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Hepatoprotective and antinociceptive effects of terpinolene in streptozotocin-induced diabetic peripheral neuropathic rats

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ABSTRACT

Diabetes induced oxidative stress impairs hepatic function, contributing to peripheral nerve injury. Growing evidence indicates that overactivation of NOX4 brings metabolic derangements in hepatic tissue, which concomitantly alter thermal behavior in experimental rats. This study investigated the hepatoprotective and antinociceptive effects of terpinolene in streptozotocin (STZ)-induced DPN rats. Male SD rats were made diabetic with STZ (55 mg/kg, b.w. i.p) prepared in 0.1 M citrate buffer (pH 4.5). After six weeks, diabetic animals were treated daily with terpinolene at doses of 12.5, 25, and 50 mg/kg for four weeks. Diabetic control rats exhibited a significant decrease in the levels of endogenous antioxidants (enzymic and non-enzymic), along with elevated lipid peroxidation products and transaminase (AST, ALT) activities, indicating severe hepatic dysfunction. An alteration in thermal behavior was observed in diabetic control rats. Oral administration of terpinolene dose-dependently improved hepatic antioxidant status and reduced lipid peroxidation markers. Histological examination revealed that terpinolene restored the architecture of hepatic tissue, and the effect was more pronounced at 50mg/kg b.w than the other two doses. Further *in silico* analysis revealed a strong binding interaction between terpinolene and NOX4 with a binding energy of -5.2 kcal/mol. This indicates that terpinolene effectively inhibits NOX4-mediated oxidative stress in the hepatic tissue and ameliorates thermal nociception in DPN rats. The efficacy of terpinolene was comparable to the standard drug, α-lipoic acid.

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INTRODUCTION

The liver is an active organ that regulates diverse processes to maintain metabolic homeostasis (Gan *et al.*, 2025). Insulin resistance and persistent hyperglycemia overproduce reactive oxygen species (ROS) in hepatocytes through glucose auto-oxidation, activation of NADPH oxidase, and mitochondrial dysfunction (González *et al.*, 2023). This redox imbalance depletes endogenous antioxidants, resulting in lipid peroxidation and histopathological changes that contribute to the development of secondary complications in multiple organs. Among these, diabetic peripheral neuropathy (DPN) is the common complication affecting nearly 50% of diabetic patients during their lifetime (Savelieff *et al.*, 2025). It manifests as pain, numbness, or reduced sensation in the extremities and is mainly caused by hyperglycemia-induced nerve damage, which is driven by oxidative stress, increased polyol pathway activity, accumulation of advanced glycation end-products (AGEs), and neuroinflammation (Mangarov *et al.,* 2025). Thus, curtailing oxidative stress in hepatic tissue will minimize neuroinflammation and pain associated with the complication of DPN.

Phytochemicals have gained attention for their potential in preventing and managing diabetes induced hepatocellular damage (Kibibi Wairimu, 2025). Further, phytochemicals that improve liver function can also alleviate the sensory abnormalities associated with DPN. Terpinolene is a monoterpene present in ginger, cardamom, and parsley (Yu *et al.*, 2022; Arista *et al.*, 2023; Petropoulos *et al.*, 2010). Several studies have documented the anticancer, antioxidant, antifungal, anti-inflammatory and analgesic properties of terpinolene in experimental models (Aydin *et al.*, 2013; Turkez *et al.,* 2015 and Macedo *et al.*, 2016). However, there are no studies in the literature on the role of terpinolene in diabetes induced hepatic dysfunction and nociception in neuropathic rats. In this context, the study investigated the hepatoprotective effect of terpinolene and on behavioral outcomes by focusing on oxidative stress markers and histological changes in STZ induced diabetic rats.

MATERIALS AND METHODS

Chemicals

Terpinolene and STZ were acquired from Sigma-Aldrich (St. Louis, MO, USA). All analytical grade chemicals were purchased from SD Fine Chemicals and Hi-Media, India.

Animal purchase and ethical statement

A total of forty-two male Sprague Dawley rats (180–220 g) obtained from Mass Biotech, Chengalpattu, Tamil Nadu, India, were maintained under laboratory conditions (22 ± 2°C temperature, 75 ± 5% humidity, and a 12-hour light/dark cycle) had free access to pellet diet and water. All procedures are in line with the guidelines of the Indian National Animal Care and approved by the Institutional Animal Ethics Committee (IAEC), Government Medical College, Chidambaram (Proposal No: AU-IAEC/1365/9/23).

