RESEARCH ARTICLE

Acute and sub-chronic toxicity study of *Musa paradisiaca* leaf extracts in mice

Sumita Bera[1], Sanjib Bhattacharya[2*], J. N. Pandey[1], Moulisha Biswas[1]

[1]Bengal Institute of Pharmaceutical Sciences, Kalyani, Nadia 741235, West Bengal, India
[2]Bengal School of Technology, Delhi Road, Sugandha, Hooghly 712102, West Bengal, India.

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ABSTRACT

In the present study, the safety profile of *Musa paradisiaca* (L.) (Musaceae) leaf was evaluated by acute and sub-chronic toxicity study of the petroleum ether, ethyl acetate and methanol extracts of *M. paradisiaca* leaf in 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, the three extracts were administered at the single daily dose of 200 mg/kg body weight orally for 28 consecutive days and at the 29th day, different 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 observed biochemical parameters in treated groups when compared to vehicle control group after 28 days. The results of the present study therefore indicated that *M. paradisiaca* leaf is safe in adult male albino mice demonstrating no noticeable toxicity.

Key words: Acute toxicity, biochemical, *Musa paradisiaca,* leaf, sub-chronic toxicity.

INTRODUCTION

Musa paradisiaca (L.) (Musaceae) commonly known as banana in English, *Kola-gachh* in Bengali and *Kela* in Hindi, is a perennial tree like herb grown indigenously in tropics and subtropics. It is widely found throughout Indian subcontinent and commercially cultivated in various parts of India for its consumable fruits. All parts of this plant have been traditionally used in India for several medicinal purposes. Ayurvedic physicians of Karnataka and Kerala states of India recommended *M. paradisiaca* for the treatment of urinary stones. [1]. The stem juices of *M. paradisiaca* have been reported for dissolving pre-formed stones and in preventing the formation of stones in the urinary bladder of rats. [2, 3] Its stem juice has also been used in nervous affectations like epilepsy, hysteria and in dysentery and diarrhoea. Several oligosaccharides comprising fructose, xylose, galactose, glucose and mannose occur naturally in banana, making it an excellent prebiotic for the selective growth of beneficial bacteria in the intestine. [4] Previous workers reported several experimental studies on *Musa paradisiaca.* [5-9] However, the acute and sub-chronic safety profile of its leaf is still not reported. Therefore, in the present investigation we attempted these studies on the leaf extracts of *M. paradisiaca.*

Address for correspondence

Sanjib Bhattacharya,
Pharmacognosy Division, Bengal School of Technology (A College of Pharmacy), Delhi Road, Sugandha, Hooghly 712102, West Bengal, India
E-mail: sakkwai@yahoo.com

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MATERIALS AND METHODS

Plant material

The mature leaves of *Musa paradisiaca* (L.) (Musaceae) were collected during November 2012 from Nadia, West Bengal, India. The plant material was taxonomically identified at the Central National Herbarium, Botanical Survey of India, Howrah, West Bengal, India. The voucher specimen [CNH/5/2013/Tech.II/963] was maintained at our research laboratory for future reference. The 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 extracted with petroleum ether, ethyl acetate and methanol

successively using simple percolation technique. The solvents were almost removed from the extracts in a hot water bath leaving the semisolid masses (yields: 1.32, 2.12 and 6.11 % w/w respectively) and stored in conical flasks wrapped with aluminum foil. Preliminary phytochemical analysis was performed on all three extracts to identify the phytoconstituents present in the extracts. [10]

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), reduced nicotinamide adenine dinucleotide (NADH) and reduced glutathione (GSH) from SISCO Research Laboratory, Mumbai, India; potassium dichromate and glacial acetic acid from Ranbaxy, Mumbai, India. 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 study

The acute toxicity of three test extracts in Swiss albino mice was studied as per reported method. [11] Each extract was given 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 study

The adult Swiss albino mice were divided into four groups containing 6 animals per group. The first group received normal saline (5 ml/kg body weight, p.o.) and the other three groups received the three 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 organ weights. [12]

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, kidney and pancreas weights of all mice were measured immediately after post treatment sacrifice.

