SUB-CHRONIC TOXICITY STUDIES OF THE ALCOHOLIC EXTRACT OF *Litsea polyantha* JUSS BARK

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Summary

The acute and sub-chronic toxicity studies of alcoholic extract of *Litsea polyantha* Juss *(L. polyantha)* bark extract in albino mice and rats were investigated. Phytochemical analysis was also carried out. The LD_{50} was found to be 562.1 mg/kg b.w. *(i.p.)* in mice. In sub-chronic toxicity studies 50, 75 and 100 mg/kg b.w. of the alcoholic extracts (MELP) of the bark were administered orally to the test groups while distilled water was given to the control group. The parameters measured include food and fluid intake, body weight, absolute and relative weight of various organs, haematological parameters [total white blood cell (WBC) and packed cell volume (PCV)], and tests for liver function: Serum glutamic oxaloacetic transaminase (SGOT), Serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase and total bilirubin. Rats treated with MELP in the therapeutic dose level had no progressive increase in body weight or fluid intake. There were no significant changes in both the absolute and relative organ weights between the control and the test groups. The liver enzymes and haematological parameters were statistically equal in all the groups. In dose higher than LD_{50}, animal were dying after sudden jumping and jerking movement indicating that the extract have toxic effect on central nervous system.

Key words: *Litsea polyantha,* sub-chronic toxicity, liver function, packed cell volume, absolute weight, relative weight.

Introduction

Litsea, a large genus comprising of 400 species of evergreen trees or shrubs, distributed chiefly in tropical and subtropical Asia, Australia and the Pacific Islands, belongs to the family Lauraceae. *Litsea polyantha* Juss is found throughout North, East and Central India and to an altitude of 1,200 m[1,2]. It is often planted as ornamental plant. It is mostly grown as a good source of fodder for the livestock. It is a small to medium sized evergreen tree attaining a height of 21 m and a girth of 1.8m. Barks are dark grey or pale brown color, exfoliating in quite small polygonal corky scales[2,3].

In Folklore medicines - Bark, Stem, Roots and Leaves are used to treat various diseases and disorders. The bark of *L. polyantha* Juss is mildly astringent and is reported to be used for diarrhea. Powdered bark and roots are used in external applications for pains, bruises and contusions; they are also used for fractures in animals[1]. The present study was undertaken to investigate the acute and sub-chronic toxicity studies of the alcoholic extract of *L. polyantha* Juss. in rats.

Materials and Methods

Plant material:
The bark and the aerial parts of *L. polyantha* Juss (Lauraceae) were collected from Kanke, Khunti, Tamar and BIT, Mesra of Ranchi District. The parts were authenticated by Birsa Agriculture University, Kanke, Ranchi, India and Department of Pharmaceutical Sciences, BIT, Mesra, Ranchi, India. The voucher specimen (BIT 417; 2008-09) was preserved in the Department of Pharmaceutical Sciences, BIT, Mesra for future references.

Preparation of Extract:
The stem bark was dried under shade and then powdered with a mechanical grinder and stored in airtight container. The dried and powdered plant material (Bark) was subjected to hot extraction in a Soxhlet continuous extraction apparatus with Methanol. The extraction time period was 72 hours. The extract was then filtered through whatmann filter paper and the filtrate obtained was lyophilized. The yield of Methanol extract (MELP) was 10.21% w/w.

Phytochemical Screening:
The extract was subjected to preliminary phytochemical analysis for major group of phytoconstituents[4-6]. In each test 10% (w/v) solution of the methanolic extract was used unless otherwise specified for an individual test. Phytochemical screening of the extracts revealed the presence of alkaloid(s), terpenoid(s), steroid(s), tannin(s) & flavonoid(s).

Animals:
Studies were carried out using albino rats and albino mice of either sex weighing between 180-200 g & 20 to 25 g respectively. They were obtained from the animal house, Department of Pharmaceutical Sciences, BIT, Mesra, Ranchi, India. The animals were grouped and housed in polyacrylic cages with not more than six animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2 °C) with dark and light cycle (14/10 h). Animals were allowed free access to standard pellet (Hindustan Lever, Mumbai, India) food and drinking water *ad libitum.* The rats were acclimatized to laboratory condition for 10 days before commencement of experiment[7]. All procedures described were reviewed and approved by the Institute animal ethical committees (621/02/ac/CPCSEA) and (1053/07/ac/CPCSEA).

