

Hepatoprotective activity of *Cnidoscolus Phyllacanthus* leaves against D-galactosamine induced hepatotoxicity in Rats

Radheshyam Sharma*, Bharat Tyagi, Pradeep Chauhan

ABSTRACT

Introduction: Plants have been utilized as a natural source of medicinal compounds for thousands of years. Humans use numerous plants and plant-derived products to cure and relieve various physical and mental illnesses. These plants are used in traditional Chinese, Ayurveda, Siddha, Unani, and Tibetan medicines. Ancient literature such as Rigveda, Yajurveda, Atharvaveda, CharakSamhita, and Sushrut Samhita also describes the use of plants for the treatment of various health problems. In recent times, the focus on plant research has increased worldwide, and a large body of evidence has been collected to show the immense potential of medicinal plants used in various traditional systems.

Materials and Methods: The present study was carried out to evaluate the hepatoprotective activity of ethanolic extract of *Cnidoscolus Phyllacanthus* (ECP) leaves against D-galactosamine induced liver damage in Wistar rats. Hepatotoxicity was induced by D-Galactosamine (270 mg/kg body weight) administered intraperitoneally (i.p.) on the 14th day of a total two-week experiment. In contrast, the extract of the investigated plant was given orally throughout the whole experiment at 200 and 400 mg/kg body weight. Silymarin (100 mg/kg body weight) was given orally as a standard hepatoprotective drug.

Results and Discussion: Histological studies showed that 400 mg/kg CP attenuated hepatocellular necrosis in d-GalN intoxicated rats. It was concluded from the results that the ethanolic extract of *Cnidoscolus Phyllacanthus* leaves reduces D-galactosamine-induced hepatotoxicity in rats.

Keywords: *Cnidoscolus Phyllacanthus*, D-Galactosamine, ECP, Silymarin, Hepatotoxicity.

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INTRODUCTION

The liver is one of the largest organs in the human body that regulates metabolism, secretion, storage, and detoxification in our body in which hepatic damage is often linked with alterations of these functions.^[1] Most hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation (LPO) or by oxidative damage.^[2] Hepatotoxicity is a common disease which leads to serious consequences ranging from metabolic disorders to even death.^[3] In this respect, different toxic agents may induce hepatic injury. d-Galactosamine (d-GalN) is a well-known hepatotoxic agent which induces liver injury with close resemblance to human viral hepatitis, showing necrosis, inflammation, and regeneration.^[4] Noxiousness of d-GalN is mostly associated with reducing uridine pools linked to inadequate ribonucleic acid and protein synthesis, thus changing hepatocellular function.^[5] Subsequently, organelle damage and necrosis of hepatocytes take place. Along this line, research findings indicated that d-GalN directly triggers mast cells to release histamine and Kupffer cells to release tumor necrosis factor-alpha, which potentiates cell death in numerous ways, including elevation of oxidative stress and inflammatory procedure.^[6] The liver is the largest glandular organ in the body,^[7] which is involved with multiple functions like carbohydrate, protein and fat metabolism, detoxification, bile secretion, and storage of vitamins.^[8] But these functions are prevented by toxic chemicals, xenobiotics, alcohol consumption, malnutrition, anemia, medications, autoimmune disorders, viral infections

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(hepatitis A, B, C, D, etc.), and microbial infections, which cause liver damage through lipid peroxidation and other oxidative events. As a result, serum levels of many biochemical markers like AST, ALT, ALP, TB levels are increased.^[9] But several chemicals have been used to induce experimental hepatotoxicity in laboratory animals, such as carbon tetrachloride (CCl₄), D-galactosamine, thioacetamide, antitubercular drugs, paracetamol, arsenic, etc.^[10] Among all these chemicals, D – Galactosamine-induced hepatotoxicity is the most commonly used model for the sort out of drugs having hepatoprotective activities. For this reason, D-galactosamine-induced hepatotoxicity was selected as the experimental model.^[11] The investigated extract is collected from the leaves of *Cnidoscolus Phyllacanthus* belonging to the family Euphorbiaceae, traditionally used for bronchitis, biliousness, jaundice, liver enlargement,

cough, and blood diseases. The pretreatment with T.dioica extracts showed profound histopathological protection to liver cells as evident from histopathological studies from ferrous sulfate (FeSO_4) induced hepatotoxicity in experimental rats. Plants of the genus *Cnidoscopus* are considered forage for the Caatinga biome animals, mainly during the drought. These plants are still used as herbal medicine, with many properties, e.g., antitumor agent, genitourinary system, antiseptic agent, hematomas, fractures, wounds, warts, analgesic, dysentery, antibiotic, and hemorrhage. Hence the present study is focused on evaluating the hepatoprotective potentials of ethanolic extract of the leaves of *Cnidoscopus Phyllacanthus* against D-galactosamine-induced liver injury in rats.

