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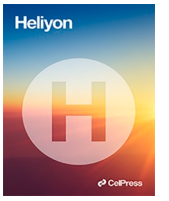

Effect of Lauric acid against ethanol-induced hepatotoxicity by modulating oxidative stress/apoptosis signalling and HNF4α in Wistar albino rats

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ABSTRACT

Ethanol (EtOH) is most widely used in alcoholic beverages to prepare alcohol. As EtOH is mainly metabolised in the liver, the excessive consumption of EtOH forms a primary toxic metabolic product called acetaldehyde, as the gradual increase in acetaldehyde leads to liver injury, as reported. Lauric acid (LA) is rich in antioxidant, antifungal, antibacterial, anticancer, and antiviral properties. LA is an edible component highly present in coconut oil. However, no report on LA protective effects against the EtOH-instigated hepatotoxicity exists. Therefore, the experiment is carried out to investigate the potency effects of LA on EtOH-instigated hepatotoxicity in thirty male albino rats. Rats were divided into five groups (n-6): control DMSO alone, EtOH -intoxicated, EtOH + LA 180 mg/kg, EtOH + LA 360 mg/kg, and LA alone were administered orally using oral gavage. The study measured body weight every weekend in all rat groups. The rats were sacrificed and assessed for serum markers (alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase), antioxidant activity (superoxide dismutase, reduced glutathione, glutathione peroxidase), lipid peroxidation (malondialdehyde), histopathological, cytokine levels (TNF-α, IL-1β and IL-6), protein expression (caspase 3 and caspase 8 and Bcl-2 and HNF4α) were evaluated after the 56-days study period. The impact of EtOH intoxication reduces the rat's body weight by 90 g, upregulates the liver enzyme markers, depletes the antioxidant levels, produces malondialdehyde, changes the histoarchitecture (periportal inflammation and hepatocyte damage), downregulates the Bcl-2 expressions and HNF4α, and elevates the expression of cytokines and apoptotic markers. LA alleviated EtOH-induced liver toxicity by significant ($p < 0.05$) modulation of biochemical levels, caspase-8/3 signalling, reducing pro-inflammatory cytokines, and restoring the normal histoarchitecture, upregulating the Bcl-2 and HNF4α Expressions. In conclusion, LA treatment can protect the liver against EtOH-induced hepatotoxicity, evidenced by alleviating Oxidative stress, lipid peroxidation, inflammation, apoptosis, and upregulation of HNF4α.

1. Introduction

Excessive alcohol intake leads to liver damage, stimulates extensive hepatocyte injury, and mainly causes liver dysfunction [1,2]. Approximately 3 million cases of deaths result from alcohol consumption across the globe [3]. It accounts for 5.3 % of all other death

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conditions. Around the globe, 13.5 % of people involved in alcohol consumption are between 20 and 39 years old [4]. The early reversible stages of alcoholic liver disease (ALD) are hepatosteatosis, fatty liver, and alcoholic hepatitis. However, the continuous intake of alcohol leads to further irreversible stages like fibrosis, cirrhosis, and hepatocellular carcinoma, which might result in severe liver damage [5,6].

The normal metabolism of alcohol involves a catalase reaction where the acetaldehyde changes to form acetate and enters the circulatory system [7]. The cytochrome p450 predominantly regulates this. Excessive alcohol consumption leads to oxidative stress and increases lipid accumulation, inflammation, and liver apoptosis [8,9]. The oxidative stress in the liver leads to the elevation of hepatic biomarkers like alanine transaminase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) [10]. Lipid biomarkers like triglyceride (TG), total cholesterol (TC), and low-density lipoproteins (LDL) would increase, and high-density lipoproteins (HDL) could decrease [11]. Under the oxidative stress condition induced by alcohol, cytokine levels like IL-6, IL-1β, and TNF-α would elevate. The hepatocyte nuclear factor-4 alpha (HNF4α) is majorly present in all regular metabolism pathways of the liver like bile acid synthesis, fatty acid metabolism, carbohydrate metabolism, xenobiotic metabolism, ureagenesis, and blood coagulation [12–14]. Therefore, the decreased level of HNF4α expression leads to hepatic dysfunction [15]. Overconsumption of alcohol leads to liver tissue damage, organ dysfunction, and hepatocyte death [1,16].

Inflammatory mediators act as an enhancer of the tissue-affected region and cause pain in the tissue or organ in response to the stimuli. Molecules called inflammatory mediators are essential for starting and controlling the inflammatory response. Various cells create them in response to an infection, an injury, or other stimuli, including immune cells, endothelial cells, and tissue-resident cells. Hence, inflammatory mediator inhibitors are used as the therapeutic approach. Several anti-inflammatory drugs are being used for ALD, but the therapeutic efficiency is hindered by adverse side effects on long-term usage [17,18]. Prednisolone is a Corticosteroid drug that doctors mostly prescribe to treat alcoholic hepatitis patients. Still, long-term drug consumption would cause more side effects such as leg swelling, increased blood pressure, weight gain, psychological changes, and behavior [19]. The drugs most commonly used to treat ALD are Methotrexate, Azathioprine, Disulfiram, and Acetaminophen. Those Non-steroidal anti-inflammatory drugs must be used with warning in ALD patients because they can be toxic to the liver if taken in large doses or for an extended treatment period. This study focuses on natural drug discovery applications to overcome these side effects.

Natural and edible product plays a major role in drug discovery. In recent days, research has been focused on phytonutrient-based disease management. Lauric acid (LA) is a medium-chain fatty acid compound with twelve carbon atoms that is highly present in virgin coconut oil, about (46–52 %) [20]. LA is also present (20–40 %) in food varieties like human milk, fruits, kernel palm oil, and laurel [21,22]. The LA was chosen for our study based on its anti-inflammatory, antioxidant, and antimicrobial properties [20]. In recent studies, LA treatment is protective against testosterone-induced toxicity that causes prostatic hyperplasia [23]. In addition, LA is used as an additive to reduce bacterial formation in meats [24]. LA also reduces cardiovascular diseases by increasing HDL, reducing LDL, and decreasing blood pressure [25,26]. No studies have been carried out on this model. Hence, this study is investigating the potency effects of LA on EtOH-induced hepatotoxicity by suppressing oxidative stress and apoptosis by promoting HNF4α.

2. Materials and methods

2.1. Animals

Thirty male albino Wistar rats weighing an average of 250 g from the Vellore Institute of Technology in Vellore, Tamil Nadu, India, were used. The ethical committee approved the experimental protocol (VIT/IAEC/18/Dec2020/01). It was completed in VIT, Vellore, in compliance with Indian CPCSEA standards. Initially, animals were kept in a pathogen-free environment at a constant temperature of 20–25 °C, lab conditions, and a 12-h dark/light cycle for one week. The animals were subsequently fed with commercial animal feed pellets purchased from Hindustan Lever Ltd. in Mumbai, India, and were allowed unrestricted access to processed water.

2.2. Drugs and chemicals

EtOH with ≥99.8 % and Lauric acid (purity >98 %) (MKCF8837) was purchased from Sigma-Aldrich Chemical Pvt Limited from Bangalore, India. The lauric acid was dissolved in the Dimethyl sulfoxide (DMSO) with the pathogen-free liquid with prior administration to rats. All other chemicals, reagents, and kits required for the experiment were of analytical grade. Based on previous study research, optimal doses of Lauric acid (180 mg/kg b/w and 360 mg/kg b/w) [27], EtOH (56%v/v 5 ml/kg b/w) [28], and DMSO 10 % alone [29].

2.3. Experimental design

After a week of acclimatization, rats were divided into five groups of six each, and for the fifty-six days of the study period, they received the following care:

Group 1: control vehicle (DMSO 10 % alone) for the last 14 days.
Group 2: EtOH (5 ml/kg b/w) given Orally for 56 days.
Group 3: EtOH (5 ml/kg b/w) treated Orally for 56 days + Lauric acid low dose 1 (180 mg/kg b/w) (LAD1) for the last 14 days.
Group 4: EtOH (5 ml/kg b/w) treated Orally for 56 days + Lauric acid high dose 2 (360 mg/kg b/w) (LAD2) for last 14 days.
Group 5: LAD2 drug alone (360 mg/kg b/w) for the last 14 days.
Drugs were administered by oral route to the rats using oral gavage. The rats were euthanized with ketamine and xylazine with a

ratio of 4:1 (1 ml/rat *i.m*) after the final day of 24-h treatment. A cardiac puncture was done to remove the blood from the unconscious rat with sterile syringes, which were then centrifuged for 15 min at 3000 RPM to separate the serum for biochemical analysis. The liver was then taken from the rat and cleaned with phosphate-buffered saline (PBS). Then, the liver tissue sample is rapidly frozen with the liquid nitrogen stored in the −80 °C freezer for further analysis. The part of liver tissue was preserved in formalin for further histological inspection.

2.4. Weight assessment

Rats' body weight was assessed at 0–8 weeks to measure the weight of the rat's digital weighing balance. Water and food consumption was also monitored.

2.5. Serum biochemical analysis

ALP, AST, and ALT hepatic liver functions were measured using serum obtained from the blood of rats from each group were determined, and the units were given in U/L. Lipid function tests like TC, TG, LDL, and HDL were done [30], and units were given in mg/dL. Assays were performed as followed by manufacturer instructions (Auto span diagnostics).

