Pravara Med Rev; March 2025, 17 (01), 79 – 84
DOI: 10.36848/PMR/2025/22111.60100

Original article

Ameliorating potential of ethanolic extract of Terminalia paniculata bark on liver function biomarkers in experimental animals.

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Abstract

Background- Alcohol usage has been associated with a variety of negative effects out of which the liver suffers the most. Plants due to their rich phytochemicals can be a source to treat different pathologies.
Aim - The present study was conducted to evaluate the hepatoprotective activity of the ethanolic extract of Terminalia Paniculata against ethanol-induced liver damage in rats.
Materials & Methods- The ethanolic extract of Terminalia Paniculata (100mg/kg) was administered orally to the animals with hepatotoxicity induced by Ethanol (5gm/kg). Silymarin (25mg/kg) was given as a reference standard. All the test drugs were administered orally.
Results - The plant extract effectively protected the liver against the injury induced by Ethanol in rats. This was evident from a significant reduction in serum enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP).
Conclusion- It was concluded from the result that the ethanolic extract of Terminalia Paniculata possesses hepatoprotective activity against ethanol-induced hepatotoxicity in rats.
Keywords - ethanolic extract , Terminalia paniculata bark , liver function biomarkers

Introduction

Globally, the most prevalent addiction worldwide is alcohol consumption. According to the National Survey on Drug Use and Health, 85.6% of people over the age of 18 have admitted to drinking alcohol at some point in their lives[1]. The most significant organ in the human body is the liver, which is crucial for metabolism, detoxification, and excretion of various endogenous and foreign substances[2]. The most severe illness is liver disease, which is primarily brought on by toxic chemicals (such as excessive alcohol usage, high paracetamol doses, carbon tetrachloride, chemotherapeutic drugs, peroxidized oil, etc.)[3]. Alcohol consuming has been linked to several side effects, including cancer, gastrointestinal problems, genitourinary system alterations, myopathies, psychosis, and neurological conditions [1, 4]. In experimental hepatopathy, alcohol is frequently utilized as a liver toxin. There is evidence that ethanol-induced liver injury is caused by oxidative stress, which results in fibrosis and reduced liver functions, even though the aetiology of alcohol-induced liver disease is not fully understood[5, 6].

Despite the allopathic medical system's explosive growth, synthetic antioxidants and organ-protectants are not readily available. As a result, researchers worldwide are looking for hepatoprotective and organ-protective herbal medicines [2] and its application in the prevention and treatment/cure of various illnesses is gaining more and more attention[7, 8]. The beneficial effects of plants are attributed to the presence of phytochemicals metabolites such as flavonoids, polyphenols, tannins, terpenoids, alkaloids, and ellagic acids [9]. Numerous

studies have demonstrated the protective effects of antioxidant-rich natural substances against various toxicant-mediated tissue injuries [10, 11, 12.] Specifically, only few studies have demonstrated the protective effects of antioxidants such as Curcumin, quercetin, resveratrol and pumpkin oil, against alcohol-induced tissue injury indicating there is a need of remedy[13-17].

Terminalia paniculata (commonly known as Kindal tree) is a tropical tree, found in the Western Ghats. This plant has been utilized in traditional Ayurvedic medicine since ancient times to cure a variety of illnesses, such as fever, inflammation, cholera, and issues with menstruation. This plant is known to have hepatoprotective activity against hepatotoxins like paracetamol, and carbon tretrachloride[18, 19]. The plant also possesses antioxidant, anti-inflammatory, and anti-diabetic activities[20].

However, since ALD leads to liver failure, there are few medications available for treating it; liver transplantation is the sole treatment available for ALD sufferers.

There is no information on the hepatoprotective effects of *Terminalia paniculata* in ethanol-induced hepatotoxicity in rats so in this study examines the plant's hepatoprotective potential by assessing a variety of biochemical parameters in alcohol-induced hepatoxicity.

Materials and Methods

The present study was carried out in the Department of Pharmacology, Teegala Ram Reddy College of Pharmacy, Hyderabad, Telangana. The chemical reagents required for the study were procured from SRL laboratories and Sigma-Aldrich. The protocol of the study was approved Institutional Ethics Committee.

1. **Preparation of plant extract**.

One kilogram of *Terminalia paniculata* bark was dried in the shade and ground in a suitable grinder to a coarse powder. The resulting powder was employed in the extraction procedure (Soxhlet extractor) and kept in a hygienic, sealed glass container. The resulting extract was concentrated and evaporated under a vacuum to entirely eliminate the solvent, yielding a dark crimson semisolid residue The plant was authenticated by Professor, Department of Pharmacognosy, Challa Malla Reddy College of Pharmacy, Hyderabad.

