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Evaluation of acute oral toxicity of methanolic fractions of *Sesbania grandiflora* Linn. roots in albino mice

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ABSTRACT

Introduction: Although *Sesbania grandiflora* Linn. roots have valuable pharmacological effects, the comprehensive awareness about its toxicity potential has been lacking. **Objectives:** The objectives of the study were to assess the acute oral toxicity of methanolic fraction of *S. grandiflora* root (MFSGR). **Materials and Methods:** The female albino mice were divided into two groups (n = 5), one group served as normal control while the other treated with MFSGR (2000 mg/kg, p.o). Both the groups of mice were observed for 14 days for various parameters according to the Organization for Economic Cooperative and Development guidelines 425 TG. **Results:** Oral administration of MFSGR at a dose of 2000 mg/kg did not result in mortality and treatment-related adverse reactions such as variation in water and food consumption, alteration in body weight, relative organ weights, hematological and biochemical parameters. However, treatment showed mild changes in behavioral patterns such as sleeping and itching during initial time point. Further, histopathological evaluation of vital organs exhibited normal architecture and this observation provided good support to general and biochemical measurements. **Conclusion:** The present study revealed that the MFSGR did not show any signs of toxicity in female albino mice. Hence, the methanolic fraction can be used for the pharmacological studies by calculating dose based on toxicity studies.

Keywords: Acute toxicity, mortality, toxicity

INTRODUCTION

Nature is an origin for traditional herbal remedies against many ailments since primordial period. Due to the diverse culture and rich plant flora in Asia and Africa, medicinal plants are used as folklore medicines in health-care system.[1] The medicinal plants have ability to synthesize variety of chemical compounds which are used to heal variety of human aliments. According to the WHO, about 80% of the world population relies on plant medicines for primary health care and they are being used on large scale, so many researchers are carrying out the experiments on medicinal plants for their efficacy and safety.[1]

Sesbania grandiflora Linn., (Fabaceae), is extensively distributed in Karnataka state in India. Conventionally, it is employed to treat various conditions including ulcer, cardiac disorders, liver disorders, and inflammation.[2] In addition, it is used by tribal people to cure bruises, sore throat, catarrh, and stomatitis. The aqueous decoction of the powdered roots is used to cure rheumatic swellings.[3]

S. grandiflora has been reported to contain grandiflora, arginine, cysteine, histidine, isoleucine, phenylalanine, tryptophan, valine, threonine, alanine, asparagine, aspartic acid, a saponin yielding oleanolic acid, galactose, rhamnose, and glucuronic acid.[3,4] It also contains flavonol glycoside and kaempferol.[5]

Literature survey revealed that the aerial parts of *S. grandiflora* have been reported for various scientific activities such as antibacterial,[6] antioxidant,[7] anticancer,[8]

anthelmintic,[9] anti-inflammatory and anti-arthritis[10] hepatoprotective,[11] anxiolytic, anticonvulsive,[12] wound healing,[13] antiulcer,[14] and anti-tuberculosis activity.[15]

Although the roots of *S. grandiflora* have been traditionally claimed to be used in treating rheumatic conditions, the systemic approach in evaluating their efficacy and safety data is needed. Therefore, the present study aimed to evaluate the acute oral toxicity potential of methanolic fractions of *S. grandiflora* roots (MFSGR) following Organization for Economic Cooperative and Development (OECD) TG 425 in female albino mice.

MATERIALS AND METHODS

Chemicals and Reagents

Complete Freund's adjuvant, Carrageenan, potassium dihydrogen orthophosphate, sodium dihydrogen orthophosphate, disodium hydrogen orthophosphate, trichloroacetic acid, acetic acid, N-1-naphthylethylenediamine-di-HCl, sodium nitrite and Folin's phenol reagent, and Tris-hydrochloric acid were procured from Sigma-Aldrich, St. Louis, USA. 5,5-Dithio-bis-2-nitrobenzoic acid, thiobarbituric acid and potassium sodium tartrate, and ethylenediaminetetraacetic acid (EDTA) were purchased from HiMedia Laboratories, Mumbai, and diclofenac sodium by Wexford Laboratory, Tumakuru, Karnataka. Commercially available reagent kits for the determination of meters were purchased from ERBA Diagnostic, Mannheim, Germany. All other chemicals and reagents used were of analytical grade from S. D. Fine-Chem Ltd., Mumbai.