2.6. Reactive oxygen species and lipid peroxidation

Stored rats' liver tissue samples were cut into small pieces, added to PBS (7.4 PH), and then homogenized with homogenizer. Then, the homogenized samples were centrifuged at 3000 RPM for 20 min. Then the supernatant was collected in the fresh tube and analysed for malondialdehyde (MDA) was estimated to determine lipid peroxidation Esterbauer et al. [31] and reactive oxygen species (ROS) level in each group was assessed using an ELISA kit. According to the manufacturer's protocol from Sigma-Aldrich, Bangalore, India, the results units were determined by the RFU/mg protein.

2.7. Liver antioxidant activity

The liver was homogenized with PBS, centrifuged for 15 min at 1700 RPM, and the supernatant was collected in the fresh tube. Then the supernatant was used to analyze the antioxidant assays like reduced glutathione peroxidase (GPx), glutathione (GSH), and superoxide dismutase (SOD), which were measured by methods described by Rotruk et al. [32], Moron et al. [33], and Marklund and Marklund [34].

2.8. Histopathological analysis

After animal sacrifice, the rat liver samples were washed in 0.1 M ice-cold PBS buffer and immersed in 10 % fixative formalin. The tissues were dehydrated with the serial concentration of alcohol and embedded in wax, and then tissues were cut into 5–10 mm thick tissue slice sections using a rotatory microtome (Leica Biosystems, India). Then, the sliced tissue is floated through a water bath to remove creases and deformity before being captured by a slide. The tissue section should be preserved by drying labelled slides at 37 °C for a few hours to allow the extra paraffin wax to melt away. The cytoplasm is counterstained pink to red (eosin), and the nuclei are counterstained blue to purple (hematoxylin) using the hematoxylin and eosin (H&E) staining method. The samples are then examined under an optical microscope (Labomed 2000 Vision, India) for histopathological anomalies, which are noted and photographed.

2.9. ELISA

The ELISA protocol for HNF4α and inflammatory markers involves homogenizing tissue samples in PBS, collecting the supernatants through centrifugation, preparing the ELISA assay according to the manufacturer's instructions, adding samples to a microplate, incubating the microplate for specific durations, washing it with PBS, adding detection antibody (HNF4α, TNF-α, IL-1β, and IL-6) and 3,3′,5,5′-Tetramethylbenzidine (TMB) substrate solution, stopping the reaction with a stop solution, and reading the microplate at using an iMark microplate absorbance reader. The concentration of HNF4α, TNF-α, IL-1β, and IL-6 can be calculated using a formula that involves the OD values of the sample and blank and the slope of the standard curve.

$$\text{Concentration (pg / ml)} = (\text{OD of sample} - \text{OD of blank}) \,/\, (\text{Slope of the standard curve})$$

2.10. RNA isolation and qPCR

Using the RNA iso plus from Takara Biosciences, Japan, RNA was extracted from liver tissue. cDNA synthesis was done using the prime script cDNA synthesis kit (Takara Biosciences, Japan). Real-time PCR was used to perform mRNA analysis of gene targets. Real-time PCR was performed using SYBR Green reagent and BIORAD CFX96 Real-Time system, and the primers β-actin and HNF4α were designed and produced from the Eurofins, Bangalore. The primer sequence was 5′ ACCCTCACCTGATGCAAGAAC and 3′ GCCCTTGTAGAGTTGACCCCA.

2.11. Western blot analysis

Liver samples were homogenized using a cold radioimmunoprecipitation assay (RIPA) buffer. Homogenization was done, followed by the tissue sample being centrifuged at 13000 rpm for 20 min at 4 °C. Soluble proteins in the supernatant phase were collected in a contaminant-free Eppendorf tube and stored under cold conditions. The same number of protein samples were run through the 10 % SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis). The purified protein was then moved to a polyvinylidene difluoride membrane, and the well was then blocked with 5 % skim milk overnight at 4 °C before being subjected to the primary antibody treatments. Secondary antibodies were blotted in the membrane for 1 h with the horseradish peroxidase (HRP), enhancing the blot formation. Later, the protein band intensity was visualized with the western blotting equipment, and the image of protein intensity was saved as an image using the image software, as per the protocol given by the manufacturer. Finally, all protein bands were normalized with vinculin control protein, and protein graphs were plotted.

2.12. Statistical analysis

Using the GraphPad Prism software (version 8.02), one-way analysis of variance (ANOVA) and Tukey's post hoc test were used to determine the statistical significance between the groups. Data were represented as Mean ± SEM. A P-value of 0.05 or less was considered significant.

3. Results

3.1. Lauric acid's effect on body weight changes in an EtOH-induced model

EtOH intoxication (56 %) for 56 days caused a body weight loss in the 8th week compared with the other weeks. EtOH + LAD1 and EtOH + LAD2 groups showed a significant ($p < 0.05$) higher body weight of 238.2 ± 19.9 and 245.1 ± 23.7. In the LAD2 group rats (244.9 ± 25.8), almost similar body weight was compared to the normal vehicle group rats (242.9 ± 23.3) (Fig. 1).

3.2. Lauric acid's effect on hepatic markers in the EtOH-induced model

Administration of EtOH (56 %) for 56 days significantly elevated ($p < 0.05$) the liver marker enzymes like ALP (209.4 ± 2.4), AST (168.1 ± 2.9), and ALT (180.5 ± 3) compared with the control vehicle group of rats ALP (103 ± 2.5), AST (83.2 ± 2.6), ALT (91.3 ± 3.8). Thus, elevated liver enzyme markers show that the hepatic cells were damaged. In the treatment group, EtOH + LAD1 and EtOH + LAD2 showed a decrease in serum liver enzyme markers ALP (158.8 ± 2.7 and 138.1 ± 2.6), AST (118 ± 2.9 and 96 ± 2.9), and ALT (130.4 ± 3.1 and 112.1 ± 4.5) when compared with EtOH group rats. In the LAD2-treated group, rats showed normal serum liver enzyme markers ALP (108.2 ± 2.7), AST (88 ± 2.6), and ALT (99.3 ± 3.7) compared to the normal control vehicle group (Fig. 2A, B&C).

3.3. Lauric acid's effect on lipid profile in the EtOH-induced model

Constant administration of EtOH (56 %) group rats for 56 days considerably increased the serum TC (96.3 ± 3), LDL (102.7 ± 2.2),

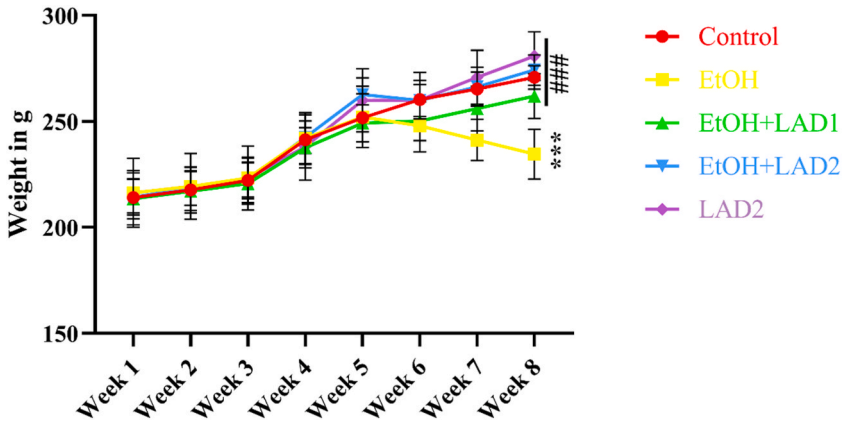

Fig. 1. The experimental Wistar rats were administered LA (180 & 360 mg/kg) for 14 days after the EtOH intoxication. The data are represented as means ± SE (n = 6). (Vehicle group), EtOH 56 %/day for 56 days, EtOH + LAD1 (lauric acid (180 mg/kg) from 43rd day), EtOH + LAD2 (lauric acid (360 mg/kg) from 43rd day), and LAD2 (lauric acid (360 mg/kg) from 43rd day). '*' represents the group compared with vehicle, and '#' represents the group compared with the EtOH group, where statistical significance shows */# indicates $p < 0.05$, **/## indicates $p < 0.01$and ***/### indicates $p < 0.001$.

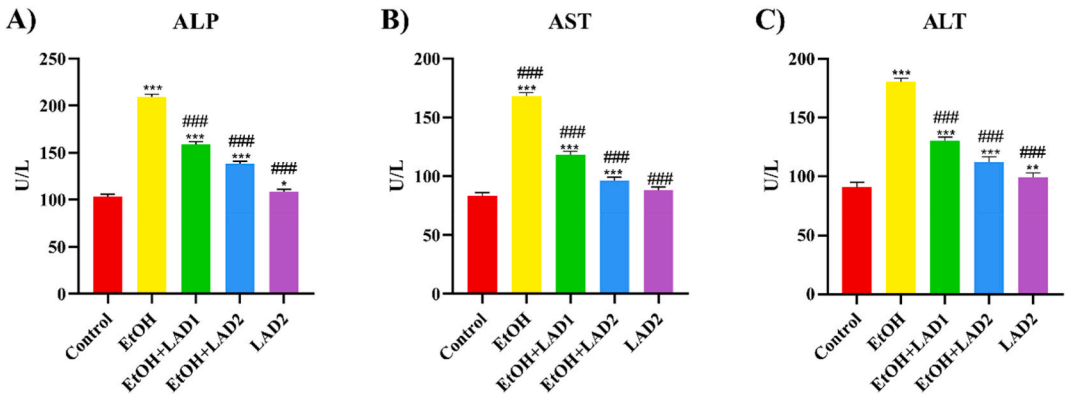

Fig. 2. The experimental Wistar rats were administered LA (180 & 360 mg/kg) 14 days after the EtOH intoxications, where the parameters represent ALT(A), AST(B)&ALT(C). The data are represented as means ± SE (n = 6). (Vehicle group), EtOH 56 %/day for 56 days, EtOH + LAD1 (lauric acid (180 mg/kg) from 43rd day), EtOH + LAD2 (lauric acid (360 mg/kg) from 43rd day), and LAD2 (lauric acid (360 mg/kg) from 43rd day). '*' represents the group compared with the vehicle, and '#' represents the group compared with the EtOH group, where statistical significance shows */# indicates $p < 0.05$, **/## indicates $p < 0.01$and ***/### indicates $p < 0.001$.