Induction and assessment of diabetes

Following a week of acclimatization, diabetes was induced in experimental rats after an overnight fast by administering STZ (55 mg/kg b w/ i.p) as described by Visnagri *et al.*, 2014. To prevent the risk of hypoglycemic shock-related mortality, glucose solution (5%) was provided to STZ injected rats for 24h. Control rats received citrate buffer and were maintained alongside the diabetic groups. Seventy-two hours post-injection, using a glucometer, fasting glucose levels (FBG) were measured and animals with FBG >250 mg/dl were included in the study.

Study design

Six weeks post-STZ administration, based on body weight, glucose levels and pain sensitivity responses, animals were grouped (n=6) and treated as follows:

Group 1: Vehicle treated normal control

Group 2: Normal control rats treated with terpinolene (50 mg/kg *p.o*) daily for four weeks.

Group 3: Vehicle treated diabetic control

Group 4: Diabetic rats treated with terpinolene (12.5 mg/kg *p.o*) daily for four weeks.

Group 5: Diabetic rats treated with terpinolene (25 mg/kg *p.o*) daily for four weeks.

Group 6: Diabetic rats treated with terpinolene (50 mg/kg p.o) daily for four weeks.
Group 7: Diabetic rats treated with α-lipoic acid (100 mg/kg *p.o*) daily for four weeks.

After treatment, animals were euthanized and sciatic nerves were harvested and placed at −80°C for analysis. By cardiac puncture, blood samples were collected and stored for biochemical assays.

Drug preparation

Terpinolene was freshly prepared in 2% Tween 80 in 0.9% saline and orally administered for 4 weeks following 6th week of STZ injection. Animals in group 7 were treated with α-lipoic acid, the standard drug.

Behavioral assessments in experimental animals

Parameters related to thermal hyperalgesia and allodynia were measured in experimental rats at different (1, 3, 5, 7 and 9 wks) time intervals of the experimental period.

Tail immersion test

The time to withdraw the tail placed in hot water (55 ± 0.5°C) was measured as thermal response (Ramabadran *et al.*, 1989).

Acetone drop test

In this test, a drop of acetone was applied five times at regular intervals (5 mins) onto the plantar surface of the left hind foot of experimental animals. Withdrawal is considered as positive and the responses are counted. Animals with two or more withdrawal responses are considered to have cold allodynia (Kuhad and Chopra, 2009).

Assay of liver marker enzymes

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined following the method of Reitman and Frankel (1957). Reagents were prepared according to standard protocols using commercially available diagnostic kits obtained from Sigma Diagnostics (I) Pvt. Ltd., Baroda, India. Briefly, 250 µl of Reagent 1 was dispensed into two test tubes labeled as sample and blank. To the sample tube, 50 µl of the serum was added, while 50 µl of distilled water was added to the blank. The contents were mixed thoroughly and incubated at 37 °C for 60 min. Subsequently, 250 µl of Reagent 2 was added to each tube, mixed, and incubated for an additional 20 min at 25 °C. Thereafter, 2.5 ml of working sodium hydroxide reagent was added, mixed thoroughly, and allowed to stand for 10 min. Absorbance was measured at 540 nm against the reagent blank.

Preparation of liver homogenate

At the end of the study, after euthanasia, the liver was carefully collected and stored. After homogenization (Tris–HCl buffer; 0.1 M; pH 7.4), the supernatant was utilized for various assays. A part of the tissue was used for histopathological analysis.

Determination of superoxide dismutase activity

Briefly, liver homogenate (0.5 mL), distilled water (1 mL), ethanol (2.5 mL), and chloroform (1.5 mL) were mixed at 4°C and centrifuged. To an aliquot of supernatant (0.1 mL), sodium pyrophosphate buffer (0.025 M; pH 8.3; 1.2 mL), phenazine methosulfate (186 µM; 0.1 mL), nitroblue tetrazolium (30 µM; 0.3 mL), and NADH (780 µM; 0.2 mL) were added and incubated at 30°C for 90 sec. After arresting the reaction using glacial acetic acid, the absorbance was measured in the n-butanol layer at 560 nm (Kakkar *et al.*, 1984).

Estimation of catalase activity

To liver homogenate (0.2 mL), phosphate buffer (0.01 M, pH 7.0; 1.0 mL) and H_2O_2 (0.2 M; 0.5 mL) were added and after 60 sec incubation, the reaction was arrested using 2.0 mL of dichromate–acetic acid reagent and boiled for 10 minutes. The absorbance was read at 620 nm (Sinha, 1972).