2. Animal experiments.

Adult male albino Wistar rats (180-220 g) were procured from Gen-Tox Bio Services, Hyderabad. The animals received water at regular times and standard laboratory food. The handling and usage of rats in the study were rigorously followed as governed by CPCSEA. Rats were divided into 5 groups for the experimental investigation. 6 rats per group were encased. All drugs were given orally.

Group I (control): Rats treated with normal saline (5ml/kg body weight).

Group II: (E) Rats treated with ethanol (5g/kg body weight) each day for 28 days using intragastric tubes.

Group III: (E-TP) Rats receiving ethanol (5g/kg body weight) along with Terminalia paniculata (100 mg/kg body weight) regularly for 28 days.

Group IV: (E-SM) Rats receiving ethanol (5g/kg body weight) along with reference drug silymarin (25 mg/kg body) every day for 28 days.

Group V: (E-TP-SM) Rats receiving ethanol (5 g/kg body weight,) along with reference drug silymarin (25 mg/kg body weight) and extract of Terminalia paniculata (100 mg/kg body weight,) for regularly for 28 days.

Sample Preparation

On 29th day, all rats were euthanized by isoflurane anaesthesia, and the blood samples (2ml) were collected from retro-orbital venous plexus in the collection tubes. The serum was separated through centrifugation at 3000(rpm) for 15 minutes and stored at -20 °C and used for the analysis of various biochemical liver parameters like AST, alanine aminotransferase (ALT), ALP and tissue homogenate for analysis of ADH(Alcohol dehydrogenase).

Biochemical liver parameters analysis

The analysis of AST, ALT, and ALP enzyme activities was done according to the instructions available in the commercial diagnostic kits (Enzo lab diagnostic kits). The method of Mohur et al, (1975) was used for SGOT (AST-aspartate aminotransferase), and SGPT (ALT-alanine transaminase). The method of King et al, 1965 was used to carry out an assay of alkaline phosphatase. ADH assay (Ahd *et al*., 2019) was carried out in a microtiter plate. About 50 µl of dilution buffer, 50µl of NADH (2 mM) added in diluted buffer and then 50 µl of sample to be assayed was then added. The reaction was initiated by addition of 150 µl of reagent solution (330nM NAD+; 330nM NBT; 8nM PMS in dilution buffer and 0.13% gelatine). This preparation was incubated in dark at room temperature after which absorbance was determined spectrophotometrically at the wavelength of 590 nm.

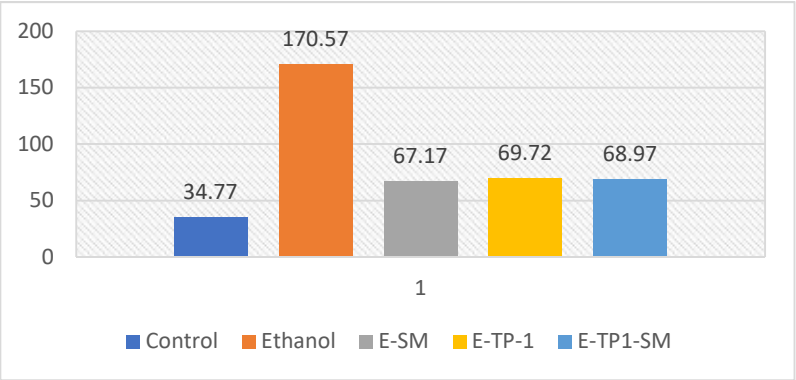
Enzyme activity (single unit) was the amount of enzyme liberated 1 /xmol of NADH/min where standard assay conditions were maintained.

Statistical analysis

The data of this study were analyzed using a one-way analysis of variance and with Tukey post hoc comparison tests to assess the significance of individual variations between the control and treatment groups using a computer-based software (SPSS 22 using Windows student version). The significance was considered at the level of $p< 0.001$.

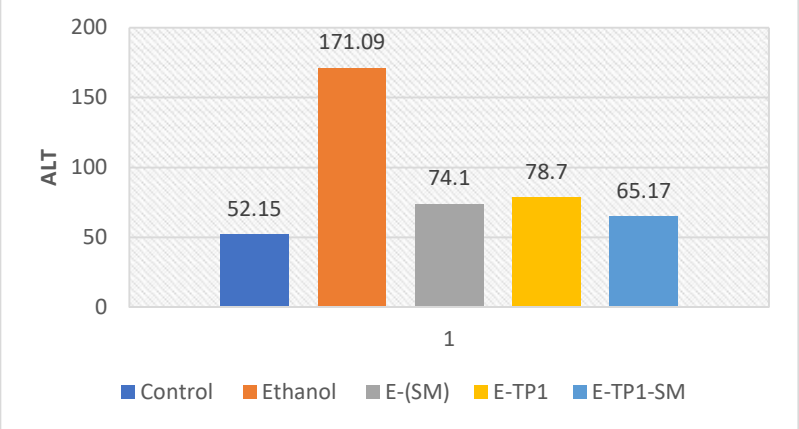
RESULTS.

