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Research Article

INSIGHTS INTO THE MOLECULAR AND BIOCHEMICAL ROLE OF QUINIC ACID IN ALLEVIATING ETHANOL-INDUCED LIVER TOXICITY IN A RAT MODEL: EXPLORING OXIDATIVE STRESS, INFLAMMATION, AND APOPTOSIS SIGNALING PATHWAYS

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ABSTRACT

Objective: The study aimed to evaluate the effects of quinic acid, a natural bioactive compound, on tissue and circulatory antioxidant status, lipid peroxidation, and its anti-apoptotic and anti-inflammatory mechanisms in ethanol-induced hepatotoxicity in rats.

Methods: The rats were divided into four groups. Groups 1 and 4 were administered isocaloric glucose. Groups 2 and 3 received 30% ethanol at a dose of 5 g/kg body weight daily. In addition, Groups 3 and 4 were treated with quinic acid (50 mg/kg body weight) dissolved in 2% dimethyl sulfoxide.

Results: The results demonstrated significantly elevated levels of tissue thiobarbituric acid reactive substances (TBARS), conjugated dienes (CD), and lipid hydroperoxides (LOOH), along with significantly reduced enzymatic and non-enzymatic antioxidant activities, including superoxide dismutase (SOD), catalase (CAT), and glutathione-related enzymes such as glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione-S-transferase (GST), as well as reduced levels of glutathione (GSH), Vitamin C, and Vitamin E in ethanol-treated rats compared to the control group. Administration of quinic acid to rats with ethanol-induced liver injury significantly reduced the levels of TBARS, LOOH, and CD while markedly increasing the activity of SOD, CAT, GPx, GR, GST, and levels of GSH, Vitamin C, and Vitamin E in liver tissues compared to untreated ethanol-exposed rats. In addition, ethanol-treated rats showed increased mast cell accumulation, which was reduced by quinic acid treatment, along with elevated expressions of inflammatory and apoptotic markers, including Bax, Caspase-9, tumor necrosis factor-alpha, Nuclear factor kappa B, and interleukin-6, and a decreased expression of Bcl2 in the liver. Quinic acid supplementation in ethanol-fed rats reversed these ethanol-induced changes. Immunohistochemical studies further supported these findings.

Conclusion: Quinic acid, with its antioxidant, anti-inflammatory, and anti-apoptotic properties, may offer a therapeutic option for protecting against ethanol-induced hepatotoxicity.

Keywords: Antioxidants, Ethanol, Lipid peroxidation, Liver disease, Quinic acid.

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INTRODUCTION

Alcohol toxicity primarily affects organs involved in excretion and metabolism, particularly the liver. The morbidity and mortality rate among heavy alcohol drinkers has reached approximately 6.1% [1]. Alcoholic liver disease (ALD) is one of the most severe outcomes of chronic alcohol abuse worldwide [2]. According to Tuma and Casey (2003), factors contributing to ALD include acetaldehyde production, oxidative stress, hypoxia, immune response, and membrane alterations [3]. The liver is particularly vulnerable to alcohol toxicity because around 80% of ingested alcohol is metabolized there, producing numerous reactive oxygen species (ROS) in the process [4]. Initially, alcohol is converted to highly toxic acetaldehyde by alcohol dehydrogenase in the liver. Acetaldehyde is then oxidized to acetate by aldehyde oxidase or xanthine oxidase, generating ROS through cytochrome P450 2E1 [5]. Chronic alcohol consumption also elevates nitric oxide levels, which may contribute to toxicity through the formation of peroxynitrite, a potent oxidant [6]. Consequently, an overproduction of reactive nitrogen species and ROS can occur when their generation exceeds the system's capacity to neutralize and eliminate them.

Recognizing this pathogenic process, Chambers concluded that hepatic oxidative stress from chronic alcohol consumption results from a reduction in the liver's antioxidant capacity, which can lead to cell membrane and organelle damage, accompanied by the release of reactive aldehydes [7].

Ethanol intake can disrupt the fragile equilibrium between the body's prooxidant and antioxidant systems, resulting in oxidative stress. An increased production of free radicals originating from oxygen and ethanol has been noted in microsomes (particularly in the ethanol-inducible cytochrome P450 isoform), cytosolic enzymes such as xanthine and/or aldehyde oxidase, as well as within the mitochondrial respiratory chain [8].

In cases of chronic ethanol exposure, increased lipid peroxidation may arise from heightened free radical production within microsomal membranes [9]. Ethanol and its metabolites can shift the liver's balance toward oxidative processes, either by acting as prooxidants, lowering antioxidant levels, or both [10]. Lipid peroxidation driven by free radicals is regarded as a key mechanism, leading to cell membrane disruption and tissue damage [11]. To neutralize these oxidants, cells rely on various antioxidant enzymes, including superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT). Dietary antioxidants, especially Vitamins E and C, play a significant role, and poor nutrition can lead to deficiencies in these vitamins, thereby increasing the liver's vulnerability to ethanol-induced oxidative stress [12]. Apoptosis, a form of programmed cell death, is essential for maintaining tissue balance [13]. Mast cells, primarily associated with allergic reactions, can undergo apoptosis to control their numbers and limit excessive inflammatory responses [14]. Alcohol intake can provoke an inflammatory response by disrupting the gut barrier, allowing

endotoxins to enter the bloodstream and activate immune responses. This process triggers the release of pro-inflammatory cytokines, which can lead to tissue damage and contribute to conditions such as ALD. Persistent alcohol-induced inflammation can intensify organ damage and elevate the risk of various health complications [15].

