0.26	0.75 ± 0.39	1.74 ± 0.66	1.73 ^{c*} ± 0.56
Total protein (g/dL)	7.06± 0.57	5.90 ± 0.87	6.63 ± 2.53	6.38 ^{b*}± 0.46	5.49 ^{b*}± 1.22	6.63 ± 1.12
Albumin (g/dL)	1.13 ± 0.15	1.07 ± 0.25	0.83 ± 0.06	0.83 ± 0.15	0.97 ± 0.25	1.07 ^{c*} ± 0.21
ALT (U/L)	37.33 ± 6.66	43.33 ± 3.51	27.00 ± 5.29	31.00 ± 4.00	43.67 ^{c*} ± 5.51	40.33 ± 6.11
AST (U/L)	76.67 ± 8.14	76.83 ± 14.57	77.00 ± 19.31	98.33 ± 14.57	94.33 ^{b*}± 13.05	95.67 ± 16.50
ALP (U/L)	105.67 ^{b*}± 11.59	130.00 ^{c*} ± 7.94	107.67 ^{b*}± 5.13	118.67 ± 6.66	102.33 ± 5.86	97.00 ^{c*} ± 4.58
Bilirubin (mg/dL)	1.17 ^{b*}± 0.02	1.13 ± 0.03	1.48 ± 0.58	1.79 ± 0.54	1.47 ± 0.56	1.09 ± 0.07
Total cholesterol (mg/dL)	76.94± 27.31	56.59 ± 0.90	45.20 ± 4.22	40.16 ^{b*}± 8.27	46.59 ± 2.71	48.51 ± 1.19
Triglycerides (mg/dL)	89.79 ^{b*}± 4.08	92.23 ^{b*}± 23.63	69.36 $^{b*, c*}$± 3.36	64.48 ± 6.91	53.82 ^{c*} ± 3.86	67.18 ^{b*}± 10.15
Urea (mg/dL)	19.67 ± 0.58	25.00 ^{b*}± 6.56	28.33 ± 6.66	22.00 ± 4.58	19.33 ± 3.21	23.33 ± 4.93
Calcium (mg/dL)	9.90 ± 0.46	10.23 ± 0.15	9.50 ^{b*}± 0.36	9.87 ± 0.15	10.30 ± 0.87	9.93 ± 0.81
Sodium (mmol/L)	131.00 ± 5.00	138.67 ± 9.07	142.67 ^{c*} ± 8.33	139.33 ± 9.29	146.33 ± 6.11	144.00 ± 4.36
Potassium (mmol/L)	3.60 ^{b*}± 0.20	5.33 $^{b*, c*}$± 0.21	4.00 ± 0.30	4.23 ± 0.15	4.37 ± 0.55	4.93 ± 0.65

Values are expressed as Mean ± S.D. (n = 3). b = measurement in comparison with baseline value on Day 1, c= measurement in comparison with control group on Day 29, **p < 0.01 (highly significant) and *p < 0.05 (significant).

control group. A significant increase in creatinine and albumin at 400 mg/kg doses and ALT at 200mg/kg doses was observed. All other parameters were similar to the control group.

The urine analyses of male and female rats from all groups do not report any alteration in urine parameters compared to the control group and baseline measurements (Table S3). The observation of these parameters on Day 29 is displayed in Table 3.

The Functional Observational Battery (FOB) observations are collated in Table 4, whereas FOB scores for neurotoxicological investigation are included in Table S5. A significant difference ($p < 0.01$) was observed for the ease of handling parameter as compared to the control group on Day 1. The animal response to touch was found to be significantly decreased ($p < 0.05$) compared to the control group on Day 1; however, on Day 29, it was found normal. Most of the FOB parameters were found normal during mid-week also (Table S4).

Figure 4 represents the tracing of animals in an open-field test. The distance travelled, velocity of travel, and time spent in the center by male rats at 200 and 400 mg/kg doses was found to be increased significantly ($p < 0.05$) compared to the control group. Female rats also reported a more significant velocity increase ($p < 0.05$) than group 1.

Figure 5 represents the locomotor activity of the control group and 200 and 400 mg/kg doses of extracts. The female rats from 400mg/kg doses showed a significant increase ($p < 0.01$) in locomotor activity at 30min during mid-week compared to the control group. The spontaneous motor activity was more in male rats during the week of Day 14 and Day 28, whereas a significant increase was observed at 200 mg/kg doses ($p < 0.05$) and 400 mg/kg doses ($p < 0.01$).

Table 3. Effect of plant extracts on urine parameters on Day 29

Parameters	Observations	Groups (Male)			Groups (Female)		
		Control	200 (mg/kg)	400 (mg/kg)	Control	200 (mg/kg)	400 (mg/kg)
Colour	Colourless	0	0	0	0	0	0
	Pale yellow	1	2	2	2	1	1
	Yellow	1	0	1	0	0	1
	Amber	0	0	0	1	2	1
	Dark Yellow	1	1	0	0	0	0
Appearance	Clear	3	3	3	3	3	3
pH	Less than 6	0	0	0	0	0	0
	6-8	3	3	3	3	3	3
	More than 8	0	0	0	0	0	0
Specific gravity	Less than 1.015	1	0	3	1	1	0
	1.015 - 1.020	2	2	0	2	2	1
	1.021 - 1.025	0	1	0	0	0	2
	1.026 - 1.030	0	0	0	0	0	0
Urobilinogen	0.2	3	2	1	1	3	2
	1	0	1	2	2	0	1
Protein	-ve	0	1	1	1	1	1
	Trace	2	1	2	1	0	1
	High	1	1	0	1	2	1
Bilirubin	(-ve) / (+ve)	3 (-ve)	3 (-ve)	3 (-ve)	3 (-ve)	3 (-ve)	3 (-ve)
Ketone	(-ve) / (+ve)	3 (-ve)	3 (-ve)	3 (-ve)	3 (-ve)	3 (-ve)	3 (-ve)
Glucose	(-ve) / (+ve)	3 (-ve)	3 (-ve)	3 (-ve)	3 (-ve)	3 (-ve)	3 (-ve)
Epithelial cells	(-ve) / (+ve)	3 (-ve)	3 (-ve)	3 (-ve)	3 (-ve)	3 (-ve)	3 (-ve)
PC	(-ve) / (+ve)	3 (-ve)	3 (-ve)	3 (-ve)	3 (-ve)	3 (-ve)	3 (-ve)
Occult blood	-ve/+10	3 (-ve)	3 (-ve)	3 (-ve)	3 (-ve)	3 (-ve)	3 (-ve)
Casts and crystals	(-ve) / (+ve)	3 (-ve)	3 (-ve)	3 (-ve)	3 (-ve)	3 (-ve)	3 (-ve)

