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Hepatoprotective activity of *Trichosanthes dioica* Roxb. leaves against D-galactosamine induced hepatotoxicity in rats

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Abstract

The present study was carried out to evaluate the hepatoprotective activity of ethanolic extract of the leaves of *Trichosanthes dioica* Roxb. (ETD) against D-galactosamine induced liver damage in Sprague-Dawley rats. Hepatotoxicity was induced by D-Galactosamine (270 mg/kg body weight) administered intraperitoneally (i.p.) at 14th day of total two week experiment whereas the extract of investigated plant was given orally throughout the whole experiment at 250 and 500 mg/kg body weight. Silymarin (100 mg/kg body weight) was given orally as standard hepatoprotective drug. Pre-treatment with ethanolic extract of leaves of *Trichosanthes dioica* Roxb. was effective in protecting the liver against the injury induced by D-galactosamine in rats. The degree of hepatoprotection was evident from significant reduction in serum enzymes like alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and total bilirubin. Histopathological observations of livers sections were also supplemented these biochemical observations. It was concluded from the results that the ethanolic extract of *Trichosanthes dioica* Roxb. leaves reduces D-galactosamine induced hepatotoxicity in rats.

Keywords: ETD, D-Galactosamine, silymarin, hepatotoxicity

Introduction

The liver is the largest glandular organ in the body [1] which is involved with multiple functions like carbohydrate, protein and fat metabolism, detoxification, bile secretion and storage of vitamin [2]. But these functions are prevented by toxic chemicals, xenobiotics, alcohol consumption, malnutrition, anaemia, medications, autoimmune disorders, viral infections (hepatitis A, B, C, D, etc.) and microbial infections which cause liver damage through lipid peroxidation and other oxidative events³. As a result, serum levels of many biochemical markers like AST, ALT, ALP, TB levels are increased [3]. But several chemicals have been used to induce experimental hepatotoxicity in laboratory animals such as carbontetrachloride (CCl₄), D-galactosamine, thioacetamide, antitubercular drugs, paracetamol, and arsenic etc [4]. Among all these chemicals, D – Galactosamine induced hepatotoxicity is most commonly used models for the sort out of drugs having hepatoprotective activities. For this reason, D-galactosamine induced hepatotoxicity was selected as the experimental model [5]. The investigated extract is collected from the leaves of *Trichosanthes dioica* Roxb. belonging to family Cucurbitaceae, which is used traditionally for bronchitis, biliousness, jaundice, liver enlargement, cough and blood diseases. It is also used as antipyretic, diuretic, cardiogenic, laxative and immunoadjuvants. The leaf juice is used in fever and is rubbed over the chest in the liver congestion [6]. In spite of tremendous advances in modern medicine, there are no effective drugs available that stimulate liver function, offer protection to the liver from damage or help to regenerate hepatic cells. In absence of reliable liver-protective drugs in modern medicine, there are a number of medicinal preparations in ayurveda recommended for the treatment of liver disorders and their usage is in vogue since centuries and are quite often claimed to offer significant relief. The pretreatment with *T.dioica* extracts showed profound histopathological protection to liver cells as evident from histopathological studies from ferrous sulphate (FeSO₄) induced hepatotoxicity in experimental rats [7]. Hence the present study is focused to evaluate the hepatoprotective potentials of ethanolic extract of the leaves of *Trichosanthes dioica* Roxb. Against D-galactosamine-induced liver injury in rats.

2. Material and Methods

2.1 Collection of Plant material

The fresh leaves of *Trichosanthes dioica* Roxb. were collected from Dhamrai, Savar, Dhaka, where the plant was cultivated during month of November. The leaves washed thoroughly in tap water; shade dried and smashed into fine powder by laboratory mixer grinder.

2.2 Preparation of plant extract

The fine powder of leaf (300gm) was successfully treated with 1000 ml of ethanol for five days in soxhlet apparatus at elevated temperature (65°C). Extraction was considered to be completed when cycles of colourless liquid siphoning of the plant materials in the Soxhlet apparatus were confirmed. The resultant extract was concentrated by evaporation of solvents through rotary vacuum evaporator. The extract was weighed 24.8gm as an ethanolic extract of *Trichosanthes dioica* (ETD) which was 8.2% w/w. The extract was kept in suitable container with proper labeling and stored in cold and dry place.

