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Original Article

Acute and Sub-acute Toxicity Studies of Ethanol and Chloroform Extracts of *Solanum giganteum* Jacq. Leaves

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ABSTRACT

Objectives: To investigate the acute and subacute oral toxicity of ethanol and chloroform extracts *Solanum giganteum* Jacq (SG) leaves in a rodent for evaluating its safety profile. **Methods:** *Solanum giganteum* Jacq leaves extract for the acute oral toxicity (300 to 5000 mg/kg) and subacute oral toxicity SG-Chloro (SGC 50, 200, 500 mg/kg) and SG-Alcohol (SGA 100, 500, 1000 mg/kg) studies were administered orally according to the guidelines 423 and 407 of OECD, respectively. **Results:** In the acute oral toxicity study, doses are administered by 300 mg/kg to 5000 mg/kg. SGA does not show toxicity up to 5000 mg/kg, SGC shows toxicity at a dose of 2000 mg/kg. In the subacute toxicity study, the daily oral treatment with extracts of SGA 100, 500, 1000 mg/kg, SGC 50, 200, 500 mg/kg of extracts for 28 days did not produce any death or hazard. Likewise, SGA 100, 500, 1000 mg/kg, SGC 50, 200 mg/kg of *Solanum giganteum* Jacq no significant changes were recorded in food intake, body weight gain, the biochemical and haematological parameter of control and treated rats. SGC 500 mg/kg of significant changes were recorded in food intake and body weight gains were decreased compared to the control group. SGC 500 mg/kg of extract treaded group saw mild liver pathological findings characterized by abnormal hepatic configuration was observed with treated animals. **Conclusion:** The acute and subacute toxicity study *Solanum giganteum Jacq* alcohol extract is considered relatively safe on acute and subacute oral exposure. *Solanum giganteum Jacq* chloroform extract shows acute toxicity at dose 2000 mg/kg and in subacute toxicity study, higher dose 500 mg/kg.

Key words: *Solanum giganteum,* Acute toxicity, Aubacute toxicity, OECD, SG-Chloro, SG-alcohol.

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INTRODUCTION

In recent years, there has been an indication of the increasing popularity of complementary and alternative medicine approaches to health in India. Even with rapid growth, there is inadequate evidence about the efficiency and toxicity of alternative medicine. Much more needs to be done to increase the evidence base for herbals, botanicals, and dietary supplements. Since ancient times, plants have been a basic source of drugs, but scientific medicine tends to no attention to the importance of herbal medicine.[1]

Approximately any substance can be harmful at a few doses but, at the same time, can be without harmful effects at some lower dose. Between these two limits, there is a variety of possible effects, from delicate long-term chronic toxicity to instant lethality. The large range of toxic chemicals formed by plants (phytotoxins), commonly referred to as secondary plant compounds, is often held to have developed as defence mechanisms against animals, mainly insects and mammals. Many toxic chemicals are constituents of plants that form part of the human diet.[2]

The many varieties in the plant of the genus Solanaceae. There may be up to around 1,500 species worldwide. With a few 800 conventional specific and infraspecific taxa of the more than 4,000 described, the genus Solanum contains more species than any other genus in the *Solanaceae* family and it is one of the majors between the angiosperms.

Solanum giganteum (Family: Solanaceae) Much-branched shrub or small tree up to 6 m high. Branchlets with white, woolly hairs and stout, straight prickles up to 5 mm long. Leaves elliptic, margin entire, large, up to 250 x 90 mm, softly textured, dark green, glabrescent. Flowers in many-flowered, branched, dense, terminal corymbs faintly scented; corolla white, mauve to blue or purple, 15 mm in diameter, anthers yellow. Distributed in almost all parts of the world and abundantly found in Tropical Africa, India (Maharashtra; Gujarat; Punjab and Rajasthan). In India, it is widely distributed. Traditionally it is used treat as an anti-inflammatory, antibacterial, antifungal, and much more.[3-8]

Hence, in the existing work**,** Acute and subacute oral toxicity estimation of ethanol and chloroform extracts of *Solanum giganteum* Jacq leave. No clinical proof or studies for the oral acute and sub-acute oral toxicity investigation for this plant are available in the literature. Therefore, acute and sub-acute oral toxicity studies were carried out by the OECD guidelines to verify and set up the safety for its use in clinical practice. The purpose of this study was to estimate the safety of *Solanum giganteum* in animals' models.