-ve: Negative; +ve: Positive

Table 4. Effect of plant extracts on functional observation battery parameters

FOB Endpoints	Male						Female					
	Day 1			Day 29			Day 1			Day 29		
	Control	200mg/kg	400mg/kg	Control	200mg/kg	400mg/kg	Control	200mg/kg	400mg/kg	Control	200mg/kg	400mg/kg
Home cage assessment												
Body posture	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Convulsions/tremors	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Biting	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Vocalization	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Hand-held observations												
Ease of removal from cage	1.67 ± 0.58	2.0 ± 0.00	3.0 ± 1.00	1.67 ± 1.15	1.67 ± 0.58	1.33 ± 0.58	2.0 ± 1.00	1.33 ± 0.58	2.33 ± 0.58	3.0 ± 1.00	2.33 ± 1.53	1.67 ± 0.58
Ease of Handling	3.33 ± 0.58	4.33 ± 0.58	3.67 ± 1.53	2.33 ± 1.53	2.0 ± 1.00	1.33 ± 0.58	3.33 ± 0.58	2.67 ± 1.15	1.67 ** ± 0.58	2.0 ± 1.00	1.33 ± 0.58	2.33 ± 1.53
Lacrimation	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Salivation	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Piloerection	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Open field observations												
Arousal	3.33 ± 0.58	3.67 ± 1.15	4.33 ± 1.53	5.0 ± 1.00	5.0 ± 1.00	5.33 ± 0.58	4.0 ± 1.00	3.67 ± 0.58	3.67 ± 0.58	3.67 ± 0.58	4.33 ± 1.53	4.33 ± 1.53
Gait score	1.67 ± 0.58	2.0 ± 1.00	1.67 ± 0.58	2.0 ± 1.00	1.33 ± 0.58	2.0 ± 1.00	1.33 ± 0.58	1.33 ± 0.58	1.67 ± 1.15	1.67 ± 0.58	2.33 ± 0.58	1.33 ± 0.58
Mobility	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Stereotypical behavior	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Diarrhea	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sensorial responses												
Tail pinch response	1.33 ± 0.58	1.0 ± 0.00	1.33 ± 0.58	2.0 ± 1.00	1.33 ± 0.58	2.0 ± 1.00	1.0 ± 0.00	1.33 ± 0.58	1.33 ± 0.58	1.33 ± 0.58	1.67 ± 1.15	1.33 ± 0.58
Touch response	4.67 ± 0.58	3.33* ± 0.58	2.67* ± 1.15	2.67 ± 1.15	3.0 ± 1.00	1.67 ± 0.58	3.0 ± 1.00	3.67 ± 1.15	3.00 ± 1.73	2.33 ± 0.58	2.33 ± 0.58	3.0 ± 1.00
Click response	4.33 ± 0.58	4.33 ± 0.58	3.67 ± 0.58	3.0 ± 1.00	3.67 ± 1.53	1.67* ± 1.15	3.33 ± 1.15	3.67 ± 0.58	3.67 ± 1.53	3.33 ± 0.58	2.33 ± 0.58	2.33 ± 0.58
Righting reflex	2.33 ± 1.15	1.33 ± 0.58	1.67 ± 1.15	1.67 ± 1.15	2.33 ± 1.15	1.33 ± 0.58	1.33 ± 0.58	1.33 ± 0.58	2.33 ± 1.53	1.33 ± 0.58	1.67 ± 0.58	1.33 ± 0.58
Pupil response	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

Values are expressed as Mean ± SD (n = 3). **p < 0.01 compared to control group; *p < 0.05 compared to control group.

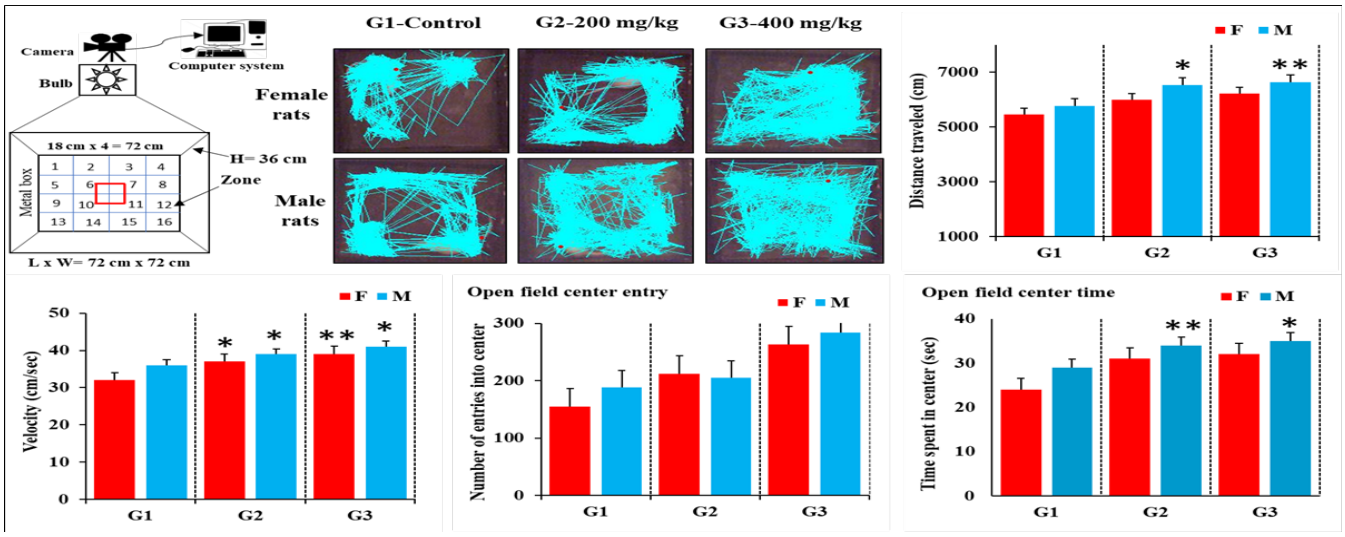


Figure 4. Assessment of open field test parameters. Overall study set-up organized for an open field test along with a video recording of the rat's moments inside the box and a graphical presentation of various open field parameters assessed of the control group and treated with SCPOHAE at the doses of 200 and 400 mg/kg in the sub-acute toxicity study. Values are presented as Mean ± SD, n = 3; Significant in relation to the control group at $^{*}p < 0.05$, $^{**}p < 0.01$ using one-way ANOVA followed by Dunnett's test.

There was a significant increase ($p < 0.05$) in absolute liver and spleen weight at 200 and 400 mg/kg doses of plant extract, respectively. The relative organ weight of the spleen and brain was also significantly increased ($p < 0.05$) in male and female rats, respectively, at 400mg/kg of extract. However, this increase was not dose-dependent.

Microscopic examination of all the animals treated with test items did not show any lesion of pathological significance when compared with the control group. However, microscopic examination of the liver showed a mild vacuolation in male rats in treatment groups (Figure 6). Relatively, female rats showed normal architecture of all vital organs (Figure 7). The incidence of abnormal findings having a minimal severity was comparable across the control and the plant extract-treated groups and did not reveal any treatment-related findings.

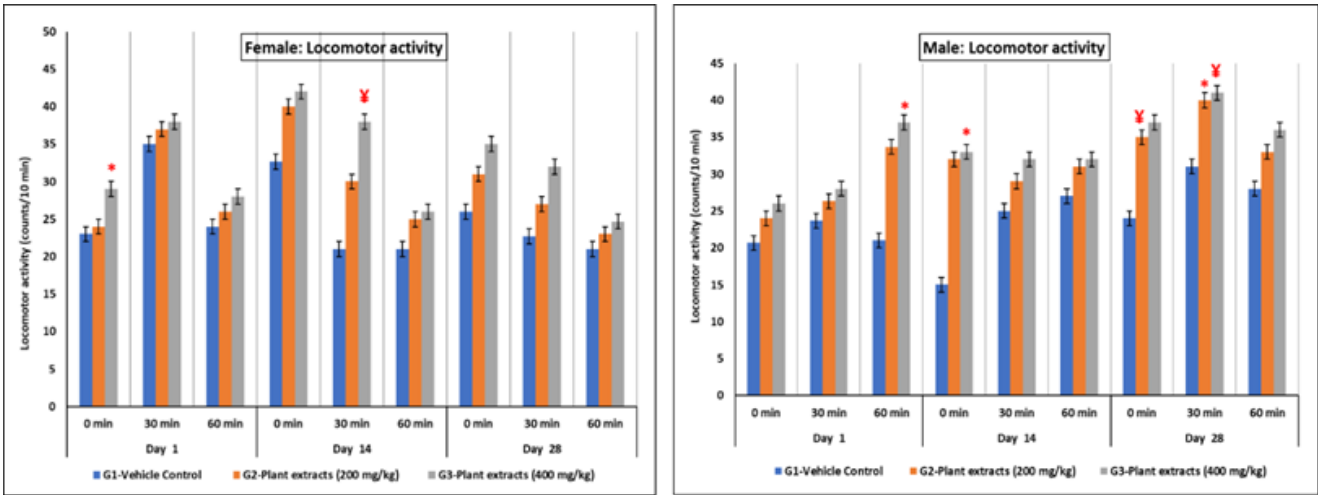


Figure 5. The effect on locomotor activities. Locomotor activity was measured by actophotometer of male and female rats of the control group and treated with SCPOHAE at 200 and 400mg/kg doses in the sub-acute toxicity study. Values are presented as Mean ± SD, n = 3; Significant in relation to control group at $^{*}p < 0.05$, ¥ $p < 0.01$ using one-way ANOVA followed by Dunnett's test.

