

Original Research Article

Acute and Subchronic Oral Toxicity Assessment of the Ethanolic Extract of the root of *Oncoba spinosa* (Flacourtiaceae) in Rodents

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Abstract

Purpose: To investigate the toxicological profile of the ethanol extract of *Oncoba spinosa* (EEOS) after acute and sub-chronic administration to rodents.

Methods: In the acute toxicity study, a single administration of the extract at doses of 2000 and 5000 mg/kg, respectively, was given to the mice. Mice were observed for general behavioural changes, adverse effects and mortality up to 14 days post-treatment. In sub-acute toxicity studies, EEOS was given orally to male and female rats at doses of 250 mg/kg, 500 mg/kg and 1000 mg/kg for 28 days. Body weight, food intake and water intake were monitored throughout the experimental period; hematological parameters, biochemical parameters of the blood and histopathology of various organs were also evaluated at the end of the experiment.

Results: In the acute toxicity study, both the doses used did not cause any mortality or significant behavioural changes. Daily oral administration of EEOS for 28 days, in the sub-chronic toxicity study, did not show any treatment-related abnormalities with regard to hematological and biochemical parameters. There were no significant differences in body weight and organ weight between the control and treated groups ($p < 0.05$). Histological analysis did not show any morphological changes in the major vital organs (liver, kidney, stomach, spleen, brain and heart) tested.

Conclusion: These results demonstrate that the extract may not have any single dose toxicity. The LD50 value is greater than 5000 mg/kg. The no-observed adverse effects level (NOAEL) was considered to be 1000 mg/kg for male and female rats, respectively.

Keywords: *Oncoba spinosa*, Acute and sub-acute toxicity, Hematological, Biochemical parameters

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INTRODUCTION

Medicinal plants constitute an important part of the health care system for the prevention and treatment of various disorders, since the ancient civilization. Previous studies have shown that 30 % of the marketed drugs contain active principles that were isolated for the first time from plants [1]. Herbal preparations prepared from medicinal

plants can bring about a solution to certain disorders, mostly in developing countries where the commercial drugs are expensive and not easily available to the poor people [2]. In fact, there is lack of evidence on quality, safety and efficacy of many herbal preparations. Although many herbal preparations are non-toxic, many plants currently used for medicines have been shown to be highly toxic when given either

acutely or sub-chronically [3,4]. The increasing number of plant based medication users around the globe and lack of experimental reports on their safety make it basic to direct toxicological investigation on natural herbal products [5]. In this respect, an earlier study revealed severe hepatotoxicity with the utilization of some herbal plants [6].

The concern for herbal toxicity has raised anxiety among different national and international regulatory authorities to figure and execute different guidelines for surveying, monitoring and preventing toxicity emerging due to herbal products. For example, Uppsala Monitoring Centre in WHO collates and distributes data with respect to unfavourable responses of herbal products whereas Organization for Economic Cooperation and Development (OECD) sets various guidelines and procedures for conducting toxicity studies. Many herbal products in the market are frequently identified with toxic effects.

Oncoba spinosa, the snuff-box tree, fried egg tree or fried-egg flower, is a spiny shrub that belongs to family of Flacourtiaceae which has about 95 genera and 800-1000 species. The leaves are dark, glossy green in colour and somewhat leathery and hairless. The fruits are grown up to 60 mm in diameter and consists of hard shell that becomes dark-reddish brown when mature with shiny seeds embedded in a dry yellowish pulp [7]. To the best of our knowledge, no reports were available on the safety profile of this plant despite its traditional use in the treatment of diabetes, tumour, dysentery and bladder complaints [8].

As a part of safety evaluation, acute and sub acute oral dose toxicity studies were carried out to investigate the potential toxicity after single oral dosing of extract in mice and 28-day repeated oral dosing of extract in rats.

EXPERIMENTAL

Collection and identification of plant materials

Fresh roots of *Oncoba spinosa* were collected from Tirupathi, Andhrapradesh. The plant was identified and authenticated taxonomically by Dr K Madhava Chetty of the Department of Botany, S.V. University, Tirupathi, AndhraPradesh, India. A voucher specimen of the collected sample was deposited in the herbarium of the institution for future reference.

