Hepatoprotective activity of alcoholic and aqueous extracts of leaves of *Tylophora indica* (Linn.) in rats

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ABSTRACT

Objective: To investigate the hepatoprotective activity of alcoholic (ALLT) and aqueous (AQLT) extracts of leaves of *Tylophora indica* (asclepiadaceae) against ethanol-induced hepatotoxicity.

Materials and Methods: Leaf powder of *Tylophora indica* was successively extracted with alcohol and water. Preliminary phytochemical tests were done and the LD_{50} values for both extracts determined. The hepatoprotective activity of the ALLT and AQLT were assessed in ethanol-induced hepatotoxic rats.

Results: The ALLT showed presence of alkaloids, carbohydrates, steroids, saponins and triterpenes, while alkaloids, carbohydrates and saponins were present with AQLT. The ALLT did not produce any mortality even at 5000 mg/kg while LD_{50} of AQLT was found to be 3162 mg/kg. Ethanol produced significant changes in physical (increased liver weight and volume), biochemical (increase in serum alanine transaminase, aspartate transaminase, alkaline phosphatase, direct bilirubin, total bilirubin, cholesterol, triglycerides and decrease in total protein and albumin level), histological (damage to hepatocytes) and functional (thiopentone-induced sleeping time) liver parameters. Pretreatment with ALLT or AQLT extract significantly prevented the physical, biochemical, histological and functional changes induced by ethanol in the liver.

Conclusion: The present study indicates that ALLT and AQLT extracts possessed hepatoprotective activity. The alcoholic extract was found to exhibit greater hepatoprotective activity than the aqueous extract.

KEY WORDS: Ethanol, hepatoprotective activity, leaf extracts, *Tylophora indica*.

Introduction

The liver is the key organ regulating homeostasis in the body. It is involved with almost all the biochemical pathways related to growth, fight against disease, nutrient supply, energy provision and reproduction.[1] The liver is expected not only to perform physiological functions but also to protect against the hazards of harmful drugs and chemicals.

In spite of tremendous scientific advancement in the field of hepatology in recent years, liver problems are on the rise. Jaundice and hepatitis are two major hepatic disorders that account for a high death rate.[2,3] Presently only a few hepatoprotective drugs and that too from natural sources (there is not a single effective allopathic medication), are available for the treatment of liver disorders.

Tylophara indica is a branching climber or shrub that grows up to 1.5 meter found in the eastern and the southern regions of India. The leaves are ovate-oblong to elliptic-oblong, 3-10 cm long and 1.5-7 cm wide.[4] The plant has been traditionally used for the treatment of bronchial asthma, jaundice and inflammation.[4,5] Its antitumor,[6] immunomodulatory,[7-9] antioxidant,[10] anti-asthmatic,[11] smooth muscle relaxant, antihistaminic, hypotensive, antiinflammatory, analgesic, anticonvulsant, and antirheumatic[12] activities are scientifically proven. Although the leaf and root of this plant are widely used for treating jaundice in Northeastern Karnataka,[4] there is a paucity of scientific evidence regarding its usage in liver disorders. Hence, the present study was aimed to investigate the hepatoprotective activity of leaf extracts of *T. indica* in ethanol-induced hepatotoxic model in rats.

Materials and Methods

Drugs and chemicals

Silymarin was obtained from Micro Labs, Bangalore. The

kits for all biochemical estimations were purchased from Transasia Biomedicals Ltd., Daman, India. The solvents and other chemicals used were of analytical grade.

Plant material and extracts

The leaves of *T. indica* were collected during June-July of 2005 from the fields of Raichur, Karnataka; were authenticated by a botanist, Professor Srivatsa of LVD College, Raichur and a voucher specimen (C-2516) deposited. The leaves were shade-dried at room temperature and the alcoholic extract (ALLT) was obtained with 95% v/v alcohol for 18 hour, using soxhlet apparatus. The aqueous extract (AQLT) was prepared with the remaining mass by a maceration process for 7 days. The extracts were dried at 50°C in a water bath. The percentage yield of ALLT and AQLT were 11.16% and 18.22%, respectively.

Phytochemical screening

A preliminary phytochemical screening of ALLT and AQLT was carried out as described by Khandewal.[13]

Animals

Swiss albino mice (18-20 gram) and Wistar albino rats (100-150 gram) of either sex were procured from Sri Venkateshwara Enterprises, Bangalore and were acclimatized for 10 days under standard housing conditions maintained at a room temperature of 24±1°C; relative humidity 45-55% with 12:12 hour light/dark cycle. The animals had free access to rat food (Lipton Gold Mohr, India) and water. The animals were habituated to laboratory conditions for 48 hour prior to the experimental protocol to minimize any nonspecific stress. The Institutional Animal Ethics Committee of V.L College of Pharmacy, Raichur, India, approved the experimental protocol in accordance with the guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) with registration number 557/02/C/CPCSEA.

