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Preclinical Safety Evaluation of Aqueous Bark Extract of *Saraca Asoca Roxb*: Acute and Subacute Oral Toxicity

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Abstract

The present study was aimed to investigate the potential toxicity of the aqueous extract of the stem bark of *Saraca asoca* (SA) in Wistar rats following the OECD guidelines. In acute oral toxicity, female rats received a single dose of 2000 mg/kg of SA and were observed for 14 days. In sub-acute toxicity, doses of 250, 500, 1000 mg/kg/day of SA were administered to rats (males and females) for 28 days. No mortality rate was found in acute toxicity testing but some behavioural changes were recorded in animals. In sub-acute oral toxicity no significant changes in body weight, body temperature, food and water consumption and urine parameters were observed. However, significant alterations in haematological and serum biochemical parameters were noticed in animals receiving higher doses. A significant variation in relative organ weight of liver, spleen and kidney was observed in all test groups of 28 day and 90 day repeated dose oral toxicity testing. Such variation was dose dependent and hence, the present toxicity study indicates that orally administered aqueous bark extract of *Saraca asoca* may have some toxic effects.
Keywords: *Saraca asoca*, acute toxicity, subacute oral toxicity, subchronic oral toxicity

1. Introduction

Toxicological study includes tests such as acute, subacute and subchronic toxicity and special toxicities that are impossible to detect clinically such as carcinogenicity, genotoxicity and reproductive toxicity. These tests help in the identification of possible target organs involved and the toxic symptoms. Studies of special toxicology such as carcinogenesis are very important if the plants contain compounds with known mutagenic or carcinogenic activities [1]. The primary goals of the preclinical safety evaluation are to identify a starting dose in human volunteers or patients, a dose escalation scheme, relevant clinical parameters be monitored. Furthermore, the animal studies give us the understanding of molecular pharmacological action and possible toxic effects. Toxicological data from laboratory studies helps in fixing the safety margin for therapeutic use of compound. This information serves as an important part of the basis for health & regulatory decision related to toxic substances [2].

Saraca asoca (Roxb.) belonging to family Caesalpinaceae, is widely distributed in evergreen forests of India up especially in Himalaya, Kerala, Bengal and whole south region. Bark, Flower and Seeds are used traditionally for the treatment of diseases and aliments such as skin diseases, haemorrhoids, wounds, diarrhoea, worms, ulcers and bone fractures. It is also used in the treatment of uterine disorders which includes menstrual disorders and menorrhagia [3]. It is a major herb for the uterus, uterine/ ovarian fibroids, menorrhagia, bleeding haemorrhoids, bleeding dysentery. The bark is bitter, astringent, sweet, refrigerant, anthelmintic, styptic, stomachic, constipating, febrifuge and demulsant. In India, Sri Lanka and Pakistan it has been used in the treatment of internal bleeding haemorrhoids and haemorrhagic dysentery. It has proven for uterine sedative and uterine affections activities as well as used in several preparations associated with menstrual cycle in females [4]. The plant *Saraca asoca* has been used in various traditional medicine systems and has been reported to possess many pharmacological activities like spasmogenic, oxytocic, uterotonic, anti-bacterial, anti-implantation, anti-tumour, anti-progestational, antiestrogenic activity against menorrhagia and anti-cancer [5].

As *Saraca asoca* plant has many therapeutic uses and various reported pharmacological activities, so it becomes essential to focus on its safety and efficacy with the aid of modern science. However, the general toxicity study of *Saraca asoca* has not been intensively studied. The present study was designed to investigate the safety of the aqueous extract of bark of *Saraca asoca* by determining its behavioural and pharmaco-toxicological effects after acute and 28 days repeated dose toxicity study.

2. Material and Methods

2.1 Plant extract

Saraca asoca bark extract (SA) was purchased from Amsar Pvt. Ltd. Indore as dry aqueous extract (Batch No. 9172, No. F/D- 645).

2.2 Preliminary Phytochemical screening

After obtaining of dry extract, qualitative preliminary phytochemical screening was performed to find out the presence of various phytochemicals such as steroids, saponins, alkaloids, flavonoids, tannins, phenolic compounds, and glycosides [6].

