Acute and sub-acute toxicity studies on the effect of *Senna alata* in Swiss Albino mice

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*Cogent Biology* (2016), 2: 1272166
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S. Roy¹, B. Ukil¹ and L.M. Lyndem¹*

**Abstract:** *Senna alata* has attracted the attention of many researchers due to its numerous medicinal properties. This study aims to test the acute and sub-acute toxicity of its leaf extracts in Swiss albino mice. Studies were carried out with a fixed dose of 1,000, 2,000, and 3,000 mg/kg body weight through oral administration daily. Signs of toxicity in terms of behavior and mortality were noted after every two hours till 24 h of administration for acute toxicity and further administration of extracts till 15 days to analyze the physical, biochemical, hematological parameters, and histopathological studies in liver, kidney, and spleen for sub-acute study. The highest dose administered did not produce mortality or changes in the general behavior of the test animals. All parameters were unaltered throughout the study. The present study revealed no obvious toxicity in mice treated with *S. alata*. These results indicate the safety of the oral administration of leaf extract.

**Subjects:** Pharmaceutical Science; Pharmacy; Drug Design & Development; Natural Products

**Keywords:** toxicity; *Senna alata*; extracts; non-toxic; mice

ABOUT THE AUTHOR

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PUBLIC INTEREST STATEMENT

Helminthiasis is a neglected disease and has shown resistance to some available marketed drugs. Scientists continue the search for new anthelmintic especially from natural sources agents and plants have proved to be a potential source for this purpose. There are few species of *Senna* plant that showed to have medicinal properties, three species viz. *S. alexandrina*, *S. alata* and *S. occidentalis* leaf extracts have been reported for the first time from our laboratory to have cestocidal property. Amongst the three plants, *S. alata* leaf extracts showed to have more anthelmintic efficacy and is apparently believed to be nontoxic, but detail pre-clinical toxicological evaluation in animals have not been evaluated. Moreover, not all medicinal plants are safe for consumption in the crude form. Some level of toxicity arises from the potent toxic compounds present in it and nontoxic compounds can also behave like a toxic compound even at a lower dose, and can produce an adverse effect in human or animal cells. Thus, it is required to examine the toxicity profile of *S. alata* leaf extract, given its widespread consumption by human.
1. Introduction

According to World Health Organization (2003), more than 80% of the world’s population rely on traditional medicine for their primary health care and more than 30% of the plant species have been used for this purpose. A major population of the world are attracted to this type of traditional medicine due to scarcity and high costs of available drugs (Hudaib et al., 2008) and an easy access to these plants in some regions of the world (Humber, 2002). Besides, a large amount of evidences has shown immense potential of medicinal plants for prevention, diagnosis, and treatment of various diseases (Abera, 2014; Ghosh, Sahoo, Das, Duley, & Palhy, 2014). In India, over 3,000 plants were officially recognized for their medicinal value, but it is generally estimated that over 6,000 plants are being used in traditional, folklore, and herbal medicines. Although the scientific study of some medicinal plants clearly validates the effectiveness and reliability of ethno-medical knowledge and traditional use in managing diseases, however herbal medicines are complex mixtures of many bioactive phytochemicals which may differ in different mechanisms (Sengupta, Sharma, & Chakraborty, 2011). Some level of toxicity arises from the potent toxic compounds present in it, and nontoxic compounds can also behave like a toxic compound even at a lower dose, and can produce an adverse effect by interacting with human or animal cells. Therefore, not all medicinal plants are safe for consumption in the crude form. Thus, such plants should be investigated to better understand their properties, safety and efficiency.

*Senna alata* Linn. (Family Fabaceae) has been recognized in traditional medicine for its medicinal activities (Lim, 2013; Karthika, Manivannan, & Mohamed, 2016) and was also recently reported to have anthelmintic activity (Kundu, Roy, & Lyndem, 2012, 2014). Though this plant is apparently believed to be nontoxic, but detail pre-clinical toxicological evaluation in animals have not been evaluated. Thus, it is required to examine the toxicity profile of *S. alata* leaf extract given its widespread consumption by human.

