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EVALUATION OF TOXICITY STUDIES OF SESBANIA GRANDIFLORA LEAVES EXTRACTS IN WISTAR ALBINO RATS

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ABSTRACT
Objective: *Sesbania grandiflora* is a well-known medicinal plant belonging to the family Fabaceae, and it is used to treat several disorders. The current investigation aims to analyze the negative consequences of short and long-term administration of hydroalcoholic extract of *S. grandiflora* leaf in experimental animals. **Materials and Methods:** Acute and subacute toxicity were the two phases in which the entire examination was completed. In phase first, acute toxicity was performed at the dose of (2000, mg/kg) and adverse effect was recorded. However, in sub-acute toxicity the effect of different doses of (1000, 2000 and 5000 mg/kg) were studied for twenty-eight days. Animals were euthanized on the last day of the investigation, and selected internal body organs and samples of the blood were taken from each animal for histological, biochemical, and haematological analysis. **Results:** The result of the current investigation showed that the LD50 of *S. grandiflora* was observed more than 2000 mg/kg, Furthermore, experimental animals did not experience any mortality or alterations in their behavioral patterns when *S. grandiflora* was administered repeatedly at 1000, 2000, and 5000 mg/kg or in a single dose of 2000 mg/kg. Besides this, S. *grandiflora* also did not significantly modify any of the biochemical or haematological markers, or the histological analyses of selected organs. **Conclusion:** The results of the above research revealed that the orally administration of *S. grandiflora* extract did not exhibit any apparent harmful effects in experimental animals. Hence, *S. grandiflora* could be regarded as a safe and can be used for the therapeutic purpose in human being.

INTRODUCTION

Herbal remedies have been a continuous source of medicines since ancient times to treat several diseases and injuries [1]. They are an extensive reservoir of several biologically active substances, providing infinite possibilities for developing novel medications [2]. The plethora of information available in several traditional healthcare systems worldwide is the basis for selecting medicinal plants and discovering new medicinal products. According to WHO, approximately 80% of people rely on medicinal plants to meet their basic medical needs due to their

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greater cultural acceptability, compatibility with the human body, and fewer adverse effects [3]. Nearly 25% of modern medications are from plants worldwide [4]. Plants produce a wide range of metabolites, some of which may be valuable or harmful to humans [5].

Most people believe that folk medicine has more negative consequences than modern medicine. However, this general assumption that natural remedies are completely safe without any negative consequences is not true [6]. Approximately one-third of therapeutic candidates in the current drug development process require expensive toxicity studies [7], and evaluation of acute toxicity studies is the initial footstep in determining the negative consequences of drugs [8]. Numerous plant extracts have demonstrated multiple beneficial effects in managing or preventing various chronic diseases [9]. According to Yujing et al. (2020), toxicological research is necessary to forecast the potential negative consequences of herbal medications on human health. Therefore, acute and sub-acute toxicity studies should be used to determine the toxicological properties of plant-derived compounds and evaluate their safety [10]. In sub-acute toxicity studies, the test substance is administered daily from three weeks to three months in experimental animals [11]. However, in sub-acute toxicity, the test substances are administered for one to 3 months in experimental animals, The liver and kidneys are the primary metabolic organs that are adversely targeted by the metabolic responses of harmful substances [12] and can be used to forecast the toxicity effects of medications or phytotherapeutic agents [13].