2.3 Animals

Eight week old male and female wistar rats weighing between 150-200 g were purchased from National Toxicology Centre, Pune, India. Females were nulliparous and non-pregnant. Animals were randomly assigned to control and test groups (n=5/sex/group). The animals were housed under standard environment conditions (12 h light/ 12 h dark cycle), at a temperature 22ºC (±3ºC) and 30-70% humidity. Animals had free access to standard pellet diet (Amrut laboratory animal feed, Sangli-Maharashtra) and water *ad libitum*. Laboratory animal handling and experimental procedures were performed according to the guidelines of CPCSEA and experimental protocol was approved by the Institutional Animal Ethics Committee with approval no DYPIPSR/IAEC/10-11/P-05. Ethical guidelines were strictly followed during all the experiments.

2.4 Acute Oral Toxicity (14 days)

The acute oral toxicity of *Saraca asoca* was evaluated in rats by using the Organization of Economic Co-operation and Development (OECD) guidelines for testing of chemicals. Healthy adult female Wistar rats (n=3) were selected for this study and body weights were measured before dosing. Animals were received single high dose of SA Extract 2000 mg/kg orally. The animals were observed individually once during first 30 minutes after oral administration and periodically during the first 24 hours, with special attention during first 4 hours and thereafter daily for 14 days for observations like sign of toxicity or mortality, body weight and food- water consumption. Animals were observed for behavioural, neurological and autonomic profiles such as changes in skin, fur, eyes, mucus membrane, tremor, convulsions, salivation, diarrhoea, lethargy, sleepiness, piloerection, lacrimation, excessive grooming, changes in posture continuously for the first 4 h after dosing and occasional there after a period of 24 and 72 h for any mortality/death. Finally, animals were kept under observations maintained for further 13 days with sign of toxicity or mortality, body weight and food- water consumption. On the 15th day, all fasting rats were sacrificed for necropsy examination. The internal organs were excised and weighed [7].

2.5 Sub-acute oral toxicity test (28 days)

2.5.1 Experimental Setup

Repeated dose oral toxicity study was performed as per Organization for Economic Co-operation and Development. Wistar rats weighing 200-250 g of either sex were divided into four groups (n=10, 5 male and 5 females). Animals from Group I served as control and received distilled water 0.1 ml/kg orally. Animals from Group II, III & IV served as test and received a SA Extract at 250, 500 & 1000 mg/kg p.o., respectively for 28 days in subacute oral toxicity study [8-10].

2.5.2 General clinical observations:

Toxic manifestations like abnormal motor activity, writhing, sedation, diarrhoea, piloerection, exophthalmos spasm, and tremors were observed at least once a day throughout the 28 days of dosing. All animals were observed for morbidity and mortality for a period of 28 days.

2.5.3 Haematological Parameters:

At the end of 29th day animals were fasted overnight and blood was collected by cardiac puncture for haematological study. Haematological parameters like Hemoglobin concentration (Hb), Red blood cell count (RBC), White blood cell count (WBC), Differential leukocyte count (DLC), Leukocytes, Neutrophils, Eosinophils, Monocytes and platelet count were performed.

2.5.4 Biochemical Parameters

At the end of 29th day animals were fasted overnight and blood was collected by cardiac puncture and the serum was separated from blood by centrifugation for assessment of biochemical parameters. Biochemical parameters like Total protein, Total Bilirubin, SGOT, SGPT, Creatinine, Blood Urea Content, Total cholesterol and Blood Glucose were done using auto analyser.

2.5.5 Relative Organ Weight:

At the end of 29th day animals were sacrificed and dissected. The organs such as liver, lungs, heart, spleen, brain, kidneys, testes and ovaries were removed and weighed. The relative organ weights were then calculated.

2.5.6 Statistical Analysis

The comparison will be made against the control group and the data was expressed as mean ± SEM. The data was analysed by one way ANOVA followed by Dunnett's multiple tests and unpaired student's-t test.