and TG (241.7 ± 4.3) levels compared with the control group rats TC (60.1 ± 3.1), LDL (49.8 ± 2.2), and TG (171.7 ± 4.8) levels. In the treatment LA group, EtOH + LAD1 and EtOH + LAD2 showed a decrease in the serum TC (67.4 ± 2.9 and 71.3 ± 3.1), LDL (52.7 ± 2.3 and 49.7 ± 2.2), and TG (186.6 ± 5 and 191.3 ± 4.9) levels compared with the EtOH group rats, and LAD2 group rats showed normal serum TC (59.2 ± 3.1), LDL (48.6 ± 2.9), and TG (172.7 ± 3.7) levels almost similar to control vehicle group rats. HDL level (23.9 ± 2.2) significantly decreased ($p < 0.05$) in the EtOH (56 %) administered group rats compared to the EtOH + LAD1 and EtOH + LAD2, LAD2, and control groups (38 ± 2.2, 35.1 ± 2.2, 41 ± 2.3 and 42.8 ± 2.1) as shown in (Fig. 3A and B, C&D).

3.4. Lauric acid's effect on oxidative stress and lipid peroxidation in an EtOH-induced model

The ROS level in the liver sample was significantly increased ($p < 0.05$) in the EtOH-administered group (409.9 ± 90) for 56 days compared with a control group (223.8 ± 24.3). The LA treatment groups EtOH + LAD1 and EtOH + LAD2 significantly decreased ($p < 0.05$) the liver's ROS level (255.5 ± 23.1 and 233.8 ± 16). LAD2 showed normal ROS levels (229.4 ± 20.1) compared with the control vehicle group. Lipid peroxidation analysis showed a significant increase($p < 0.05$) in MDA levels in the EtOH groups (2.74 ± 21) compared to the control vehicle group (1.72 ± 0.23). Treatment groups EtOH + LAD1 and EtOH + LAD2 significantly ($p < 0.05$) dropped the MDA level (1.8 ± 0.2 and 2.1 ± 0.26). LAD2 alone treated group showed almost similar MDA level (1.59 ± 0.29) as the control vehicle group, as shown in (Fig. 4 A&B).

3.5. Lauric acid's effect on hepatic antioxidants in EtOH-induced model

The antioxidant activity was evaluated in the rat's liver homogenate after sacrifice. The constant administration of EtOH (56 %) group rats for 56 days significantly decreased ($p < 0.05$) endogenous antioxidants like SOD (47.8 ± 3.1), GSH (58.2 ± 2.8), and GPx (69.2 ± 3.5) compared with the normal control vehicle SOD (83.6 ± 2.5), GSH (101.1 ± 2.8), and GPx (102 ± 4). Shows that toxins created by the EtOH administration the antioxidants were utilized to suppress toxins created by EtOH. Treatment groups EtOH + LAD1 and EtOH + LAD2 significantly increased the SOD (73.0 ± 2.9 and 74.5 ± 2.5), GSH (99.1 ± 2.8 and 101 ± 2.9), and GPx 97.7 ± 2.8 and 100.2 ± 3.8). The antioxidant levels of the groups were restored to normal control group levels. It shows that LAD2 impacts the treatment of EtOH-administered groups, which could give better points. The LAD2 drug alone group showed normal antioxidant levels of SOD (85.9 ± 2.9), GSH (103 ± 2.8), and GPx (103.3 ± 3.8), almost similar to the control vehicle group of rats, as shown in (Fig. 5A, B&C).

3.6. Lauric acid's effect on liver histoarchitecture in EtOH-induced model

Fig. 6A illustrates the normal liver histoarchitecture in vehicle control rats, with the normal portal vein, bile duct, hepatocytes, sinusoids, and prominent nuclei. Portal vein inflammation, sinusoid dilations, hepatocyte necrosis, and bile duct expansions changed the hepatic architecture in the EtOH-instigated rats (Fig. 6 B). LA-treated group shows effect in changes in the portal vein inflammation, sinusoid dilations, moderate hepatocyte damage, and bile duct expansions in LAD1 and LAD2 almost restored to the normal hepatocytes (Fig. 6C&D). The LAD2-only treated group rats had normal liver tissue histoarchitecture with normal hepatocytes (Fig. 6E).

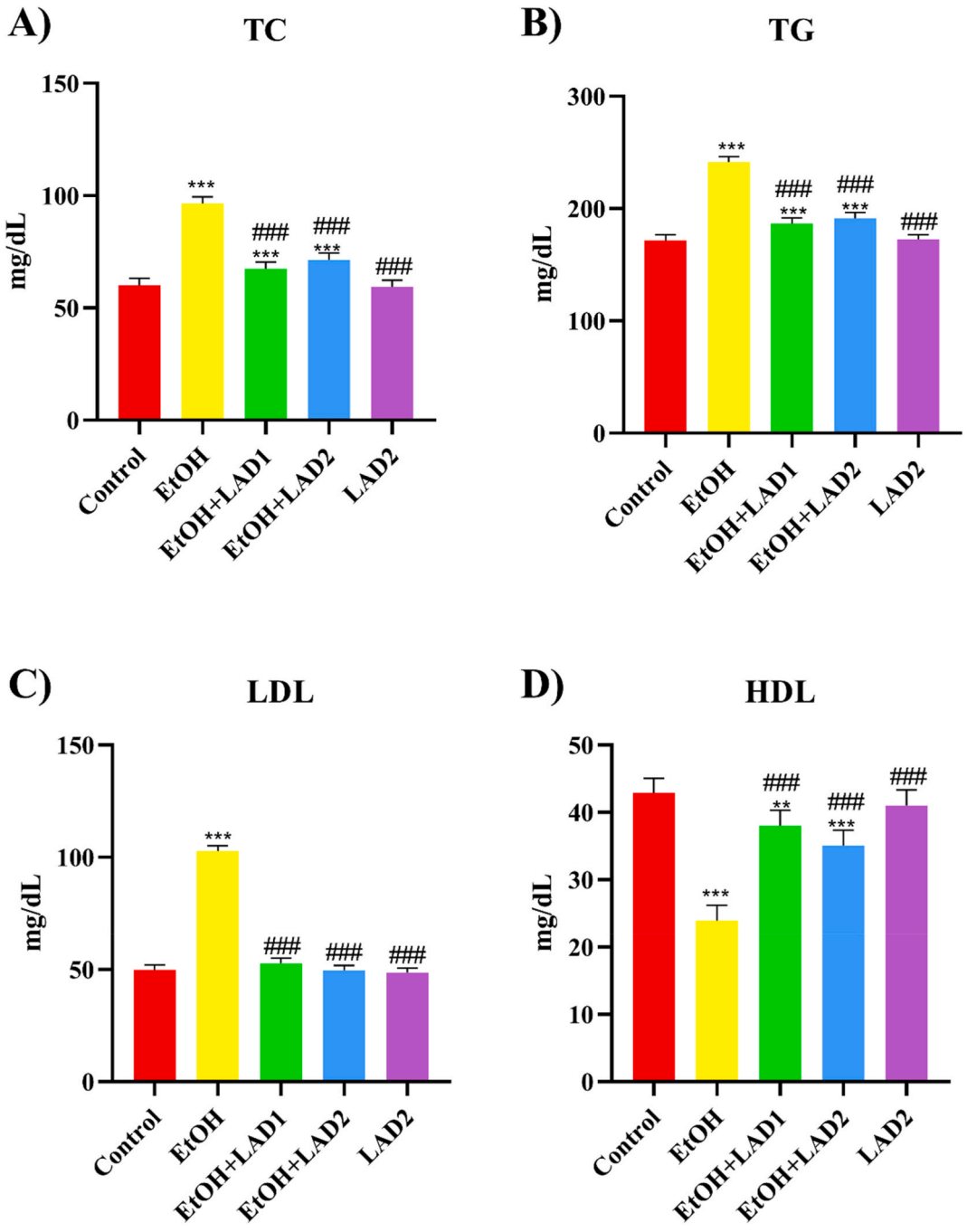

Fig. 3. The experimental Wistar rats were administered LA (180 & 360 mg/kg) 14 days after the EtOH intoxication, where the parameters represent TC(A), TG(B), LDL(C)&HDL(D). The data are represented as means ± SE (n = 6). (Vehicle group), EtOH 56 %/day for 56 days, EtOH + LAD1 (lauric acid (180 mg/kg) from 43rd day), EtOH + LAD2 (lauric acid (360 mg/kg) from 43rd day), and LAD2 (lauric acid (360 mg/kg) from 43rd day). '*' represents the group compared with the vehicle, and '#' represents the group compared with the EtOH group, where statistical significance shows */# indicates $p < 0.05$, **/## indicates $p < 0.01$and ***/### indicates $p < 0.001$.