Glutathione peroxidase activity

The measurement involves incubating Tris–HCl (0.4 M; pH 7.0; 0.2 mL), EDTA (0.4 mM; 0.2 mL),

sodium azide (10 mM; 0.1 mL), liver homogenate (0.5 mL), glutathione (2 mM; 0.2 mL), and H_2O_2 (0.2 mM; 0.1 mL) for 10 minutes at 37°C. The contents were centrifuged after the addition of TCA (10%; 0.5 mL). To the supernatant (1.0 mL), Ellman's reagent (0.5 mL) and phosphate buffer (3 mL) were added and the absorbance was read at 420 nm (Rotruck *et al.*, 1973).

Assessment of glutathione S-transferase activity

The mixture contained phosphate buffer (0.3 mM; pH 6.5; 1.0 mL), CDNB (30 mM; 0.1 mL), liver homogenate (0.1 mL) and distilled water (0.7 mL) held at 37°C for 5 minutes. After initiating the reaction by the addition of glutathione (30 mM; 0.1 mL), the change in absorbance was spectrophotometrically read at 340 nm for 5 min against a blank (Habig *et al.*, 1974).

Estimation of Glutathione reductase activity

A reaction mixture containing liver homogenate (0.1 mL), phosphate buffer (2.0 mL) and 0.1 mL each of oxidized glutathione (GSSG) and FAD was incubated at 37°C for 15 minutes. Subsequently, NADPH (0.1 mL) was added and the absorbance was recorded at 340 nm for 5 minutes (Carlberg *et al.*, 1975).

Measurement of reduced glutathione

Briefly, liver homogenate (0.5 mL) was mixed with TCA (10%; 2.0 mL) and centrifuged. To the supernatant (1.0 mL), Ellman's reagent (0.5 mL) and phosphate buffer (0.2M; 3 mL) were added and the absorbance was read at 420 nm (Ellman, 1959).

Estimation of lipid peroxidation markers

The lipid peroxidation markers were measured in the hepatic tissue as per Niehius and Samuelsson, and Jiang *et al.* (1992) respectively. To estimate TBARS, liver homogenate.

(0.1 mL) was mixed with TBA–TCA–HCl reagent (2 mL; 1:1:1) and heated for 15 minutes, cooled and following centrifugation, the absorbance of supernatant was measured at 535 nm. Similarly, by incubating liver homogenate (0.2 mL) at 37°C for 30 minutes with FOX reagent (1.8 mL), the levels of HP were measured at 540 nm.

Histological analysis

After fixing in formalin and embedding in paraffin, thin sections (5 µm) of hepatic tissues were made using microtome and stained with hematoxylin and eosin.

Molecular docking

Binding studies between terpinolene and ALA with NOX4 (PDB ID: 8WEJ) were analyzed using AutoDock (V. 4.0) in Pyrx software. The interactions were visualized by Biovia Discovery Studio Visualizer 2020 and binding energy (kcal/mol) was calculated.

Statistical analysis

Statistical analysis between groups was evaluated using one-way ANOVA, and data are presented as mean ± SD. A p-value < 0.05 was considered statistically significant and Duncan's Multiple Range Test (DMRT) was applied for post-hoc comparisons (SPSS version 26.0).

RESULTS

Behavioral responses in experimental animals

In tail immersion test, the response and withdrawal latency were decreased in diabetic control compared to normal, confirming the onset of DPN. Administration of terpinolene for 4 weeks dose dependently improved response latency compared to diabetic control rats (Fig. 1A). In acetone drop test, an increased withdrawal response was observed in diabetic rats which were ameliorated in terpinolene-treated diabetic rats. A similar response was observed in ALA-treated groups (Fig. 1B).

Influence of terpinolene on serum transaminase activities

Transaminases activities were elevated in the serum of diabetic rats, which were dose dependently decreased in terpinolene-treated group. A similar effect was observed in ALA ALA-treated groups (Fig. 2 and 3).