MATERIALS AND METHODS

Plant material

The plant *Solanum giganteum* Jacq was collected in November 2020, from the area of Satara Maharashtra, India, the specimen was authenticated by comparing it with the voucher specimen deposited in the Department of Botany by Y.C. Institute of Science, Satara, Maharashtra, India

Preparation of extract

Freshly collected leaves of the plant *Solanum giganteum* Jacq. were dried at room temperature for three weeks. The dried plant material was made a coarse powder and weighed the quantity of the powder (500g)

was subjected to hot extraction in a soxhlet apparatus using petroleum ether, chloroform, ethanol, at a temperature range of 40-80°C. The marc was completely dried and weighed, before and after every extraction. At room temperature, the extract was concentrated by evaporation of the solvent.[9,10]

Animals

Adult healthy albino mice weighing 25–30 g and Wister rat 150- 200 g were used and kept in the animal house of the Department of Pharmacology, Satara College of Pharmacy, Satara. The animals were kept in plastic cages (34 × 47 × 18 cm3) at the animal house, in an air-conditioned environment with T in each cage and maintained at room temperature of (25 ± 2)°C with relative humidity (60 % ± 10%) under 12 hr night and light cycle. The experimental protocol was permitted by the Institutional Animal Ethics Committee of Satara College of Pharmacy, Satara and was carried out according to the CPCSEA guidelines for laboratory animal facilities. Protocol sanction number Protocol No.: SCOP/IAEC/102/2020 (CPCSEA registration number-1314/PO/Re/S/2009/CPCSEA)

Acute toxicity studies

The acute oral toxicity study of extracts of SG-Chloro and SG-Alcohol of *Solanum giganteum* Jacq. was estimated according to the OECD guideline 423 on mice (20–30 g), where the maximum test dose of 5000 mg/kg was used.

All the animals were set aside at overnight fasting before every experiment with free excess to water. The animals were separated into four groups, each comprising 3 animals. The 1st group, 2nd, 3rd and 4th were considered as tested groups administrated orally extracts (dissolved in normal saline) extract at a dose of 300 mg/kg, 1000 mg/kg, 2000 mg/kg and 5000 mg/kg. Before the dose was received, the bodyweight of each animal was estimated, and the dose was measured according to the body weight.

The animals were observed for any toxic signs and symptoms for the first 4 h after the treatment period. Additionally, animals were examined for 14 days for any toxic effect. Behavioural changes and other factors such as body weight, urination, food intake, water intake, respiration, convulsion, tremor, temperature constipation, changes in eye and skin colours, etc.[11,12]

Subacute toxicity studies

The subacute oral toxicity study of extracts of SG-Chloro, SG-Alcohol of *Solanum giganteum* Jacq was estimated according to OECD guideline 407 on Wister rats (150-200 g).

The Wistar rats were randomly allocated to three groups by sex (n = 6, M=3, F=3). The first group was provided distilled water orally (control group). Groups 2 to 7 were orally treated with (low, medium, high Doses) extracts of SG-Chloro (50, 200, 500 mg/kg) and SG-Alcohol (100, 500, 1000 mg/kg) of *Solanum giganteum* Jacq respectively, for 28 consecutive days. All animals were supplied with feed and water *ad libitum* throughout the testing periods. The clinical sign was monitored daily for physiological and behavioural changes. Toxic symptoms such as toxicity and death were observed for signs of abnormalities. Bodyweight changes were recorded weekly and food consumption and water intake were observed daily. At the finish of treatment, the animals were fasted overnight but permitted administered to water *ad libitum*. They were then anaesthetized and blood samples were collected by retro-orbital puncture using capillary tubes with or without the anticoagulant.[13]

