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RESEARCH ARTICLE

Evaluation and Assessment of the Acute Toxic Potential of *Sansevieria cylindrica* and *Plumeria obtusa* Plant Extracts in Wistar Albino Rats

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Abstract

Sansevieria cylindrica (SC) Bojer ex Hook. (Asparagaceae) and *Plumeria obtusa* (PO) L. (Apocynaceae) are indoor and outdoor ornamental plants respectively. These plants are traditionally used by the local healers during accidental injuries. However, their toxicological properties are very poorly explored over folkloric usage. Therefore, the present study evaluated the toxic potencies of SC leaves and PO seed Hydro-Alcoholic Extract (SCPOHAE) through acute oral dose (14-days) administration in female Wistar rats. Safety of the SCPOHAE was evaluated as per Organization for Economic Co-operation and Development (OECD) Acute Oral Toxicity study guidelines 423. The female Wistar rats were divided into three groups (n=3). A single oral dose of 2000 mg/kg of body weight of individual extract and 1:1 blend was administered to each animal. The animals were closely observed for clinical signs, neurobehavioral changes, morbidity, and mortality if any for the first half an hour and then every hour for the first four hours followed by observation every 24–hours for 14 days. Changes in food and water consumption, body weight were monitored daily during the study. On day 1 and day 15 blood samples were collected to evaluate changes in the hematology and biochemistry parameters. The urine samples were also collected for urine analysis parameters. Animals were sacrificed on day 15 and organ samples of liver and kidney were collected for histopathological findings. The SCPOHAE individually and also as 1:1 blend at the limit dose (2000 mg/kg, body weight) 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 statistically non-significant small differences in the body weight were observed.

Conclusion: The oral administration of SCPOHAE did not cause any systemic toxic effects. In conclusion, the No-observed-Adverse-Effect Level (NOAEL) of these extracts in rats was found to be greater than 2000 mg/kg.

Keywords: Acute Toxicity, *Plumeria obtusa*, Safety Assessment, *Sansevieria cylindrica*

1. Introduction

Worldwide many plants in the form of basic plant extracts/formulations are utilized for their medicinal properties against various diseases. As per World Health Organization (WHO), nearly 80 % of the world's population uses traditional herbal medicines to

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treat their health problems[1]. Due to their long history of usage by humans, there is a widespread belief that herbal products are safe and non-toxic[2]. Plant usage, on the other hand, has the potential to generate negative impacts and health difficulties in people and animals[3,4]. According to a survey, several ayurvedic medications used in traditional medicine have been linked to negative side effects[5]. Some historically used therapeutic herbs, e.g. *Crotalaria, Senecio jacobaea*, *Heliotrpium*, *Symphytum officinale* (comfrey), *Teucrium chamaedrys* (germander), *Chelidonium majus* (greater celandine), *Piper methysticum* (kava-kava), *Cassia angustifolia* (senna), *Larrea tridentata* (chaparral) and others, contain pyrrolizidine alkaloids and they have been linked to hepatotoxicity[6]. As medicinal plant safety remains a major concern, it is necessary to properly assess their safety and efficacy to determine their advantages to humanity[7]. Furthermore, the United States Food and Drug Administration (USFDA) has indicated that animal testing of any new drug for determination of its toxicity and pharmacological efficacy is critical[8].

SC plant belongs to the Asparagaceae family and is commonly found in the African continents whereas it is also grown for ornate reasons in Egypt, India and other countries[9,10]. 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, subcylindrical in shape with a smooth striped surface and greenish-gray in colour[11]. *Sansevieria* species exert a wide range of biologic activities like antitumor, antibacterial, scavenging free radicals, antidiabetic effects, etc. They have also demonstrated suppression of capillary permeability which might help in anti-inflammatory activity[12].

PO plant is geographically distributed in Greater Antilles, Florida, northern Central America and southern Mexico[13,14]. 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[15]. It can develop as a large tree or small shrub with a height ranging from 0.9 to 6.1 meters. The leaves grow in clusters at the branch tips and are arranged alternatively on the branch. They are dark green obovate shaped, around 6-22 cm in length and 2-7 cm in width. This species' fruit is a pair of pods containing dry follicles. The pods are joined in the centre. The fruit splits on one side to release the wing-shape seeds. The *Plumeria* has many medicinal uses such as emmenagogue, febrifuge, hemostatic, laxative, purgative, rubefacient, stimulant, vermifuge[16,17].

