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Original Article

Effects of Naringenin on Lipid Peroxidation, Antioxidant Defense, and Membrane Enzyme Activity in Rats With Alcohol-Induced Toxicity

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Abstract

Introduction: Alcohol-induced toxicity causes heightened oxidative stress, leading to increased lipid peroxidation and impaired antioxidant defenses, which damage cellular membranes and enzyme functions. Naringenin, a flavonoid in citrus fruits, has demonstrated strong antioxidant properties. This study investigated the potential protective effects of naringenin against alcohol-induced toxicity in rats, focusing on lipid peroxidation, antioxidant status, and membrane enzyme activity.

Methods: Four groups of male Wistar rats were used: Control (saline), Alcohol (ethanol), Naringenin (50 mg/kg), and Naringenin + Alcohol. Ethanol was administered for four weeks to induce toxicity, and naringenin was given orally during the final two weeks. Lipid peroxidation (LPO), antioxidant state, and membrane enzyme activity were evaluated in blood samples.

Results: Compared with the Control group, the Alcohol group showed notably greater lipid peroxidation and decreased antioxidant defenses. The Naringenin + Alcohol group exhibited a significant decrease in LPO and a marked increase in antioxidant enzyme activity compared with the Alcohol group. Membrane-bound enzymes, including lactate dehydrogenase (LDH) and alkaline phosphatase (ALP), also exhibited noticeably greater activity in the Naringenin + Alcohol group than in the Alcohol group.

Conclusion: Naringenin mitigates alcohol-induced damage by reducing LPO, increasing antioxidant defenses, and improving membrane enzyme activity. It has a protective role against alcohol-related oxidative stress and may help manage alcohol-induced damage, warranting further research for potential clinical applications.

Keywords: Alcohol, Biochemical assessment, Enzyme, Naringenin

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Introduction

Regular consumption of alcohol is a major contributing factor to diseases related to oxidative stress, including liver damage and cardiovascular disorders. Alcohol metabolism generates reactive oxygen species (ROS) in the body, resulting in lipid peroxidation and the formation of harmful byproducts, such as malondialdehyde (MDA). These compounds damage cellular membranes, leading to various health problems (1). Alcohol-induced oxidative stress also weakens antioxidant defenses by impairing the activity of key enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPX) (2,3). This results in considerable oxidative stress, which damages cells, disrupts their functions, and exacerbates the adverse effects of chronic alcohol consumption on health (4).

Despite being vital for cellular metabolism and homeostasis, membrane-bound enzymes such as lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) are extremely susceptible to oxidative damage, especially under alcohol-induced oxidative stress conditions. LDH catalyzes the conversion of lactate to pyruvate, a crucial step in cellular energy metabolism, while ALP

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participates in dephosphorylation reactions essential for membrane transport and signal transduction (5). ROS produced during excessive alcohol metabolism pose a serious threat to the lipid bilayer of cellular membranes, where these enzymes are anchored or tightly connected (6). ROS-mediated lipid peroxidation disrupts the microenvironment necessary for maintaining enzyme structure and function by altering membrane fluidity and integrity (7). The catalytic activity of ALP and LDH can be directly hampered by oxidative changes such as carbonylation or nitration, which lowers enzymatic efficiency (8). Furthermore, altered serum levels of ALP and LDH are regarded as reliable indicators of cellular injury and dysfunction following alcohol-induced oxidative damage, as damaged membranes may cause these enzymes to leak into the bloodstream (9,10). Monitoring these enzyme activities provides valuable insight into the degree of oxidative stress and the development of tissue damage caused by alcohol toxicity.

Naringenin, a flavonoid predominantly found in citrus fruits, has recently gained considerable attention for its antioxidant and anti-inflammatory properties. Recent research has highlighted its capacity to reduce oxidative stress and restore antioxidant enzyme activity across different conditions (11,12). Additionally, naringenin plays a key role in modulating signaling pathways involved in fatty acid metabolism by promoting fatty acid oxidation and preventing lipid accumulation in the liver, thereby offering protection against fatty liver disease (13). It has also been demonstrated to effectively lessen lipoprotein accumulation and plasma lipids (14).

Specifically, naringenin has shown promise in reducing lipid peroxidation and enhancing endogenous antioxidant defenses, making it a potential therapeutic agent for alcohol-induced oxidative damage (15,16). However, its protective effects in the context of alcohol-induced toxicity remain insufficiently explored.

