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Research Article

Evaluation of acute and sub acute hepatotoxic activity of *Trichodesma indicum* aqueous methanolic extract in mice

Nadia Perveen[1], Khwaja Zafar Ahmed[1], Tehseen Irshad[2], Nayab Latif[3], Zainab Kaleem[4], Faiza Naseer[1*]

[1] College of Pharmacy, Government College, University, Faisalabad, Pakistan
[2]Foundation University Medical College, Rawalpindi, Pakistan
[3]Department of Pharmacy, The University of Lahore, Faisalabad, Pakistan
[4]Department of Physiology and Pharmacology, Agriculture University, Faisalabad, Pakistan

ABSTRACT

Objective: Present study was carried out to evaluate acute and subacute hepatotoxicity of *Trichodesma indicum* (whole plant).

Methods: Toxicity study of *Trichodesma indicum* was carried out in Swiss mice after ingestion of the aqueous methanolic extract, during one day (acute toxicity) and after fifteen days (subacute toxicity).

Results: The results showed that the LD_{50} of the extract was higher than 4000 mg/kg and subacute treatment showed no change in weight of the liver and ALT, ALP, AST, no marked effect on bilirubin, albumin, protein decreased and globulin values increased significantly. Histopathological studies also showed necrosis and excessive vacuolation in maximum dose.

Conclusion: So, the extract showed a ladder like dose related hepatotoxicity. This showed that liver function showed mild injury by *Trichodesma indicum* in this study.

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***Correspondence to:**
Dr. Faiza Naseer
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faiza.naseer@ymail.com

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INTRODUCTION:

In Pakistan medicinal plants are great source of economic values. Nature awarded us with botanical wealth and trees grow in large number in different parts of Pakistan [1]. In Pakistan thousands of plants have medicinal values used in different parts to cure many diseases since ancient times. Herbal medicine is still used by 80% of the whole population in primary health care because of less side effects better cultural acceptability and better compatibility with the human body [2]. Herbs preparations are being used to cure of different diseases due to strong believe that all the plant preparations are safe. Due to this belief in the rural population use of herbal preparations is found abundantly. In different ethnic groups plant based preparations caused serious adverse effects and mortality [3]. Now it has become necessary to rule out the toxicity profile of herbal preparations. *Trichodema indicum* (synonyms; *Borago indica L* and *T. hirsutum*) belongs to family Borginaceae. It has worldwide distribution in Afghanistan, India, Mauritius, Philippines and in Pakistan at low altitude in Sindh, Karachi, Baluchistan, Swat, Chitral, Texila, Jhelum, Hazara, Rawalpindi, Dhamyal, Poonch, and Kashmir regions [4]. *Trichodesma indicum* herb has been used as emollient, carminative, cough suppressant [5], diuretic [6] and anti-inflammatory [7]. Extract used in ophthalmic and urinary diseases [8]. Leaves are effective in snake bite and its infusion used as depurative [9]. Root extract is potent analgesic and antipyretic [10]. Dysentery in children is treated by its oral root paste and this paste is also applied on swelled joints [4]. Dead fetus expelled out with the help of plant decoction. Flower has shown a sudorific and pictorial effect.

Material and methods

Collection and identification:
Whole plant used in experimental work was collected from Taxila Rawalpindi, Pakistan and identified by Professor Dr. Masood, Botany Department, Agriculture University, Faisalabad, Pakistan. The plant *Trichodesma indicum* was compared with a voucher specimen (No. 254-1-13) deposited at the Botany Department Herbarium at University of Agriculture Faisalabad, Pakistan.

Plant material and extraction:
The plant was washed with tap water; shade dried, powdered and soaked in the aqueous methanolic extract for seven days. The extract was prepared by a simple maceration process using 5L of aqueous methanol. The extract was evaporated under reduced pressure using a rotary evaporator at 65ºC for aqueous methanol extract. The concentrate was stored at 4ºC in a dark amber colored bottle [10].

Experimental Animals:
Swiss albino female mice approximately of the same age group having weight of 25-30g were purchased from National Institute of Health Sciences Islamabad Pakistan. They were maintained in Animal House of College of Pharmacy Govt. College, University, Faisalabad, Pakistan. They were kept in the cages, had free access to food and water and maintained under temperature controlled environment (23±2°C) with 12 hrs light –dark cycle [11].

