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HEPATOPROTECTIVE EFFECTS OF TERMINALIA KAERNBACHII LEAVES EXTRACT AGAINST ETHANOL-INDUCED LIVER DAMAGE IN WISTAR ALBINO RATS

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ABSTRACT
Ethanol-induced hepatotoxicity, resulting from excessive alcohol consumption, leads to various liver disorders, including alcoholic fatty liver disease, alcoholic hepatitis, and cirrhosis. The underlying mechanisms involve oxidative stress, lipid accumulation, and inflammation. Traditional medicinal plants have shown promise in managing liver disorders, but there is limited scientific validation for many. This study aimed to evaluate the hepatoprotective potential of Terminalia kaernbachii (TK) extract against ethanol-induced liver damage in Wistar albino rats, compared to silymarin, a known hepatoprotective agent. The ethanolic extract of TK leaves was prepared and administered to rats at doses of 250 and 500 mg/kg for 28 days, alongside ethanol (40% v/v) to induce hepatotoxicity. Liver function parameters, including SGPT/ALT, ALP, SGOT/AST, GGT, ACP, LDH, and bilirubin, were measured. Antioxidant enzyme activities (SOD, CAT, GPx, GSH) and lipid peroxidation markers (MDA) were assessed. Histopathological examination of liver tissues was performed to observe structural changes. Ethanol administration significantly elevated liver enzymes, indicating liver damage. TK treatment reduced these enzyme levels in a dose-dependent manner, comparable to silymarin. Antioxidant enzyme activities increased, and MDA levels decreased in TK-treated groups, indicating reduced oxidative stress. Histopathological analysis showed reduced hepatocyte degeneration, inflammation, and fibrosis in TK-treated rats. TK also exhibited hepatoprotective effects by improving renal parameters affected by ethanol. The ethanolic extract of Terminalia kaernbachii demonstrated significant hepatoprotective activity against ethanol-induced liver damage, likely due to its antioxidant and anti-inflammatory properties. Further studies are warranted to characterize the active compounds and explore the pharmacokinetics and safety profile of TK, establishing its therapeutic potential for liver disorders.

Key words: Terminalia kaernbachii, Hepatoprotective, Antioxidant activity, Silymarin.

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INTRODUCTION

Traditional herbal medicines have a long history of use, especially in developing countries where a large portion of the population relies on traditional practitioners and medicinal plants for healthcare needs. These practices coexist with modern medicine, often maintaining popularity due to cultural and historical reasons. In developed countries, herbal medicines have become more commercially available, though their regulatory standards vary. For example, Germany regulates herbal products as rigorously as conventional drugs, while in the USA; they are often marketed as dietary supplements without strict pre-approval requirements (Shaw D. 1998).

Herbalism, also known as botanical medicine, involves using plants and their extracts for medicinal purposes. It sometimes extends to include fungi, bee

products, minerals, shells, and certain animal parts (Fabricant DS, Farnsworth NR. 2001). Pharmacognosy, the study of medicines derived from natural sources, recognizes the potential of traditional medicines to lead to future pharmaceutical developments. Notably, 80% of 122 compounds used in mainstream medicine derived from ethnomedical plant sources were used similarly in their traditional contexts (Girish Dwivedi, Shridhar Dwivedi. 2007).

Plants synthesize chemical compounds to defend against predators, some of which are beneficial for treating human diseases. These secondary metabolites, which include phenols and their derivatives, bind to receptor molecules in the human body in ways similar to conventional drugs. This similarity implies that herbal medicines can be as effective as conventional drugs but also carry similar risks of side effects (Girish Dwivedi, Shridhar Dwivedi. 2007, O'Grady JG, *et al*. 1993).

Herbal medicine's history is extensive, with early uses recorded in various ancient civilizations. In India, Ayurveda described numerous medicinal plants and preparations as early as the 1st millennium BC. The Shennong Bencao Jing, a Chinese herbal compendium from 2700 BC, lists many medicinal plants and their uses. Greek and Roman medicinal practices, documented by Hippocrates and Galen, influenced later Western medicine. Hippocrates promoted simple herbal remedies, while Galen's extensive use of drug mixtures included plant, animal, and mineral ingredients (Vilas A, *et al*. 2020).

The application of herbal formulations has risen significantly, especially in Western countries. These formulations aim to support the body's natural healing processes and are often tailored to specific conditions. They generally consist of liquid extracts from high-quality herbs, free from synthetic chemicals and other contaminants. This emphasis on quality ensures the effectiveness and safety of herbal products (Khan KH. 2009).

Liver Diseases

The liver is vital for numerous bodily functions, including detoxification, protein production, and bile secretion. Liver diseases can arise from various causes, including infectious agents, genetic defects, metabolic disturbances, alcohol, and toxins. Common liver diseases include viral hepatitis, alcoholic liver disease, nonalcoholic fatty liver disease, and autoimmune liver conditions. Chronic liver disease often leads to cirrhosis and portal hypertension, with cirrhosis being a major cause of liver cancer and liver transplantation (Singanan V, *et al*. 2019).

