European Review for Medical and Pharmacological Sciences 2024; 28: 1259-1271

Oleuropein, a phenolic component of *Olea europaea* L. ameliorates CCl_4-induced liver injury in rats through the regulation of oxidative stress and inflammation

A.H. RAHMANI[1], A. ALMATROUDI[1], K.S. ALLEMAILEM[1], H.O.A. ALHARBI[1], A.Y. BABIKER[1], S.A. ALTHWAB[2], N. ALSUHAYMI[3], M.H. ALSUGOOR[3], A.A. KHAN[4], W.A.I. AL-MEGRIN[5]

[1]Department of Medical Laboratories, College of Applied Medical Sciences, Qassim University, Buraydah 51452, Saudi Arabia
[2]Department of Food Science and Human Nutrition, College of Agriculture and Veterinary Medicine, Qassim University, Buraydah 51452, Saudi Arabia
[3]Department of Emergency Medical Services, Faculty of Health Sciences, Umm Al-Qura University, AlQunfudah, Makkah, Saudi Arabi
[4]Department of Basic Health Sciences, College of Applied Medical Sciences, Qassim University, Buraydah, Saudi Arabia
[5]Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, P.O. Box 84428, Riyadh 11671, Saudi Arabia

Abstract. – OBJECTIVE: This study aimed to assess the hepatoprotective role of oleuropein (Olp), a phenolic compound found in olive, against carbon tetrachloride (CCl_4)-induced liver damage in rats.

MATERIALS AND METHODS: The research involved male albino rats, which received intraperitoneal injections of 100 mg/kg b.w. of oleuropein for 8 consecutive weeks before being subjected to carbon tetrachloride (CCl_4) at a dosage of 1.0 ml/kg b.w. Changes induced by CCl_4 in antioxidant and inflammatory marker levels were assessed using ELISA assay kits. Moreover, CCl_4-induced liver tissue architecture alteration, fibrosis, and expression pattern of protein were evaluated by performing H&E, Sirius red, Masson trichrome, and immunohistochemistry staining.

RESULTS: Increased serum transaminases and massive hepatic damage were observed by this liver toxicant. The hepatic injury was further evidenced by a significant decrease in antioxidant enzyme activity [superoxide dismutase (SOD), glutathione peroxidase (GPx), Glutathione (GSH) and Total Antioxidant Capacity (T-AOC)]. The administration of CCl_4 resulted in an increased inflammatory response, which was measured by C-reactive protein, interleukin-6, as well as tumor necrosis factor-alpha. Olp as a curative regimen led to significant attenuation in the inflammatory response and oxidative/nitrosative stress. This polyphenol treatment improved the hepatic tissue architecture and decreased fibrosis. In the CCl_4 treatment group, the expression pattern of IL-6 protein was high, whereas expression was decreased after Olp, as evidenced by immunohistochemistry staining.

CONCLUSIONS: The study suggests that oleuropein treatment has the potential to reduce liver damage caused by CCl_4 induction by inhibiting oxidative stress and inflammation and maintaining liver tissue architecture. This could make it a promising treatment option for liver pathogenesis.

Key Words:
Hepatotoxicity, CCl_4, Oleuropein, Liver function test, Antioxidant enzymes, Inflammatory markers.

Introduction

The liver is a vital organ and plays a significant role in the detoxification of toxic materials. Various agents, such as drugs, toxins, alcohol, and viruses, cause liver injury. Paracetamol, also known as acetaminophen, and alcohol cause liver toxicity[1,2] and long-term exposure to carbon tetrachloride (CCl_4) causes chronic liver pathogenesis.

Carbon tetrachloride (CCl_4) is considered one of the environmental hepatotoxins and its administration can result in an overproduction of free radicals and oxidative stress, which can lead

Corresponding Author: A.H. Rahmani, Ph.D; e-mail: ah.rahmani@qu.edu.sa

to liver damage[3]. It is a common industrial solvent used in the synthesis of chlorinated organic compounds, semiconductor production, processing of oils, and laboratory applications, and is used as an agricultural fumigant[4,5]. The covalent binding of reactive intermediates of CCl_4 to different cellular components promotes increased lipid peroxidation and leads to lipid destruction, particularly of unsaturated phospholipids. The plasma membrane and intracellular membranes of hepatocytes are severely damaged by CCl_4[6]. It is well known that breakdown products, including reactive aldehydes, can cause significant damage to cells. These breakdown products can lead to increased membrane permeability of lysosomes and mitochondria, ultimately resulting in cell death[7,8].

CCl_4 has been widely used to induce liver injury in experimental animal models to examine the hepatoprotective potential of natural compounds. The liver is a vital organ that can be injured by a variety of toxins, including CCl_4. CCl_4 is known to cause liver injury through several mechanisms, including oxidative stress, inflammation, and programmed cell death[9,10].

The role of natural phenolic compounds/flavonoids has been widely reported to ameliorate oxidative stress-mediated liver damage and inhibit various pathogenesis[11-14]. Olive leaves have a long history of use in traditional medicine throughout Europe and countries in the Mediterranean[15]. Oleuropein (Ole) is the main phenolic compound in the olive tree, *Olea europaea* L., and is chiefly abundant in unprocessed olive fruit and leaves, with concentrations up to 140 mg g^{-1} on a dry matter basis in young olives[16], and 60-90 mg g^{-1} of dry matter in the leaves[17] and component of various medicines[18]. The pharmacological activity of oleuropein is diverse, including anti-atherosclerotic, anti-inflammatory, anti-cancer, antimicrobial, and antiviral activity[19,20]. Besides, Oleuropein has strong dose-dependent antioxidant potential; it can scavenge nitric oxide, reduce levels of reactive oxygen species (ROS) and reactive nitrogen species (RNS), and decrease lipid peroxidation levels in different models of ischemia[21].

It was found that oleuropein has anti-inflammatory properties, which may be attributed to its antioxidant effects[22]. The mechanism behind this anti-inflammatory effect involves the inhibition of several factors, such as NF-κβ and its translocation to the nucleus, COX-2, caspase-3, and iNOS, as reported by Domitrović et al[23]. Further, its role in cancer management has been reported, and recent studies have shown that oleuropein has the ability to induce apoptosis in MCF-7 cells through a p53-dependent pathway, which is mediated by the up-regulation of Bax and down-regulation of Bcl2 gene expression[24].

In this study, it was aimed to evaluate the potential hepatoprotective effects of oleuropein against liver damage induced by carbon tetrachloride (CCl_4).

Materials and Methods

Materials

CCl_4 and oleuropein were purchased from Sigma-Aldrich Inc. (lot no.: 12247, St. Louis, MO, USA). Liver function diagnostic kits [aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP)] were purchased from Abcam Company, UK. The antioxidant enzyme assay kits, superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione (GSH) and Total Antioxidant Capacity (T-AOC) were purchased from Abcam, UK. The kits of inflammatory markers C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) ELISA-based kits were also procured from Abcam, UK. Procured tissue staining kits, such as the Picro Sirius red stain kit and trichrome stain kit, from Abcam, UK, to examine connective tissue. Additionally, IL-6 primary antibody was purchased from the same company.

Experimental Animals

The healthy male albino Wistar rats used in this study weighed between 150-200 g and were 5-6 weeks old and placed under a controlled temperature of 23 ± 2°C, 12 h light/dark cycle, and 55 ± 5% relative humidity. The animals were acclimatized for one week to ease any transportation stress and were properly observed for any weakness. The animals were given free access to standard laboratory food and water *ad libitum*. The laboratory animal ethics committee, CAMS, and Qassim University permitted the study, and rats were treated as per the guidelines from this committee.