Haematological Analysis

The blood samples collected with the anticoagulant were used immediately for the determination of haematological parameters such as red blood corpuscles (RBC) count, white blood corpuscles (WBC) count, haemoglobin (Hb) and platelet count were performed using a blood cell counter.[14]

Biochemical analysis

The blood samples collected without the anticoagulant was centrifuged at 4,000 rpm for 10 min to separate the serum. The serum was investigated for biochemical constraints such as glucose, urea, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total protein employing standard diagnostic kits.[15]

Necropsy

At the finish of the protocol, the animals were anaesthetized and necropsy was performed on randomly preferred animals of each group per sex to investigate the macroscopic external features of vital organs such as the liver. The organ is cautiously detached and weighed. Organ weights were recorded in comparative terms (gram per 100 g of body weight). The collected organs were fixed in 10 % buffered formalin and fixed in paraffin. Histology sections (5 µm thick) were stained with hematoxylin and observed under a light microscope[16].

Statistical analysis

Statistical analysis was done by using one way ANOVA followed by Dunnett's Comparison Test for comparison between different groups using Graph pad prism-5 software. A $p < 0.05$ was considered statistically significant

RESULTS

Acute toxicity studies

Acute toxicity study of extracts of SG-Alcohol, SG-Chloro of Solanum giganteum Jacq.

Oral acute toxicity was estimated according to the guidelines of the OECD. Doses are administered by the 300 mg/kg, 1000 mg/kg, 2000 mg/kg and 5000 mg/kg (OECD guideline no. 423). SG-Alcohol does not show toxicity up to 5000 mg/kg, SG-Chloro shows toxicity at a dose of 2000 mg/kg.

Subacute toxicity studies

Effect of extracts of SG-alcohol and SG-chloro of solanum giganteum jacq *on general signs and bodyweight*

The daily oral treatment with extracts of SG-Alcohol (SGA 100, 500, 1000 mg/kg), SG-Chloroform (SGC 50, 200, 500 mg/kg) of *Solanum giganteum* Jacq extracts for 28 days did not produce any death or hazardous sign such as piloerection, alteration in the locomotor activity and other physiological activities as compared to the control animals. Likewise, SGA 100, 500, 1000 mg/kg, SGC 50, 200 mg/kg of *Solanum giganteum* Jacq no significant changes were recorded in food intake and body weight gain of control and treated rats (Table 1 and 2). SGC 500 mg/kg of *Solanum giganteum* significant changes were recorded in food intake and body weight gains are decrease compared to control group.

Haematological and biochemical parameters

The effects of subacute administration of SG-Alcohol (SGA 100, 500, 1000 mg/kg) and SG-Chloroform (SGC 50, 200, 500 mg/kg) of *Solanum giganteum* Jacq extracts on haematological and biochemical parameters are presented in Tables 3 and 4. Most haematology measures

Table 1: Effect of *Solanum giganteum* extracts on food intake.

Days	Groups						
	Control	SGC mg/kg			SGA mg/kg		
		50	200	500	100	500	1000
7	30 ± 0.25	29 ± 0.28	28 ± 0.14	27 ± 0.12	27 ± 0.26	26 ± 0.22	28 ± 0.26
14	32 ± 0.24	31 ± 0.32	27 ± 0.42	29 ± 0.22	30 ± 0.35	28 ± 0.14	31 ± 0.32
28	33 ± 0.44	34 ± 0.14	32 ± 0.45	27 ± 0.42*	33 ± 0.48	34 ± 0.35	33 ± 0.54

Effect of SGC and SGA extracts on food intake in rats. Data are mean SEM values (n = 6 in each group). Data were analyzed by one way ANOVA followed by Dunnett test Comparisons test. *P<0.05 compared with normal control.