Preparation of the extract

The roots were shade dried and made into coarse powder and extracted with 70 % ethanol by cold maceration method for 72 h with intermittent shaking. The extract was filtered and concentrated at high vacuum and then stored in the refrigerator till used.

Animals

Swiss albino mice (25-30 g) were selected for acute toxicity studies and Wistar albino rats of both sexes and weighing 130 - 200 g were selected for sub-acute toxicity studies. The animals had free access to food and water and were maintained under standard laboratory conditions which included 12-hour light-dark cycle and temperature of 28-30 °C. Animals were allowed a one-week acclimatization period prior to the study. The experimental protocol was approved by the Animal Ethical Committee of the Jawaharlal Nehru Technological University, Hyderabad, Telangana.

Acute toxicity studies

Acute toxicity studies of ethanolic extract of *Oncoba spinosa* (EEOS) was carried out in female mice by using Organisation for Economic Co-operation and Development (OECD) guideline 425 [9]. Before oral administration of a single dose of the test samples, the mice were deprived of food for 3 h. Doses of 2000 and 5000 mg/kg of the test samples were given using oral gavage to mice of Group I and Group II respectively. All the mice were observed for general behavioral changes; symptoms of toxicity and mortality after treatment for the first four (critical) hours, then over a period of 24 h, thereafter daily for 14 days.

Sub-acute toxicity studies

Sub-acute toxicity study (28-day repeated oral toxicity study) was carried out according to OECD 407 guidelines [10]. Both sexes of rats (130-200 g) were divided into four groups with 10 animals (5 males plus 5 females in each). The group I received 1 % carboxymethylcellulose (CMC) vehicle orally at a dose of 10 ml/kg body weight and served as a control group whereas the rats in groups II, III and IV were treated with EEOS at the doses of 250 mg/kg, 500 mg/kg and 1000 mg/kg body weight continuously up to 28 days by suspending in 1 % CMC. All the groups of rats were observed twice daily for clinical signs and the time of onset, duration of these symptoms, mortality and morbidity till the completion of the experiment. Body weights of

the rats in all groups were recorded once before the start of dosing, once weekly during the treatment period and finally on the day of sacrifice. The quantities of food and water intake were recorded daily and the data were expressed as 7 days cumulative value. At the end of the experiment (on 29th day), blood samples were collected from the rats after overnight fast (but with drinking water allowed) by retro-orbital bleeding into heparinized and non-heparinized tubes for hematological analysis and biochemical analysis, respectively

Hematological parameters

The heparinised blood was used for the analysis of hematological parameters such as hemoglobin, red blood cell count, white blood cell count, platelet count using fully automated hematology analyser (PE 6000).

Biochemical parameters

The serum was separated from non-heparinized blood and the serum biochemical parameters including total cholesterol, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), blood urea nitrogen (BUN), triglycerides, total cholesterol, albumin, bilirubin and total protein were analysed by using semi-automatic biochemical analyser (Star 21plus, India).

Histopathology

After blood collection on day 29, all the animals were euthanized for gross pathological examinations of all major internal organs. Organs such as liver, kidney, stomach, brain, heart and spleen were collected from all the animals for histopathology. The collected organs were weighed and preserved in 10 % neutral buffered formalin, trimmed and a 5 μ thickness of tissue sections was stained with hematoxylin and eosin for histopathological study.

Statistical analysis

Results are expressed as mean \pm SEM. One way ANOVA followed by Dunnett's test was used for data comparison and any $p < 0.05$ was considered as statistically significant.

RESULTS

Acute toxicity studies

Oral administration of the EEOS at 2000 mg/kg and 5000 mg/kg did not produce any clinical signs of toxicity or deaths of the mice. As there

were no mortality and clinical signs of toxicity in both the tested doses, LD₅₀ value of EEOS was found to be greater than 5000 mg/kg.