Experimental Design

Thirty-two rats were randomly divided into four groups (group I to group IV), with each group having (n = 8) animals (Table I). All animals received water and standard laboratory food

Table I. Rats grouping and treatment plan.

Group number	Group name	Brief designation	Treatment plan
I	Normal control	NC	The animals were given free access to water and standard laboratory food, and were orally administered 1.0 ml/kg b.w. of normal saline as a placebo.
II	Disease control	CCl_4	Intraperitoneal (I.P.) injection of 1.0 ml/kg b.w. of CCl_4 (1:1 in olive oil)[25], twice weekly.
III	Diseased animal + Oleuropein treatment group	Olp + CCl_4	CCl_4 treatment, same as group II animals followed by one hour break and i.p. dose of oleuropein 100 mg/kg b.w.[23]
IV	Oleuropein treatment	Olp	I.P. dosage of oleuropein, same as group III treatment

ad libitum. Group I was the control group and received 1.0 ml/kg. b.w. normal saline as a placebo. In group II, animals were assigned as the disease control group, intoxicated with an i.p. dose of 1.0 ml of CCl_4 (50% v/v in olive oil) twice weekly for eight weeks consecutively. In group III, animals were assigned to the diseased animal treatment group and received 100 mg/kg b.w. Olp one hour before CCl_4 dosage for eight weeks consecutively. Group IV animals received only oleuropein, 100 mg/kg. b.w. same as group III animals to investigate the effects of Olp only.

Animal Sacrifice, Liver Isolation, and Blood Sampling

After a total of 10 weeks of CCl_4 and oleuropein treatment, all animals were sacrificed after anesthesia by chloroform inhalation. Animals were then dissected, and blood was collected in plain tubes. The collected blood was then kept at room temperature for 30 minutes to allow it to clot. Once clotted, the blood was centrifuged at 3,000 rpm for 10 minutes to obtain the serum. This serum was then collected and divided into smaller aliquots, which were stored at -20°C for further analysis. The liver was cut from each animal at the time of sacrifice, washed in normal saline, and stored in 10% formalin for further analysis.

Measurement of Liver Function Enzyme (LFT) Activity

The activity of LFT, such as AST, ALT, and ALP in sera samples, was determined using an automatic biochemical analyzer as directed by the manufacturer. The activity of these liver function enzymes was determined using commercial kits (Abcam), and all the results were interpreted correspondingly.

Measurement of Lipid Profile

The level of lipids, like cholesterol and triglycerides, in sera of different animal groups was determined using an automatic biochemical analyzer as directed by the manufacturer.

Measurement of Antioxidant Enzyme Activity

At the time of sacrifice, the liver was quickly removed from each animal and washed in phosphate buffer saline (PBS) with a pH of 7.4. One portion of the liver from each animal was homogenized quickly in PBS, and the homogenate was centrifuged at 4,500 g for 20 minutes.

The supernatant from each homogenate sample was preserved at -20°C for subsequent analysis of antioxidant enzymes, following storage at 4°C. The activity of hepatic antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione (GSH) and total antioxidant capacity was checked using Abcam kits following the manufacturer's instructions.

Measurement of Inflammatory Markers

The measurement of CRP, IL-6, and TNF-α in serum/tissue samples was estimated using ELISA kits from Abcam, following the manufacturer's instructions.

Histopathological Analysis by Haematoxylin and Eosin Staining

The histopathological analysis of liver samples was performed by Hematoxylin and Eosin (H&E) Staining. The liver samples from each animal were extracted and processed. The samples were washed with phosphate buffer saline (PBS) and were then treated with 10% formalin till further processing. Different grades of ethanol were then used to dehydrate the samples, cleaned in xylene,

and embedded in paraffin by using an embedding station (Leica embedding unit, Leica EG1160). The block of all liver tissue samples was then processed for cutting 5 µM tissue sections using a microtome (Leica RM2125). The tissue sections were mounted on polylysine-coated glass slides. H&E staining was performed, and tissue architecture was examined under a light microscope.

Masson Trichrome Staining

This staining was performed as per the manufacturer's instruction provided with the kit with slight modification. Briefly, deparaffinising of tissue sections was performed, followed by adding preheated Bouin's fluid to each tissue section for 1 hr. It was followed by washing the slides in minimum flow running tap water till all the dye was removed satisfactorily. The slides were then stained with Wiegert's Iron hematoxylin for 5 minutes. To ensure accurate differentiation of tissue samples, we utilized a highly effective phosphomolybdic/phosphotungstic acid solution for 10-15 minutes, or until the collagen was visibly red. After the initial staining process, we applied an aniline blue solution to the slides using a dropper for 5-10 minutes, without any rinsing. This was followed by the use of a 1% acetic acid solution for 3-5 minutes to complete the staining process. Dehydration of liver sections was performed using ethyl alcohol, and xylene was used to clear slides, followed by mounting each slide.

Sirius Red Staining

Sirius red staining was performed as per the manufacturer's instruction provided with the kit with slight modification. Fibrosis was evaluated in different areas, and a photograph was captured.

Immunohistochemistry

Immunohistochemistry was done to examine the expression of IL-6 as per the previously described protocol[26]. In short, the liver tissue sections were deparaffinised by xylene, followed by the use of 0.3% H_2O_2 to block the endogenous peroxidase activity. Antigen retrieving was performed by boiling the slides in citrate butter under high pressure for a few minutes. The blocking step was performed by using a standard blocking agent. Monoclonal antibodies against IL-6 protein were added to each slide under humid conditions at 4°C overnight by using an acrylic closed chamber. For optimal results, we added a secondary antibody for 2 hours, followed by incubation with streptavidin-biotin enzyme complex for 45 minutes. We then utilized diaminobenzidine (DAB) chromogen to complete the staining process. Finally, we counterstained the sections with hematoxylin to enhance the visibility of the stained samples.

Statistical Analysis

To ensure accurate analysis of the data obtained, all results as mean ± standard deviation were evaluated. We then utilized a one-way analysis of variance (ANOVA) with a statistical package (SPSS) version 19, (IBM Corp., Armonk, NY, USA) to evaluate the difference among the mean values. The p-value, $p < 0.05$, was considered statistically significant.

Results

Effect of Oleuropein on Liver Function Enzymes

To evaluate the status of liver injury and the protective role of oleuropein, serum aminotransferases (ALT, AST) and ALP were estimated in all experimental animals. The CCl_4-treated animal group exhibited an enhanced level of ALT (180.5 ± 9 U/L), AST (190.6 ± 7 U/L), and ALP (203.7 ± 6 U/L) level in comparison to the control group ($p < 0.05$), due to hepatocyte cell membrane breakdown. However, treatment of animals with oleuropein significantly reduced the level of these aminotransferases and ALP, when compared with the CCl_4-treated (disease control) group ($p < 0.05$) (Figure 1). The animals treated with oleuropein alone showed no significant difference compared to the control group. These results advocate oleuropein's hepatoprotective role through maintaining cell membrane integrity.

Effect of Oleuropein on Lipid Profile

The lipid profile (total cholesterol and triglycerides) was estimated in all treatment groups. The results presented that serum cholesterol (196.37 ± 8.7 mg/dl), and triglycerides (239.7 ± 7.2 mg/dl) were significantly raised in CCl_4-intoxicated animals as compared to the control group ($p < 0.05$). The animals treated with oleuropein exhibited a significant decrease in these parameters as compared to the disease-control (CCl_4-treated) animals ($p < 0.05$) (Figure 2). The animals treated with oleuropein only showed no significant difference compared to the control group.