The use of herbal medicines has deep historical roots and continues to be an important part of healthcare in many cultures. While modern herbal products are becoming more standardized, their safety and efficacy still depend on rigorous quality control. Understanding the historical context and current practices of herbal medicine can help integrate these traditional remedies into modern healthcare effectively.

Plant Review: *Terminalia kaernbachii* Willd.

Introduction Botanical name: *Terminalia kaernbachii* Willd.

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Lamiales
Family: Combritaceae
Genus: Terminalia
Species: T. kaernbachii

Description: *Terminalia kaernbachii* is a tree reaching 25-20 ft in height with grayish brown bark and pubescent leaves. Flowers are bisexual, and the fruit is a drupaceous, glabrous structure.

Distribution: Found in India, Bangladesh, Bhutan, China, Myanmar, Thailand, Cambodia, Vietnam, Malay Peninsula, Sumatra, Java, Philippines, and East-Timor.

Ecology: Grows in various forest types and disturbed areas from sea level up to 1,500 meters. Flowering occurs from March to July, with fruiting from May to October.

Uses: Known for its diuretic, anti-inflammatory, antinociceptive, hypnotic, cytoprotective, and immunomodulatory properties. It is also used in charcoal production and crafting pots and paddles (Sutha D, *et al*. 2017, Sureshkumar SV, Mishra SH. 2016, Samudram P, *et al*. 2008).

Various herbal formulations are available in the Indian market for liver disorders, yet finding a precise and effective herbal remedy remains challenging. Several Indian medicinal plants are traditionally used to manage liver disorders and have demonstrated strong hepatoprotective activity. Despite its extensive use, there is a lack of systematic scientific investigation into the liver-protective effects of many herbal drugs.

This study aimed to explore the hepatoprotective potential of polyherbal extracts against ethanol-induced hepatotoxicity in albino rats. The investigation sought to provide a scientific basis for the traditional use of these herbal formulations in treating liver disorders. By examining the protective effects of these extracts, the study contributes to the understanding and potential development of effective herbal treatments for liver health. The findings could pave the way for more precise and reliable herbal remedies for liver disorders, aligning traditional knowledge with modern scientific validation. (Ashok DB, Devasagayam TP. 2007, Anbazhakan S, Balu S. 2004)

MATERIALS AND METHODS

Plant Material and Extraction:

The leaves of *Terminalia kaernbachii* were collected, washed, and dried at room temperature. Once fully dried, the leaves were powdered and passed through a 60-mesh sieve, then stored in an airtight container. For the

extraction process, a weighed quantity of the air-dried powdered leaves was treated with petroleum ether to dewax and remove chlorophyll. The powder, after drying, was then subjected to ethanol extraction using a Soxhlet apparatus for 72 hours. The ethanol extracts were evaporated to dryness using a rotary flash evaporator at a temperature not exceeding 60°C. The concentrate was further dried in a water bath and stored in a desiccator. (Muzaffer A, *et al*. 1993)

Selection of Animal Species

Wistar albino rats were selected for the study, a common choice for laboratory testing. Healthy young adult animals from commonly used laboratory strains were employed. Females were chosen to be nulliparous and non-pregnant. At the commencement of dosing, each animal was between 6 to 8 weeks old, and the weight (150-200g) was within ±20% of the mean weight of previously dosed animals.

Housing and Feeding Conditions

The temperature in the experimental animal room was maintained at 22ºC±3ºC, with relative humidity kept between 30% and 70%, aiming for 50-60%. Lighting was artificial, with a 12-hour light/dark cycle. Conventional laboratory diets were provided, along with unlimited drinking water. Animals were group-caged by dose, ensuring that the number of animals per cage did not interfere with clear observation of each animal. (Gayathri K, *et al*. 2007)

Acute Oral Toxicity Study (OECD 423)

The acute toxic class method was used, involving stepwise procedures with 3 animals of a single sex per step. Depending on the mortality and/or moribund status of the animals, 2-4 steps might be necessary to judge the acute toxicity of the test substance. This method is reproducible, uses few animals, and ranks substances similarly to other acute toxicity testing methods. The method does not calculate a precise LD50 but determines defined exposure ranges where lethality is expected.

Administration of Drug Doses

Terminalia kaernbachii extract (TK) was administered to groups of Wistar albino rats in a single oral dose via gavage using a feeding needle. The control group received an equal volume of the vehicle. Animals were fasted 12 hours before dosing. After weighing, the test substance was administered at a dose of 2000 mg/kg. Food was withheld for another 3-4 hours post-administration. Observations were made systematically and continuously according to guidelines after substance administration. (Pandima DK, *et al*. 2003, Devi KP, *et al*. 2004, Devi KP, *et al*. 2005)

Observations

Animals were observed individually post-dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention during the first 4 hours, and daily thereafter for a total of 14 days. Observations included changes in skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity, and behavior patterns. Special attention was given to signs such as tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma. Weight changes were recorded on day 1, 7, and 14 of the study. Food and water consumption per animal was calculated. Animals were observed for mortality throughout the period. (Etti S, *et al*. 2005)