Collection of Plants

S. grandiflora roots were collected during the month of August 1st week from the rural areas of Tumkur, Karnataka. The specimen was identified and authenticated by Dr. Chidananda, Sree Siddaganga Women's College, Tumkur, Karnataka. Voucher specimen (SSCP/PC/2018-05) was deposited in the department.

Preparation of Crude Extract

Air-dried powdered roots (1.2 kg) of S. grandiflora were extracted with 90% aqueous methanol (2 × 4.5 L) at room temperature for a period of 7 days. The extract was concentrated to a volume of 100 mL under reduced pressure using a rotary evaporator (Buchi R-300) at a bath temperature of 40°C and then partitioned with hexane to afford a hexane-soluble fraction (*Fr. A,* 1.5 g). The aqueous methanol-soluble fraction was suspended in water and partitioned with chloroform to afford chloroform soluble fraction (*Fr. B*, 1.2 g). The aqueous methanol-soluble fraction was resuspended in water and repartitioned with ethyl acetate to afford ethyl acetate-soluble fraction (*Fr. C*, 15 g) and remaining methanol fraction (*Fr. D* 10.2 g) was designed as MFSGR [Figure 1].[15] Further, phytochemical investigation was carried out on MFSGR fraction to know the presence of bioactive secondary metabolites.

Approval from Animals Ethics Committee

Acute oral toxicity studies were carried out after receiving prior approval from the Institutional Animal Ethics Committee (IAEC) of Sree Siddaganga College of Pharmacy (SSCPT/IAEC.Clear/184/2018-19 Date 09/03/2018).

Acute Oral Toxicity Study

This study was performed in accordance to OECD test guidelines 425 (up and down procedure). Nulliparous and non-pregnant female albino mice, weighing 30–32 g with age 6–8 weeks, were randomly selected. Animals were kept under standard conditions for 5 days and had free access to water *ad libitum*, temperature at 24 ± 2°C, and relative humidity 55–65%, with 12 h light and dark cycle in a standard animal house facility.

The acute toxicity test was performed at 2000 mg/kg single oral dose of MFSGR. Mice were kept without food for 5–6 h before dosing but had free access to water *ad libitum*. The dose of MFSGR was prepared in water and administered to a single female mouse according to the body weight. The animal was closely observed for the first 30 min, then for 4 h and 24 h. Food was provided after 1 h of dosing. After survival of the treated mice, four additional mice were given with the same dose under similar conditions. The same procedure was followed for vehicle (normal control) treated groups containing five mice. Both the groups were observed closely for any toxic effect and behavioral changes within the first 4 h and then different time interval for a total of 14 days. Animals were weighed at different time points. Survived animals were observed to determine the toxic reaction onset. At the end of the study, blood samples were collected by cardiac puncture under isoflurane anesthesia and serum was used for biochemical estimation. Further, the remaining blood samples were stored in EDTA container tubes for hematological estimation. After sacrificing the mice, vital organs such as heart, kidney, liver, lungs, and spleen were excised and weighed. For histopathological examinations, these organs were preserved in 10% formalin solution.[16]

Hematological analysis

The complete blood count parameters containing hemoglobin (Hb), total red blood cell (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), platelet count, white blood cells, neutrophils, lymphocytes, monocytes, eosinophils, mean corpuscular hemoglobin (MCH), and hematocrit test[17] were determined using autoanalyzer.