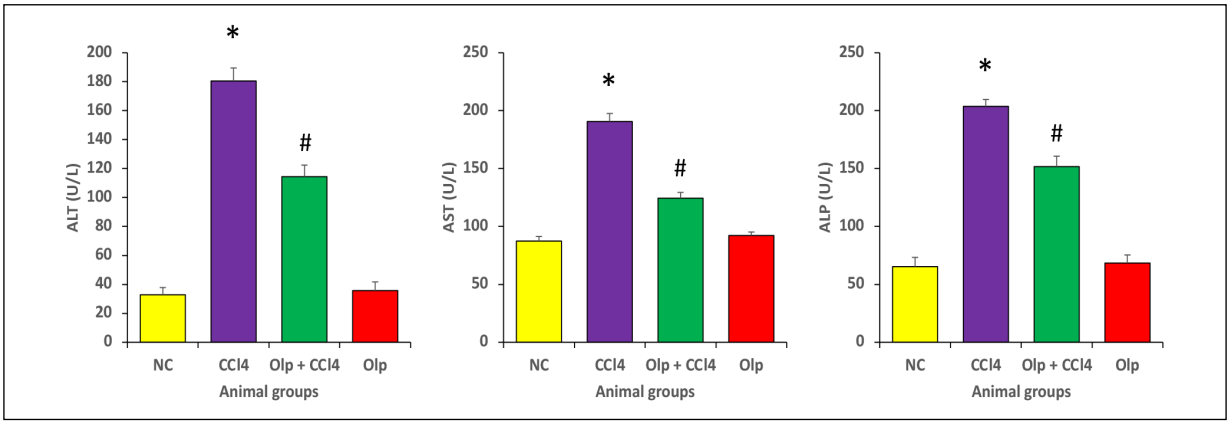

Figure 1. The liver function enzyme activity (ALT, AST, and ALP) in different animal groups, with 8 rats per group, was measured. The values are represented as the standard error of the mean (± SEM), ensuring accurate and reliable analysis of the results. Higher enzyme activity was observed in rats treated with CCl_4 only as compared to control group. The oleuropein treatment for CCl_4-intoxicated animals (Olp + CCl_4) showed a significant decrease in these liver function enzyme activities. The sign asterisk (*) ($p < 0.05$) represents the statistical difference in comparison to the control group, while the sign hashtag (#) ($p < 0.05$) represents the statistical difference in comparison to the CCl_4-treated group.

Effect of Oleuropein on Antioxidant Enzymes and Non-Enzymatic Antioxidant Potential

The antioxidant potential of oleuropein was analyzed by estimating the antioxidant enzymes and antioxidant potential in liver homogenates from different experimental animals. The antioxidant enzymes like SOD, GSH, GPx and TAC were estimated in all experimental groups (Figure 3). The results advocated that the animals treated with CCl_4 exhibited a significant decrease in these parameters as compared to the control group ($p < 0.05$) as (SOD: 42.5 *vs.* 77.9 U/mg protein; GPs: 32.4 *vs.* 67.8 U/mg protein; GSH: 21.9 *vs.* 45.9 mmol/g protein and TAC: 98.8 *vs.* 167.5 nM). However, the animals treated with oleuropein showed a significant enhancement in all these parameters as compared to the disease control group (CCl_4-intoxicated animals) ($p < 0.05$) as (SOD: 65.7 *vs.* 42.5 U/mg protein; GPs: 52.3 *vs.* 32.4 U/mg protein; GSH: 31.8 *vs.* 21.9 mmol/g protein and TAC: 115.7 *vs.* 98.8 nM). These re-

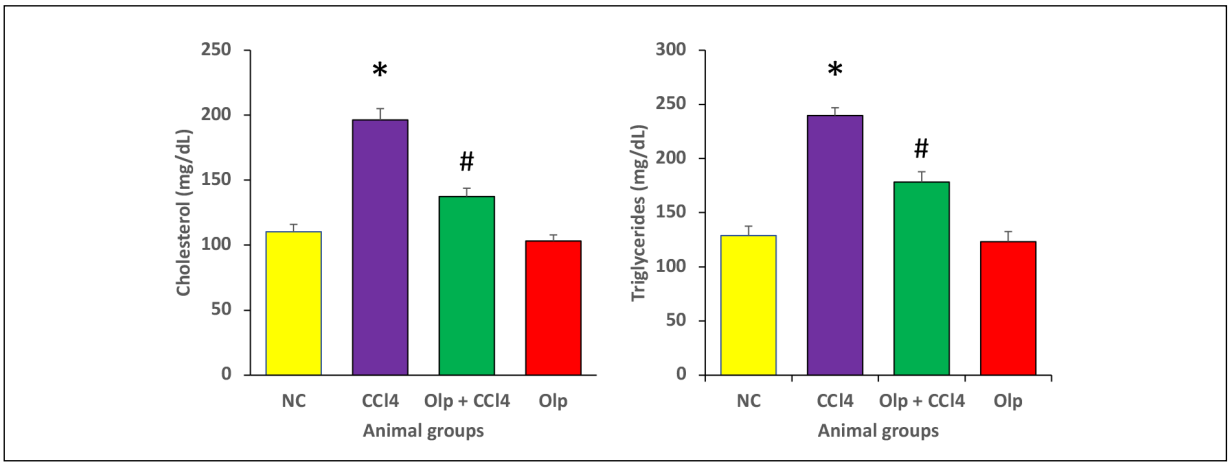

Figure 2. Lipid profile (cholesterol and triglycerides) in different animal groups. The values represent the standard error of the mean (± SEM), with 8 rats per group. A higher lipid profile was observed in rats treated with CCl_4 only as compared to the control group. The oleuropein treatment to CCl_4-intoxicated animals (Olp + CCl_4) showed a significant decrease in lipid profile. The sign asterisk (*) ($p < 0.05$) represents the statistical difference in comparison to the control group, while the sign hashtag (#) ($p < 0.05$) represents the statistical difference in comparison to the CCl_4-treated group.