Table 5. Effect of plant extracts on absolute and relative organ weight of rats

Sex	Organ	Absolute Organ Weight (g)			Relative Organ Weight (g)		
		Control	200(mg/kg)	400(mg/kg)	Control	200(mg/kg)	400(mg/kg)
Male [Day 29 Body wt.] G1=296.33 mg G2=296.67 mg G3=307.33 mg	Liver	10.37 ± 0.71	11.47 ± 0.65*	11.94 ± 1.22	3.50 ± 0.26	3.87 ± 0.22	3.89 ± 0.46
	Kidney	2.89 ± 0.12	2.99 ± 0.31	3.09 ± 0.29	0.97 ± 0.05	1.01 ± 0.10	1.00 ± 0.08
	Brain	2.16 ± 0.10	2.15 ± 0.17	2.15 ± 0.04	0.73 ± 0.04	0.72 ± 0.06	0.70 ± 0.01
	Spleen	0.95 ± 0.05	0.97 ± 0.12	1.05 ± 0.07*	0.32 ± 0.02	0.33 ± 0.04	0.34 ± 0.03*
	Testis	3.69 ± 0.36	3.39 ± 0.49	3.85 ± 0.37	1.24 ± 0.11	1.14 ± 0.17	1.25 ± 0.10
Female [Day 29 Body wt.] G1=220.00 mg G2=244.33 mg G3=278.67 mg	Liver	7.09 ± 0.51	7.92 ± 0.80	8.19 ± 0.88	3.22 ± 0.20	3.24 ± 0.33	2.94 ± 0.29
	Kidney	1.68 ± 0.19	1.84 ± 0.18	1.91 ± 0.15	0.76 ± 0.08	0.75 ± 0.10	0.69 ± 0.06
	Brain	1.96 ± 0.10	1.97 ± 0.12	1.84 ± 0.18	0.89 ± 0.04	0.81 ± 0.06	0.66 ± 0.07*
	Spleen	0.74 ± 0.05	0.80 ± 0.07	0.86 ± 0.07	0.34 ± 0.03	0.33 ± 0.03	0.31 ± 0.02
	Ovary	0.64 ± 0.04	0.66 ± 0.07	0.71 ± 0.08	0.29 ± 0.02	0.27 ± 0.03	0.26 ± 0.03
	Uterus	0.54 ± 0.06	0.64 ± 0.19	0.77 ± 0.34	0.25± 0.03	0.26 ± 0.08	0.28 ± 0.13

Values are expressed as Mean ± S.D. (n = 3). **p < 0.01 (highly significant) and *p < 0.05 (significant) in comparison with control group.

4. Discussion

Sub-acute toxicity study provides evidence for the plausible adverse effects likely to arise by longer exposure to test substances. This study was a continuation of a previous acute toxicity study of SC and PO plant extract, where the single oral dose of 2000 mg/kg was found to be safe without any toxic symptoms and mortality during and at the end of 14 days. The results of the acute toxicity study showed that the LD_{50} value of plant extracts was greater than 2000mg/kg[15]. Hence, 200mg/kg and 400mg/kg doses were considered to be safe and selected to conduct a sub-acute toxicity study[28,29].

In sub-acute toxicity study, administration of plant extracts up to a maximum dose of 400 mg/kg did not cause any mortality among rats and did not show any notable toxicological effects or abnormal clinical toxicities such as changes in the skin and fur, respiration rate, eyes, autonomic (sweating, salivation, and piloerection), or stereotypical behaviors. Body weight, food, and water intake are indicators of general health. Therefore, these parameters are crucial to assess the safety of the drug during the sub-acute toxicity study[30]. In general, an increase or decrease in an animal's body weight has been used as an indicator of an adverse effect of drugs and chemicals[31]. Oral repeated dosing of both plant extracts showed minor fluctuation in body weight, which might be observed because of the measurement of body weights on alternate days, which could have reflected this result. However, the percentage of body weight gain was not affected adversely. Hence, alteration in body weight was considered a common finding in rats. Similarly, the water and food intake did not show any undesirable change. The sporadic incidences of statistically significant food consumption were considered incidental and not related to treatment, as the percentage increase was minimal (~5-7 %). Hence there was no impact on the growth and physiological functions of rats.

The toxic effect on the hematopoietic system is one of the most sensitive targets for hazardous chemicals in humans and animals, as well as a significant indicator of pathological damages caused by the compound[32]. Hematological parameters can be utilized to determine the extract's harmful effects on the hematopoietic system[33]. After treatment with extracts, the hematological profile revealed values that were mostly non-significant and within the normal range. Most of the reported observations were within the limits of historical control data at the test facility and/or pathological reference ranges, were devoid of any dose dependence, lacked any toxicological significance, and therefore safe to take for a short period of time. This statement has been supported by other similar published literature[34]. Evaluation of serum biochemical parameters is regarded as a crucial marker for preliminary assessment of toxicity