Figure1. Comparison of AST activity of the Liver.



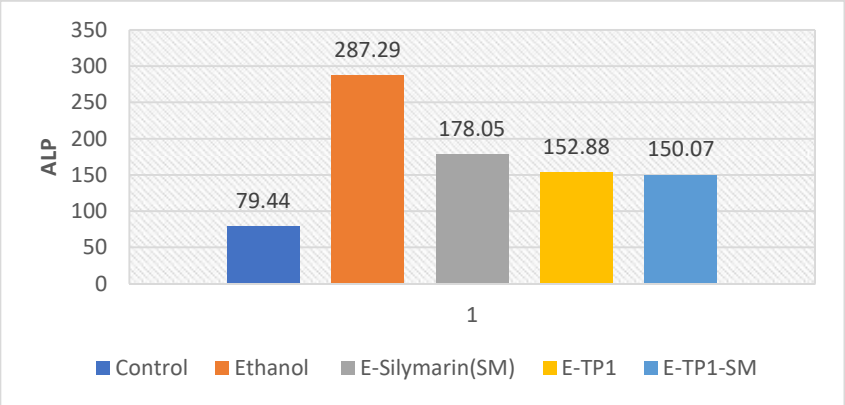
The comparative account of AST levels in various groups is shown in Figure 1. Aspartate aminotransferase (AST) levels in the E group was significantly increased compared to the control group whereas it was significantly decreased in E-SM, E-TP, and E-TP-SM groups compared to the E group ($F=87.09$, $P<0.001$ one-way ANOVA)

Figure 2. Comparison of ALT activity of the Liver.



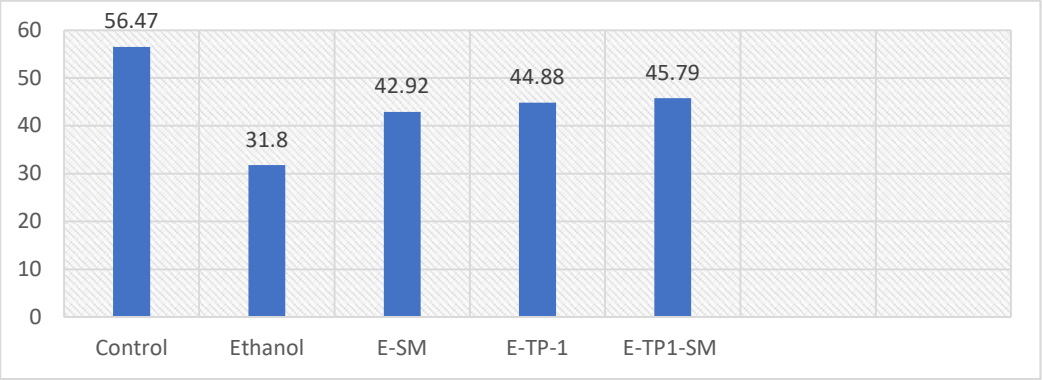
The comparative account of ALT levels in various groups is shown in Figure 2. Alanine transaminase (ALT) levels in the E group was significantly increased compared to the control group whereas it was significantly decreased in E-SM, E-TP, and E-TP-SM groups compared to the E group ($F=221.29$, $P<0.001$ one-way ANOVA).

Figure3. Comparison of ALP activity of the Liver.



The comparative account of ALP levels in various groups is shown in Figure 3. Alkaline phosphatase (ALP) levels in the E group were significantly increased compared to the control group whereas it was significantly decreased in E-SM, E-TP, and E-TP-SM groups compared to the E group (F=91.02, P<0.001 one-way ANOVA).

Figure 4. Comparison of ADH in Liver.



The comparative account of ADH levels in various groups is shown in Figure 4. ADH levels in the E group were significantly lowered compared to the control group whereas it was significantly increased in E-SM, E-TP, and E-TP-SM group compared to the E group (F=91.02, P<0.001 one-way ANOVA).