The toxicity profile of SC or other related species is not well established and reported in only few literature[18,19]. Similarly, according to current literature, the toxicity studies of the PO are sparse[15]. As a result, an appropriate assessment of the toxicity profile of these plants is lacking. The purpose of this acute toxicity study was to assess the acute toxicological profile of SCPOHAE alone and in combination in rats.

2. Materials and Methods

2.1 Collection, Authentication of Plant Material

The fresh whole plant of SC and PO was collected from the Mehenduri village of Tehsil-Akole, District-Ahmednagar, Maharashtra, India. The plant materials were sent to Western Regional Center, Pune, Maharashtra, India which is affiliated to Botanical Survey of India (BSI), the apex taxonomic research organization of the country under the Ministry of Environment, Forest and Climate Change, Government of India.

2.2 Chemicals and Reagents

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

2.3 Preparation of Extracts

The leaves and seeds of SC and PO plants respectively were dried using shade drying in the laboratory at room temperature (25 ± 2 °C) for 15-20 days. After that, they were crushed in an electrical grinder, powder, and then sieved by using 20 mesh and stored in an air tight container for long-term use. The powdered plant material of both plants was then extracted using a reflux process with a hydroalcoholic solvent (H_2O 40 units: Ethanol 60 units). To acquire SC leaves and PO seed extract, the crude solutions were filtered by Whatman filter paper No. 42, then the excess solvents were evaporated using a rotary vacuum evaporator and

concentrated on a water bath. Before analysis, the crude hydroalcoholic extracts were kept at 4 °C till further execution of the study.

2.4 Preliminary Phytochemical Tests

Qualitative analysis of both the plants extracts was performed for the presence of phytochemicals viz. alkaloids, tannins, flavonoids, glycosides, saponins, terpenoids, steroids, etc.[20,21].

2.5 Experimental Animals

The acute toxicity investigation was conducted on healthy adult female Wistar albino rats (age 8–12 weeks, body weight 180–250 g). Crystal Biological Solutions, Pune (Reg. No. 2030/PO/RcBiBt/S/18/CPCSEA) provided the rats. The female rats chosen were nulliparous and were not pregnant. Prior to 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 12 hours light/12 hours dark cycle). Care and handling of animals was done as per 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, Pune, India) and drinking water during the study.

2.6 Study Design

The acute oral toxicity study was carried out on Wistar albino rats in accordance with OECD standards 423, with a few minor adjustments[22]. The Institutional Animal Ethics Committee (IAEC) of the CPCSEA registered (regd. no.: 198/PO/Re/S/2000/CPCSEA) DPU's Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Pune has approved this study (Protocol no.: DYPIPSR/IAEC/Feb/20-21/P-23).

2.7 Grouping of Animals and Dosing Regime

The study included nine healthy female rats which were randomly divided into three groups (n=3) (Table 1) maintaining a mean body weight variation of less than 25 %. The test substances (extracts) were suspended in carboxy methyl cellulose (CMC) (1%, v/v) and orally administered to rats in Groups I and II at doses of 2000 mg/kg, body weight respectively. Rats in Group III were administered 2000 mg/kg, body weight of a combination of both plant extracts in a 1:1 ratio.

2.8 Experimental Assessment

2.8.1 Clinical Observations and Survival

Prior to the initiation of the study, the body weight of all rats was measured and identification marks were given to each rat. The rats were abstained from food; however easy access to water was provided. Following dosing with test extracts, each rat was observed closely for changes in skin color, hair and eye, food consumption and water intake, lethargy, tremors, diarrhoea, salivation, respiration, abnormal behaviour, convulsions, sleep, motor activity, coma, changes in gait for first 4 hours and then for 24 hours for any mortality or aberrant changes. The rats were then monitored for 14 days for any neurobehavioral or other remarkable changes until the planned necropsy.

2.8.2 Body Weight and Food Intake

The body weight and food intake of each rat was recorded prior to dosing (day 1) and on the 7th and 14th day post-dosing of the test extracts.