3.7. Lauric acid's effect on liver pro-inflammatory cytokines in an EtOH-induced liver damage model

The pro-inflammatory effects of LA against the EtOH (56 %) for 56 days of intoxication were evaluated. The pro-inflammatory cytokines TNF-α, IL-6, and IL-1β levels were measured. The normal control vehicle group showed decreased TNF-α (301.9 ± 27.4), IL-6 (64.1 ± 11.3), and IL-1β (93.7 ± 10.4) levels compared with the EtOH toxic group TNF-α (386.4 ± 18.9), IL-6 (93.6 ± 11.4) and

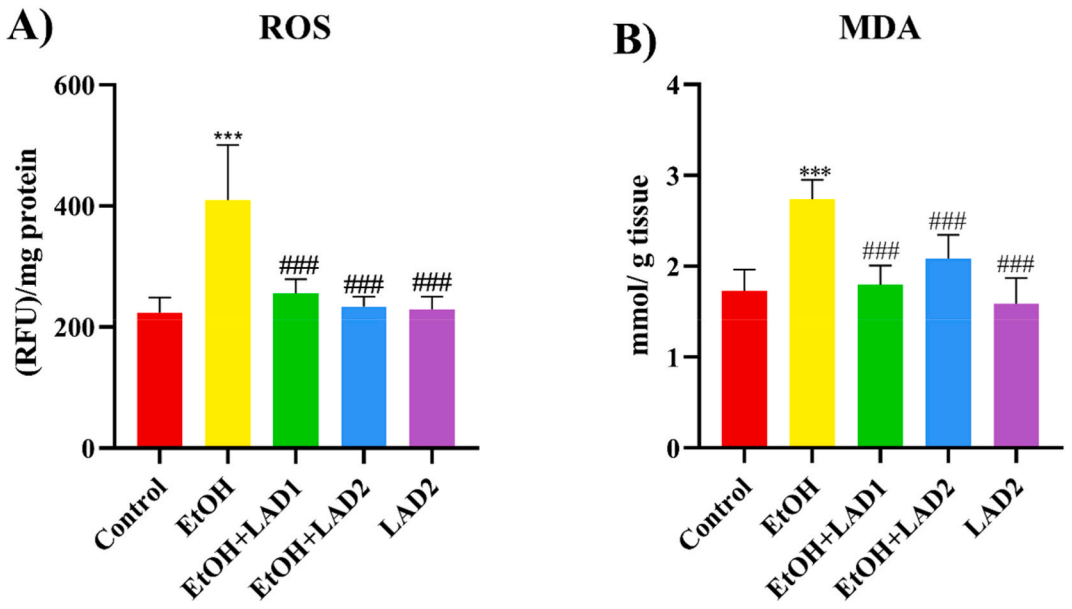

Fig. 4. The experimental Wistar rats were administered LA (180 & 360 mg/kg) 14 days after the EtOH intoxication, where the parameters represent ROS(A)&MDA(B). The data are represented as means ± SE (n = 6). (Vehicle group), EtOH 56 %/day for 56 days, EtOH + LAD1 (lauric acid (180 mg/kg) from 43rd day), EtOH + LAD2 (lauric acid (360 mg/kg) from 43rd day), and LAD2 (lauric acid (360 mg/kg) from 43rd day). '*' represents the group compared with the vehicle, and '#' represents the group compared with the EtOH group, where statistical significance shows */# indicates $p < 0.05$, **/## indicates $p < 0.01$and ***/### indicates $p < 0.001$.

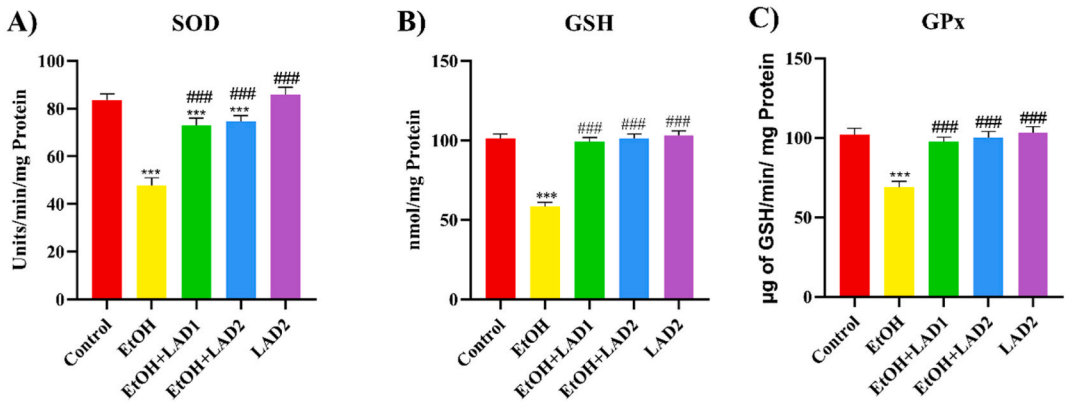

Fig. 5. The experimental Wistar rats were administered LA (180 & 360 mg/kg) 14 days after the EtOH intoxication, where the parameters represent; SOD(A), GSH(B)&GPx(C). The data are represented as means ± SE (n = 6). (Vehicle group), EtOH 56 %/day for 56 days, EtOH + LAD1 (lauric acid (180 mg/kg) from 43rd day), EtOH + LAD2 (lauric acid (360 mg/kg) from 43rd day), and LAD2 (lauric acid (360 mg/kg) from 43rd day). '*' represents the group compared with the vehicle, and '#' represents the group compared with the EtOH group, where statistical significance shows */# indicates $p < 0.05$, **/## indicates $p < 0.01$and ***/### indicates $p < 0.001$.

IL-1β (121.7 ± 12.3). Whereas the treatment group EtOH + LAD1 and EtOH + LAD2 showed a significant decrease ($p < 0.05$) in cytokine levels TNF-α (334.8 ± 21.6 and 310.7 ± 20.8), IL-6 (57.9 ± 11.2 and 54.7 ± 10.4) and IL-1β (91.7 ± 9.1 and 88.6 ± 9.6), LA has reduced hepatotoxicity caused by EtOH. The rats administered with the drug alone in the LAD2 group showed similar TNF-α (297.6 ± 27.6), IL-6 (63.3 ± 9.9), and IL-1β (92.6 ± 9.1) levels with the control vehicle rats. The analysis showed that LA reduced cytokine levels (Fig. 7A, B& C).

3.8. Lauric acid's effect on liver HNF4 α in EtOH-induced model

HNF4α protein levels of each group were analysed in the liver homogenate using an ELISA kit. The EtOH group showed a significantly lower ($p < 0.05$) HNF4α level (2648.5 ± 149.5) when compared with the treated group. In the EtOH group, the HNF4α was reduced due to the loss of metabolic activity of the liver induced by EtOH toxin content. In the treatment group, there was a

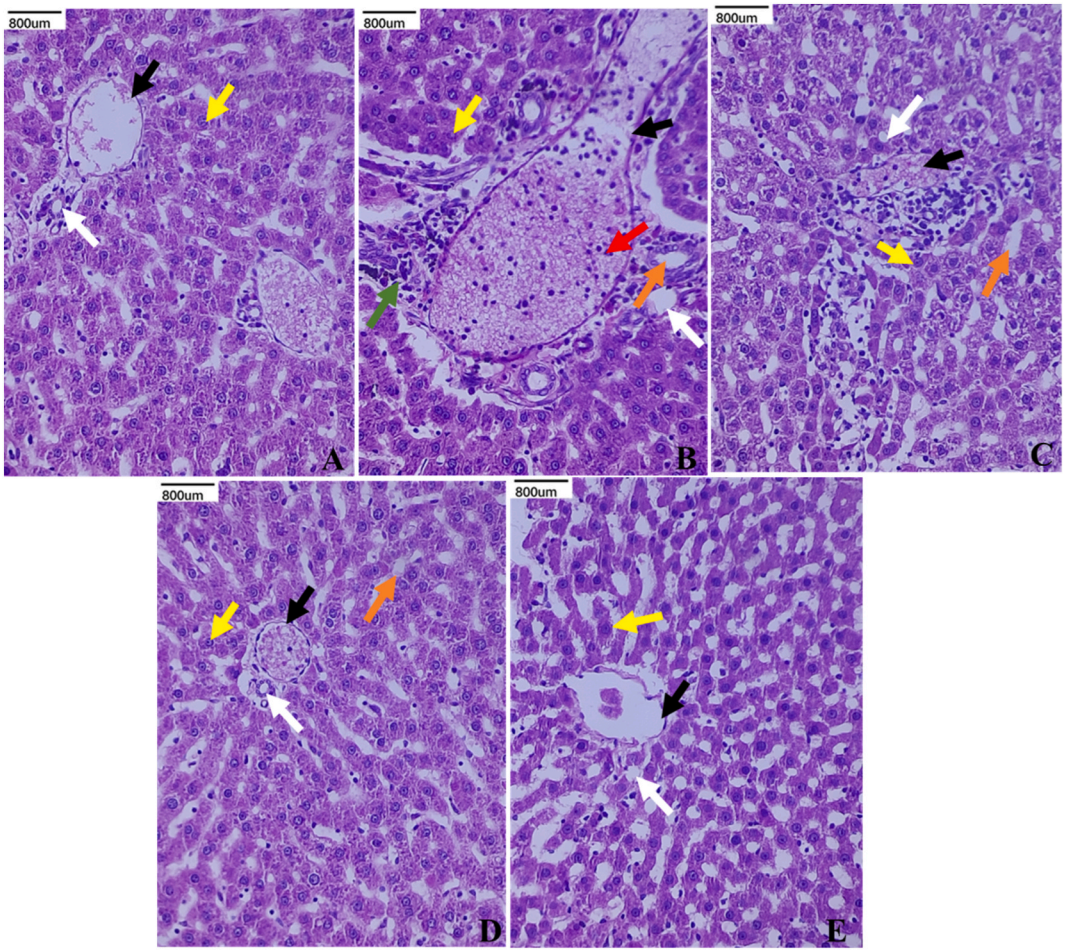

Fig. 6. Histopathological analysis was done in the experimental Albino male Wistar rats. Animals were administered LA (180 & 360 mg/kg) for 14 days after the EtOH challenge. Control vehicle A, EtOH 56 %/day for 56 days B, EtOH + LAD1 (lauric acid (180 mg/kg) from 43rd day) C, EtOH + LAD2 (lauric acid (360 mg/kg) from 43rd day) D and LAD2 (lauric acid (360 mg/kg) from 43rd day) E. in the control vehicle group A shows black arrow shows the normal portal vein, the yellow arrow shows normal hepatocytes and white arrow shows the normal bile duct, EtOH group B black arrow indicates the abnormal portal vein. The yellow arrow indicates the changing structure of hepatocytes, the white arrow indicates the expansion of bile ducts, the red arrow shows the periportal inflammation, the green arrow indicates the centrilobular inflammation and the orange arrow indicates the sinusoidal changes, group C&D shows the mild and moderate architecture change without portal vein inflammation, sinusoid dilations, hepatocyte necrosis, and bile duct expansions. Group E shows normal histoarchitecture as the same control vehicle group. The black arrow shows the normal portal vein, the yellow arrow shows normal hepatocytes, and the white arrow represents the normal bile duct.

