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Research article
Hepatoprotective Activity of *Martynia annua* L. Leaves Against CCl_4-induced Hepatic Damage in Rats

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Abstract

Background and Objective: In modern medicine, there is a need for effective and safe hepatoprotective drugs to treat and prevent drug-induced liver damage. Leaves of *Martynia annua* L., (Martyniaceae) are used traditionally for their hepatoprotective effect. In the present study, methanol extract of *Martynia annua* L. leaves was selected for the evaluation of hepatoprotective activity owing to its traditional use. **Materials and Methods:** Hepatic injury in rats was carried out using the CCl_4-induced hepatotoxic model. Methanolic extracts of *Martynia annua* L. were administered orally at two different doses (200 and 400 mg kg^{-1}) daily. The biochemical parameters (SGOT, SGPT, ALP and serum bilirubin) were estimated using Reitman and Frankel's method in addition with Kind King's method. **Results:** The preliminary phytochemical studies confirmed the existence of glycosides, phenols, carbohydrates, tannins, anthocyanins and flavonoids. The CCl_4 treated group showed noteworthy boost the concentrations of Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT), alkaline phosphate (ALP) and serum bilirubin as compared to control group (rats treated with vehicle). The methanolic extract of plant 400 mg kg^{-1} and Silymarin 100 mg kg^{-1} indicated major and equipotent ($p<0.01$) decrease in raised levels of the enzymes. The histopathological examinations of liver sections of rats were supplemented with biochemical observations. **Conclusion:** The results clearly indicate that *Martynia annua* L. leaves have notable hepatoprotective activity in rats against hepatic damage induced by CCl_4.

Key words: Hepatoprotective, histopathology, *Martynia annua* L., serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphate (ALP), serum bilirubin

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Liver is one of the largest organs in human body and the chief site for intense metabolism and excretion. So it has a surprising role in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to grow, fight against disease, nutrient supply, energy provision and reproduction[1]. The major functions of the liver are carbohydrate, protein and fat metabolism, detoxification, secretion of bile and storage of vitamin. Thus, to maintain a healthy liver is a crucial factor for overall health and well being. But it is continuously and variedly exposed to environmental toxins and abused by poor drug habits and alcohol and prescribed and over-the-counter drug which can eventually lead to various liver ailments like hepatitis, cirrhosis and alcoholic liver disease[2-3]. Thus liver diseases are some of the fatal diseases in the world today. They pose a serious challenge to international public health. Modern medicines have little to offer for alleviation of hepatic diseases and it is chiefly the plant based preparations which are employed for their treatment of liver disorders. But there is not much drug available for the treatment of liver disorders[4-5]. Therefore, many folk remedies from plant origin are tested for its potential antioxidant and hepatoprotective liver damage in experimental animal model. Carbon tetrachloride (CCl_4) induced hepatotoxicity model is widely used for the study of hepatoprotective effects of drugs and plant extracts[6-7].

According to literature survey, the plant *Martynia annua* L. shows significant anthelmintic activity, anti-fertility and anti-hypertensive, wound healing, antinociceptive and CNS depressant activity, antibacterial, anti-convulsant, analgesic and antipyretic and antioxidant activity[8]. The aim of this study was to evaluate the hepatoprotective activity of *Martynia annua* L.

MATERIALS AND METHODS

Plant material: The leaves of the plant *Martynia annua* L. were collected from the Herbal Nature Park, Chuharpur, forest Division, Yamunanagar, Haryana in the month of September 2018. Taxonomically, the plant was authenticated by Department of Botany, Kurukshetra University, Kurukshetra. After collection, the leaves were washed thrice with water to remove dust or debris and air-dried. After total dryness, the leaves were ground to a coarse powder using a blender, then passed through the sieve of 40 mesh and stored in a well-closed container.

Extraction and fractionation: The powdered plant material of *Martynia annua* L. leaves was extracted in a soxhlet apparatus using petroleum ether (60-80°C) as solvents for defatting. The defatted dried powder material was again extracted with methanol. The solvent was then removed at 40°C under reduced pressure in a rota evaporator.