Table 1. Experimental groups

Group	Group Name	Dose-volume	Dosage	No. of rats	Gender
G1	*Sansevieria cylindrica* (PE 1)	10 mL/kg	2000 mg/kg	3	F
G2	*Plumeria obtusa* (PE 2)		2000 mg/kg	3	F
G3	SCPOHAE [PE 1+2 (1:1 ratio)]		1000 mg/kg/PE	3	F

PE: plant extract; SCPOHAE: Sansevieria cylindrica and Plumeria obtusa hydroalcoholic extract, F: female rats

2.8.3 Clinical Pathology

The rats were fasted overnight before blood sample collection. The blood samples were collected on day 1 and day 15 for hematological analysis through retro-orbital puncture using the capillary in pre-calibrated tubes containing anticoagulant (EDTA) for assessment of hematology and without anticoagulant for assessment of biochemistry parameters.

2.8.3.1 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 (HGB), hematocrit (HCT), Mean Corpuscular Volume (MCV), mean corpuscular hemoglobin (MCH), Platelets Count (PLT), Mean Platelet Volume (MPV), Platelet Distribution Width (PDW), Platelet Crit (PCT), Platelet Large Cell Ratio (PLCR), Platelet Large Cell Count (PLCC) were evaluated.

2.8.3.2 Biochemical Analysis

The blood samples were centrifuged at 1000 rpm for 20 minutes and serum was separated. The separated serum samples were 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), Triglyceride (TG), Total Cholesterol (TC), etc. An automated biochemistry analyzer (Epoch Micro-plate Spectrometer, SN/Model-1505231C, Biotech Instruments Inc.) and standard diagnostic test kits (Pathozyme Diagnostics, Maharashtra, India) were used for analyses of these parameters.

2.8.3.3 Urinalysis

The qualitative analysis of urine samples was carried out prior to 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 250 µL of a urine sample.

2.8.4 Necropsy and Histopathological Studies

All the animals fasted overnight were euthanized with Ketamine hydrochloride (100 mg/kg, i.m.) The external surfaces, all orifices, and the thoracic, abdominal, cranial, and pelvic cavities were all examined macroscopically. The livers and kidneys of rats were removed during necropsy. These vital organs were thoroughly cleaned using water and kept in a 10 % neutral buffered formalin solution. These organs were also examined under the microscope for the development of lesions or other remarkable changes. Histopathological assessment of the liver and kidney was undertaken afterward by using surgically removed tissue samples. They were dehydrated in a graded sequence of ethanol (70-99.9 %), rinsed with toluene, and then 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)[23]. Microscopically, the parts were examined for pathological tests, and photomicrographs were taken. The entire acute dose toxicity study schema is shown in Figure 1.

2.8.5 Statistical Analysis

The data was tallied and reported as mean and standard deviation. GraphPad Prism; v 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 (< 0.01) and significant (< 0.05) p-value were determined for comparative analysis.

3. Observations and Results

3.1 Authentication of Plant

Based on the supplied herbarium and the accessible database, the BSI has authenticated both plants. BSI