gradual increase in the HNF4α levels in EtOH + LAD1 and EtOH + LAD2 as it restored the normal metabolism of the liver (3102 ± 142.2 and 3803.1 ± 178.1) as compared with EtOH alone group. In the normal group and LAD2 drug alone group, the normal HNF4α protein levels were shown (4198.8 ± 159.2 and 4272.6 ± 139.5) because liver metabolism is not affected (Fig. 8A).

The mRNA liver expression levels of HNF4α were evaluated using qPCR. The EtOH toxic group showed lower gene expression (0.62 ± 0.02) of HNF4α than the control vehicle group (1.17 ± 0.03) and other treated groups. It is due to the disruption of the liver metabolism through EtOH administration. It led to a decrease in the HNF4α mRNA gene levels. β-actin primer was used as a control marker in qPCR. There was a gradual increase in the HNF4α mRNA gene levels in EtOH + LAD1 and EtOH + LAD2 (0.81 ± 0.09 and 1.04 ± 0.02) because LA acts as the inhibitor to restore the liver's normal metabolism. The normal group and LAD2 drug-alone group showed normal HNF4α mRNA gene levels (1.18 ± 0.03) (Fig. 8B).

3.9. Lauric acid's effect on pro/anti-apoptotic markers in the EtOH-induced model

The attenuating effect of LA on EtOH-induced toxicity was observed using the Western blot analysis, in which caspase 3, 8 pro-apoptotic markers and Bcl-2 anti-apoptotic marker gene expression levels were seen in liver tissue (Fig. 9A). For control, vinculin was used. The Bcl-2 expression level significantly decreased ($p < 0.05$) in the EtOH group (0.09 ± 0.02), whereas the EtOH + LAD1 and EtOH + LAD2 treatment groups significantly increased ($p < 0.05$) Bcl-2 expression (0.84 ± 0.01 and 1.16 ± 0.05). The control

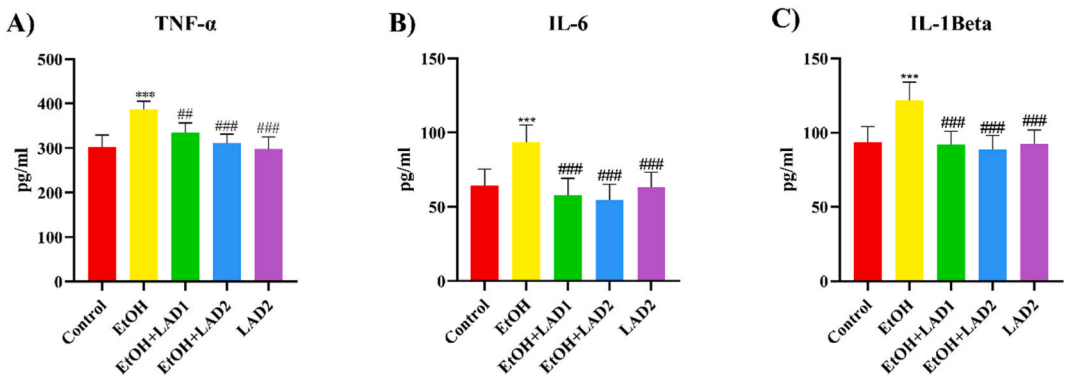

Fig. 7. The experimental Wistar rats were administered LA (180 & 360 mg/kg) 14 days after the EtOH intoxication, where the parameters represent TNF-α(A), IL-6(B)&IL-1Beta (C). The data are represented as means ± SE (n = 6). (Vehicle group), EtOH 56 %/day for 56 days, EtOH + LAD1 (lauric acid (180 mg/kg) from 43rd day), EtOH + LAD2 (lauric acid (360 mg/kg) from 43rd day), and LAD2 (lauric acid (360 mg/kg) from 43rd day). '*' represents the group compared with the vehicle, and '#' represents the group compared with the EtOH group, where statistical significance shows */# indicates $p < 0.05$, **/## indicates $p < 0.01$and ***/### indicates $p < 0.001$.

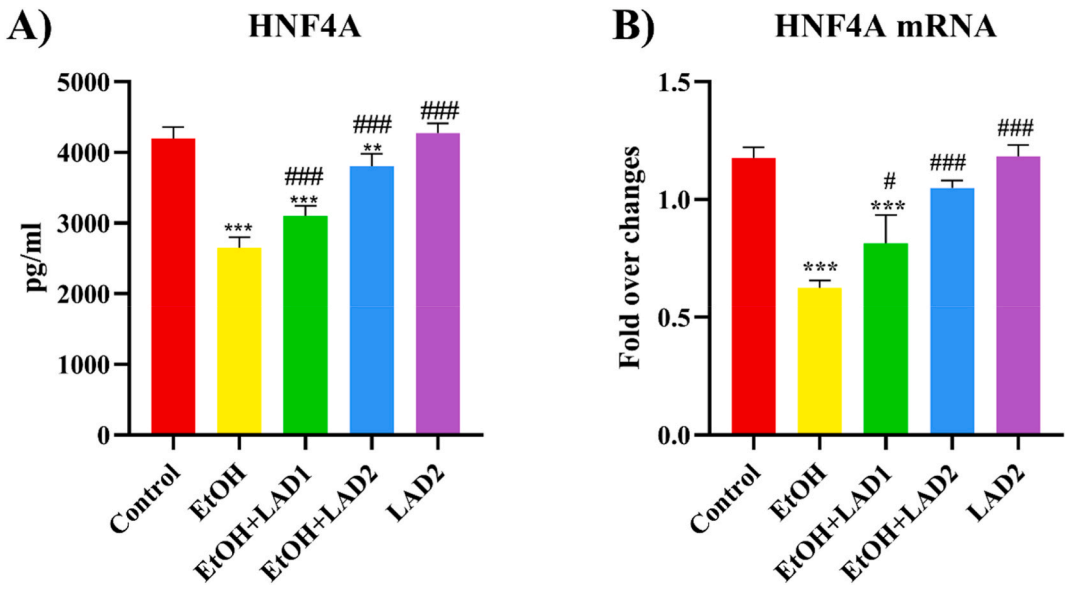

Fig. 8. The experimental Wistar rats were administered LA (180 & 360 mg/kg) 14 days after the EtOH intoxication, where the parameters represent HNF4A(A) &HNF4A mRNA(B). The data are represented as means ± SE (n = 6). (Vehicle group), EtOH 56 %/day for 56 days, EtOH + LAD1 (lauric acid (180 mg/kg) from 43rd day), EtOH + LAD2 (lauric acid (360 mg/kg) from 43rd day), and LAD2 (lauric acid (360 mg/kg) from 43rd day). '*' represents the group compared with the vehicle, and '#' represents the group compared with the EtOH group, where statistical significance shows */# indicates $p < 0.05$, **/## indicates $p < 0.01$and ***/### indicates $p < 0.001$.

vehicle and LAD2 drug alone group showed a normal Bcl-2 gene expression (1.04 ± 0.08 and 1.21 ± 0.02) (Fig. 9B). Caspase 3 (1.69 ± 0.01) and caspase 8 (1.85 ± 0.01) were significantly elevated ($p < 0.05$) in the toxic EtOH alone group. In contrast, the EtOH + LAD1 and EtOH + LAD2 treatment groups show significantly($p < 0.05$) decreased expression Caspase 3 (1.24 ± 0.01 and 0.99 ± 0.01) and caspase 8 (1.34 ± 0.02 and 1.01 ± 0.07). In the drug-alone group, LAD2 shows (1.04 ± 0.01 and 1.05 ± 0.02) a similar expression to the control group (1 ± 0.02 and 0.99 ± 0.03) (Fig. 9C&D).

4. Discussion

In younger generations all over the world, consumption of alcohol has increased. As a result, alcoholism can lead to liver damage in most people [35,36]. This research illustrates the importance of lauric acid in reverting the effect of EtOH-instigated hepatotoxicity. LA, a highly rich antioxidant component present in coconut oil [20], was studied to examine the ability to protect the alcoholic liver in EtOH-instigated hepatotoxicity of rat models.