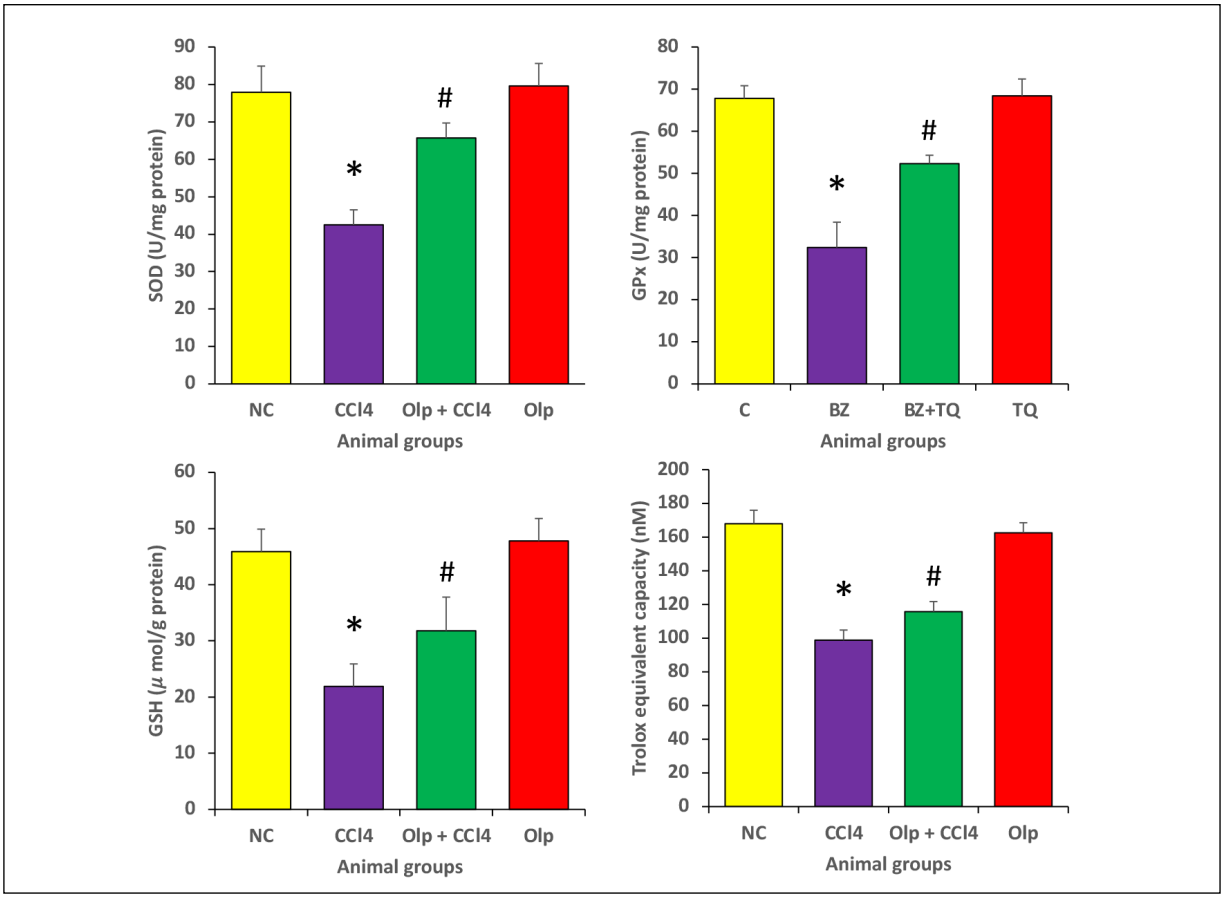

Figure 3. Antioxidant enzyme activity and non-enzymatic antioxidant status in different animal treatment groups. The values represent the standard error of mean (± SEM), with 8 rats per group. A significant decrease in antioxidant enzymes (SOD and GPx) and non-enzymatic antioxidant potential (GSH and TAC) was observed in rats treated with CCl_4 only as compared to control group. The oleuropein treatment to CCl_4-intoxicated animals (Olp + CCl_4) showed a significant increase in these parameters. To ensure accurate analysis of the data obtained, we used the sign asterisk (*) ($p < 0.05$) to represent the statistical difference in comparison to the control group, while the sign hashtag ($^{\#}$) ($p < 0.05$) was used to represent the statistical difference in comparison to the CCl_4-treated group.

sults evidently support that oleuropein exhibits significant hepatoprotective activity against CCl_4 intoxication by employing its antioxidant properties.

Effect of Oleuropein on Pro-Inflammatory Markers

An imbalance between antioxidant defense and oxidative stress is associated with the inflammatory response. The CCl_4-treated animals showed a significant increase in inflammatory markers like CRP (1.44 ± 0.07 ng/ml), IL-6 (95.6 ± 6 pg/ml), and TNF-α (107.5 ± 7 pg/ml) as compared to the control group ($p < 0.05$). However, the animals treated with oleuropein showed a significant decrease in all these pro-inflammatory markers as compared to the disease-control (CCl_4-treated) animals ($p < 0.05$). These results further support the anti-inflammatory and therapeutic role of oleuropein (Figure 4).

Effect of Oleuropein on Liver Tissue Architecture

The toxic potential of CCl_4 and the therapeutic role of oleuropein were further extended by studying liver histology in all animal groups (Figure 5). The liver sections from control animals showed a normal hepatocyte architecture with appropriate liver sinusoids. The liver sections from disease-control animals (CCl_4-treated animals) exhibited several changes, such as inflammation, congestion, and infiltration of lymphocytes. These sections further showed. However, the histopathological examination of the

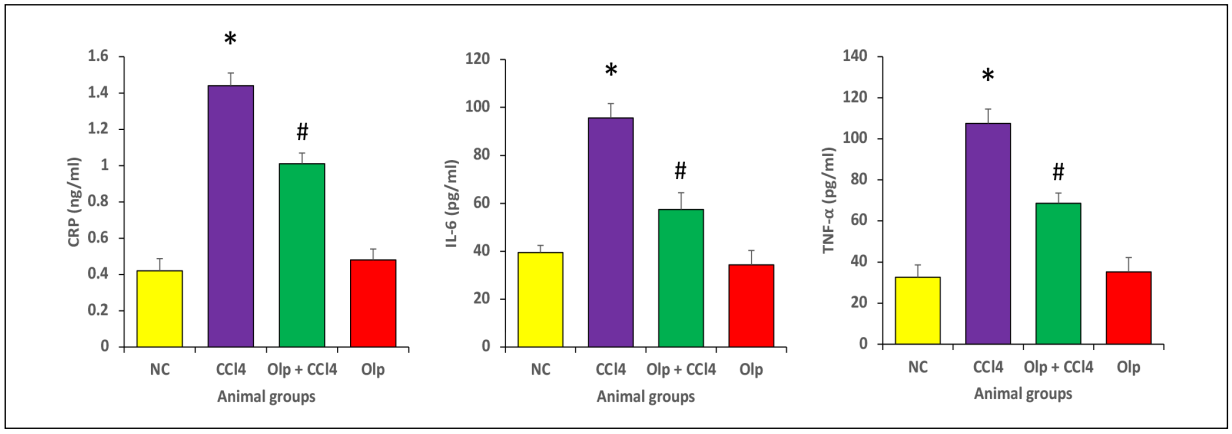

Figure 4. The pro-inflammatory markers (CRP, IL-6, and TNF-α) levels in different animal treatment groups. The values represent the standard error of the mean (± SEM), with 8 rats per group. A significant increase in these pro-inflammatory markers was observed in rats treated with CCl_4 only as compared to the control group. The oleuropein treatment for CCl_4-intoxicated animals (Olp + CCl_4) showed a significant decrease in these parameters. The sign asterisk (*) ($p < 0.05$) represents the statistical difference in comparison to the control group, while the sign hashtag ($^{\#}$) ($p < 0.05$) represents the statistical difference in comparison to the CCl_4-treated group.