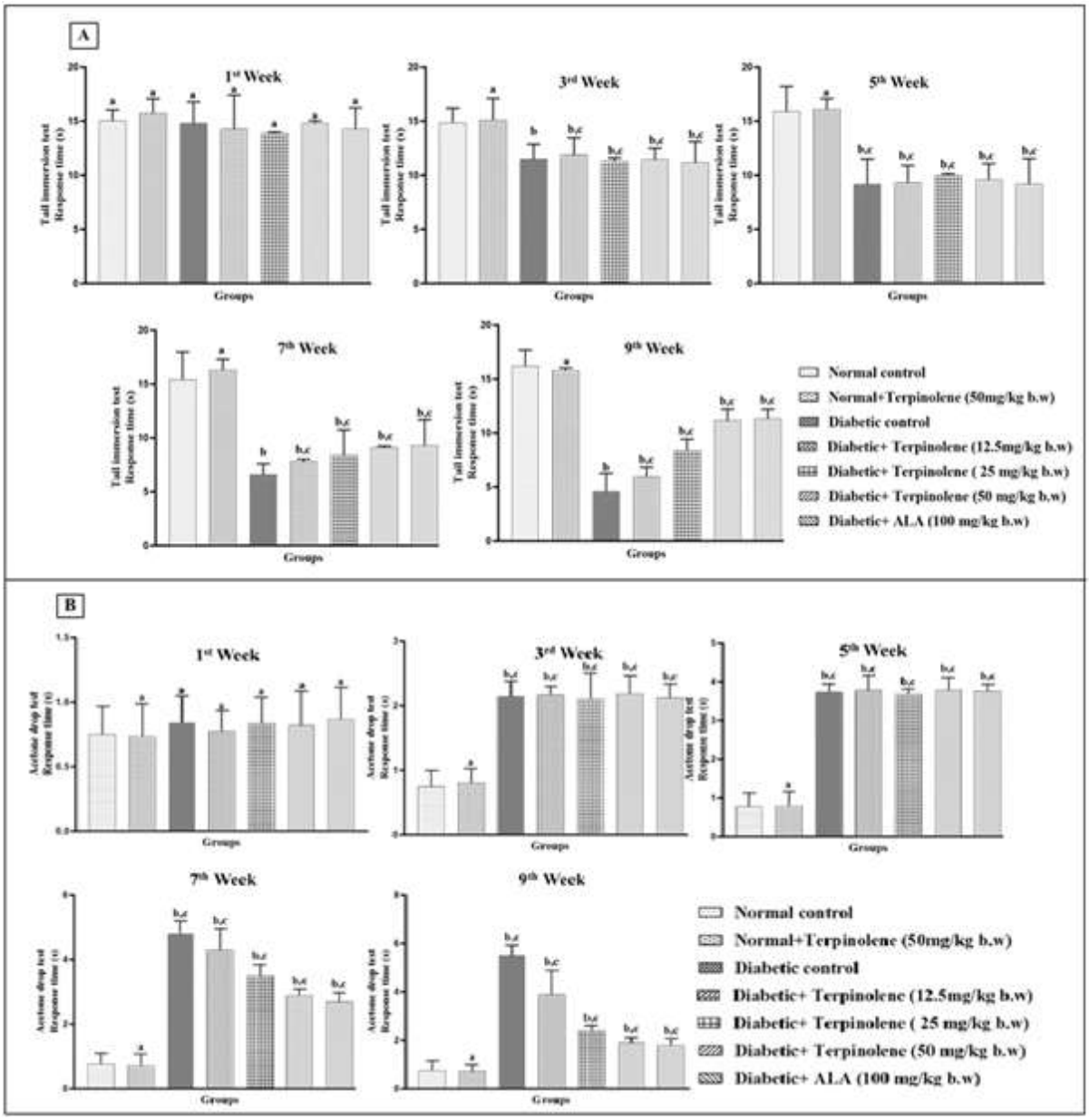

Fig. 1. Effect of terpinolene on diabetes induced hyperalgesia and allodynia in experimental rats. (A) Tail immersion test (B) Acetone drop test

Data are expressed as mean ± SD (n=6) ($p<0.05$; One way ANOVA, DMRT); [a] Not significant as compared to Normal control; [b] Significant as compared to Normal control; [c] Significant as compared to Diabetic control.

Role of terpinolene on enzymic antioxidants in the hepatic tissue of experimental rats

A significant reduction in enzymic antioxidants (SOD, CAT, GPx, GR and GST) was observed in the hepatic tissue of diabetic rats compared to normal rats. Terpinolene treatment for 4 weeks significantly improved their activities towards near normal. These findings are comparable with ALA-treated diabetic rats (Table 1).

Effect of terpinolene on hepatic GSH levels in experimental rats

The levels of GSH in the hepatic tissue of experimental rats are shown in Fig. 4. The decreased GSH observed in diabetic control rats was significantly increased on treatment with terpinolene for four weeks. Notably, terpinolene at 50 mg/kg exhibited a marked improvement over the other two doses. The results are comparable with ALA-treated diabetic groups.