3. Results

Animals receiving single oral dose of SA Extract (2000mg/kg) showed no changes in skin, fur, eyes, mucous membrane, respiratory, circulatory and autonomic nervous system. Also other toxic signs such as tremors, convulsions, salivation, diarrhea, piloerection, lacrimation, grooming and coma were not seen in both groups. Whereas symptoms like lethargy and sleep were seen in SA Extract treated group and control group animals.

No significant changes were observed in the relative organ weights of vital organs such as liver, lung, heart, spleen, kidney, brain and uterus with ovary as compared with control group.

Table 1: Effect of SA Extract treatment on body weight, body temperature and food- water consumption in acute toxicity study in rats

Treatment	Day	Body temperature (ºC)	Body weight (gm)	Food intake (gm)	Water intake (ml)
Control	0^{th}	38.08±0.094	161.66±7.27	16.7	36.7
	7^{th}	37.76±0.58	180±5.77	13.4	35
	14^{th}	37.84±0.023	185±2.89	14	40
SA Extract (2000 mg/kg)	0^{th}	37.67±0.30	168.33±4.41	16.6	34
	7^{th}	38.62±0.20	180±5.77	13.3	31.2
	14^{th}	38.03±0.14	181.67±4.41	14	33

Data are expressed as mean± S.E.M., n = 5. No statistical difference between control and test group ($p > 0.05$), analyzed by using student t-test.

Table 2: Effect of SA Extract treatment on relative organ weight in acute toxicity study in female rats

Relative Organ Weight (gm)	Control	SA Extract (2000mg/kg)
Liver	4.79±0.052	4.37±0.212
Lungs	0.77±0.049	0.71±0.049
Kidney	0.38±0.021	0.41±0.018
Spleen	0.56±0.017	0.55±0.008
Heart	0.43±0.028	0.40±0.035
Brain	0.86±0.05	0.78±0.02
Uterus+Ovary	0.57±0.010	0.55±0.0088

The data are expressed as mean± S.E.M., n = 5. No statistical difference between control and test group, analyze by using student t-test.

Table 3: Effect of SA Extract treatment on hematological parameters in subacute oral toxicity study in male rats

Parameters	Control	250mg/kg	500mg/kg	1000mg/kg
Haemoglobin g/dl)	10.1± 0.184	8.4± 0.0858**	7.4±2.1053**	6.73±0.087**
RBC ($\times 10^6$ mm $^{-3}$)	6.034±0.14	5.386±0.168	5.24±0.230*	4.686±0.189**
Platelet($\times 10^3$mm^{-3})	345200±22637	272650±5612.5**	227500±12722**	187000±4242.6**
WBC($\times 10^6$ mm $^{-3}$)	3.51±0.37	4.31±0.37*	5.75±0.49**	8.23±0.32**
Lymphocytes (%)	49.9± 4.78	64.5±0.93**	75.64±4.42**	78.26± 1.87**
Neutrophils (%)	34.8±5.42	22.8±0.86**	12.8±0.332**	9.1±1.46**
Eosinophils (%)	7.26±0.35	4.6±0.50**	1.8±0.22**	1.5±0.37**
Monocytes (%)	2.8± 0.58	2.2± 0.40*	1.0± .00**	1.0± .00**

The data are expressed as Mean ± SEM (n=5). The data was analysed using One-way Analysis of Variance (ANOVA) followed by *Dunnett's- test*. No statistical difference between control and test groups, statistically significant at*($p < 0.05$), ** ($p<0.01$).

Table 4: Effect of SA Extract treatment on hematological parameters in subacute oral toxicity study in female rats

Parameters	Control	250mg/kg	500mg/kg	1000mg/kg
Haemoglobin(g/dl)	10.29±0.11	8.48±0.08**	7.068±0.16**	6.7±0.24**
RBC ($\times 10^6$ mm $^{-3}$)	4.854±0.007	4.126±0.32	3.628±0.42*	2.936±0.024**
Platelet($\times 10^3$mm^{-3})	361200±6922	271050±1237**	229750±3891**	181490±2148**
WBC($\times 10^6$ mm $^{-3}$)	3.23±0.24	3.38.4±0.13*	3.64.6±0.37**	6.33±0.37**
Lymphocytes (%)	49.9±4.78	64.5±1.871**	75.64±0.93**	78.26±4.42**
Neutrophils (%)	42.6±4.523	29.2±1.772**	20.6±3.03**	13.8±1.53**
Eosinophils (%)	6.64±0.18	3.36±0.51**	2.6±0.40**	1.6±0.60**
Monocytes (%)	4±0.18	3.66±0.44	1.8±0.37**	1.6±0.4**