Quinic acid, a cyclic polyol, is widely found in plants, especially fruits, vegetables, and coffee beans. Structurally, it comprises a cyclohexane ring with four hydroxyl groups and one carboxylic acid group (Fig. 1) [16]. This compound contributes to the flavor and aroma of fruits like apples, cherries, and berries, as well as vegetables such as tomatoes and carrots. In coffee beans, quinic acid is a primary constituent formed from chlorogenic acids during the roasting process. Pharmacologically, quinic acid exhibits antioxidant activity by scavenging ROS and preventing lipid peroxidation [17]. It also has anti-inflammatory effects, modulating inflammatory signaling pathways and reducing the production of pro-inflammatory cytokines [18].

Quinic acid has anti-apoptotic properties, as it inhibits pathways that lead to programmed cell death, thus providing cellular protection against apoptosis. This effect is advantageous for minimizing tissue damage in various conditions associated with inflammation and oxidative stress [19]. In addition, quinic acid inhibits mast cell activation, thereby reducing the release of histamine and other inflammatory mediators, which helps alleviate allergic reactions and lower inflammation [20]. Quinic acid also shows antimicrobial activity, effectively targeting bacteria, fungi, and parasites, positioning it as a potential therapeutic agent for oxidative stress-related diseases, inflammatory conditions, and microbial infections [21]. Given its diverse pharmacological properties and abundance in natural sources, quinic acid holds promise for further research and therapeutic development.

It is now established that alcohol-induced hepatotoxicity is partly due to the production of ROS and that quinic acid contains active antioxidant compounds. We also propose that quinic acid treatment may offer protective benefits by modulating inflammatory pathways, apoptotic processes, and oxidative mechanisms. Consequently, this study was conducted to assess the efficacy of quinic acid in countering ethanol-induced hepatotoxicity in rats, to develop a safe and effective hepatoprotective agent.

METHODS

Chemicals and reagents

Chemicals and reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA). Ethanol was sourced from E.I.D Parry India Ltd. (Nellikuppam, Cuddalore District, India). All other analytical-grade chemicals and reagents were purchased from Himedia Laboratory Ltd. (Mumbai, India).

Preparation and administration of quinic acid

Quinic acid was dissolved in 2% dimethyl sulfoxide immediately before treatment. The solution was administered orally through intragastric intubation daily at a dose of 50 mg/kg body weight (b.w.).

Experimental animals

Male albino Wistar rats (150–180 g) were procured from the Central Animal House, Rajah Muthiah Medical College and Hospital, Annamalai University.

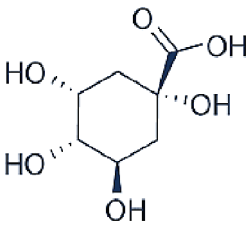

Fig. 1: Quinic acid structure

Animal care and housing

The rats were cared for under the guidelines of the Institutional Animal Ethics Committee (IAEC) of Annamalai University (AU-IAEC/1259/11/19), adhering to the Indian National Law on Animal Care. Housing conditions included Plastic cages with paddy husk bedding, a temperature of 27±2°C, and a light-dark cycle of 12 h light and 12 h dark.

Experimental procedures

All experiments complied with the "Guide for the Care and Use of Laboratory Rats."

Experimental design

The study consisted of four groups, each comprising eight male albino Wistar rats. The experiment lasted 60 days.

Group distribution

- Control group (Group 1): Rats received a standard pellet diet and isocaloric glucose (40% glucose in drinking water) orally for 60 days.
- Ethanol group (Group 2): Rats received 30% ethanol (equivalent to 50 g/kg body weight, orally) daily for 60 days.
- Ethanol + Quinic acid group (Group 3): Rats received 30% ethanol daily and Quinic acid (50 mg/kg body weight, orally) from day 31 to 60.
- Quinic acid group (Group 4): Rats received a standard pellet diet, isocaloric glucose, and Quinic acid (50 mg/kg body weight, orally) from day 31 to 60.

Tissue collection and processing

After 60 days, rats were sacrificed by cervical dislocation. Blood was collected in heparinized tubes, and plasma was separated. Livers were removed, cleaned with ice-cold saline (0.9% sodium chloride), and homogenized, and the supernatant was used for biochemical estimations and molecular studies.

Biochemical analysis

Lipid peroxidation was determined by measuring thiobarbituric acid reactive substances (TBARS) in tissues using Ohkawa *et al.*'s method [22], where the reaction between secondary lipid peroxidation products and thiobarbituric acid produces a pink chromogen quantified at 532 nm. Tissue lipid hydroperoxides (LOOH) concentration was estimated using Jiang *et al.*'s method [23], while conjugated dienes (CD) were estimated according to Rao and Recknagel's method [24], involving formation of CDs from polyunsaturated fatty acids with an absorbance maximum at 233 nm.

SOD (EC 1.15.1.1) activity was determined using Kakkar *et al.*'s method [25], measuring 50% inhibition of NADH-phenazine methosulfate nitroblue tetrazolium formazan formation at 520 nm. CAT (EC 1.11.1.6) activity was assessed using Sinha's method [26], involving conversion of dichromate to perchromic acid and chromic acetate upon heating in the presence of H_2O_2, with resulting chromic acetate quantified at 620 nm.

