ACUTE AND SUB-CHRONIC TOXICITY STUDY OF *DIOSPYROS CORDIFOLIA* STEM BARK IN SWISS ALBINO MICE

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Summary

In the present study, the safety profile of *Diospyros cordifolia* stem bark was evaluated by acute and sub-chronic toxicity study of the methanol extract of *Diospyros cordifolia* (MEDC) in Swiss albino mice. In sub-chronic toxicity study, MEDC was administered at the single daily dose of 50 mg/kg b.w., i.p., for 28 consecutive days and at the 29th day, the hematological, histological, serum and hepatic biochemical parameters were evaluated by sacrificing the animals. No mortality was observed during the course of whole study period. No detectable alterations were found in hematological, biochemical and histological parameters in MEDC treated group when compared to vehicle control group after 28 days. The results of the present study therefore indicated that *D. cordifolia* is safe in Swiss mice demonstrating no noticeable toxicity.

Key words: Sub-chronic toxicity, *Diospyros cordifolia*, stem bark, biochemical.

Introduction

Diospyros cordifolia Roxb. (Ebenaceae), commonly known as Indian ebony, is a deciduous tree and used traditionally in India for several medicinal purposes. The plant holds very important position in Indian folk medicine where it is mostly used as the treatment of liver disorders, whooping cough, leprosy, dysentery, abdominal pain, wounds, gonorrhoea, fever, inflammation, as emetic and anthelmintic (1, 2).

Previous researchers reported hepatoprotective activities of its stem bark (3). Also the present authors have reported anticonvulsant, anti-inflammatory and antidiabetic activities of its stem bark extract in experimental animal models (4-6). Ursolic acid, α-amyrin, β-amyrin, lupeol, taraxerol, nentriacontane, hentriacontanol and β-sitosterol were isolated from the leaves of *D. cordifolia* (7). Therefore, the present study was aimed to investigate the acute and sub-chronic toxicity profile of methanol extract from *Diospyros cordifolia* stem bark (MEDC) in Swiss albino mice to establish its safety profile in rodents.

Materials and methods

Plant material: The plant *Diospyros cordifolia* Roxb. (Ebenaceae), was collected in the month of November 2008 from the forest region of West Bengal, India. The taxonomical identification of the plant was done by Botanical Survey of India, Shibpur, India and the voucher specimen (PMU-5/JU/2008) has been preserved in Pharmacology Research Laboratory, Jadavpur University, Kolkata for future reference.

Preparation of extract: The stem bark of the *Diospyros cordifolia* was dried under shade and then powered with mechanical grinder. The powdered plant material was extracted with 80% methanol using soxhlet extraction apparatus. The solvent was completely removed under reduced pressure and semisolid mass was obtained (MEDC, yield: 14.5 % w/w).

Drugs and chemicals: Bovine serum albumin from Sigma Chemical Co., St. Louis, Mo, USA; Trichloroacetic acid (TCA) from Merck Ltd., Mumbai, India; Thiobarbituric acid (TBA), 5,5′-dithio *bis*-2-nitro benzoic acid (DTNB), Phenazonium methosulphate (PMS), Nicotinamide adenine dinucleotide (NADH) and reduced glutathione (GSH) from SISCO Research Laboratory, Mumbai, India. Potassium dichromate and glacial acetic acid from Ranbaxy, Mumbai. All the other reagents used were of analytical reagent grade obtained commercially.

Animals: Adult male Swiss albino mice weighing 18-25 g were used for the present investigation. They were housed in a clean polypropylene cage and maintained under standard laboratory conditions (temperature 25 ± 2ºC with dark/light cycle 14/10 h). They were fed with standard pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. The animals were acclimatized to laboratory conditions for one week prior to experiment. All experimental procedures described were reviewed and approved by the Jadavpur University Animal Ethical Committee.

Acute toxicity: The acute toxicity of MEDC in male Swiss albino mice was studied as reported method (8).

Sub-chronic toxicity: The Swiss albino mice were divided into two groups containing 6 animals per groups. The first group received normal saline (5 ml/kg body weight, i.p.) and the

second group received MEDC at 50 mg/kg body weight i.p., daily for 28 consecutive days. Food and water intake of animals were observed during this period. Twenty four hours after the last dose (i.e., at the 29th day), blood was collected from overnight fasted rats of each group by cardiac puncture for estimation of hematological and serum biochemical parameters. Then the rats were sacrificed by cervical dislocation for the study of liver biochemical parameters and organ weights.

Body weight and organ weights: The body weight of mice of each group were measured just before and 28 days after MEDC treatment respectively. Heart, lung, liver and kidney weights of all mice were measured immediately after post treatment sacrifice.

Hematological studies: Collected blood was used for the estimation of hemoglobin (Hb) content; red blood cell count (RBC) and white blood cell counts (WBC) as per standard procedures (9).

Estimation of serum biochemical parameters: Collected blood was used for the estimation of serum biochemical parameters viz. serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP), serum cholesterol and total protein contents by using commercially available reagent kits (Span Diagnostics, Surat, India).

Estimation of liver biochemical parameters: Lipid peroxidation i.e., thiobarbituric acid reactive substances (TBARS) was estimated by the previously reported method and expressed as mM/100 g of liver tissue (10). Reduced glutathione (GSH) was determined by the reported method and was expressed as mg/100 g of liver tissue (11). Catalase (CAT) activity was assayed according the method described by standard method and expressed as μmoles of H_2O_2 consumed/min/mg of liver tissue (12).