In our study, the body weight of intoxicated rats was reduced notably, which signifies that EtOH had a toxic effect. EtOH alters food

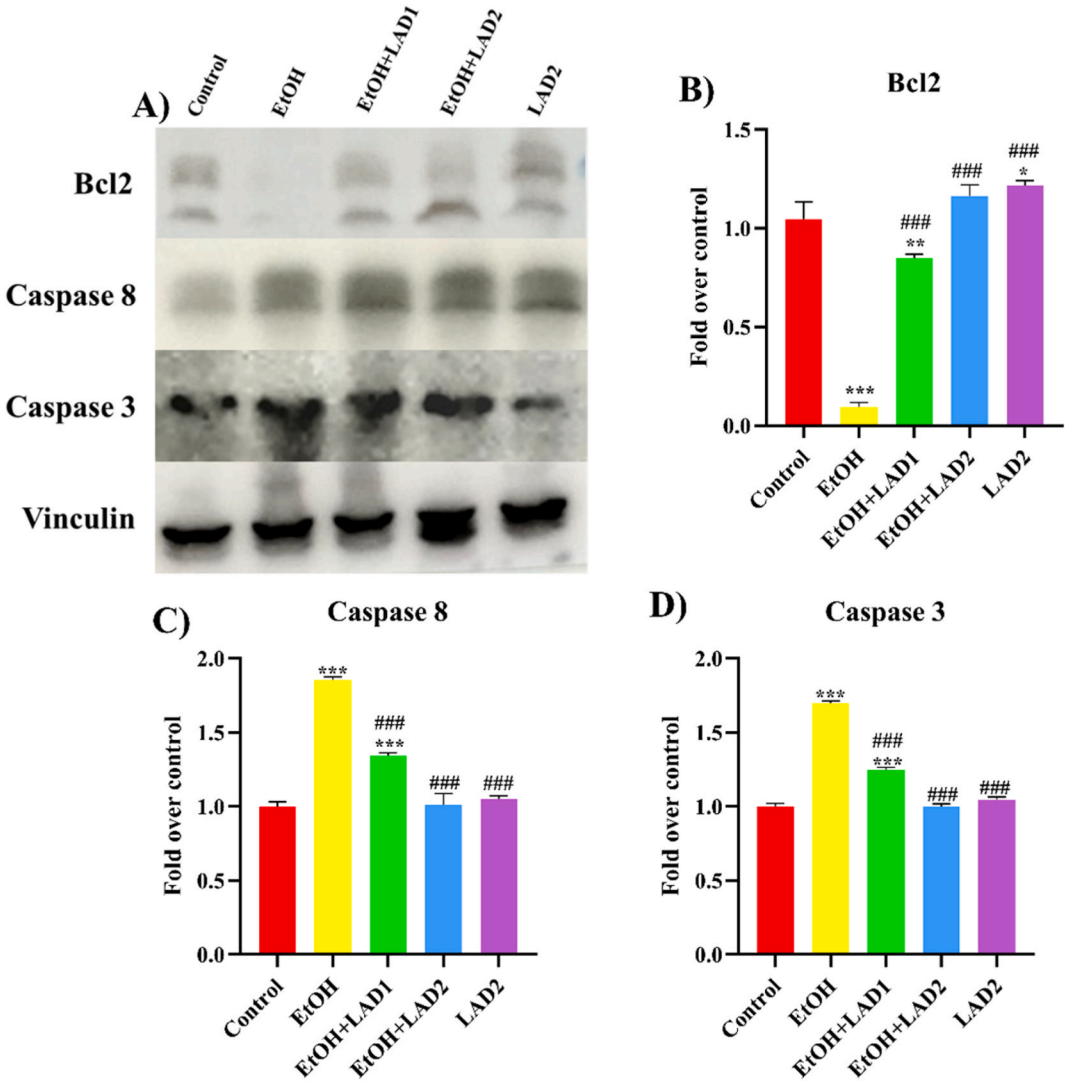

Fig. 9. The experimental Wistar rats were administered LA (180 & 360 mg/kg) 14 days after the EtOH intoxication, where the parameters represent Western blot gel image(A), Bcl2(B), Caspase-8(C) & Caspase(D). The data are represented as means ± SE (n = 6). (Vehicle group), EtOH 56 %/day for 56 days, EtOH + LAD1 (lauric acid (180 mg/kg) from 43rd day), EtOH + LAD2 (lauric acid (360 mg/kg) from 43rd day), and LAD2 (lauric acid (360 mg/kg) from 43rd day). '*' represents the group compared with the vehicle, and '#' represents the group compared with the EtOH group, where statistical significance shows */# indicates $p < 0.05$, **/## indicates $p < 0.01$and ***/### indicates $p < 0.001$.

and water consumption by administering EtOH reducing body weight. In contrast, the control group continuously gained body weight for 56 days without any cause of death in other groups. Aleksandra Kołota et al. reported similarly [37]. On the other side, comparing the treated group is almost similar to that of a control group, which is different from the EtOH-intoxicated group, which shows LA restored the effect created by EtOH in treated groups.

AST, ALT, and ALP are the serum enzyme markers known for liver damage [38,39]. Serum biomarkers (AST, ALT, and ALP) were highly elevated in the intoxicated group due to the indication liver damage leaked in the stream with the hepatocytes. Whereas treatment with LA considerably maintains the optimum levels of hepatic function markers with different doses. In which LAD2 almost maintained the hepatic function compared with control. Then, in the EtOH-intoxicated group of rats, lipid markers such as triglycerides, LDL, and total cholesterol were significantly higher, whereas HDL level was significantly decreased. As in previous studies, it is reported it would be because of an imbalance in the lipid synthesis and inhibition of bile synthesizes [40]. The recovery of these damages could be attributed to LA's capacity to prevent necrotic damage caused by EtOH by inhibiting hepatic enzyme release, stabilizing cell membranes, and improving biliary cell function, producing the synthesis of bile acids [41].

Liver damage occurs in the cell membrane by oxidation process, and oxidative stress plays an essential role in the ALD. Drinking alcohol increases ROS, a free radical production in the liver, depleting the antioxidant level mechanism, which fails to maintain the ROS at the cellular level [38]. In the EtOH intoxicated group, there is a significant increase in ROS, showing that the oxidative stress is

taken, compared with the LA-treated group, which significantly reduced the oxidative stress by increasing the antioxidant defense level mechanism.

EtOH intoxication alters the antioxidant enzyme levels like SOD, GSH, and GPx were depleted by oxidative stress [42]. Those enzymes are the initial defender against the oxidative stress-free radicals created [43]. Lipid peroxidation is one of the primary causes of liver damage, and MDA, its end product, is a significant reactive aldehyde used as an oxidative stress marker [44]. In the present study, EtOH-intoxicated shows increased MDA levels and decreased antioxidant activities in rats. So, a rise in the MDA levels suggests that liver lipid peroxidation has occurred, resulting in liver tissue damage and the depletion of the antioxidant defense mechanism system [45]. The current study shows that the LA prevented the lack of antioxidant enzymes and reduced the MDA levels. The oxidative damage brought on by EtOH overdose was decreased significantly by LA treatment. Because LA functions as a natural antioxidant, limiting lipid peroxidation [46] and preventing antioxidant depletion, we can conclude that it protects against free radical-mediated oxidative stress by scavenging free radicals, which directly neutralizes biological superoxides. Supportive of our findings, another study shows that liver damage by drug metabolites triggers oxidative stress and compromises the antioxidant activity in animal models [47]. Also, hepatoprotective activity of LA was demonstrated previously through promoting antioxidants [22].

Moreover, the histoarchitecture data also supported these findings, proving that LA has protective effect properties. Upon EtOH administration, rats showed portal vein inflammation, sinusoid dilations, hepatocyte necrosis, and bile duct expansions, and these changes were neutralized by the LA treatment. Similar results were reported by Wang et al. on the Hepatoprotective effects of chamazulene against alcohol-induced liver damage by alleviation of oxidative stress in rat models [48]. LA protected the toxic changes with normal histology.

As discussed earlier, reducing antioxidant levels leads to oxidative stress, activating cytokines like IL-1β, TNF-α, and IL-6. During the stress and inflammatory conditions, the TNF-α and IL-6 cytokine levels were elevated [49,50]. IL-1β plays an essential part in cell differentiation, cellular inflammation, and apoptosis, and it is reported that IL-1β is an autocrine growth mediator in different cancers [51,52]. This observation study in the EtOH-administered group has imbalanced levels of pro-inflammatory cytokines, leading to liver toxicity. Moreover, the LA-treated group showed normal IL-1β, IL-6, and TNF-α compared with the EtOH group. Our study showed that LA was anti-inflammatory against the EtOH-induced liver toxicity rats. It correlated with the previous study where the LA reduced the inflammatory mediators in Lipopolysaccharides (LPS) induced liver injury through myeloid differentiation primary response 88 (MyD88) and Toll-like receptor 4 (TLR4) pathway [22]. It confirms the anti-inflammatory properties of LA. With the support of our findings, another study reported that drug metabolites trigger the cytokines by depleting the antioxidant levels and inducing hepatotoxicity [53].

According to reports, EtOH activates caspases, causing DNA fragmentation [54]. This study used western blotting method to analyze apoptosis signaling markers like caspase-8- 8 as the initiator, caspase-3 as the effector, and Bcl-2 as an anti-apoptotic control marker in rat liver tissue samples [55,56]. Caspase 3 gets activated when the cell is injured. The significant rise in the Caspase 3 level indicates cellular destruction had occurred in the EtOH-administered group. EtOH has also been suggested to regulate mitochondria-induced apoptosis through increasing caspase-3 levels [57] in the EtOH-intoxicated group. Caspase-8 is a cysteine protease involved in extrinsic apoptotic signaling through death receptors [58]. The level of caspase3, caspase-8, and anti-apoptotic marker Bcl-2 was significantly downregulated in the EtOH group. Apoptotic markers were normal in LA-treated groups compared with the control vehicle group. Our study suggests that LA alleviated the apoptosis caused by the EtOH in the liver by reducing caspase-8 and caspase-3. It also maintained the Bcl-2 level like the normal control vehicle group. Our study suggests that LA alleviated the apoptosis caused by the EtOH in the liver by reducing caspase-8 and caspase-3. Manisha et al. reported the similar findings [59].