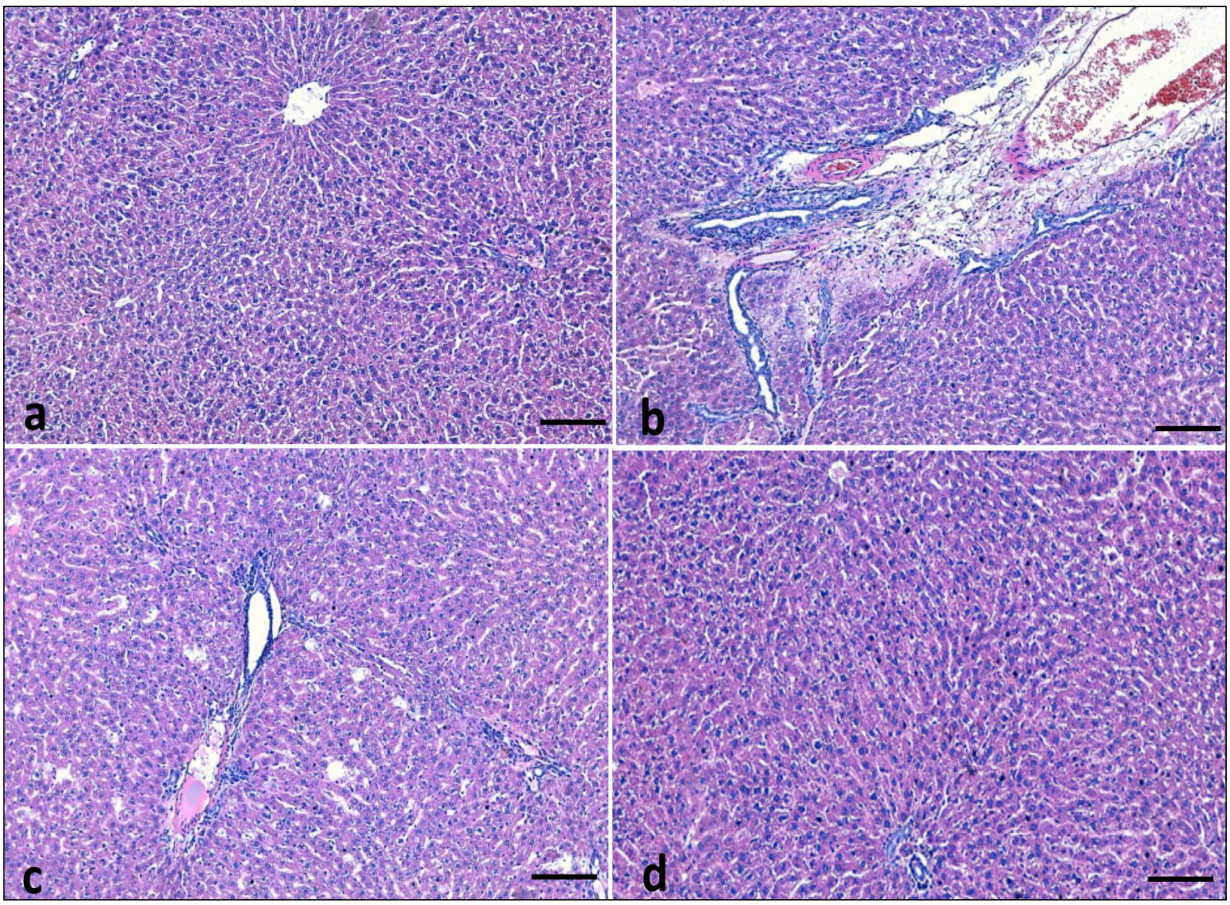

Figure 5. Liver tissue architecture, (**a**) normal tissue architecture of hepatocytes of control group animals, (**b**) CCl4-treated (disease control) rat liver tissue presents different alterations such as blood vessel dilation, congestion, and increased inflammatory cells, (**c**) CCl4 + Olp treatment group revealed a significant decrease in liver tissue architecture as compared to the disease control group, (**d**) Olp treatment only group animals showed normal tissue architecture (Original magnification 100×).

oleuropein treatment group exhibited preservation of hepatocytes, hemorrhage, and reduced infiltration of lymphocytes.

Effect of Oleuropein on Liver Fibrosis

The toxic potential of CCl_4 and the therapeutic role of oleuropein were further extended by studying liver histology in all animal groups (Figure 6). The liver sections from disease-control animals (CCl_4-treated animals) exhibited more fibrosis. However, the oleuropein treatment group exhibited less fibrosis by Masson trichrome staining (Figure 6).

Furthermore, Sirius red staining established that the fibrosis levels were higher in CCl_4-treated liver tissue rats, whereas Olp administration group rats showed less fibrosis formation (Figure 7). These findings reveal that CCl_4-treated rat liver tissue causes accumulation of fibrosis, and oleuropein showed a protective role against liver injury damage and fibrosis formation caused by CCl_4 treatment.

Effect of Oleuropein on IL-6 Protein Expression

The effect of Olp on IL-6 protein expression was used to assess the anti-inflammatory protective effect on the expression of any inflammatory markers in hepatocytes. Here, the expression of IL-6 was checked in liver tissues of all the animal groups (Figure 8). Our analysis revealed that the expression of this protein was not observed in the control group animals, while the disease control animals showed a significant increase ($p < 0.05$) in

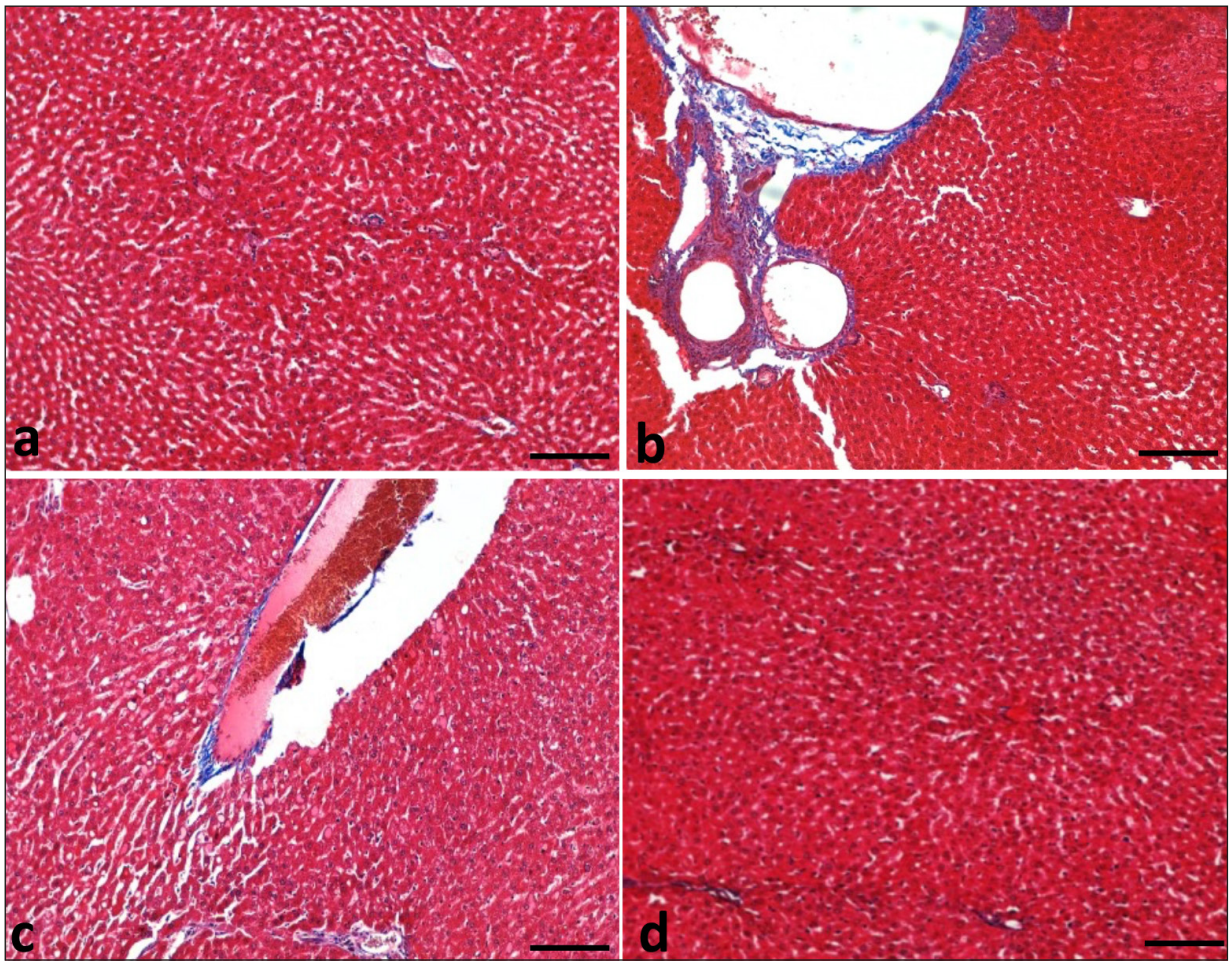

Figure 6. Masson trichrome staining for fibrosis analysis. **a**, The control animal liver tissues presented normal architecture of hepatocytes, (**b**) CCl_4 only (disease control) animal liver tissues presented significant fiber formation, (**c**) Group III animal (CCl_4+Olp) liver tissue architecture presented significantly lower collagen fiber formation, and (**d**) Olp treatment animal liver tissue presented normal hepatocyte and minimal fibers (Original magnification 100×).

