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Pharmacological Evaluation Of Turnera Aphrodisiaca Leaves For Hepatoprotective Activity In Albino Rats

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Abstract

Objective: The present study is done to evaluate the hepatoprotective activity of ethanolic extract of Turnera aphrodisiaca leaves against ethanol - induced liver damage in male Wistar rats.

Methods: Rats were divided into five different groups each having six. Group 1 served as a Normal control, Group 2 received 40% ethanol (2 ml/100 g, oral), in sterile water, Groups 4 and 5served as extract treatment groups and received a dose of 200 mg/kg, and 400 mg/kg, orally, ethanolic leaf extract of Turnera aphrodisiaca (ELTA) and Group 3 served as standard group and received silymarin at a dose of 25 mg/kg orally, treatment protocols followed 21 days, and after which rats were sacrificed, the liver was taken for biochemical and histological studies, respectively.

Results: It was found that ethanolic leaf extract of Turnera aphrodisiaca (ELTA) at a dose of 200 and 400 mg/kg body weight showed significant hepatoprotective activity by lowering the alkaline phosphate (ALP), Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), and total bilirubin contents and also significantly increased the levels of total protein. The results of histopathological studies further supported this hepatoprotective activity. Ethanolic leaf extract of Turnera aphrodisiaca at dose of 400 mg/Kg showed significant hepatoprotective activity that is equivalent to silymarin.

Conclusion: It is concluded that the alcoholic extract of Turnera aphrodisiaca leaves protects against ethanol-induced oxidative liver injury in rats.

Keywords: Ethanol, Turnera aphrodisiaca, hepatoprotective, ELTA.

1. Introduction

Complementary and alternative medicines paly prominent role in the treatment of various tropical diseases. Plant based products i.e herbal extracts or phytochemicals significantly contribute for the treatment of hepatic disorders[1]. Liver is a vital organ responsible for drug metabolism, that is a sensitive target site for biotransformation of substances[2]. Excessive consumption of alcohol, exposure to toxic chemicals, infections and autoimmune disorders are the main etiological factors for hepatic disorders.

The hepatotoxic chemicals by inducing lipid peroxidation and other oxidative stress in liver damages liver cells[3]. Ethanol by hepatic microsomal cytochrome P-450 converts into reactive toxic metabolites which results in hepatotoxicity[4].

Hepatic diseases have become one of the major causes of morbidity and mortality due to alcohol abuse over the counter drugs other toxicants. All these make the liver vulnerable to a variety of disorders such as hepatitis, cirrhosis and jaundice[5]. Therefore, in the present study, the ethanol induced acute model have been used to assess hepatoprotective activity.

Phytochemical studies on T. aphrodisiaca revealed that the plant contains tetraphyllin B (cyanoglycoside), gonzalitosin I (flavonoid), arbutin (phenolic glycoside), damianin, tricosan-2-one, hexacosanol (hydrocarbons); a volatile oil containing α-pinene, β-pinene, p-cymene and 1, 8-cineole and β-sitosterol (phytosterol) [6-13].

2. Materials and Methods

2.1 Collection, authentication and extraction of Plant material:

Turnera aphrodisiaca leaves were collected from a local market and authenticated by Botanical Survey of India, Deccan Regional Centre, Hyderabad. Shade dried leaves were powdered and extracted using 80 % ethanol in Soxhlet apparatus for 8 h[14]. Then, the extract was evaporated to dryness and the final dry extract was stored in dark at -20 °C until used for the experiments. The percentage yield of extract was 17.7 % (w/w).

2.2 Chemicals

All chemicals, solvents used for this research were procured from Ranbaxy Fine Chemicals Ltd., Mumbai. Silymarin was obtained as gift sample from Cadila Pharma Ltd. Standard kits for SGOT, SGPT, ALP, and bilirubin were obtained from Span Diagnostics Ltd, India.

3. Experimental animals

Wistar albino rats, weighing about 150-180 g of either sex were used. Animals were acclimatized with 12 h light / dark cycle; 25^{o} ± 2^{o} C, 45-60% RH and were fed standard rat feed and water ad libitum for a week before commencement of experiment. This experimental protocol was reviewed and approved by the Institutional Animal Ethical Committee (IAEC) and the care of the laboratory animals was taken as per the CPCSEA (1475/PO/Re/S/11CPCSEA) regulations.