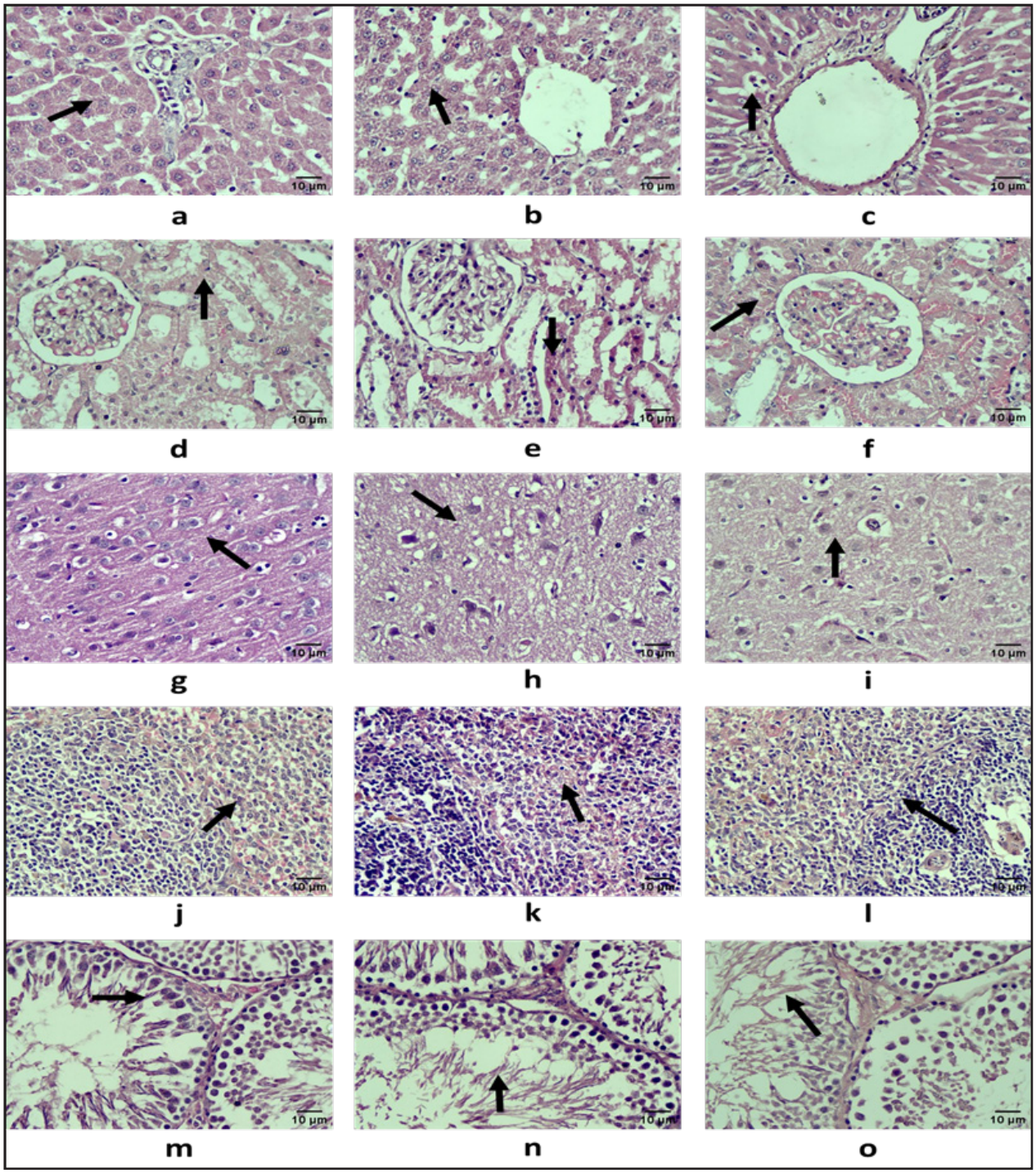


Figure 6. Histopathological assessment of vital organs of male rats. Photomicrographs of sections of (**a**) the Liver of albino rats from the control group; (**b**) the Liver of albino rats from 200 mg/kg b.wt. treatment group; (**c**) Liver of albino rats from 400 mg/kg b.wt. treatment group; **d**) Kidney of albino rats from the control group; (**e**) Kidney of albino rats from 200 mg/kg b.wt. treatment group; (**f**) Kidney of albino rats from 400 mg/kg b.wt. treatment group; (**g**) Brain of albino rats from the control group; (**h**) Brain of albino rats from 200 mg/kg b.wt. treatment group; (**i**) Brain of albino rats from 400 mg/kg b.wt. treatment group; (**j**) Spleen of albino rats from the control group; (**k**) Spleen of albino rats from 200 mg/kg b.wt. treatment group; (**l**) Spleen of albino rats from 400 mg/kg b.wt. treatment group; (**m**) Testis of albino rats from the control group; (**n**) Testis of albino rats from 200 mg/kg b.wt. treatment group; (**o**) Testis of albino rats from 400 mg/kg b.wt. treatment group

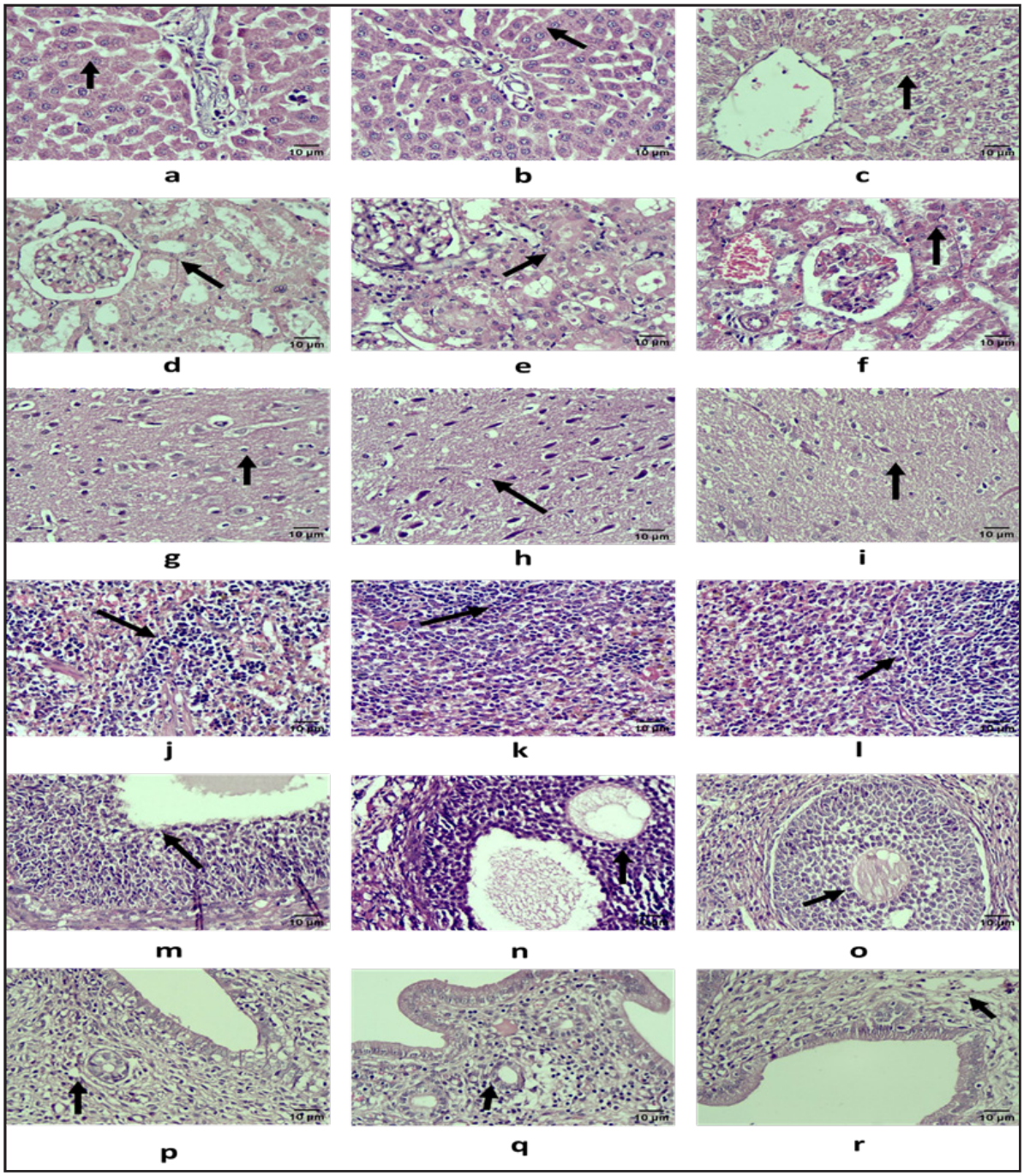


Figure 7. Histopathological assessment of vital organs of female rats. Photomicrographs of representative sections of **(a)** Liver of albino rats from the control group; (**b**) Liver of albino rats from 200 mg/kg b.wt. treatment group; (**c**) Liver of albino rats from 400 mg/kg b.wt. treatment group; (**d**) Kidney of albino rats from the control group; (**e**) Kidney of albino rats from 200 mg/kg b.wt. treatment group; (**f**) Kidney of albino rats from 400 mg/kg b.wt. treatment group; (**g**) Brain of albino rats from the control group; (**h**) Brain of albino rats from 200 mg/kg b.wt. treatment group; (**i**) Brain of albino rats from 400 mg/kg b.wt. treatment group; (**j**) Spleen of albino rats from the control group; (**k**) Spleen of albino rats from 200 mg/kg b.wt. treatment group; (**l**) Spleen of albino rats from 400 mg/kg b.wt. treatment group; (**m**) Ovary of albino rats from the control group; (**n**) Ovary of albino rats from 200 mg/kg b.wt. treatment group; (**o**) Ovary of albino rats from 400 mg/kg b.wt. treatment group; (**p**) Uterus of albino rats from the control group; (**q**) Uterus of albino rats from 200 mg/kg b.wt. treatment group; (**r**) Uterus of albino rats from 400 mg/kg b.wt. treatment group.

to vital organs[35]. Almost all the biochemical parameters were normal with no significant changes; however, alterations in a few parameters were deemed to be of little toxicological significance since they were of a small size and/or were within the normal physiological range or not dose progressive, and/or comparable to respective pre-treatment values. It is important to assess the toxic effects on the renal system as a significantly large volume of blood passes through the renal system, and the toxins get concentrated in the renal tubules[36]. Proteinuria is a marker of renal pathology caused by various renal diseases and toxic kidney injury[37]. Qualitative urine analysis was found to be normal without any alteration in parameters.