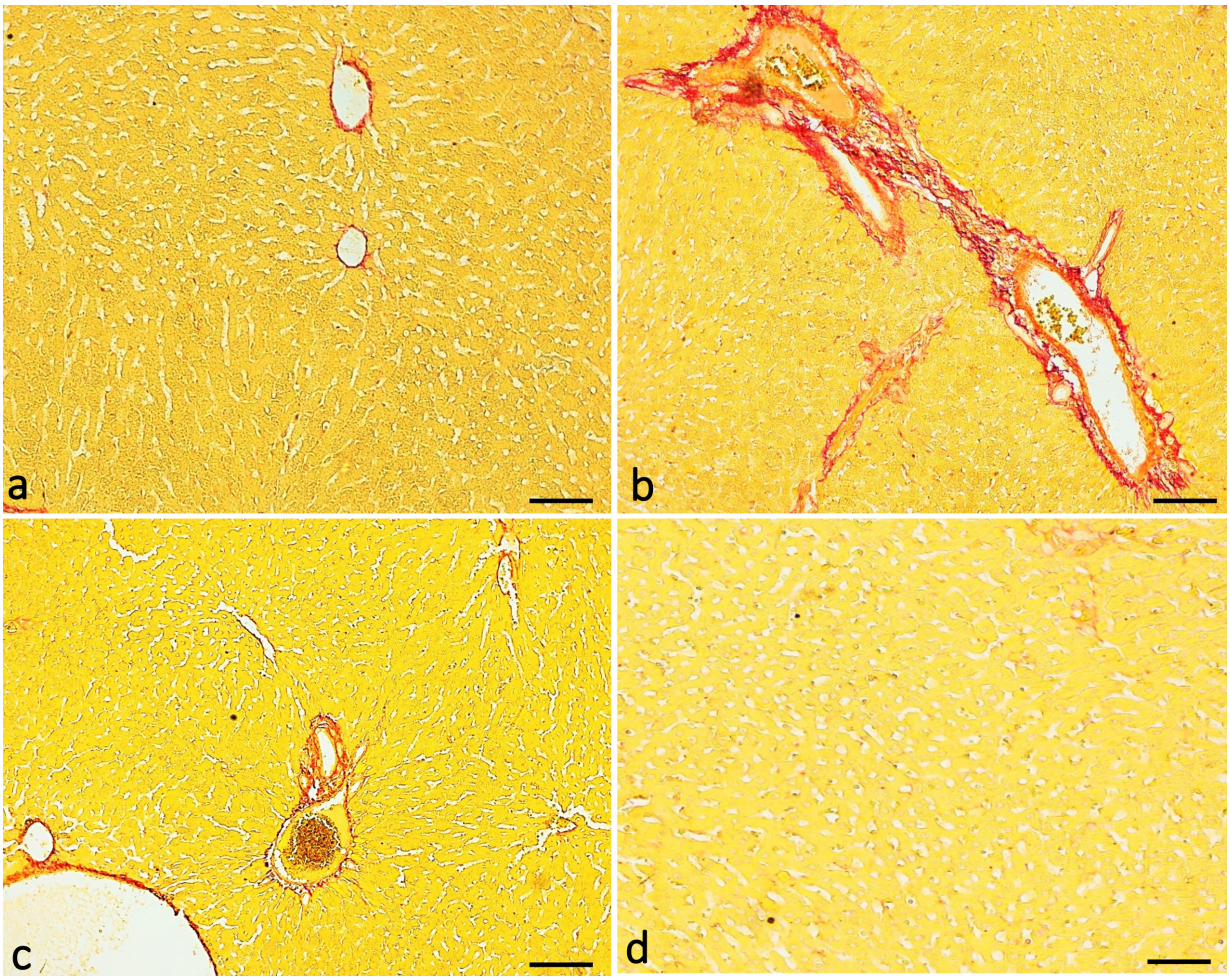

Figure 7. Siris red staining for fibrosis analysis. **a**, The control animal liver tissues presented normal fiber, (**b**) CCl_4 only (disease control) animal liver tissues presented significant fiber formation, (**c**) Group III animal (CCl_4+Olp) liver tissue architecture presented significantly lower collagen fiber formation, and (**d**) Olp treatment animal liver tissue presented normal hepatocyte and minimal fibers (Original magnification 100×).

expression of IL-6. Our detailed analysis revealed that treatment with Olp to CCl_4-treated animals led to a significant reduction in the expression of this inflammatory marker protein as compared to the disease control group ($p < 0.05$). Moreover, Olp treatment only does not have any effect on inflammatory marker protein expression.

Discussion

The liver is the major organ performing essential biological functions such as bile acid secretion, synthesis of different proteins and blood clotting factors, destruction of bacteria, and detoxification of various xenobiotics. Different drugs, microbes, and toxins can trigger liver injury, including acute and chronic[25,26]. Different types of toxic compounds lead to oxidative stress and inflammation, which is the prime cause of liver damage[27,28]. In this research work, we demonstrated that oleuropein significantly suppressed oxidative liver damage in CCl_4-intoxicated rats, as evidenced by an increase in antioxidant potential and a decrease in inflammation. The liver oxidative stress and inflammation were also significantly reduced in the presence of oleuropein. Giner et al[29] have demonstrated that the NF-κB pathway is significantly inhibited by oleuropein. NF-κB pathway leads to the induction of ROS, which modulates liver injury by enhancing the cytotoxicity through the production of different cytokines, such as TNF-α[25]. It was noticed that these results are in agreement with previous findings[30]. It was shown that the hepatoprotective effect of oleuropein-rich extract