Biochemical analysis

The kidney function test (serum creatinine and urea), liver function test (serum alanine aminotransferase [ALT], aspirate aminotransferase [AST], alkaline phosphate [ALP], total bilirubin, direct bilirubin, indirect bilirubin, total protein, albumin, and globulin), and lipid profiles (serum cholesterol, triglycerides, high-density lipoprotein [HDL], low-density lipoprotein [LDL], and very low-density lipoprotein) were performed using ERBA diagnostic kits.

Histopathological study

The vital organs such as heart, kidney, liver, lungs, and spleen were isolated from sacrificed mice which were fixed in 10% formalin in buffer, then after processing embedded in paraffin wax. Sections were made at 5 mm and stained with hematoxylin and eosin (H and E). The slides were studied

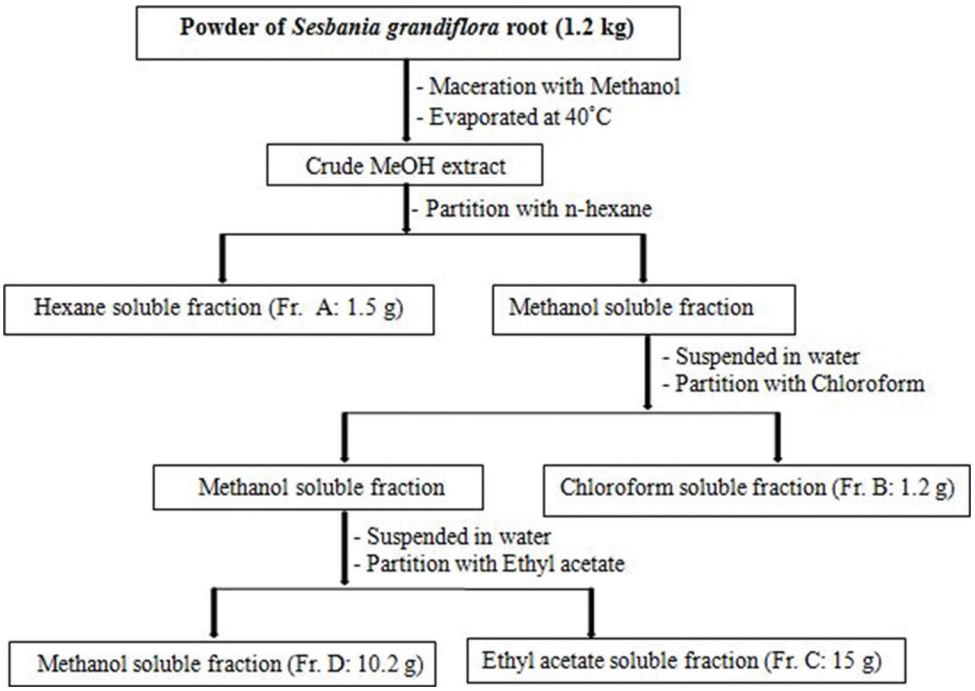

Figure 1: Flowchart of extraction and fractionation of *Sesbania grandiflora* roots

under a light microscope and captured the magnified images of tissues structure for further study.[16]

Statistical Analysis

Experimental results were expressed as Mean ± SEM and the statistical analysis was performed by one-way ANOVA followed by Tukey's multiple comparison test.

RESULTS

Acute Oral Toxicity Study

The single oral dose of MFSGR (2000 mg/kg) did not reveal any signs of toxicity or mortality at studied time points up to 14 days. In addition, single-dose treatment did not show any signs of behavioral abnormalities, toxicity, and mortality.

Preliminary Phytochemical Profile of MFSGR

Phytochemical investigation of MFSGR using various secondary metabolite test revealed the presence of phenolic compounds (++), flavonoids (++), glycosides (+), tannins (++), terpenoids (++), carbohydrate (++), and proteins (++). In addition, alkaloids and steroids were absent in tested fraction.