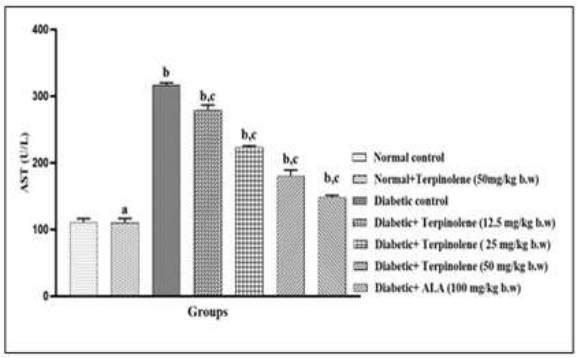

Fig 2. Influence of terpinolene on serum AST levels in experimental rats

Data are expressed as mean ± SD (n=6) ($p<0.05$; One way ANOVA, DMRT); [a] Not significant as compared to Normal control; [b] Significant as compared to Normal control; [c] Significant as compared to the Diabetic control.

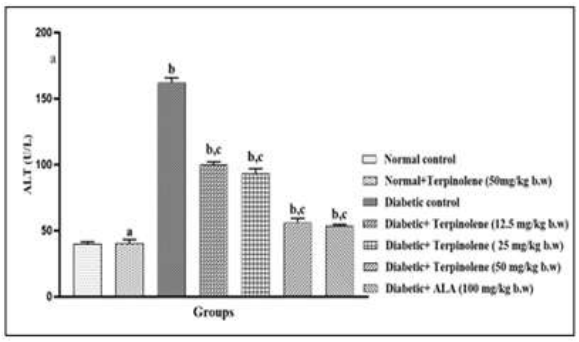

Fig 3. Role of terpinolene on serum ALT levels in experimental rats

Data are expressed as mean ± SD (n=6) ($p<0.05$; One way ANOVA, DMRT); [a] Not significant as compared to Normal control; [b] Significant as compared to Normal control; [c] Significant as compared to Diabetic control.

Table 1. Effect of terpinolene on enzymic antioxidant activities in the hepatic tissue of experimental

Groups	Normal control	Normal + Terpinolene (50 mg/kg/b. w)	Diabetic control	Diabetic + Terpinolene (12.5 mg/kg/b.w)	Diabetic + Terpinolene (25 mg/kg/b.w)	Diabetic + Terpinolene (50 mg/kg/b.w)	Diabetic + ALA (100 mg/kg/b.w)
SOD	19.31 ± 0.72	18.93 ± 1.31 [a]	5.18 ± 0.61 [b]	6.6 ± 1.43 [b,c]	8.3 ± 2.90 [b,c]	13.85 ± 1.95 [b,c]	14.88 ± 0.77 [b,c]
CAT	82.83 ± 1.12	83.5 ± 1.74 [a]	42.9 ± 0.27 [b]	50.16 ± 2.24 [b,c]	53.5 ± 1.64 [b,c]	65.5 ± 1.04 [b,c]	71.86 ± 1.61 [b,c]
GPx	14.73 ± 0.51	15.05 ± 1.54 [a]	5.75 ± 1.06 [b]	6.69 ± 2.54 [b,c]	8.9 ± 1.77 [b,c]	11.36 ± 0.87 [b,c]	13.43 ± 1.73 [b,c]
GST	227.4 ± 1.67	225.8 ± 2.63 [a]	162.3 ± 3.67 [b]	173.9 ± 1.78 [b,c]	189.8 ± 3.06 [b,c]	194.65 ± 4.17 [b,c]	199.9 ± 2.33 [b,c]
GR	76.16 ± 0.62	76.3 ± 1.25 [a]	34..3 ± 2.83 [b]	48.9 ± 2.10 [b,c]	56.81 ± 1.14 [b,c]	65.6 ± 2.65 [b,c]	66 ± 2.96 [b,c]

SOD – One unit refers to 50% inhibition of nitroblue tetrazolium reduction in one min/mg protein; CAT - µM of H_2O_2 consumed/minute; GR - µM of NADPH oxidized/min; GPx - µg of glutathione consumed/minute/mg protein; GST - µM of 1-chloro 2,4-dinitrobenzene-GSH conjugate formed/minute/mg protein. Data are expressed as mean ± SD (n=6) ($p<0.05$; One-way ANOVA, DMRT).

[a]Not significant as compared to Normal control; [b]Significant as compared to Normal control; [c]Significant as compared to the Diabetic control.

