Journal of Phytology 2010, 2(8): 06–10
An Open Access Journal
ISSN: 2075-6240
Available Online: www.journal-phytology.com

REGULAR ARTICLE

ACUTE AND SUB-CHRONIC TOXICITY STUDY OF *DREGEA VOLUBILIS* FRUIT IN MICE

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SUMMARY

In present study, the safety profile of *Dregea volubilis* fruits was evaluated by acute and sub-chronic toxicity study of the petroleum ether extract of *D. volubilis* fruit (PEDV) in Swiss albino mice. The oral median lethal dose (LD50) of PEDV was found to be 900 mg/kg body weight. For sub-chronic toxicity study PEDV was administered at the single daily dose of 200 mg/kg for 28 consecutive days and at 29th 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. No detectable alterations were found in hematological biochemical and histological parameters in PEDV treated group when compared to vehicle control group after 28 days. The results of present study therefore indicate that *D. volubilis* fruit is safe in Swiss mice demonstrating no noticeable toxicity.

Key words: Sub-chronic toxicity, *Dregea volubilis*, biochemical, LD_{50}

Moulisha Biswas et al. Acute and Sub-chronic Toxicity Study of *Dregea volubilis* Fruit in Mice. J Phytol 2/8 (2010) 06-10.
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1. Introduction

Dregea volubilis Benth. (Asclepiadaceae), commonly known as *Jukti* in Bengali is a tall woody climber with densely lenticulate branches, occurring throughout the warmer regions of India and Car Nicober Islands ascending to an altitude of 1500 m. The parts of the plant have been traditionally used for medicinal purposes. The juice of the plant is used as a sternutatory and leaves are employed in application for boils and abscesses. The roots and tender stalks are used as emetic and expectorant. It is reported that an alcohol (50%) extract of the plant showed activity on the central nervous system as well as anti-cancer activity against Sarcoma 180 in mice. Two pregnane glycosides dregeosides were isolated from this plant collected from Thailand showed antitumor activities against melanoma B-16 in mice [1]. The isolation and characterization of twelve polyhydroxy C/D cis-pregnane glycosides were reported from the same plant collected from Thailand [2, 3]. Isolation of β-sitosterol, kaempherol-3-galactoside, a 2- deoxy sugar, drevogenin A, drevogenin P, D-cymarose and L-olendrose from the plant was also reported [4]. The authors reported the isolation and characterization of a novel pentacyclic triterpenoid designated as taraxerone having anti-leishmanial and anti-cancer activity on K562 leukemic cell line [5]. Present study was aimed to investigate the acute and sub-chronic toxicity profile of petroleum ether extract of *Dregea volubilis* fruit (PEDV) in Swiss albino mice to establish its safety profile in rodents.

2. Materials and Methods

Plant material

The fruits of *D. volubilis* were collected during August 2008 from South 24-Paraganas, West Bengal, India. The plant

material was taxonomically identified by Dr. Lakhmi Narashimhan, Scientist, Botanical Survey of India, Central National Herbarium, Howrah, West Bengal, India. The voucher specimen [CNH/I-I/(267)/2008/Tech.II/267] was maintained in our laboratory for future reference. The fruits 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 (450 g) was extracted with petroleum ether (60-80°C) for 72 h in the cone shaped percolator at 33°C. The solvent was distilled in reduced pressure and resulting semisolid mass was vacuum dried using rotary flash evaporator to yield a solid residue (PEDV, 5.33 % w/w). Preliminary phytochemical studies on PEDV revealed the presence of alkaloids, triterpenoids, and steroids [6].

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 ± 2°C 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 PEDV in male Swiss albino mice was studied as reported method [7], 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 PEDV 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 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 PEDV 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) [8] and white blood cell count (WBC) [9].

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 [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].

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.

3. Results

The oral LD_{50} value of the petroleum ether extract of *D. volubilis* fruit (PEDV) 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 PEDV 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 PEDV 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 PEDV 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 PEDV 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.

Table 1: Effect of PEDV 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
PEDV(200 mg/kg)	20±0.9	26±1.15	0.12±0.06	0.14±0.05	1.16±1.14	0.29±0.78	0.18±0.05

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

Table 2: Effect of PEDV 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
PEDV(200 mg/kg)	13.58 ± 0.45	6.29 ± 0.33	3.29 ± 0.54

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

Table 3: Effect of PEDV 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
PEDV (200 mg/kg)	43.09 ±1.29	37.33 ±1.45	86.92 ±1.83	0.94 ±0.29	153.45 ±10.8	7.13 ±1.8	43.06 ±1.24	7.29 ±1.33	1.27 ±0.27

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

Table 4: Effect of PEDV 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
PEDV (200 mg/kg)	1.18±0.9	42.45±1.3	80.15±1.6

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

4. Discussion

Present study was aimed to investigate the possible toxic effects of the petroleum ether extract of *D. volubilis* fruit (PEDV) 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 PEDV on long term use. The results showed no deleterious effects on blood cell counts and haemoglobin content thereby suggesting that PEDV 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 PEDV. 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 PEDV 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 PEDV 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 [13]. Antioxidants can retard or stop the uncontrolled generation of ROS, thus help to reduce oxidative stress-induced diseases [14]. 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 PEDV 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 PEDV 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 PEDV when administered orally in Swiss mice offering no obvious toxicity.

Acknowledgements

The authors are thankful to the authority of Jadavpur University, Kolkata 700032, India, for providing necessary facilities for the present study.

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