The data are expressed as Mean ± SEM (n=5). The data was analysed using One-way Analysis of Variance (ANOVA) followed by *Dunnett's- test*. No statistical difference between control and test groups ($p > 0.05$), statistically significant at *($p < 0.05$), ** ($p<0.01$)

Table 5: Effect of SA Extract treatment on biochemical parameters in subacute oral toxicity study in male rats

Parameters	Control	250mg/kg	500mg/kg	1000mg/kg
Total protein (gm/dl)	7.39± 0.28	7.57±0.16	7.89±0.54	7.61±0.21
Total Bilirubin (mg/dl)	0.764±0.009	0.904±0.0375*	0.95±0.018**	1.13±0.064**
SGPT (IU/L)	103.7±3.367	95.46±2.43	112.4± 1.86	81.5± 1.871**
SGOT (IU/L)	108.8±7.658	105.2±1.497	100.5±0.224	91.5±9.35
Creatinine (mg/dl)	1.56±0.075	1.46±0.075	0.78±0.080**	0.78±0.08**
BUL (mg/dl)	37.6±0.748	43.36±3.56	27.5±2.12**	19.6±0.51**
Cholesterol (mg/dl)	120.7±2.62	118.1±4.12	118.3±5.52	117.86±0.37*
Blood Glucose (mg/dl)	110.7±2.44	92.2±2.81**	66±5.81**	45.2±2.72**

The data are expressed as Mean ± SEM (n=5). The data was analyzed using One-way Analysis of Variance (ANOVA) followed by *Dunnett's- test*. No statistical difference between control and test groups ($p > 0.05$), statistically significant at *($p < 0.05$), ** ($p<0.01$).

Table 6: Effect of SA Extract treatment on biochemical parameters in subacute oral toxicity study in female rats

Parameters	Control	250mg/kg	500mg/kg	1000mg/kg
Total protein (gm/dl)	6.3±0.3742	6.906±0.108	7.5±0.114	7.084±0.5705
Total Bilirubin (mg/dl)	0.824±0.03	0.816±0.034	1.09±0.002**	1.27±0.11**
SGPT(IU/L)	96.6±4.49	96.56±5.03	106.7±5.073	74.4±3.265**
SGOT(IU/L)	95.9±1.122	93±3.742	96.8±2.245	88.96±0.563
Creatinine(mg/dl)	1.366±0.018	1.36±0.080	0.9±0.0374**	0.9 ±0.051**
BUL (mg/dl)	38.56±1.311	37.14±5.130	25.1±0.900**	25.2±1.319**
Cholesterol (mg/dl)	114.3±1.6	101.6±2.62	106.26±4.12	99.1±4.68*
Blood Glucose (mg/dl)	107±5.407	88.0±4.30**	61.0±1.497**	47.0±0.400**

The data are expressed as Mean ± SEM (n=5). The data was analyzed using One-way Analysis of Variance (ANOVA) followed by *Dunnett's- test*. No statistical difference between control and test groups ($p > 0.05$), statistically significant at *($p < 0.05$), ** ($p<0.01$).

Table 7: Effect of SA Extract treatment on relative organ weights in subacute oral toxicity study in male rats

Organ weight (gm)	Control	250mg/kg	500mg/kg	1000mg/kg
Heart	0.658±0.015	0.664±0.012	0.64±0.007	0.646±0.015
Brain	1.182±0.005	1.157±0.018	1.154±0.019	1.168±0.012
Kidney	0.712±0.018	0.648±0.003	0.648±0.003	0.618±0.013
Liver	6.324±0.118	5.96±0.064	6.29±0.022	5.76±0.050
Lungs	1.416±0.036	1.392±0.016	1.356±0.016	1.326±0.009
Spleen	0.65±0.007	0.58±0.005	0.642±0.003	0.633±0.005
Testis	0.744±0.012	0.764±0.011	0.728±0.013	0.758±0.014

The data are expressed as Mean ± SEM (n=5). The data was analysed using One-way Analysis of Variance (ANOVA) followed by *Dunnett's- test*. No statistical difference between control and test groups ($p > 0.05$).