Statistical analysis: The all experimental data were expressed as mean ± standard error of mean (SEM).

Results

In the present study, there were no significant changes in body weights and organ weights of mice of MEDC treated group (after 28 days) from saline control group (Table 1). No mortality was evident from the experimental results in mice. The food and water intake of MEDC treated group was found comparable to the control group without showing significant alteration in body weight and growth rate. From the present study it was seen that there was no significant changes in the number of WBC, RBC and Hemoglobin in the MEDC treated group compared to normal control group (Table 2). After 28 days of treatment no significant alterations were observed in all serum and hepatic biochemical parameters in animals of MEDC treated group when compared to normal control group (Tables 3 and 4).

Discussion

The present study was aimed to investigate the possible toxic effects of the methanol extract of *Diospyros cordifolia* stem bark (MEDC) in Swiss mice. Various physical, chemical and histological parameters were thoroughly studied in the sub-chronic toxicity study.

The body weights, food and water intakes were found to be unaltered during the 28 days treatment period when compared to control group. Similarly there were no significant changes in different organ weights also. No mortality was observed during this period. Also in the study of hematological parameters there was no alteration of the normal levels of RBC, WBC and hemoglobin count with MEDC treated group. Therefore, MEDC had no toxic effect on the blood and haematopoetic system.

The serum biochemical parameters were studied to evaluate the possible alterations in hepatic and renal functions influenced by MEDC. Biochemical parameters related to hepatic vital functions viz. SGPT, SGOT, SALP, bilirubin, cholesterol contents exhibited no significant alterations as compared with the normal control mice. It is well known that almost all drugs, chemicals and xenobiotics are eliminated through renal excretion hence it was found necessary to estimate the effects of MEDC on kidney functions. Serum biochemical parameters related to kidney functions viz. urea, uric acid creatinine and total protein demonstrated no significant differences with respect to normal control group of animals. Therefore, it can be inferred that MEDC did not affect the normal hepatic and renal functions on 28 days treatment.

Free radicals or reactive oxygen species (ROS) are regarded to be involved in the pathogenesis of several degenerative diseases and toxic reactions (13). Antioxidants can retard or stop the uncontrolled generation of ROS, thus help to reduce oxidative stress-induced diseases (14). In the present study, liver endogenous antioxidant parameters viz. lipid peroxidation (TBARS), reduced glutathione (GSH) and catalase activity (CAT) were estimated to ascertain the functioning of normal liver endogenous antioxidant defense systems, and it was found that no alterations in these parameters took place thereby implying maintenance of normal hepatic non-enzymatic and enzymatic antioxidant mechanisms during MEDC treatment.

From the present investigation, it can be concluded that MEDC exhibited excellent safety profile in acute and sub-chronic toxicity studies. The present study establishes the reliable safety profile of MEDC in Swiss mice offering no obvious toxicity.

Table1: Effect MEDC on body weight and weight of major organs in mice.

Treatment	Initial body wt (g)	Body wt (g)	Heart wt (g)	Lung wt (g)	Liver wt (g)	Kidney wt (g)
Normal control (0.9% NaCl)	18±1.12	25±1.11	1.51±0.14	1.82±0.19	2.18±0.12	0.66±0.02
MEDC(50 mg/kg)	20±0.7	25±0.9	1.53±0.15	1.91±0.17	2.18±0.14	0.68±0.02

Values are expressed as mean ± SEM (n = 6).

Table 2: Effect MEDC on hematological parameters in mice.

Treatment	Hemoglobin (g/dl)	RBC (10^6 cells/ml)	WBC (10^3 cells/ml)
Normal control (0.9% NaCl)	13.82 ± 1.14	6.76 ± 0.16	3.92 ± 0.42
MEDC (50 mg/kg)	13.68 ± 1.26	6.45 ± 0.42	4.27± 0.28

Values are expressed as mean ± SEM (n = 6).

Table 3: Effect of MEDC on serum biochemical parameters in mice.

Treatment	SGOT (IU/dl)	SGPT (IU/dl)	SALP (IU/dl)	Bilirubin (mg/dl)	Cholesterol (mg/dl)	Total protein (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/ml)
Normal control (0.9% NaCl)	42.48 ±1.27	39.45 ±1.32	85.37 ±1.76	0.92 ±0.13	96.33 ±6.52	7.54 ±1.8	41.126 ±1.12	6.96 ±1.48	0.96 ±0.11
MEDC(50 mg/kg)	44.15 ±1.14	40.12 ±1.32	84.25 ±1.64	0.97 ±0.25	98.63 ±9.56	7.26 ±1.9	46.37 ±1.20	6.75 ±1.58	0.98 ±0.26

Values are expressed as mean ± SEM (n = 6).

Table 4: Effect MEDC on liver biochemical parameters in mice.

Treatment	TBARS (mM/100 g of wet liver tissue)	GSH (mg/ 100 g of wet liver tissue)	CAT (µmoles of H_2O_2 consumed/min/mg of wet liver tissue)
Normal control (0.9% NaCl)	1.14±0.6	46.26±1.7	84.36±2.8
MEDC (50 mg/kg)	1.15±0.4	45.22±1.8	86.45±2.1

Values are expressed as mean ± SEM (n = 6).

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