The present study evaluated the effects of naringenin on lipid peroxidation, antioxidant defense mechanisms, and membrane enzyme activity in rats with alcohol-induced toxicity. It sought to uncover the potential protective mechanisms of naringenin against alcohol-induced oxidative damage.

Materials and Methods

Reagents and Chemicals

Naringenin (Figure 1) was obtained from Sigma Chemical Co. (St. Louis, Missouri, USA). Ethanol was supplied by E.I.D. Parry India Ltd., Nellikuppam, Cuddalore District, Southern India. The remaining reagents and chemicals were all obtained from Central Drug House Pvt. and were of analytical quality.

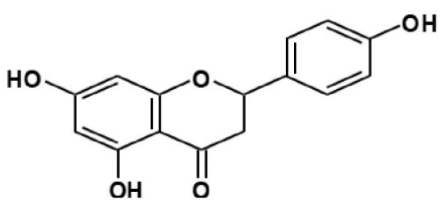


Figure 1. Chemical Structure of Naringenin

Animals

Male Albino Wistar rats weighing between 150 and 200 grams were purchased from Rajah Muthiah Medical College and Hospital (RMMC&H), Annamalai University. The animals were housed under controlled environmental conditions: temperature of 28 ˚C, a 12-hour light/dark cycle, and relative humidity of 50%. Rats were provided with unrestricted access to water and fed a standard pellet diet. All experimental procedures were reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of Annamalai University (Registration No: 160/1999/CPCSEA/557).

Study Design

The rats were randomly divided into four groups, each consisting of eight rats. Groups 3 and 4 were administered ethanol via intubation for 30 days, whereas groups 1 and 2 received glucose with 0.5% carboxymethyl cellulose (CMC). After 30 days, groups 1 and 3 continued their respective regimens. However, group 2 rats received naringenin, while group 4 rats received both ethanol and naringenin for an additional 30 days. The entire trial lasted 60 days. The research design is illustrated in Figure 2. Prior to euthanasia, rats were anesthetized using inhalational isoflurane. Once deeply anesthetized, euthanasia was performed by cervical dislocation.

Preparation of Plasma and Hemolysate

Blood samples containing Heparin were centrifuged to extract plasma, and the red blood cells (RBCs) were washed thoroughly with saline. To assess antioxidant enzyme activity in RBCs, a hemolysate was prepared by lysing the RBCs with a hypotonic phosphate buffer at pH 7.4.

Preparation of Erythrocyte Membrane

Hemoglobin-free ghost erythrocytes, also referred to as erythrocyte membrane, were obtained according to Dodge and colleagues' (17) method, with buffer modifications suggested by Quist (18). The packed cells were washed with isotonic Tris-HCl buffer and then subjected to hemolysis using a hypotonic Tris–HCl buffer, yielding ghosts harvested by high-speed centrifugation. After removing the hemolysate, the ghost pellet was reconstituted and washed three times until a colorless appearance was obtained. The final pellets were suspended in Tris–HCl buffer for further analyses. Overall, the process involved sequential washing, hemolysis, and centrifugation steps.

Biochemical Estimations

Various enzymatic activities were evaluated using different biochemical methods from multiple studies. ALP activity was determined by measuring its ability to

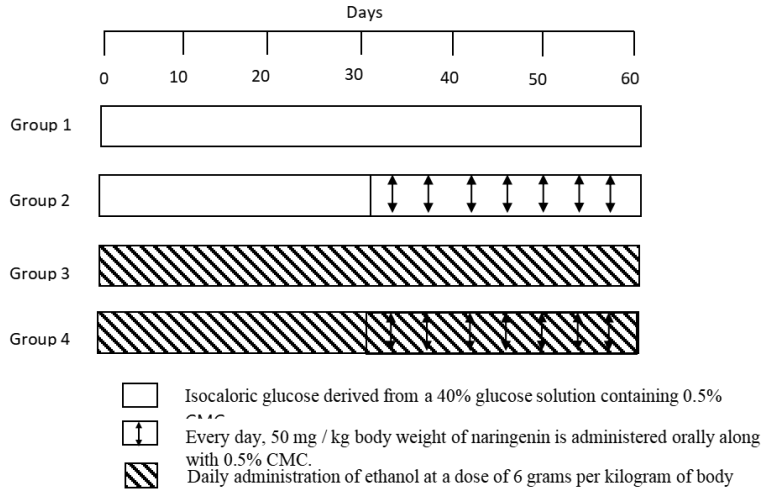


Figure 2. Study Design

hydrolyze phosphate esters, according to the methods of Kind & King (19) and King (20). LDH activity was quantified by converting lactate to pyruvate. Bilirubin activity was assessed through the formation of a colored complex with diazotized sulfanilic acid, while thiobarbituric acid reactive substances (TBARS) in tissue samples were estimated at 532 nm following Ohkawa et al (21). Lipid hydroperoxides (LOOH) were measured using the reagent described by Jiang et al (22), while conjugated dienes (CD) were determined by absorbance at 233 nm based on Rao and Recknagel (23).

