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Acute and Sub-Chronic Toxicity Study of *Manilkara zapota* Leaf Extracts in Mice

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Abstract: In the present study, the safety profile of *Manilkara zapota* leaf was evaluated by acute and sub-chronic toxicity study of the ethyl acetate and methanol extracts of *M. zapota* leaf in adult male Swiss albino mice. In acute toxicity study, each extract up to 2000 mg/kg body weight orally did not produce any toxic effect or death. In sub-chronic toxicity study, both the extracts were administered at the single daily dose of 200 mg/kg body weight orally for 28 consecutive days and at the 29th day, the hematological and serum 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 and serum biochemical parameters in treated groups when compared to vehicle control group after 28 days. The results of the present study therefore indicated that *M. zapota* leaf is safe in adult albino mice demonstrating no noticeable toxicity.

Key words: Acute Toxicity · Biochemical · *Manilkara zapota* · Leaf · Sub-Chronic Toxicity

INTRODUCTION

Manilkara zapota (L.) P. Royen (Sapotaceae), commonly known as the Sapodilla in English, *Sabeda* in Bengali, is a glabrous, evergreen tree Indigenous to Southern Mexico, Central America and the Caribbean islands. It is grown in large quantities in India, Thailand, Malaysia, Cambodia, Indonesia, Bangladesh and Mexico. It is cultivated throughout India for its consumable fruits. The fruits are edible, sweet, and nutritious with agreeable flavour. Traditionally the plant has been used for several medicinal purposes. The seeds are aperients, diuretic tonic and febrifuge. The bark is carrying antibiotic, astringent and febrifuge effects. Bark is used as tonic and the decoction is given in diarrhoea and peludism. Bark is also used in treatment of diarrhoea and dysentery. The leaves are used to treat cough, cold, and diarrhoea [1, 2]. Antimicrobial and antioxidant activities have been reported from the leaves [3, 4]. The present study aimed to establish the safety of the said plant by assessing the possible acute and sub-chronic toxicity profile of extracts from *M. zapota* leaf in adult Swiss albino mice.

MATERIALS AND METHODS

Plant Material: The mature leaf of the plant *Manilkara zapota* (L.) P. Royen (Sapotaceae) was collected in the month of December 2013 from North 24 Parghanas, West Bengal, India. The plant species was taxonomically identified at the Central National Herbarium, Botanical Survey of India, Howrah, West Bengal, India. The voucher specimen (CNH/16/2014/Tech. II/073) was maintained in the research laboratory of Bengal Institute of Pharmaceutical Sciences for future reference. The leaves were collected and washed with running tap water. Then plant material was shade-dried with occasional shifting and then powdered with mechanical grinder, passing through sieve no. 40, and stored in an air-tight container.

Preparation of Extracts: The powdered plant material was first defatted with petroleum ether, and then extracted separately with ethyl acetate and methanol using simple percolation technique. The solvent were almost removed from the extracts in a hot water bath leaving the semisolid masses (EAMZ and MEMZ) and stored in small conical flasks wrapped with aluminum foil and kept in refrigerator.

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Drugs and Chemicals: Bovine serum albumin from Sigma Chemical Co., St. Louis, Mo, USA. All the other reagents used were of analytical reagent grade obtained commercially.

Experimental Animals: Adult Swiss albino mice of either sex weighing 18-25 g were used for the present investigation. They were housed in clean polypropylene cages and maintained under standard laboratory conditions (temperature 25±2°C 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 were reviewed and approved by the Institutional Animal Ethics Committee.

Acute Toxicity: The acute toxicity of EAMZ and MEMZ in Swiss albino mice was studied as per previously reported method [4]. Both the extracts were administered to four groups (n = 6) of mice at 50, 500, 1500 and 2000 mg/kg body weight, *p.o*. The treated animals were kept under observation for 3 days, for mortality and general behaviour. No death was observed till the end of the study.

Sub-Chronic Toxicity: The adult male Swiss albino mice were divided into three groups containing 6 animals per group. The first group received normal saline (5 ml/kg body weight, *p.o.*) and the other two groups received the two extracts each at 200 mg/kg body weight *p.o.*, respectively 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 mice of each group by cardiac puncture for estimation of hematological and serum biochemical parameters. Then the mice were sacrificed by cervical dislocation for the study of liver biochemical parameters and vital organ weights [6].

Body Weight and Organ Weights: The body weight of mice of each group were measured just before and 28 days after the extract treatment, respectively. Heart, lung, liver, pancreas 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) by standard recommended procedures [7, 8].

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, bilirubin, total protein, urea, uric acid and creatinine contents by using commercially available reagent kits (Span Diagnostics, Surat, India).