2.3 Experimental Animals

Adult female rats (Sprague-Dawley strain) were collected from central animal house of the department of Pharmacy, Jahangirnagar University, Savar, Dhaka-1342. The animals were randomized and separated into normal and experimental groups of body weight ranging from 60 to 140 g.

The experimental animals were housed in plastic cages (having dimension of 30×20×13 cm and bedding was soft wood chips) under controlled conditions (temperature: 27.0±1.0°, relative humidity: 55-65%) and 12 hours dark-light cycles. They all received a basal diet of food preparation formulated by the Bangladesh Council of Scientific and Industrial Research (BCSIR) and tap water ad libitum. All the experimental animals were placed in a well-ventilated hygienic experimental animal house where adequate nutritional supply was also maintained. All experimental procedures involving animals were conducted in accordance to ethical guidelines of the Department of Pharmacy, Jahangirnagar University and approved by the Institutional Ethical Committee of Department of Pharmacy, Jahangirnagar University.

2.4 Acute Toxicity Study

The acute toxicity study was conducted to find out LC₅₀ of the test samples. The test samples were administered orally to the test animals at different concentrations (100, 250, 500, 1000, 2000, 3000 and 4000 mg/kg body weight) of the extract. After administration of the extract solutions mortality or sign of any toxicity was observed for 1 hour. Then the test animals were observed every hour for next 5-6 hours. The animals were kept under observation for 1 week^[8].

2.5 Experiment Protocol

Experimental animals were divided into following five groups. Each group consists of five rats.

Group 1: Normal Control: The animals were given normal saline water (1 ml/ kg p.o.) for 14 days.

Group 2: Positive control: The animals were given normal saline water for 13 days and D – galactosamine (270 mg/kg by I.P. route) at 14th day.

Group 3: Standard: The animals were given Silymarin (100 mg/kg p.o.) for 13 days and D - galactosamine 270 mg/kg by I.P. route at 14th day.

Group 4: *Trichosanthes dioica* Roxb. (ETD - 250 mg/kg p.o.): The animals were given ETD (250 mg/kg p.o.) for 13 days and D - galactosamine (270 mg/kg by I.P. route) at 14th day.

Group 5: *Trichosanthes dioica* Roxb. (ETD - 500 mg/kg p.o.): The animals were given ETD (500 mg/kg p.o.) for 13 days and D - galactosamine (270 mg/kg by I.P. route) at 14th day.

Rats were treated as per the treatment protocol. Body weights of these rats were monitored sequentially in control and experimental animals for a period of 14 days.

D - Galactosamine solution was prepared in distilled water and given by intra-peritoneal (I.P.) route^[8] whereas ethanolic extract solution of *Trichosanthes dioica* and Silymarin were also prepared in distilled water and given by orally.

2.6 Identification of Animals during Experiment

To identify individual animal of a group during the treatment, they were marked or coded I, II, III, IIII and none (for no. five) on their tails.

2.7 Preparation of the samples for biochemical Studies

From the post vena cava of the animal, blood sample were collected and immediately blood was transferred to the tubes having heparin. Blood samples were centrifuged for 10 minutes at 3000 rpm to separate serum for biochemical analysis i.e. Alanine aminotransferase (ALT)^[9], Aspartate aminotransferase (AST)^[10], Alkaline phosphatase (ALP)^[11], bilirubin (B)^[12] and total protein (TP)^[13-14] and albumin^[15] which were assessed by Dimension RXL (Max)/vittros-250 auto analyzer using kits in Medinova Hospital. The liver was dissected out and part of it was taken for lipid peroxidation test.

2.8 Histopathological investigation

The liver from each animal was removed after dissection. The liver lobes were fixed for 48h in 10% formalin and were embedded in paraffin. Subsequently, 5 u sections of livers were stained with haematoxylin and eosin. These sections were observed under light microscope for histological changes and compound to normal liver physiology.