Agathi, a well-known plant in the traditional system of medicine known as *Sesbania grandiflora*, belongs to the family Fabaceae [14]. It is a fast-growing, loosely branched plant that reaches 8 to 15 m and 25 to 30 cm (d) [15]. Although native to North America and Malaysia, it is now cultivated as an ornamental plant worldwide. Leaves are dark green with a pinnate arrangement. Leaflets have a blunt apex and are oblong-elliptical in shape. Blades typically have a scorching intensity and a bitter, acidic, and pungent flavor [16]. The Indian traditional medical systems of Sidha and Ayurveda also used this plant to treat a range of acute and chronic ailments [17]. The leaves of *S. grandiflora* are highly nutritious and contain vast amounts of protein, vitamins, carbohydrates, fats, fiber, and minerals like calcium and iron. The dried leaves are thought to have strong antibacterial, anticancer, and contraceptive effects. The plant's leaves, petals, and seeds are all edible due to its high nutritional content. The raw flowers and young leaves are edible and consumed as salad [18]. Flowers are reportedly used as an emollient, astringent, and antipyretic property. Additionally, flowers can treat intermittent fevers, running nose, inflammation of the mucous membrane inside the nose, and liver and spleen disorders [19]. The leaves of *S. grandiflora* are highly nutritious and contain several vitamins (vitamins A, E, and C), minerals, and amino acids. It contains glycosides, flavanoids, saponin, triterpenoid, tannin, pectin, and grandiflorol (α-5-methyl-5-pentacosanol). Despite the broader use of *S. grandiflora*, little information on the plant's safety profile is available. Therefore, investigating toxicological studies is essential to gather information regarding its safety and potential toxicity. Hence, taking all into consideration, the current investigation aims to explore the potential acute and subacute harmful effects of *S. grandiflora* leaf extract in experimental animals [20].

MATERIALS AND METHODS

Collection of plant material and preparation of extract:

The Chief Scientist of CSIR-NISCAIR, New Delhi, verified the *S. grandiflora* leaves collected from the Mathura, Uttar Pradesh. A voucher specimen was kept in the herbarium (NISePR/RHMD/Consult2022/4023-24) for future reference. The leaves of *S. grandiflora* were dried at room temperature until they were moisture-free, and afterward, these leaves were examined for their physicochemical and histochemical composition. The dried leaves of *S. grandiflora* were powdered and successively extracted in a soxhlet apparatus and with a pet—Ether, Chloroform, and hydroalcoholic solvent. The individual extract was concentrated in a rotary evaporator (Hi com) and stored in an airtight container for various studies.

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Chemicals and Reagents: Petroleum ether, Chloroform, ethanol, Hydrochloric acid, and all other analytical grade chemicals are used in the study.

Experimental Animals: Female Wistar rats weighing 180-230g were purchased from the National Institute of Biologicals Noida Uttar Pradesh. Animals were kept in polypropylene cages with regular 12-hour light and dark cycles and unrestricted access to water and food (Aishrwad Feed Pvt. Ltd). The Institutional Animal Ethics Committee approval was taken to carry out the experimental work.

Acute Toxicity Studies: The OECD guidelines evaluated acute toxicity in rats. Twelve female Wistar rats were divided into two groups, each comprising six rats, which served as control and test groups. Animals in the treatment group received a dose of 2000 mg/kg body weight of the hydroalcoholic extract of *S. grandiflora*. In contrast, the animals in the control group were administered a dose of 1 ml/100 g body weight of distilled water. After oral treatment administration, the rats were continuously observed for four hours for their general behavior and any visible signs of toxic effects. They were then observed for a whole day afterward. After that, the rats were monitored once daily for fourteen days to look for their behavioral changes, as well as symptoms of toxicity and fatality. On the fourteenth day, all the animals were kept fasted overnight, and on the 15th day, after recording the body weights, all the animals were euthanized, and samples of the blood were collected by puncturing the heart for hematological and biochemical studies. The organs were isolated and stored in a 10% formalin solution for histological analyses.

Subacute Toxicity Studies: Following OECD guidelines, a subacute toxicity study of *S. grandiflora* was performed on female Wistar rats. Twenty-four experimental animals were divided into four groups, containing six rats in each. Group A served as a control group and received 1ml tap water/100g body weight, and Group B, C, and D test groups received 1000, 2000, and 5000mg/kg body weight of *S. grandiflora* hydroalcoholic extract for 28 days once daily. On the first day of every week, an electronic weighing balance was used to record the body weight of every animal. On the last day of the experiment, after recording the body weights, all the animals were euthanized by inhalation of a high dose of anesthetic ether. In order to conduct biochemical and hematological analyses, the blood samples were collected into test tubes. After collecting blood samples, the kidney, liver, heart, and lungs were removed and weighed to determine the relative organ weight. The isolated organs were preserved in a 10% buffered formaldehyde solution for histological analysis [21].