SOD activity was estimated following Kakkar et al (24) and expressed as the amount of enzyme needed to inhibit the reduction of NBT by 50% per minute per mg of protein. The Ellman method (25) was used to quantify catalase (CAT) activity, which was then expressed as micromoles of H_2O_2 degraded per minute per mg of protein. Ellman reported that glutathione S-transferase (GST) activity was assessed by measuring the rate of 1-chloro-2,4-dinitrobenzene (CDNB) conjugate formation per minute per mg of protein at 340 nm, while glutathione reductase (GR) activity was determined using the rate of NADPH oxidation per minute per mg of protein. According to Roe and Kuether (26), reduced glutathione (GSH) levels were determined by measuring the formation of a yellow-colored complex with 5,5'-dithiobis (2-nitrobenzoic acid). As described by Baker et al (27), ascorbic acid levels were estimated by measuring the reduction of 2,6-dichlorophenolindophenol, and protein concentration was determined according to Lowry et al (28).

The measurement of membrane-bound enzymes in erythrocytes involved several assays. Released phosphorus was quantified, and colorimetric analysis at 640 nm was used to determine the rate of phosphorus liberation per minute per mg of protein at 37 °C. Mg^{2+}-ATPase activity, an incubation mixture containing buffer, magnesium sulfate, NaCl, EDTA, and ATP was prepared, and the RBC membrane suspension was added. Similarly, Ca^{2+}-ATPase activity was ascertained by measuring phosphorus release per minute per mg of protein, following Hjerken and Pan's (29) method.

Reverse Transcriptase Polymerase Chain Reaction

To assess the expression levels of membrane-bound enzymes in blood samples, biochemical tests were performed. Glassware was cleaned with diethyl pyrocarbonate (DEPC)-treated water to prevent RNase activity. Total RNA was extracted utilizing the guanidium thiocyanate-chloroform-phenol method described by Chomczynski and Sacchi (30). Tissue samples were minced and homogenized with an RNA isolation buffer containing sodium acetate, phenol, and a $CHCl_3$. Isoamyl alcohol mixture. After centrifugation of the resulting homogenate, RNA was precipitated using isopropanol, washed with ethanol, and dissolved in DEPC-treated water. Spectrophotometry was used to quantify the RNA, which was then utilized in reverse transcription to produce cDNA. Subsequently, PCR was performed to study caspase-3 expression, with GAPDH acting as the internal standard. The primer sequences are presented in Table 1. This process was effective in extracting and examining RNA from diverse tissue samples, offering valuable insights into gene expression levels such as *caspase-3*.

Statistical Analysis

In addition to using Duncan's multiple range tests as a supplementary method, analysis of variance (ANOVA) was used to statistically analyze the data. All analyses were conducted using SPSS version 11.0 software (SPSS Inc., Chicago, IL, USA). A *P* value of less than 0.05 was considered statistically significant.

Results

As shown in Table 2, rats fed alcohol exhibited significantly higher serum levels of bilirubin, LDH, and ALP compared to the control group. There was no significant variation between the control and control + naringenin groups (107.22 ± 10.32 U/L vs. 112.99 ± 10.87 U/L), suggesting

Table 1. Primer Sequences and the Resulting Polymerase Chain Reaction Products

PCR Products	Primer Sequences
Caspase-3	Sense: 5′- AAAGACTATCCATCCATGGAAGCAAG -3′ Antisense: 5′- TGCTCCTTTTGCTGTGATC -3′
GAPDH	Sense: 5′- TGT GTC CGT CGT GGA TCT GA-3′ Antisense: 5′- CCT GCT TCA CCA CCT TCT TGA -3′

Note. PCR: Polymerase chain reaction: GAPDH: Glyceraldehyde 3-phosphate dehydrogenase

Table 2. Impact of Naringenin on Liver Enzymes in Rats Exposed to Ethanol Compared to a Control Group