Sub acute toxicity studies

There were no treatment related toxicity signs and mortality observed in both sexes of rats treated at 250 mg/kg, 500 mg/kg and 1000 mg/kg orally during the 4 weeks of treatment. No significant differences in body weight were observed between the initial and final body weight of the rats treated with EEOS and control rats (Figure 1). A similar absence of toxic effect was observed in the case of food consumption (Table 1). There were also no significant differences between control and EEOS treated groups in organ weight (Table 2). The hematological profile of treated and control group are summarized in Table 3. All hematological parameters such as total red blood cell count, total white blood cell count, platelet count, hemoglobin, hematocrit and differential leukocyte count were within normal range in both control and treated groups during the experimental period. Sub-acute administration of EEOS did not show any significant changes in biochemical parameters such as creatinine, urea, triglycerides, total cholesterol, total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total bilirubin when compared to control groups (Table 4). There were no statistically significant differences in the hematological parameters and biochemical parameters measured between control and EEOS treated groups. Histopathological examinations of the brain, heart, liver, kidney, stomach and spleen of the control group mice and the mice that received high dose of EEOS revealed no abnormalities. The No-Observed Adverse Effect level (NOAEL) of the extract was estimated to be greater than 1000 mg/kg/day in rats.

DISCUSSION

Toxicological evaluation has revealed that in both acute and sub-acute toxicity test, EEOS did not produce any toxic effect in mice. In the acute toxicity study, no morbidity or mortality were observed in any mice, which survive throughout the 14 days of observation. LD₅₀ value of the extract was found to be greater than 5000 mg/kg. This finding, therefore, suggests that the extract at the limit dose tested is essentially non-toxic and safe after oral administration.

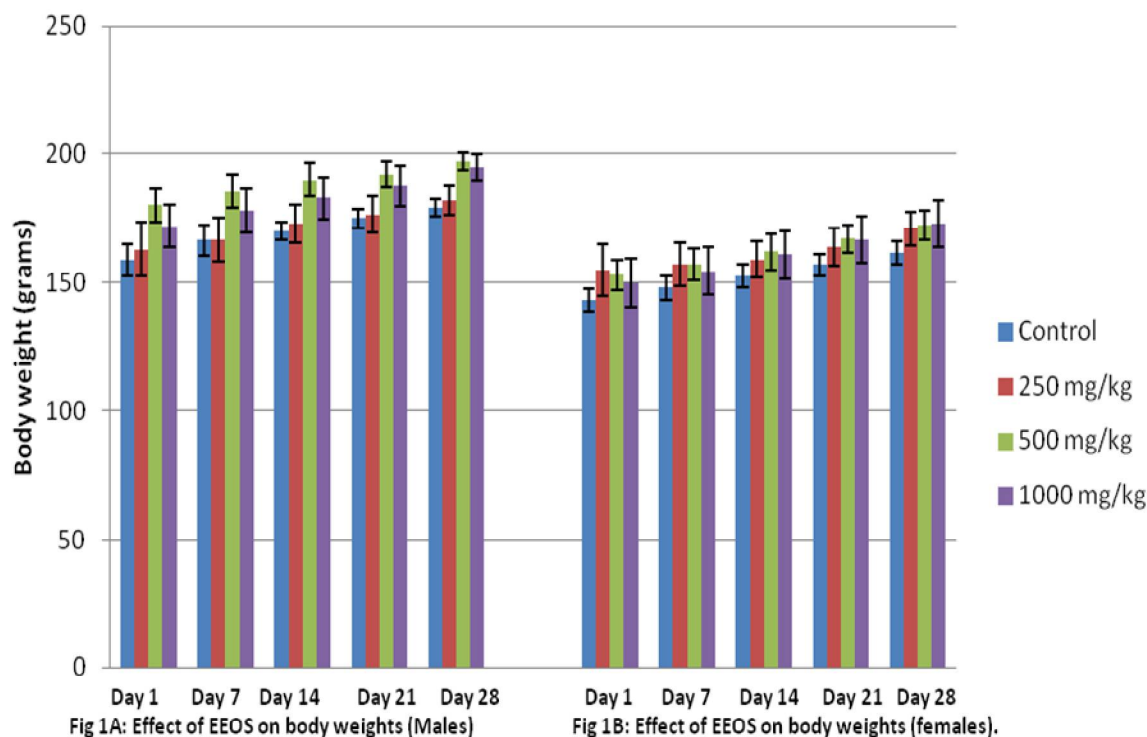


Figure 1: Effect of ethanol extract of *Oncoba spinosa* (EEOS) on body weights in experimental rats in sub-acute toxicity study