Estimation of biochemical and hematological parameters

The blood samples collected in plain Eppendorf tubes were centrifuged for 10 min at 3,000 rpm, and afterward, separated serum was used to analyze liver function tests [22]. Alkaline phosphatase, Aspartate aminotransferase, and Alanine aminotransferase were evaluated as markers of liver injury using enzyme marker kits (Span Diagnostic Pvt Ltd) and a Clinical Chemistry Auto-analyzer (Robotnik). Besides this, the syntax-automated hematology analyzer was used to perform hematological analyses [23].

Relative Organ Weights and Histopathology: The histopathology of major metabolic organs was performed just after the animals were sacrificed. The heart, Liver, Lungs, and kidneys were removed by surgical procedure and weighed on a digital balance, and relative organ weight was calculated using the formula.

$$Relative\ organ\ weight\ (\%) = \frac{Organ\ weight}{Body\ weight} \times 100$$

After the ROW calculation, all the isolated organs were fixed in a 10% formalin solution and embedded in paraffin wax. Using a microtome, 4 µm thick sections were prepared and stained with hematoxylin and eosin. The stained slides were examined under a light microscope (using × 10 X objective lenses) for tissue integrity, necrosis, apoptosis, and infiltration of WBC cells. The photomicrograph was captured using a digital camera embedded in a microscope [24].

Statistical Analysis. The results were expressed as mean ± S.E.M. Statistical analysis was performed using Graph Pad Prism 5.2® software with one-way analyses of variances (ANOVA) followed by Dunnett's test.

RESULT

Acute Toxicity: Oral administration of an *S. grandiflora* hydroalcoholic extract up to 2000 mg/kg body weight did not cause any death among the experimental animals. Furthermore, during the observational period of 14 days, none of the treated rats demonstrated any sign of changes in the behavioral patterns of the animals, the color of skin and fur, eyes, salivation, and diarrhea, as depicted in Table 1. The average body weight of the treatment and control groups of animals is depicted in Table 2. No statistically significant ($p < 0.05$) alteration in the average body weight of experimental animals between the treatment and placebo groups was observed.

Effects of *S. grandiflora* hydroalcoholic extract on liver biochemical and hematological parameters: Table 3 demonstrates the impact of *S. grandiflora* hydroalcoholic extract 2000mg/kg on experimental animals' blood and liver biochemical parameters. The results of the statistical analysis

revealed that there were no remarkable variations ($p< 0.05$) among the biochemical and hematological parameters of the treatment and control group was observed.

Effects of *S. grandiflora* hydroalcoholic extract on Relative organ weight: The impact of *S. grandiflora* hydroalcoholic extract in a single dose (2000mg/kg) on ROW is depicted in Table 4. According to statistical analysis, no discernible variation ($p<0.05$) was observed between the ROW of the control and the treated group. Furthermore, the microscopic study of treated rats' hearts, livers, lungs, and Kidneys did not exhibit any apparent pathological abnormalities, and all the organs were almost identical to the non-treated group.

Subacute Toxicity Study

Effects of *S. grandiflora* hydroalcoholic extract on General behavior and mortality: Oral administration of *S. grandiflora* hydroalcoholic extract at different dose levels, 1000, 2000, and 5000mg/kg continuously for 28 days, did not produce any behavioral abnormalities or mortality in the treated rats.

Effects of *S. grandiflora* hydroalcoholic extract on Body weight and relative organ weight: The body weight of rats gradually increased during the treatment (depicted in Table 5 and 6). Continuous treatment of *S. grandiflora* extract for 28 days did not significantly affect the overall body weight of experimental animals between the test and control groups. Both groups of animals were found to be normal during the 28-day trial period. However, no significant ($p<0.05$) effect was observed between the ROW of the control and the treated group.

Effects of *S. grandiflora* hydroalcoholic extract on Biochemical and Haematological parameters: Table 7 demonstrates the impact of *S. grandiflora* hydroalcoholic extract on hematological and serum biochemical markers of the liver. The analysis of hematological parameters revealed that treating *S. grandiflora* hydroalcoholic extract at 1000, 2000, and 5000mg/kg for twenty-eight days did not cause any remarkable change ($p<0.05$) compared to the control group. However, the treatment of 5000mg/kg slightly changes the hepatic biochemical markers, but these changes were insignificant compared to the control group.