Reduced glutathione (GSH) in tissues was assayed using Ellman's method [27], relying on the yellow color development when 5,5-dithiobis (2-nitro benzoic acid) reacts with sulfhydryl-containing compounds. The activities of GPx (GPx, EC 1.11.1.9) and glutathione reductase (GR, EC 1.6.4.2) were determined using Rotruck *et al.*'s [28] and Carlberg and Mannervik's methods [29], respectively, involving incubation of enzyme preparations with H_2O_2 in the presence of GSH, with H_2O_2 utilization measured using Ellman's method [27], and enzyme activity expressed as mmol GSH consumed/min per mg protein. Glutathione S-transferase (GST, EC 2.5.1.18) activity was assayed according to Habig *et al.*'s method [30], recording absorbance changes at 340 nm, with enzyme activity calculated as μmol of 1-chloro-2,4-dinitrobenzene conjugate formed/min per mg protein, using a molar extinction coefficient of 9.6 × 10^3 min/cm. In addition, tissue ascorbic acid (Vit C) was estimated using Roe and Kuether's method [31], and α-tocopherol (Vit E) was estimated using Baker *et al.*'s method [32].

Mast cell staining
Mast cell staining was performed according to Khan *et al*.'s method (2013) [33]. Toluidine blue staining enabled mast cell identification, as mast cell granules exhibit metachromatic staining, characterized by deep purplish-blue granular cytoplasmic staining.

Immunohistochemistry
Immunohistochemistry was conducted on formalin-fixed, paraffin-embedded liver sections, following Elshopakey and Elazab's protocol (2021) [34]. After deparaffinization, antigen retrieval was performed using potassium citrate buffer (pH 6). The sections were then incubated overnight at 4°C with polyclonal primary antibodies targeting rat antigens. Subsequent steps involved washing with tris-buffered saline and incubating with horseradish peroxidase-conjugated secondary antibodies: Donkey anti-mouse (1:1000), goat anti-rabbit (1:1000) for 1 h at room temperature. Visualization was achieved through 1.5-min development with 3,3'-diaminobenzidine tetrahydrochloride followed by hematoxylin counterstaining and coverslipping. Tissues were photographed (40× magnification) using an Axio Scope A1 light microscope (Carl Zeiss, Jena, Germany).

Real-time quantitative polymerase chain reaction (RT-qPCR)
Liver tissue was utilized for total RNA extraction using TRIzol reagent, following the manufacturer's instructions. Briefly, tissue lysate was incubated at room temperature for 5 min, followed by centrifugation at 15,000 g for 10 min at 4°C, collection of the aqueous phase into an RNase-free tube followed by the addition of chloroform. Subsequent centrifugation at 15,000 g for 15 min at 4°C led to: Collection of the aqueous phase into another RNase-free tube and the addition of isopropanol. The RNA pellet obtained was washed with 75% ethanol and air dried. DNase treatment was conducted using the DNase I, RNase-Free kit, according to the manufacturer's instructions. cDNA synthesis was performed using 2 µg of total RNA with the ReverAid First Strand cDNA Synthesis Kit (Thermo), following the provided protocol. RT-qPCR was conducted using Hieff qPCR SYBR Green Master mix (Yeasen) and LightCycler 96 System (Roche). Reaction conditions were 1.2 µL cDNA template, 0.2 µM specific forward and reverse primers and Final volume: 20 µL. Cycling conditions were initial denaturation at 95°C, 5 min. 40 amplification cycles: 95°C, 10 s and annealing/extension: 60°C, 30 s. Melt curve analysis: 65°C–97°C, increments of 0.2°C every second, 95°C, 10 s, 65°C, 60 s. Experiments, including negative controls lacking cDNA, were conducted in triplicate. RT-qPCR primer sequences are provided in Table 1.

Quantitative analysis of target gene expression was performed using the 2–ΔΔCt method, with β-actin serving as the housekeeping gene for standardization. The calculation formula was: Ratio = 2–ΔΔCt where: ΔCt = Ct (target gene) - Ct (β-actin) ΔΔCt = ΔCt (experimental group) - ΔCt (control group) this method allowed for the relative quantification of target gene expression levels between experimental and control groups, normalized to the internal reference gene, β-actin.

Statistical analysis
Data are presented as mean ± standard error of the mean. Statistical analysis was performed using Statistical Package for the Social Sciences software to determine statistical significance. An analysis of variance was conducted, followed by Duncan's Multiple Range Test to identify significant differences between groups. Statistical significance was established at a $p<0.05$. The values are presented as the mean accompanied by the standard error of the mean.

Table 1: Apoptotic and inflammatory primer

Markers	Forward primer	Reverse primer
NF-κB	ACAAATGGGCTACACCGAAG	ATGGGGCATTTTGTTGAGAG
TNF-α	AGCCCATGTTGTAGCAAACC	GCTGGTTATCTCTCAGCTCCA
IL-6	AGATGATAAGCCCACTCTACAG	ACATTCAGCACAGGACTCTC
Bax	TGCTGATGGCAACTTCAACT	ATGATGGTTCTGATCAGCTCG
Bcl-2	GGTGGAGGAACTCTTCAGGGA	GGTTCAGGTACTCAGTCATCCA
Caspase-9	CGACATGATCGAGGATATTCAG	TGCCTCCCTCGAGTCTCA
β-actin	GGGAAATCGTGCGTGACATT	GCGGCAGTGGCCATCTC

NF-κB: Nuclear factor kappa B, TNF-α: Tumor necrosis factor-alpha, IL-6: Interleukin-6

RESULTS

Lipid peroxidation and enzymic and non-enzymic antioxidants in the livers of the control and experimental groups were analyzed, and the results are presented in Tables 2-5.