LD_{50} determination

Acute oral toxicity (AOT) of ALLT and AQLT were determined using nulliparous, non-pregnant female mice. The animals were fasted for 3 hour prior to the experiment and were administered with single dose of extracts dissolved in 2% W\V Tween 80 and observed for mortality for upto 48 hour (short-term toxicity). Based on the short-term toxicity, the dose of the next animal was determined as per OECD guideline 425.[14] All the animals were also observed for long-term toxicity (14 days). The LD_{50} of the test extracts were calculated using 'AOT 425' software provided by Environmental Protection Agency, USA.

Hepatoprotective activity

Hepatoprotective study was carried out as described by Shukla *et al*.[15] Albino rats of either sex (150-200 gram) were selected and divided into seven groups of six animals each. The animals were pretreated twice daily with vehicle (2% v/v tween 80)/ALLT (200 and 500 mg/kg)/AQLT (125 and 300 mg/kg)/silymarin (100 mg/kg) orally, 1 hour before ethanol administration. All the animals, except normal control group, received ethanol (3.76 g/kg, p.o) twice daily for a period of 25 days. On day 26, thiopentone sodium (40 mg/kg, i.p) was injected and the sleeping time recorded in all the animals.

The same animals were then anesthetized using anesthetic ether, 1 hour after complete recovery from thiopentone sodium effect and blood collected by retro orbital puncture. The levels of serum alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), direct and total bilirubin, cholesterol, triglycerides, total proteins and albumin were estimated as per the standard procedures prescribed by the manufacturer (Transasia Biomedicals Ltd., Daman, India). Immediately after the collection of, blood the animals were euthanized with an overdosage of ether; their livers removed, washed in saline and the wet weight and volume determined. Histopathology of liver was carried out by a modified method of Luna.[16] In brief, the autopsied livers were washed in normal saline and fixed in 10% formalin for 2 days followed with bovine solution for 6 hour. Then the livers were paraffin embedded and 5 μ thickness microtome sections made. The sections were processed in alcohol-xylene series and stained with haematoxylin and eosin. The slides were studied under a light microscope for any histological damage/protection.

Statistical analysis

The data are expressed as mean±SEM. Statistical differences between means were determined by one-way ANOVA followed by Tukey Kramer's *post hoc* test. Values of $P<0.05$ were considered as significant.

Results

Preliminary phytochemical studies revealed the presence of alkaloids, carbohydrates, steroids, saponins, and triterpenes in ALLT while alkaloids, carbohydrates, and saponins were noticed in AQLT. The ALLT was found to be nontoxic up to a dose of 5000 mg/kg and the LD_{50} of AQLT was found to be 3162 mg/kg.

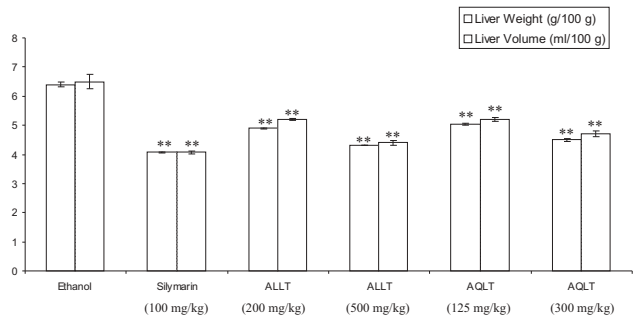
Treatment of rats with ethanol produced an increase in the weight and volume of wet liver. Rats pretreated with silymarin, ALLT and AQLT showed significant decrease in wet-liver weight and volume compared to control (toxic) group [Figure 1].

Ethanol administration resulted in significant elevation of AST, ALT, SALP, triglycerides, cholesterol, direct bilirubin and total bilirubin levels, while total protein and albumin were found to be decreased compared to normal control group. Pre-treatment with silymarin, ALLT or AQLT significantly prevented the biochemical changes induced by ethanol. The hepatoprotective effect offered by ALLT was found to be significantly greater than AQLT treatment [Table 1].

A significant reduction in thiopentone-induced sleep time was observed with both the extracts as compared to the ethanol-treated control group [Table 2]. The ALLT treatment greatly reduced the thiopentone sleep time as compared to AQLT [Figure 2].