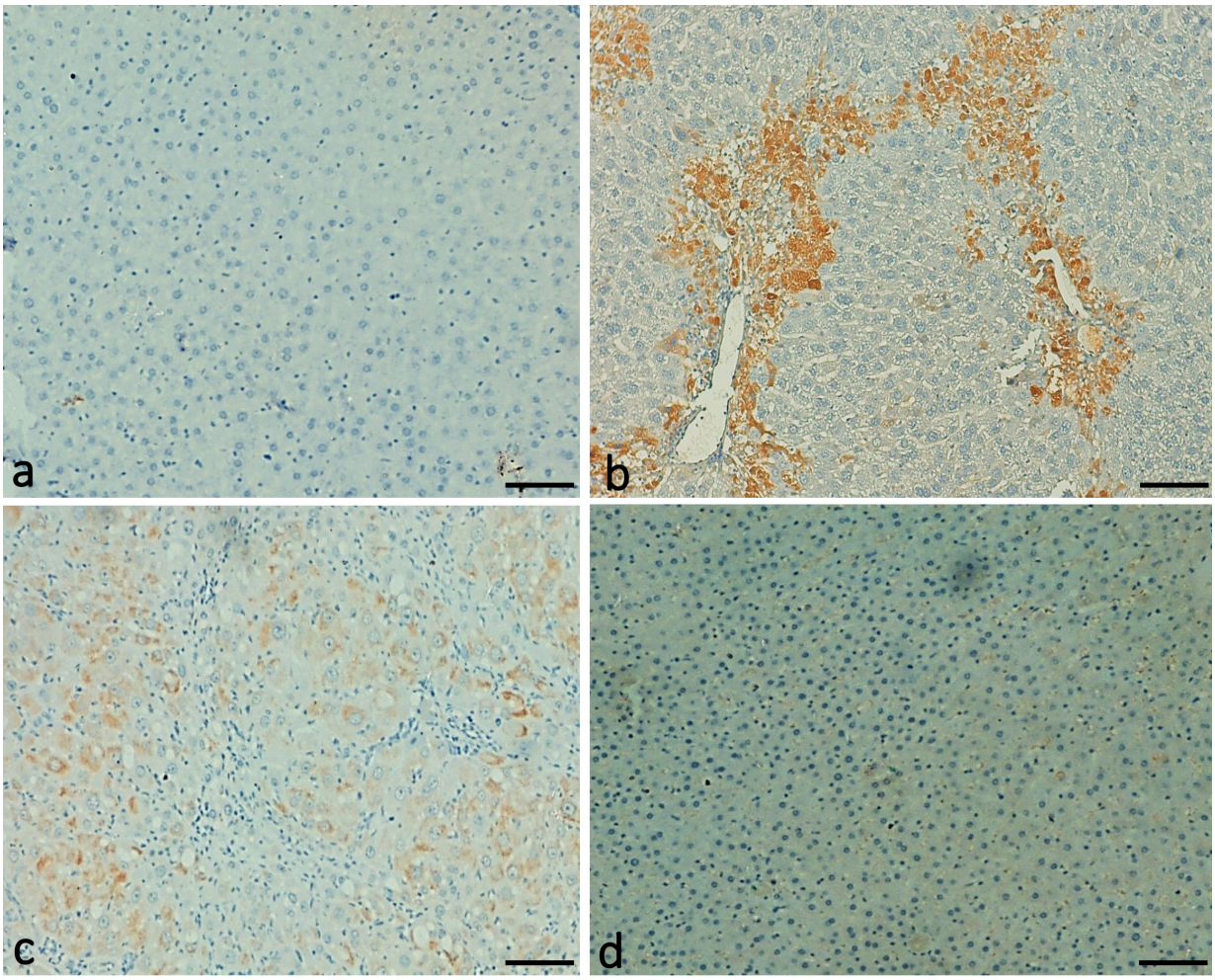

Figure 8. Immunohistochemistry analysis of IL-6 protein expression. IL-6 protein expression was evaluated in all animal treatment groups. **a**, The control animal group did not show any IL-6 protein expression, (**b**) the disease control (CCl_4-treated) animals showed significantly high expression of IL-6 proteins, (**c**) the IL-6 protein expression was also seen in Olp-treated CCl_4-intoxicated animal group tissue, and (**d**) Olp only treatment animal group did not show any IL-6 protein expression (Original magnification 100×).

from olive leaves against cadmium-induced toxicity in mice leads to an improvement in the antioxidant defense system and a reduction in lipid peroxidation. Moreover, oleuropein-rich extract in Cd-treated mice restored these values with a more pronounced effect on the SOD activity[30]. These results are in agreement with our findings, as we have demonstrated that CCl_4-intoxicated rats treated with oleuropein maintained their cell membrane integrity with a significant decrease in liver function enzymes such as ALT, AST, and ALP. The cell membrane integrity is preserved by reducing the level of lipid peroxides[31]. It is worth noting that oleuropein has been shown to protect against liver damage induced by high-fat diet-induced metabolic disorders in rats[32].

In our study, we demonstrated that CCl_4-treated rats demonstrated a significant decrease in some antioxidant enzymes like SOD, GPx, and GSH, and these parameters were significantly reversed by the treatment of oleuropein. Similar results were demonstrated with a reduction in oxidative stress and a significant decrease in catalase and GSH levels. However, the antioxidant potential of dry olive leaf extract, significantly rich in oleuropein, when pre-treated to CCl_4-intoxicated rates, demonstrated a higher level of antioxidant enzymes[33-35].

Besides, in CCl_4-treated rats, total antioxidant capacity was significantly low as compared to the control group, whereas oleuropein group rats showed an increased level of total antioxidant

capacity. The current findings are consistent with a previous study[36] that observed the effects of cadmium on mice; the total antioxidant capacity values displayed a noteworthy decrease in liver tissue as compared to controls. On the other hand, oleuropein given in cadmium-treated mice caused a significant positive effect on the hepatic antioxidant potential.

In the current study, it was shown that CCl_4-treated rats demonstrated a significant increase in inflammatory markers, and these parameters were significantly decreased by the treatment of oleuropein. In this context, in the literature it is reported that lipopolysaccharide stimulated a noteworthy release of pro-inflammatory cytokines. Oleuropein pretreatment diminished these cytokines levels and these things increased with increasing oleuropein dose[37]. A recent report[38] based on flavonoids reported that this compound shows anti-inflammatory effects.

Chronic administration of CCl_4 causes liver fibrosis, cirrhosis as well as hepatocellular carcinoma[38]. Reactive metabolites were recognized to participate in CCl_4-induced liver toxicity *via* adduct formation, lipid peroxidation, calcium homeostasis loss, and finally, cell death[39,40]. To examine the possible liver tissue architecture maintenance ability of oleuropein, it was demonstrated that the CCL_4-induced hepatotoxicity group depicted various changes in the liver tissues compared to the control group. The oleuropein-treated group showed a role in the maintenance of liver tissue architecture, such as less inflammation, congestion, hemorrhages, and fibrosis. Another study[37] also reported that oleuropein pretreatment ameliorated LPS-induced liver histological changes.

Furthermore, another discovery aligned with the present finding, indicating that mice treated with CCl_4 exhibited significant necrosis of hepatocytes in the centrilobular region. The livers of CCl_4-intoxicated mice getting oleuropein treatment showed improvement in liver morphology[23].

Our study showed that the expression of IL-6 was high in CCl_4-intoxicated rats, whereas the expression of this marker was restored in Olp-treated rats. This suggests that Olp treatment may have a protective effect against the inflammatory response induced by CCl_4 intoxication. This finding is similar to the previous study based on another natural compound, as intraperitoneal injection of CCl_4 significantly increased the IL-6 expression in the inflamed liver parts. However, treatment with *Jasminum grandiflorum* extracts inhibited the CCl_4-induced up-regulation of IL-6 as compared with the model group[41]. A similar study[28] based on curcumin supplementation showed suppression of CCl_4-induced IL-6 production *via* the prevention of pro-inflammatory cytokine secretion.

Conclusions

The present study demonstrates that oleuropein possesses significant hepato-protective potential, as evidenced by the reduction of inflammatory marker proteins, lipid profile, and liver function enzymes. This study also revealed that Olp possesses a high antioxidant potential and can overshadow the oxidative stress induced by the CCl_4 intoxication. Furthermore, Olp treatment restores the liver tissue architecture, reduces fibrosis, and decreases histopathological changes that occur by induction of CCl_4. This study supports the consumption of olives, fruits, and oils as food additives that can reduce liver pathogenesis *via* the regulation of inflammation, oxidative stress, and maintenance of liver tissue architecture. However, further research needs to be done through *in vivo* and *in vitro* studies to explore thoroughly the molecular mechanism and protective role of oleuropein.

Conflict of Interest

The authors declare no conflicts of interest.