HNF4α is a master key regulator of liver cell differentiation in hepatocytes, and it also plays a major role in embryonic development [60]. Studies reported that HNF4α forced expression on cancer cells decreases the cancer growth in hepatocellular carcinoma (HCC) [61]. HNF4 α affects the liver's bile acid synthesis and metabolism of fatty acids [14]. The liver-specific deletion of HNF4α leads to the spontaneous proliferation of hepatocytes, leading to HCC in KO mice [13,62,63]. In the EtOH-induced toxicity group, rats showed decreased HNF4α levels in protein and mRNA gene fold expressions. The LA-treated group showed a significant increase in the HNF4α protein level and the mRNA gene fold expression. LA may act as an agonist for activating HNF4α in the treated groups. In contrast, the LA and control vehicle group shows normal HNF4α protein levels, and the mRNA gene level folds expression.

5. Conclusion

The results suggest that EtOH, on consistent administration, increases the free radical activity that causes ROS and initiates the inflammatory mechanism and apoptosis. Moreover, it was the first study to observe that the treatment of LA reduced free radicals produced by EtOH. The mechanism behind LA's therapeutic process is inflammatory markers downregulation and increased antioxidant levels in the liver. In addition, LA ameliorated the pro-apoptotic markers like IL-1β, IL-6, and TNF-α. The restoration of protein and gene expression of HNF4α was observed in the liver of LA-treated groups. Therefore, we conclude that the LA treatment has a protective effect against EtOH-induced hepatotoxicity by modulating oxidative stress, apoptosis signalling, and HNF4α in Wistar albino rats.

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Ethics statement

The Institutional Animal Ethical Committee approved the experimental protocol (VIT/IAEC/18/Dec2020/01). It was completed at VIT, Vellore, in compliance with Indian CPCSEA standard protocol.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Arunraj Namachivayam: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Abilash Valsala Gopalakrishnan:** Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e21267.

References

[1] N.A. Osna, T.M. Donohue Jr., K.K. Kharbanda, Alcoholic liver disease: pathogenesis and current management, Alcohol Res 38 (2) (2017) 147–161.
[2] K. Ohashi, M. Pimienta, E. Seki, Alcoholic liver disease: a current molecular and clinical perspective, Liver Res 2 (4) (2018) 161–172.
[3] B. Gao, R. Bataller, Alcoholic liver disease: pathogenesis and new therapeutic targets, Gastroenterology 141 (5) (2011) 1572–1585.
[4] S.H. Park, D.J. Kim, Global and regional impacts of alcohol use on public health: emphasis on alcohol policies, Clin. Mol. Hepatol. 26 (4) (2020) 652–661.
[5] K.G. Ishak, H.J. Zimmerman, M.B. Ray, Alcoholic liver disease: pathologic, pathogenetic and clinical aspects, Alcohol Clin. Exp. Res. 15 (1) (1991) 45–66.
[6] M. Roerecke, A. Vafaei, O.S.M. Hasan, B.R. Chrystoja, M. Cruz, R. Lee, M.G. Neuman, J. Rehm, Alcohol consumption and risk of liver cirrhosis: a systematic review and meta-analysis, Am. J. Gastroenterol. 114 (10) (2019) 1574–1586.
[7] Y. Jiang, T. Zhang, P. Kusumanchi, S. Han, Z. Yang, S. Liangpunsakul, Alcohol metabolizing enzymes, microsomal ethanol oxidizing system, cytochrome P450 2E1, catalase, and aldehyde dehydrogenase in alcohol-associated liver disease, Biomedicines 8 (3) (2020).
[8] Y. Yu, Z.Q. Tian, L. Liang, X. Yang, D.D. Sheng, J.X. Zeng, X.Y. Li, R.Y. Shi, Z.P. Han, L.X. Wei, Babao Dan attenuates acute ethanol-induced liver injury via Nrf2 activation and autophagy, Cell Biosci. 9 (2019) 80.
[9] A. Namachivayam, A. Valsala Gopalakrishnan, A review on molecular mechanism of alcoholic liver disease, Life Sci. 274 (2021), 119328.
[10] K. Renu, A. Saravanan, A. Elangovan, S. Ramesh, S. Annamalai, A. Namachivayam, P. Abel, H. Madhyastha, R. Madhyastha, M. Maruyama, V. Balachandar, A. Valsala Gopalakrishnan, An appraisal on molecular and biochemical signalling cascades during arsenic-induced hepatotoxicity, Life Sci. 260 (2020), 118438.
[11] R.R. Kathak, A.H. Sumon, N.H. Molla, M. Hasan, R. Miah, H.R. Tuba, A. Habib, N. Ali, The association between elevated lipid profile and liver enzymes: a study on Bangladeshi adults, Sci. Rep. 12 (1) (2022) 1711.
[12] F.J. Gonzalez, Regulation of hepatocyte nuclear factor 4 alpha-mediated transcription, Drug Metabol. Pharmacokinet. 23 (1) (2008) 2–7.
[13] I. Huck, E.M. Morris, J. Thyfault, U. Apte, Hepatocyte-specific hepatocyte nuclear factor 4 alpha (HNF4) deletion decreases resting energy expenditure by disrupting lipid and carbohydrate homeostasis, Gene Expr. 20 (3) (2021) 157–168.
[14] K.W. Huang, V. Reebye, K. Czysz, S. Ciriello, S. Dorman, I. Reccia, H.S. Lai, L. Peng, N. Kostomitsopoulos, J. Nicholls, R.S. Habib, D.A. Tomalia, P. Sætrom, E. Wilkes, P. Cutillas, J.J. Rossi, N.A. Habib, Liver activation of hepatocellular nuclear factor-4α by small activating RNA rescues dyslipidemia and improves metabolic profile, Mol. Ther. Nucleic Acids 19 (2020) 361–370.
[15] F. Parviz, C. Matullo, W.D. Garrison, L. Savatski, J.W. Adamson, G. Ning, K.H. Kaestner, J.M. Rossi, K.S. Zaret, S.A. Duncan, Hepatocyte nuclear factor 4alpha controls the development of a hepatic epithelium and liver morphogenesis, Nat. Genet. 34 (3) (2003) 292–296.
[16] S. Zakhari, T.K. Li, Determinants of alcohol use and abuse: impact of quantity and frequency patterns on liver disease, Hepatology 46 (6) (2007) 2032–2039.
[17] V. Oliviu, Adverse effects and drug interactions of the non-steroidal anti-inflammatory drugs, in: A.-k. Ali Gamal Ahmed (Ed.), Non-steroidal Anti-inflammatory Drugs, IntechOpen, Rijeka, 2017. Ch. 3.
[18] V. Subramaniyan, S. Chakravarthi, R. Jegasothy, W.Y. Seng, N.K. Fuloria, S. Fuloria, I. Hazarika, A. Das, Alcohol-associated liver disease: a review on its pathophysiology, diagnosis and drug therapy, Toxicol Rep 8 (2021) 376–385.
[19] H.J. Kwon, Y.S. Won, O. Park, D. Feng, B. Gao, Opposing effects of prednisolone treatment on T/NKT cell- and hepatotoxin-mediated hepatitis in mice, Hepatology 59 (3) (2014) 1094–1106.
[20] N.A.A. Ghani, A.A. Channip, P. Chok Hwee Hwa, F. Ja'afar, H.M. Yasin, A. Usman, Physicochemical properties, antioxidant capacities, and metal contents of virgin coconut oil produced by wet and dry processes, Food Sci. Nutr. 6 (5) (2018) 1298–1306.
[21] T.C. Wallace, Health effects of coconut oil-A narrative review of current evidence, J. Am. Coll. Nutr. 38 (2) (2019) 97–107.
[22] H.U. Khan, K. Aamir, P.R. Jusuf, G. Sethi, S.P. Sisinthy, R. Ghildyal, A. Arya, Lauric acid ameliorates lipopolysaccharide (LPS)-induced liver inflammation by mediating TLR4/MyD88 pathway in Sprague Dawley (SD) rats, Life Sci. 265 (2021), 118750.

