

## Phytochemical Screening and Toxicity Study of *Saraca asoca* Bark Methanolic Extract

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### ABSTRACT

The study was conducted to screen for the phytochemicals constituents of bark of *Saraca asoca* (Roxb.), one of the folk medicinal plants found in West Bengal, Bangladesh and Sri Lanka. The acute and sub-acute toxicity of methanolic extract of *S. asoca* bark on mice was studied. Phytochemical analysis of *S. asoca* (bark) methanolic extract revealed the presence of high percentage of flavonoids and reducing sugar in addition with saponins, glycosides, steroids, anthraquinones and volatile oil. In mice, daily single oral doses of *S. asoca* methanolic extract (0.3 and 1.2 g/Kg body weight) were well tolerated and no adverse behavioral effect was found as used for 30 days (LD<sub>50</sub>=6.526 g/Kg for acute administration) and induced no significant changes in body and organs weights. However, hematological parameters showed a significant decrease in platelet count (432±98.3, p<0.05) when the dose is 1.2 g/Kg body weight in relation to control animals, suggesting disturbances in platelet production whereas no effect was found on serum biochemical parameters like serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (ALP), and bilirubin, total protein, uric acid, urea, creatinin. This study indicates that *Saraca asoca* is non-toxic for both acute and sub-acute oral administrations.

**Keywords:** *Saraca asoca*, acute and sub acute toxicity, bark extract, toxicity profile.

### Introduction

Phytochemicals are secondary plant metabolites that can protect plants from predators, act on animal cells and tissues to affect DNA formation and destroy cell membranes [1]. It is also known that phytochemicals confer pharmacological relevance on plant generally. The growing interest in herbal medicine demands information on various plant preparations used in the treatment of diseases [2, 3]. Scientific evaluation of medicinal plants is important to the discovery of novel drugs and also helps to assess toxicity risks associated with the use of either herbal preparations or conventional drugs of plant origin. *Saraca asoca*

(Roxb.) De Wilde or *S. indica* Linn. (Family: Caesalpiniaceae; local names: Ashok, Anganapriya, etc.) is a medicinal plant of West Bengal, Bangladesh and Sri Lanka. It is a medium sized evergreen tree up to 9 m height with numerous branches. Bark extract is bitter in taste and has a stimulatory effect on endometrium and ovarian tissue and used in uterine fibrosis, menorrhagia, bleeding hemorrhoids and hemorrhagic dysentery and also as astringent [4]. Flowers are used in regulation of inflammation, dysentery, and hemorrhoids [5]. A preliminary study showed the presence of catechols, sterols, tannins, glycosides, leukocyanidins and leukopelargonidin in bark extract of *Saraca asoca* [6]. *Saraca asoca* is

popular in many communities as being used as herbal medicine [7]. But scientific information on its medicinal use and side effects is however lacking. The objective of this study is to determine the phytochemical constituents of *Saraca asoca* bark and to establish the acute and sub-acute toxicity levels of these chemicals in mice model.

## Materials and methods

### Animals:

Six to eight weeks old Swiss albino mice (*Mus musculus*) of body weight 15-20 gm were purchased (of the same strain) and were housed in a stainless steel wire cages (Tarsons, India), maintained on a 12 hour light-dark cycle in the animal house of Department of Zoology, University of Kalyani. Pellet diet (West Bengal Dairy and Poultry development corp. Ltd., Kalyani Industrial Area, Kalyani) and water was provided ad libitum. Water was supplied ad libitum through the tubing throughout the study. The animals were grouped as 5-6 in number per cage and provided with normal environmental conditions (temperature at  $25\pm 1^\circ\text{C}$  and humidity at  $55\pm 5\%$ ). These mice were kept for a minimum of one week prior to oral administration at the Departmental animal room to allow for their acclimatization to the laboratory conditions.

### Bark collection and extraction of phytochemicals:

*S. asoca* bark was harvested during the month of February-March. The family and species of *S. asoca* were confirmed by eminent taxonomists using standard methods. Pieces of bark were air-dried at room temperature until complete drying. They were kept away from direct sun light to avoid destruction of active compounds. The bark pieces were pounded to powder using metallic motor and pestle.