Table 2 shows tissue levels of TBARS, LOOH, and CD in control and experimental animals. Notably, ethanol-treated liver (Group 2) exhibited significantly elevated TBARS, LOOH, and CD levels compared to controls (Group 1) ($p<0.05$). Quinic acid supplementation in ethanol-treated rats (Group 3) substantially reduced TBARS, LOOH, and CD levels compared to unsupplemented ethanol-treated rats (Group 2). Conversely, quinic acid treatment in control rats (Group 4) did not significantly alter TBARS, LOOH, and CD levels. Lipid peroxidation, enzymic, and non-enzymic antioxidants in the liver of control and experimental groups were examined, and the data are given in Tables 2-5.

The activities of SOD and CAT in the liver of control and experimental animals are presented in Table 3. Ethanol treatment (Group 2) significantly decreased SOD and CAT activity in the liver compared to controls (Group 1) ($p<0.05$). However, quinic acid supplementation in ethanol-treated rats (Group 3) significantly increased SOD and CAT activity compared to ethanol treatment alone (Group 2). In contrast, quinic acid administration to control rats (Group 4) did not significantly affect SOD and CAT activity compared to normal control rats (Group 1).

Table 4 presents the activities of glutathione (GSH) and glutathione-related enzymes, including GPx, GR, and GST, in control and experimental animal tissues. Ethanol treatment (Group 2) significantly reduced GSH levels and GPx, GR, and GST activities in the liver compared to controls (Group 1) ($p<0.05$). However, co-administration of quinic acid (50 mg/kg) with ethanol (Group 3) significantly increased GSH levels and GPx, GR, and GST activities compared to ethanol treatment alone (Group 2). In contrast, quinic acid treatment in control rats (Group 4) did not significantly affect GSH levels or GPx, GR, and GST activities compared to normal control rats (Group 1).

Table 5 reveals a decline in hepatic non-enzymic antioxidants, specifically Vitamins C and E, in ethanol-exposed rats (Group 2) compared to the control group (Group 1). Conversely, quinic acid supplementation in ethanol-treated rats (Group 3) substantially increased Vitamin C and E levels compared to those receiving ethanol alone (Group 2). Quinic acid administration to control rats (Group 4) did not significantly impact Vitamin C and E levels compared to the untreated control group (Group 1).

Effect of quinic acid on mast cells population
Fig. 2 illustrates the histopathological analysis of mast cells using Toluidine blue staining. Ethanol treatment resulted in a significant increase in mast cell accumulation compared to control rats. However, quinic acid administration to ethanol-treated rats markedly reduced mast cell accumulation, approaching levels similar to those of control rats. In contrast, quinic acid treatment in control rats did not induce significant changes in mast cell accumulation compared to untreated control rats.

Effect of quinic acid on apoptotic and inflammatory markers by immunohistochemistry
Figs. 3 and 4. show immunohistochemical staining of liver tissue. Ethanol-induced apoptosis and inflammation, increasing Bax, Caspase-9, tumor necrosis factor-alpha (TNF-alpha), nuclear factor kappa B (NF-κB), and interleukin-6 (IL-6), while decreasing Bcl-2. Quinic acid treatment reversed this effect, upregulating Bcl-2 and downregulating pro-apoptotic and inflammatory proteins.

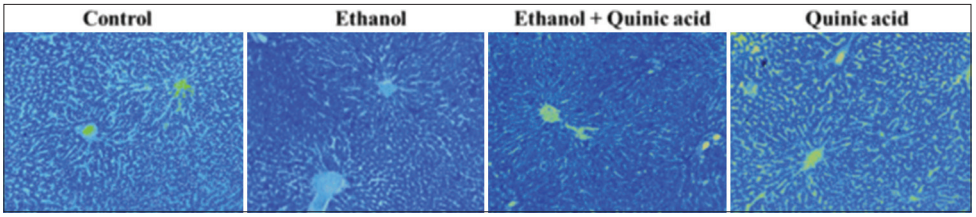

Fig. 2: Photomicrographs depicting mast cell granules by toluidine blue staining. Original magnification: 20×