MATERIAL AND METHODS

Collection of Plant Material

The fresh leaves of *Cnidoscopus Phyllacanthus* were collected from in and around the sheapur (MP) district, where the plant was cultivated during the month of November. And it was authenticated by Dr. R.A.S. Chauhan, H.O.D., Department of Botany, Ambah Post-Graduation College, Ambah, Morena, M.P. A voucher specimen (Bot/Sci/189/15) was identified by comparison with specimens available at the Herbarium of the Ambah Post Graduation college, Ambah, Morena, M.P. All the materials used in this study were of analytical grade purchased from Himedia Lab Limited, India.

Preparation of Plant Extract

For alcoholic extraction, the crude drug was shade dried for thirty days and then coarsely powdered by a laboratory mixer grinder. The coarse powder was loaded in a thimble made of Whatman filter no.1 and extracted in the soxhlet extraction column with 1-2 L of 95% alcohol for 30-40 cycles for each batch. The extract so obtained was thick and syrupy with a characteristic odor. The resultant extract was concentrated by evaporation of solvents through a rotary vacuum evaporator. The extract was weighed 24.8 gm as an ethanolic extract of *Cnidoscopus Phyllacanthus* (ECP), which was 8.2% w/w. The extract was kept in a suitable container with proper labeling and stored in a cold and dry place.

Experimental Animals

Healthy albino Wistar rats of either sex weighing 180-220 g were used. The animals were kept in the standard polypropylene cages at $25 \pm 20^\circ\text{C}$ and 60% relative humidity in normal day and night photo cycles (12:12 h). The animals were acclimatized for a period of 14 days prior to experimenting. 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 following ethical guidelines approved by the Institutional Animal Ethics Committee, Institute of Professional Studies, Gwalior.

Acute Toxicity Study

The acute toxicity study was conducted to find out the 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 the next 5-6 hours. The animals were kept under observation for 1-week.^[12]

Experiment Protocol

Experimental animals were divided into the 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 IP route) on the 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 IP route on the 14th day.

Group 4: *Cnidoscopus Phyllacanthus* (ECP-200 mg/kg p.o.): The animals were given ECP (200 mg/kg p.o.) for 13 days and D - galactosamine (270 mg/kg by IP route) on the 14th day.

Group 5: *Cnidoscopus Phyllacanthus* (ECP - 400 mg/kg p.o.): The animals were given ECP (400 mg/kg p.o.) for 13 days and D - galactosamine (270 mg/kg by IP route) at the 14th day. Rats were treated as per the treatment protocol. The 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 intraperitoneal (IP) route.^[12] In contrast, ethanolic extract solution of *Cnidoscopus Phyllacanthus* and Silymarin was also prepared in distilled water and given orally.

Identification of Animals during Experiment

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

Preparation of the samples for biochemical Studies

From the post vena cava of the animal, blood samples 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),^[13] Aspartate aminotransferase (AST),^[14] Alkaline phosphatase (ALP),^[15] bilirubin (B)^[16] and total protein (TP)^[17-18] and albumin^[19] which were assessed by Dimension RXL (Max)/vittros-250 auto analyzer using kits in Hospital. The liver was dissected out, and part of it was taken for lipid peroxidation test.

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 μ sections of livers

were stained with hematoxylin and eosin. These sections were observed under a light microscope for histological changes and compounds to normal liver physiology.

Statistical analysis

The Statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests. The values are represented as Mean + SEM. The probability value at $p < 0.01$ was considered statistically significant.

RESULTS AND DISCUSSIONS

The current study was designed to investigate the protective effects of *Cnidoscolus Phyllacanthus* on experimentally induced hepatic toxicity in rats. Hepatic injury in the study was induced using a single dose of D-Gal, a hepatotoxicant and inducer in the hepatic injury model in vivo.^[20] It is known that D-Galactosamine causes serum enzymes elevation through leakage from the cell by disrupting the permeability of the plasma membrane.^[21] Numerous tests have been developed and employed to evaluate liver function or diseases that are based on several pathological mechanisms. Damaged hepatocytes or biliary epithelium may release cell constituents (e.g., enzymes) into the 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).

