ORIGINAL ARTICLE

Hepatoprotective activity of the methanolic extract of *Tylophora indica* (Burm. f.) Merill. leaves

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The methanolic extract of *Tylophora indica* leaves was screened for hepatoprotective activity in carbon tetrachloride induced hepatotoxicity in albino rats. The degree of protection was measured by estimating biochemical parameters like Serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, total protein and level of serum bilirubin (both total and direct). Hepatoprotective activity of methanolic extract at a dose of 200 mg/kg and 300 mg/kg body weight, i.p., was compared with Silymarin (25 mg/kg, i.p.) treated animals. *Tylophora indica* leaves (200 and 300 mg/kg) exhibited significant reduction in serum hepatic enzymes when compared to rats treated with carbon tetrachloride alone. Furthermore, histopathological studies were also done to support the study.

Key words: Carbon tetrachloride, hepatoprotective activity, Silymarin, *Tylophora indica*

INTRODUCTION

Tylophora indica (Burm. f.) Merill. (Family; Asclepidaceae) commonly known as Antamool in Ayurveda is a twining perennial plant distributed throughout southern and eastern part of India in plains, forests, and hilly places.[1] The plant has been reported to contain 0.2-0.46% alkaloids viz. Tylophorine, tylophorinine,[2,3] tylophorinidine, (+)-septicine, isotylocrebrine,[4] tylophorinicine, sterols, flavonoids,[5] wax, resins and tannins.

In Ayurveda, the plant has been used in treatment of asthma, dermatitis, and rheumatism.[1,6] The other reported activities include immunomodulator activity,[7] anti-inflammatory activity,[8] anticancer activity,[9] and antiamoebic activity.[10]

In the present study, the hepatoprotective effects of methanolic extract (flavonoid rich fraction) are investigated in a scientific manner to validate its use as alternative and complimentary herbal medicine.

MATERIALS AND METHODS

Plant Material

Tylophora indica leaves were collected from herbal garden, Jamia Hamdard and authenticated by the taxonomist of Department of Botany, Faculty of Science, Hamdard University. The voucher specimen was deposited in the herbarium of university for future reference.

Preparation of Extract

The air-dried and coarsely powdered leaves of plant (1 kg) were Soxhlet extracted with methanol for 72 h and the methanolic extract was concentrated on a water bath and dried under reduced pressure to get a dark brown mass (80 g).

HPTLC Fingerprint and Preliminary Phytochemical Screening

Methanolic extract of plant leaves (1 g/ml) was applied (2 µl) on silica gel G F_{254} High Performance Thin Layer Chromatography (HPTLC) plate in duplicate with bandwidth of 5 mm using Linomat V applicating device (Camag, Switzerland). The chromatogram was developed in Twin trough chamber using solvent system Toluene: Chloroform: Ethyl acetate (1:5:3) saturated with 10% acetic acid and scanned in scanner III at 366 nm wavelength using mercury lamp in fluorescence mode. On preliminary phytochemical screening of leaves of *T. indica*, extract showed positive tests for alkaloids, glycosides, steroids, and flavonoids.

Animals

The hepatoprotective activity was carried out on Wistar albino rats of either sex (120-150 g), supplied by Central animal house facility of Jamia Hamdard, New Delhi (Registration no. 173/CPCSEA). They were maintained in a 12 h light/dark cycle at 25 ± 2°C. They were allowed free access to standard pellet diet (Amrut Laboratory Rat Feed, Navamaharashtra, Pune, India) and water *ad libitum*.

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The study was approved by ethics committee CPCSEA and ethical norms were strictly followed during all experimental procedures.

Drugs and Dosing Schedule

Animals were divided into five groups; Group I (control), Group II (CCl_4 treated), Group III (CCl_4 + Silymarin treated), Group IV and V (CCl_4 + extract). Animals of group II, III, IV, and V were administered 50% (v/v) CCl_4 in olive oil at a single dose of 2 ml/kg body weight per day for 4 days by Subcutaneous (S.C.) route. Simultaneously but at different hours of the day, animals of group III, IV, and V were fed with Silymarin suspension (25 mg/kg body weight, i.p.), methanolic extract at a dose of 200 mg/kg and 300 mg/kg body weight, i.p. for 4 days, respectively. Animals of group I was given distilled water in a volume of 10 ml/kg body weight.

Serum Analysis and Histopathological Examination

On 5th day, after treatment period the animals of all groups were anaesthetized and sacrificed. Blood was withdrawn from heart and serum was separated by centrifugation at 3000 rpm at 30°C for 15 min and analyzed for various biochemical parameters; Serum transaminases viz. Serum glutamate oxaloacetate transaminase (SGOT),[11] serum glutamate pyruvate transaminase (SGPT),[11] total protein and bilirubin (direct and total).[12] The histopathological studies were also carried out by reported method.[13] Liver was removed from sacrificed animal, sliced, and washed in normal saline. Liver pieces were processed in 10% formaldehyde solution. The pieces of liver were processed and embedded in paraffin wax. Sections made were 4-6 μm in thickness. They were stained with hematoxylin and eosin and lastly photographed.

Statistical Analysis

Results of biochemical parameters are reported as mean±S.E.M. statistical significance was determined by one way analysis of variance (ANOVA) followed by Dunnet's *t*-test.[14] *P* value <0.05 was considered statistically significant.