### Extraction process

Ten grams of dried crude powder of *S. asoca* bark was used for extraction by Soxhlet extractor. Plant material was defatted by petroleum ether before extraction in methanol. Methanol was evaporated using vacuum evaporator under reduced pressure from the extract. The residue was left to air dry at room temperature for 72 hour. The dry residue was stored at  $4^\circ\text{C}$  in air-tight bottle. The extraction yield was 12% (W/W).

### Qualitative tests on bark extracts of *S. asoca*

The method of Parekh et al. [8] was used to test different classes of compounds in the bark extracts. As already reported that tested compounds are protective agents of plants against predators are as follows: alkaloids, steroids, terpenoids, tannins, cardiac glycosides, flavonoids, carbohydrates and saponins. Chloroform, ethanol, methanol and water extracts of dry *S. asoca* bark powder were used to determine the qualitative and quantitative presence of these compounds. Hydrochloric acid, Dragendoff's reagent and Meyer's reagent were used to detect the presence of alkaloids, indicated by yellowish precipitate. To detect the presence of steroids and terpenoids, a mixture of acetic anhydride, chloroform in presence of concentrated sulphuric acid were used. The appearance of brown-red ring at the interface between the two liquids indicated the presence of steroid and a blue-green ring indicated the presence of terpenoids. A mixture of methanol and 0.1% ferric chloride were used to identify the presence of tannins. The appearance of a blackish blue color indicated the presence of tannins while formation of foam after shaking with water showed the presence of saponins. One milligram of dry extract was dissolved in 1 ml of methanol containing metallic magnesium at  $50^\circ\text{C}$  and concentrated hydrochloric acid was added to maintain the pH at acidic range. Formation of a tomato red color solution indicated the presence of flavonoids. Ethanolic bark extract after mixing with Molisch reagent, was added slowly with a small amount of concentrated sulphuric acid slowly, leading to the formation of purple ring at the interface. This result indicates the presence of carbohydrate in the sample. 2ml of ethanolic extract was shaken with 10 ml of benzene, filtered and added with 5 ml 10 % ammonia solution. The mixture was shaken; the presence of a pink color in the ammoniacal (lower) phase indicated the presence of anthraquinones [9]. Petroleum ether extract was added with the same amount of copper acetate solution, the mixture was shaken vigorously and allowed to the formation of a green colour solution, indicated the presence of resin [10].

### **Chemicals:**

Chemicals used in this study were purchased from MERCK, India. All the chemicals were of analytical grade.

### **Acute and sub-acute oral toxicity study:**

Toxicity study was performed according to the Organization of Economic Co-operation and Development (OECD) guideline for testing of chemicals (OECD 2001). In order to study acute toxic effect of the plant extract 7 groups of 10 animals (5 male and 5 female) were used. Group I used as control and II to VII used as treatment groups. Last six groups were received a single oral dose of 1, 2, 3, 4, 5 and 6 g/Kg body weight of the methanolic extract respectively for acute toxicity study. All the mice were fasted overnight prior to treatment and body weight of each of them were recorded. All the animals of the last six groups were administered the respective amount of methanolic extract dissolved in 1 ml of distilled water, the control group of animals was administered only distilled water through gastric tubing. On the 15<sup>th</sup> day, all mice were kept fasted for 15 h, and then sacrificed for necropsy examination. The internal organs were excised and weighed. The gross pathological changes of the tissues were studied and LD50 value was determined. The selection of doses for mice used to study sub-acute toxicity was determined from the equivalence of 15-120 times of normal human dose (10-20 mg/Kg) that also in accordance to the information collected from herbalists. The low dose was 0.3g/kg and the higher dose was selected as 1.2g/kg. The extract was administered daily for 30 days to the mice of both low dose group and higher dose group (each containing 5 male and 5 female animals) through gastric tubing and the control group containing the vehicle. On the 31<sup>st</sup> day mice were made fasted for 15 h and sacrificed. Blood samples for hematological blood analyses were taken by puncturing heart. The internal organs were weighed to determine relative organs weights, and examined for gross lesions. All tissues were fixed in Buins solution for histological examination.

