ACUTE AND SUB-CHRONIC TOXICITY STUDY OF *TERMINALIA ARJUNA* LEAF IN SWISS ALBINO MICE

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Summary

In the present study, the safety profile of *Terminalia arjuna* leaf was evaluated by acute and sub-chronic toxicity study of the methanol extract of *T. arjuna* leaf (META) in Swiss albino mice. The oral median lethal dose (LD_{50}) of META was found to be 900 mg/kg body weight. For sub-chronic toxicity study META was administered at the single daily dose of 200 mg/kg for 28 consecutive days and at 29^{th} day, the hematological, histological, serum and liver 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 META treated group when compared to vehicle control group after 28 days. The results of the present study therefore indicated that *T. arjuna* leaf is safe in Swiss mice demonstrating no noticeable toxicity.

Key words: Sub-chronic toxicity, *Terminalia arjuna,* biochemical, leaf, LD_{50}.

Introduction

Terminalia arjuna Roxb. (Combretaceae), commonly known as Arjuna, is a large tree grown on the banks of rivers, streams, and dry watercourses throughout the Indian peninsula. In India, the plant has been traditionally used for several medicinal purposes. The fruits of the plant are used as a tonic (1). Externally its leaf paste is used as a cover on sores and ulcers. The bark is antidysenteric, antipyretic, astringent, cardiotonic, lithotriptic, and tonic; a powder of the bark acts as diuretic in cirrhosis of liver and gives relief in symptomatic hypertension (2, 3). A decoction of bark made with milk is given every morning on an empty stomach, or its powder with milk, as a cardiotonic (4). The powder of the bark is also given with honey in fractures and contusions with echymosis. Beside this, the extract of the bark as astringent is used for cleaning sores, ulcers, cancers etc (5). The extract of the bark is prescribed in scorpion-stings and lowering blood glucose (6). No pharmacological investigation is still less reported on *T. arjuna* leaf. Present study was aimed to investigate the acute and sub-chronic toxicity profile of methanol extract of *Terminalia arjuna* leaf (META) in Swiss albino mice to establish its safety profile in rodents.

Materials and methods

Plant material: The leaves of *T. arjuna* were collected during January 2008 from Nadia, West Bengal, India. The plant species was authenticated by Dr. Lakshmi Narashimhan, Scientist, Botanical Survey of India, Central National Herbarium, Howrah, West Bengal, India. The voucher specimen [no. CNH/I-I/(216)/2008/Tech.II/216] was maintained in our laboratory for future reference. The leaves were shade-dried with occasional shifting and then powdered with mechanical grinder passing through sieve no. 40, and stored in an air-tight container.

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.

Preparation of extract: The powdered plant material (400 g) was macerated at room temperature (24-26ºC) with methanol (850 ml) for 4 days with occasional shaking, followed by re-maceration with the same solvent for 3 more days. The macerates were combined, filtered and distilled off in reduced pressure. The resulting concentrate was vacuum dried at 40°C to yield the dry extract (META, yield: 21.45% w/w). The dry extract was kept in a vacuum desiccator until use. Preliminary phytochemical studies of META (7) revealed the presence of alkaloids, triterpenoids, tannins and flavonoids.

Animals: Adult male Swiss albino mice weighing 18-22 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 12/12 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 University Animal Ethics Committee, Jadavpur University.

Acute toxicity: The acute oral toxicity of META in male Swiss albino mice was studied as reported method (8), and its median lethal dose (LD_{50} value) was determined.

Sub-chronic toxicity: The animals were divided into two groups (n = 6). The first group received normal saline (5 ml/kg body weight p.o. and the second group received META at 200 mg/kg body weight p.o. daily for 28 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 haematological 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 META treatment. Heart, lung, liver, kidney and pancreas weights of all rats were measured immediately after post treatment sacrifice.

Hematological parameters: Collected blood was used for the estimation of hemoglobin (Hb) content; red blood cell count (RBC) (9) and white blood cell count (WBC) (10).

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

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 (11). Reduced glutathione (GSH) was determined by the reported method and was expressed as mg/100 g of liver tissue (12). 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 (13).