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Discussion

Alcohol addiction has a negative impact on a person's general physical and mental health in addition to major social and financial losses. Alcoholism in excess increases the risk of problems involving multiple organ systems[21]. Alcohol consumption has a significant negative impact on the liver since it can lead to advanced liver disease. Alcoholic cirrhosis, alcoholic hepatitis, and fatty liver are the three types of liver illnesses that are most frequently formed. Most cases of alcoholic hepatitis are asymptomatic, and 90% of alcoholics are known to have a fatty liver[22].

Ethanol-induced liver damage is always attributed to increased serum liver enzyme levels like ALT, ALP, and AST. ALT is a liver-specific enzyme that seeps into the bloodstream from the cytoplasm of hepatic cells after causing cell damage. Muscle injury as well as aberrant hepatic cell function may be due to leakage of ALP into blood[23]. When organelles, such as mitochondria, are injured, soluble enzymes such as AST are released, indicating membrane damage [24].

Based on existent scientific literature, Combretaceae plants possess an abundance of phytochemicals with diverse physiological and pharmacological effects. These plants have drawn the interest of numerous researchers due to their possible therapeutic qualities, since they may be used to treat a variety of chronic and incurable illnesses, including HIV/AIDS, cancer, hepatitis, diabetes mellitus, and cardiac conditions. Phytochemicals of *T. paniculata*, a plant belonging to the Combretaceae family, are responsible for its therapeutic properties[25].

Hepatotoxicity is induced not only by high doses of medications and substances but also by typical therapeutic dosages. This mechanism occurs primarily because when organic agents, inorganic substances, and medications are taken, metabolism occurs in the liver, producing free radicals and increasing oxidative stress. Approximately seventy-five percent of idiosyncratic reactions result in hepatotoxicity, necessitating a liver transplant or death. Hepatotoxic medications and substances such as paracetamol, alcohol, methotrexate, rifampicin, isoniazid, azathioprine, mercuric chloride, carbon tetrachloride, and galactosamine increase the effective biomarker of liver enzymes. And concluded that these studies with hepatotoxicity help to develop new hepatoprotective drugs[27]

The hepatoprotective effect of *Terminalia paniculata* seen after paracetamol and carbon tetrachloride administration on rat hepatitis and it was discovered that the dosage of paracetamol and carbon tetrachloride increased the biochemical markers of AST, ALT, and ALP, which then returned to near-normal levels after treatment with *T.paniculata* [18, 19]. Whereas, in this study, toxicity was induced with an ethanol dose of 5g/kg body weight which markedly raised the serum level of enzymes such as AST, ALT, and ALP in rats, and their levels were restored after administering the plant extract. The reversal could be due to its membrane stabilizing function preventing intracellular enzyme leakage. This is consistent with the widely held acceptance that serum transaminase levels revert to normal after hepatic parenchymal repair and hepatocyte regeneration[26]. Ethanol is metabolized in the liver by both oxidative and non-oxidative mechanisms. The two key components of the oxidative metabolism route are the enzymatic conversion of acetaldehyde to acetate by acetaldehyde dehydrogenase (ALD) and acetaldehyde to acetate by alcohol dehydrogenase (ADH). Alcohol increases the activity of the ADH enzyme, which then transforms alcohol into aldehyde. All treatment groups demonstrated a notable change in levels of enzyme ADH when compared with control group. In our study male wistar rats in the ethanol group had lower levels of ADH than the control animal group, which is in line with observations made by many researchers. Low levels of ADH can impact alcohol metabolism, causing it to circulate more freely in liver tissue and raising the risk of ALD. ADH levels stabilized in groups which received *T. paniculata* alone or in combination with silymarin, and results were equivalent to the control group, highlighting the hepatoprotective effect of this plant.

This plant's phytochemical characterization had previously been studied, and the presence of components such as gallic acid and ellagic acid had been documented. The current study's findings are consistent with the phytochemical components of the plant, as gallic acid and ellagic acid are established hepatoprotective agents that function by maintaining specific cellular homeostasis.[20]. Thereby from the present study, it can be suggested that *Terminalia paniculata* as a hepatoprotective agents which proved to be effective against ethanol-induced hepatotoxicity. The results of this study indicated there is a significant difference between the ethanol group, *Terminalia paniculata* and Silymarin groups. Hence it can be concluded that *Terminalia paniculata* is effective in treating ethanol-induced hepatotoxicity either individually or in combination with Silymarin.

CONCLUSION

Long-term alcohol consumption is linked with severe health concerns, including multiple organ disorders. Besides conventional allopathic medicine, alternative and complementary medical approaches have been gaining recognition and popularity in recent years. As *Terminalia paniculata* is a natural product and generally considered safe for human consumption, on the basis of the results of the present study, it can be looked upon as a promising hepatoprotective agent in ethanol-induced hepatic damage.

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