Acute toxicity studies:
The LD_{50} was determined using the graphical method of Litchfield and Wilcoxon (1949) in mice[8]. Briefly, geometric doses of MELP (100-1000 mg/kg) was administered i.p. to 10 groups of mice (n=6). Control group received normal saline (10 ml/kg i.p.) and vehicle control received 10% propylene glycol (PG) (10 ml/kg i.p.). After administration of dose the animals were observed continuously for first 4 h for behavioral changes and signs of toxicity and mortality within 24-72 h were noted. The values thus obtained were plotted against the corresponding log dose. The antilog of log dose corresponding to probit 5 gave the value of LD_{50}.

Sub-chronic Toxicity Study:
A total of twenty four mature Wister rats were used in this study. They were divided into four groups of six rat each. Three of the groups received 50, 75 and 100 mg/ kg body weight of the MELP *(p.o.),* respectively, while the control group received distilled water only.

Food and water intake were monitored daily. After 30 days of exposure, blood was collected from the animals, by cardiac puncture, for haematological and biochemical analysis. Thereafter, the animals were sacrificed and the following organs isolated and weighed: heart, lungs, kidney, liver, spleen and pancreas. Relative weight of the respective organs was calculated from each organ's wet weight and the animal's body weight.

Effect of Extract on Liver Function:
About 5 ml of whole blood collected into a plain tube was centrifuged at 3500 rpm for 5 min using table centrifuge (Remi, India) and the serum separated and analyzed for the liver enzymes. Serum glutamic oxaloacetic transaminase (SGOT) and Serum glutamic pyruvic transaminase (SGPT) were assayed using the methods of Reitman and Frankel, alkaline phosphatase (ALP) was analysed by the method of King and Armstrong, while total bilirubin level was determined by the method of Malloy and Evelyn. All assay methods employed were as reported by Varley *et al.*

Haematological Assay:
EDTA-anticoagulated tubes were used to collect whole blood for these investigations. Packed cell volume (PCV) was determined by the microhaematocrit method, while total WBC was determined by visual method.[10]

Statistical Analysis:
Data were analyzed using Student's t-test.

Results and Discussion

The acute and subchronic toxicity studies of the methanolic extract (MELP) of *L. polyantha* Juss bark were carried out. Phytochemical tests indicate MELP contains alkaloids, terpenoids, steroids, tannins & flavonoids. The LD_{50} was found to be 562.1 mg/kg b.w. *(i.p.)* in mice. Table 1 shows the effect of various therapeutic doses of MELP on weekly food and fluid intake. MELP did not increase the food or water intake of the test animals compared to control at $p < 0.05$ throughout the three weeks of exposure. Rats treated with the various therapeutic doses of MELP (50, 75 and 100 mg/kg) had no significant change in body weight. No statistically significant differences existed in the absolute and relative weight of all the isolated organs between the treated and the control rats (Table 2). Kluwe documented that the absolute organ weight has been observed to be a relative sensitive indicator of nephrotoxicity for known nephrotoxicants. An increase in kidney weight (either absolute or relative) indicates nephrotoxicity[11]. MELP did not induce any toxic effect on the kidney and the other organs going by this indicator, since the absolute and relative weight of the organs were not significantly different from control values.

Table 1: Effect of MELP on weekly Food (g) and Fluid* (ml) intake in Rats

	Weeks		
Treatments	1	2	3
Control	297.48 ± 3.69 (825.52 ± 4.09)	295.72 ± 4.09 (832.96 ± 3.33)	295.64 ± 4.69 (831.81 ± 3.41)
50 mg/kg	295.03 ± 2.76 (835.66 ± 2.09)	299.37 ± 2.22 (833.14 ± 2.70)	300.97 ± 4.03 (834.63 ± 3.08)
75 mg/kg	304.76 ± 1.47 (831.15 ± 4.44)	299.43 ± 2.70 (826.61 ± 3.77)	292.01 ± 8.00 (827.54 ± 2.86)
100 mg/kg	297.32 ± 3.37 (831.61 ± 3.56)	301.65 ± 3.23 (827.17 ± 2.39)	300.61 ± 3.09 (829.88 ± 3.26)

* Values in parenthesis indicate volume of fluid ingested. Each value is mean ± S.E.M (n = 6)