Table 2: Effect of *Solanum giganteum* Extracts on Bodyweight (gm).

Days	Groups						
	Control	SGC mg/kg			SGA mg/kg		
		50	200	500	100	500	1000
7	164.33 ± 3.33	161.3 ±1.73	158.3 ± 1.84	162.16 ± 1.2	160.1 ± 1.3	156.3 ± 1.21	158.17 ± 1.3
14	168.43 ± 2.38	166.3 ±1.93	162.3 ± 2.43	163.7 ± 0.6	167.4 ± 2.44	160.4 ± 2.11	162.1 ± 2.44
28	173.23 ± 1.39	171.6 ± 1.15	168.6 ± 0.15	154.1 ± 0.6**	170.5 ± 1.18	170.3 ± 2.41	168.1 ± 1.18

Effect of SGC and SGA extracts on Body weight in rats. Data are mean SEM values (n = 6 in each group). Data were analyzed by one way ANOVA followed by Dunnett test Comparisons test. **P<0.01 compared with normal control.

Table 3: Effect of *Solanum giganteum* extracts on haematological parameters in a subacute oral toxicity study.

Parameter	Groups						
	Control	SGC mg/kg			SGA mg/kg		
		50	200	500	100	500	1000
Hb (g/dL)	12.5 ±1.2	12.8 ± 3.8	12.66 ± 3.8	11.6 ± 1.28	12.4 ± 0.95	12.9 ±1.65	12.1 ± 1.62
RBC (10^6/mm3)	6.5 ± 2.3	6.3 ± 3.6	6.87 ± 2.8	5.9 ± 2.3	6.4 ± 1.3	6.7 ± 1.3	7.01 ± 2.0
WBC (10^3/mm^3)	7.6 ± 0.32	7.6 ± 1.3	7.9 ± 2.3	9.92 ± 0.7*	7.3 ± 1.6	7.1 ± 1.6	7.4 ± 1.85
Platelets 10^5/mm^3	6.74 ±1.24	6. 32 ± 2.2	6. 52 ± 3.2	6.15 ± 1.73	6.44 ± 1.54	6.84 ± 2.84	6.26 ± 1.41

Effect of SGC and SGA extracts on the haematological parameter in rats. Data are mean SEM values (n = 6 in each group). Data were analyzed by one way ANOVA followed by Dunnett test Comparisons test. *P<0.05 compared with normal control.

(haemoglobin, total red blood cells, total white blood cells, and platelet count) in treated rats were not significantly different from the controls group, except marginal variations in certain parameters of SGA 100, 500, 1000 mg/kg, SGC 50, 200 mg/kg of *Solanum giganteum* Jacq treated group. SG-Chloroform (SGC 500 mg/kg)) there was significant (P < 0.05) initiation in WBC (24 %) counts in the treated animals compared to that of the control group.

Table 4: Effect of *Solanum giganteum* extracts on biochemical parameters in subacute oral toxicity study.

Parameter	Groups						
	Control	SGC mg/kg			SGA mg/kg		
		50	200	500	100	500	1000
ALT (IU/L)	36.6 ± 3.3	39.14 ± 1.2	41.24 ± 1.8	53.83 ± 1.5**	37.5 ± 3.1	38.5 ± 3.1	39.8 ± 2.7
AST (IU/L)	89.97 ± 5.15	93.4 ± 2.6	94.4 ± 2.8	108.9 ± 4.6**	88.40 ± 3.17	91.80 ± 7.77	94.12 ± 6.46
ALP (IU/L)	101.4 ± 4.2	105.5 ± 2.4	106.8 ± 3.2	115.5 ± 2.1**	103.8 ± 4.6	100.5 ± 3.1	106.4 ± 2.8
Total protein (gm/dL)	6.9 ± 1.3	6.6 ± 2.4	7.6 ± 4.3	9.4 ± 2.2**	6.7 ± 4.7	6.4 ±1.7	6.8 ± 2.4

Effect of SGC and SGA extracts on Biochemical parameters in rats. Data are mean SEM values (n = 6 in each group). Data were analyzed by one way ANOVA followed by Dunnett test Comparisons test. **P<0.01 compared with normal control.