Table 1: Impact of acute oral administration *S. grandiflora* leaves extract on behavioral responses of rats during an acute oral toxicity study

Parameter	30 Min		4h		12H		24 h		Day 2		Day7		Day 14	
	CG	TG	CG	TG	CG	TG	CG	TG	CG	TG	CG	TG	CG	TG
Fur and Skin	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Salivation	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Lacrimation	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Urine Colour	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Fecal Consistency	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Sleep	N	Y	N	Y	N	Y	N	N	N	N	N	N	N	N
Itching	N	Y	N	Y	N	N	N	N	N	N	N	N	N	N
Coma	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
Mortality	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
Convulsion	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF

CG: Control group, TG: Treatment group treated with (2000 mg/kg, p.o)., N: Normal, R: regular, Y: Yes, NF: Not found

Table 2: Impact of acute oral administration *of S. grandiflora* leaves extract on change in body weight of Rats during acute oral toxicity study

Treatment Group	Body weight		
	Day-1	Day-7	Day-14
Control	182.56±5.26	183.10±5.11	184.92±4.1
S. grandiflora 2000mg/kg	190.10±7.10	190.88±6.62	192.66±5.15

Table 3: Impact of acute oral administration of *S. grandiflora* leaves extract on hematological and liver biochemical parameters of rats

Parameters	**Control group**	**Treatment Group (2000mg/kg)**
Red blood cells (10^{12}/L)	7.92±0.19	8.45±0.66
Haemoglobin (g/dL)	12.22±1.28	13.68±1.28
Mean corpuscular Haemoglobin (pg)	15.4±1.3	16.2±1.0
Haematocrit (%)	41.45±0.25	39.81±0.12
White blood cells (10^6/µL)	4.68±0.38	4.52±0.38
Platelets (10^3/µL)	1031±42.16	1054±22.16
Aspartate aminotransferase U/L	78.66±8.16	84.66±8.16
Alanine aminotranferase U/L	42.66±3.16	40.66±5.16
Alkaline phosphatase U/L	111±10.16	1115±7.16

Table 4: Impact of acute administration of *S. grandiflora* leaves extract on absolute and relative organ weights of rats

Organ	**Absolute weight**		**Relative weight**	
	Control group	***S. grandiflora* 2000mg/kg**	**Control group**	***S. grandiflora* 2000mg/kg**
Heart	1.3±0.23	1.2±0.08	0.70±0.01	0.64±0.02
Liver	5.85±0.04	6.01±0.0	3.16±0.03	3.25±0.04
Lungs	1.1+0.03	1.1+0.03	0.59±0.0	0.59±0.0
Kidney	0.9±0.05	0.7±0.05	0.43±0.01	0.37±0.01

Table 5. Impact of subacute oral administration of *S. grandiflora* leaves extract on change in body weight of Rats during subacute oral toxicity study

Treatment Group	**Body weight**				
	Day-1	**Day-7**	**Day-14**	**Day-21**	**Day-28**
Control	212.80±11.26	215.10±9.15	216.92±10.1	219.56±10.1	221.95±9.5
S. grandiflora 1000mg/kg	225.10±8.17	224.10±11.17	227.65±10.5	229.95±10.1	232.37±7.7
S. grandiflora 2000mg/kg	213.99±5.17	215.1±5.1	216.5±5.1	219.76±4.5	220.10±6.5
S. grandiflora 5000mg/kg	227.20±8.0	230.50±7.6	237.1±8.0	237.9.6±8.0	239.97±8.6

Table 6: Impact of subacute oral administration *of S. grandiflora* leaves extract on absolute and relative organ weight

Organ	**Absolute weight**				**Relative weight**			
	Control group	**1000 mg/kg**	**2000 mg/kg**	**5000 mg/kg**	**Control group**	**1000 mg/kg**	**2000 mg/kg**	**5000 mg/kg**
Heart	1.7±0.23	1.39±0.23	1.57±0.68	1.28±0.21	0.77±0.19	0.59±0.021	0.72±0.024	0.55±0.031
Liver	7.48±0.36	7.21±0.11	7.77±0.12	8.23±0.38	3.41±0.42	3.10±0.031	3.59±0.12	3.56±0.18
Lungs	1.5+0.03	1.42+0.03	2.0+0.02	1.49+0.03	0.68±0.02	0.61±0.018	0.92±0.036	0.64±0.00
Kidney	1.02±0.05	1.52±0.11	1.42±0.05	1.59±0.05	0.46±0.0	0.65±0.041	0.65±0.01	0.68±0.022