RESULTS

High Performance Thin Layer Chromatography fingerprints of methanolic extract of *T. indica* leaves showed presence of 20 spots, confirming the presence of different class of phytoconstituents as revealed by preliminary phytochemical screening.

Administration of CCl_4 led to significant hepatocellular damage as evident from increase in serum activities of Serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) (268.39 U/ ml, 262.2 U/ml), and bilirubin (total; 3.36 mg/dl and direct; 2.16 mg/dl, respectively) concentration as compared to normal control group (46.23 U/ ml, 34 U/ml, 0.68 mg/ dl and 0.20 mg/dl, respectively). Treatment of rats with methanolic extract of leaves at a dose of 200 mg/ kg and 300 mg/kg body weight, i.p. exhibited significant reduction (P<0.05) in CCl_4 induced elevation of serum glutamate oxaloacetate transaminase (SGOT) (129.27-150.26 U/ml), serum glutamate pyruvate transaminase (SGPT) (127.6- 147.6 U/ml) and bilirubin (total;1.95-2.17 mg/ dl, direct; 1.28-1.44 mg/dl, respectively) and increased the level of TP (total protein) (3.69-4.2 g/ dl) [Table 1] in a dose dependant manner. Treatment with Silymarin also reversed the hepatotoxicity significantly. Histopathological studies of liver sections in control animals showed normal hepatic cells with prominent nucleus and central vein [Table 2]. In CCl_4 treated animals the sections showed hydropic changes in centrilobular hepatocytes with single cell necrosis, congestion of central vein and sinusoids were seen with acute and chronic inflammatory cells mainly in central zone. Pretreatment of the animals with Silymarin and methanolic extract exhibited a significant recovery of hepatocytes in different sections of the liver.

DISCUSSION

CCl_4 is one of the most commonly used hepatotoxin in the

Table 1: Effects of methanolic extract of *T. indica* leaves on SGOT, SGPT, TP and Bilirubin level in CCl_4 induced hepatotoxicity in rats

Group	Treatment	SGOT (U/ml)	SGPT (U/ml)	Bilirubin (mg/dl)		TP (g/dl)
				Total	Direct	
I	Control	46.23 ± 4.28	34 ± 2.38	0.68 ± 0.3	0.2 ± 0.05	7.42 ± 0.36
II	CCl_4 treated	268.39 ± 9.70	264 ± 11.01	3.36 ± 1.50	2.28 ± 0.19	2.16 ± 0.25
III	CCl_4 + silymarin	53.06 ± 3.94*	42.4 ± 1.43*	1.03 ± 0.46*	0.37 ± 0.02*	6.82 ± 0.48*
IV	CCl_4 + extract (200 mg/kg)	150.26 ± 6.81*	147.6 ± 3.12*	2.17 ± 0.97*	1.44 ± 0.12*	3.69 ± 0.25*
V	CCl_4 + extract (300 mg/kg)	129.27 ± 4.19*	127.6 ± 4.44*	1.95 ± 0.87*	1.28 ± 0.08*	4.22 ± 0.22*

Values are mean ± S.E.M. (n = 5), *P < 0.05 Vs CCl_4. One way analysis followed by Dunnet's *t*-test. SGOT= Serum glutamate oxaloacetate trasaminase, SGPT= Serum glutamate pyruvate transaminase, TP= Total protein

Table 2: Results of histopathological studies on the liver of CCl_4 intoxicated rats

Group	Observation
Control	Control group showed normal hepatocytes in cord pattern with portal triads and central vein
CCl_4 treated	Toxic group showed diffuse fatty changes across hepatic lobule and marked congestion with lymphocytic infiltrate. CCl_4 + silymarin standard group showed normal appearance of liver parenchyma in the periportal of mild zonal areas
CCl_4 + extract (200 mg/kg)	Extract treated livers showed mild fatty changes restricted to centribular zone with sparing of periportal hepatocytes
CCl_4 + extract (300 mg/kg)	Extract treated livers showed mild fatty changes

experimental study of liver diseases.[15] The hepatotoxic effects of CCl_4 are largely due to its active metabolite, trichloro methyl radical.[16] These activated radicals bind covalently to the macromolecules and induce peroxidative degradation of membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids. This leads to the formation of lipid peroxides, which in turn give products like malondialdehyde (MDA) that cause damage to the membrane. This is evidenced by an elevation in the serum marker enzymes. The increase in the levels of serum bilirubin reflected the level of jaundice and increase of transaminases was the clear indication of cellular leakage and loss of functional integrity of cell membrane.[17] Methanolic extract has significantly reduced these liver enzyme levels and has increased the level of total protein in the serum in a dose dependant manner, which indicates hepatoprotection. Furthermore, results of hepatocellular damage caused by CCl_4 and its recovery by methanolic extract suggest that the drug might be considered a potential source of natural hepatoprotective agents, which could be related to free radical scavenging properties of flavonoids present in the high concentration in the methanolic extract of the plant.

Histopathological studies revealed that CCl_4 caused steatosis and hydropic degeneration of the liver tissue. *T. indica* pretreatment exhibited protection, which confirmed the result of biochemical studies. Also all the effects of methanolic extract of *T. indica* were comparable with those of Silymarin, a proven hepatoprotective drug. Further isolation of active principles responsible for hepatoprotective activity is currently under progress in our lab.

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