2.9 Chemicals and Reagents

Table 1: Sources of Chemicals and Reagents that required for the tests

Chemicals and Reagents	Sources
D - Galactosamine	Atomax Chemicals Co.Ltd, China.
Silymarin	Square Pharmaceuticals Ltd., Bangladesh
Heparin	Rotex Medica, Germany
Pentyl IM/IV Injection (Ketamine HCl, USP, 50 mg/ml)	ACI Pharmaceuticals Ltd., Bangladesh
Bilirubin Liquicolor-Human, Total Protein Liquicolor- Human, Albumin Protein Liquicolor- Human, ALP Liquicolor- Human, AST Liquicolor-Human, ALT Liquicolor-Human	HUMAN GmbH, Germany

2.10 Statistical analysis

All the grouped data were statistically evaluated with SPSS version 16.0 software. The result of biochemical estimation was expressed as mean ±SEM (Standard error of mean)

values for five animals in each group. Means were compared by one way analysis of variance (ANOVA) followed by Dunnett.'s multiple comparison tests. Probability (p) value of 0.05 or less ($p < 0.05$) was considered as significant. Here ^a

$P \leq 0.001$ and $^b p \leq 0.05$ compared to Group II (water + D-Galactosamine). $^c P \leq 0.001$, $^d P \leq 0.01$ and $^e P \leq 0.05$ compared to Group I (Normal water).

3. Results and Discussions

The current study was designed to investigate the protective effects of *Trichosanthes dioica* Roxb. On experimentally induced hepatic toxicity in rats. Hepatic injury in the study was induced by using a single dose of D-Gal, which is a hepatotoxicant, and inducer in hepatic injury model *in vivo* [16]. It is known that, D-Galactosamine causes serum enzymes

elevation through leakage from the cell by disrupting the permeability of plasma membrane [17].

Numerous tests have been developed and employed to evaluate liver function or diseases which are based on several pathological mechanisms. Damaged hepatocytes or biliary epithelium may release cell constituents (e.g. enzymes) into blood resulting in increased levels of these analytes. The more commonly measured 'liver' enzymes are alanine aminotransferase (ALT, formerly known sGPT), aspartate aminotransferase (AST, formerly known sGOT) and alkaline phosphatase (ALP) [18-21].

Table 2: Measurement of biochemical parameters

Groups + Treatment	Total Bilirubin (IU/L)	ALT/GPT (IU/L)	AST/GOT (IU/L)	ALP (IU/L)	Total Protein (IU/L)	Albumin (IU/L)
Group I Normal water	0.22± 0.02	33.2± 6.629	57.4± 6.2817	112.0± 9.2358	47.60± 1.776	26.60± 0.9274
Group II Normal water + D-Gal.	1.824± 0.202 ^c	1498.0± 815.807 ^e	1744.8± 1040.304	136.6± 141.699 ^d	46.00± 1.7321	27.20± 1.2806
Group III Standard Silymarin + D-Gal.	0.26± 0.04 ^a	91.2± 25.158	139.4± 35.9883	443.0± 8.14002 ^b	41.40± 4.7392	27.00± 0.7071
Group IV <i>Trichosanthes dioica</i> Roxb. (ETD 250mg/kg b.w) + D-Gal	0.18± 0.02 ^a	70.2± 17.388	101.0± 28.2807	118.2± 10.2098 ^b	46.40± 1.7205	26.20± 1.0677
Group V <i>Trichosanthes dioica</i> Roxb. (ETD-500mg/kg b.w) + D-Gal.	0.25± 0.087 ^a	53.75± 7.284	64.75± 6.0467	107.5± 11.1243 ^b	46.50± 0.9574	24.25± 0.8539

Values are mean ± SEM (n = 5). ^a $P \leq 0.001$ and ^b $p \leq 0.05$ compared to Group II (water + D-Galactosamine).

^c $P \leq 0.001$, ^d $P \leq 0.01$ and ^e $P \leq 0.05$ compared to Group I (Normal water). One way ANOVA followed by Dunnett.'s multiple comparison tests.

3.1 Effects of *Trichosanthes dioica* Roxb. Leaves on Bilirubin Level

Serum bilirubin level was significantly increased in all the animals of Group II (Normal water + D-Galactosamine) in comparison with all other groups (Table 2). Treatment with ethanolic extract of *Trichosanthes dioica* Roxb. Significantly (^a $P \leq 0.001$) reduces bilirubin level although these groups (group 4 & 5) also treated with same quantity of hepatotoxic reagent, D-Galactosamine. Extract mediated suppression of the increased bilirubin level suggests the possibility of the extract being able to stabilize biliary dysfunction.