Drugs and chemicals: Carbon tetrachloride (CCl_4) was procured from E. Merck, Germany. Silymarin was purchased from Sigma Co. (St. Louis, MO). All other chemicals and reagents used in this study were of analytical grade.

Phytochemical screening: The preliminary qualitative phytochemical screening of methanolic extract of *Martynia annua* L. was conducted to detect the presence and/or absence of various chemical constituents like alkaloids, cardiac glycosides, flavonoids, tannins, anthraquinones, saponins, volatile oils, cyanogenic glycosides, coumarins, sterols and/or triterpenes[9].

Animals: Wistar albino rats of either sex (100-150 g) were selected for the experimental study. The procurement of rats was done from the animal house of Guru Gobind Singh College of Pharmacy, Yamuna Nagar, Haryana. The animals were kept and maintained under laboratory control conditions of humidity (60±1%), temperature (22±2°C) and 12 hour light or dark cycle. Free access to water and food was allowed to rats. Experiment protocols and procedures employed in the study were approved by the Institutional Animal Ethics Committee of Guru Gobind Singh College of Pharmacy, Yamuna Nagar, Haryana, India and confirmed with the guideline of Committee for the purpose of Control and Supervision on experiments on animals.

Acute toxicity test: The acute toxicity study[10] of methanolic extract of leaves of *Martynia annua* L. was performed by using adult rats of either sex (100-150 g) maintained under approved husbandry conditions. In accordance with the up and down method of CPCSEA for toxicity studies, the animals have fasted for 3 hours before the start of the experiment. *Martynia annua* L. extract was dissolved in distilled water and administered orally at different doses (50-1000 mg kg^{-1}) to rats. The animals kept under observation for behavioural changes from the first hour of drug intake up to seven days. The parameters, for example, hyperactivity, convulsions, hypothermia, sedation, mortality and grooming were recorded for the doses of 200 and 400 mg/kg/day.

Carbon tetrachloride-induced liver toxicity: Albino rats weighing between 150-200 g were divided into five groups (1, 2, 3, 4 and 5) (N = 6 animals/group). Group 1 animals were kept as control who received the vehicle for ten days at a dose of 1 mL kg^{-1} (p.o.). Group 2 animals were considered as positive and received dose for ten days every 72 hours. Group 3 received standard drug silymarin (100 mg kg^{-1}) and CCl_4 dispersed in sterile olive oil (1:1 v/v, 2 mL kg^{-1}, i.p.) for 10 days. Group IV-V received methanolic extract at the dose 200 and 400 mg/kg/day, respectively (dispersed in 0.5% sodium carboxymethyl cellulose) and carbon tetrachloride dispersed in sterile olive oil (1:1 v/v, 2 mL kg^{-1}) for 10 days. The rats were fasted overnight and sacrificed by using ether anesthesia at the end of the experiment. The blood samples were collected separately into the sterilized dry centrifuge tubes and separated serum was analyzed for determination of the liver enzymes markers. For histopathological examination, the liver is removed and kept in 10% formalin solution[11].

Estimation of marker enzymes and bilirubin: Reitman and Frankel's method is used for the estimation of Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT). Kind King's method is used for the estimation of alkaline phosphatase (ALP) and serum bilirubin. Jendrassik and Grofs method and cholesterol oxidase/peroxidases method are used for the determination of total bilirubin and total protein, respectively[12-15].

Histopathological evaluation: Liver tissue samples were fixed in neutral buffered formalin for 24 h. Sections of the liver tissue were histopathologically examined to study the hepatoprotective activity. The tissues were fixed in 10% buffered formalin and processed using a VIP tissue processor. The processed tissues were further dehydrated with alcohol and embedded in paraffin. For light microscope analysis, fine sections mounted on glass slides were counterstained with hematoxylin and eosin. The slides were examined microscopically for pathomorphological changes such as congestion, hemorrhage, edema and erosions using an arbitrary scale for severity assessment of these changes.