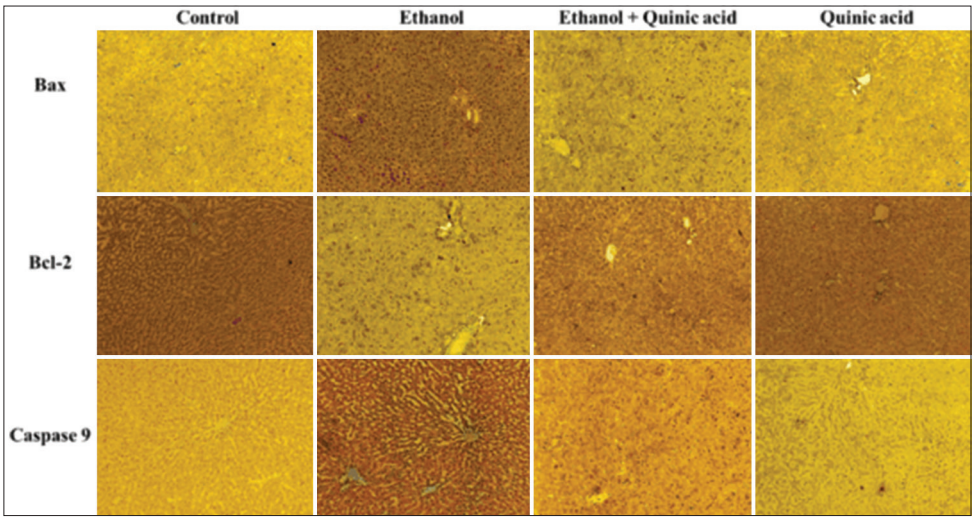

Fig. 3: Effect of quinic acid on apoptotic markers in the liver tissue of control and experimental rats. Immunohistochemical staining of Bax, Bcl2 and Caspase 9. Original magnification: 20×

Table 2: Effect of quinic acid and ethanol on the lipid peroxidation markers of the control and experimental liver tissue

Groups	TBARS (nmol/g tissue)	LOOH (mmol/g tissue)	CD (mmol/g tissue)
Control	28.56±2.45[a]	64.73±4.72[a]	104.73±6.72[a]
30% Ethanol alone	58.72±4.15[b]	85.26±4.08[b]	135.26±8.08[b]
30% Ethanol+Quinic acid (50 mg/kg. bw)	33.94±2.32[c]	65.16±4.21[c]	105.16±7.21[c]
Quinic acid (50 mg/kg. bw)	27.38±1.03[a]	62.09±4.20[a]	92.09±5.20[a]

Values are means±SD of eight rats from each group. [a-c]values with different superscript letters differ significantly at p<0.05 (ANOVA followed by Duncan's multiple range test). TBARS: Thiobarbituric acid reactive substances, LOOH: Lipid hydroperoxides, ANOVA: Analysis of variance, CD: Conjugated dienes

Table 3: Effect of quinic acid and ethanol on Superoxide dismutase (SOD) and Catalase (CAT) of the control and experimental liver tissue

Groups	SOD	CAT
Control	6.73±0.72[a]	74.73±4.72[a]
30% Ethanol alone	3.26±0.18[b]	48.26±3.19[b]
30% Ethanol+Quinic acid (50 mg/kg.bw)	6.16±0.41[c]	72.16±5.21[c]
Quinic acid (50 mg/kg. bw)	7.09±0.40[a]	79.09±5.27[a]

Values are means±SD of eight rats from each group. [a-c]Values with different superscript letters differ significantly at p<0.05 (ANOVA followed by Duncan's multiple range test). For superoxide dismutase the units are: 50% inhibition of NBT reduction/min per mg protein for the liver; enzyme required for 50% inhibition of NBT reduction/min per mg hemoglobin for lysate. For catalase the units are: µmol H_2O_2 utilized/min per mg protein for liver; µmol H_2O_2 u tilized/min per mg hemoglobin for lysate. NBT: Nitroblue tetrazolium, ANOVA: Analysis of variance

Effect of quinic acid on mRNA expression of apoptotic and inflammatory markers by RT-qPCR

Figs. 5 and 6. illustrate that ethanol induction resulted in downregulated Bcl2 mRNA expression and upregulated mRNA expression of Bax, Caspase-9, TNF-alpha, NF-κB, and IL-6 Conversely, quinic acid supplementation during ethanol induction led to upregulated Bcl2 mRNA expression and downregulated mRNA expression of Bax, Caspase-9, TNF-alpha, NF-κB, and IL-6.

DISCUSSION

Ethanol-induced tissue damage may result from oxidative stress and nutritional deficiency [35]. Our laboratory's studies support this hypothesis, as ethanol-treated rats exhibited decreased food intake and increased oxidative stress [36]. Ethanol, with 7.1 kcal/g, lacks nutrients and affects various organs due to its tissue-permeable and water- and fat-soluble properties. Ethanol administration generates excessive free radicals, including hydroxyl ethyl radicals, superoxide radicals, hydroxyl radicals, peroxyl radicals, and hydrogen peroxide [37], which rapidly react with lipids, leading to lipid peroxidation [38], a toxic manifestation of ethanol ingestion. This enhanced lipid peroxidation causes tissue damage through free radical-mediated lipid peroxidation, resulting in membrane disorganization and decreased membrane fluidity [39].

Numerous researchers have reported excessive lipid peroxidation, measured by TBARS, LOOH, and CD formation, in ethanol-treated rats [40]. Consistent with these findings, our study showed increased TBARS, CD, and LOOH levels in ethanol-treated rats compared to controls. However, quinic acid administration significantly decreased

Table 4: Effect of quinic acid and ethanol on of GSH and glutathione-related enzymes of the control and experimental liver tissue

Groups	GSH	GPx	GR	GST
Control	17.56±1.45[a]	15.76±1.42[a]	24.59±1.72[a]	8.65±0.54[a]
30% Ethanol alone	12.69±0.15[b]	7.99±0.15[b]	12.63±0.34[b]	4.27±0.51[b]
30% Ethanol+Quinic acid (50 mg/kg.bw)	16.94±0.32[c]	14.94±0.32[c]	21.94±1.32[c]	6.48±0.23[c]
Quinic acid (50 mg/kg .bw)	19.38±1.13[a]	16.85±0.93[a]	25.76±1.42[a]	7.83±0.39[a]