Table 1: Effect of ethanol extract of *Oncoba spinosa* (EEOS) on food intake in rats in sub-acute toxicity study

Treatment group	Sex	Gms			
		First week	Second week	Third week	Fourth week
Control (1% CMC)	Males (n=5)	39.30±1.88	36.55±1.71	42.77±1.87	40.62±1.99
	Females(n=5)	32.50±1.92	37.20±0.98	45.88±1.46	47.51±1.18
250 mg/kg	Males (n=5)	39.07±0.89	36.84±1.18	39.25±1.68	42.04±1.69
	Females(n=5)	38.95±1.50	39.95±1.00	44.94±1.76	47.15±1.12
500 mg/kg	Males (n=5)	35.24±1.40	40.17±2.75	40.22±1.52	42.62±1.60
	Females(n=5)	33.30±1.30	39.08±0.39	42.70±1.47	43.72±1.87
1000 mg/kg	Males (n=5)	37.87±1.47	39.34±1.00	40.20±1.31	45.22±0.47
	Females(n=5)	33.92±0.90	39.92±1.35	43.75±1.32	45.67±1.44

Values are expressed as mean ± SEM, n = 5 females and 5 males

Table 2: Effect of Ethanolic extract of *Oncoba spinosa* (EEOS) on organ weights in rats in sub-acute toxicity study

Organ weight (gms)	Treatment group							
	Control (1% CMC)		250 mg/kg		500 mg/kg		1000 mg/kg	
	Males (n=5)	Females (n=5)	Males (n=5)	Females (n=5)	Males (n=5)	Females (n=5)	Males (n=5)	Females (n=5)
Liver	4.28±0.31	4.19±0.26	4.75±0.27	4.68±0.31	4.58±0.37	4.28±0.24	5.07±0.49	3.83±0.25
Brain	1.45±0.03	1.47±0.05	1.54±0.11	1.65±0.06	1.51±0.09	1.59±0.11	1.72±0.06	1.40±0.07
Spleen	0.47±0.05	0.45±0.02	0.49±0.05	0.47±0.01	0.46±0.04	0.42±0.04	0.42±0.04	0.41±0.02
Kidney	1.01±0.06	1.05±0.07	1.01±0.05	1.07±0.04	1.19±0.09	1.08±0.06	1.07±0.07	1.02±0.03
Heart	0.71±0.03	0.69±0.02	0.61±0.02	0.65±0.01	0.68±0.02	0.70±0.02	0.67±0.05	0.71±0.04
Stomach	2.57±0.18	2.69±0.17	3.22±0.09	3.25±0.14	2.87±0.21	3.11±0.15	2.80±0.18	3.10±0.17

Values are expressed as mean±SEM, n=5 females and 5 males

Table 3: Effect of Ethanolic extract of *Oncoba spinosa* (EEOs) on hematological parameters in sub-acute toxicity study