Histopathological studies: After sacrifice the organs like heart, lung, liver, kidney and pancreas of animals from each group were subjected for histopathological examinations. After fixing the tissues in 10% formaldehyde the tissues were dehydrated and paraffin blocks were made. Then sectioning was done at about 5-7μ. Routine histopathology was performed by using the haemotoxylin stain.

Results

The oral LD_{50} value of the methanol extract of *T. arjuna* leaf (META) in Swiss mice was found to be 900 mg/kg body weight. In sub-chronic study there were no significant changes in body weights and organ weights of mice of META 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 META treated group was found comparable to the control group without showing significant alteration in body weight and growth rate. The heamatological parameters were found practically unaltered in animals of META treated group as compared to the control group (Table 2). After 28 days of treatment no significant alterations were observed in all hepatic and renal biochemical parameters in animals of META treated group when compared with those of control group (Tables 3 and 4). Histopathological studies revealed that there were no detectable histopathological changes in all organs of treated group with respect to the control group.

Discussion

The present study was aimed to investigate the possible toxic effects of the methanol extract of *T. arjuna* leaf (META) in Swiss mice. In acute toxicity study, the oral median lethal dose (LD_{50} value) was determined. Various physical, chemical and histological parameters were studied in 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. Haematological parameters were evaluated to assess haematological toxicity of META on long term use. The results showed no deleterious effects on blood cell counts and haemoglobin content thereby suggesting that META had no toxic effect on blood and haemapoetic system.

The serum biochemical parameters were studied to evaluate the possible alterations in hepatic and renal functions influenced by META. Biochemical parameters related to hepatic functions viz. SGPT, SGOT, SALP, bilirubin, cholesterol contents exhibited no significant alterations as compared to the control mice. It is well known that almost all drugs, chemicals, xenobiotics are eliminated through renal excretion hence its was found necessary to estimate the effects of META on kidney functions. Serum biochemical parameters related to kidney function viz. urea, uric acid creatinine and total protein demonstrated no significant differences with respect to control group animals. Therefore, it can be inferred that META 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 (14). Antioxidants can retard or stop the uncontrolled generation of ROS, thus help to reduce oxidative stress-induced diseases (15). In present study, liver antioxidant parameters viz. lipid peroxidation (TBARS), reduced glutathione (GSH) and catalase activity (CAT) were estimated to ascertain the functioning of normal liver 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 META treatment.

The above mentioned findings were well supported by histopathological outcomes and no signs of histotoxicity were observed in any organ in histopathological analysis. Therefore histopathological studies definitively ascertained the sub-chronic safety data.

From the present study, it can be concluded that META although not showing very high oral LD_{50} in Swiss mice, exhibited excellent safety profile in sub-chronic toxicity at moderately higher dose as compared to the LD_{50}. Present study establishes the reliable safety profile of META when administered orally in Swiss mice offering no obvious toxicity.

Table 1: Effect of META on body weight and weight of major organs in mice.

Treatment	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
META (200 mg/kg)	20±0.9	26±1.18	0.13±0.06	0.14±0.08	1.17±1.15	0.30±0.78	0.19±0.09

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

Table 2: Effect of META on hematological parameters in mice.

Treatment	Hemoglobin (g/dl)	RBC (10^6 cells/ml)	WBC (10^3 cells/ml)
Normal control (0.9% NaCl)	13.96 ± 0.85	6.63 ± 0.54	3.15 ± 0.42
META (200 mg/kg)	13.78 ± 0.36	6.03 ± 0.36	3.36 ± 0.63

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

Table 3: Effect of META 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)	42.51 ±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
META (200 mg/kg)	42.96 ±1.18	37.33 ±1.33	85.78 ±1.9	0.96 ±0.29	153.63 ±11.6	6.90 ±1.8	42.63 ±1.29	7.15 ±1.63	1.18 ±0.29

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

Table 4: Effect of META 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)	1.15±0.5	45.54±1.8	83.27±3.3
META (200 mg/kg)	1.17±0.6	42.27±1.8	82.18±3.3

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

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