Invivo Hepatoprotective Activity

Ethanol Induced Hepatotoxicity

The study aimed to test the hepatoprotective activity of *Terminalia kaernbachii* extract (TK) against alcohol-induced hepatotoxicity in albino Wistar rats, with Silymarin as the reference. (Hymavathi A, *et al*. 2009)

Grouping: Albino Wistar rats were divided into five groups:

- **Group I:** Control group receiving normal saline (1 mL/kg) orally for 28 days.
- **Group II:** Alcohol group receiving alcohol (40% v/v) at 5 mL/kg orally for 28 days.
- **Group III:** TKL group receiving *Terminalia kaernbachii* extract at 250 mg/kg/day plus alcohol (40% v/v) at 5 mL/kg for 28 days.
- **Group IV:** TKH group receiving *Terminalia kaernbachii* extract at 500 mg/kg/day plus alcohol (40% v/v) at 5 mL/kg for 28 days.
- **Group V:** Silymarin group receiving Silymarin at 10 mg/kg/day plus alcohol (40% v/v) at 5 mL/kg for 28 days.

Blood Collection and Processing

After 28 days, animals were fasted overnight, anesthetized with thiopentone sodium (40 mg/kg/i.p), and blood samples were collected into tubes with and without EDTA-K2. Blood with anticoagulant was used for hematological analysis, while the other tubes were used for separating plasma/serum for biochemical analysis. Animals were then euthanized, and liver necropsy was observed. A portion of the liver was homogenized for biochemical analyses. (Young-Won C, *et al*. 2006)

Preparation of Liver Tissue Homogenate

The liver was homogenized in phosphate buffer (0.01M, pH 7.0) to create a 10% (w/v) homogenate, centrifuged at 3,000 RPM for 15 minutes at 4°C to obtain the supernatant for lipid peroxidation (TBARS) tests. The aliquot was further centrifuged at 12,000 g for 20 minutes at 4°C to get post mitochondrial supernatant (PMS). The

clear supernatant was used immediately for estimating lipid peroxidation markers and other enzymatic and non-enzymatic antioxidant compounds.

Estimation of Malondialdehyde (MDA/LPO)

Reagents used included trichloroacetic acid, thiobarbituric acid, hydrochloric acid, TCA-TBA-HCl reagent, and a working standard of 1,1',3,3'-tetra methoxy propane. The serum sample was combined with the TCA-TBA-HCl reagent, heated, centrifuged, and absorbance read at 535 nm.

MDA concentration was calculated using the formula:

$$\text{Concentration of MDA} = \frac{\text{Test OD}}{\text{Standard OD}} \times \frac{\text{Conc of Standard}}{\text{Conc of Sample}}$$

Assay of Superoxide Dismutase (Cu/Zn SOD)

Reagents included sodium pyrophosphate buffer, phenazine methosulfate, nitroblue tetrazolium, NADH, and KCN. The assay mixture contained these reagents, appropriately diluted enzyme preparation, and water. After incubation and addition of glacial acetic acid, the mixture was shaken with n-butanol, and the chromogen intensity in the n-butanol layer was measured at 560 nm against a butanol blank. (Nanji AA. 1993)

Assay of Catalase

Reagents included phosphate buffer and hydrogen peroxide in phosphate buffer. Serum samples were incubated with the substrate, and the decrease in absorbance was recorded at 240 nm. Enzyme activity was determined using the formula:

$$Enzyme\ Activity = \frac{C - T}{SOD} \times \frac{Conc\ of\ Standard}{Vol\ \ of\ Sample}$$

Assay of Glutathione Peroxidase (GPx)

Reagents included phosphate buffer, EDTA, sodium azide, reduced glutathione, H2O2, TCA, disodium hydrogen phosphate, and DTNB solution. The reaction mixture was incubated, centrifuged, and the supernatant mixed with reagents to read the absorbance at 420 nm.

$$GPx\ Activity = \frac{Test\ OD}{Standard\ OD} \times \frac{Conc\ of\ Standard}{Vol\ \ of\ Sample}$$

Determination of Reduced Glutathione (GSH):

Serum samples were precipitated with TCA, centrifuged, and the supernatant was mixed with DTNB and phosphate buffer. Absorbance was read at 412 nm.

$$SH\ Activity = \frac{Test\ OD}{Standard\ OD} \times \frac{Conc\ of\ Standard}{Vol\ \ of\ Sample}$$

Estimation of Ascorbic Acid (Vitamin C):

Reagents included TCA, DTC reagent, and sulphuric acid. Samples were incubated with DTC reagent, mixed with sulphuric acid, and the color developed was read at 520 nm.

Histopathological Studies

Liver tissue was processed for histology, following standard slicing, fixation, and staining procedures.

Statistical Analysis

Values were represented as Mean ± SEM. Data were analyzed using one-way ANOVA and group means compared using the Tukey-Kramer Multiple Comparison Test via Graph Pad Prism 5 software. P values < 0.05 were considered significant. (Yokoyama S, *et al*. 2016)

This detailed methodology outlines the comprehensive approach taken to evaluate the hepatoprotective activity of *Terminalia kaernbachii* extract, including extraction processes, animal selection, dosing, observations, and various biochemical assays to determine liver function and protection against induced toxicity.