Table 2. Effect of terpinolene on the levels of lipid peroxidation markers in hepatic tissue of experimental rats

Groups	LIVER	
	TBARS (mM/100g) tissue)	LHP (mmol/100 g tissue)
Normal control	0.87 ± 0.16	72.21 ± 5.50
Normal+ Terpinolene (50 mg/kg b.w)	0.90 ± 0.22[a]	74.20 ± 5.94[a]
Diabetic control	4.55 ± 1.27[b]	151.66 ± 1.43 [b]
Diabetic + Terpinolene (12.5 mg/kg b.w)	3.89 ± 2.32 [b,c]	119.9 ± 0.99 [b,c]
Diabetic + Terpinolene (25 mg/kg b.w)	3.09 ± 1.43 [b,c]	99.99 ± 0.77 [b,c]
Diabetic + Terpinolene (50mg/kg b.w)	2.62 ± 0.90 [b,c]	89.99 ± 0.99 [b,c]
Diabetic + ALA (100 mg/kg b.w)	1.59 ± 0.72 [b,c]	79.99 ± 2.10 [b,c]

Data are expressed as mean ± SD (n=6) ($p<0.05$; One-way ANOVA, DMRT).

[a]Not significant as compared to Normal control; [b]Significant as compared to Normal control; [c]Significant as compared to the Diabetic control.

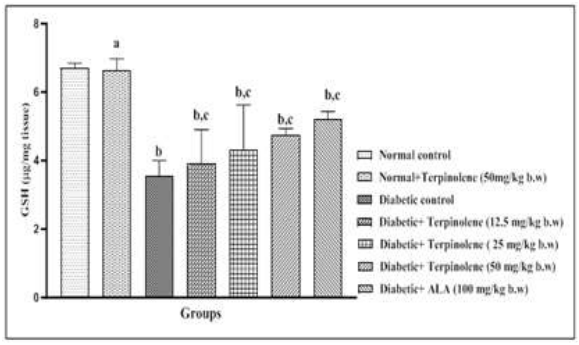

Fig. 4. Influence of terpinolene on non-enzymic antioxidant in hepatic tissue of experimental rats

Data are expressed as mean ± SD (n=6) ($p<0.05$; One way ANOVA, DMRT).

[a] Not significant as compared to Normal control; [b] Significant as compared to Normal control; [c] Significant as compared to Diabetic control.

Role of terpinolene on lipid peroxidation markers in experimental rats

Lipid peroxidation markers (TBARS, LOOH) were increased in the hepatic tissue of diabetic control rats. Treatment with terpinolene for four weeks in diabetic rats reduced TBARS and LOOH when compared to the diabetic control group (Table 2). Administration of ALA showed a similar effect in diabetic treated rats.

Influence of terpinolene on histological changes in the liver of experimental rats

In the histological analysis, liver sections from the control rats displayed a normal concentric organization of hepatocytes with well-defined sinusoidal cords surrounding the portal tracts and central vein.

In contrast, STZ-induced diabetic rats exhibited disrupted hepatocyte architecture, marked periportal fat accumulation, sinusoidal congestion near the central vein, micro-vesicular vacuolation, granular degeneration, and areas of focal necrosis. Mild dilatation of sinusoids with reduced accumulation of fats was observed in terpinolene (50mg/kg b.w) treated rats, signifying the hepatoprotective nature. The findings are comparable with ALA-treated diabetic rats (Fig. 5).

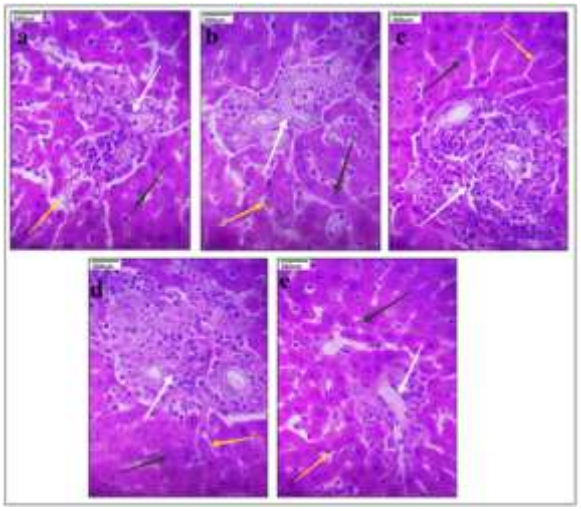

Fig. 5. Histopathological changes in the liver of experimental rats

(a) Normal control (b) Normal + Terpinolene (50mg/kg b.w) Normal hepatocytes (black arrow), normal appearing portal triad (white arrow), sinusoids (yellow arrow), normal appearance of central vein. (c) Diabetic control: Hepatocytes showed dense peri portal inflammation and fibrosis (white arrow) with bile duct proliferation, sinusoidal dilation (yellow arrow), congested appearance of central vein. (d) Diabetic + Terpinolene (50mg/kg b.w) Hepatocytes with no specific pathology (black arrow), reduced inflammation in the portal region (white arrow), mild dilation of sinusoids (yellow arrow) (e) Diabetic + ALA (100mg/kg b.w) Normal hepatocytes (black arrow), mild inflammation in the portal triad (white arrow), normal appearing sinusoids (yellow arrow).