The International Conference on Harmonization's (ICH) Tripartite Guideline on Safety Pharmacology Studies for Human ICH S7A recommends a neurobehavioral assessment (usually a Functional Observational Battery (FOB), which is generally undertaken in the rat to measure, at a minimum, the effects of the substance on general motor activity, behavioural changes, coordination, sensory/motor reflex responses for effects on the central nervous system prior to "first dose in man[38,39]. This test is a non-invasive procedure carried out to detect the functional changes in healthy adult rats. This test may not be useful to determine all neurotoxicity parameters, but it can be used along with neuropathologic evaluation and/or general toxicity testing[40,41]. In our FOB, no major neurotoxicological findings were reported compared to the control group. The increase in the frequency of locomotor activity indicates an increase in locomotion exploration, whereas the reduced form could reflect reduced exploration as it is accompanied by reduced rearing frequency[42,43]. Consistent with these studies, results from the current study showed an increase in locomotion in treatment groups compared to the control group. This might indicate protection against hyperlocomotion and neuroprotective effects[44].

The presence of any macroscopic damage to important organs such as the liver, kidney, brain, spleen, testis, ovary, uterus, pancreas, heart, lungs, and stomach was investigated using gross morphology. No incidence of any gross pathological finding in any of the organs/tissues of the rats was observed. Relative organ weight is an important parameter to study the degree of toxicity of any substance[45]. The increase in absolute and relative spleen weight was observed in male rats. However, it may not be considered toxic and can be linked to body weight changes[46]. The increase in brain weight in female rats was sporadic and non-dose dependent because the brain histopathological was found to be normal in these rats. Moreover, the strain of animals, age, sex, environmental and experimental conditions were reported to be influencing animal organ weights[47]. As these findings were negligible, it can be concluded that the plant extracts did not cause any toxic effect on vital organs. A histopathological investigation is useful for the detailed assessment of any toxic effects on vital organ tissue[48].

On histopathological examination, no incidence of any microscopic pathological findings in any of the vital organs/tissues was observed. Microscopic examination of the liver showed no severe hepatic changes, infiltration, and hepatocellular degeneration. The hepatic biopsy sections of the control group rats reported normal findings. The multiple sections from renal biopsies showed the normal glomeruli, tubules, renal corpuscles, medullary ray, intestinal, nephrons, and blood vesicles in both male and female rats. The spleen tissue excised from all three groups had integral red and white pulp and did not report any structural changes. The cerebral cortex section of the brain showed healthy neurocytes containing central large vesicular nuclei that contained one or more nucleoli and peripheral distribution of Nissl granules. The testis histology was fully preserved, showing the normal arrangement and shape of seminiferous tubules supported by a basement membrane with myoid cells and lined by stratified primary spermatocytes, spermatids, and Sertoli cells. The ovaries from all groups indicate intact ovarian follicles with normal oocytes surrounded by cubical granulosa cells, normal blood vessels, and stroma cells, whereas the tissue section from the uterus shows common uterine histology with regular columnar epithelial cells lining the uterine lumen and glands. The incidence of abnormal findings having a minimal severity was comparable across the control and the plant extract-treated groups and did not reveal any treatment-related findings. These findings were considered to be incidental. These results, in fact, supported the other evidence related to body weight, hematology, biochemistry, and urine analysis findings.

5. Conclusion

The results of the current study stated that both plant extracts had no deleterious effects on Wistar albino rats when a repeated dose was administered orally under the test condition for the treatment period of 28 days. The NOAEL of these extracts in rats was found to be greater

than 400mg/kg per rat. Based on the evidence, it can be concluded that repeated administration of plant extracts separately and in combination is safe and does not cause systemic toxicity as a result of long-term exposure. The sub-chronic and chronic toxicity research of both these plants may be useful for further evaluation.

6. Acknowledgment

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7. References

1. Takawira R, Nordal I. The genus of *Sansevieria* (family Dracaenaceae) in Zimbabwe. Acta Hortic. 2003; 572:189-98. https://doi.org/10.17660/ActaHortic.2002.572.22
2. Ahamad T, Singh D, Khan MF. Phytochemical analysis, total phenolic content, antioxidant and antidiabetic activity of *Sansevieria cylindrica* leaves extract. J Nat Prod Resour 2017; 3(2:6):134-36.
3. Herbal and Natural Medicine, *Sansevieria cylindrica*. The most complete medicinal herbs database backed by science. Available from: https://www.herbal-organic.com/en/herb/27992
4. Da Silva Antunes A, Da Silva BP, Parente JP, Valente AP. A new bioactive steroidal saponin from *Sansevieria cylindrica*. Phytother Res. 2003; 17(2):179-82. https://doi.org/10.1002/ptr.1059 PMid:12601684
5. Herbal and Natural Medicine, *Plumeria obtusa*. The most complete medicinal herbs database backed by science. Available from: https://www.herbal-organic.com/en/herb/20274
6. Narwariya P, Nabi J. Comprehensive overview of *Plumeria obtusa*. World J Pharmac Res. 2017; 6(4):664-76. https://doi.org/10.20959/wjpr20174-8212
7. Bihani T, Tandel P, Wadekar J. *Plumeria obtusa* L.: A systematic review of its traditional uses, morphology, phytochemistry and pharmacology. Phytomed Plus. 2021; 1(2):100052. https://doi.org/10.1016/j.phyplu.2021.100052
8. Dogra NK. Phytochemical analysis and in-vitro antioxidant studies of *Plumeria obtusa* L. Leaves. Indian J Pharm Sci. 2016; 78(1):169-71. https://doi.org/10.4103/0250-474X.180256 PMid:27168698 PMCid:PMC4852569
9. Shinde PR, Patil PS, Bairagi VA. Phytopharmacological review of *Plumeria* species. Sch Acad J Pharm. 2014; 3(2):217-27.
10. Guldiken B, Ozkan G, Catalkaya G, Ceylan FD, EkinYalcinkaya I, Capanoglu E. Phytochemicals of herbs and spices: Health versus toxicological effects. Food Chem Toxicol. 2018; 119:37-49. https://doi.org/10.1016/j.fct.2018.05.050 PMid:29802945
11. Shewale S, Undale V, Chitlange S, Sharma H. Ophidian bite: The Balance between perception, idealism and realism. Trop J Nat Prod Res 2021; 5(7):1166-78. https://doi.org/10.26538/tjnpr/v5i7.1
12. Mirza AC, Panchal SS. Safety evaluation of syringic acid: Subacute oral toxicity studies in Wistar rats. Heliyon. 2019; 5(8):e02129. https://doi.org/10.1016/j.heliyon.2019.e02129 PMid:31463381 PMCid:PMC6706588
13. Hayes AW, Claire LK. Principles and methods of toxicology. London: CRC Press; 2015.
14. Greaves P. Histopathology of preclinical toxicity studies: Interpretation and relevance in drug safety evaluation. USA: Academic Press; 2011.
15. Shewale S, Undale V, Bhalchim V, Desai S, Shelar M, Padole S. Chitlange S, Wavale V, Parekh S, Pujari P. Evaluation and assessment of the acute toxic potential of *Sansevieria cylindrica* and *Plumeria obtusa* plant extracts in Wistar albino rats. J Nat Remedies 2022; 22(2):209-20. https://doi.org/10.18311/jnr/2022/28768
16. Agarwal A, Chaudhari S, Nariya M, Galib R, Prajapati PK. Acute and 90 days repeated dose toxicity of *Rasa parpati* (an ayurvedic mercurial formulation) in Charles Foster albino rats. Toxicol Int. 2020; 27(3&4):149-57.
17. Organization for Economic Co-operation and Development (OECD). Guidelines for testing of chemicals: Repeated dose 28-day oral toxicity study in rodents. Test No. 407, Adopted 3rd October 2008. Avaialbe from: https://www.oecd-ilibrary.org/docserver/9789264070684-en.pdf?expires=1639822040&id=id&accname=guest&checksum=85342A6F05F880D32E536C03AB0CA362
18. Wu Z, Ma Y, Zhao L, Cai S, Cheng G. Acute and subchronic toxicities of the ethanol and hot-water extracts from Chinese sumac (*Rhus chinensis* Mill.) fruits by oral administration in rats. Food Chem Toxicol. 2018; 119:14-23. https://doi.org/10.1016/j.fct.2018.06.009 PMid:29886233
19. Ugwah-Oguejiofor CJ, Okoli CO, Ugwah MO, Umaru ML, Ogbulie CS, Mshelia HE, Umar M, Njan AA.