Groups	ALP (U/L)	LDH (U/L)	Bilirubin (mg/dL)
Control	107.22 ± 10.32a	127.05 ± 12.23a	0.49 ± 0.048a
Control + Naringenin	112.99 ± 10.87a	132.83 ± 12.78a	0.45 ± 0.044a
Ethanol	162.63 ± 15.65b	306.23 ± 29.47b	0.86 ± 0.083b
Ethanol + Naringenin	117.75 ± 11.33a	135.32 ± 13.02a	0.58 ± 0.176a

Note. ALP: Alkaline phosphatase; LDH: Lactate dehydrogenase.
For each group, the mean ± standard deviation values were computed for eight rats. According to Duncan's multiple range test, values with distinct superscript letters represent significant differences at a level of $P < 0.05$.

that naringenin does not notably affect ALP activity in the absence of ethanol. ALP activity was significantly higher in the ethanol-administered group (162.63 ± 15.65 U/L) compared to the ethanol + naringenin group (117.75 ± 11.33 U/L), indicating that naringenin had a protective effect by reducing the elevated ALP levels induced by ethanol. Similarly, LDH levels were comparable between the control and control + naringenin groups (127.05 ± 12.23 U/L vs. 132.83 ± 12.78 U/L), showing no significant effect of naringenin alone on LDH activity. However, the ethanol group shows administration caused a marked increase in LDH activity (306.23 ± 29.47 U/L) compared to the ethanol + naringenin group (135.32 ± 13.02 U/L). This finding implies that naringenin significantly mitigates the increase in LDH activity, reflecting its potential protective role against ethanol-induced liver damage. Bilirubin levels also followed a similar trend. No notable difference was found between control and control + naringenin groups (0.49 ± 0.048 mg/dL versus 0.45 ± 0.044 mg/dL), suggesting that naringenin has no impact on bilirubin levels in the absence of ethanol. The bilirubin levels in the group consuming ethanol were noticeably elevated at 0.86 ± 0.083 mg/dL, whereas those in the ethanol + naringenin group were lower at 0.58 ± 0.176 mg/dL**.** This suggests that naringenin reduces bilirubin levels elevated by ethanol administration, highlighting its role in ameliorating hepatic dysfunction.

Table 3 presents comparable TBARS values for the control and control + naringenin groups (3.21 ± 0.309 nmol/mL vs. 3.21 ± 0.309 nmol/mL), suggesting that naringenin has no impact on TBARS levels in the absence of ethanol. The administration of ethanol resulted in a significant increase in TBARS levels (5.10 ± 0.49 nmol/mL) compared to the ethanol + naringenin group (3.28 ± 0.316 nmol/mL). This suggests that naringenin effectively mitigates lipid peroxidation, as evidenced by

Table 3. Impact of Naringenin on Plasma Levels of TBARS, LOOH, and CD

Groups	TBARS (nmol/mL)	LOOH (nmol/mL)	CD (μmol/mL)
Control	3.21 ± 0.309a	0.69 ± 0.066b	0.98 ± 0.095a
Control + Naringenin	3.21 ± 0.309a	0.64 ± 0.066a	0.96 ± 0.093a
Ethanol	5.10 ± 0.49b	2.97 ± 0.28a	3.26 ± 10.314c
Ethanol + Naringenin	3.28 ± 0.316a	0.76 ± 0.07a	1.28 ± 0.12b

Note. TBARS: Thiobarbituric acid reactive substances; LOOH: Lipid hydroperoxides; CD: Conjugated dienes.
The mean ± standard deviation values were calculated for eight rats per group. Values with unique superscript letters indicate significant differences at $P < 0.05$ according to Duncan's multiple range test.

lower TBARS levels in its presence.

Under normal conditions, naringenin appears to have no effect on LOOH levels, as reflected in the similar measurements from both groups (0.69 ± 0.66 nmol/mL vs. 0.64 ± 0.066 nmol/mL). In contrast, the ethanol group exhibited significantly higher LOOH levels (2.97 ± 0.28 nmol/mL) compared to the ethanol + naringenin group (0.76 ± 0.07 nmol/mL). This demonstrates that naringenin effectively reduces the ethanol-induced increase in LOOH, highlighting its capacity to lessen oxidative stress.

The results of the agarose gel electrophoresis (Figure 3) display bands corresponding to *caspase-3* mRNA levels across different experimental groups. Lanes 1–4 represent the Control, Control + Naringenin, Ethanol, Ethanol + Naringenin groups A densitometer was utilized to examine the intensity of the bands on the agarose gel. The relative *caspase-3* mRNA levels were normalized to the housekeeping gene *GAPDH*. The *caspase-3/GAPDH* ratio was then expressed as a percentage and presented as mean ± standard deviation for three independent experiments.