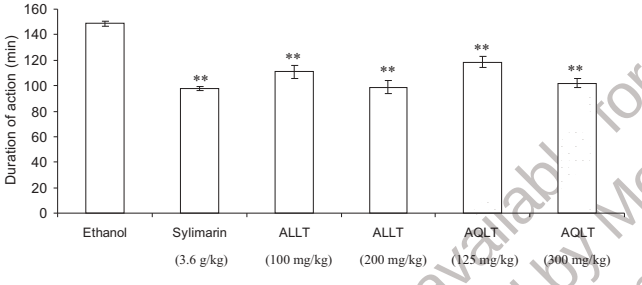
Hepatocytes of the normal control group showed a normal lobular architecture of the liver. In the ethanol-treated group the liver showed microvascular fatty changes and the hepatocytes were surrounded by large number of fat droplets. Silymarin-, ALLT- and AQLT-pretreated groups showed minimal fatty changes [Figure 3] and their lobular architecture was normal, indicating the hepatoprotective effect of these extracts. However, AQLT showed more microvascular fatty changes

Figure 1: Effect of ALLT and AQLT on liver weight and volume in ethanol induced hepatotoxic rats



Results are expressed as Mean ±SEM obtained from 6 animals. ***P*<0.01 vs ethanol treated group.

Figure 2: Effect of ALLT and AQLT on thiopentone induced sleeping time in ethanol induced hepatotoxic rats



Results are expressed as Mean ±SEM obtained from 6 animals. ***P*<0.01 vs ethanol treated group.

[Figure 3E] than ALLT. The hepatoprotective activity of the extracts were in the order of Silymarin > ALLT > AQLT.

Discussion

The liver can be injured by many chemicals and drugs. In the present study ethanol was selected as a hepatotoxicant to induce liver damage, since it is clinically relevant. Ethanol produces a constellation of dose-related deleterious effects in the liver.[17] In chronic alcoholics, hepatomegaly occurs due to accumulation of lipids and proteins in hepatocytes,[18] with an impaired protein secretion by hepatocytes.[19] Water is retained in the cytoplasm of hepatocytes leading to enlargement of liver cells, resulting in increased total liver mass and volume[20] as observed in the present study. This alcohol-induced increase in total wet-liver weight and volume was prevented by pretreatment with *Tylophora indica* leaf extracts, thus indicating a hepatoprotective effect.

During hepatic damage, cellular enzymes like AST, ALT and ALP present in the liver cells leak into the serum, resulting in increased concentrations.[21] Ethanol administration for