2. Results and discussion

During the 15-day period of toxicity study, mice showed no signs of behavioral distress or change in skin color, no changes in the eyelids, sleep, food, and water intake, and no observable toxicity symptoms or death. The experimented mice survived till the completion of the experimental duration at all levels of treatment (Table 1). Thus, this indicates there was no disturbance in carbohydrate, protein, or fat metabolism (Klaassen, 2001).

Though the body weight gradually increased in control and treated groups, there was no significant difference in mean body weight amongst the different treated groups and the control (Table 2) which indicated that the extract has negligible levels of toxicity on the growth of the animals as also observed by Mir, Sexena, and Malla (2013) and Rajalakshmi, Jayachitra, Gopal, and Krithiga (2014). There were no significant changes in organ weight and relative organ weight of liver, kidney, and spleen with respect to the body weight as well (Table 3). Kluwe (1981), documented that the increase in organ weight had been observed to be a relative sensitive indicator of nephrotoxicity. Thus, *S. alata* did not induce any toxic effect on the kidneys and the other organs going by this indicator.

Hematological tests showed no significant differences in hemoglobin, RBC, WBC (total and differential), and platelet count in all doses as compared to control. In WBC differential count, lymphocytes showed slight variation in the dose of 2,000 and 3,000 mg/kg body weight compared to 1,000 mg/kg body weight and also to control, while eosinophil count showed no significant differences at all dose levels compared to control (Table 4). According to Onyeiili, Iwuoha, and Akininiyi (1998), administration of an agent can result in loss of blood cells and/or inhibition of blood cell
### Table 1. Acute toxicity study of ethanol extract of *Senna alata* in mice

| Observations            | Control 1,000 mg/kg body wt. | 2,000 mg/kg body wt. | 3,000 mg/kg body wt. |
|-------------------------|-------------------------------|----------------------|----------------------|
| Consciousness           | +                             | +                    | +                    |
| Grooming                | −                             | −                    | −                    |
| Touch response          | +                             | −                    | −                    |
| Sleeping duration       | +                             | +                    | +                    |
| Movement                | +                             | +                    | +                    |
| Gripping strength       | +                             | +                    | +                    |
| Righting reflex         | +                             | +                    | +                    |
| Food intake             | +                             | +                    | +                    |
| Water consumption       | +                             | +                    | +                    |
| Tremors                 | −                             | −                    | −                    |
| Diarrhea                | −                             | −                    | −                    |
| Hyper activity          | −                             | −                    | −                    |
| Pinna reflex            | +                             | +                    | +                    |
| Corneal reflex          | +                             | +                    | +                    |
| Salivation              | +                             | +                    | +                    |
| Skin color              | +                             | +                    | +                    |
| Lethargy                | −                             | −                    | −                    |
| Convulsion              | −                             | −                    | −                    |
| Marbidity               | −                             | −                    | −                    |
| Sound response          | +                             | +                    | +                    |

Notes: + = normal, − = absent.

### Table 2. Body weight of mice during sub-acute toxicity study after administration of *S. alata* extract

| Body weight          | Initial day | After 5 days | After 10 days | After 15 days |
|----------------------|-------------|--------------|---------------|--------------|
| Control              | 26.3 ± 0.35 | 27.45 ± 0.5  | 29.95 ± 1.51  | 34.34 ± 4.33 |
| 1,000 mg/kg          | 24.67 ± 0.3 | 26.20 ± 0.54 | 28.14 ± 0.41  | 30.87 ± 2.38 |
| 2,000 mg/kg          | 24.63 ± 0.25| 26.59 ± 0.35 | 27.51 ± 0.35  | 29.32 ± 0.66 |
| 3,000 mg/kg          | 25.02 ± 0.32| 26.94 ± 0.51 | 28.11 ± 0.5   | 30.44 ± 1.79 |

Note: All values are expressed as mean ± SD of 6 animals.