### **Behavioral study:**

The animals were observed for gross behavioral changes related to CNS depression after dosing (hypo activity, passivity, relaxation and ataxia), CNS stimulation (hyperactivity, irritability, convulsion, straub tail and analgesia) and ANS stimulation (ptosis and exophthalmia) at 1, 2, 3, 4, 12, 48 hours of interval for 14 days after each treatment [11]. The change in body weight and rate of mortality were also recorded for 14 days of observation to test acute toxicity. The deviations in normal behavior, coat conditions, discharge, movement and mortality were also studied for 30 days to test sub-acute toxicity.

### **Ethical clearance:**

The protocol used in this study was approved by the animal ethical committee, Department of Zoology, University of Kalyani (under Committee for the Purpose of Control and Supervision of Experiments on Animals, India).

### **Hematological parameters:**

Collected blood was used to determine hemoglobin concentration (g %) using Sahil's hemoglobinometer and total WBC, RBC and platelets were counted using hemocytometer chamber [12].

### **Body weight and organ weights:**

The body weight of experimental animals was measured for both acute and sub-acute treatment group. Heart, lung, liver, kidney and pancreas were considered for measurement immediately after sacrifice.

### **Serum biochemical parameters:**

Blood collected from sub-acute treatment group was used for the estimation of serum biochemical parameters like: serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (ALP), serum total cholesterol, total protein, urea, uric acid and creatinine contents by using reagent kits (Span Diagnostics, Surat, India), following instruction of manufacturer.

**Histological studies:**

After sacrifice different organs like heart, lung, liver, kidney and pancreas of animals from each group were subjected for histological examinations. After fixing the tissues in Buins solution the tissues were dehydrated by passing through ethanol gradation and made ready for paraffin block preparation. The tissues were sectioned at the thickness of about 5 $\mu$ . Routine histological studies were performed by using Haemotoxylin stain.

**Data analysis:**

All values are expressed as mean $\pm$  SEM. The statistical comparisons were made using students-t test. Values were considered significant at  $p < 0.05$  level. From the graph equation of probit against log dose of the methanol extracts, the log dose that responded to probit 5 (50% deaths) was calculated and its anti-log gave the LD50 value [13].

**Results****Preliminary phytochemical analysis of *Saraca asoca* bark:**

The results of qualitative analysis of phytochemicals present in bark of *Saraca asoca*, is summarized in Table 1 that shows mild, moderate or substantial amount of presence or absence of the phytochemicals.

**Table 1: Phytochemicals present in bark of *Saraca asoca*.**

Sl. No.	Phytochemicals	Presence
1	Alkaloids	-
2	Anthraquinones	+
3	Flavonoids	+++
4	Resins	-
5	Saponins	-
6	Glycosides	++
7	Reducing sugars	+++
8	Tannins	++
9	Steroids	+
11	Terpinoids	-
12	Volatile oil	+

(-) = absent (<10mg/Kg),

(+) = mild presence (10mg/Kg to 50mg/Kg),

(++) = moderate presence (50mg/Kg to 100mg/Kg),

(+++)= substantial presence (>100mg/Kg).

**Acute oral toxicity:**

The effects of *S. asoca* methanolic extract in mice after oral administration are summarized in Table 2. There was an irregular dose-dependent increase in mortality. The first mouse died at a dose of 3 g/kg, 3 mice died at 4 g/kg, 3 died at 5 g/kg and seven died at 6 g/kg body weight within 2 days of dosing. However, the observed symptoms associated with ingestion of *S. asoca* methanolic extract included minor to more noticeable convulsions, disorientation, hypoactivity, hyperventilation and pilo-erection. The body weights were not significantly different during day to day measurement. The non-observed-adverse effect-level was 2 g/kg, the maximum tolerated dose was 5 g/kg and the calculated LD50 = 6.526 g/Kg body weight.