3.1 Ethanol induced hepatotoxicity

Treatment protocol [15]

Thirty Wistar Albino male rats of weight 150-250 g were selected for this study. Animals were divided into five groups of six animals each.

Group 1: Normal Control group (distilled water) for 21 days.

Group 2: Diseased control group , Inducer (Ethanol 2 ml/100 g body weight, p.o.) for 21 days

Group 3: Silymarin 25 mg/kg body weight p.o. + ethanol 2 ml/100 g body weight, p.o. for 21 days

Group 4: Ethanolic extract of the leaves of Turnera aphrodisiaca (ELTA) 200 mg/kg body weight p.o. + ethanol 2 ml/100 g body weight, p.o. for 21 days
Group 5: Ethanolic extract of the leaves of Turnera aphrodisiaca (ELTA) 400 mg/kg body weight p.o. + ethanol 2 ml/100 g body weight, p.o. for 21 days.

3.2 Assessment of hepatoprotective activity

As per the treatment protocol, all animals were treated for 21 days. On 22nd day, blood was collected from animals by puncturing retro orbital plexus. Serum was separated by centrifugation at 2500 rpm at 30°C for 15 min and utilized for the estimation of various biochemical parameters including SGOT & SGPT [16], ALP [17], serum bilirubin [18] and serum protein [19]. All the animals were then sacrificed and weight of liver, weight of animals, were observed to evaluate the protective effect of the drug. Hepatoprotective drugs reduces liver weight/100 gm body weight of rats [20, 21].

3.3 Histopathology studies

A small portion of liver tissue was excised from all the animal groups. The excised liver tissues were kept in 10% buffered formalin for 48h and then fixed in bovine solution for 6 h, followed by processing with paraffin embedding. The sections of 5µ thickness were taken and mounted with eosin and hematoxylin. These samples were examined under magnification of 100X using a light microscope [22].

4. Statistical analysis

The results are expressed as mean ± standard error of the mean. The data was analysed using one-way ANOVA followed by Dunnett's multiple comparisons tests. $p<0.05$ were considered statistically significant.

5. RESULTS

5.1 Effect of ELTA on SGOT, SGPT and ALP levels

There was a significant elevation in the levels of serum marker enzymes like SGOT, SGPT etc, content of ethanol intoxicated animals. In contrast, pretreatment with ELTA (400 & 200 mg/kg, po) and silymarin (25 mg/kg, po) exhibited an ability to counteract the hepatotoxicity by decreasing serum marker enzymes. The results were depicted in Figure1.