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

RESULTS

In acute toxicity study, EAMZ and MEMZ up to 2000 mg/kg body weight orally exhibited no toxic effect or death in mice. There were no significant changes in body weights and vital organ weights of mice of extract treated groups (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 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 counts of WBC, RBC and hemoglobin content in the treated group compared to normal control group (Table 2). After 28 days of treatment no significant alterations were observed in all serum biochemical parameters in animals of extract treated group when compared to those of normal control group (Table 3).

DISCUSSION

The present study was aimed to investigate the possible toxic effects of the ethyl acetate and methanol extracts of *M. zapota* leaf (EAMZ, MEMZ) in adult male Swiss albino mice. The results of acute toxicity study revealed that the extracts may be regarded as safe in Swiss albino mice up to the highest single dose administered via oral route.

Various parameters were thoroughly studied in the sub-chronic toxicity study. Body weight is regarded as a non-specific indicator of general wellbeing of animals. Reduction in body weight is an indicator of decline in general health conditions. The body weights, food and water intakes were found to be unaltered during the 28 days treatment period when compared to control group mice. Similarly there were no significant changes in different vital organ weights also. No mortality was observed during this period.

Table 1: Effect of leaf extracts of *M. zapota* on body weight and weight of organs in mice.

Treatments	Initial body wt (g)	Final body wt (g)	Final Heart wt (g)	Final Lung wt (g)	Final Liver wt (g)	Final Kidney wt (g)	Final Pancreas wt (g)
Normal control (0.9% NaCl)	19±0.13	26±1.13	0.12±0.05	0.14±0.08	1.19±1.13	0.29±0.95	0.19±0.09
EAMZ (200 mg/kg)	20±0.19	25±1.17	0.13±0.05	0.13±0.08	1.18±1.09	0.28±0.63	0.18±0.16
MEMZ (200 mg/kg)	20±0.07	24±1.09	0.13±0.05	0.14±0.09	1.18±1.29	0.29±0.81	0.19±0.15

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

Table 2: Effect of leaf extracts of *M. zapota* on hematological parameters in mice

Treatments	Hemoglobin (g/dl)	RBC (10^6 cells/ml)	WBC (10^3 cells/ml)
Normal control (0.9% NaCl)	13.96±0.85	6.63±0.54	3.15±0.42
EAMZ (200 mg/kg)	12.78±0.63	6.14±0.54	3.92±0.26
MEMZ (200 mg/kg)	13.68±0.15	6.63±0.68	3.26±0.66

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

Table 3: Effect of leaf extracts of *M. zapota* on serum biochemical parameters in mice

Treatments	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)	42.51±1.29	35.99±1.27	83.29±1.89	0.91±0.15	151.33±9.6	7.22±1.7	42.15±1.13	6.92±1.89	0.95±0.13
EAMZ (200 mg/kg)	44.64±1.28	36.82±1.43	88.54±1.79	0.97±0.18	154.15±9.27	6.33±1.8	43.33±1.29	7.93±1.45	1.75±0.27
MEMZ (200 mg/kg)	43.36±1.57	35.23±1.81	86.27±1.17	0.91±0.32	153.36±10.26	6.65±1.9	43.79±1.19	7.29±1.33	1.29±0.18

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

Haematological parameters were evaluated to assess haematological toxicity of the test extracts on long term use. Also in the study of hematological parameters there was no alteration of the normal levels of RBC, WBC and hemoglobin compared with the extract treated groups. Therefore, the test extracts had no toxic effect to the blood and haematopoetic system of extract treated mice.

The serum biochemical parameters were studied to evaluate the possible alterations in hepatic and renal functions influenced by the extracts. Liver is the key organ of metabolism and detoxification of drugs and xenobiotics [9]. Serum biochemical parameters related to hepatic function namely SGPT, SGOT, SALP, serum bilirubin and cholesterol contents exhibited no significant alterations as compared to the normal control mice.

It is well known that almost all drugs, chemicals and xenobiotics are principally eliminated through renal excretion hence it was found necessary to estimate the effects of the extracts on kidney functions [10]. Serum biochemical parameters related to kidney functions viz. urea, uric acid, creatinine and total protein demonstrated no significant differences with respect to control group animals. Therefore, it can be inferred that all the three extracts did not affect the normal hepatic and renal functions of mice on 28 days treatment.

From the present investigation, it can be concluded that EAMZ and MEMZ exhibited excellent safety profile in acute and sub-chronic toxicity studies in mice. The present study establishes the reliable safety profile of *M. zapota* leaf extracts in adult male Swiss albino mice offering no obvious toxicity.

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