GSH: Reduced glutathione, GPx: Glutathione peroxidase, GR: Glutathione reductase, GST: Glutathione *S*-transferase. Values are means±SD of eight rats from each group. [a-c]Values with different superscript letters differ significantly at p<0.05 (ANOVA followed by Duncan's multiple range test). For GSH the units are: mmol/g tissue for liver; mmol/mg protein for plasma. For GPx the units are µg of GSH utilized/min per mg protein for liver; µmol of GSH utilized/min per mg hemoglobin for lysate. For GR the units are: nmol of NADPH utilized/min per mg protein for liver; µmol of CDNB-GSH conjugate formed/min per mg hemoglobin for lysate. For GST the units are: µmol of CDNB-GSH conjugate formed/min per mg protein; µmol CDNB-GSH conjugate formed/min per mg hemoglobin for lysate. CD: Conjugated dienes, ANOVA: Analysis of variance

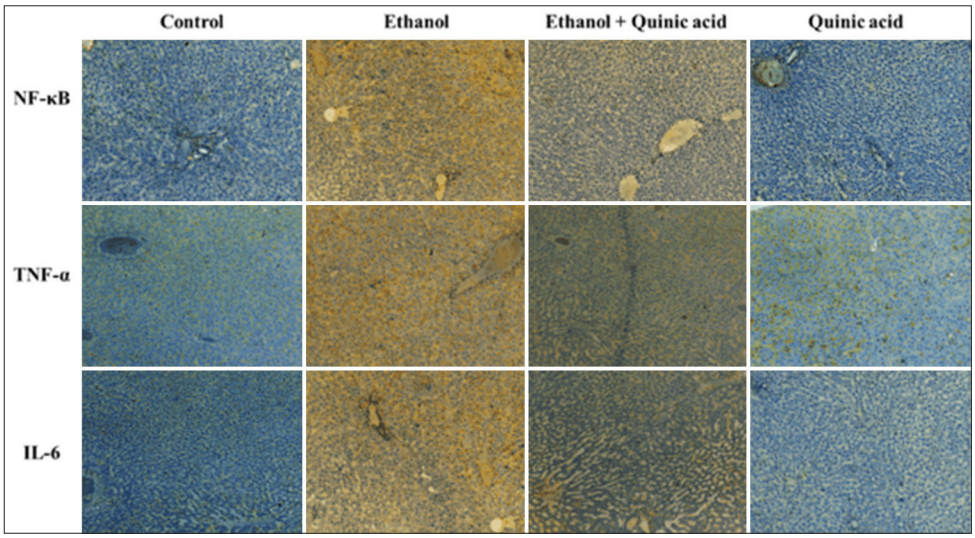

Fig 4: Effect of quinic acid on inflammatory markers in the liver tissue of control and experimental rats. Immunohistochemical staining of Nuclear factor kappa B, tumor necrosis factor-alpha and interleukin-6. Original magnification: 20×

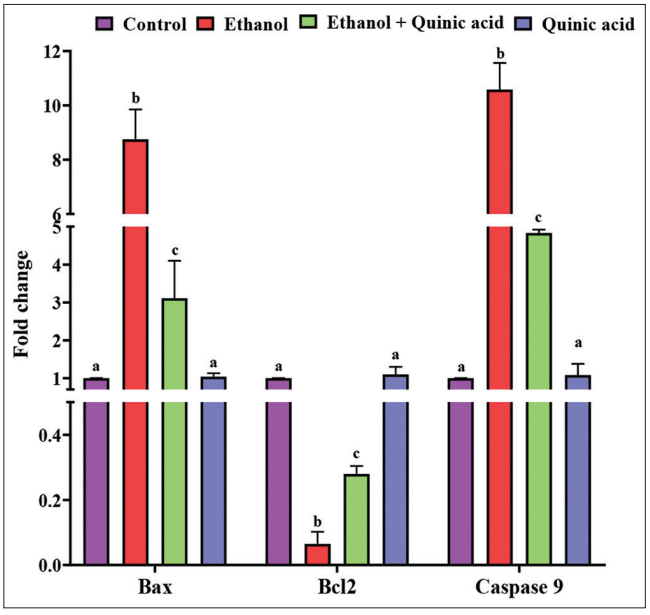

Fig. 5: Effect of quinic acid on apoptotic markers in the liver tissue of control and experimental rats. Values not sharing common alphabets as superscripts are significantly different from each other at the level of p<0.05 (analysis of variance followed by Duncan's multiple range test)

these lipid peroxidation markers in ethanol-treated rats. This decrease correlates with elevated antioxidant levels [41], likely due to quinic acid's free radical scavenging properties, attributed to its five hydroxyl groups. Quinic acid's ability to enhance antioxidant levels and inhibit lipid peroxidation suggests its potential in mitigating free radical-mediated injury associated with alcohol-induced liver damage, making it a promising compound for counteracting alcohol abuse-related liver damage.

