

Hepatoprotective Effect of *Tribulus terrestris* L against Acetaminophen-Induced Liver Damage in Wistar Rats

Javed Akhtar Ansari, Abdul Mateen Sayyed, R Manavalan, Balamurgan

ABSTRACT

Background: Nowadays liver diseases due to exposure of different drug molecules are very common. The aim of the present study was to investigate the effect of methanolic extract of *Tribulus terrestris* L (TT) against acetaminophen (AA)-induced liver damage in Wistar rats.

Materials and methods: Animals were divided into five groups of six rats each.

Results and discussion: Oral administration of TT (500 mg/kg) along with AA (2.5 gm/kg) showed a significant decrease in serum enzyme aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, direct bilirubin and alkaline phosphatase (ALP) levels with a decrease in total protein levels ($p < 0.01$). The results were comparable with silymarin (SL), a standard hepatoprotective drug. The present study reveals that the administration of TT can be beneficial for the suppression of liver damage. Based on present findings, it can be concluded that methanolic extract of TT protects the liver from AA-induced liver damage. The present study for the first time reports the hepatoprotective potential of TT in AA-induced liver damage in Wistar rats.

Keywords: Paracetamol, Hepatotoxicity, Liver enzymes.

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INTRODUCTION

Nowadays liver diseases due to exposure of different drug molecules are very common.¹ An attention must be paid to cure these hepatic disorders. The mainstay of treatment is supportive care, with careful monitoring for signs of acute liver failure or progression to chronic liver disease.²

The use of plants, plant extracts or plant-derived pure chemicals to treat disease is a therapeutic modality, which has stood the test of time.³ Despite phenomenal growth of modern medicine; there are almost no drugs available for the treatment of drug-induced hepatic diseases. However, there are numerous herbs/herbal formulations claimed have to possess valuable activity in treating hepatic disorders. Several hundred plants have been examined for use in a wide variety of liver disorders. Just a handful has been fairly well evaluated. The latter category of plants includes: *Silybum marianum* (milk thistle), *Picrorhiza kurroa* (kutki),

Curcuma longa (turmeric), *Camellia sinensis* (green tea), *Chelidonium majus* (greater celandine), *Glycyrrhiza glabra* (licorice), and *Allium sativa* (garlic).⁴

Tribulus terrestris L (TT; Zygophyllaceae) is an annual plant native of Mediterranean region, but now extensively distributed in the warm regions all over the world.⁵ It is employed in the folk medicine of India, China, Bulgaria and South Africa against sexual impotence, edemas, abdominal distention and cardiovascular diseases.^{5,6}

There is no scientific basis or reports in the modern literature regarding its usefulness as hepatoprotective agent against acetaminophen (AA)-induced liver damage in Wistar rat. Therefore, the present study was conducted to evaluate the effect of the methanolic extract of the TT fruits against AA-induced liver damage in Wistar rats.

MATERIALS AND METHODS

Chemicals

AA, silymarin (SL) (Micro Labs Ltd, Bengaluru, India), ethanol (SD Fine Chemicals Ltd, Mumbai, India). AA was suspended in 0.5% gum acacia before use. All other chemicals and reagents were of the highest analytical grades available.

Plant

The fruits of TT were procured from Chidambaram, Cuddalore, Tamil Nadu, India, during the month of November. The plant was identified and authenticated by Prof. Dr R Selvaraj, Chief Botanist, Department of Botany, Annamalai University, Annamalai Nagar, Chidambaram, Cuddalore, Tamil Nadu, India. A voucher specimen has been kept at the herbarium of the University.

Preparation of Extract

The fruits of TT were dried in shade, powdered and passed through a 40-mesh sieve. Dried powder (500 gm) was taken and subjected to successive extraction with petroleum ether, chloroform, methanol and water in Soxhlet apparatus. The extracts were concentrated to dry residue by distillation (temperature 60°C without vacuum) and dried completely in desiccators and weighed. The yield of the methanol extract was 19.5% w/w. The extract was freeze dried and

stored at -80°C until further use. The dried mass (yield = 50.2 gm) was diluted with normal saline and used in experiments. The methanol extract was found to be effective as hepatoprotective.