### Table 3. Absolute organ weight (g) and relative organ weight (g) during sub-acute toxicity study of *S. alata* extract

| Liver | Kidney | Spleen |
|-------|--------|--------|
| Absolute organ weight | Relative organ weight | Absolute organ weight | Relative organ weight | Absolute organ weight | Relative organ weight |
| Control | 1.58 ± 0.11\textsuperscript{a} | 4.59 ± 0.23\textsuperscript{a} | 0.25 ± 0.014\textsuperscript{a} | 0.72 ± 0.03\textsuperscript{a} | 0.23 ± 0.02\textsuperscript{a} | 0.66 ± 0.05\textsuperscript{a} |
| 1,000 mg/kg | 1.62 ± 0.02\textsuperscript{a} | 5.23 ± 0.28\textsuperscript{a} | 0.25 ± 0.02\textsuperscript{a} | 0.79 ± 0.03\textsuperscript{a} | 0.20 ± 0.03\textsuperscript{a} | 0.61 ± 0.06\textsuperscript{a} |
| 2,000 mg/kg | 1.57 ± 0.09\textsuperscript{a} | 5.33 ± 0.20\textsuperscript{a} | 0.23 ± 0.02\textsuperscript{a} | 0.79 ± 0.10\textsuperscript{a} | 0.21 ± 0.03\textsuperscript{a} | 0.72 ± 0.10\textsuperscript{a} |
| 3,000 mg/kg | 1.50 ± 0.14\textsuperscript{a} | 5.00 ± 0.54\textsuperscript{a} | 0.22 ± 0.03\textsuperscript{a} | 0.73 ± 0.05\textsuperscript{a} | 0.20 ± 0.02\textsuperscript{a} | 0.67 ± 0.08\textsuperscript{a} |

Notes: Values are expressed as mean ± SD of 6 animals. A rows means followed by a common superscript are not significantly at 5% by using DMRT.
synthesis and decrease in such hematological parameters in experimental animals has been associated with anemia. The above results suggest the nontoxicity of *S. alata* in mice. A similar observation was reported by Ping, Darah, Chen, Sreeramanan, and Sasidharan (2013), after oral administration of *Euphorbia hirta*, *Carica papaya*, *Petroselinum crispum*, and *Lygodium flexuosum*.

The serum analyses showed no significant difference in calcium and chloride level between the control and experimental group. However, there is a less significant level of difference in phosphorus at 1,000 and 2,000 mg/kg body weight compared to control group (Table 5). Similarly, levels of creatinine and uric acid were not significantly different between the control and the experimental group of mice (Table 6). This observation was also made by Ping et al. (2013). Moreover, levels of total protein, albumin, SGPT, total bilirubin, direct bilirubin, cholesterol, triglyceride, alkaline phosphatase (ALP), and aspartate transaminase (AST) also showed no significant difference, however slight variation in the latter two was observed at 1,000 mg/kg body weight of the control group (Table 7). Liver injury is characterized as hepatocellular when there is predominant elevation of the ALT, while AST is a mitochondria enzyme whose increased activity reflects severe tissue injuries (Martin, 2006). Hypo-proteinemia, a common finding in liver damage (Larrey, 2002), was also not observed in the present study. This indicates that the extract did not cause any overt liver damage at the dose levels studied. Further, there was a low level of glucose in the treated group as compared to the control group (Table 7) which may be due to inadequate insulin secretion that indicates normal functioning of the liver. Similar observations were made by Rajalakshmi et al. (2014), Nabukenya

| Hematological parameters | Group 1 control | Group 2 1,000 mg/kg body wt. | Group 3 2,000 mg/kg body wt. | Group 4 3,000 mg/kg body wt. | Unit of values |
|--------------------------|----------------|-----------------------------|-----------------------------|-----------------------------|----------------|
| Hemoglobin               | 14.03 ± 0.45^a| 13.7 ± 0.41^a               | 14 ± 0.43^a                 | 14.16 ± 0.67^a              | gm/dl          |
| RBC count                | 7.07 ± 0.81^b | 7.07 ± 0.31^b               | 7 ± 0.57^b                  | 7.1 ± 0.51^b                | Million/Cu mm  |
| Total lymphocytes        | 11.71 ± 1^c   | 11.68 ± 0.4^c               | 11.57 ± 0.23^c              | 11.53 ± 0.5^c               | 10^3/μl        |
| Neutrophils              | 25.5 ± 3.78^d | 24.83 ± 3.82^d              | 25.17 ± 3.31^d              | 25.17 ± 2.56^d              | %              |
| Lymphocytes              | 66.55 ± 2.91^e| 65.1 ± 3.89^e               | 64.79 ± 2.35^e              | 64.45 ± 3.34^e              | %              |
| Eosinophils              | 1.33 ± 0.52^f | 1.33 ± 0.52^f               | 1.33 ± 0.52^f               | 1.33 ± 0.52^f               | %              |
| Monocytes                | 0             | 0                           | 0                           | 0                           | %              |
| Basophils                | 0             | 0                           | 0                           | 0                           | %              |
| Platelet count           | 273 ± 7.35^g  | 268.17 ± 11.34^h            | 268.83 ± 6.33^g             | 271.67 ± 25.57^h            | 10^3/μl        |