Table 5: Effect of quinic acid and ethanol on Vitamin C and E of the control and experimental liver tissue

Groups	Vitamin C (mg/100 g tissue)	Vitamin E (mg/100 g tissue)
Control	0.87±0.04[a]	6.76±0.42[a]
30% Ethanol alone	0.63±0.05[b]	4.81±0.45[b]
30% Ethanol + Quinic acid (50 mg/kg. bw)	0.79±0.06[c]	5.99±0.52[c]
Quinic acid (50 mg/kg. bw)	0.83±0.03[a]	6.85±0.93[a]

Values are means±SD of eight rats from each group. [a-c]Values with different superscript letters differ significantly at p<0.05 (ANOVA followed by Duncan's multiple range test). ANOVA: Analysis of variance

SOD, a universally present chain-breaking antioxidant in aerobic organisms, plays a vital protective role against oxidative damage triggered by ROS [42]. SOD catalyzes the conversion of superoxide ion (O_2–) into hydrogen peroxide (H_2O_2), which is subsequently broken down by CAT and GPx. CAT, concentrated in the liver and erythrocytes [43], helps mitigate tissue damage caused by superoxide ions (O_2^-) and hydroxyl radicals (OH•) [44]. Compounds with antioxidant properties can alleviate liver damage, and scavenging superoxide ions and hydroxyl radicals is a key defense mechanism against various diseases [45]. Ethanol-treated rats showed significantly lowered SOD

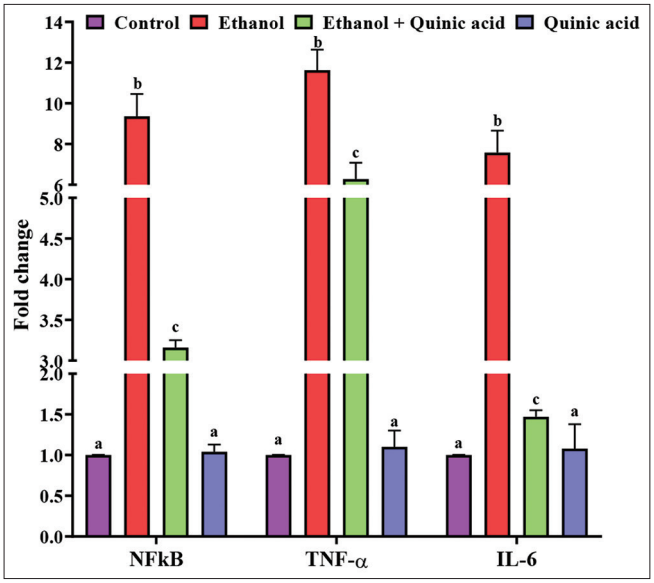

Fig. 6: Effect of quinic acid on inflammatory markers in the liver tissue of control and experimental rats. Values not sharing common alphabets as superscripts are significantly different from each other at the level of p<0.05 (analysis of variance followed by Duncan's multiple range test)

and CAT activities compared to control rats, leading to accumulation of reactive free radicals and deleterious effects like loss of cell membrane integrity [46]. Our laboratory's previous studies reported decreased hepatic SOD and CAT activity with ethanol treatment [47-49], consistent with our present findings. Reduced SOD and CAT activity may be linked to elevated intracellular H_2O_2 concentrations [50], which can inactivate SOD [51]. Oxidative inactivation or α-hydroxy ethyl radical generation from ethanol may also contribute to decreased SOD activity [52,53]. Notably, quinic acid supplementation to ethanol-treated rats elevated liver SOD and CAT activity, highlighting quinic acid's antioxidant and hepatoprotective properties.

GSH, a pivotal tripeptide composed of L-γ-glutamylcysteinylglycine, plays a central role in cellular defense, safeguarding cells through its antioxidant properties, detoxifying ROS, conjugating and removing harmful molecules, and regulating inflammatory cytokine responses [54]. GSH depletion impairs cellular defense against ROS, potentially leading to peroxidative injury [55]. Consistent with previous reports [54,56,57], our study showed significantly decreased GSH levels in ethanol-treated rats. As a direct free radical scavenger and substrate for GPx and GST [58], GSH's depletion likely contributed to reduced GPx and GST activities observed with ethanol treatment. Quinic acid administration to ethanol-treated rats increased GSH levels and GPx and GST activities. In addition, GR, which restores GSH by reducing its oxidized product, showed lower activity in ethanol-treated rats compared to controls [59], potentially inactivating SH-group-containing enzymes and inhibiting protein synthesis. Quinic acid supplementation, however, helped restore GR activity, highlighting its antioxidant and hepatoprotective effects.

Quinic acid, identified as a powerful free radical scavenger, boasts five hydroxyl groups that confer its remarkable antioxidant properties [60]. This inherent antioxidant capacity enables quinic acid to preserve other antioxidants, including SOD, CAT, GSH, GPx, GST, and GR, thereby maintaining their cellular levels. Consequently, quinic acid supplementation in ethanol-treated rats resulted in elevated levels of these enzymic and non-enzymic antioxidants in the liver, highlighting quinic acid's protective role in mitigating ethanol-induced oxidative stress.

Non-enzymatic antioxidants play a key role in shielding against oxidative stress. Our research uncovered a substantial decline in vitamins C and E, essential non-enzymatic antioxidants, likely attributable to intensified oxidative stress. This discovery concurs with preceding studies documenting an 18% reduction in Vitamin E concentrations among alcoholic patients [61-63]. Vitamin E functions as a chain-breaking antioxidant, preventing lipid peroxidation, and its depletion culminates in free radical-mediated cellular damage. Vitamin E halts lipid peroxidation by capturing free radicals, transforming into α-tocopheroxyl radicals, while Vitamin C rejuvenates α-tocopherol from α-tocopheroxyl radicals, thereby sustaining antioxidant defenses [64,65]. Notably, quinic acid, Vitamin C, and Vitamin E exhibit a synergistic relationship in their antioxidant activities, enhancing the body's defense against oxidative stress. Their combined effect amplifies protection against cellular damage, supporting overall health [66].