Table 2: Effect of Various Doses of MELP on the Relative (%) and Absolute Weights* (g) of Organs

	Treatment			
Organ	Control	50 mg/kg	75 mg/kg	100 mg/kg
Heart	0.41 ± 0.02 (0.77 ± 0.04)	0.41 ± 0.03 (0.78 ± 0.04)	0.41 ± 0.01 (0.79 ± 0.02)	0.41 ± 0.02 (0.79 ± 0.03)
Lung	0.85 ± 0.02 (1.60 ± 0.06)	0.84 ± 0.04 (1.59 ± 0.08)	0.82 ± 0.02 (1.58 ± 0.04)	0.81 ± 0.04 (1.56 ± 0.06)
Kidney	0.79 ± 0.02 (1.49 ± 0.02)	0.80 ± 0.02 (1.51 ± 0.03)	0.78 ± 0.01 (1.50 ± 0.02)	0.77 ± 0.02 (1.49 ± 0.02)
Liver	4.08 ± 0.21 (7.69 ± 0.39)	4.08 ± 0.24 (7.71 ± 0.32)	4.12 ± 0.17 (7.90 ± 0.32)	4.02 ± 0.07 (7.77 ± 0.1 7)
Pancreas	0.27 ± 0.02 (0.52 ± 0.04)	0.28 ± 0.02 (0.54 ± 0.03)	0.28 ± 0.01 (0.53 ± 0.03)	0.27 ± 0.02 (0.53 ± 0.03)
Spleen	0.47 ± 0.03 (0.88 ± 0.06)	0.48 ± 0.03 (0.91 ± 0.06)	0.46 ± 0.04 (0.88 ± 0.07)	0.46 ± 0.02 (0.88 ± 0.04)

* Values in parenthesis indicate absolute weight. Values are expressed as mean ± S.E.M (n = 6)

The effect of MELP on liver enzymes and bilirubin is shown on Table 3. The levels of bilirubin and the liver enzymes: SGOT, SGPT and ALP were not significantly affected by the therapeutic dose of the extract. Certain drugs and other substances are known to affect and influence circulating bilirubin levels and elevation in bilirubin levels suggests increase in haemolysis[12]. MELP however, did not alter significantly, the bilirubin levels of the exposed rats, as well as other liver enzymes compared to the control.

Table 3: Dose Effect Relationship of MELP on the Liver Function of Rats

Treatment	Analyte			
	SGOT (iu/l)	SGPT (iu/l)	ALP (iu/l)	Total Bilirubin (mg/dl)
Control	56.99 ± 0.82	22.87 ± 0.16	178.69 ± 0.46	0.075 ± 0.003
50 mg/ kg	56.51 ± 0.93	23.05 ± 0.24	179.11 ± 0.40	0.085 ± 0.003
75 mg/kg	56.83 ± 0.62	23.23 ± 0.13	179.05 ± 0.47	0.078 ± 0.003
100 mg/kg	56.84 ± 0.65	23.06 ± 0.16	179.55 ± 0.49	0.082 ± 0.003

Values are expressed as mean ± S.E.M (n = 6) (SGOT = Serum Glutamic Oxaloacetic Transaminase, SGPT = Serum Glutamic Pyruvic Transaminase, ALP = Alkaline Phosphatase)

According to Onyenyili and co-workers (13), anemia following administration of an agent can be as a result of lysis of blood cells and/or inhibition of blood cell synthesis by the active constituents of the extract, and decrease in hematological parameters in experimental animals has been associated with anemia[13].

There was no significant change in haematological parameters in the extract-treated animals compared to the control (Table 4), which indicates that there is no lysis of blood cells and/or inhibition in blood cells synthesis by the active constituents of MELP. The above results suggest no toxicity of MELP in rats.

Table 4: Dose Effect Relationship of MELP on the Haematological Parameters of Rats

Treatment	PCV (%)	WBC (cells/mm^3)
Control	60.05 ± 0.21	7446.50 ± 27.48
50 mg/kg	59.70 ± 0.24	7415.83 ± 32.24
75 mg/kg	60.27 ± 0.15	7379.50 ± 34.32
100 mg/kg	60.09 ± 0.17	7447.83 ± 37.52

Values are expressed as mean ± S.E.M (n = 6)

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