Hematological Parameter	Sex	Treatment group			
		Control	250 mg/kg	500 mg/kg	1000 mg/kg
Hemoglobin (g/dl)	Males (n=5)	13.32±0.73	14.62±0.34	14.36±0.66	14.58±0.83
	Females(n=5)	14.52±0.71	15.34±0.41	15.74±0.50	16.38±0.40
RBC count (x10 ⁶ /μl)	Males (n=5)	4.76±0.33	4.96±0.31	4.80±0.43	5.22±0.34
	Females(n=5)	5.46±0.30	6.02±0.24	6.54±0.20	6.40±0.32
WBC count (x10 ³ /μl)	Males (n=5)	9.10±0.49	8.52±0.61	8.68±0.37	9.78±0.69
	Females(n=5)	10.58±0.40	10.18±0.50	9.29±0.52	10.72±0.36
Platelet count (x10 ³ /μl)	Males (n=5)	827.51±38.04	866.03±40.54	886.38±33.22	903.89±31.38
	Females(n=5)	845.51±42.00	852.03±39.42	860.38±38.97	874.14±18.75
Hematocrit (%)	Males(n=5)	44.32±1.67	46.46±2.22	45.76±1.25	49.74±1.83
	Females(n=5)	40.46±0.84	41.24±1.99	40.56±0.99	42.14±1.83
Neutrophils (%)	Males (n=5)	48.84±2.43	51.50±2.52	54.74±1.89	53.48±1.80
	Females(n=5)	43.84±1.52	44.80±0.53	46.76±0.80	45.36±1.14
Lymphocytes (%)	Males	34.92±1.41	29.62±1.62	34.70±1.80	33.54±2.33
	Females(n=5)	35.66±1.66	31.04±1.02	31.92±1.43	33.98±2.09
Eosinophils (%)	Males(n=5)	3.26±0.48	3.78±0.47	4.06±0.37	4.00±0.34
	Females(n=5)	3.50±0.31	3.76±0.35	3.70±0.36	4.40±0.37
Monocytes (%)	Males(n=5)	5.66±0.39	5.70±0.57	5.82±0.59	5.24±0.19
	Females(n=5)	4.16±0.26	3.96±0.39	3.74±0.67	4.40±0.27
Basophils (%)	Males(n=5)	0.94±0.19	1.30±0.10	1.0±0.22	1.06±0.24
	Females(n=5)	0.84±0.17	0.96±0.24	0.50±0.27	0.66±0.20

Values are expressed as mean±SEM, n=5 females + 5 males

Table 4: Effect of ethanol extract of *Oncoba spinosa* (EEOs) on biochemical parameters-in sub-acute toxicity study

Biochemical Parameter	Sex	Treatment group			
		Control	250 mg/kg	500 mg/kg	1000 mg/kg
Creatinine (mg/dl)	Males (n=5)	1.40±0.19	1.14±0.27	1.26±0.19	1.02±0.24
	Females(n=5)	1.14±0.19	1.32±0.29	1.00±0.20	1.24±0.28
Glucose (mg/dl)	Males (n=5)	72.18±3.34	81.80±3.24	75.90±2.44	84.38±5.37
	Females(n=5)	91.40±3.68	89.58±3.27	90.62±2.73	101.98±1.34
Total protein (g/dl)	Males (n=5)	7.18±0.34	6.68±0.27	6.84±0.31	7.12±0.36
	Females(n=5)	6.50±0.36	6.94±0.35	6.76±0.24	7.24±0.37
ALP (U/L)	Males (n=5)	71.18±5.49	82.02±4.39	78.62±4.62	84.62±6.92
	Females(n=5)	81.10±5.33	87.24±3.65	80.28±4.57	95.84±2.35
AST (U/L)	Males(n=5)	51.44±2.63	55.30±2.97	55.44±1.82	53.76±3.53
	Females(n=5)	48.94±2.99	53.90±3.13	54.02±1.68	49.76±2.72
ALT (U/L)	Males (n=5)	44.88±2.86	49.52±1.39	44.26±2.57	45.78±1.46
	Females(n=5)	40.24±0.60	44.62±1.55	43.94±2.56	46.40±2.34
BUN (mg/dl)	Males(n=5)	28.86±2.21	35.90±1.88	35.86±2.65	33.98±2.03
	Females(n=5)	27.24±1.96	32.72±1.03	31.80±0.74	31.98±0.62
Triglycerides (mg/dl)	Males(n=5)	66.18±3.13	55.30±2.13	57.68±4.19	67.50±3.69
	Females(n=5)	65.66±2.23	59.30±3.91	60.08±2.42	68.46±2.07
Total cholesterol (mg/dl)	Males(n=5)	89.46±6.39	78.04±3.56	101.68±1.05	98.40±2.42
	Females(n=5)	80.54±3.53	80.62±2.78	90.90±2.91	90.76±4.02
Albumin (g/dl)	Males(n=5)	3.92±0.28	4.70±0.25	4.64±0.33	4.70±0.18
	Females(n=5)	4.36±0.38	4.24±0.27	4.42±0.24	4.40±0.22
T.Bilirubin (mg/dl)	Males(n=5)	0.84±0.18	1.20±0.13	0.72±0.24	1.26±0.22
	Females(n=5)	0.66±0.18	0.76±0.17	0.50±0.16	0.78±0.25

Values are expressed as mean ± SEM, n = 5 females + 5 males

The sub-acute dose was selected based on the rats LD₅₀ value which kept rats alive, i.e. 1/5, 1/10 and 1/20 of LD₅₀ value of 5000 mg/kg. In repeated dose 28-day oral toxicity study, there were no deaths and treatment-related signs were observed in all the groups of animals.