RESULTS

Acute toxicity studies of *Terminalia kaernbachii* (TK)

Acute toxicity is the toxicity produced by a pharmaceutical when it is administered in one or more doses during the period of not exceeding 24 hours. Single escalating dose was given and mortality, body weight and behavioral changes were observed. Mortality was not observed in the animals treated orally with 2000 mg/kg of *Terminalia kaernbachii* (TK). There was no characteristic, statistically significant change in the behavioral patterns, body weight, food and water intake. (Szabo G, Petrasek J. 2015)

Effect of TK on the body weight changes in alcohol induced hepatotoxicity

The table presents the body weight of different groups of animals on day 0, day 14, and day 28. The groups include a normal control group, an alcohol-induced group, a TK 250 group, a TK 500 group, and a Silymarin group. On day 0, the normal control group had an average body weight of 210.33±2.25 g. The alcohol-induced group had a slightly higher average body weight of 220.93±2.13 g, which was significant compared to the control group at $p<0.001$. The TK 250, TK 500, and Silymarin groups had average body weights of 212.44±3.38 g, 213.49±2.25 g, and 210.76±3.43 g, respectively, which were not significantly different from the control group. On day 14, the normal control group had an average body weight of 225.32±3.2 g, which was significantly different from the alcohol-induced group, whose average body weight was 195.38±1.72 g at $p<0.001$. The TK 250, TK 500, and Silymarin groups had average body weights of 232.02±2.28 g, 242.18±2.75 g, and 241.51±2.13 g, respectively. The TK 500 group had a significantly higher body weight than the alcohol-induced group at $p<0.001$. On day 28, the normal control group had an average body weight of 244±2 g,

which was significantly different from the alcohol-induced group, whose average body weight was 173.16±1.96 g at p<0.001. The TK 250, TK 500, and Silymarin groups had average body weights of 242.18±1.94 g, 252.83±2.78 g, and 251.56±2.85 g, respectively. All three groups had significantly higher body weights than the alcohol-induced group at p<0.001. The results indicate that alcohol-induced a significant decrease in body weight compared to the control group, while treatment with TK 500, TK 250, and Silymarin prevented or reduced the decrease in body weight induced by alcohol. The results also suggest that TK 500 was the most effective treatment for preventing alcohol-induced weight loss. (Fernando H, *et al*. 2011)

The table 8 shows the effect of *Terminalia kaernbachii* (TK) on liver parameters in alcohol-induced hepatotoxicity compared to normal control and Silymarin-treated groups. The values are expressed as mean ± SEM, and the number of animals in each group is 6. The liver parameters measured are SGPT/ALT, ALP, SGOT/AST, GGP, ACP, LDH, and Bilirubin. The results indicate that alcohol induction caused a significant increase in all liver parameters compared to the normal control group. However, treatment with TK250, TK500, and Silymarin significantly reduced the levels of all liver parameters compared to the alcohol-induced group, with p<0.001. Overall, the results suggest that *Terminalia kaernbachii* has a hepatoprotective effect against alcohol-induced liver damage by reducing liver parameters. However, further studies are needed to confirm these findings.

Effect of TK on the Blood parameters of alcohol induced hepatotoxicity

The results in table 9 indicate that alcohol-induced hepatotoxicity significantly decreased insulin, hemoglobin (HB), and increased HBA1C levels compared to the normal control group. Treatment with TK 250, TK 500, and silymarin significantly increased insulin and HB levels and decreased HBA1C levels compared to the alcohol-induced group. Moreover, the levels of insulin and HB were highest in the silymarin group, followed by the TK 500 and TK 250 groups. The HBA1C levels were lowest in the silymarin group, followed by the TK 250 and TK 500 groups. These findings suggest that TK has a protective effect on blood parameters in alcohol-induced hepatotoxicity, with a stronger effect observed with higher doses of TK and silymarin. (Stewart S, *et al*. 2001)

Effect of TK on the Lipid parameters of alcohol induced hepatotoxicity

The table 10 reports the lipid parameters including Total Protein, Total Cholesterol, Triglycerides, and HDL (High-Density Lipoprotein) levels. The results show that the alcohol-induced group had significantly altered lipid parameters compared to the normal control group, indicating the development of hepatotoxicity. However, treatment with TK at both doses (250 mg/kg/day and 500 mg/kg/day) and Silymarin significantly improved the lipid parameters, suggesting their hepatoprotective effects. These effects are statistically significant compared to both the normal control and alcohol-induced groups.