Ethanol is known to induce hepatotoxicity and increase apoptosis. Elevated caspase-3 mRNA levels reflect enhanced apoptotic activity. If lane 3 (Ethanol) shows significantly higher band intensity for *caspase-3* compared to the control, it indicates that ethanol treatment upregulates *caspase-3*, thereby promoting apoptosis in hepatocytes. Naringenin, a flavonoid known for its antioxidant and anti-apoptotic properties, may help reduce ethanol-triggered cell death. If lane 4 (Ethanol + Naringenin) shows a reduced *caspase-3/GAPDH* ratio compared to the ethanol-only group, it suggests that naringenin reduces *caspase-3* mRNA levels, thereby potentially protecting against ethanol-induced hepatocyte apoptosis.

Table 4 displays no notable discrepancy in SOD levels between these groups (10.43 ± 1.00 units vs. 10.52 ± 1.01 units), suggesting that naringenin does not impact SOD activity under normal conditions. Ethanol administration leads to a significant decrease in SOD activity (6.34 ± 0.61 units) compared to the ethanol + naringenin group (9.4 ± 0.91 units). This suggests that naringenin helps restore SOD activity, which is suppressed by ethanol-induced oxidative stress. The ethanol + naringenin group demonstrated a substantial increase in SOD activity

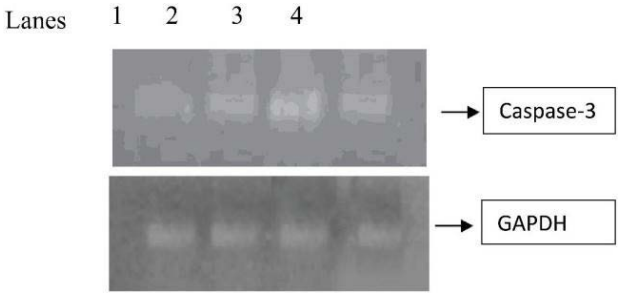


Figure 3. Effect of Naringenin on Caspase-3 mRNA Level in the Hepatotoxicity Rats

(9.4 ± 0.91 units) compared to the ethanol-only group (6.34 ± 0.61 units) after administration. This indicates that naringenin helps restore SOD activity, which is decreased due to ethanol-induced oxidative stress.

CAT levels were similar between the control and control + naringenin groups (46.206 ± 4.44 μmol H_2O_2 utilized/min vs. 45.79 ± 4.40 μmol H_2O_2 utilized/min), indicating that naringenin does not affect CAT activity in the absence of ethanol. Ethanol administration significantly reduced CAT activity (25.50 ± 2.45 μmol H_2O_2 utilized/min) compared to the ethanol + naringenin group (44.67 ± 4.3 μmol H_2O_2 utilized/min). This suggests that naringenin effectively counteracts the decrease in CAT activity induced by ethanol, underscoring its protective role against oxidative damage.

Table 5 shows that the control groups exhibit comparable GPX activity, which slightly rises in the presence of naringenin. However, exposure to ethanol notably decreases GPX activity compared to the control, suggesting possible oxidative stress or enzymatic harm. Administration of naringenin to ethanol-treated rats partially restores GPX activity, though it remains below control levels. These results suggest that naringenin has a protective effect against ethanol-induced inhibition of GPX activity, even if it does not completely normalize enzyme activity.

GR activity in the control groups was similar, with a slight decrease noted following naringenin administration. Ethanol exposure significantly reduced GR activity, reflecting possible glutathione depletion or impaired enzymatic function. Naringenin treatment in ethanol-exposed rats partially restored GR activity, approaching, but not reaching, control levels. This indicates that naringenin has a beneficial effect in mitigating ethanol-induced reduction in GR activity.

GST activity remained similar between the control and control + naringenin groups. Ethanol administration decreased GST function; however, when ethanol-treated rats received naringenin, GST activity increased notably, even exceeding that of the control + naringenin group. This indicates that naringenin can enhance GST performance more efficiently in the presence of ethanol-induced stress.