Notes: Values are expressed as mean ± SD of 6 animals. A column means followed by a common superscript are not significant at 5% by using DMRT.

| Serum biochemical parameter | Group 1 control | Group 2 1,000 mg/kg body wt. | Group 3 2,000 mg/kg body wt. | Group 4 3,000 mg/kg body wt. | Unit of values |
|-----------------------------|----------------|-----------------------------|-----------------------------|-----------------------------|----------------|
| Phosphorus                  | 5.5 ± 0.5^a   | 5.1 ± 0.83^b               | 5.0 ± 0.4^a                 | 5.4 ± 0.3^a                 | mg/dl          |
| Chloride                    | 102.82 ± 3.96^c| 102.77 ± 3.36^c            | 102.4 ± 1.6^c               | 100.13 ± 1.88^c             | mmol/lit       |
| Calcium                     | 10.47 ± 0.52^a| 10 ± 0.01^c                | 10.06 ± 0.2^a               | 10.1 ± 0.5^a                | mg/dl          |

Notes: Values are expressed as mean ± SD of 6 animals. A column means followed by a common superscript are not significant at 5% by using DMRT.
et al. (2014), Priyadarshini, Mazumder, and Choudhury (2014) and Bello et al. (2016). Cholesterol and triglyceride levels have no significant difference in treated animals which concurs that this plant extract does not present any risk of hypercholesterolemia or artherosclerosis at a high level of doses (Bello et al., 2016).

Histological studies revealed no abnormalities in liver, kidney and spleen tissue in treated mice. The liver tissue displayed normal hepatocytes without any enlargement in sinusoidal vein, central vein, and portal triad in all treated groups compared to control (Figure 1). Similar type of observation was also seen by Bello et al. (2016) in rat liver. Kidney micrograph revealed normal architecture of glomerulus and Bowman's capsules with no degeneration, necrosis, or inflammation (Figure 2), which are comparable with the study made by Ping et al. (2013) and Nabukenya et al. (2014). Histological features of spleen showed normal splenocytes with prominent nucleus in both treated and control groups (Figure 3). These observations agreed with that of Ping et al. (2013) in rat model that have been treated with Euphorbia hirta. Thus, histopathological evaluation indicated that the extract did not have any adverse effect on morphology of the tissues and these observations supported the biochemical results mentioned above. Therefore, it is concluded that S. alata did not produce any toxic effect in male albino mice.
Figure 1. Histology study of liver from of mice: (a) control group; (b) 1,000 mg/kg; (c) 2,000 mg/kg and (d) 3,000 of S. alata leaf extract in a 15-day sub-acute toxicity.

Notes: No significant damage was detected in any treatment group. Indicators: Bowman’s capsule (BW), glomerulus (G), proximal collecting tubule (P), distal collecting tubule (D).

Figure 2. Histology study of kidney of mice: (a) control group; (b) 1,000 mg/kg; (c) 2,000 mg/kg and (d) 3,000 of S. alata leaf extract in a 15-day sub-acute toxicity.

Notes: No significant damage was detected in any treatment group. Indicators: Portal Triad (pt); Central Vein (CV).
3. Materials and methods

3.1. Preparation of plant extract

*S. alata* leaves were collected from in and around the University campus of Visva-Bharati, Santiniketan. Young leaves were washed with distilled water, allowed to dry in an oven at 50°C, and crushed to powder. About 250 g of the powdered form was extracted with 1 L of ethanol (90%) in a Soxhlet apparatus for 7–8 h, and the final crude extract was recovered using rotary evaporator and stored at 4°C until further use.