[23] J.K. Fauser, G.M. Matthews, A.G. Cummins, G.S. Howarth, Induction of apoptosis by the medium-chain length fatty acid lauric acid in colon cancer cells due to induction of oxidative stress, Chemotherapy 59 (3) (2013) 214–224.
[24] K. Zeiger, J. Popp, A. Becker, J. Hankel, C. Visscher, G. Klein, D. Meemken, Lauric acid as feed additive – an approach to reducing Campylobacter spp. in broiler meat, PLoS One 12 (4) (2017), e0175693.
[25] N.F. Alves, T.M. de Queiroz, R. de Almeida Travassos, M. Magnani, V. de Andrade Braga, Acute treatment with lauric acid reduces blood pressure and oxidative stress in spontaneously hypertensive rats, Basic Clin. Pharmacol. Toxicol. 120 (4) (2017) 348–353.
[26] M.A. Alfhili, G.S. Aljuraiban, Lauric acid, a dietary saturated medium-chain fatty acid, Elicits Calcium-Dependent Eryptosis, Cells 10 (12) (2021).
[27] S.V. Veeresh Babu, B. Veeresh, A.A. Patil, Y.B. Warke, Lauric acid and myristic acid prevent testosterone induced prostatic hyperplasia in rats, Eur. J. Pharmacol. 626 (2–3) (2010) 262–265.
[28] C. Lu, F. Zhang, W. Xu, X. Wu, N. Lian, H. Jin, Q. Chen, L. Chen, J. Shao, L. Wu, Y. Lu, S. Zheng, Curcumin attenuates ethanol-induced hepatic steatosis through modulating Nrf2/FXR signaling in hepatocytes, IUBMB Life 67 (8) (2015) 645–658.
[29] M. Ouédraogo, K. Konaté, A.N. Lepengué, A. Souza, B. M'Batchi, L.L. Sawadogo, Free radical scavenging capacity, anticandicidal effect of bioactive compounds from Sida Cordifolia L., in combination with nystatin and clotrimazole and their effect on specific immune response in rats, Ann. Clin. Microbiol. Antimicrob. 11 (1) (2012) 33.
[30] E. Seo, H. Kang, H. Choi, W. Choi, H.S. Jun, Reactive oxygen species-induced changes in glucose and lipid metabolism contribute to the accumulation of cholesterol in the liver during aging, Aging Cell 18 (2) (2019), e12895.
[31] H. Esterbauer, K.H. Cheeseman, M.U. Dianzani, G. Poli, T.F. Slater, Separation and characterization of the aldehydic products of lipid peroxidation stimulated by ADP-Fe2+ in rat liver microsomes, Biochem. J. 208 (1) (1982) 129–140.
[32] J.T. Rotruck, A.L. Pope, H.E. Ganther, A.B. Swanson, D.G. Hafeman, W.G. Hoekstra, Selenium: biochemical role as a component of glutathione peroxidase, Science 179 (4073) (1973) 588–590.
[33] M.S. Moron, J.W. Depierre, B. Mannervik, Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver, Biochim. Biophys. Acta 582 (1) (1979) 67–78.
[34] S. Marklund, G. Marklund, Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase, Eur. J. Biochem. 47 (3) (1974) 469–474.
[35] X. Niu, L. Zhu, Y. Xu, M. Zhang, Y. Hao, L. Ma, Y. Li, H. Xing, Global prevalence, incidence, and outcomes of alcohol related liver diseases: a systematic review and meta-analysis, BMC Publ. Health 23 (1) (2023) 859.
[36] P.D. Axley, C.T. Richardson, A.K. Singal, Epidemiology of alcohol consumption and societal burden of alcoholism and alcoholic liver disease, Clin. Liver Dis. 23 (1) (2019) 39–50.
[37] A. Kołota, D. Głąbska, M. Oczkowski, J. Gromadzka-Ostrowska, Influence of alcohol consumption on body mass gain and liver antioxidant defense in adolescent growing male rats, Int. J. Environ. Res. Publ. Health 16 (13) (2019).
[38] M. Das, S. Basu, B. Banerjee, A. Sen, K. Jana, G. Datta, Hepatoprotective effects of green Capsicum annum against ethanol induced oxidative stress, inflammation and apoptosis in rats, J. Ethnopharmacol. 227 (2018) 69–81.
[39] X. Wang, K. Dong, Y. Ma, Q. Jin, S. Yin, S. Wang, 1, Hepatoprotective effects of chamazulene against alcohol-induced liver damage by alleviation of oxidative stress in rat models, 15, 2020, pp. 251–258.
[40] N. Ge, H. Liang, Y.Y. Zhao, Y. Liu, A.J. Gong, W.L. Zhang, Aplysin protects against alcohol-induced liver injury via alleviating oxidative damage and modulating endogenous apoptosis-related genes expression in rats, J. Food Sci. 83 (10) (2018) 2612–2621.
[41] L. Xu, Y. Yu, R. Sang, J. Li, B. Ge, X. Zhang, Protective effects of taraxasterol against ethanol-induced liver injury by regulating CYP2E1/nrf2/HO-1 and NF-κB signaling pathways in mice, Oxid. Med. Cell. Longev. (2018) (2018), 8284107.
[42] Y. Zhang, C. Wang, B. Yu, J.D. Jiang, W.J. Kong, Gastrodin protects against ethanol-induced liver injury and apoptosis in HepG2 cells and animal models of alcoholic liver disease, Biol. Pharm. Bull. 41 (5) (2018) 670–679.
[43] O.M. Ighodaro, O.A. Akinloye, First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): their fundamental role in the entire antioxidant defence grid, Alexandria J. Med. 54 (4) (2018) 287–293.
[44] A. Ayala, M.F. Muñoz, S. Argüelles, Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal, Oxid. Med. Cell. Longev. 2014 (2014), 360438.
[45] M.D. Shah, U.J.A. D'Souza, M. Iqbal, The potential protective effect of Commelina nudiflora L. against carbon tetrachloride (CCl4)-induced hepatotoxicity in rats, mediated by suppression of oxidative stress and inflammation, Environ. Health Prev. Med. 22 (1) (2017) 66.
[46] I. Belghit, R. Waagbø, E.-J. Lock, N.S. Liland, Insect-based diets high in lauric acid reduce liver lipids in freshwater Atlantic salmon, Aquacult. Nutr. 25 (2) (2019) 343–357.
[47] S. Nithiyanandam, S.E. Prince, Caesalpinia bonducella counteracts paracetamol-instigated hepatic toxicity via modulating TNF-α and IL-6/10 expression and bcl-2 and caspase-8/3 signalling, Appl. Biochem. Biotechnol. 195 (10) (2023) 6256–6275.
[48] X. Wang, K. Dong, Y. Ma, Q. Jin, S. Yin, S. Wang, Hepatoprotective effects of chamazulene against alcohol-induced liver damage by alleviation of oxidative stress in rat models, Open Life Sci. 15 (1) (2020) 251–258.
[49] S. Saha, B. Buttari, E. Panieri, E. Profumo, L. Saso, An overview of Nrf2 signaling pathway and its role in inflammation, Molecules 25 (22) (2020).
[50] M. Mittal, M.R. Siddiqui, K. Tran, S.P. Reddy, A.B. Malik, Reactive oxygen species in inflammation and tissue injury, Antioxid Redox Signal 20 (7) (2014) 1126–1167.
[51] S. Kany, J.T. Vollrath, B. Relja, Cytokines in inflammatory disease, Int. J. Mol. Sci. 20 (23) (2019).
[52] C. Rébé, F. Ghiringhelli, Interleukin-1β and cancer, Cancers 12 (7) (2020).
[53] N.K. Panchal, P. Swarnalatha, S.E. Prince, Trichopus zeylanicus ameliorates ibuprofen inebriated hepatotoxicity and enteropathy: an insight into its modulatory impact on pro/anti-inflammatory cytokines and apoptotic signaling pathways, Inflammopharmacology 30 (6) (2022) 2229–2242.
[54] J. Liu, Ethanol and liver: recent insights into the mechanisms of ethanol-induced fatty liver, World J. Gastroenterol. 20 (40) (2014) 14672–14685.
[55] T. Miyata, L.E. Nagy, Programmed cell death in alcohol-associated liver disease, Clin. Mol. Hepatol. 26 (4) (2020) 618–625.
[56] C. Li, J. Li, G. Xu, H. Sun, Influence of chronic ethanol consumption on apoptosis and autophagy following transient focal cerebral ischemia in male mice, Sci. Rep. 10 (1) (2020) 6164.
[57] S.J.S. Flora, P. Gautam, P. Kushwaha, Lead and ethanol Co-exposure lead to blood oxidative stress and subsequent neuronal apoptosis in rats, Alcohol Alcohol 47 (2) (2012) 92–101.
[58] C. Wang, R.J. Youle, The role of mitochondria in apoptosis, Annu. Rev. Genet. 43 (2009) 95–118.
[59] M. Parthasarathy, S.E. Prince, Andrographis Paniculata (Burm.f.) Nees Alleviates Methotrexate-Induced Hepatotoxicity in Wistar Albino Rats, Life, 2023.
[60] E.N. Tafaleng, A. Mukherjee, A. Bell, K. Morita, J. Guzman-Lepe, N. Haep, R.M. Florentino, R. Diaz-Aragon, C. Frau, A. Ostrowska, J.R. Schultz, P.G.V. Martini, A. Soto-Gutierrez, I.J. Fox, Hepatocyte nuclear factor 4 alpha 2 messenger RNA reprograms liver-enriched transcription factors and functional proteins in end-stage cirrhotic human hepatocytes, Hepatol Commun 5 (11) (2021) 1911–1926.
[61] C. Yin, Y. Lin, X. Zhang, Y.X. Chen, X. Zeng, H.Y. Yue, J.L. Hou, X. Deng, J.P. Zhang, Z.G. Han, W.F. Xie, Differentiation therapy of hepatocellular carcinoma in mice with recombinant adenovirus carrying hepatocyte nuclear factor-4alpha gene, Hepatology 48 (5) (2008) 1528–1539.
[62] I. Huck, S. Gunewardena, R. Espanol-Suner, H. Willenbring, U. Apte, Hepatocyte nuclear factor 4 alpha activation is essential for termination of liver regeneration in mice, Hepatology 70 (2) (2019) 666–681.
[63] C. Walesky, S. Gunewardena, E.F. Terwilliger, G. Edwards, P. Borude, U. Apte, Hepatocyte-specific deletion of hepatocyte nuclear factor-4α in adult mice results in increased hepatocyte proliferation, Am. J. Physiol. Gastrointest. Liver Physiol. 304 (1) (2013) G26–G37.