No statistically significant differences in liver function parameters (ALT, AST, and alkaline phosphatase and Total protein) were noted except for marginal variations seen in SGA 100, 500, 1000 mg/kg and SGC 50, 200 mg/kg of *Solanum giganteum* Jacq extracts treated group.

SGC 500 mg/kg of *Solanum giganteum* Jacq and extracts treaded group significant (P<0.05) induction (ALT, AST, and alkaline phosphatase and Total protein).

Histopathological studies

In the subacute oral toxicity study, microscopic analysis of the histological sections of the liver (Figure 1) did not show evidence of any histological abnormalities such as inflammation, necrosis, degeneration, cellular infiltration, vascular changes and haemorrhage or cellular abnormalities in both normal and treated animals of SG-Alcohol (SGA 100, 500, 1000 mg/kg), and SG-Chloroform (SGC 50, 200 mg/kg) of *Solanum giganteum* Jacq extracts.

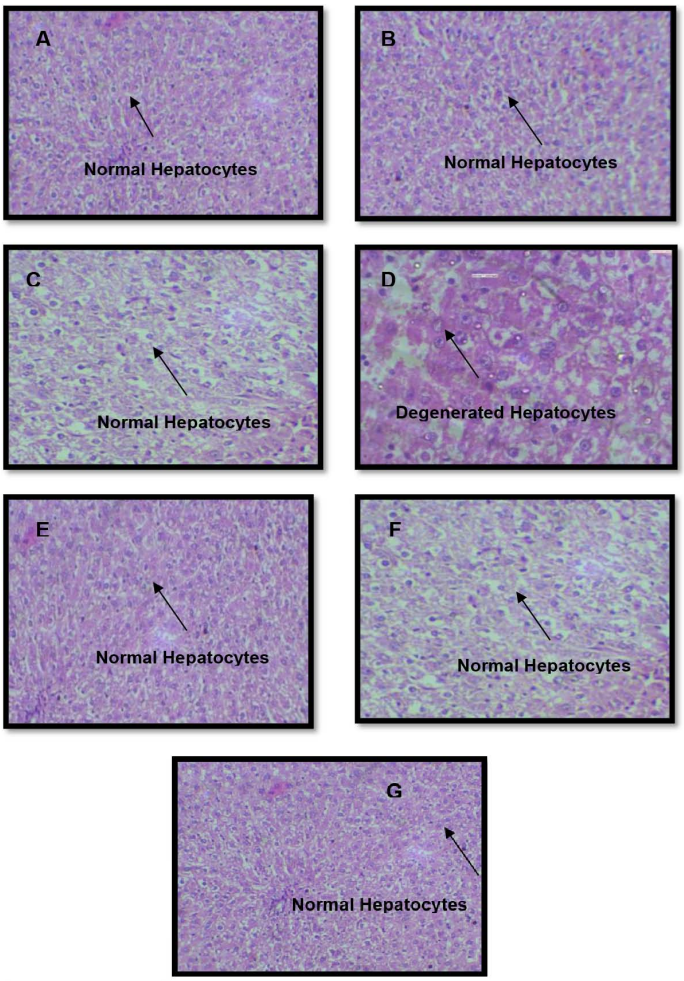
SGC 500 mg/kg of *Solanum giganteum* Jacq extract treaded group seen mild liver pathological findings characterized by abnormal hepatic configuration were observed with treated animals.