Evaluation of hematological parameters

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 [13, 14].

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

Statistical analysis

All the experimental data were expressed as mean ± standard error of mean (SEM).

RESULTS

Preliminary phytochemical studies showed the presence of steroids in the petroleum ether extract;

steroids, triterpenoids, in the ethyl acetate extract; and alkalods, saponins, glycosides and carbohydrates in the methanol extract. In acute toxicity study, all the three extracts 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 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 groups were 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 haematological parameters in the treated groups 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 groups when compared to those of normal control group animals (Table 3).

DISCUSSION

The present study was aimed to investigate the possible toxic effects of the petroleum ether, ethyl acetate and methanol extracts of *M. paradisiaca* leaf in adult Swiss albino mice. The results of acute toxicity study revealed that the extracts may be safe in Swiss albino mice. Various 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 vital 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 compared with the extract treated groups. Therefore, the test extracts had no toxic effect to the blood and haematopoetic system of mice.

The serum biochemical parameters were studied to evaluate the possible alterations in hepatic and renal functions influenced by the extracts. It is well known that most of the drugs, chemicals and xenobiotics are metabolized in liver. [15] Serum biochemical parameters related to hepatic function namely SGPT, SGOT, SALP, bilirubin and cholesterol contents exhibited no significant alterations as compared to the control mice. It is also well known that almost all drugs, chemicals and xenobiotics are eliminated through renal excretion hence it was found necessary to estimate the effects of the extracts on kidney functions. [16] 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 the petroleum ether, ethyl acetate and methanol extracts of *M. paradisiaca* leaf exhibited excellent safety profile in acute and sub-chronic toxicity studies in mice. The present study establishes the reliable safety profile of *M. paradisiaca* leaf extracts in adult Swiss albino mice offering no obvious toxicity.

Table 1: Effect of *M. paradisiaca* leaf extracts 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
Pet. ether (200 mg/kg)	19±0.18	23±1.27	0.12±0.07	0.13±0.09	1.17±1.15	0.27±0.78	0.18±0.07
Ethyl acetate (200 mg/kg)	20±0.19	25±1.18	0.13±0.06	0.13±0.07	1.18±1.08	0.28±0.73	0.18±0.06
Methanol (200 mg/kg)	20±0.09	24±1.08	0.13±0.04	0.14±0.08	1.19±1.27	0.29±0.88	0.19±0.05

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

Table 2: Effect of *M. paradisiaca* leaf extracts 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
Pet. ether (200 mg/kg)	12.08 ± 0.36	5.82 ± 0.33	4.33 ± 0.81
Ethyl acetate (200 mg/kg)	12.98 ± 0.65	6.13 ± 0.57	3.91 ± 0.36
Methanol (200 mg/kg)	13.78 ± 0.16	6.73 ± 0.78	3.36 ± 0.69

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

Table 3: Effect of *M. paradisiaca* leaf extracts 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
Pet. ether (200 mg/kg)	47.81 ±1.19	37.69 ±1.61	88.38 ±1.81	1.04 ±0.27	156.63 ±10.06	5.91 ±1.8	45.61 ±1.29	8.15 ±1.69	2.05 ±0.27
Ethyl acetate (200 mg/kg)	44.94 ±1.18	36.92 ±1.53	87.74 ±1.89	0.98 ±0.15	155.15 ±10.27	6.32 ±1.5	44.32 ±1.27	7.91 ±1.55	1.79 ±0.29
Methanol (200 mg/kg)	43.96 ±1.67	35.29 ±1.83	86.29 ±1.18	0.92 ±0.35	154.36 ±10.16	6.95 ±1.6	43.69 ±1.18	7.19 ±1.33	1.19 ±0.18

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

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