Preliminary Phytochemical Screening

Petroleum ether, chloroform, methanol and aqueous extracts of TT were subjected to preliminary phytochemical screening for their presence or absence of active constituents utilizing standard method of analyses.⁷

Animal

The study was conducted after obtaining institutional ethical committee clearance bearing the number 160/1999/CPCSEA (Committee for Control and Supervision on Experiments on Animals). Male Wistar rats (100-150 gm; 4-6 weeks old) were maintained under controlled conditions of light (12 h/12 h), temperature ($26 \pm 2^{\circ}\text{C}$) and relative humidity (44-56%) for 1 week before and during the experiments. The animals had access to standard laboratory feed (Gold Mohur, Hindustan Lever Ltd, Mumbai, India) and water ad libitum. For experimental purposes, animals were kept fasting overnight but were allowed free access to water.

Experimental Procedure

AA was suspended in 0.5% gum acacia and administered orally at a single dose of 2.5 gm/kg. This dose is known to induce liver damage in rats.⁸ Animals were divided into five groups of six animals each. Group I (normal control, NC) received saline (0.9% NaCl, oral) 1 ml/kg and group V (TT *per se*) received TT 500 mg/kg, orally, for 6 days. The group II (AA control), group III (TT + AA), group IV (SL + AA) received 0.5% gum acacia in distilled water (1 ml/kg, po), methanolic extract of TT (500 mg/kg, orally) and SL (100 mg/kg, orally) respectively, once a day for 6 days. On the fourth day, 30 minutes after the administration of the respective treatments, all the animals of groups II, III and IV were administered with AA (2.5 gm/kg, orally). On the sixth day after 2 hours of respective treatments, under ether anesthesia, blood samples were collected in centrifuge tubes via retro-orbital route, and the serum was separated.

Biochemical Analysis

The serum was separated after centrifuging the blood sample at 3,000 rpm for 15 minutes and the following enzyme analysis were quantified: Aspartate aminotransferase (AST), alanine aminotransferase (ALT),⁹ total bilirubin,¹⁰ alkaline phosphatase (ALP)¹¹ and total protein.¹²

STATISTICAL ANALYSIS

The obtained raw data in each experimental group was computed into mean and standard error of mean (SEM). Groups of data were compared with the analysis of variance (ANOVA) followed by Dunnett's test for multiple comparison of the two treatment groups with the control. Values were considered statistically significant when $p < 0.01$.

RESULTS

Preliminary Phytochemical Screening

Alkaloids, carbohydrates, cardiac glycosides, flavonoids, saponins, tannins and proteins were found to be present in methanolic and aqueous extract while steroids were absent in all extracts. Chloroform extracts also showed the presence of alkaloids.

Biochemical Observations

Effect of AA on Serum Biochemical Parameters

As shown in Table 1, administration of AA (2.5 gm/kg, orally) induced a marked increase in the serum enzyme AST, ALT, total bilirubin, direct bilirubin and ALP levels as compared to the NC group ($p < 0.01$). Total protein levels were significantly reduced in the AA control group as compared to the NC group ($p < 0.01$).

Effect of TT on Serum Biochemical Parameters

TT + AA group showed decrease in serum enzyme AST, ALT, total bilirubin, direct bilirubin and ALP levels as compared to the AA control group ($p < 0.01$). TT + AA group significantly increased the total protein levels as compared to the AA control group ($p < 0.01$) (Table 1).

Effect of SL on Serum Biochemical Parameters

SL + AA group showed decrease in serum enzyme AST, ALT, total bilirubin, direct bilirubin and ALP levels as compared to the AA control group ($p < 0.01$). SL + AA group significantly increased the total protein levels as compared to the AA control group ($p < 0.01$) (Table 1).

Effect of TT per se on Serum Biochemical Parameters

TT *per se* group showed nonsignificant changes in serum enzyme AST, ALT, total bilirubin, direct bilirubin and ALP levels as compared to AA control group ($p > 0.05$). TT *per se* group did not show a significant change in total protein levels as compared to NC group ($p > 0.05$) (Table 1).

Table 1: Effect of *Tribulus terrestris* L administration on biochemical parameters in rats exposed to acetaminophen