Acute and sub-acute toxicity of aqueous extract of aerial parts of *Caralluma dalzielii* NE. Brown in mice and rats. Heliyon. 2019; 5(1):e01179. https://doi.org/10.1016/j.heliyon.2019.e01179 PMid:30775575 PMCid:PMC6356088

20. Kim Y, Jaja-Chimedza A, Merrill D, Mendes O, Raskin I. A 14-day repeated-dose oral toxicological evaluation of an isothiocyanate-enriched hydro-alcoholic extract from *Moringa oleifera* Lam seeds in rats. Toxicol Rep. 2018; 5:418-26. https://doi.org/10.1016/j.toxrep.2018.02.012 PMid:29854612 PMCid:PMC5977371
21. Luna LG. Manual of histological staining methods of the armed forces Institute of Pathology. New York: Blakiston Division, McGraw Hill; 1968.
22. Gadaga LL, Tagwireyi D, Dzangare J, Nhachi CF. Acute oral toxicity and neurobehavioural toxicological effects of hydroethanolic extract of *Boophone disticha* in rats. Hum Exp Toxicol. 2011; 30(8):972-80. https://doi.org/10.1177/0960327110384524 PMid:20889580
23. Pattanayak SP, Mazumder M. Assessment of neurobehavioral toxicity of *Dendrophthoe falcata* (L.f) Ettingsh in rats by functional observational battery after a subacute exposure. Pharmacogn Mag. 2009; 5(18):98-105. http://www.phcog.com/text.asp?2009/5/18/98/57965
24. Ghosh D, Mondal S, Ramakrishna K. Acute and sub-acute (30-day) toxicity studies of *Aegialitis rotundifolia* Roxb., leaves extract in Wistar rats: Safety assessment of a rare mangrove traditionally utilized as pain antidote. Clin Phytosci. 2019; 5(13):1-16. https://doi.org/10.1186/s40816-019-0106-2
25. Abubakar AR, Haque M. Anxiolytic testing of medicinal plants in Nigeria: Frequently used experimental models. Med Med J. 2019; 34(1):83-98.
26. Arika WM, Kibiti CM, Njagi JM, Ngugi MP. Effects of DCM leaf extract of *Gnidia glauca* (Fresen) on locomotor activity, anxiety, and exploration-like behaviors in high-fat diet-induced obese rats. Behav Neurol 2019; 2019:7359235. https://doi.org/10.1155/2019/7359235 PMid:31933694 PMCid:PMC6942765
27. Asuquo OR, Ottoh MO, Eluwa MA, Oko OK, Ekanem TB. Locomotor activity of Ethanolic extract of *Spondias mombin* leaf. Int J Pharm Sci Invent. 2013; 2(10):31-5. https://doi.org/10.1016/S2305-0500(13)60141-4
28. Kale OE, Awodele O, Akindele AJ. Subacute and subchronic oral toxicity assessments of *Acridocarpuss meathmannii* (DC.) Guill. and Perr. root in Wistar rats. Toxicol Rep. 2019; 6:161-75. https://doi.org/10.1016/j.toxrep.2019.01.005 PMid:30766799 PMCid:PMC6360914
29. Awotunde OS, Adewoye SO, Hawumba J. Subacute toxicity study of aqueous root extract of *Terminalia schimperiana* in male Wistar rats. Toxicol Rep. 2019; 6:825-32. https://doi.org/10.1016/j.toxrep.2019.07.006 PMid:31463203 PMCid:PMC6709380
30. Wang J, Cai Y, Li X, Zhao X, Wang J, Shi YF, Shi YX. Experimental investigation of high-power light-emitting diodes' thermal management by ionic wind. Appl Therm Eng. 2017; 122:49-58. https://doi.org/10.1016/j.applthermaleng.2017.04.084
31. Kharchoufa L, Bouhrim M, Bencheikh N, El AS, Amirou A, Yamani A, Choukri M, Mekhfi H, Elachouri M. Acute and subacute toxicity studies of the aqueous extract from *Haloxylon scoparium* pomel (*Hammada scoparia* (Pomel)) by oral administration in rodents. Biomed Res Int. 2020; 2020:4020647. https://doi.org/10.1155/2020/4020647 PMid:33123573 PMCid:PMC7582073
32. Mukinda JT, Syce JA. Acute and chronic toxicity of the aqueous extract of *Artemisia afra* in rodents. J Ethnopharmacol. 2007; 112(1):138-44. https://doi.org/10.1016/j.jep.2007.02.011 PMid:17367969
33. Yakubu M, Akanji M, Oladiji A. Hematological evaluation in male albino rats following chronic administration of aqueous extract of *Fadogia agrestis* stem. Pharmacogn Mag. 2007; 3(9):34-8.
34. Damjuti W, Kwansang J, Boonruab J. Evaluation of haematological and biochemical activity of Thai traditional antinausea remedy in normal albino rats. Toxicol Int. 2021; 28(4):411-19. https://doi.org/10.18311/ti/2021/v28i4/28112
35. Suganthy N, Muniasamy S, Archunan G. Safety assessment of methanolic extract of *Terminalia chebula* fruit, *Terminalia arjuna* bark and its bioactive constituent 7-methyl gallic acid: *In vitro* and *in vivo* studies. Regul Toxicol Pharmacol. 2018; 92:347-57. https://doi.org/10.1016/j.yrtph.2017.12.019 PMid:29288719
36. Al-Attar AM, Alrobai AA, Almalki DA. Protective effect of olive and juniper leaves extracts on nephrotoxicity induced by thioacetamide in male mice. Saudi J Biol Sci. 2017; 24(1):15-22. https://doi.org/10.1016/j.sjbs.2015.08.013 PMid:28053566 PMCid:PMC5198929
37. Wolf G, Ziyadeh FN. Cellular and molecular mechanisms of proteinuria in diabetic nephropathy. Nephron Physiol. 2007; 106(2):26-31. https://doi.org/10.1159/000101797 PMid:17570945
38. International Conference on Harmonization (ICH). ICH Guidelines S7A Safety Pharmacology Studies for Human Pharmaceuticals. 2001. Available from https://www.ema.europa.eu/en/documents/scientific-guideline/ich-s-7-

safety-pharmacology-studies-human-pharmaceuticals-step-5_en.pdf
39. Gauvin DV, Yoder JD, Holdsworth DL, Harter ML, May JR, Cotey N, Dalton JA, Baird TJ. The standardized functional observational battery: Its intrinsic value remains in the instrument of measure: The rat. J Pharmacol Toxicol Methods. 2016; 82:90-108. https://doi.org/10.1016/j.vascn.2016.08.001 PMid:27534836
40. Moser VC. Functional assays for neurotoxicity testing. Toxicol Pathol. 2011; 39(1):36-45. https://doi.org/10.1177/0192623310385255 PMid:21075917
41. Mondal S, Ghosh D, Anusuri KC, Ganapaty S. Toxicological studies and assessment of pharmacological activities of *Abrus precatorius* L. (Fabaceae) ethanolic leaves extract in the management of pain, psychiatric and neurological conditions: An *in-vivo* study. J Appl Pharm Sci. 2017; 7(2):207-16.
42. Asuquo OR, Ottoh MO, Eluwa MA, Oko OK, Ekanem TB. Locomotor activity of ethanolic extract of *Spondias mombin* leaf. International Journal of Pharmaceutical Science Invention. 2013; 2(10):31-5.
43. Voigt JP, Hörtnagl H, Rex A, van Hove L, Bader M, Fink H. Brain angiotensin and anxiety-related behavior: The transgenic rat TGR (ASrAOGEN) 680. Brain Res. 2005; 1046(1-2):145-56. https://doi.org/10.1016/j.brainres.2005.03.048 PMid:15869747
44. Gill NS, Kumar N. Neuroprotective effect of *Juniperus communis* on Chlorpromazine induced parkinson disease in animal model. Chinese J Bio. 2015:542542. https://doi.org/10.1155/2015/542542
45. Balogun SO, da Silva IF, Colodel EM, de Oliveira RG, Ascêncio SD, Martins DT. Toxicological evaluation of hydroethanolic extract of *Helicteres saca* rolha A. St.-Hil. *et al*. J Ethnopharmacol. 2014; 157:285-91. https://doi.org/10.1016/j.jep.2014.09.013 PMid:25239833
46. Choi JH, Kim S. Mechanisms of attenuation of clot formation and acute thromboembolism by syringic acid in mice. J Funct Foods. 2018; 43(5):112-22. https://doi.org/10.1016/j.jff.2018.02.004
47. Piao Y, Liu Y, Xie X. Change trends of organ weight background data in sprague dawley rats at different ages. J Toxicol. 2013; 26(1):29-34. https://doi.org/10.1293/tox.26.29 PMid:23723565 PMCid:PMC3620211
48. Zhang Q, Mao Z, Zhang Q, Qiu J, Jia Z, Qin L. Acute and sub-chronic toxicological studies of the iridoid glycosides extract of *Lamiophlomis rotata* (Benth.) kudo in rats. Regul Toxicol Pharmacol. 2018; 92:315-23. https://doi.org/10.1016/j.yrtph.2017.12.018 PMid:29287802