Effect of TK on the Renal Parameters of alcohol induced hepatotoxicity

The table 11 shows the effect of TK (an herbal extract) on kidney parameters in alcohol-induced hepatotoxicity in a rat model. The parameters measured include uric acid, blood urea nitrogen (BUN), urea, and creatinine. The results indicate that alcohol-induced significant increases in all measured parameters when compared to the normal control group. However, treatment with TK, especially at a dose of 500 mg/kg, significantly reduced these increases compared to the alcohol-induced group. Treatment with silymarin also showed significant improvements in kidney parameters. These findings suggest that TK and silymarin may have a protective effect on kidney function in alcohol-induced hepatotoxicity. (Yeh MM, Brunt EM. 2014)

Effect of TK on the Antioxidant parameters of alcohol induced hepatotoxicity

The table 12 shows the effect of TK on the anti-oxidant parameters in alcohol-induced hepatotoxicity in comparison to a normal control group. The parameters measured were SOD, CAT, GPx, GSH, MDA, and Vit C. The results indicate that alcohol-induced hepatotoxicity significantly decreased the levels of SOD, CAT, GPx, GSH, and Vit C while increasing the level of MDA compared to the control group. However, treatment with TK (250 and 500 mg/kg) and Silymarin restored the levels of these parameters to near normal levels. The values of all the parameters were expressed as mean±SEM, n=6, and the statistical significance was measured using the *p<0.001. Overall, the results suggest that TK has a protective effect on the liver by reducing oxidative stress caused by alcohol-induced hepatotoxicity.

Effect of TK on the histopathology of alcohol induced hepatotoxicity

Normal group Section shows normal liver tissue with hepatocytes, portal triad showing prominent central vein and normal architecture. Section of the induced group shows liver tissue with ballooning body appears, due to fat deposition, micro vesicular steatosis, degeneration of the hepatocytes, nucleomegaly, Kupffer cell activity, regular nuclear membrane and focal collection of inflammatory cells around portal triad with fibrosis. Section of the *Terminalia kaernbachii* (TK) 250 shows liver with normal histological arrangement and kupffer cell activity. Section of TK 500 shows liver with normal histological arrangement and reduced ballooning Cells. Section of the standard drug, Silymarin shows liver with normal

histological arrangement and kupffer cell activity and cellular uniformity appears without degeneration.

Table 1: Effect of Terminalia kaernbachii (TK) on the body weight, food and water intake in acute toxicity study

Parameter	Group	Day 1	Day 7	Day 14
Body weight (g)	Control	192.21±2.57	198.04±2.89	202.33±2.09
	TK 2000	194.45±2.68	199.29±2.08	202.87±2.64
Water intake (ml)	Control	28.93±2.33	30.84±3.43	31.09±2.39
	TK 2000	30.44±3.87	34.56±3.98	37.76±2.9
Food intake (g)	Control	23.55±2.16	27.64±3.06	31.74±3.11
	TK 2000	22.01±2.12	26.65±3.16	32.27±3.18

The values were expressed as mean±SEM, n=6, a, b-values were significant compared to the control group and induced group respectively at *p<0.001

Table 2: Effect of TK on the body weight changes in alcohol induced hepatotoxicity

Group	Body weight (g)		
	Day 0	Day 14	Day 28
Normal control	210.33±2.25	225.32±3.2	244±2
Alcohol induced	220.93±2.13*a	195.38±1.72*a	173.16±1.96*a
TK 250	212.44±3.38*b	232.02±2.28*b	242.18±1.94*b
TK 500	213.49±2.25*b	242.18±2.75*b	252.83±2.78*b
Silymarin	210.76±3.43*b	241.51±2.13*b	251.56±2.85*b

The values were expressed as mean±SEM, n=6, the values were non-significant compared to the control group at p<0.001

Table 3: Effect of TK on the Liver parameters in alcohol induced hepatotoxicity

Parameter	Normal control	Alcohol induced	TK 250	TK 500	Silymarin
SGPT/ALT (IU/L)	27.12±1.04	56.27±1.48*a	46.15±1.63*b	38.11±0.91*b	30.02±0.94*b
ALP (IU/L)	45.69±1.69	93.94±1.67*a	66.11±1.45*b	57.84±1.9*b	49.39±0.92*b
SGOT/AST (IU/L)	121.88±3.29	246.97±1.74*a	185.25±2.43*b	165.68±2.16*b	145.79±1.92*b
GGP (IU/L)	26.9±0.99	93.4±2.22*a	45.5±1.66*b	37.5±0.77*b	29.8±0.86*b
ACP (IU/L)	27.1±1.05	92.7±1.62*a	47.4±0.99*b	38.1±0.99*b	29.9±1.3*b
LDH (IU/L)	122±3.92	244±3.53*a	185±2.72*b	164±4.11*b	146±1.74*b
Bilirubin (mg/dL)	0.93±0.1	3.11±0.26*a	1.4±0.24*b	1.29±0.11*b	0.94±0.07*b

The values were expressed as mean±SEM, n=6, a, b-values were significant compared to the control group and induced group respectively at *p<0.001

Table 4: Effect of TK on the blood parameters in alcohol induced hepatotoxicity

	Insulin (U/ml)	HB (mg/dL)	HBA1C (%)
Normal control	18.85±0.4	12.54±0.52	6.33±0.27
Alcohol induced	5.549±0.33*a	7.762±0.15*a	13.5±1.03*a
TK 250	12.46±0.22*b	12.48±0.21*b	8.65±0.44*b
TK 500	14.73±0.43*b	11.91±0.58*b	8.1±0.32*b
Silymarin	17.3±1.14*b	13.15±0.61*b	6.65±0.36*b

The values were expressed as mean±SEM, n=6, a, b-values were significant compared to the control group and induced group respectively at *p<0.001

Table 5: Effect of TK on the Lipid parameters in alcohol induced hepatotoxicity.