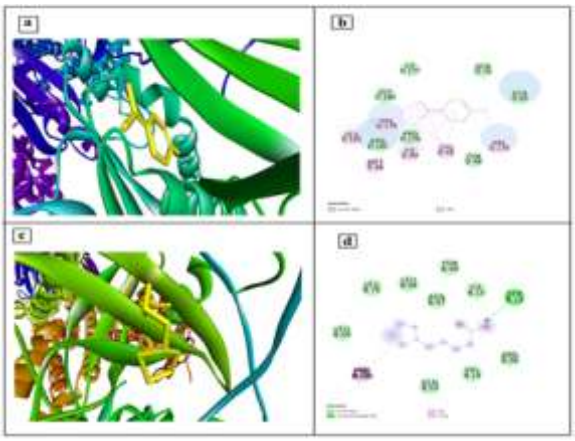

Fig. 6. Molecular interaction between terpinolene (a and b) and ALA (c and d) with NOX4

Table 3. Binding energy and interacting residues between terpinolene and ALA with NOX4

Target	Compounds	ΔG kcal/mol	Interacting residues
NOX4	Terpinolene	-6.2	ILE 177, GLN76, GLN83, GLY180, VAL176, THR48, CYS45, ILE49, PRO178, MET46, ARG181, LEU182, VAL179, GLY180.
	ALA	-5.2	THR99, ILE15, ASN97, LEU94, LEU76, LEU69, ARG96, VAL14, GLN93

Binding interaction between terpinolene and NOX4

Molecular docking studies revealed that terpinolene binds to Nox4 by interacting with key amino acid residues such as ILE 177, GLN76, GLN83, GLY180, VAL176, THR48, CYS45, ILE49, PRO178, MET46, ARG181, LEU182, VAL179 and GLY180 with a binding energy of −6.2 kcal/mol (Fig. 6 and Table 3). Similar interaction with a binding energy of −5.2 kcal/mol was observed with ALA.

DISCUSSION

Type 2 diabetes mellitus disrupts hepatic function, which significantly impairs the clearance of blood glucose and increases gluconeogenesis, contributing to hyperglycemia (Tănase *et al.*, 2023). Chronic hyperglycemia progressively damages peripheral nerves, which impairs nerve conduction and alters behavioral responses to sensory stimuli, leading to heightened pain sensitivity (Ye *et al.*, 2022). The tail immersion test is a sensitive and reliable tool for assessing thermal nociception in DPN rats.

In the present study, DPN rats exhibited reduced withdrawal latencies, reflecting hyperalgesia due to heightened sensitivity of damaged nociceptive fibers. Similarly, in the acetone drop test, an exaggerated withdrawal reaction to the mild cooling sensation produced by acetone evaporation was observed in DPN rats. These findings illustrate the interconnectedness between metabolic impairment in the liver to pain pain-related behavioral changes in diabetic rats. Hyperglycemia-induced oxidative stress and inflammatory responses divert glycolytic intermediates into multiple molecular pathways that exacerbate hepatic injury (Chen *et al.*, 2022). An imbalance between pro-oxidant and antioxidant systems is a well-established risk factor for hepatic dysfunction (Park *et al.*, 2022). Excessive electron flow through complexes I and III of ETC leads to electron leakage and overproduction of superoxide anion ($O_2^{-}\cdot$) in the liver (Tabassum *et al.*, 2020). Superoxide dismutase (SOD), an enzymatic antioxidant that serves as the first line of defence against free radicals, converts this highly reactive radical into molecular oxygen (O_2) and hydrogen peroxide (H_2O_2) (Engwa *et al.*, 2022).

Inactivation of superoxide dismutase through glycation and oxidative modification exacerbate oxidative stress and hepatocellular injury. In diabetic hepatopathy, loss or oxidation of essential metal cofactors destabilizes SOD and mediates liver injury in diabetes mellitus (Fontes *et al.,* 2024). Chronic hyperglycemia drives excessive production of H_2O_2, particularly through mitochondrial fatty acid β-oxidation and peroxisomal metabolism. Another important enzymatic source of H_2O_2 is NOX4, a constitutively active enzyme widely expressed in liver, kidney, vasculature, and adipose tissue.