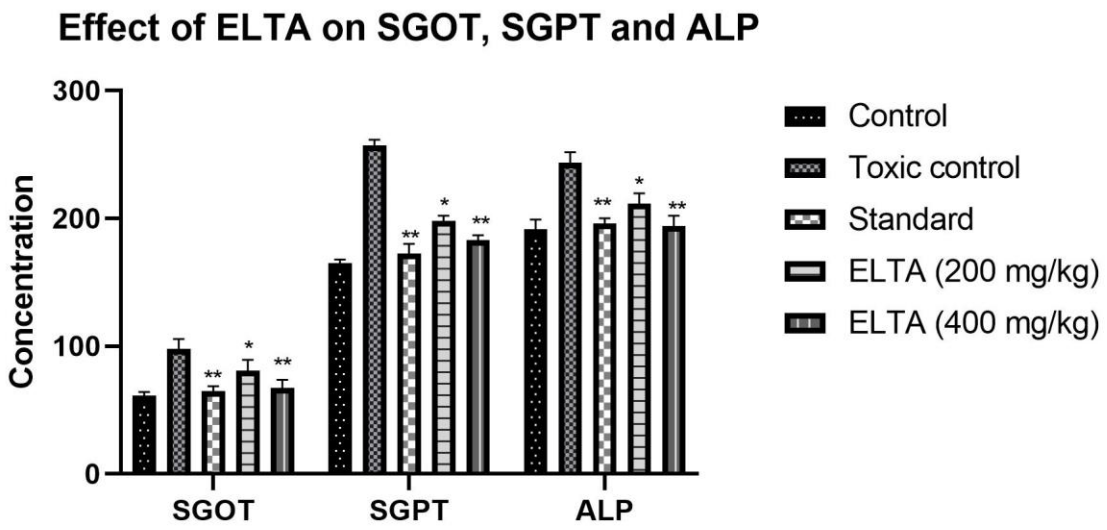

Figure 1: Effect of ELTA on SGOT, SGPT and ALP

Values are mean ±SEM, n= 6. * P<0.05, ** P<0.00, when compared with Diseasedcontrol

5.2 Effect of ELTA on Total bilirubin and Total protein

In ethanol treated groups, there was a significant increase in total bilirubin and significant reduction in total protein content. Whereas, pretreatment with ELTA (400 & 200 mg/kg, po) caused significant reduction in total bilirubin and significant increase in total protein. The results were showed in Figure 2.

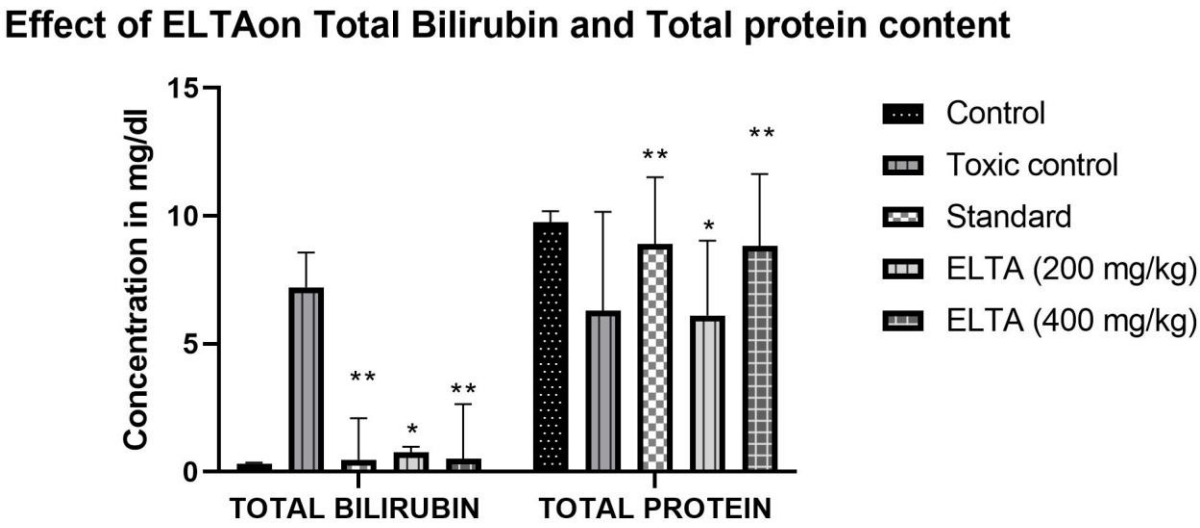

Figure 2: Effect of ELTA on Total bilirubin and Total Protein

Values are mean ±SEM, n= 6. * P<0.05, ** P<0.01, when compared with Diseasedcontrol

5.3 Effect of ELTA on liver weight

Ethanol intoxicated group of animals, weight of the liver was significantly increased, but it was normalized in ELTA (400 & 200 mg/kg, po) treated groups of animals. A significant reduction in liver supports this finding. The results were showed in Figure 3.