**Table 2: Determination of LD50 values by Miller and Tainter method [22] of the methanolic extract of *Saraca asoca* bark in mice.**

Oral dose (mg/kg)	Log dose	Dead/total	% Death	Probit
1000	0	0/10	0	0
2000	3.3	0/10	0	0.078
3000	3.47	1/10	10	0.796
4000	3.6	3/10	30	2.448
5000	3.69	3/10	30	4.463
6000	3.77	7/10	70	6.252

**Sub-acute oral toxicity:****Effect of the oral *S. asoca* methanolic extract on the general behaviour of the mice:**

No significant changes in general behaviour of mice were observed during this study. No significant changes were recorded in body weight (Table 3) and daily food intake in the treated mice as compared to the control (0 mg/Kg). Both the control and treated mice appeared consistently healthy throughout the 30-day period of study. However, mice receiving high dose (1.2 g/Kg) of extract exhibited some minor deviations (symptoms of toxicity), which included salivation, intermittent diarrhea, and aggressiveness, while no such deviation was observed in the mice treated with lower dose (0.3 g/Kg). No death also was recorded in both the control and treated groups suggesting that the LD50 for sub-acute oral dosing

with *S. asoca* methanolic extract was much higher than 1.2 g/kg.

**Table 3: Effect of *Saraca asoca* bark extract on hematological parameters and body weight after sub-acute administration. Data are presented as mean± SEM, \* (p<0.05).**

Parameters	Control	Lower dose(0.3 g/Kg body weight)	Higher dose (1.2 g/Kg body weight)
RBC (X10 <sup>12</sup> /l)	6.8±0.12	6.5±0.16	6.6±0.13
Hb(g/dl)	12.8±0.22	13.1±0.28	12.9±0.31
WBC(X10 <sup>9</sup> /l)	31±0.42	31.2±0.0.38	31.1±0.26
Platelet (X10 <sup>9</sup> /l)	760±41.2	742±46.1	432±98.3*
Body weight (g)	23±1.2	23±1.5	23±1.4

#### Effect of oral *S. asoca* methanolic extract on the hematological parameters of the mice:

The hematological parameters (i.e. RBC, haemoglobin, and WBC) showed no significant changes (P > 0.05) in the treated mice compared to the control mice. However, as observed, in the hematological analysis, a significant decrease (P < 0.05) in the platelets count (Table 3) was observed. These statistical differences (i.e. increases and decreases) appeared to be biologically relevant and consistent within the group suggesting they were treatment-related.

#### Effect of oral *S. asoca* methanolic extract on organ weights of mice:

The wet weights of mice organs of both treated and control groups are presented in Table 4. The sub-acute oral ingestion of *S. asoca* methanolic extract over 30 days caused no significant changes in the weights of the organs (i.e. heart, lung, liver, kidney and pancreas) in the treated as compared to the control mice (0 mg/Kg). The slight differences were due to normal biological growth of mice with time.

**Table 4: Wet weights of Kidney, Liver, Heart, Lung and Pancreas of sub-acutely treated mice (n=5) with *Saraca asoca* bark extract. All data are represented as mean± SEM.**

Dose group	Kidney (g)	Liver (g)	Heart(g)	Lung(g)	Pancreas(g)
Control	1.21±0.63	4.23±1.19	0.12±0.09	0.14±0.08	0.19±0.09
Lower dose(0.3g/Kg)	1.12±0.21	4.21±1.23	0.13±0.05	0.14±0.07	0.18±0.08
Higher dose(1.2g/Kg)	1.19±0.32	4.13±1.08	0.13±0.06	0.15±0.02	0.19±0.04

**Table 5: Effect of *Saraca asoca* bark extract on serum biochemical parameters after sub-acute administration. Data are represented as mean± SEM.**

Parameters	Control	Lower dose(0.3 g/Kg body weight)	Higher dose (1.2 g/Kg body weight)
SGOT (IU/dl)	41.75±1.12	42.08±1.19	41.96±1.15
SGPT (IU/ dl)	36.22±1.19	35.98±1.16	36.11±1.13
ALP (IU/dl)	80.12±1.78	78.56±1.76	79.01±1.72
Bilirubin (mg/dl)	0.83±0.13	0.81±0.11	0.82±0.12
Cholesterol (mg/dl)	152.25±9.3	153.33±8.7	153.69±8.9
Total protein (mg/dl)	6.98±2.09	6.92±2.01	7.01±2.33
Urea (mg/dl)	43.66±1.32	42.89±1.21	43.67±1.31
Uric acid (mg/dl)	5.57±1.77	5.23±1.56	5.18±1.67
Creatinine (mg/ml)	0.95±0.12	0.92±0.21	0.94±0.18



**Effect of oral *S. asoca* methanolic extract on tissue histopathology of mice:**

Histological studies of different mice organ revealed that there were no detectable alterations in treated groups with respect to the control group (data not shown).