Table 7: Impact of subacute oral administration of *S. grandiflora* leaves extract on complete blood count and liver biochemical marker

Parameters	Control group	*S. grandiflora* 1000mg/kg	*S. grandiflora* 2000mg/kg	*S. grandiflora* 5000mg/kg
Red blood cells (10^{12}/L)	7.92±0.19	8.95±0.37	8.31±0.66	8.67±0.2
Haemoglobin (g/dL)	12.22±1.28	14.92±1.28	14.26±3.1	13.45±1.28
Mean corpuscular Haemoglobin(pg)	17.9±1.3	16.7±1.0	17.2±0.5	15.5±0.8
Haematocrit (%)	41.45±0.25	39.81±0.12	40.21±0.0	39.81±0.18
White blood cells 10^6/µL)	4.51±0.87	4.52±0.38	4.62±0.28	4.89±0.15
Platelets (10^3/µL)	1131±42.16	1254±22.16	1231±18.16	1240±21.16
Aspartate aminotransferase U/L	102±18.16	98±10.16	105±9.23	118±13.22
Alanine aminotranferase U/L	71.22±4.23	74.25±5.12	79.86±4.28	88.22±6.66
Alkaline phosphatase U/L	122±7.98	128±10.12	138±6.78	154±8.98

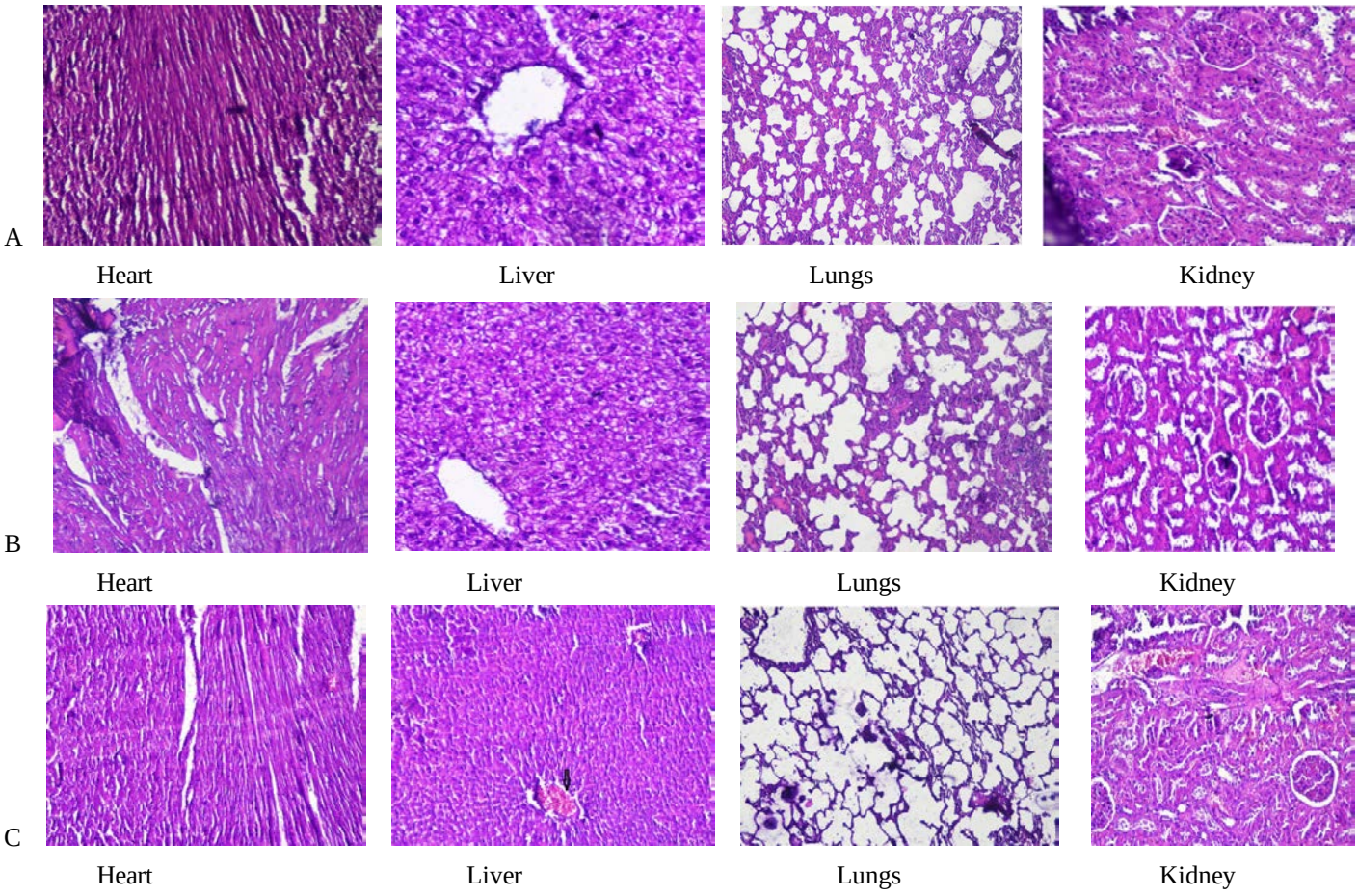

Figure 1: Effect of *S. grandiflora* 2000 and 5000 mg/ kg on heart, liver, lungs, and Kidney

Histopathology: Histopathological examination of Kidney tissue revealed the typical architecture in the control group and animals treated with 1000mg/kg. Additionally, macroscopic examination of the treated rats did not reveal any change in the texture or color of the isolated organ. However, the kidney tissue of rats treated with hydroalcoholic extract of *S. grandiflora* at the dose of 2000 and 5000 mg/kg showed small irregularities in their histoarchitecture, such as glomerular injury, interstitial inflammation, and infiltration of plasma cells depicted in Figure 1. The histopathological examination of liver tissue demonstrated the visible central veins and standard hepatocellular architecture in the control group. Rats treated