Experimental Protocol:
50 animals were divided into one control and four treated (aqueous methanol extract) groups. 10 animals were placed in each group [12].

Group I: Control group was administered 1ml/kg normal saline per oral for 15 days.
Group II: 500mg/kg by gavage for 15 days (once a day).
Group III: 1000mg/kg by gavage for 15 days (once a day).
Group IV: 2000mg/kg by gavage for 15 days (once a day).
Group V: 4000mg/kg by gavage for 15 days (once a day).

Selection and preparation of dose for pharmacological screening
The aqueous methanol extract of the plant was suspended in normal saline to prepare four doses of 500, 1000, 2000, and 4000 mg according to animal body weight.

Preparation of stock solutions:
For 500mg/kg of plant extract, by dissolving 50mg of plant extract per one ml of aqueous methanol the stock solution was prepared; dose was administered as 1ml/100g. For 1000mg/kg dose of each plant extract, 100mg of plant extract was dissolved per 1ml of normal saline. Similarly 2000 and 4000mg/kg of plant extract was prepared by dissolving 200 and 400mg of plant extract per 1ml of normal saline respectively. Further filtration was done to remove any impurities.

Acute toxicity
The animals were observed for 24h after administering the extract serial dilutions. No change in physiological activities, general behavior or death was observed.

Sub acute toxicity
Sub acute toxicity was observed in all the groups received 500, 1000, 2000 and 4000mg/kg by gavage for 15 days (once a day). The animals were weighed on a daily basis. At the end of experimental animals were sacrificed and blood sample was collected for biochemical analysis. After blood collection, the animals' livers were removed for histopathology. The biochemical parameters evaluated include alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate amino transferase (AST), bilirubin total, albumin, protein and globulin.

Statistical analysis
The results are presented as mean ± S.D., and the statistical significance between the groups was analyzed by means of the analysis of variance (one way ANOVA).

Results and Discussions:
Rural communities of Pakistan use herbal preparations abundantly for the treatment of different diseases. *Trichodesma indicum* has been used commonly in cough remedies, snake bite, dead fetus expulsion, inflammatory, antibacterial, dysentery and diarrhea [4, 11]. The plant is important in folk medicine due to pharmacological properties, so hepatotoxic study gained importance as most of the drugs are metabolized in the liver.

Oral administration of the aqueous methanol extract of *Trichodesma indicum* in doses from 500-4000mg/kg did not produce significant changes in breathing, behavior, gastrointestinal effects and sensory nervous system effects in female albino mice. Same gender was selected to evaluate the same pattern of alteration of selected parameters and to avoid reproduction during experimental time period. All previously mentioned effects were observed during the experimental period of 24 hours. No death occurred in any of the group during the first 24h of the experiment. These results showed that there was no adverse effect of *T. indicum* in acute doses, indicating that the median lethal dose (LD50) was higher than 4000mg/kg for mice.

The results showed that aqueous methanol extract of *T. indicum* was safe in oral administration in acute doses in mice. The treatment with the extract did not decrease water and food consumption (data not shown). The body weight of animals treated with aqueous methanol extract/day during 15 days (subacute treatment) did not show any significant change when compared with the control group. Estimation of ALT, AST, ALP, protein, bilirubin and globulin commonly measured parameters for liver function test. Microscopic analysis of target organ of different animals showed changes in liver structure in comparison with the control group.

The aqueous methanol extract of *Trichodesma indicum* also showed a significant decrease in ALT at 500mg/kg to 19.60±4.51 but values of ALT increased gradually at 1000, 2000 and 4000mg/kg to 63.40±2.074. The group treated with 4000mg/kg showed highest liver necrosis as compared to control group and control group was provided 1ml/kg normal saline for 15 days [12]. Whereas AST values at a dose of 500 and 1000mg/kg increased up to 22.4±4.72 and at 2000 and 4000mg/kg decreased up to 20±5 and 18.2±2.049. ALP value increased gradually in 500 to 4000mg/kg up to 392±7.58 as shown in Table I but there was no marked increase in bilirubin as compared with the control group. Albumin, protein value decreased globulin value increased. It was also in accordance with the histopathological study, in which control group was compared to the *Trichodesma indicum* aqueous methanol extract at 500mg/kg showed minor changes hepatic tissue pattern. Figure I (B and C) had shown cell aggregates, nuclear variations and minor cell necrosis, but Figure I (D and E) had shown the disappearance of hepatocytes, hydropic degeneration, vascular degeneration, karyorrhexis, more prominent necrotic changes and chromatolysis. But this necrosis was mild and reversible.