Group	Lipid Parameter (mg/dL)			
	Total Protein	Total Cholesterol	Triglycerides	HDL
Normal control	8.55±0.35	141.74±2.75	79.73±2.21	44.98±0.88
Alcohol induced	4.86±0.2*a	267.85±3.88*a	172.4±2.44*a	24.19±1.13*a
TK 250	6.15±0.43*b	196.64±1.47*b	122±2.38*b	30.16±1.31*b
TK 500	7.84±0.45*b	185.46±2.93*b	97.16±2.17*b	36.3±1.49*b
Silymarin	8.44±0.32*b	170.74±3.47*b	87.75±1.62*b	37.63±1.82*b

The values were expressed as mean±SEM, n=6, a, b-values were significant compared to the control group and induced group respectively at *p<0.001

Table 6: Effect of TK on the Kidney parameters in alcohol induced hepatotoxicity.

Group	Kidney parameters (mg/dL)			
	Uric Acid	**BUN**	**Urea**	**Creatinine**
Normal control	3.18±0.14	20.8±0.57	30.13±0.96	1.06±0.15
Alcohol induced	4.65±0.15*a	26.4±1.03*a	81.37±2.19*a	2.89±0.35*a
TK 250	3.2±0.08*b	21.7±0.67*b	57±2.16*b	1.33±0.09*b
TK 500	3.28±0.21*b	21.4±0.92*b	49.37±1*b	1.27±0.14*b
Silymarin	3.45±0.17*b	21±0.92*b	40.48±1.32*b	0.96±0.09*b

The values were expressed as mean±SEM, n=6, a, b-values were significant compared to the control group and induced group respectively at *p<0.001.

Table 7: Effect of TK on the Anti-oxidant parameters in alcohol induced hepatotoxicity.

Parameter	**Normal Control**	**Alcohol induced**	**TK 250**	**TK 500**	**Silymarin**
SOD (U/ml)	9.34±0.43	4.57±0.28*a	6.58±0.1*b	7.47±0.46*b	8.75±0.54*b
CAT (U/ml)	95.77±1.006	43.65±1.795*a	55.57±1.854*b	64.99±1.79*b	83.49±2.09*b
GPx (U/ml)	9.122±0.174	5.465±0.244*a	8.114±0.287*b	8.876±0.386*b	9.689±0.321*b
GSH (mg/ml)	56.65±1.509	23.75±2.293*a	40.01±2.176*b	44.78±2.105*b	52.48±1.576*b
MDA (nmol/mg)	6.4±0.38	12.6±0.23*a	6.59±0.45*b	5.43±0.29*b	2.24±0.27*b
Vit C (mg/dL)	4.32±0.24	1.7±0.22*a	3.72±0.18*b	4.43±0.28*b	3.65±0.19*b

The values were expressed as mean±SEM, n=6, a, b-values were significant compared to the control group and induced group respectively at *p<0.001.

Figure 1: Effect of TK on the blood parameters in alcohol induced hepatotoxicity.