Body Weight and Behavioral Changes

The body weight of MFSGR-treated animals did not significantly alter during the entire study period when compared to normal control mice, as shown in Table 1. Among behavioral parameters observed, the MFSGR (2000 mg/kg, p.o) treated animals exhibited increased sleeping time at the 4th h and mild itching was observed for initial period (within 30 min), as summarized in Table 2.

Table 1: Effect of MFSGR on the body weight of mice in acute oral toxicity study

Treatment groups	Body weight (g)		
	1st day	7th day	14th day
Normal control	32.40±1.07	32.20±0.58	32.80±0.80
MFSGR (2000 mg/kg, p.o)	31.00±0.77	28.20±1.28	29.80±1.93

Values are represented as Mean±SEM, n=5. MFSGR: Methanolic fraction of *Sesbania grandiflora* roots

Organ to Body Weight Index

Organ weight is an important parameter of physiological and pathological changes in man and animals. At the end of the experiment, there was no significant difference in organ to body weight index which was noticed between MFSGR treated and normal control group of mice, Table 3.

Hematological Analysis

Hematological parameter analysis is more relevant test to predict toxicity of druggable candidates from both synthetic and natural sources. The data of hematological parameter of normal control and treated mice are tabulated in Table 4. The MFSGR-treated group did not produce any alteration in all tested blood parameters when compared with the normal control group.

Biochemical Analysis

In the renal function test, no significant changes were observed in serum creatinine and urea levels in MFSGR-treated group, when compared with the normal control group, as shown in Table 5. Single oral administration of MFSGR to group of mice did not significantly alter the tested liver function parameters [Table 5]. Furthermore, treatment of MFSGR did not cause

Table 2: Effect of MFSGR on behavioral pattern of mice in acute oral toxicity study

Parameter	30 min		4 h		24 h		48 h		7th day		14th day	
	NG	TG	NG	TG	NG	TG	NG	TG	NG	TG	NG	TG
Fur and skin	N	N	N	N	N	N	N	N	N	N	N	N
Eyes	N	N	N	N	N	N	N	N	N	N	N	N
Salivation	N	N	N	N	N	N	N	N	N	N	N	N
Respiration	N	N	N	N	N	N	N	N	N	N	N	N
Urination (color)	N	N	N	N	N	N	N	N	N	N	N	N
Feces consistency	N	N	N	N	N	N	N	N	N	N	N	N
Somatomotor activity and behavior pattern	N	N	N	N	N	N	N	N	N	N	N	N
Sleep	N	N	N	N	N	N	N	N	N	N	N	N
Mucous membrane	N	N	N	N	N	N	N	N	N	N	N	N
Convulsions and tremors	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F
Itching	N.F	P	N.F	N	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F
Coma	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F
Mortality	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F

NG: Normal control group treated with vehicle, TG: Treatment group treated with MFSGR (2000 mg/kg, p.o). N: Normal, P: Present, ↑: Increased,N.F: Not found. MFSGR: Methanolic fraction of *Sesbania grandiflora* roots

Table 3: Effect of MFSGR on organ to body weight indices in mice in acute oral toxicity study

Organs	Normal control	MFSGR (2000 mg/kg)
Heart	0.60±0.02	0.63±0.07
Kidney	1.44±0.08	1.39±0.14
Liver	5.47±0.10	5.01±0.32
Lungs	1.14±0.05	1.10±0.09
Spleen	0.58±0.02	0.47±0.09

Values are represented as Mean±SEM, $n=5$. Organ to body weight index=(Organ weight/body weight)×100. MFSGR: Methanolic fraction of *Sesbania grandiflora* roots

significant changes in lipid profiles compared to normal group of mice [Table 5].