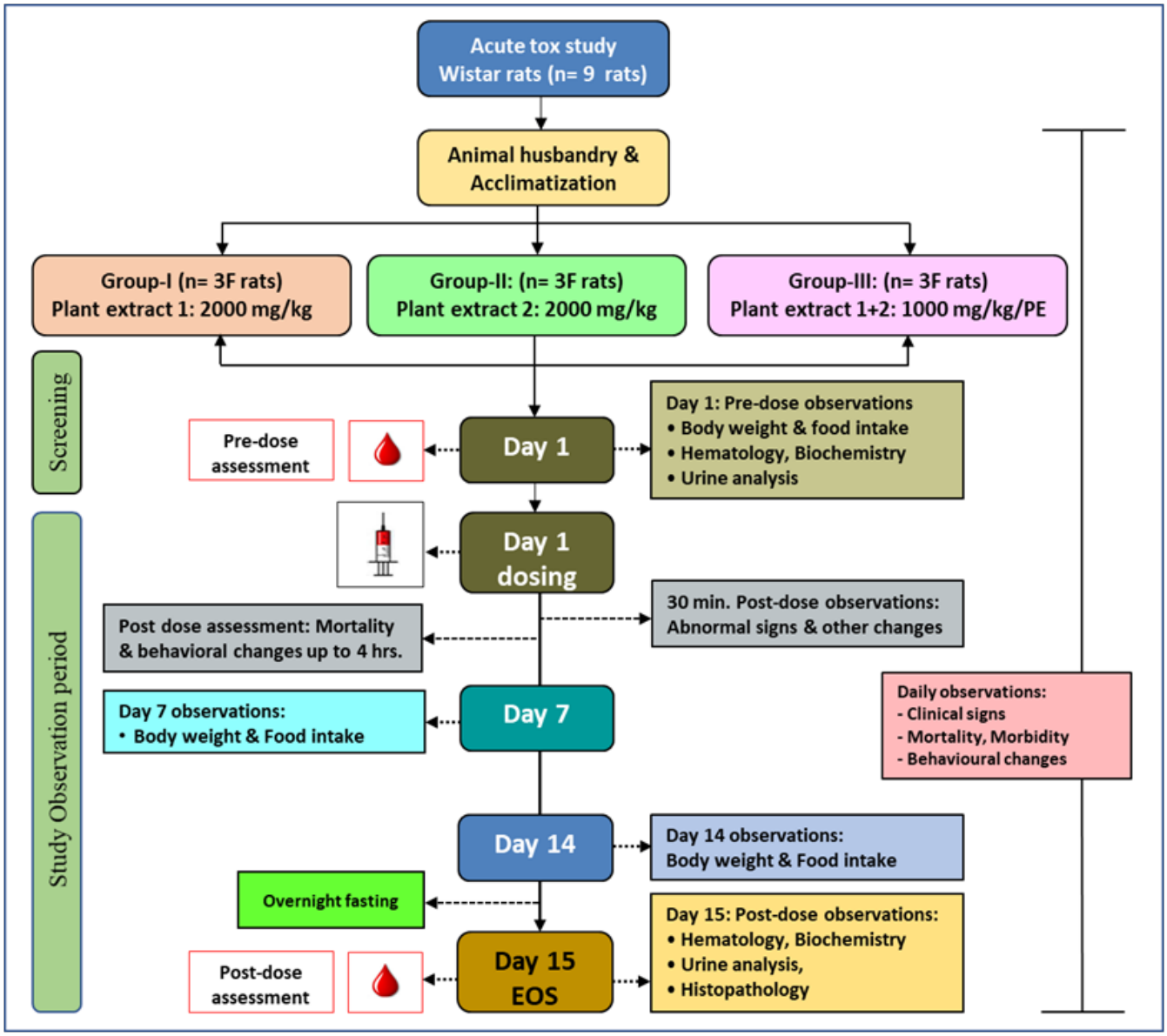


Figure 1. Acute dose toxicity study schema.

has issued an authenticity certificate with the number BSI/WRC100-1/TECH/2019/62 dated December 19, 2019, confirming that, the submitted plant species are *Sansevieria cylindrica* Bojer ex Hook and *Plumeria obtusa* L. belonging to a family of Asparagaceae and Apocynaceae respectively.

3.2 Preliminary Phytochemical Analysis

The plant extracts showed the presence of alkaloids, flavonoids, saponins, glycosides, terpenoids, and saponins glycosides, according to the preliminary phytochemical investigation.

3.3 In-life Clinical Observations and Animal Survival

Over the course of 15 days after the single oral dose, no death was reported in any of the treatment groups. None of the rats developed any remarkable morbidity or clinical toxicity symptoms such as changes in the skin and fur, respiration rate, eyes, autonomic (sweating, salivation and piloerection), or stereotypical behaviors.

3.4 Body Weight and Food Consumption

The body weight of few rats was found to be increased in each subsequent observation. Throughout the trial,

the body weight of all animals in group 1 decreased. Nevertheless, the hematology, biochemistry and urine analysis parameters were found to be normal in this group. Hence, the decrease in body weight was considered as an incidental finding. In any other group studied, no significant differences in mean body weights or net body weight gains were reported. During the trial, also, no significant adverse effects of the therapy on food consumption were identified (Figure 2).

3.5 Hematological Analysis

Table 2 shows the effect of plant extracts on hematological parameters. When the parameters of female rats were compared to the baseline measurements, some significant alterations were noticed. Group 1 had a higher WBC count; however, the difference was not significant. MCV and MCH were found to be higher in all three groups, with significant changes in both parameters in group 2 ($P < 0.01$ and < 0.05, respectively), but group 3 only had a significant rise in MCH ($P < 0.01$). In group 1, the MCV and MCH values were impacted in a non-significant way. In female rats from groups 1 and 2, a non-significant rise in hemoglobin was detected. When compared to baseline data, RBC in group 3 and platelet count in groups 2 and 3 both revealed a significant ($P < 0.05$) decrease. In all three groups, a significant lowering trend was seen for PDW, PCT, and PLCC. When compared to pre-dose levels, all other results revealed no significant changes.