Parameters	NC	AA	TT + AA	SL + AA	TT per se
AST (U/L)	54.30 ± 0.315	140.02 ± 0.278 ^{##}	65.43 ± 0.304 ^{**}	59.60 ± 0.352 ^{**}	52.14 ± 0.571 ^{**}
ALT (U/L)	25.21 ± 0.496	62.11 ± 0.287 ^{##}	27.36 ± 0.450 ^{**}	20.81 ± 0.344 ^{**}	23.45 ± 0.401 ^{**}
ALP (U/L)	96.10 ± 0.194	392.13 ± 0.211 ^{##}	241.26 ± 1.353 ^{**}	228.45 ± 0.697 ^{**}	95.20 ± 0.073 ^{**}
Total bilirubin (mg/100 ml)	0.88 ± 0.008	2.16 ± 0.025 ^{##}	0.95 ± 0.010 ^{**}	0.90 ± 0.008 ^{**}	0.87 ± 0.011 ^{**}
Direct bilirubin (mg/100 ml)	0.25 ± 0.004	0.69 ± 0.023 ^{##}	0.28 ± 0.001 ^{**}	0.26 ± 0.003 ^{**}	0.25 ± 0.005 ^{**}
Total protein (gm /100 ml)	5.14 ± 0.035	3.01 ± 0.023 ^{##}	5.02 ± 0.026 ^{**}	5.10 ± 0.045 ^{**}	5.13 ± 0.068 ^{**}

NC: Normal control (0.9% NaCl, 1 ml/kg, po); AA: Acetaminophen control (2.5 gm/kg, po); TT: *Tribulus terrestris* L (500 mg/kg, po); TT + AA: *Tribulus terrestris* L (500 mg/kg, po) + acetaminophen (2.5 gm/kg, po); SL + AA: SL (100 mg/kg, po) + acetaminophen (2.5 gm/kg, po); Values are mean ± SEM of six animals in each group; ^{##}p < 0.01, as compared to NC group; ANOVA followed by Dunnett test; ^{**}p < 0.01, as compared to AA control group, ANOVA followed by Dunnett test

DISCUSSION

The present study focused on the hepatoprotective effect of TT on AA-induced liver damage in Wistar rats and found that administration of TT suppressed significantly the increases in serum enzyme AST, ALT, total bilirubin, direct bilirubin and ALP, and stimulated the decreases in total protein levels (p < 0.01).

AA, also known as paracetamol, is extensively used as an analgesic and antipyretic drug, though proved safe at therapeutic doses; AA is toxic at higher doses and causes acute liver failure characterized by centrilobular hepatic necrosis. AA can also induce renal failure and ultimately death in severe cases.¹³

Serum AST, ALT and bilirubin are the most susceptible biomarkers employed in the diagnosis of liver diseases.¹⁴ Throughout hepatocellular damage, varieties of enzymes normally located on the cytosol are released into the blood flow. Their quantification in serum is a valuable biomarker of the extent and type of hepatocellular damage.¹⁴ As expected, a single oral dose of AA (2.5 gm/kg, po) showed significant hepatotoxicity, as evidenced by a dramatic elevation of AST, ALT, ALP, bilirubin and suppression of total protein. Pretreatment along 2 days with TT showed a significant protective effect against AA-induced acute hepatotoxicity in rats. Serum aminotransferase activities have long been considered as sensitive markers of hepatic injury.¹⁵ Injury to the hepatocytes changes their transport function and membrane permeability, causes leakage of enzymes from the cells. Consequently, the significant release of ALT and ALP into the circulation indicates severe damage to hepatic tissue membranes during AA intoxication. The amplified activities of AST, ALT and ALP in the present study may be interpreted as a result of the liver cell destruction or alters in the membrane permeability representing the severity of hepatocellular damage induced by AA, which is in accordance with previous report.¹⁶ The increase in ALT activity is almost always due to

hepatocellular injury and is usually accompanied by an increase in AST. An elevation in ALP reflects the pathological alteration in biliary flow.¹⁷

CONCLUSION

The results of the present investigation confirmed that AA caused changes in the levels of several liver components. The TT as well as SL when given orally was able to target the liver enzymes, and found to improve liver enzyme contents in AA-induced Wistar rats. These results present initial evidence that TT may be useful for the treatment of liver damage and raise the possibility of a new application as a hepatoprotective therapeutic modality.

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ABOUT THE AUTHORS

Javed Akhtar Ansari (Corresponding Author)

Department of Pharmacology, MESCO College of Pharmacy Mustaidpura, Hyderabad-500006, Andhra Pradesh, India, e-mail: javed.ansari47@gmail.com

Abdul Mateen Sayyed

Department of Pharmacology, MESCO College of Pharmacy Mustaidpura, Hyderabad, Andhra Pradesh, India

R Manavalan

Faculty of Pharmacy, Annamalai University, Chidambaram Cuddalore, Tamil Nadu, India

Balamurgan

Faculty of Pharmacy, Annamalai University, Chidambaram Cuddalore, Tamil Nadu, India