Histopathology Study

Histopathology of kidney

Microscopic slides of histopathology of control mice showing regular architecture with no pathological changes. From Figure 2, it can be seen that section showing good normal glomerulus marked as yellow arrow and Bowman's capsule as blue arrowhead, clear distal convoluted tubules and proximal convoluted tubules seen in normal control mice. Following administration with MFSGR fraction for a period of 14 days reveals that some of the insignificant pathological changes in renal histology [Figure 2e]. Administration of MFSGR fraction causes little tissue hemorrhagic strikes in some regions marked as black arrow, mild tubular degeneration, and distorted glomeruli can be seen in some parts. Only minor pathological lesions were seen in histology of kidney of mice treated with 2000 mg/kg of MFSGR fraction.

Histopathology of liver

Histopathological assessment of liver of normal control mice showed normal hepatocytes, central vein, and portal triads. Pathological observations of the liver histology of treatment with MFSGR fraction for 14 days showed slight enlarged central vein with mild hemorrhagic strikes with mild distorted architecture (marked as black arrowhead). However, these observations were insignificant compared to liver histology of normal mice [Figure 2].

Histopathology of spleen

Splenic slice of normal mice exhibited normal architecture with regular white pulp and red pulp arrangement [Figure 2c]. However, treatment of MFSGR fraction for 14 days did not alter the normal structural constructions of spleen appreciably [Figure 2g].

Histopathology of heart

Histological slides of heart of normal mice exhibited regular architecture [Figure 2d]. Whereas, treatment of MFSGR fraction showed slight degeneration and maintained normal structural architecture of heart [Figure 2h].

DISCUSSION

The phytotherapeutic products derived from herbal floras have become popular universally in community health care, chiefly in developing nations, and few have been incorrectly considered as harmless as they obtained from natural origin. Yet, these bioactive products from herbal plants are assumed to be safe without any deleterious effects on health and therefore extensively used to treat oneself.[18] The herbal preparations are generally administered for a long period of time without correct dosage monitoring by the professionals and lack of knowledge of the adverse effects that might be due to their sustained use.[19] Therefore, the toxicological outcomes of many medicinal plants used worldwide have been documented.[20] Careful evaluations of safety profiles of these medicinal plants are need of the hour to popularize the acceptance.[21] Before the pharmacological validation and

Table 4: Effect of MFSGR on complete blood count of mice in acute oral toxicity study

Parameters	Units	Normal control	MFSGR (2000 mg/kg, p.o)
Hemoglobin	g/dL	13.9±0.17	14.50±0.16
Red blood cells	$\times 10^6/\mu L$	8.68±0.14	9.11±0.31
Hematocrit test	%	45.80±0.44	47.88±0.67
Mean corpuscular volume	fL	52.64±0.58	51.06±0.38
Mean corpuscular hemoglobin concentration	g/dL	30.06±0.71	28.50±0.45
Platelet count	$\times 10^3/\mu L$	1230±93.71	1198±77.01
White blood cells count	$\times 10^3/\mu L$	6.42±1.24	5.34±0.27
Neutrophils	%	13.20±2.03	11±1.89
Lymphocytes	%	83.20±2.59	84.80±2.08
Monocytes	%	2.20±0.58	2.40±0.40
Eosinophils	%	1.40±0.24	1.80±0.20
Mean corpuscular hemoglobin	Pg	15.94±0.30	15.18±0.30

Values are expressed as Mean±SEM. MFSGR: Methanolic fraction of *Sesbania grandiflora* roots

Table 5: Effect of MFSGR on liver function test in acute oral toxicity study in mice