3.6 Biochemical Analysis

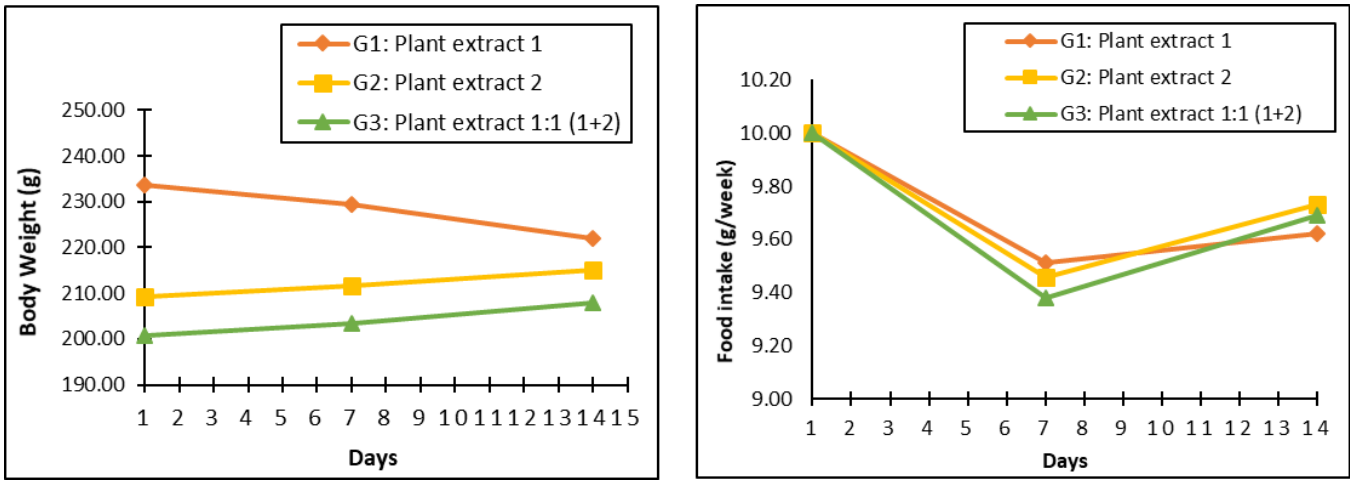
Table 3 shows the biochemical parameters measured during the experiment. In all three groups, glucose and creatinine levels decreased non-significantly, with the exception of group 3, where creatinine levels remained unchanged after the dose. In groups 1 and 3, the total protein was significantly increased compared to the pre-dose level. Similarly, albumin levels declined non-significantly from pre to post-dose. The minor fluctuation was observed for bilirubin. The level of ALT was reduced in all groups in a non-significant way, however, the levels of AST and ALP increased significantly in group 1 and group 2, respectively. In all 3 groups, there was very little difference in urea levels between pre and post-dosage observation. Triglycerides and cholesterol were found to be significantly increased from the baseline measurement in groups 1 and 2.

3.7 Urine Analysis

The urine analysis of rats from all three groups did not report any alteration in urine parameters. The observation of these parameters is displayed in Table 4.

3.8 Histopathological Examinations

Figures 3 and 4 show the histopathological examination results of the liver and kidney organ samples respectively. Microscopic examination of the liver showed no severe



(a) Body weight **(b) Food intake**

Figure 2. Graphical presentation of body weight and food intake.