3.2. Experimental designs

Twenty-four Swiss albino male mice weighed 24–28 g were divided into four groups of six animals each (Group 1–4). Group 1 is control group, fed daily with only normal laboratory diet and water. Group 2–4 were treated with a dose of 1,000, 2,000, and 3,000 mg/kg body weight, respectively, for 15 days through an oral needle following a period of 10-h fasting. All animals were maintained on standard laboratory diets with water *ad libitum*. The experimental protocol and procedures used in this study were approved by the Institutional Animal Ethical Committee (IAEC), Visva-Bharati, Santiniketan.

3.3. Acute oral toxicity study

After administration of the extract, animals were monitored continuously for every two hours for a day to detect acute changes in morphological and behavioral responses, spontaneous activity, irritability, corneal reflex, tremors, convulsion, salivation, diarrhea, lethargy if any, and also monitored for any mortality during the course of toxicity study.
3.4. Sub-acute oral toxicity study

Body weight of each animal was recorded every five days interval till the last day of experiment. After the 15th day, all animals were sacrificed after light chloroform inhalation of anesthesia and different hematological and biochemical studies were performed.

3.4.1. Hematological assay

About 1.5–2 ml of blood was drawn directly with a hypodermic syringe to minimize the damage of serum contamination through a cardiac puncture (Jochems, Valk, Staflaeu, & Boumans, 2002). About 100 μl of the collected blood sample was used for the determination of hematological parameters like hemoglobin concentration, total RBC count, total WBC count, WBC differential count, and total platelet count following the methods of Smith (1995) and Kjeldsberg (1998).

3.4.2. Analysis of serum biochemical parameters

The rest of the collected blood sample was prepared for serum isolation according to the method of Singh and Rana (2007). In brief, blood was kept for 20 min at room temperature of 30°C and then centrifuged at 2,500 rpm for 5 min at 4°C. The serum obtained as supernatant, was collected in an eppendorf tube and kept at 4°C for use. Determination of glucose, calcium, phosphorus, chloride, total protein, albumin, ALP, AST, SGPT, total bilirubin, direct bilirubin, creatinine, cholesterol, triglyceride, and uric acid were analyzed by different assay kits following the manufacturer protocol.

3.4.3. Measurement of relative organ weight

Liver, kidneys, and spleen were carefully dissected out and weighed separately. The relative organ weight of each animal was then calculated as follows:

\[
\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rat on sacrifice day (g)}} \times 100
\]

3.4.4. Histological examination

Each selected organs were cut into small pieces and kept in Bouin’s fixative for 24 h, and processed for histological study following methods of Mayer (1896) with slight modification and later observed under a light microscope.

3.5. Chemicals

All the chemicals used were of analytical grade. Ethanol was supplied by Bengal Chemicals, Kolkata. Stains and fixatives were purchased from Sigma–Aldrich. All biochemical assay kits were purchased from Coral clinical system company, Goa, India, and all other reagents were obtained from Merck Life Science Pvt. Ltd., Merck India.

3.6. Statistical analysis

Data are expressed as a mean ± SD. Total variations present in a set of data were estimated by one way Analysis of Variance (ANOVA) comparisons were made between the treated groups. All data were analyzed using Duncan’s Multiple Range Test (DMRT). \( p < 0.05 \) was considered as the level statistical significance.

4. Conclusions

The absence of gross and histopathological lesions in the organs as well as no significant differences in hematological and biochemical test in the treated groups from the control could suggest the level of safety of the leaf alcoholic extract on the animals. In conclusion, to our knowledge, this is the first investigation of the various parameters of toxicity studies made on the \( S. \text{alata} \) at higher dose. This study has shown that sub-acute administration of the alcoholic leaf extract of \( S.\text{alata} \) may be safe and thereby provide a support to the use of \( S.\text{alata} \) leaves as an alternative system of medicine.
Acknowledgments
We also wish to thank the Department of Zoology, Centre for Advanced Studies, Visva-Bharati for providing infrastructural support.

Funding
The authors gratefully acknowledge the University Grants Commission (UGC), New Delhi for providing financial assistance through a major research project (No: UGC/ SR/40-385/2011) sanctioned to Lorisha M. Lyndem.

Competing Interests
The authors declare no competing interest.