9. Supplementary Tables

Sub-acute toxicity profiling of *Sansevieria cylindrica* and *Plumeria obtusa* plant extracts in Wistar albino rats

Table S1. Assessment of baseline hematological parameters on day 1

Parameters	Groups (Male)			Groups (Female)		
	Control	200 (mg/kg)	400 (mg/kg)	Control	200 (mg/kg)	400 (mg/kg)
WBC (10^9/L)	8.55 ± 0.30	8.03 ± 0.75	8.07 ± 0.95	7.67 ± 0.15	8.77 ± 0.67	7.60 ± 0.44
LYM (#)	6.43 ± 1.25	5.93 ± 0.72	6.00 ± 0.60	5.87 ± 0.31	6.27 ± 0.67	5.33 ± 0.40
LYM (%)	70.73 ± 1.62	73.78 ± 4.54	74.49 ± 1.41	70.13 ± 8.80	72.62 ± 2.73	70.16 ± 2.93
MID (#)	0.67 ± 0.06	0.57 ± 0.25	0.50 ± 0.10	0.47 ± 0.15	0.67 ± 0.06	0.57 ± 0.12
MID (%)	7.81 ± 0.86	7.18 ± 3.62	6.53 ± 0.32	6.07 ± 1.92	7.63 ± 0.81	7.51 ± 1.83
GRAN (#)	6.84 ± 2.67	6.59 ± 1.30	7.02 ± 2.44	5.58 ± 2.10	6.37 ± 3.46	9.10 ± 2.86
GRAN (%)	9.77 ± 4.76	16.73 ± 6.59	10.83 ± 6.60	9.27 ± 4.31	17.10 ± 7.33	18.50 ± 5.26
NEUT (#)	1.70 ± 0.10	1.53 ± 0.23	1.57 ± 0.25	1.37 ± 0.21	1.83 ± 0.06	1.70 ± 0.20
NEUT (%)	21.45 ± 0.77	19.04 ± 1.48	19.35 ± 0.91	17.42 ± 2.90	21.00 ± 1.78	22.34 ± 1.79
RBC (10^12/L)	8.74 ± 0.66	7.63 ± 1.99	8.36 ± 0.13	8.66 ± 0.87	8.50 ± 0.96	9.22 ± 0.24
HGB (g/dL)	16.00 ± 1.15	17.03 ± 0.25	16.53 ± 0.25	17.33 ± 1.50	16.13 ± 0.68	16.57 ± 0.57
HCT (%)	65.17 ± 4.20	58.80 ± 6.66	63.07 ± 2.89	63.50 ± 4.85	63.13 ± 1.55	64.53 ± 2.34
MCV (fL)	75.30 ± 3.35	74.33 ± 2.67	73.73 ± 0.92	76.00 ± 2.95	76.80 ± 3.24	75.83 ± 2.21
MCH (pg)	17.40 ± 2.23	16.53 ± 0.85	19.23 ± 1.78	18.67 ± 2.34	17.93 ± 1.50	17.73 ± 2.42
MCHC (g/dL)	26.70 ± 2.57	26.40 ± 0.61	26.37 ± 0.40	27.77 ± 0.71	27.47 ± 1.85	28.23 ± 1.36
RDW-CV (%)	26.93 ± 1.00	28.33 ± 1.23	27.70 ± 1.31	28.13 ± 1.46	27.80 ± 2.07	28.00 ± 2.23
RDW-SD (fL)	13.80 ± 2.43	13.63 ± 1.99	13.20 ± 0.40	14.33 ± 1.66	12.00 ± 1.73	13.43 ± 1.10
PLT (10^9/L)	828.00 ± 37.32	844.67 ± 49.17	870.00 ± 36.06	874.00 ± 45.90	846.00 ± 30.51	804.00 ± 51.39
MPV (fL)	8.30 ± 0.01	8.37 ± 0.93	7.97 ± 0.59	7.93 ± 0.60	8.77 ± 0.40	6.97 ± 1.12
PDW (fL)	13.50 ± 1.77	12.67 ± 1.45	12.67 ± 0.91	12.33 ± 0.96	13.27 ± 0.70	11.57 ± 1.36
PCT (%)	0.66 ± 0.08	0.58 ± 0.16	0.61 ± 0.03	0.73 ± 0.13	0.64 ± 0.09	0.69 ± 0.05
P-LCR (%)	16.27 ± 1.36	16.93 ± 1.79	17.53 ± 0.51	15.07 ± 0.90	16.33 ± 0.76	16.83 ± 1.33
P-LCC (10^9/L)	57.00 ± 7.55	69.67 ± 4.73	61.00 ± 5.29	57.67 ± 4.16	59.67 ± 8.14	76.67 ± 7.57

Table S2. Assessment of baseline biochemical parameters on day 1

Parameters	Groups (Male)			Groups (Female)		
	Control	200 (mg/kg)	400 (mg/kg)	Control	200 (mg/kg)	400 (mg/kg)
Glucose (mg/dL)	77.52 ± 8.35	113.69 ± 5.11	123.02± 13.77	84.53 ± 13.55	106.35 ± 13.76	129.90 ± 10.65
Creatinine (mg/dL)	1.30 ± 1.07	1.22 ± 0.68	1.44 ± 0.80	1.96 ± 0.94	2.33 ± 1.44	2.48 ± 0.26
Total protein (g/dL)	4.17± 2.78	7.58 ± 1.36	7.74 ± 0.23	9.32 ± 0.75	9.58 ± 1.24	8.74 ± 0.13
Albumin (g/dL)	0.97 ± 0.15	1.03 ± 0.15	1.10 ± 0.10	0.87 ± 0.15	0.87 ± 0.15	0.97 ± 0.15
ALT (U/L)	45.33 ± 5.13	51.67 ± 9.71	47.00 ± 2.65	38.67 ± 4.16	42.67 ± 2.89	50.00 ± 5.57

Table S2. *Continued...*

Parameters	Groups (Male)			Groups (Female)		
	Control	200 (mg/kg)	400 (mg/kg)	Control	200 (mg/kg)	400 (mg/kg)
AST (U/L)	66.00 ± 6.00	80.00 ± 11.27	68.00 ± 6.56	81.33 ± 9.29	73.33 ± 8.50	77.67 ± 8.08
ALP (U/L)	144.67 ± 12.66	126.00± 13.08	180.00 ± 34.60	117.00 ± 7.21	139.33 ± 15.89	145.67 ± 9.45
Bilirubin (mg/dL)	1.49 ± 0.07	0.75 ± 0.64	0.78 ± 0.53	1.50 ± 0.08	0.51 ± 0.20	1.08 ± 0.61
Total cholesterol (mg/dL)	61.29± 3.00	63.20 ± 4.20	73.89 ± 17.55	68.88 ± 6.35	72.71 ± 14.84	56.72 ± 3.89
Triglycerides (mg/dL)	117.67 ± 9.96	135.34 ± 31.87	117.17 ± 23.04	108.70 ± 13.32	107.37 ± 38.83	117.64 ± 31.07
Urea (mg/dL)	18.00 ± 2.65	18.67 ± 3.06	16.00 ± 2.65	14.33 ± 2.08	19.00 ± 2.00	24.00 ± 3.00
Calcium (mg/dL)	10.60 ± 0.44	10.67 ± 0.67	10.90 ± 0.30	10.37 ± 0.51	10.27 ± 0.40	10.50 ± 0.44
Sodium (mmol/L)	139.00 ± 1.00	141.00 ± 1.00	142.00 ± 2.65	141.67 ± 1.53	140.33 ± 2.52	141.33 ± 3.21
Potassium (mmol/L)	5.00 ± 0.20	4.63 ± 0.25	4.80 ± 0.53	5.17 ± 0.12	4.87 ± 0.42	4.90 ± 0.10