3.2 Effects of *Trichosanthes dioica* Roxb. Leaves on ALT Level

Elevation of ALT activity is found in cirrhosis of liver, obstructive jaundice, hepatic congestion and myocardial infarction. The ALT enzyme level was significantly very high (^e $P \leq 0.05$) (Table 2) in Group II (Normal water + D-Galactosamine) compared to Group I (Normal water) that was not seen in the standard and extract treated groups (Group 4 & 5).

3.3 Effects of *Trichosanthes dioica* Roxb. Leaves on AST Level

In this study, it was seen that the serum AST level is high in Group II (Normal water + D-Galactosamine) compared to

other groups. Organs rich in AST are heart, liver and skeletal muscles. Hence, plasma AST rises in myocardial infarction, muscle necrosis and/or hepatic disorders. Pre-treatment with ETD extract (at different doses level 250 and 500 mg/kg) attenuated the increased activities of AST enzyme in serum caused by D-Galactosamine.

3.4 Effects of *Trichosanthes dioica* Roxb. Leaves on ALP Level

The mean value of ALP in Group II (Normal water + D-Galactosamine) was significantly high (^d $P \leq 0.01$) compared to Group I (Normal water) whereas pre-treatment with ETD extract (at different doses level 250 and 500 mg/kg) attenuated the increased activities of this enzyme significantly (^b $p \leq 0.05$) compared to Group II (water + D-Galactosamine). Recovery towards normalization suggests that ETD extract causes parenchymal cell regeneration in liver, thus protecting membrane fragility, thereby, decreasing enzyme leakage.

3.5 Effects of *Trichosanthes dioica* Roxb. leaves on Total Protein and Albumin Level

The mean value of total protein and albumin in all experimented groups were almost same. No significant result obtained among any groups.