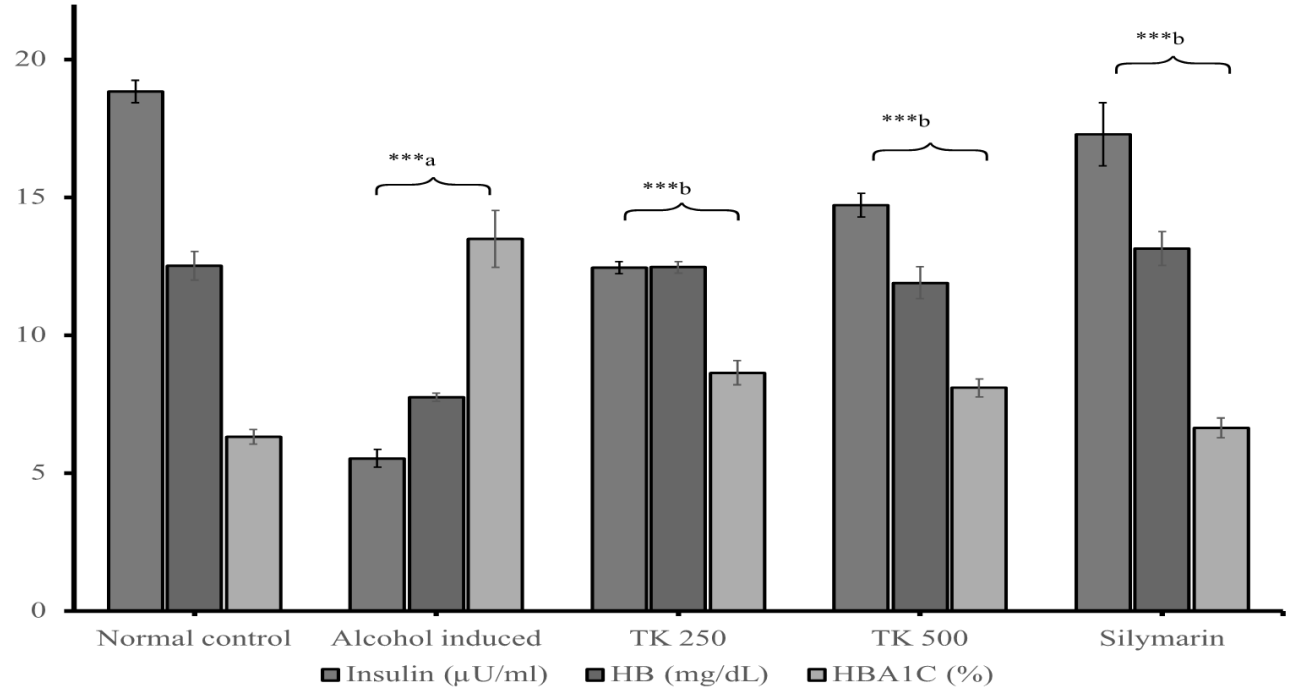
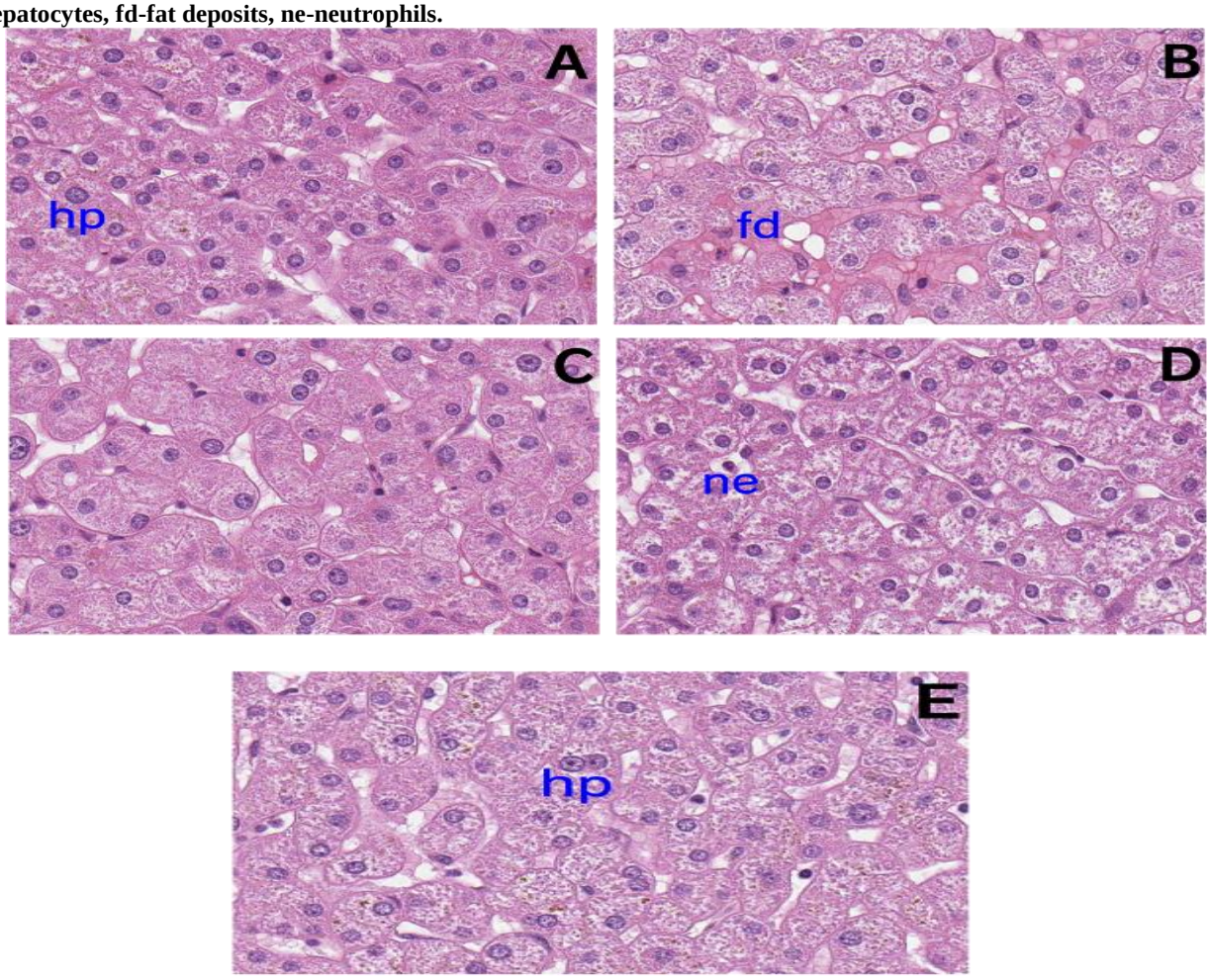


Figure 2: Histopathology of Liver.
A. Normal group, B. Alcohol Induced group, C. TK250, D. TK500, E. Silymarin
hp-hepatocytes, fd-fat deposits, ne-neutrophils.