Test	Parameter	Unit	Normal control	MFSGR (2000 mg/kg, p.o)
Liver function	Serum glutamic-pyruvic transaminase (alanine aminotransferase)	IU/L	65.40±2.46	66.40±1.50
	Serum glutamic-oxaloacetic transaminase (aspirate aminotransferase)	IU/L	206.2±3.76	211.8±0.60
	Alkaline phosphatase	IU/L	127.6±2.24	130.2±1.53
	Bilirubin total	mg/dL	0.68±0.03	0.76±0.05
	Direct bilirubin	mg/dL	0.16±0.02	0.14±0.02
	Indirect bilirubin	mg/dL	0.50±0.04	0.62±0.03
	Total protein	g/dL	6.74±0.16	7.10±0.32
	Albumin	g/dL	1.78±0.14	1.87±0.11
	Globulins	g/dL	5.44±0.07	5.48±0.11
Kidney function	Serum creatinine	mg/dL	5.0±0.15	5.1±0.20
	Serum urea	mg/dL	39.60±0.98	40.18±0.02
Lipid profile	Cholesterol	mg/dL	58.60±1.36	60.14±0.77
	Triglycerides	mg/dL	64.80±0.66	63.50±0.40
	High-density lipoprotein (Cholesterol)	mg/dL	12.0±0.54	13.16±0.24
	Low-density lipoprotein (cholesterol)	mg/dL	33.80±0.58	32.20±0.96
	Very low-density lipoprotein	mg/dL	12.80±0.48	11.20±0.12

Value are expressed as Mean± SEM, $n=5$. MFSGR: Methanolic fraction of *Sesbania grandiflora* roots

the development of a phytomedicine from traditional plants, toxicological evaluation is compulsory according to standard guidelines.[20] In addition, preclinical dose determination of medicinal plant is also an essential practice.[20] Overall, toxicity profiling provides an precise information on potentially related adverse effects medicinal products.

Despite the beneficial pharmacological uses of *S. grandiflora* roots, there is a lack of detailed data about acute oral toxicity study. Hence, the study was undertaken to assess and focus the acute oral toxicity of MFSGR in albino mice as per the OECD 425TG. In the acute toxicity study, administration of a single dose of MFSGR (2000 mg/kg) to five mice did not reveal any signs of toxicity or mortality throughout the observation period and some mild changes in behavioral patterns such as increased sleep time and itching were noticed for initial period. The increased sleeping tendency of mice treated with MFSGR might be due to the calming effect on CNS. Further, based on this observation, MFSGR may be a good candidate to evaluate anticonvulsant activity. The LD_{50} of the MFSGR may be considered to be higher than 2000 mg/kg. According to the Globally Harmonized System (GHS) of Classification and Labelling of Chemicals, the substances having an LD_{50} value >2000 mg/kg are considered as relatively safe. In some associated studies, the LD_{50} values of therapeutically used plant extracts have been found to be >2000 mg/kg, and as per the GHS standards, these extracts on acute exposure are considerably safe.[22]

During 14 days of acute oral toxicity study, the intake of water and food was observed to be normal and showed