Table 2. Effect of plant extracts on hematological parameters

Parameters	Group 1		Group 2		Group 3	
	Pre	Post	Pre	Post	Pre	Post
WBC (10^9/L)	8.07 ± 1.65	10.80 ± 4.10	7.03 ± 0.99	6.87 ± 0.93	8.77 ± 1.94	8.30 ± 1.76
LYM (#)	6.90 ± 0.92	9.00 ± 2.95	6.10 ± 0.72	5.83 ± 1.16	7.57 ± 1.63	7.30 ± 1.31
LYM (%)	86.20 ± 5.77	84.83 ± 6.24	87.27 ± 5.31	84.63 ± 5.75*	86.50 ± 2.86	88.13 ± 2.53
MID (#)	0.57 ± 0.21	0.90 ± 0.53	0.43 ± 0.15	0.43 ± 0.06	0.50 ± 0.10	0.53 ± 0.21
MID (%)	6.53 ± 1.57	8.10 ± 1.93	5.87 ± 1.59	6.70 ± 1.06	5.93 ± 0.59	6.10 ± 0.90
GRAN (#)	0.63 ± 0.51	0.87 ± 0.81	0.50 ± 0.36	0.53 ± 0.25	0.70 ± 0.30	0.50 ± 0.26
GRAN (%)	7.27 ± 4.20	7.07 ± 4.33	6.87 ± 3.76	8.67 ± 5.16	7.57 ± 2.50	5.77 ± 1.70
RBC (10^12/L)	8.39 ± 1.55	7.03 ± 0.51	8.29 ± 1.16	6.54 ± 0.03	8.35 ± 0.60	6.41 ± 0.36*
HGB (g/dL)	14.87 ± 1.55	17.00 ± 1.57	13.97 ± 1.46	15.87 ± 0.25	15.27 ± 1.10	15.73 ± 0.70
HCT (%)	42.87 ± 14.54	41.53 ± 3.74	33.77 ± 3.62	38.10 ± 0.89	43.57 ± 10.07	38.60 ± 1.47
MCV (fL)	58.10 ± 1.14	59.07 ± 1.10	56.70 ± 0.82	58.33 ± 1.18*	59.13 ± 1.99	60.27 ± 1.18
MCH (pg)	23.63 ± 0.46	24.13 ± 0.49	23.40 ± 0.30	24.23 ± 0.25**	23.83 ± 0.47	24.50 ± 0.35**
MCHC (g/dL)	40.83 ± 0.15	40.87 ± 0.15	41.33 ± 0.12	41.60 ± 0.46	40.43 ± 0.51	40.73 ± 0.32
RDW-CV (%)	11.50 ± 0.50	11.93 ± 0.21	12.77 ± 0.93	11.47 ± 0.29	11.63 ± 0.15	12.30 ± 0.66
RDW-SD (fL)	29.47 ± 1.50	30.33 ± 1.50	29.47 ± 3.00	32.07 ± 1.50	31.20 ± 2.60	32.07 ± 1.50
PLT (10^9/L)	596.33 ± 71.82	529.67 ± 67.42	580.33 ± 60.88	424.33 ± 18.01*	617.67 ± 74.01	443.67 ± 22.94*
MPV (fL)	5.47 ± 0.12	5.60 ± 0.44	5.63 ± 0.67	5.43 ± 1.00	5.63 ± 0.55	5.33 ± 0.67
PDW (fL)	8.30 ± 1.32	5.63 ± 0.12*	7.17 ± 0.12	5.47 ± 0.25**	8.00 ± 1.04	5.47 ± 0.25*
PCT (%)	0.25 ± 0.05	0.29 ± 0.02	0.31 ± 0.09	0.23 ± 0.05*	0.33 ± 0.09	0.23 ± 0.03
P-LCR (%)	1.03 ± 0.21	1.23 ± 0.29	1.20 ± 0.46	1.20 ± 0.66	1.20 ± 0.35	1.10 ± 0.44
P-LCC (10^9/L)	48.00 ± 10.15	65.33 ± 8.02	66.33 ± 33.50	50.33 ± 28.92*	71.00 ± 31.22	48.33 ± 16.44

Values are expressed as *Mean ± S.D. (n = 3). **P < 0.01 and *P* < 0.05 compared to baseline measurements.