Table 6 demonstrates that the ethanol-treated group had significantly lower GSH levels than the control

Table 4. Effect of Naringenin on SOD and CAT Levels in Hemolysate

Groups	SOD (units)	CAT (μmol H2O2 utilized/min)
Control	10.43 ± 1.00b	46.206 ± 4.44b
Control + Naringenin	10.52 ± 1.01b	45.79 ± 4.40b
Ethanol	6.34 ± 0.61a	25.50 ± 2.45a
Ethanol + Naringenin	9.4 ± 0.91b	44.67 ± 4.3b

Note. SOD: Superoxide dismutase; CAT: Catalase.
For each group, the mean ± standard deviation values were computed for eight rats. According to Duncan's multiple range test, values with distinct superscript letters represent significant differences at $P < 0.05$.

group, suggesting that ethanol induces oxidative stress and depletes this critical antioxidant. Administration of naringenin to ethanol-treated rats resulted in a significant increase in GSH levels compared to the ethanol-only group, although these levels still fell short of those in the control group. This implies that naringenin does not entirely restore GSH to baseline levels after ethanol-induced depletion but mitigates it to some extent. This finding highlights naringenin's ability to support antioxidant defenses and lessen oxidative damage.

Ethanol treatment leads to a significant decrease in vitamin C levels, suggesting oxidative stress-induced depletion. Naringenin treatment prevented a significant reduction in vitamin C levels in ethanol-treated rats, bringing levels closer to control values. This indicates that naringenin helps preserve vitamin C levels under oxidative stress conditions.

Vitamin E levels in the ethanol-treated group are significantly lower than in the control group, reflecting increased oxidative stress and lipid-soluble antioxidant utilization. Vitamin E levels increased when naringenin was given to ethanol-exposed rats, although not to the extent observed in the control group. This suggests that naringenin protects vitamin E by lessening ethanol-induced oxidative damage.

Table 7 presents the effects of ethanol and naringenin on various ATPase activities in the RBC membrane of experimental and control animals, including total ATPase, Na/K-ATPase, Ca-ATPase, and Mg-ATPase. Exposure to ethanol notably reduced the total ATPase activity, indicating a decline in overall ATPase function. However, administration of naringenin to ethanol-exposed rats led to a significant rise in total ATPase activity compared to the ethanol-only group, though not completely restoring it to control levels. This indicates that naringenin positively influences ATPase activity, partially counteracting ethanol-induced inhibition.

Ethanol exposure significantly reduced Na^+/K^+-ATPase activity, reflecting disruption of ionic homeostasis and cellular function. Naringenin treatment partially restored Na^+/K^+-ATPase activity in ethanol-treated rats, although it did not reach control levels. This indicates that naringenin helps mitigate ethanol's adverse effects on ionic regulation without completely reversing the damage. Similarly, ethanol significantly decreased Ca^{2+}-ATPase activity, which could potentially affect calcium

Table 5. Effect of Naringenin on GPX, GR, and GST Levels in Hemolysate

Groups	GPX (µg of GSH utilized/min)	GR (µmol of CDNB-GSH conjugate formed/min)	GST (µmol of CDNB-GSH conjugate formed/min)
Control	15.50 ± 1.49b	16.60 ± 1.59b	15.56 ± 1.49b
Control + Naringenin	15.61 ± 1.50b	15.62 ± 1.50b	15.96 ± 1.53b
Ethanol	8.83 ± 0.85a	9.18 ± 0.88a	10.40 ± 1.00a
Ethanol + Naringenin	14.28 ± 1.37b	15.27 ± 1.47b	16.18 ± 1.55b

Note. GPX: Glutathione peroxidase; GR: Glutathione reductase; GST: Glutathione S-transferase; GSH: Reduced glutathione; CDNB: 1-chloro-2,4-dinitrobenzene.
For each group, the mean ± standard deviation values were computed for eight rats. According to Duncan's multiple range test, values with distinct superscript letters represent significant differences at $P < 0.05$.

Table 6. Effect of Naringenin on GSH, Vitamin C, and Vitamin E Plasma Levels

Group	GSH (mmol/mg protein)	Vitamin C (mg/dL)	Vitamin E (mg/dL)
Control	33.25 ± 3.20c	4.59 ± 0.44a	50.79 ± 4.88b
Control + Naringenin	32.84 ± 3.16c	4.40 ± 0.37a	51.28 ± 4.93b
Ethanol	18.46 ± 1.77a	3.27 ± 0.315b	35.70 ± 3.43a
Ethanol + Naringenin	28.86 ± 2.27b	4.33 ± 0.417a	46.14 ± 4.43b

Note. GSH: Reduced glutathione.
For each group, the mean ± standard deviation values were computed for eight rats. According to Duncan's multiple range test, values with distinct superscript letters represent significant differences at $P < 0.05$.

homeostasis and contribute to cellular dysfunction. Naringenin administration increased Ca^{2+}-ATPase activity in ethanol-treated rats, although it did not fully restore it to control levels. This indicates that naringenin can help improve Ca^{2+}-ATPase function but cannot completely reverse ethanol-induced impairment. Ethanol treatment also resulted in a significant decrease in Mg^{2+}-ATPase activity, suggesting impairment in cellular processes dependent on this enzyme. Naringenin treatment significantly increased Mg^{2+}-ATPase activity in ethanol-treated rats, though it did not fully reach control levels. This indicates that naringenin helps preserve Mg^{2+}-ATPase function, which is crucial for maintaining cellular processes.