Effects of *Cnidoscolus Phyllacanthus* 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 1). Treatment with ethanolic extract of *Phyllacanthus Cnidoscolus* Significantly ($a p \leq 0.001$) reduces bilirubin level although these groups (group 4 and 5) also treated with the same quantity of hepatotoxic reagent, D-Galactosamine. Extract mediated suppression of the increased bilirubin level suggests the possibility of the extract stabilizing biliary dysfunction.

Effects of *Cnidoscolus Phyllacanthus* Leaves on ALT Level

Elevation of ALT activity is found in liver cirrhosis, 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 (Groups 4 and 5).

Effects of *Cnidoscolus Phyllacanthus* 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 the heart, liver, and

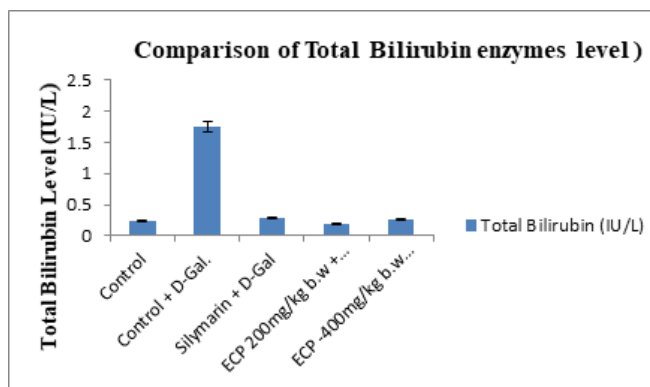


Figure 1: Comparison of Total Bilirubin enzymes level

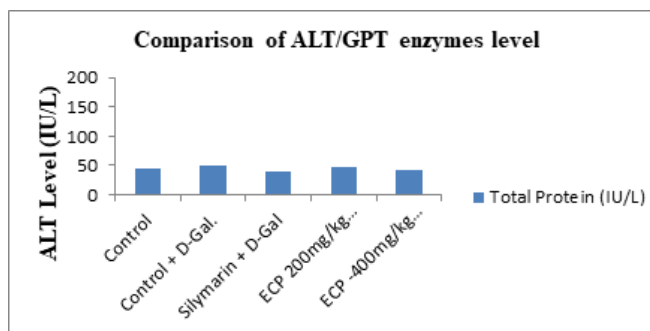


Figure 2: Comparison of ALT/GPT enzymes level

Table 1: 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	0.23 ± 0.02	31.5 ± 5.354	55.7 ± 5.748	109.1 ± 6.736	44.43 ± 1.434	25.32 ± 0.854
Group II Normal water + D-Gal.	1.745 ± 0.211c	1465.2 ± 435.81e	1624.8 ± 893.32	124.4 ± 106.78d	49.83 ± 0.942	29.73 ± 1.747
Group III Standard Silymarin + D-Gal	0.29 ± 0.07a	86.8 ± 19.19	129.7 ± 28.78	404.0 ± 6.545b	39.32 ± 3.565	25.61 ± 0.986
Group IV <i>Cnidoscolus Phyllacanthus</i> (ECP 200mg/kg b.w) + D-Gal	0.19 ± 0.01a	67.4 ± 13.74	96.3 ± 21.83	101.7 ± 8.944b	48.08 ± 1.46	29.48 ± 0.839
Group V <i>Cnidoscolus Phyllacanthus</i> (ECP -400mg/kg b.w) + D-Gal	0.26 ± 0.172a	49.73 ± 5.53	58.75 ± 4.932	99.4 ± 6.1723b	41.73 ± 0.983	22.63 ± 0.973

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.