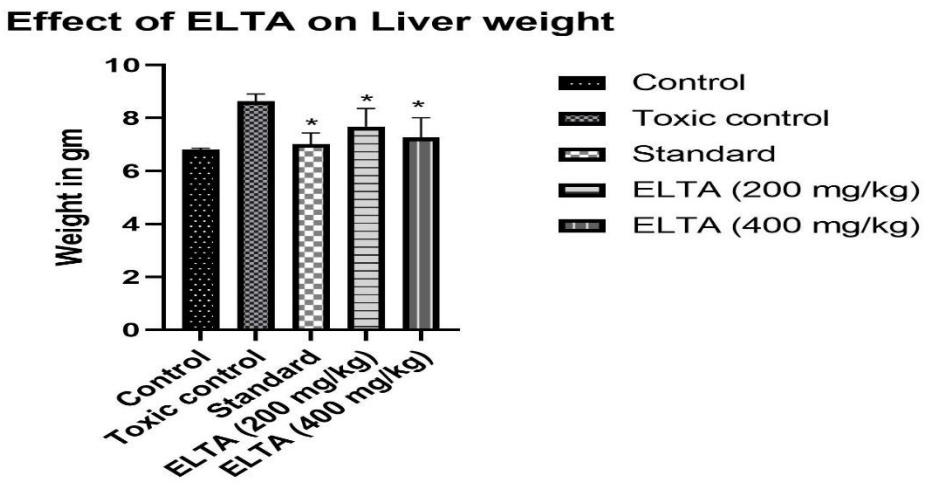

Figure 3: Effect of ELTA on Liver weight

Values are mean ±SEM, n= 6. * P<0.05, when compared with Diseased control

5.4 Histopathology

Hepatocytes are normal in cell morphology with moderate eosinophilic cytoplasm contains fine chromatin. Hepatic vein, central vein, and portal triads are in normal in position (Figure 4).

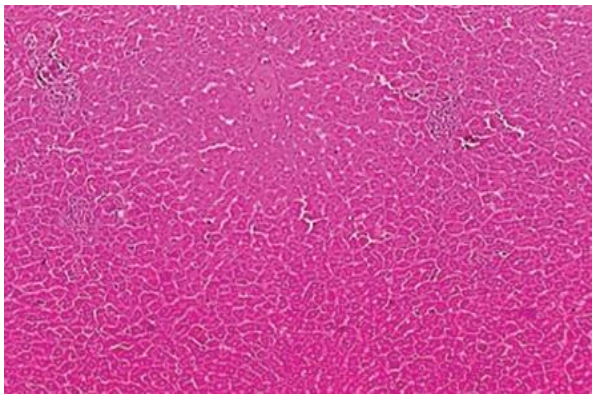

Figure 4.

There is ballooning degeneration of hepatocytes with patchy and perivascular inflammatory component comprising lymphomononuclear cell aggregates. There is mild central vein dilation, massive fatty changes and broad infiltration of the lymphocytes and the loss of cellular boundaries seen in toxicant ethanolic group [23] (Figure 5).

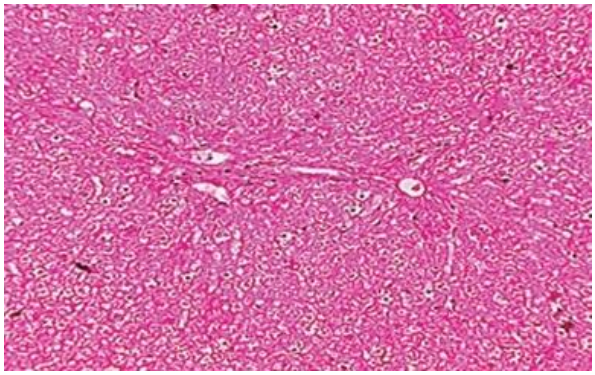

Figure 5.

The liver section show still persisting of degenerated hepatocytes with sparse inflammatory component. Bile duct, central vein, and portal triad are normal in ELTA (400 mg/kg) (Figure 6).

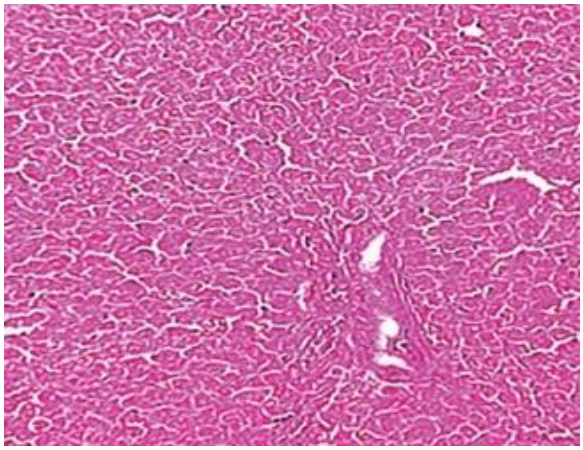

Figure 6.