Table 3. Effect of plant extracts on biochemical parameters

Parameters	Group 1		Group 2		Group 3	
	Pre	Post	Pre	Post	Pre	Post
Glucose (mg/dL)	119.81 ± 5.18	110.64 ± 8.10	128.71 ± 19.71	116.93 ± 11.89	128.44 ± 8.17	117.73 ± 18.44
Creatinine (mg/dL)	0.46 ± 0.04	0.72 ± 0.43	0.46 ± 0.04	0.39 ± 0.12	0.50 ± 0.07	0.51 ± 0.05
Total protein (g/dL)	6.00 ± 0.70	6.97 ± 0.42*	6.83 ± 0.95	7.87 ± 0.32	6.40 ± 0.30	7.20 ± 0.36*
Albumin (g/dL)	0.90 ± 0.20	1.20 ± 0.20	0.90 ± 0.10	1.07 ± 0.15	1.07 ± 0.15	1.13 ± 0.06
ALT (U/L)	52.33 ± 3.06	49.00 ± 3.00	53.00 ± 6.24	47.67 ± 9.07	55.33 ± 3.06	51.33 ± 4.51
AST (U/L)	97.67 ± 2.31	106.67 ± 6.81*	87.33 ± 5.86	97.67 ± 1.53	94.33 ± 9.71	100.12 ± 9.60
ALP (U/L)	148.00 ± 3.61	161.67 ± 21.50	149.33 ± 8.50	191.00 ± 10.54**	153.00 ± 8.89	170.33 ± 17.24

Table 3. Effect of plant extracts on biochemical parameters

Parameters	Group 1		Group 2		Group 3	
	Pre	Post	Pre	Post	Pre	Post
Bilirubin (mg/dL)	0.54 ± 0.05	0.55 ± 0.08	0.57 ± 0.06	0.76 ± 0.10	0.56 ± 0.05	0.59 ± 0.08
Total cholesterol (mg/dL)	95.60 ± 1.35	104.59 ± 2.45*	101.93 ± 2.40	118.39 ± 6.52	102.30 ± 3.70	115.61 ± 12.07
Triglycerides (mg/dL)	81.80 ± 6.92	84.36 ± 16.01	70.49 ± 9.29	86.70 ± 3.16*	94.86 ± 10.77	93.38 ± 27.22
Urea (mg/dL)	38.34 ± 2.80	39.59 ± 0.19	42.91 ± 2.39	39.72 ± 0.41	39.39 ± 4.31	39.80 ± 0.13

*The mean ± S.D. (n = 3) is used to express the values. **P < 0.01 and *P < 0.05 compared to baseline measurements.*

Table 4. Evaluation of urine profile

Parameters	Observations	Group 1		Group 2		Group 3	
		Pre	Post	Pre	Post	Pre	Post
Colour	Colourless	0	0	0	0	0	0
	Pale yellow	3	3	3	3	3	3
	Yellow	0	0	0	0	0	0
	Amber	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	2	1	1	2	2
	1.015-1.020	1	1	2	2	1	1
	1.021-1.025	0	0	0	0	0	0
	1.026-1.030	0	0	0	0	0	0
Urobilinogen	0.2	3	3	3	3	3	3
	1	0	0	0	0	0	0
Protein	-ve	0	0	0	0	0	0
	Trace	2	2	2	2	2	2
	High	1	1	1	1	1	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