Naringenin has a protective effect, partially restoring these ATPase activities, though not fully to control levels. This protective effect underscores naringenin's potential as an antioxidant compound capable of mitigating ethanol-induced cellular dysfunction.

Discussion

The research demonstrated that naringenin protects the liver from ethanol-induced harm by decreasing markers such as ALP, LDH, bilirubin, TBARS, LOOH, and CD, which are indicative of oxidative stress and liver damage. The anti-inflammatory and antioxidant characteristics of naringenin played a key role in mediating this protective effect, likely through modulation of signaling pathways involved in inflammation and oxidative stress. Moreover, naringenin enhanced hepatic detoxification enzyme activity, reduced liver fibrosis markers, and improved liver enzyme profiles, indicating its role in promoting liver health. Additionally, naringenin's regulatory effect on the gut-liver axis and gut microbiota was highlighted as a mechanism through which it helps protect the liver from ethanol-induced damage.

Overall, naringenin's ability to mitigate oxidative stress, lipid peroxidation, inflammation, and improve mitochondrial function demonstrates its potential as a therapeutic agent for preventing and treating ethanol-induced liver injury. Naringenin exerts a multifaceted protective effect on the liver, making it a promising candidate for future research and development of liver-protective treatments. The findings of this study support the concept that naringenin can effectively mitigate ethanol-induced oxidative stress and lipid peroxidation, hence underscoring its potential as a therapeutic intervention for ethanol-induced liver injury.

In particular, naringenin was found to protect antioxidant enzymes such as SOD and CAT in the hemolysate from oxidative stress induced by ethanol, counteracting the ethanol-associated reduction in their activities. This restoration of enzyme activities by naringenin aligns with its antioxidant properties and supports its efficacy in mitigating ethanol-induced oxidative damage. Additionally, the study revealed that naringenin can improve the activities of other key antioxidant enzymes, including GPX, GR, and GST, which are adversely affected by ethanol-induced oxidative stress, suggesting its ability to counteract some of the damaging effects of ethanol on antioxidant defenses. Although naringenin partially restored GSH levels depleted by ethanol exposure, it was unable to fully reverse the oxidative damage caused by ethanol. However, it effectively prevented a significant decrease in vitamin C levels induced by ethanol, indicating its role in preserving this crucial antioxidant under oxidative stress conditions.

Overall, the results emphasize how naringenin could offer therapeutic advantages by protecting against ethanol-induced oxidative stress and liver injury. This is achieved through enhancing antioxidant enzyme activities and safeguarding essential antioxidants. Recent literature supports the role of naringenin in maintaining vitamin C levels under oxidative stress conditions, as well as mitigating ethanol-induced decreases in vitamin C. Moreover, naringenin treatment has been found to increase vitamin E levels in ethanol-treated rats, thereby protecting against oxidative damage (31).

Further research confirms that naringenin helps maintain vitamin E levels in models of oxidative stress, showing its protective properties. Naringenin exhibits an overall antioxidant protection efficiency in the range of approximately 70-80%, characterized by enhanced preservation of vitamins and enzymatic antioxidants,

Table 7. Effect of Ethanol and Naringenin on RBC Membranes of Control and Experimental Animals: total ATPases, Na + K + ATPase, Ca2 + ATPase, and Mg2 + ATPase

Group	Total-ATPase (μmol Pi·min^{-1}·mg^{-1})	Na^+/K^+- ATPase (U·mg^{-1})	Ca^{2+}-ATPase (U·mg^{-1})	Mg^{2+}-ATPase (U·mg^{-1})
Control	1.95 ± 0.188b	0.918 ± 0.088c	0.47 ± 0.045a	2.34 ± 0.255c
Control + Naringenin	1.92 ± 0.185b	0.82 ± 0.069b	0.408 ± 0.039c	2.14 ± 0.206bc
Ethanol	0.91 ± 0.080a	0.45 ± 0.044a	0.255 ± 0.024b	1.63 ± 0.157a
Ethanol + Naringenin	1.78 ± 0.171b	0.79 ± 0.076b	0.357 ± 0.034b	2.01 ± 0.194b

Note. RBC: Red blood cell; ATPase: Adenosine triphosphatase; Na^+/K^+: Sodium/Potassium; Ca^{2+}: Calcium; Mg^{2+}: Magnesium; Pi: Inorganic phosphate. The mean ± standard deviation values were calculated for eight rats per group. According to Duncan's multiple range test, values with distinct superscript letters represent significant differences at $P < 0.05$.

though only partial restoration of GSH and ATPase activities (32,33).