DISCUSSION

An oral acute toxicity test estimated the adverse consequences that occur within a short time after administration of a solo dose of a test substance. This testing was carried out primarily in rodents and regularly done early in the discovery of a new chemical or product to supply information on its probable toxicity.

For acute oral toxicity study, extracts of SG-Alcohol and SG-Chloro of *Solanum giganteum* Jacq were given to mice at a dose of 300 mg/kg to 5000 mg/kg. SG-Alcohol groups did not show any signs of toxicity at all doses employed. No death was observed in the treated groups, i.e., at 300 mg/kg to 5000 mg/kg during the study period. Therefore, the LD_{50} of the extract could be more than 5000 mg/kg. The SG-Alcohol extract may, therefore, be considered comparatively safe on acute exposure.

SG-Chloro of *Solanum giganteum* Jacq extract did not produce any signs of toxicity at a dose of 300 mg/kg to 1500 mg/kg and a dose of 2000 mg/kg



A. Normal **B**. SGC 50 mg/kg **C.** SGC 200 mg/kg **D**. SGC 500 mg/kg **E.** SGA 100 mg/kg **F.** SGA 500 mg/kg

Figure 1: Photomicrographs of rat liver tissue section under low power.

shows toxicity 3 out of 2 animals deaths. Therefore SG-Chloro LD_{50} was found at a dose of 2000 mg/kg.

An acute oral toxicity study gives a guideline to decide the doses for subacute toxic study (low, medium and high) which may be a more clinically applicable group after 28 days of daily treatment. On basis of acute toxicity study for subacute administration of SG-Alcohol (SGA 100, 500, 1000 mg/kg) and SG-Chloroform (SGC 50, 200, 500 mg/kg) of *Solanum giganteum* Jacq.

In the hematopoietic system, individual one of the most susceptible targets of toxic chemicals is a significant index of the physiological and pathological category of humans and animals. In this study, the test groups of SGA 100, 500, 1000 mg/kg and SGC 50, 200 mg/kg of *Solanum giganteum* Jacq treaded group did not illustrate any significant variation in the haematological parameters except SGC 500 mg/kg there was significant ($P < 0.05$) initiation in WBC (24 %) counts in the treated animals compared to that of the control group.

The liver has an essential role in the metabolism of drugs or plant products. Exogenous chemicals and their metabolites might affect toxicity or cell damage on this organ. In the current histopathological examination of the liver, treated animals of SGA 100, 500 and 1000 mg/kg and SGC 50 and 200 mg/kg of *Solanum giganteum* Jacq. Showed no change in the microscopic structure of the liver. The common architecture of the liver, the appearance of the hepatocytes, the hepatic sinusoids, portal triads, and central veins are normal as compared with controls. The result was also accompanied by the non-adverse effects of the extracts in any of the biochemical markers (such as ALT, AST and alkaline phosphatase and Total protein), which showed statistically insignificant changes compared with a control group. SGC 500 mg/kg of *Solanum giganteum* Jacq extract treaded group seen mild liver pathological findings characterized by abnormal hepatic configuration were observed with treated animals. SGC 500 mg/kg of extract treaded group significant ($P<0.05$) induction (ALT, AST, and alkaline phosphatase and Total protein) found in the liver and this dynamic in the respected study.

CONCLUSION

The present result from the acute and subacute oral toxicity study *Solanum giganteum* Jacq. SG-Alcohol extract are considered comparatively safe on acute and subacute oral exposure. The SG-chloroform extract shows acute oral toxicity at dose 2000 mg/kg and in subacute toxicity study, higher dose 500 mg/kg shows by abnormal hepatic configuration were observed with treated animals.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

SG: *Solanum giganteum* Jacq.; **SGA:** *Solanum giganteum* alcohol extract; **SGC:** *Solanum giganteum* chloroform extract; **WBC:** White blood cells; **ALT:** Alanine transaminase; **AST:** Aspartate aminotransferase; **ALP:** Alkaline phosphatase.

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