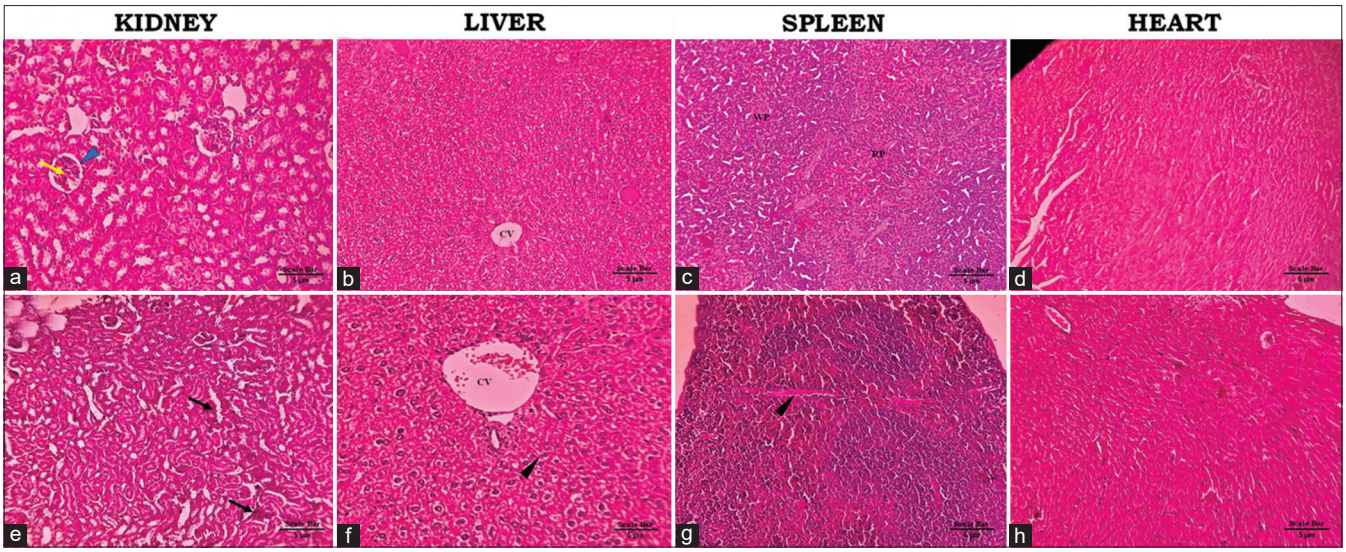

Figure 2: Histopathological sections of various organs in acute oral toxicity study of female mice. (a-d) For normal mice and (e-h) for methanolic fraction of *Sesbania grandiflora* root treated mice (Stain: Hematoxylin and eosin)

non-significant variations of body weight suggesting the usual metabolism of lipids, carbohydrates, and proteins within the body, since these nutrients have an important role in numerous physiological body functions.[16] The relative organ weight index is considered as added basic marker to measure the lethal effects of the plant constituents. The effect of lethal ingredients on the vital organs can be recognized by evaluating the relative organ weight as the index gives a preliminary perception to the inflammation or injury inflicted by any toxic agent (Pariyani *et al*., 2015). When animals were sacrificed at the end of the study, no lesions were found on macroscopic examination of heart, kidney, spleen, lungs, and liver in comparison with normal control group. Statistically, no significant variations were found in organ to body weight index of mice in MFSGR administered group when compared with vehicle control.

Hematological parameters are perceptive markers of the physiological changes in lethal stress or environmental pollutant in animals.[17] The assessment of these parameters could be used to reveal the deleterious effect of foreign compounds including plant extracts on the blood constituents of the animals. They can also be used to determine possible alterations in the levels of biomolecules, metabolic products, hematology, normal functioning, and histomorphology of the organs.[23] There were no significant alterations in white blood cell counts, HB, RBC counts, MCV, MCH, MCHC, hematocrit, and platelets between the treated groups and the control group, indicating that the MFSGR had no effect on the circulating blood cells of the treated animals.[24]

Increase in urea and creatinine concentrations are common marker of toxic agent and indicate diminished glomerular filtration rate. These increased levels are usually due to pre-renal causes or renal causes (Ramaiah *et al*., 2017). In the present study, no significant changes were observed in serum urea and creatinine levels showing that there is no renal injury inflicted by the treatment of MFSGR.

As one of the biochemical parameters analyzed, AST is normally found in cytoplasm and mitochondria of many cells, primarily in cardiac muscle, liver, and skeletal muscle. However, its concentration is much lower in the kidney, pancreas, and erythrocytes. Therefore, an increase in the serum levels of ALT, AST, ALP, or total bilirubin indicates hepatic.[25] These changes occur in the blood when the hepatic cellular permeability is changed or when necrosis and cellular injury occurs. Biochemical analysis of MFSGR-treated mice showed that there were no significant differences in these tested markers.[26] Further, the MFSGR did not elicit any significant alteration of cholesterol, HDL, LDL, and triglycerides when compared to the normal control mice. This observation indicated that treatment did not alter the normal homeostasis of lipid. Overall, the observation of biochemical estimations was well supported by histopathological assessments of liver and kidney tissues.

CONCLUSION

MFSGR did not exhibit any toxic effects and can be concluded that MFSGR is comparatively safe with its LD_{50} >2000 mg/kg. Taken together, the present study provided the platform to establish the scientific footage to traditional claims of *S. grandiflora* roots.

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