Conclusion:
Finally, it was concluded that the plant has shown some degree of hepatotoxic injury. Toxicity is directly proportional to dose i.e. the higher the dose greater will be the toxicity. To evaluate chronic effects more studies were needed.

Table I. Effect of treatment with *Trichodesma indicum* aqueous methanol extract on biochemical parameters

Dose mg/kg	Control (n=5)	500mg (n=5)	1000mg (n=5)	2000mg (n=5)	4000mg (n=5)
ALP (U/L)	243.4±1.52	249±4.18	261.0±32.1	313±7.58	392±7.58
ALT (U/L)	32.60±1.673	19.60±4.51	41.6±2.30	42.8±2.59	63.40±2.074
AST (U/L)	13.80±1.304	14.60±0.548	22.40±4.72	20.0±5.0	18.20±2.049
Bilirubin total	0.940±0.0894	0.800±0.1225	0.520±0.0837	0.700±0.0707	0.920±0.0837
Albumin	3.740±0.894	3.30±0.100	3.120±0.1095	3.060±0.0894	3.0600±0.0894
Protein	6.92±0.0837	6.840±0.0894	6.68±0.1304	6.680±0.1304	6.58±0.1095
Globulin	3.10±0.070	3.540±0.1517	3.46±0.1517	3.180±0.1304	3.540±0.0894

Mean ± SD used to show the results; the results were compared by one way ANOVA and all the values were statistically significant $p<0.001$.

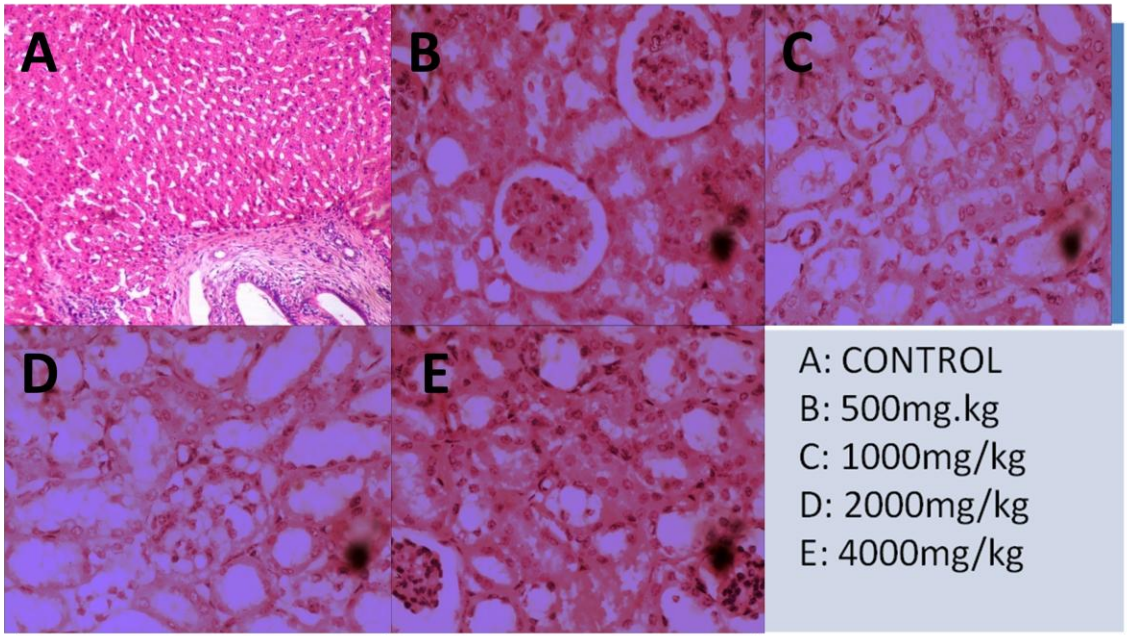

Figure 1: Histopathology of the liver sections (H&E 857X): (B and C): cell aggregates, nuclear variations and minor cell necrosis (D and E) disappearance of hepatocytes, hydropic degeneration, vacuolar degeneration, karyorrhexis, more prominent necrotic changes and chromatolysis.

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