with 2000 and 5000 mg/kg showed mild acute injury and congestion in the central portal vein as well as in the sinusoid of the liver. Furthermore, lung tissue histology demonstrated the infiltration of inflammatory cells and mild injury in the alveolar sac. However, the rats treated with 2000 and 5000 mg/kg did not exhibit any change in cardiac tissue and demonstrated a normal myofibrillar structure with striations in all the groups of animals.

DISCUSSION

Medicinal herbs and dosage forms have been recognized as safe and efficient for hundreds of years due to minimal adverse reactions. Such a view might have significantly enhanced the careless use of these drugs among the rural population [25]. Typically, these drugs are used for prolonged periods without appropriate dosage monitoring or insufficient knowledge of their potentially harmful side effects [26]. Therefore, understanding oral toxicity is essential, as this will help determine doses that may be utilized in the future and help detect potential clinical symptoms that medicines may produce [27]. Acute toxicity is the initial step in the assessment and evaluation of the pharmacological properties of medicinal plants [28], which provides preliminary information regarding the toxicity of drugs and is also helpful in determining the dose of medication in preclinical studies; besides the several pharmacological properties of this plant there is lack of information is a lack of comprehensive information regarding the toxicity of this well-known pant. Therefore, the current study was initiated to assess and analyze the acute and sub-acute toxicity of *S. grandiflora* leaf extract.