**Effect of oral *S. asoca* methanolic extract on serum biochemical parameters of mice:**

After 30 days of treatment no significant changes were observed in serum biochemical parameter of mice in *S. asoca* treated group when compared with control group (Table 5).

**Lethal dose (LD50):**

The animals having received methanol extract did not exhibit marked behavioral changes but showed weak and less active movement followed by gradual death. The LD50, calculated from equation of probit-log dose of *S. asoca* methanolic extract was 6.526 g/Kg.

**Discussion**

There is increasing concern about the safety of use of the medicinal plants. There are general and herb specific concern regarding medicinal plants and their ability to produce toxicity and adverse effects [14]. Toxicity of medicinal plants may be related to the mixture of active compounds that they contain and stability of active ingredients in tissues. Phytotherapy having its pervasive use is substantiated by the affordability, its medicinal value and the belief of their harmlessness [15]. Phytochemical analysis is an important tool to identify and screen plants for their use [6]. The analysis of this plant part (bark), table 1, shows significant presence of flavonoids and reducing sugars in addition to tannins and glycosides. The presence of very small amount of anthraquinones, steroids and volatile oil was also observed.

Generally, the reduction in body weight and internal organ weights are simple and sensitive indices of toxicity after exposure to toxic substances. Body weight changes are indicator of adverse effects of drugs and chemicals and it will be significant if body weight loss occurred is greater than 10% of initial weight [16]. No significant difference in body weight

and organ weight of the experimental animal was found as compared to the between control group of animals (table 3&4). Slight changes in weight may be due to normal physiologic changes of animals.

The haematopoietic system is one of the most responsive targets for toxic compounds and an important manifestation of physiological and pathological status in man and animal [17]. In this study, there was a statistically significant treatment-related decrease in platelet levels of mice in the high dose group when compared to the control group (table 3). This indicates that *S. asoca* bark extract had an effect on the production of platelets which may be due its modulatory effect of flavonoids on the membrane integrity and function of platelets [18, 19, 20]. All the other hematological parameters did not differ significantly when compared with the control group (table 3), discarding the possibility of anaemia or disturbance in erythrocyte or hemoglobin production.

It is well known that almost all drugs, chemicals, xenobiotics are eliminated through renal excretion hence it is necessary to estimate the effects of methanolic extract on kidney functions. Serum biochemical parameters related to kidney function viz. urea, uric acid creatinine and total protein had no significant changes in experimental groups with respect to control group animals. Other biochemical parameters related to hepatic function like SGOT, SGPT, ALP, billirubin, cholesterol showed no significant difference between control and sub-acutely treated animal group (table 5). Therefore it can be said that the bark extract had no deleterious effect on hepatic and renal function.

The results of the present study indicated that *S. asoca* methanolic bark extract was tolerated in mice up to the oral dose of 5 g/Kg. Mortality and other symptoms associated with the single oral administration of *S. asoca* methanolic extract occurred in mice receiving doses beyond 5 g/Kg (LD50 = 6.526 g/Kg) (table 2). According to Loomis and Hayes classification [21], viz. chemical substance with a LD50 within the range of 5–15 g/kg is considered as basically non-toxic. The calculated *S. asoca* LD50 being found in this range,

suggests that the plant should be considered as practically non-toxic in acute ingestion.

## Conclusion

*Saraca asoca* is highly accepted as universal solution in the ayurvedic medicine. This all-around plant is the source of various types of compounds. *Saraca asoca* is age old and consistent source of medicine. It is used in many pharmacological activities like anti cancer, anti menorrhagic, anti oxytoxic, antimicrobial activity and have extend uses in ayurveda, unani and homeopathy [7]. In conclusion, this study provides important data on the acute and sub-acute oral toxicity profile of *S. asoca*. As the molecular mechanism underlying the links between dietary intake of flavonoids and its structure-function relationship is lacking, it is difficult to conclude about the effect of *S. asoca* bark extract on platelet count. This result will be beneficial for any future studies. The *S. asoca* methanolic bark extract is non-toxic in both acute and sub-acute oral administrations for male and female mice. Determination of the effect of this plant extract on pregnant animals is needed to complete the knowledge of safety profile study of this drug.

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