Table S3. Assessment of baseline urine parameters on day 1

Parameters	Observations	Groups (Male)			Groups (Female)		
		Control	200 (mg/kg)	400 (mg/kg)	Control	200 (mg/kg)	400 (mg/kg)
Colour	Colourless	0	0	0	0	0	0
	Pale yellow	3	2	1	1	1	3
	Yellow	0	1	1	1	1	0
	Amber	0	0	1	1	1	0
	Dark Yellow	0	0	0	0	0	0
Appearance	Clear	3	3	3	3	3	3
pH	Less than 6	0	0	0	0	0	0
	6-8	3	3	3	3	3	3
	More than 8	0	0	0	0	0	0
Specific gravity	Less than 1.015	2	1	1	0	0	0
	1.015-1.020	1	1	1	2	2	3
	1.021-1.025	0	1	1	1	1	0
	1.026-1.030	0	0	0	0	0	0
Urobilinogen	0.2	3	3	3	2	2	1
	1	0	0	0	1	1	2
Protein	-ve	0	2	0	1	0	0
	Trace	3	1	2	1	1	2
	High	0	0	1	1	2	1
Bilirubin	(-ve) / (+ve)	2 (-ve), 1 (+ve)	3 (-ve)	3 (-ve)	2 (-ve), 1 (+ve)	2 (-ve), 1 (+ve)	3 (-ve)
Ketone	(-ve) / (+ve)	3 (-ve)	3 (-ve)	3 (-ve)	2 (-ve), 1 (+ve)	2 (-ve), 1 (+ve)	1 (-ve), 2 (+ve)

Table S2. *Continued...*

Parameters	Observations	Groups (Male)			Groups (Female)		
		Control	200 (mg/kg)	400 (mg/kg)	Control	200 (mg/kg)	400 (mg/kg)
Glucose	(-ve) / (+ve)	2 (-ve), 1 (+ve)	2 (-ve), 1 (+ve)	3 (-ve)	3 (-ve)	3 (-ve)	1 (-ve), 2 (+ve)
Epithelial cells	(-ve) / (+ve)	3 (-ve)	3 (-ve)	3 (-ve)	3 (-ve)	3 (-ve)	3 (-ve)
PC	(-ve) / (+ve)	3 (-ve)	3 (-ve)	3 (-ve)	3 (-ve)	3 (-ve)	3 (-ve)
Occult blood	-ve/+10	3 (-ve)	3 (-ve)	3 (-ve)	3 (-ve)	3 (-ve)	3 (-ve)
Casts & crystals	(-ve) / (+ve)	3 (-ve)	3 (-ve)	3 (-ve)	3 (-ve)	3 (-ve)	3 (-ve)

-ve: Negative; +ve: Positive

Table S4. Effect of plant extracts on functional observation battery parameters during mid-week (day 14)

FOB Endpoints	Male			Female		
	Control	200 mg/kg	400 mg/kg	Control	200 mg/kg	400 mg/kg
Home cage assessment						
Body posture	1.0	1.0	1.0	1.0	1.0	1.0
Convulsions/tremors	0.0	0.0	0.0	0.0	0.0	0.0
Biting	0.0	0.0	0.0	0.0	0.0	0.0
Vocalization	0.0	0.0	0.0	0.0	0.0	0.0
Hand-held observations						
Ease of removal from cage	2.0 ± 1.00	1.33 ± 0.58	2.33 ± 0.58	3.0 ± 1.00	2.33 ± 1.53	1.67 ± 0.58
Ease of Handling	3.33 ± 0.58	2.67 ± 1.15	1.67 ** ± 0.58	2.0 ± 1.00	1.33 ± 0.58	2.33 ± 1.53
Lacrimation	1.0	1.0	1.0	1.0	1.0	1.0
Salivation	1.0	1.0	1.0	1.0	1.0	1.0
Piloerection	0.0	0.0	0.0	0.0	0.0	0.0
Open field observations						
Arousal	4.0 ± 1.00	3.67 ± 0.58	3.67 ± 0.58	3.67 ± 0.58	4.33 ± 1.53	4.33 ± 1.53
Gait score	1.33 ± 0.58	1.33 ± 0.58	1.67 ± 1.15	1.67 ± 0.58	2.33 ± 0.58	1.33 ± 0.58
Mobility	1.0	1.0	1.0	1.0	1.0	1.0
Stereotypical behavior	0.0	0.0	0.0	0.0	0.0	0.0
Diarrhea	0.0	0.0	0.0	0.0	0.0	0.0
Sensorial responses						
Tail pinch response	1.0 ± 0.00	1.33 ± 0.58	1.33 ± 0.58	1.33 ± 0.58	1.67 ± 1.15	1.33 ± 0.58
Touch response	3.0 ± 1.00	3.67 ± 1.15	3.00 ± 1.73	2.33 ± 0.58	2.33 ± 0.58	3.0 ± 1.00
Click response	3.33 ± 1.15	3.67 ± 0.58	3.67 ± 1.53	3.33 ± 0.58	2.33 ± 0.58	2.33 ± 0.58
Righting reflex	1.33 ± 0.58	1.33 ± 0.58	2.33 ± 1.53	1.33 ± 0.58	1.67 ± 0.58	1.33 ± 0.58
Pupil response	1.0	1.0	1.0	1.0	1.0	1.0

Table S5. FOB scores for neurotoxicological investigation

Test Parameters	Score values							
	0	1	2	3	4	5	6	7
Home cage assessment								
Body posture	-	Sitting or standing (Normal)	Rearing	Asleep	Flattened	Lying on side	Crouched over	Head bobbing
Convulsions/ tremors	No	Yes	-	-	-	-	-	-
Biting	No	Yes	-	-	-	-	-	-
Vocalization	No	Yes	-	-	-	-	-	-
Hand-held observations								
Ease of removal from cage	-	Very easy	Easy	Rat flinches	Moderately difficult	Difficult	Very difficult	-
Ease of Handling	-	Low	Moderately low	Moderately high	High	Very high		
Lacrimation	-	None	Slight	-	-	-	-	-
Salivation	-	None	Slight	-	-	-	-	-
Piloerection	No	Yes	-	-	-	-	-	-
Open field observations								
Arousal	-	Very low	Low	Moderately low	Alert, normal exploratory	High (Enhanced)	Very high (excited)	
Gait score	-	Normal	Slightly abnormal	Moderately abnormal	Severely abnormal	-	-	-
Mobility	-	No impairment	Slightly impaired	Moderately impaired	Severely impaired	-	-	-
Stereotypical behavior	None	Head weaving	Body weaving	Grooming, self-mutilation	Circling, abnormal movements	Others	-	-
Diarrhea	None	Low	Moderate	High	Very high	-	-	-
Sensorial responses								
Tail pinch response	-	No reaction	Normal	Slow reaction	Energetic reaction	Exaggerated reaction	-	-
Touch response	-	No reaction	Normal	Slow reaction	Energetic reaction	Exaggerated reaction	-	-
Click response	-	No reaction	Normal	Slow reaction	Energetic reaction	Exaggerated reaction	-	-
Righting reflex	-	Normal/rat lands on feet	Slightly uncoordinated	Lands on side	Lands on back		-	-
Pupil response	No reaction	Normal	-	-	-	-	-	-