Table 8: Effect of SA Extract treatment on relative organ weights in subacute oral toxicity study in female rats

Organ weight (gm)	Control	250mg/kg	500mg/kg	1000mg/kg
Heart	0.4314±0.01	0.4714±0.019	0.468±0.003	0.455±0.001
Brain	1.24±0.02915	1.364±0.1353	1.416±0.03187	1.12±0.04572
Kidney	0.628±0.011	0.572±0.009	0.656±0.010	0.632±0.005
Liver	5.584±0.127	5.962±0.033	4.96±0.035	5.226±0.021
Lungs	1.277 ±0.022	1.26±0.006	1.232±0.002	1.232±0.017
Spleen	0.774±0.012	0.712±0.006	0.782±0.005	0.692±0.003
Ovary	0.098±0.055	0.095±0.0012	0.0788±0.017	0.077±0.016

The data are expressed as Mean ± SEM (n=5). The data was analysed using One-way Analysis of Variance (ANOVA) followed by *Dunnett's- test*. No statistical difference between control and test groups ($p > 0.05$).

4. Discussion

Various phytochemical tests were performed for presence of phytoconstituent present in *S.asoca*. Tests for Steroids, triterpenoids, glycosides, saponins, carbohydrates, alkaloids, flavonoids, tannins and Proteins found to be positive which indicate its presence in *S.asoca*. Pharmacological action of *S.asoca* is attributed to this phytochemical constituent.

In the acute toxicity study, rats fed with the SA Extract at the dose of 2000 mg/kg did not show any signs of toxicity such as tremors, convulsions, salivation, diarrhea, piloerection, lacrimation, and mortality, whereas lethargy and sleep were observed in test animal compared to control. Research showed that plant containing flavonoids, saponin, and tannin are useful in many CNS disorders same reflected in SA Extract. This may be the probable reason for the sleep and lethargy in animals [11]. No significant changes were seen in their food–water intake and utilization of food indicating normal metabolism in the animals. The reduction in body weight gain and internal organ weights is a simple and sensitive

index of toxicity after exposure to toxic substance [12,13]. There were no significant differences in body weight gain or internal organ weight such as liver, lung, heart, spleen, kidney and brain when compared with the control group. Moreover, gross morphological examinations of the internal organs revealed no abnormality.

According to the OECD guideline for testing of chemicals, the results of acute toxicity test in this study indicate that the SA Extract is fairly non-toxic after an acute exposure to the dose of 2000mg/kg. From these findings the doses 250 mg/kg, 500 mg/kg and 1000 mg/kg were selected for the further studies.

Subacute oral toxicity has been advocated as a fundamental test for assessing safety and has been applied previously in many safety assessment studies [14]. In subacute toxicity study, it appeared that the SA Extract at the doses (250 mg/kg, 500 mg/kg and 1000 mg/kg) used did not produce any marked changes in both male and female rats, as evidenced by the absence of toxic symptoms and no mortality indicating that the selected doses of SA Extract do not affect general behavior and physiology of animals.

No gross pathological alteration was found in the internal organs during long term toxicity study. The macroscopic analysis of the target organs (liver, lung, heart, spleen, brain, kidney, testis and ovary) of the animals from test group did not show any significant changes in color, texture, organ swelling and atrophy/hypertrophy as compared with that of control group. In subacute toxicity experiments, the calculation of organ weight to whole body ratio serves as useful index of toxicity [16]. The changes in the relative organ weight ratios relate to the organs getting affected due to accumulation of a toxicant or also as the organs wasted due to tissue necrosis [17]. However in the present study; no significant change was observed in relative organ weight of control and SA Extract treated animals. The functional studies in toxicology should be coupled with the appropriate histological studies, because morphological studies are useful especially during the anatomical localization of action of toxin [15]. Overall, morphological findings revealed that SA Extract was non-toxic to heart, brain, lungs, testis and ovary but it has some toxic actions on liver, spleen and kidney which are major organs of metabolism in body.