Statistical analysis: Biochemical estimation results were marked for analysis of significant intergroup difference each parameter was analyzed separately and one-way Analysis of Variance (ANOVA) was carried out. Dunnett's test was used for individual comparisons. Statistically significant results at $p<0.05$ were considered[16].

RESULTS

Phytochemical screening: Qualitative phytochemical analysis of a methanolic extract of *Martynia annua* L. leaves reveals the presence of carbohydrates, glycosides, phenolic compounds and tannins, flavonoids and saponins. The preliminary phytochemical screening of *Martynia annua* L., is depicted in Table 1.

Acute toxicity test: Oral administration of the methanolic extract of *Martynia annua* L. at a dose of 1000 mg kg^{-1} did not indicate any adverse effects or mortality when observed for 4 hrs and every-day for the next ten days. Data obtained from this study two doses i.e. 200 and 400 mg kg^{-1} were selected for further study.

Effect of biochemical markers: CCl_4 treated Group II animals showed a steep boost in the levels of enzymes i.e. SGOT, ALP, SGPT and serum bilirubin as compared to group I (untreated). However, silymarin and *Martynia annua* L. extract (200 and 400 mg kg^{-1}) treated group (III-IV) showed a sharp fall in the levels of enzymes as compared to group II i.e. CCl_4 treated group (Table 2).

Silymarin on the other hand, diminished the levels of all marker enzymes as compared to the group treated with CCl_4 only.

Effect on liver histopathology: The microscopic histopathological evaluation of livers from the control group, CCl_4 treated group, standard drug silymarin and methanolic extract have revealed the following observations.

Normal control group: The typical lobular arrangement was observed in rat liver. Hepatocytes are arranged in lobules as cords radiating around the terminal that is centrally placed hepatic veins. Hepatocytes seen are of uniform size having polyherbal shape large nuclei centrally located. The cytoplasm

Table 1: Phytochemical screening of leaves extract of *Martynia annua* L.

Components	Methanol extract
Carbohydrates	+
Proteins and amino acids	-
Fixed oil and Fats	-
Alkaloids	-
Glycosides	+
Steroids	-
Phenolic compounds and Tannins	+
Flavonoids	+
Saponins	+

+: Present, -: Absent

Table 2: Enzymatic levels of different groups

	Parameters			
Treatment	SGPT	SGOT	ALP	Serum bilirubin
Normal control	34.82±1.74	44.16±0.79	25.07±0.47	3.18±0.051
Positive control	131.7±2.25	151.1±1.95	89.34±3.19	12.6±0.63
Silymarin+CCl_4	41.82±2.71	58.32±3.30	43.12±0.91	5.23±0.55
Martynia annua L. (200 mg kg^{-1}) + CCl_4	55.82±1.70	65.65±3.38	58.73±1.02	6.29±0.28
Martynia annua L. (400 mg kg^{-1}) + CCl_4	47.66±2.36	61.16±2.77	46.67±3.21	5.85±0.43

SGPT: Serum glutamate pyruvate transaminase, SGOT: Serum glutamate oxaloacetate transaminase, ALP: Alkaline phosphate