In diabetic liver, NOX4 expression is upregulated, contributing to sustain H_2O_2, generation that overwhelms endogenous antioxidant defenses. Unscavenged H_2O_2 undergoes Fenton and Haber - Weiss reactions, generating hydroxyl radicals (OH) that drive lipid peroxidation, protein oxidation, and DNA damage (Liu *et al.*, 2012). The deleterious effect of H_2O_2 on cellular membranes is prevented by CAT, an enzyme with high catalytic efficiency and turnover rate. In the present study, SOD and CAT activities were reduced in the hepatic tissue of diabetic rats.

Glutathione peroxidases (GPx) are selenium-dependent enzymes that maintain hepatic redox homeostasis by catalyzing the reduction of H_2O_2 and lipid hydroperoxides into water and corresponding alcohols, using reduced glutathione (GSH) as a cofactor (Pei *et al.*, 2023).

This detoxification is particularly important in the liver, where intense mitochondrial respiration and β-oxidation of fatty acids generate continuous oxidative pressure (Dragoev, 2024). Glutathione reductase (GR), maintains cellular GSH levels by catalyzing the NADPH-dependent conversion of oxidized glutathione (GSSG) back to its reduced form (GSH). Excessive oxidative load depletes GSH levels and impairs the activities of glutathione-dependent enzymes (GPx, GR), thereby disrupting cellular antioxidant defense mechanisms. In the present study, a decrease in the activities of GST, GPx, GR and a reduction in GSH levels were observed in the hepatic tissue of diabetic rats. This decline resulted in the accumulation of lipid peroxides and reactive aldehydes, amplifying oxidative damage. Oral treatment with terpinolene dose dependently increased both enzymic and non-enzymic antioxidant levels, indicating an overall improvement in the oxidative balance of diabetic rats. In molecular docking studies, a binding energy of -5.2 kcal/mol was observed between terpinolene and NOX4, indicating high high-affinity interaction.

The obtained negative binding energy suggests less energy is needed for the binding of the ligand with the target. As the overexpression of NOX4 contributes to oxidative stress, compounds that can modulate its activity offer a potential strategy to curtail diabetic complications.

Lipid peroxidation arising from oxidative stress compromises the integrity of cellular membranes resulting in the accumulation of reactive aldehydes that exacerbate oxidative damage and tissue dysfunction. In this study, increased levels of thiobarbituric acid reactive substances (TBARS), lipid hydroperoxides (LOOH) were observed in the hepatic tissues of diabetic rats. Further, the observed increase in AST and ALT levels provides a measurable marker of liver damage in hyperglycemic conditions. Similarly, Ajiboye *et al.* reported that alkaloid-rich extracts of Dalbergiella welwitschia significantly restored antioxidant enzymes (SOD, CAT, GPx, GST, and GR) and normalized elevated AST and ALT levels in STZ-induced diabetic rats. A distorted arrangement of hepatocytes with fatty infiltration, vacuolization, congestion of sinusoids around the central vein and focal necrosis was observed in the liver of diabetic control rats. Studies have shown that the degree of liver stiffness and the presence of steatosis and fibrosis have been independently correlated with the prevalence and severity of diabetic peripheral neuropathy, even after adjusting for traditional risk factors (Huang *et al.*, 2021). Administration of terpinolene significantly curtailed lipid peroxidation in diabetic treated rats. In addition, mild sinusoidal dilatation with reduced fatty accumulation was observed in the hepatic tissue of terpinolene-treated diabetic rats. Thus, by restoring hepatic function and reducing oxidative stress, terpinolene alleviated sensory abnormalities by diminishing hyperalgesia and allodynia in DPN rats.

CONCLUSION

The present study demonstrated that terpinolene exerted significant hepatoprotective effects through its antioxidant properties. By restoring key enzymic and non-enzymic antioxidant defenses, reducing lipid peroxidation, and improving histopathological alterations, terpinolene mitigated diabetes-induced hepatic injury and enhanced overall redox balance. Its potential interaction with NOX4 further supported its role in suppressing ROS generation, highlighting an additional molecular mechanism underlying its protective action. Consequently, by curtailing oxidative stress, it mitigated allodynia and hyperalgesia in diabetic rats.

These findings underscore terpinolene as a promising natural therapeutic candidate for managing diabetes-related oxidative complications, with potential benefits extending to both hepatic function and peripheral nerve health.

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