The influence of ethanol and naringenin on different ATPase functions within the RBC membrane, which are essential for maintaining cellular balance, was also observed. Ethanol exposure reduced total ATPase activity, reflecting overall impairment in ATPase function due to oxidative stress. Naringenin partially restored total ATPase activity in ethanol-treated rats, indicating its beneficial effect in mitigating ethanol-induced impairment. However, the activity did not fully recover to control levels, suggesting substantial oxidative damage by ethanol. Naringenin also helped maintain Na^+/K^+-ATPase activity, which is essential for cellular function, but it could not fully counteract ethanol's inhibitory effects. Naringenin could restore Na^+/K^+-ATPase activity during oxidative stress. Similarly, ethanol decreased Ca^{2+}-ATPase and Mg^{2+}-ATPase activities, affecting calcium and magnesium regulation in cells. Naringenin treatment partially restored these activities, suggesting its ability to improve calcium and magnesium homeostasis and alleviate oxidative stress damage, showing its positive effect on Ca^{2+}-ATPase activity under stress conditions (34-36).

Studies on heavy metal contamination, where oxidative stress and enzyme dysfunction are also key components, further mirror naringenin's protective action against ethanol-induced toxicity. Exposure to metals such as cadmium and lead dramatically lowers antioxidant enzyme activities (SOD, CAT, and GPX), increases lipid peroxidation, and impairs ATPase functions in hepatic and erythrocyte membranes. Similar to its impact on ethanol-induced damage, naringenin has been demonstrated to mitigate cadmium-induced oxidative stress by lowering lipid peroxidation and restoring antioxidant enzyme activity (37). Naringenin also showed a protective mechanism against heavy metal-induced oxidative damage in mercury-induced hepatotoxicity models by reducing apoptotic indicators, preventing mitochondrial dysfunction, and elevating glutathione levels (38). These results suggest that the defense mechanisms activated by naringenin against environmental toxicants, such as heavy metals, are not exclusive to ethanol-induced toxicity. Additionally, studies on arsenic exposure reported that lipid peroxidation and oxidative stress decreased Na^+/K^+-ATPase and Ca^{2+}-ATPase activities in erythrocyte membranes, which were considerably restored following flavonoid treatment, including naringenin (40). This provides compelling evidence supporting the hypothesis that the regulation of membrane-bound enzymes by flavonoids serves as a broad protective mechanism against various harmful substances, such as heavy metals and ethanol. Furthermore, naringenin has been shown to reduce apoptosis markers such as caspase-3 and to exert anti-apoptotic effects through various mechanisms involving the restoration of Mg^{2+}-ATPase activity and reduction of apoptosis in oxidative stress models. Naringenin's anti-apoptotic properties are similar to those observed in hepatotoxic models caused by nickel and cadmium, where it decreased caspase-3 expression while maintaining mitochondrial integrity. This suggests its broader therapeutic potential as a natural antioxidant and hepatoprotective agent against multiple sources of oxidative stress.

Conclusion

Naringenin reduces ethanol-induced damage by restoring enzyme activities and reducing apoptosis, demonstrating strong hepatoprotective potential. By reducing lipid peroxidation, enhancing antioxidant defenses, restoring membrane enzyme activities, and mitigating apoptosis, naringenin targets multiple aspects of oxidative stress and cellular damage caused by ethanol. These findings highlight naringenin as a promising therapeutic agent for alleviating alcohol-induced oxidative stress and associated liver injury. Taken together, the findings suggest that naringenin's preventive effects extend beyond ethanol-induced liver damage to include harm caused by environmental contaminants such as lead, mercury, arsenic, and cadmium. Future research should further explore the mechanisms underlying these protective effects and evaluate naringenin's clinical potential in managing alcohol-related liver diseases.

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Competing Interests

The authors declare no competing interests.

Data Availability Statement

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethical Approval

Animal handling and experimental procedures were approved by the Institutional Animal. Ethical Committee, Annamalai University (Reg.No: 160/1999/CPCSEA/557), and the animals were cared for in accordance with Indian National Law on Animal Care and Use.

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