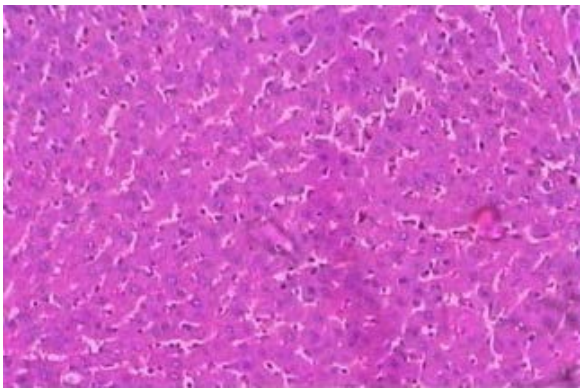

Fig. 1: Histopathology of normal control group

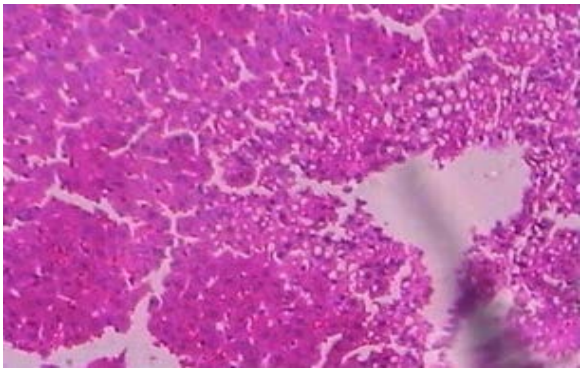

Fig. 2: Histopathology of CCL_4 treated group

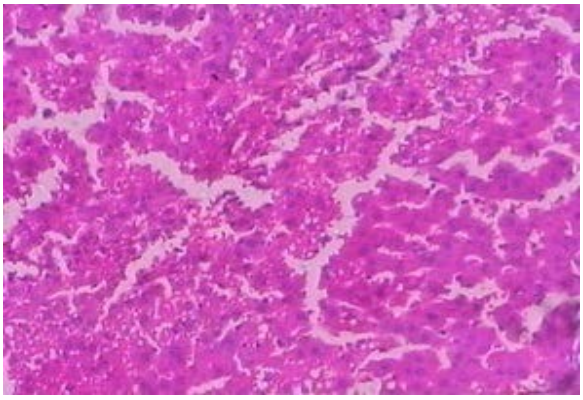

Fig. 3: Histopathology of standard silymarin group

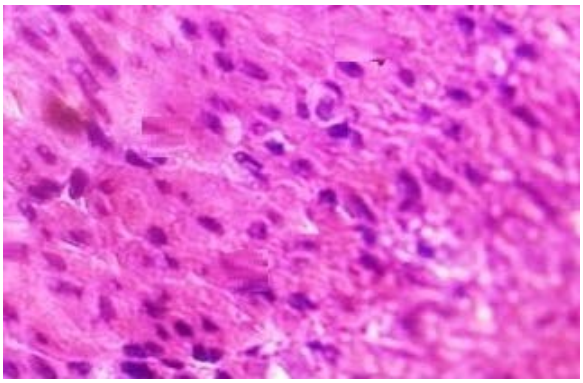

Fig. 4: Histopathology of methanol extract (200 mg kg^{-1})

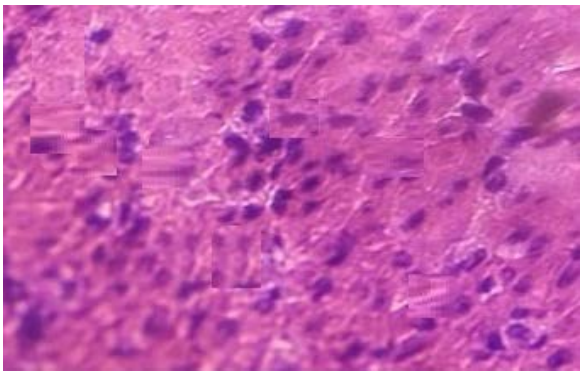

Fig. 5: Histopathology of methanol extract (400 mg kg^{-1})

is with a fine basophilic granularity is eosinophilic. Portal tracts comprising of terminal branches hepatic portal vein and hepatic artery in fibrous stoma at the periphery was observed (Fig. 1).

CCl_4 treated group: The rat liver tissue with the disturbed lobular arrangement was observed. Early necrotic and degenerative alterations extending lobules are shown in Fig. 2. There are steatotic alterations and ballooning degenerations in hepatocytes. Some amount of fibrosis seen in portal tracts. Toxic injury with fibrotic alterations and mild necrosis was observed in the liver (Fig. 2).

Standard silymarin group: Rat liver showed liver tissue with the typical lobular arrangement. Variable size hepatocytes were observed and there is an increase in fibrous connective tissues. Mild hepatotoxicity was indicated in the liver (Fig. 3).