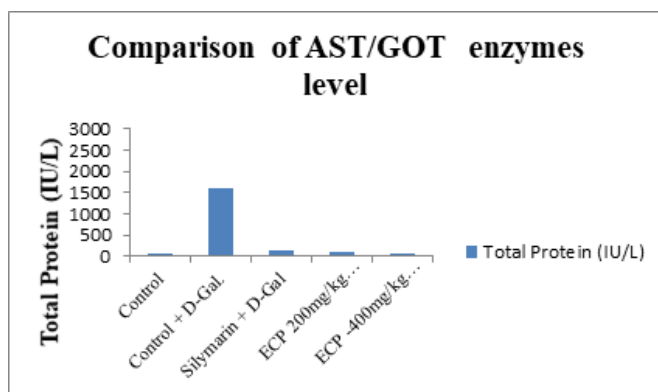


Figure 3: Comparison of AST/GOT enzymes level

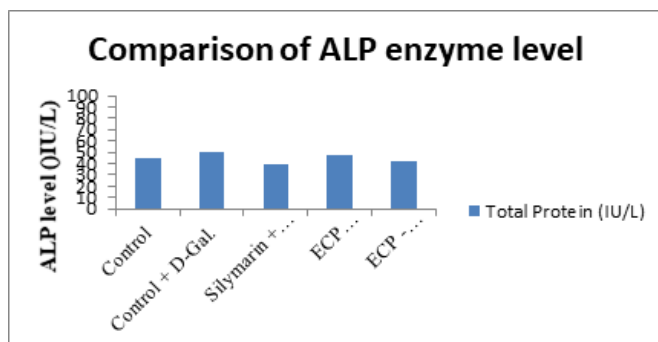


Figure 4: Comparison of ALP enzymes level

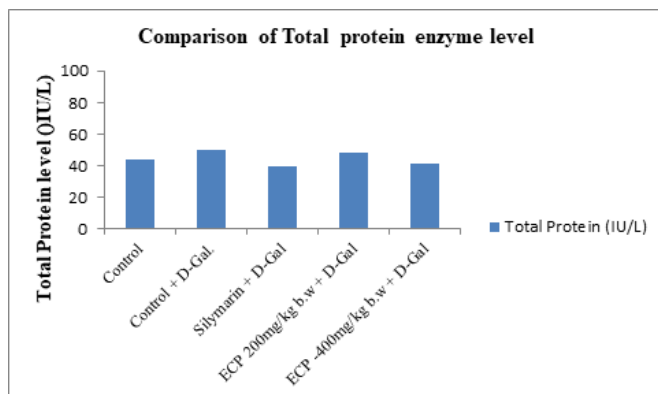


Figure 5: Comparison of Albumin enzymes level

skeletal muscles. Hence, plasma AST rises in myocardial infarction, muscle necrosis, and/or hepatic disorders. Pretreatment with ECP extract (at different doses level 200 and 400 mg/kg) attenuated the increased activities of AST enzyme in serum caused by D-Galactosamine.

Effects of *Cnidoscopus phyllacanthus* Leaves on ALP Level

The mean value of ALP in Group II (Normal water + DGalactosamine) was significantly high ($p \leq 0.01$) compared to group I (Normal water), whereas pretreatment with ECP extract (at different doses level 200 and 400 mg/kg) attenuated the increased activities of this enzyme significantly ($p \leq 0.05$) compared to Group II (water + D-Galactosamine). Recovery towards normalization suggests

that ECP extract causes parenchymal cell regeneration in the liver, thus protecting membrane fragility thereby, decreasing enzyme leakage.

Effects of *Cnidoscopus Phyllacanthus* leaves on Total Protein and Albumin Level

Total protein and albumin mean values in all experimented groups were almost identical. No significant result was obtained among any groups. Liver damage is always associated with cellular necrosis, an increase in tissue peroxidation. Silymarin reduced the lipid peroxidation (LPO) level compared to the control group. In the extract-treated groups, the LPO level was slightly reduced in group IV (*Cnidoscopus Phyllacanthus* (ECP 200mg/kg b.w) + D-Gal.) This 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 (*Cnidoscopus Phyllacanthus* (ECP-400mg/kg b.w) + D-Gal.)

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 a significant increase in serum biochemical parameters such as ALT, AST, ALP, and bilirubin, which evidenced that liver damage occurred. Pretreatment with the ethanolic extract of leaves of *Cnidoscopus Phyllacanthus* (ECP) in dosages of 200 mg/kg and 400 mg/kg attenuated the increased serum levels of hepatic enzymes. The standard hepatoprotective drug, Silymarin, used in dosage 100 mg/kg, also showed a significant reduction in elevated serum levels of hepatic enzymes. The reduction of ALT, AST, and ALP towards normal values by administering extract (ECP) indicates the repair of damaged tissues. The histopathological study also evidenced the damaged liver toward normalization. Based on these results, it can be concluded that the ethanolic extracts of *Cnidoscopus Phyllacanthus* leaves seem to have hepatoprotective effects in model rats.

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