The acute toxicity investigation aims to evaluate the harmful effects of agents on a living organism after a brief exposure. These studies are typically performed on rodents like rats and mice at the early stage of the drug development process [29]. The current investigation results demonstrated that at a dose of 2000 mg/kg, rats did not show fatalities or apparent signs of toxicological effects, except an increase in sleep time was observed in the experimental animals. A soothing effect on the central nervous system might be responsible for a higher tendency to sleep in rats, and these results are in agreement with other studies in which the ethyl acetate fraction of *S. grandflora* root was reported to be nontoxic [30]. As an acute toxicity study has a restricted clinical application, a sub-acute toxicity was carried out. Similarly, neither fatality nor behavioral changes were noted during the treatment period of sub-acute investigations. Change in an animal's body weight has been an essential parameter for the overall health of animals or to evaluate the harmful impacts of drugs on their health [31]. The findings of the current investigation demonstrated that no remarkable alteration in the change in body weight was observed among the control and treatment groups. It indicates that the extract had no adverse effects on the appetite of animals, and these results were also supported by the findings of Vinay et al. [32]. Besides this, relative organ weight is a key marker for determining animals' physiological and pathological state. The cardiovascular, respiratory, urinary, and liver are the primary fundamental organs of the body that are affected by toxic substances. Therefore, the relative organ weights of the treated animals may act as key indicators to confirm the injury caused by the test drugs. The findings of the current investigation suggested no remarkable variation in the relative organ weight of internal body parts, like heart, liver, lungs, and kidney, of the experimental animals observed in both toxicity studies.

The hematological parameters, such as the level of hemoglobin and formed elements, act as markers to identify the physiological and pathological condition of the body, and significant alterations indicate that the chemical being delivered is either harmful or protective to the hematopoietic tissue. The findings of our study are also supported by the analysis of 28, which suggested that the methanol extract of *S. grandiflora* root had no impact on the hematological parameters of experimental animals. Similarly, our study demonstrated that there were no remarkable differences in the numbers of WBC, RBC, and platelets count, as well as hematocrit and HB of the treatment and control group, which suggested that the *S. grandiflora* hydroalcoholic extract did not affect the circulating blood cells of the treated animals.

Evaluating serum biochemical markers is essential for determining organ functions, particularly the Kidney and liver. AST, ALT, and ALP are the most crucial biochemical markers to assess liver function [33]. Alterations in the level of biochemical markers of the liver are an indication of liver toxicity caused by exogenous agents. Our study's findings revealed no significant alteration in the acute toxicity study's serum level of AST ALT and ALP. However, the treatment of 2000 and 5000 mg/kg in sub-acute toxicity studies slightly alters the ALP level in experimental animals. The elevated serum ALP

level suggested higher doses of *S. grandiflora* may lead to biliary obstruction.

The liver, Kidney, lungs, and heart are the principal organs affected by the metabolic reaction of harmful substances [34]. Microscopic study of liver and kidney tissue of experimental animals treated with 2000 and 5000 mg/kg dose for 28 days demonstrated some histological changes like congestion in the central portal vein, dilation of the sinusoid, and a slight change in the architecture of the Kidney and mild inflammation, thickening of the bronchial wall as well as infiltration of plasma cells was observed. However, no sign of myocardial injury was observed in the histology of treated rats. It indicates that the *S. grandiflora* did not produce any harmful effect on the structure and functioning of the heart. Moreover, additional organ-specific studies and their mechanism are required as the prolonged use of extract harms the liver and lungs. The duration of studies and selection of animals is the major limitation of the current investigation. The studies were designed for 28 days, and only one female sex of animals was included. Therefore, in the future, the same studies should be performed on different sexes of animals with longer durations.

CONCLUSION

The current research reveals valuable insights on both acute and sub-acute toxicity. The current investigation is just preliminary research, but it may provide a foundation for further research in the future. The findings of the current investigation indicate that the hydroalcoholic leaf extract *S. grandiflora* can be considered safe or nontoxic when taken briefly. However, prolonged use of S. grandiflora at higher doses of *S. grandiflora* may cause liver toxicity and lung infection. Therefore, further investigation is required before moving on to clinical investigations of this plant. Additionally, sub-chronic and chronic toxicities of *S. grandiflora* are also required to clarify the health effects related to long-term consumption of the extract.

FINANCIAL ASSISTANCE

Nil

CONFLICT OF INTEREST

The authors declare no conflict of interest

AUTHOR CONTRIBUTION

All authors contributed equally to this work. Sharad Sharma performed the experimental work and prepared the manuscript. Bhupesh Chander Semwal developed the concept, analyzed the results, and corrected the manuscript. Avijit Mazumder checked the manuscript for grammar and plagiarism.

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