Methanolic extract treated group: Rat liver showed liver tissue with the typical lobular arrangement. Few hepatocytes show steatotic accumulation. Liver with minimal sign of hepatotoxicity was observed in Fig. 4 and 5.

DISCUSSION

Phytochemical tests of methanolic extract of leaves of *Martynia annua* L. indicated the presence of glycosides, anthocyanins, flavonoids and phenolic compounds. In the present study, the elevated levels of SGOT, SGPT, ALP and bilirubin levels have been observed in CCl_4-treated rats, which indicate the increased permeability, damage, and/or necrosis of the hepatic cells. Administration of methanol extract of *Martynia annua* L. showed a substantial decrease in SGPT, SGOT, SALP and serum bilirubin level ($p<0.05$) at a dose of 400 mg kg^{-1} in comparison with 200 mg kg^{-1} (Table 2). Similarly, treatment with hepatoprotective drug such as silymarin showed a significant decrease in SGPT, SGOT, SALP and serum bilirubin level (Table 2). Therefore it was reported that *Martynia annua* L. extract treatments normalize few morphological features of the liver in rats with CCl_4 induced hepatitis.

Normal control group showed no histopathological changes or no abnormal appearance in the liver (Fig. 1). In comparison with the normal tissues group, CCl_4 administration caused significant hepatic damage in rat liver, as represented by disturbed lobular arrangement, ballooning degeneration in hepatocytes, hepatic cell necrosis and degenerative alterations extending lobules in Fig. 2. Hepatic management with standard drug silymarin almost restored the normal architecture of liver (Fig. 3) Apart from this the treatment with methanolic extract of *Martynia annua* L. also reduced the abnormality of liver architecture caused by CCl_4 and showed a dose-dependent restoration of the altered histopathological changes (Fig. 4 and 5).

Hepatotoxicity is connected with severe impairment of cell protection mechanisms. CCl_4 induced liver cell injury involves CCl_4 biotransformation induced by cytochrome P^{450} leading to the production of trichloromethyl free radical which causes lipid peroxidation followed by metabolic leakage from mitochondria. All these changes inhibit the damage of hepatic tissue and loss of integrity of cell membrane. CCl_4, therefore, increase SGPT, SGOT and bilirubin level and induced hepatotoxicity[17-19].

In the case of hepatotoxicity, the enzymes (SGOT, SGPT, SALP and serum bilirubin) move into the bloodstream and their amount confirms the liver damage extent. Levels of bilirubin indicate the severity of necrosis and its increased levels indicate the conjugation; binding and excretory power of hepatocyte whereas the effectiveness of extracts is indicated by decreased levels of bilirubin in liver damage initiated by chemicals[20-21].

In addition, various compounds effective against liver injury induced by CCl_4 show their protective effect by either decreasing the production of free radicals generated by CCl_4 or *via* the antioxidant property of protective agents[22-23]. Moreover, the antioxidant activity of the leaves of *Martynia annua* L. was also reported in the literature which is due to the presence of flavonoids. So it may be possible that tested plant hepatoprotective activity may be due to the presence of flavonoid content. In addition, further studies are required to identify the phytoconstituents responsible for the observed hepatoprotective activity of methanol extract and to explain the hepatoprotective mechanism.

CONCLUSION

Conclusively, the methanolic extract of *Martynia annua* L. leaves possess significant hepatoprotective activity. The hepatoprotective potential of methanol extract may be due to the presence of flavonoids, rutin and quercetin or their synergistic effect. Histopathological examinations of liver through biochemical analysis provide supportive evidence against the hepatoprotective activity.

SIGNIFICANCE STATEMENT

Despite of availability of many hepatoprotective drugs in modern medicine, there is still demand of more reliable therapeutic agents to prevent and treat drug-induced liver damage. This study evaluates the hepatoprotective activity of methanol extract of *Martynia annua* L. leaves which will help the researchers to explore the phytoconstituents present in the plant responsible for the activity. Thus, the high prevalence of hepatotoxicity compels the development of new drugs; therefore, a safe and potent drug molecule to confer protection against liver damage is urgently needed.

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