The liver section show disappearance of fibrous septae and hepatocytes appeared to be normal. Very less mononuclear inflammatory infiltration is found in ELTA (200 mg/kg) (Figure 7).

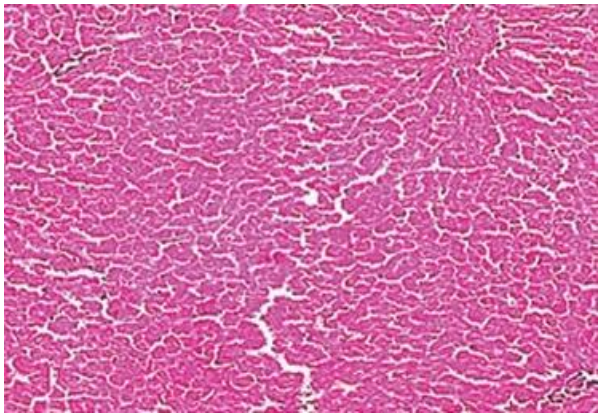

Figure 7

The liver section shows hepatocytes are normal in cell pattern and arrangement. No inflammatory component, no thromobosis, portal triad, central vein, and bile duct are more or less normal in standard silymarin (25 mg/kg) (Figure 8).

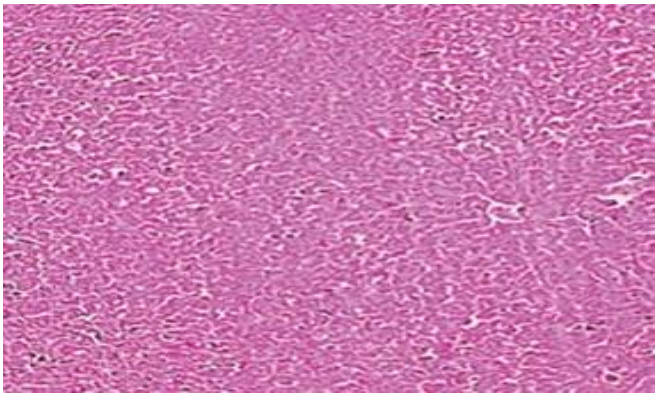

Figure 8

6. Discussion

In this research, hepatotoxicity was induced using clinically relevant ethanol. Ethanol causes dose related deleterious effects on the liver [24]. Normally liver metabolizes maximum amount of alcohol and people who abuse alcohol by routine drinking about 4 to 5 drinks of ethanol per day will be at high risk for developing alcoholic liver disease [25]. Administration of ethanol causes an increase in the formation of cytokines, which have a significant role in acute or chronic liver injury [26-28]. Liver enlargement and protein accumulation along with the development of fatty liver (steatosis), are early signs of excessive ethanol consumption that are common findings in alcoholics and heavy drinkers [29, 30].

Increased levels of SGOT, SGPT and ALP are indications of hepatocellular injury [31]. In our work, ELTA at a dose level of 200 & 400 mg/kg, po produced a significant reduction in the levels of SGOT, SGPT, and which is an indication for repair of hepatic tissue damage caused by ethanol. Similarly, with concurrent depletion of raised bilirubin level and an increase in the total plasma protein content suggests the protective effect of our extract in biliary dysfunction caused in hepatic injuries with toxicants [32].

The results of current research indicates that ELTA restored the structural uniformity of the liver cell architecture, which was confirmed by histopathological examination.

Studies related to Phytochemical screening revealed about the presence of active constituents such as phytosterols, alkaloids, flavonoids, and phenolic compounds. However, it was a known fact that phenolic compounds, flavonoids, tannins possess hepatoprotective activity[33]. Therefore, we conclude that the hepatoprotective activity of ELTA can be due to presence of these active constituents.

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