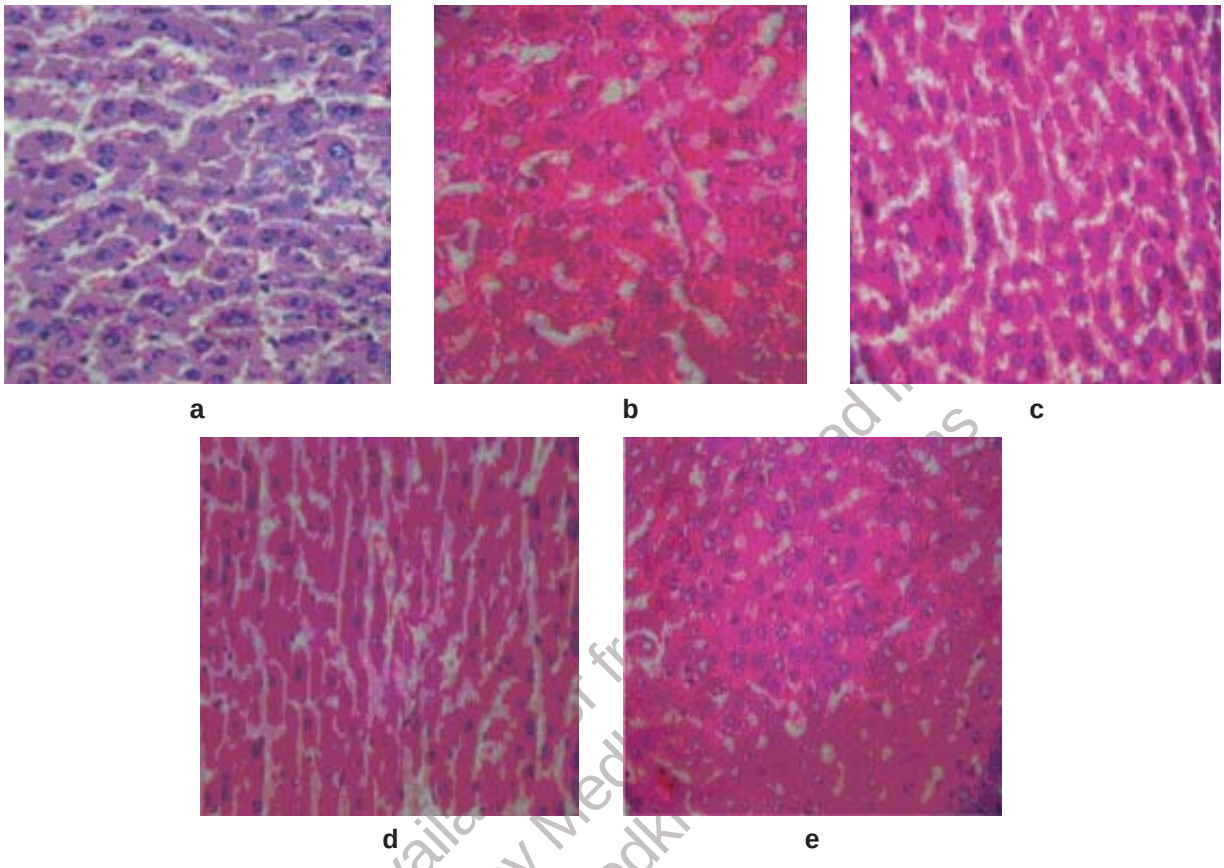
Table 1

Effect of ALLT and AQLT on different biochemical parameters in ethanol-induced hepatotoxic rats

Treatment (mg/kg)	*erum biochemical parameters*								
	Alanine transaminase U/L	*Aspartate transminase U/L*	*Alkaline phosphatase U/L*	*Direct bilirubin U/L*	*Total bilirubin U/L*	*Cholesterol U/L*	*Triglycerides U/L*	*Total protein mg/dL*	*Albumin mg/dl*
Normal control	30.94 ± 1.75	96.23 ± 10.83	96.14 ± 4.46	0.12 ± 0.014	0.19 ± 0.021	155.35 ± 12.56	30.53 ± 1.45	6.72 ± 0.16	4.90 ± 0.133
Ethanol control (3600) bid	128.24 ± 5.56	317.2 ± 11.99	215.45 ± 6.65	1.313 ± 0.23	1.75 ± 0.18	347.08 ± 5.363	227.37 ± 8.81	3.507 ± 0.18	2.15 ± 0.048
Silymarin (100) bid + ethanol	46.26 ± 1.90**	100.66 ± 3.1**	108.13 ± 1.87**	0.14 ± 0.01**	0.24 ± 0.011**	184.45 ± 9.90**	36.48 ± 3.78**	5.67 ± 0.18**	4.28 ± 0.068**
ALLT (200) bid + ethanol	47.50 ± 1.52**	178.14 ± 7.42**	143.29 ± 2.03**	0.21 ± 0.02**	0.52 ± 0.045**	225.18 ± 7.37**	73.80 ± 7.87**	4.39 ± 0.21**	3.17 ± 0.014**
ALLT (500) bid + ethanol	42.53 ± 1.98**	106.21 ± 2.56**	109.59 ± 1.93**	0.16 ± 0.01**	0.28 ± 0.025**	198.5 ± 13.7**	62.21 ± 2.89**	5.06 ± 0.26**	4.126 ± 0.13**
AQLT (125) bid + ethanol	63.39 ± 2.51**	255.87 ± 12.32**	157.95 ± 2.85**	0.24 ± 0.07**	0.72 ± 0.095**	253.3 ± 10.1**	114.05 ± 10.7**	3.79 ± 0.26**	3.10 ± 0.064**
AQLT (300) bid + ethanol	46.55 ± 2.80**	130.4 ± 3.26**	114.9 ± 1.57**	0.19 ± 0.02**	0.53 ± 0.026**	215.37 ± 3.25**	63.93 ± 1.9**	4.47 ± 0.27**	3.74 ± 0.11**
F=	127.09	106.11	140.33	22.088	45.01	41.97	113.60	25.47	100.25
df=	41	41	41	41	41	41	41	41	41
P<	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

Results are expressed as mean ±SEM, *n*=6, ***P*<0.01 *vs* ethanol-intoxicated group using one-way ANOVA followed by Tukey Kramer's *post hoc* test.

Figure 3: Histology of liver showing normal hepatocytes (a), ethanol induced microvascular fatty changes surrounded by large number of small fatty droplets (b), hepatocytes in groups treated with silymarin (c), ALLT (500 mg/kg) (d) and AQLT (300 mg/kg) (e) prior to administration of ethanol showing minimal fatty changes



25 days significantly increased all these serum enzymes, whereas the *Tylophora indica* leaf extract- (ALLT or AQLT) pretreated animals had significantly reduced AST, ALT, and SALP levels and increased total protein and serum albumin levels, indicating their hepatoprotective effect against alcohol -induced liver cell damage.