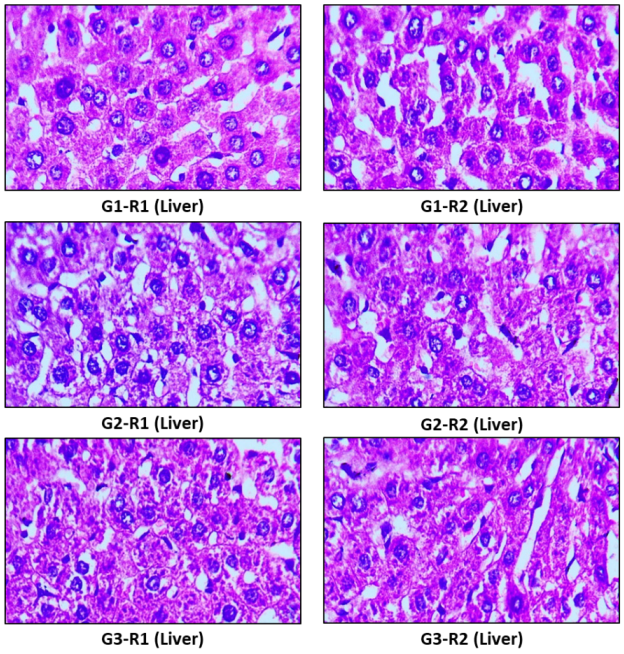


Figure 3. Histopathological evaluation of rat liver

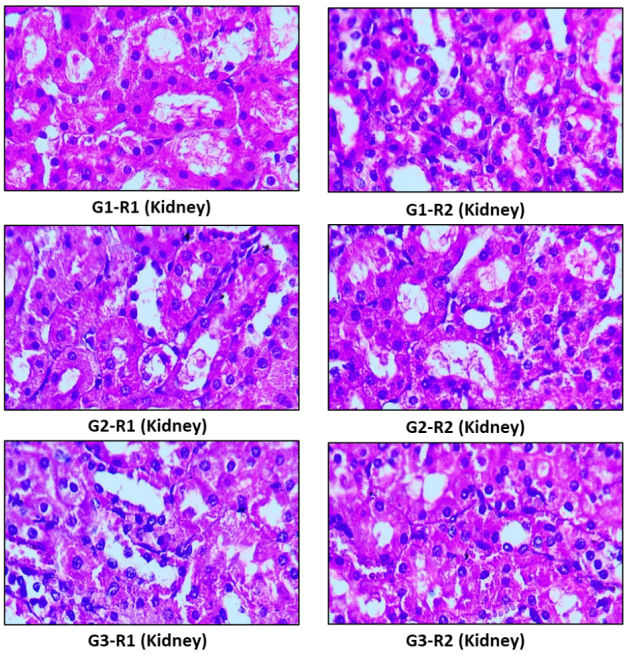


Figure 4. Histopathological evaluation of rat kidney

hepatic changes. Animals showed mild vacuolation but no infiltration and hepatocellular degeneration were observed. No pathological changes were observed in female rats in any of the treatment groups and the liver exhibited nearly intact cellular borders, hepatic cells and nucleus. However, reduced flow of bile (cholestasis infection) was noticed occasionally. Similarly, the sections from renal biopsies also showed the normal glomeruli, tubules, intestinum, nephrons and blood vesicles. No pathological significant changes such as inflammation, glomerular fibrosis, and necrosis were observed in rat kidneys.

4. Discussion and Conclusion

Acute toxicity is regarded as a preliminary investigation that lays the groundwork for classifying unknown therapeutic plants. It also gives preliminary information on a substance's method of hazardous action, allowing us to set a dose for a new molecule and aid in dose determination in animal research[24,25]. The findings of this study give valuable information and data on the toxicological qualities of *Sansevieria cylindrica* and *Plumeria obtusa*, which are historically used to treat accidental injuries and are rare and scientifically unknown. Preliminary indicators of early signs of toxicity induced by numerous substances and medicines include general behavioral changes and body weight[26]. Oral dosing of both plants extracts with doses up to 2000 mg/kg body weight had no notable toxicological effects in the acute toxicity study, except for a modest fluctuation in body weight. Alteration in body weight may occur, if plants contain the compound wherein it triggers the neurotransmitters which in turn reduces the palatability of food or other toxic effect impacting the consumption of food[27]. On the other hand, the reduction in body weight indicates a low risk of cardiovascular diseases and obesity[28–31]. Moreover, a systematic review of more than 151 short-term toxicity studies revealed that, the decrease in body weight in absence of clinical signs or expected pharmacology is justified and may be acceptable[32].

The toxic effects on the hematopoietic system is being 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[33]. Hematological parameters can be utilized to determine the extract's harmful effects on the hematopoietic system[34]. After treatment with extracts, the hematological profile revealed values that were mostly non-significant and within the normal range. Evaluation of serum biochemical parameters is regarded as a crucial marker for preliminary assessment of toxicity to vital organs like the liver and kidney[35]. Almost all the biochemical parameters were normal with no significant changes; however, modest

changes in a few parameters were deemed to be of little toxicological significance since they were of a low size and/or were within the normal physiological range. 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 presence of any macroscopic damage to important organs such as the liver, kidney, 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. A histopathological investigation is useful for the detailed assessment of any toxic effects on vital organ tissue[38]. On histopathological examination no incidence of any microscopic pathological findings in the livers and kidneys were observed.

Based on the data, it can be stated that, both the plant extracts had no deleterious effects on Wistar albino rats when a single limit dosage was administered orally under the test condition. The no-observed-adverse-effect level (NOAEL) of these extracts in rats was found to be greater than 2000 mg/kg. The sub-acute toxicity research of both these plants, on the other hand, will undoubtedly aid in the investigation and confirmation of their safety and efficacy for repeated usage.

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6. Conflict of Interest

This study was carried out as a part of Ph.D. curriculum in Pharmacology, at Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Savitribai Phule Pune University, Maharashtra. India.

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