Similarly, no significant differences in food and water intake, weight gain, biochemical and

haematological parameters were observed between control and treated groups during this period. Histopathological examination of selected vital organs (heart, brain, liver, kidney, spleen and stomach) from both treated and control animals showed normal architecture, suggesting no microscopic changes and morphological disturbances were caused due to oral administration of EEOs at highest dose level.

Although acute toxicity helps to determine the toxicity nature of substances, it is important to assess sub-acute toxicity profile because it helps to evaluate the morphological and physiological changes in organs. It is important to determine the food intake and water consumption during the investigation of safety of a product with medicinal purpose, as proper intake of supplements is important to the physiological status of the animal and to the achievement of best possible response to the compounds under investigation [11,12].

In this study, the food intake and water consumption were not affected by the administration of extract and it did not promote any appetite suppression and had no harmful effects. Thus, this indicates there was no interruption in the metabolism of carbohydrate, protein and fat [13]. After exposure to few possible toxic substances, there will be changes in body weight gain and internal organ weights which would reflect toxicity [14]. The body weight changes are markers of adverse effects of drugs and chemicals and if the body weight loss observed is more than 10 % of initial body weight, it will be considered as statistically significant [15,16]. Organ weight also is an important indicator of physiological and pathological status of animals. The relative organ weight is fundamental to confirm whether the organ weight was exposed to the injury or not. The heart, liver, kidney, spleen and lungs are the primary organs affected by metabolic reaction caused by toxicant [17]. There were no significant differences in body weight gain between control and treated groups. In the present study, organ weights in all the treated groups of both sexes were not significantly different from those of control groups. Hence it can be concluded that EEOS is almost non-toxic.

Hematological parameters are some of the most sensitive to evaluate the toxicity of medicines in humans and animals, and a blood profile normally gives important information on the reaction of body to damage or stress [18,19]. There were no statistically significant, dose-dependent adverse effects on any of the hematological parameters in both male and female rats after 4 weeks of EEOS treatment. In addition, there were no significant alterations in biochemical parameters in all the groups treated with a 4-week repeated dose of EEOS. Elevated bilirubin values are an indication of altered liver functions and a small elevation is an important indicator of liver damage in laboratory animals or could be a sign of biliary duct obstruction. Liver is

the primary site for the synthesis of plasma proteins especially albumin and alterations in the total plasma proteins may indicate liver dysfunction [20].

In this study, daily administration of EEOS for 28 days did not produce any significant changes in total protein and albumin of the treated rats from all the doses used compared to the control group. AST, ALT and ALP are the important serum biomarker enzymes usually measured to evaluate any toxic effects of the medicines on liver [21]. In the present study, no significant differences in the levels of AST, ALT and ALP in male and female rats were observed. Creatinine and BUN are known to be important markers of renal dysfunction [18]. Similarly, no significant differences in creatinine and BUN values were found in treated rats compared with the control group. These results suggest that EEOS does not have a toxic effect on the liver and kidneys. Histological analysis was done to further confirm the alteration in cell structure of the organs. The histopathological examination is the golden standard for evaluating the treatment related pathological changes in tissues and organs [22]. No abnormal findings were observed on histopathological examination of all the organs examined. Since no signs of toxicity were observed in all the tested groups with respect to hematology, biochemistry, organ weights, body weights and histopathological examination, it can be concluded that EEOS is non-toxic. Based on the results, the No Observed Adverse Effect Level (NOAEL) was found to be greater than 1000 mg/kg/day.

CONCLUSION

The ethanol extract of the root of *O. spinosa* is relatively safe, as they did not cause death or any abnormal changes in both acute and sub-chronic toxicity studies. Further studies will be required to investigate the potential medical significance of the plant.

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