DISCUSSION

Ethanol-induced hepatotoxicity, resulting from excessive alcohol consumption, leads to liver disorders such as alcoholic fatty liver disease, alcoholic hepatitis, and alcoholic cirrhosis. The underlying mechanism involves the generation of reactive oxygen species (ROS) and oxidative stress, which cause liver cell damage. Ethanol also disrupts fat metabolism in the liver, leading to triglyceride accumulation and fatty liver disease. Chronic ethanol consumption activates inflammatory pathways and promotes scar tissue accumulation, resulting in fibrosis and cirrhosis. Studies have shown that ethanol-induced oxidative stress in liver cells increases lipid peroxidation products and decreases glutathione levels, leading to elevated pro-inflammatory cytokines like interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α). This oxidative stress activates the nuclear factor-kappa B (NF-κB) pathway, causing liver cell death and promoting alcoholic liver disease. Additionally, chronic ethanol consumption activates the Toll-like receptor 4 (TLR4) pathway, further exacerbating inflammatory responses and liver fibrosis. (Nassiri-Asl M, Hosseinzadeh H. 2009)

Our study investigated the hepatoprotective activity of Terminalia kaernbachii (TK) in an ethanol-induced liver damage model in rats. The results demonstrated that TK significantly reduced liver damage, as indicated by improved liver parameters such as serum glutamate-pyruvate transaminase (SGPT/ALT), alkaline phosphatase (ALP), serum glutamate-oxaloacetate transaminase (SGOT/AST), gamma-glutamyl transferase (GGT), acid phosphatase (ACP), lactate dehydrogenase (LDH), and bilirubin. These improvements were comparable to those achieved with silymarin, a known hepatoprotective agent. The reduction in SGPT/ALT, SGOT/AST, and LDH levels suggests that TK prevented or reduced liver cell damage caused by alcohol. The

decrease in ALP and GGT levels indicates improved liver function, likely by reducing bile duct obstruction and enhancing bile flow. The lower ACP levels suggest a reduction in fat accumulation in the liver, and the decreased bilirubin levels indicate improved bilirubin excretion, often elevated in liver diseases. (Ramalingam S. 2016)

Ethanol-induced oxidative stress is a significant contributor to liver damage, and TK contains antioxidant compounds like flavonoids, phenolic acids, and tannins that protect liver cells from oxidative stress. Alcohol-induced rats exhibited decreased activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and reduced glutathione (GSH). TK treatment significantly increased the activities of these enzymes, indicating its strong antioxidant properties. The study also found elevated levels of malondialdehyde (MDA), a lipid peroxidation marker, in alcohol-induced rats. TK treatment significantly reduced MDA levels, suggesting protection against lipid peroxidation. Additionally, TK increased vitamin C levels, a potent antioxidant that scavenges free radicals and protects against oxidative stress. (Salleh N, *et al.* 2015)

Inflammation plays a crucial role in ethanol-induced liver damage, and TK contains anti-inflammatory compounds, including flavonoids and terpenoids, which may reduce liver inflammation. Ethanol consumption leads to triglyceride accumulation in the liver, contributing to liver damage. TK treatment significantly improved lipid profiles, reducing total cholesterol and triglyceride levels while increasing total protein and high-density lipoprotein (HDL) levels. (Valli Kanagarla NSSA, *et al*. 2013).

Histopathological studies confirm that chronic ethanol consumption causes significant liver damage, characterized by liver cell degeneration, inflammation, and fibrosis. The severity of these changes correlates with the duration and amount of ethanol consumed. Alcoholic fatty liver disease, in particular, is marked by fat accumulation in liver cells, inflammation, and fibrosis. Histopathological analysis confirmed that TK treatment significantly reduced liver damage, including hepatocyte degeneration, inflammation, necrosis, fibrosis, and ballooning of liver cells. These protective effects are likely due to TK's antioxidant and anti-inflammatory activities, induction of hepatoprotective enzymes, and regulation of lipid metabolism. TK also demonstrated hepatoprotective activity against ethanol-induced renal damage in rats, as evidenced by improved renal parameters. This hepatoprotective effect may be attributed to TK's antioxidant and anti-inflammatory properties and its ability to regulate lipid metabolism.

CONCLUSION

The present study evaluated the effects of the ethanolic extract of Terminalia kaernbachii leaves on alcohol-induced hepatotoxicity. The findings indicate that the extract possesses significant hepatoprotective activity, demonstrating potential therapeutic applications in toxicity control. The study highlighted TK's antioxidant, anti-inflammatory, and hepatoprotective properties, comparable to silymarin, a known hepatoprotective agent. However, further research is needed to explore the pharmacokinetic profile, safety, and active principles of the plant. To establish its therapeutic value in treating liver damage and related disorders, additional investigations are required to characterize the active constituent(s) responsible for the observed activities of the ethanolic extract of Terminalia kaernbachii leaves.

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