Past studies on natural compounds such ad flavonoids (quercetin, kaempferol) and polyphenols (resveratrol, curcumin) showed reductions in mast cell activation [67-69]. Quinic acid, a polyphenolic compound, shares anti-inflammatory properties with these compounds [70]. Ethanol-induced inflammation increased mast cell accumulation, validating the model's efficacy. Quinic acid administration reduced mast cell accumulation in ethanol-treated rats, indicating a potential protective effect against ethanol-induced inflammation. This aligns with quinic acid's known anti-inflammatory properties [71].

Our investigation revealed elevated hepatic TNF-α and IL-6 expressions in ethanol-fed rats, aligning with previous research documenting heightened plasma levels of TNF-α and IL-6 in patients with severe alcoholic hepatitis, which correlated with disease progression [72,73]. Prior studies employing the Tsukamoto French enteral feeding model also demonstrated increased TNF-α mRNA expression in liver tissue following 4 weeks of ethanol exposure [74]. Moreover, research conducted on isolated Kupffer cells showed upregulated mRNA expression of TNF-α, IL-6, and transforming growth factor-beta 1 (TGF-β1) after 17 weeks of ethanol and high-fat diet treatment [75]. Our study's heightened TNF-α and IL-6 expression likely stemmed from inflammation, necrosis, and oxidative stress triggered by ethanol consumption in rats.

We observed increased hepatic TNF-α and IL-6 expressions in ethanol-fed rats, consistent with previous studies showing elevated plasma levels of TNF-α and IL-6 in patients with severe alcoholic hepatitis, which correlated with disease progression [72,73]. Earlier research using the Tsukamoto French enteral feeding model also demonstrated increased TNF-α mRNA expression in liver tissue after 4 weeks of ethanol treatment [74]. Furthermore, studies in isolated Kupffer cells revealed upregulated mRNA expression of TNF-α, IL-6, and TGF-β1 following 17 weeks of ethanol and high-fat diet treatment [75]. The increased TNF-α and IL-6 expression in our study likely resulted from inflammation, necrosis, and oxidative stress in ethanol-fed rats.

Activation of NF-κB, coupled with upregulation of pro-inflammatory cytokines, is closely tied to necrosis and inflammation in alcoholic liver injury [77]. Research has demonstrated that elevated endotoxin and free radical formation activates NF-κB, culminating in the upregulation of pro-inflammatory cytokines and chemokines, which contributes to pathological liver injury and cirrhotic changes [78]. Notably, quinic acid supplementation significantly decreased NF-κB expression in the liver of ethanol-fed rats. As a naturally occurring flavonone possessing anti-inflammatory and immunomodulatory properties [70], quinic acid has exhibited inhibitory effects on NF-κB in macrophages [79] and reduced NF-κB expression in the lungs of acute allergic asthmatic patients [80]. This diminished NF-κB expression likely suppresses other chemokines, providing a plausible mechanism by which quinic acid confers protection against inflammation, necrosis, and ROS-induced alcoholic liver damage.

Bcl-2, a mitochondrial membrane integral protein, acts as an antioxidant and antiapoptotic factor [81], decreasing ROS production by binding to cytochrome c or inhibiting its release into the cytosol [82],

and augmenting cellular GSH content [83]. Conversely, proapoptotic proteins Bak and Bax mediate mitochondrial membrane channel opening, releasing apoptogenic proteins like cytochrome c. Our study revealed that ethanol-induced rats exhibited upregulated proapoptotic Casp-3 and Bax and downregulated antiapoptotic Bcl-2, indicating ethanol-induced hepatotoxicity through liver tissue cell death [84]. However, co-administration of quinic acid with ethanol downregulated Bax and Casp-3 and upregulated Bcl-2 expression. The significant upregulation of Bcl-2 and downregulation of Bax and Casp-3 in quinic acid-supplemented ethanol-induced rats maintained Bcl-2's antioxidant and antiapoptotic effects, underscoring quinic acid's hepatoprotective impact [84]. Notably, our findings on quinic acid's effect on ethanol-induced apoptosis are novel and promising, as no prior data exist for comparison. Quinic acid's ability to alleviate ethanol's apoptotic influences on liver tissue highlights its potential therapeutic benefits.

CONCLUSION

Our study demonstrated that quinic acid, a polyphenol, significantly prevented ethanol-induced liver toxicity by scavenging reactive free radicals, boosting the endogenous antioxidant system, inhibiting pro-inflammatory cytokines, and suppressing NFκB activation, thereby restricting the activation of its downstream effectors. These findings suggest that quinic acid may serve as a prototype for preventing ethanolic liver injury. Further research is essential to elucidate the protective effects of quinic acid in ethanol-induced liver damage, providing additional evidence for its broader therapeutic applications and potential exploitation as a remedial agent.

AUTHORS' CONTRIBUTIONS

Experimental design, guidance, supervision, and review work for the research were done by Dr. Nadanam Saravanan. Experimental work, development and optimization of the formulations, interpretation of result, and writing of this manuscript were done by Mr. Gurusamy Muthukaruppiah. Both authors read and approve the final manuscript

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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