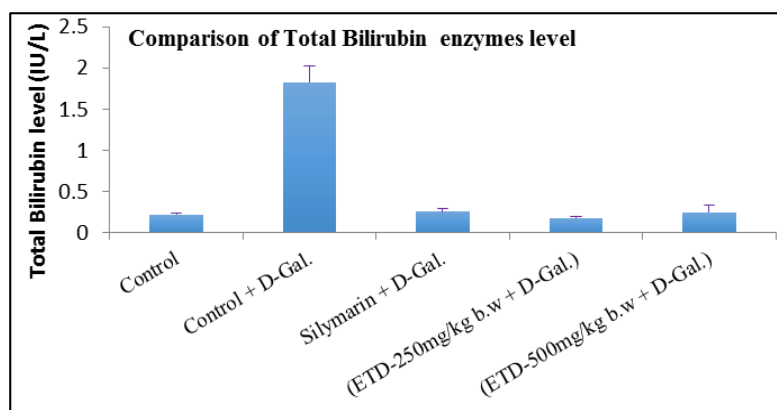


Fig 1: Comparison of Total Bilirubin enzymes level

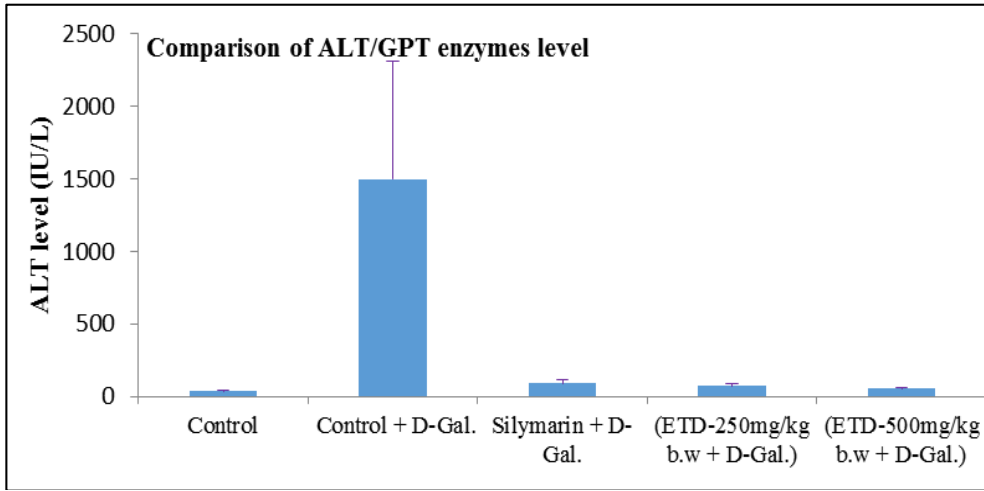


Fig 2: Comparison of ALT/GPT enzymes level

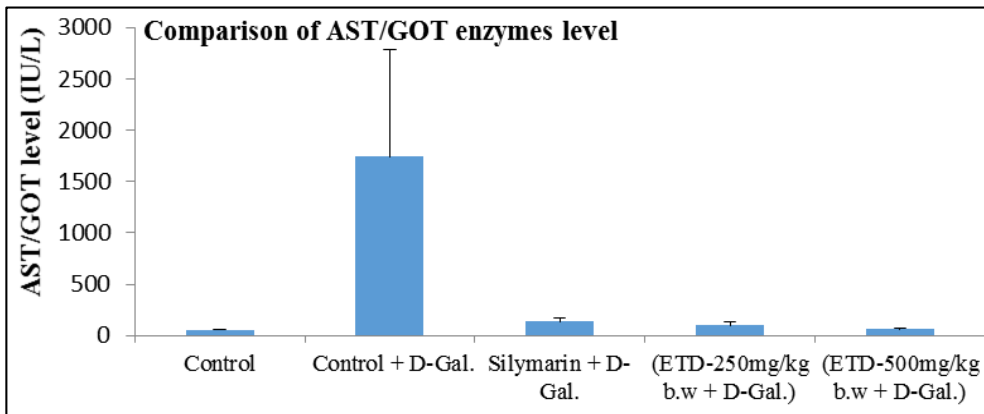


Fig 3: Comparison of AST/GOT enzymes level

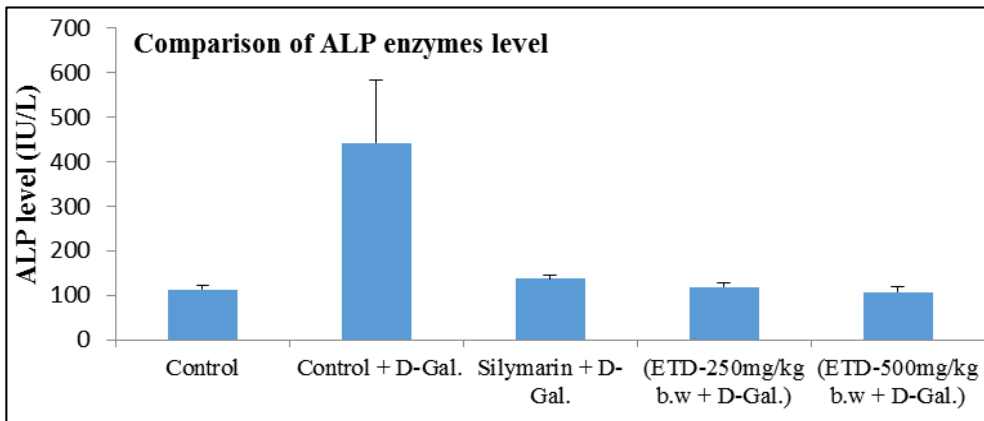


Fig 4: Comparison of ALP enzymes level

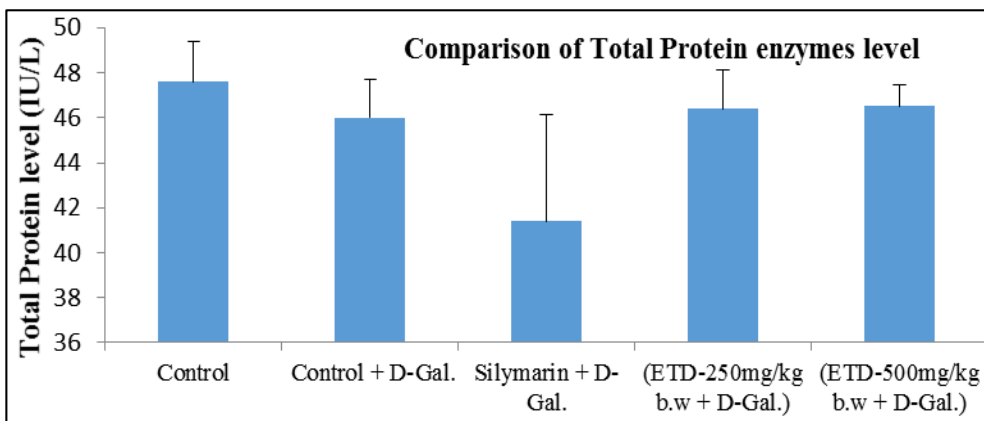


Fig 6: Comparison of Albumin enzymes level

3.6 Lipid Peroxidation Result

Table 3: Comparison of LPO level among different groups

Groups + Treatment	Mean \pm Standard Error
Group I Normal water	0.0768 \pm 0.04072
Group II Normal water + D-Gal.	0.0816 \pm 0.01334
Group III Standard Silymarin + D-Gal.	0.0430 \pm 0.00637
Group IV <i>Trichosanthes dioica</i> Roxb. (ETD 250mg/kg b.w) + D-Gal.	0.0640 \pm 0.01124
Group V <i>Trichosanthes dioica</i> Roxb. (ETD-500mg/kg b.w) + D-Gal.	0.0837 \pm 0.02438

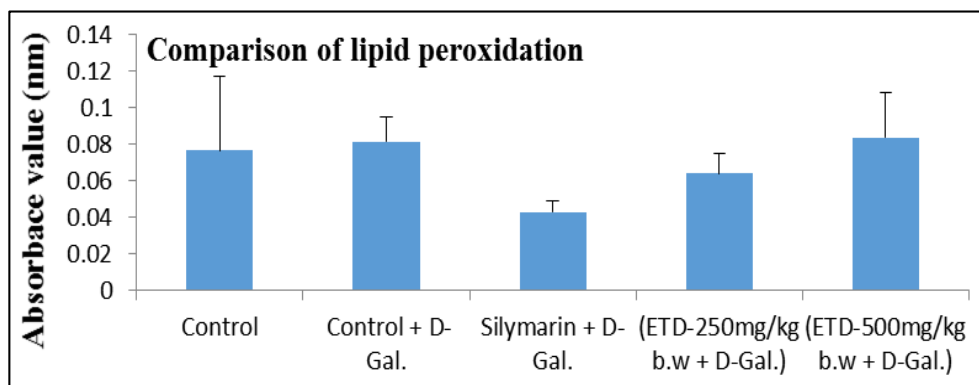


Fig 7: Comparison of LPO level among different groups

Liver damage is always associated with cellular necrosis, increase in tissue peroxidation. Silymarin reduced the lipid peroxidation (LPO) level compared to control group. In the extract treated groups, the LPO level slightly reduced in group IV (*Trichosanthes dioica* Roxb. (ETD 250mg/kg b.w) + D-Gal.) Which can be suggested due to the extract's free radical scavenging activity and the antioxidant property whereas the LPO level remained unchanged in group V (*Trichosanthes dioica* Roxb. (ETD-500mg/kg b.w) + D-Gal.).

4. Conclusion

D-Galactosamine induced hepatotoxicity is considered as an experimental model of hepatotoxicity without affecting other organs in the body. Rats treated with only D-Galactosamine showed significant increase in some biochemical parameters of serum such as ALT, AST, ALP and bilirubin which evidenced that liver damage occurred. Pre-treatment with the ethanolic extract of leaves of *Trichosanthes dioica* Roxb. (ETD) in dosages of 250 mg/kg and 500 mg/kg attenuated the increased serum levels of hepatic enzymes. Standard hepatoprotective drug, Silymarin used in dosage 100 mg/kg also showed significant reduction in elevated serum levels of hepatic enzymes. The reduction of ALT, AST and ALP towards normal values by the administration of extract (ETD) indicates the repairment of damage tissues. Histopathological study also evidenced the damaged liver toward normalization. Based on these results, it can be concluded that the ethanolic extracts of *Trichosanthes dioica* Roxb. leaves seems to have hepatoprotective effects in model rats.

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