The blood profile usually furnishes vital information on the response of the body to impairment, distress and/or stress. Certain medicinal herbal preparations or convectional drugs or chemicals adversely affect various blood components [18]. Decrease or increase in cell counts and depletion of plasma constituents or their elevation beyond reference range could equally demonstrate haematoxicity [19].

The hematological tests from 28 days toxicity study revealed a significant decrease in RBC count at 500mg/kg (26.7%) and 1000 mg/kg (33.36%) whereas at 250mg/kg (16%), which is reflected by fall in level of hemoglobin in animals from SA Extract treated group when compared to the animals from control group. Neutrophils, eosinophils, and monocytes count was reduced in animals receiving SA Extract at 500 mg/kg and 1000 mg/kg dose but at the same time percentage of lymphocyte was increased as result of elevated WBC count. Neutrophils represent an important part of total leucocyte cells. They are known to interfere with microorganisms and other foreign compounds and to destroy them and so they are responsible for innate immune response [20]. The platelet count is found to be significantly dropped in subacute and subchronic study which may lead to hematological disorders. This suggests that the SA Extract may have effect on the production of platelets or may lead to induced thrombocytopenia (reduction in number of platelets in the blood) [21]. Thus the above hematological estimation results indicate that the aqueous extract of *S.asoca* may produce some toxic effects at higher doses to the circulating red cells and platelets, interfered with their production.

All treatment animals from subacute (28 days) toxicity study shown significant change in Blood glucose level at all doses; 250 mg/kg (18.29%) 500mg/kg (43.36%), 1000mg/kg (56.3%). Decrease in blood glucose indicates the hypoglycemic effect of SA Extract on animals. Cholesterol level was reduced in the 1000 mg/kg dose level in subacute toxicity. This effect is supported by the hypolipidemic effect of SA extract [22].

SGOT level in blood is not showing any significant changes and SGPT level is decreased in animals receiving 1000mg/kg dose in subacute toxicity study when it is compared to normal. The transaminases (SGOT and SGPT) are popular enzymes used as biomarkers for anticipation of possible toxicity [23, 24]. Elevations in SGPT are usually associated with cell necrosis in many tissues. For example, pathology involving the skeletal or cardiac muscles or hepatic parenchyma induces leakage of large quantities of this enzyme into circulation [26]. Since it is one of the specific assayable liver enzymes, its elevation is associated with liver damage. It is well known that liver metabolizes a wide range of both exogenous and endogenous compounds and act as a pointer of detoxification process in the organisms. Total bilirubin content showed rise at 500 and 1000 mg/kg in subacute toxicity study, which signifies impairment in liver function; also it indicates the hemolytic action. Bilirubin production elevates in inefficient erythropoiesis, hemolysis, reabsorption of a hematoma, and rarely in muscle injury. Therefore, SA Extract may produce some hepatocellular changes as there are altered levels of SGPT, SGOT and bilirubin.

Renal function was evaluated by measuring serum urea and creatinine levels. High blood levels of urea and creatinine and other metabolites are due to impaired excretion exercised by renal diseases [25] reported that renal damage

was observed only when creatinine and blood urea are increased concomitantly. Creatinine shows decreased in level after 28 days administration at 500 and 1000 mg/kg doses. Blood urea level was decreased after 28 days administration at 500 and 1000 mg/kg doses however Total protein content remains unchanged.

In the present toxicity studies of orally administered aqueous bark extract of Saraca asoca at the doses of 250, 500 and 1000 mg/kg revealed that, there was relevance of abnormal signs and significant changes in physical, hematological and biochemical parameters as compared with the control groups. Hence from above findings it is found that SA Extract may have some toxic effects on hematopoietic and metabolic system in Wistar rats during subacute oral toxicity studies.

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