Ethanol induces hypercholesteremia and hypertriglyceridemia, which may be due to the activation of enzyme HMG CoA reductase, the rate-limiting step in cholesterol biosynthesis.[22] The increased serum triglyceride level in ethanol-treated rats may be due to the decreased activity of lipoprotein lipase, which is involved in the uptake

Table 2

Effect of ALLT and AQLT on liver weight and liver volume and thiopentone-induced sleeping time in ethanol-induced hepatotoxic rats

Treatment p.o.	*Mean liver weight (g/100g)*	*Mean liver volume (mL/100g)*	*Thiopentone induced sleeping*	
			Onset(s)	*Duration (min)*
Normal control (2% v/v tween 80, 1 mL/kg)	3.77 ± 0.070	3.05 ± 0.26	173.5 ± 4.0	87.5 ± 1.18
Ethanol control p.o.(3.76 g/kg)	6.43 ± 0.022	6.53 ± 0.043	83.67 ± 4.60	148.83 ± 2.02
Silymarin (100 mg/kg) + ethanol	4.075 ± 0.021**	4.07 ± 0.048**	159.17 ± 4.30**	97.83 ± 1.35**
Alcoholic extract (200 mg/kg) + ethanol	488 ± 0.048**	5.22 ± 0.086**	144.83 ± 2.51**	111.16 ± 5.13**
Alcoholic extract (500 mg/kg) + ethanol	4.336 ± 0.035**	4.452 ± 0.074**	154.33 ± 4.18**	98.66 ± 5.08**
Aqueous extract (125 mg/kg) + ethanol	5.05 ± 0.052**	5.27 ± 0.10**	127.5 ± 4.23**	118.67 ± 4.36**
Aqueous extract (300 mg/kg) + ethanol	4.55 ± 0.040**	4.74 ± 0.094**	142.33 ± 4.96**	102 ± 3.62**
F=	388.33	79.105	48.323	30.898
df=	41	41	41	41
P<	0.0001	0.0001	0.0001	0.0001

Results are expressed as mean ± SEM, n=6, **P<0.01 *vs* ethanol-treated group using one-way ANOVA followed by Tukey Kramer's *post hoc* test

of triglyceride-rich lipoprotein by the extrahepatic tissues.[22] Pretreatment with ALLT or AQLT reduced the elevated cholesterol and triglyceride levels, suggesting that the extracts prevented ethanol-induced hyperlipidemia probably due to their hepatoprotective activity.

Ethanol also alters the metabolic activity of hepatocytes, thereby inducing hepatic damage. Barbiturates are a class of xenobiotics that are extensively metabolized in the liver. Deranged liver function leads to delay in the clearance of barbiturates, resulting in a longer duration of hypnotic effect.[23] In the present study, administration of thiopentone sodium to rats pretreated chronically with alcohol resulted in an increased duration of thiopentone sleep time. Pre-treatment with *Tylophora indica* leaf extracts decreased thiopentone-induced sleep time, an indirect evidence of their hepatoprotective effect.

Histological changes such as steatosis (fatty changes in hepatocytes) and perivenular fibrosis were observed in ethanol-treated (toxic) control group. Both the extracts prevented these histological changes, further indicating their hepatoprotective activity. All the histological changes observed were in correlation with the physical, biochemical, and functional parameters of the liver.

Ethanol, even after short-term consumption, induces CYP2E1 enzyme activity in doses that do not cause fatty changes. This enzyme accelerates alcohol metabolism with a resultant increase in acetaldehyde production.[24] Acetaldehyde is thought to have a number of adverse effects like decreased transport and secretion of proteins owing to tubulin polymerization, enhanced vitamin metabolism and trace metals and drugs like paracetamol cause severe acute liver injury which is sometimes fatal.[25-27] Antioxidants exhibit hepatoprotective activity by blocking the conversion of ethanol to acetaldehyde.[28] From the previous studies it was found that *Tylophora indica* has exhibited an antioxidant property[10] which may be responsible for the hepatoprotective activity of these two extracts. The presence of secondary metabolites like alkaloids, steroids, saponins, and triterpenes in these extracts may be responsible for the hepatoprotective activity. Moreover, better hepatoprotective activity observed with ALLT may be due to the presence of steroids. Further work is in progress to isolate and characterize the active principles in the extracts.

It can be concluded that *Tylophora indica* leaf extracts possess a protective effect against ethanol-induced hepatotoxicity in rats, as evidenced by the physical, biochemical, functional and histological parameters.

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