Acknowledgements

Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2024R39), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

Authors' Contribution

Conceptualization, A.H.R., and A.A.A; methodology, A.H.R., A.Y.B., and A.A.A; formal analysis, W.A.A., M.H.A.; writing-original draft preparation, A.H.R., and A.A.A; writing-review and editing, A.A.; K.S.A, H.O.A.A, W.A.A.; supervision; funding acquisition, W.A.A., M.H.A, S.A.A. All authors have read and agreed to the published version of the manuscript.

Data Availability

All data have been included within the manuscript.

Ethics Approval

The animals were maintained at the animal facility of the College of Applied Medical Sciences (CAMS) by the guide-

lines of the Qassim University on Animal Care. The animal experiments were carried out as per the guidelines of CAMS, Qassim University, and approved by the Institutional Animal Ethics Committee (10194-Cams1-2020-1-3-I), CAMS, Qassim University.

Funding
This research was sponsored by Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

Informed Consent
Not applicable.

ORCID ID
Arshad Husain Rahmani: 0000-0002-1329-1391
Ahmad Almatroudi: 0000-0002-1491-6402
Khaled Allemailem: 0000-0002-6486-9835
Hajid O.A. Alharbi: 0000-0003-4566-2932
Ali Yousif Babiker: 0000-0001-5960-3381
Sami Althwab: 0000-0001-8695-4961
Naif Alsuhaymi: 0000-0002-6011-745X
Mahdi Alsugoor: 0000-0003-2106-8541
Amjad Ali Khan: 0000-0001-6030-4493
Wafa Al-megrin: 0000-0001-8011-8153

References

1) Tittarelli R, Pellegrini M, Scarpellini MG, Marinelli E, Bruti V, DI LUCA NM, Busardò FP, Zaami S. Hepatotoxicity of paracetamol and related fatalities. Europ Rev Med Pharmacol Sci 2017; 21: 95-101.
2) Scafato E, Caputo F, Patussi V, Balbinot P, Addolorato G, Testino G. The undertreatment of alcohol-related liver diseases among people with alcohol use disorder. Europ Rev Med Pharmacol Sci 2020; 24: 974-982.
3) Brattin WJ, Glende Jr EA, Recknagel RO. Pathological mechanisms in carbon tetrachloride hepatotoxicity. J Free Radic Biol Med 1985; 1: 27-38.
4) Lewis Jr RJ. Hawley's condensed chemical dictionary. 12th ed. New York: Van Nostrand Reinhold 1993; 221-222.
5) Kauppinen T, Toikkanen J, Pedersen D, Young R, Ahrens W, Boffetta P, Hansen J, Kromhout H, Blasco JM, Mirabelli D, de la Orden-Rivera V. Occupational exposure to carcinogens in the European Union. Occup Environ Med 2000; 57: 10-18.
6) Cheeseman KH, Albano EF, Tomasi A, Slater TF. Biochemical studies on the metabolic activation of halogenated alkanes. Environ Health Perspect 1985; 64: 85-101.
7) Miyazaki T, Karube M, Matsuzaki Y, Ikegami T, Doy M, Tanaka N, Bouscarel B. Taurine inhibits oxidative damage and prevents fibrosis in carbon tetrachloride-induced hepatic fibrosis. J Hepatol 2005; 43: 117-125.
8) Hassan MH, Edfawy M, Mansour A, Hamed AA. Antioxidant and antiapoptotic effects of capsaicin against carbon tetrachloride-induced hepatotoxicity in rats. Toxicol Ind Health 2012; 28: 428-438.
9) Huang HL, Wang YJ, Zhang QY, Liu B, Wang FY, Li JJ, Zhu RZ. Hepatoprotective effects of baicalein against CCl_4-induced acute liver injury in mice. World J Gastroenterol 2012; 18: 6605-6613.
10) Chen X, Meng Q, Wang C, Liu Q, Sun H, Huo X, Sun P, Yang X, Peng J, Liu K. Protective effects of calycosin against CCl_4-induced liver injury with activation of FXR and STAT3 in mice. Pharm Res 2015; 32: 538-548.
11) Oqal M, Qnais E, Alqudah A, Gammoh O. Analgesic effect of the flavonoid herbacetin in nociception animal models. Europ Rev Med Pharmacol Sci 2023; 27: 11236-11248.
12) Alsahli MA, Almatroodi SA, Almatroudi A, Khan AA, Anwar S, Almutary AG, Alrumaihi F, Rahmani AH. 6-Gingerol, a Major Ingredient of Ginger Attenuates Diethylnitrosamine-Induced Liver Injury in Rats through the Modulation of Oxidative Stress and Anti-Inflammatory Activity. Mediat Inflamm 2021; 19: 6661937.
13) Anwar S, Almatroudi A, Allemailem KS, Jacob Joseph R, Khan AA, Rahmani AH. Protective effects of ginger extract against glycation and oxidative stress-induced health complications: An in vitro study. Processes 2020; 8: 468.
14) Rahmani AH, Aldebasi YH. Ficus carica and its constituents role in management of diseases. Asian J Pharm Clin Res 2017; 10: 49-53.
15) Abd El-Rahman, H. The effect of olive leaf extract and A-Tocopherol on nephroprotective activity in rats. J Nutr Food Sci 2016; 6: 479.
16) Amiot MJ, Fleuriet A, Macheix JJ. Importance and evolution of phenolic compounds in olive during growth and maturation. J Agric Food Chem 1986; 34: 823-826.
17) Le Tutour B, Guedon D. Antioxidative activities of Olea europaea leaves and related phenolic compounds. Phytochem 1992; 31: 1173-1178.
18) Bravo L. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutr Rev 1998; 56: 317-333.
19) Barbaro B, Toietta G, Maggio R, Arciello M, Tarocchi M, Galli A, Balsano C. Effects of the olive-derived polyphenol oleuropein on human health. Int J Mol Sci 2014; 15: 18508-18524.
20) Omar SH. Oleuropein in olive and its pharmacological effects. Sci Pharm 2010; 78: 133-154.
21) Visioli F, Galli C. Oleuropein protects low-density lipoprotein from oxidation. Life Sci 1994; 55: 1965-1971.
22) Quiñones M, Miguel M, Aleixandre A. Beneficial effects of polyphenols on cardiovascular disease. Pharmacol Res 2013; 68: 125-131.

23) Domitrović R, Jakovac H, Marchesi VV, Šain I, Romić Ž, Rahelić D. Preventive and therapeutic effects of oleuropein against carbon tetrachloride-induced liver damage in mice. Pharmacol Res 2012; 65: 451-464.

24) Hassan ZK, Elamin MH, Omer SA, Daghestani MH, Al-Olayan ES, Elobeid MA, Virk P. Oleuropein induces apoptosis via the p53 pathway in breast cancer cells. Asian Pac J Cancer Prev 2013; 14: 6739-6742.

25) Al-Seeni MN, El Rabey HA, Zamzami MA, Alnefayee AM. The hepatoprotective activity of olive oil and Nigella sativa oil against CCl 4 induced hepatotoxicity in male rats. BMC Complement Alt Med 2016; 16: 1-4.

26) Almatroodi SA, Alnuqaydan AM, Babiker AY, Almogbel MA, Khan AA, Husain Rahmani A. 6-Gingerol, a bioactive compound of ginger attenuates renal damage in streptozotocin-induced diabetic rats by regulating the oxidative stress and inflammation. Pharmaceutics 2021; 13: 317.

27) Khan AQ, Nafees S, Sultana S. Perillyl alcohol protects against ethanol induced acute liver injury in Wistar rats by inhibiting oxidative stress, NF-B activation and proinflammatory cytokine production. Toxicol 2011; 279: 108-114.

28) Reyes-Gordillo K, Segovia J, Shibayama M, Vergara P, Moreno MG, Muriel P. Curcumin protects against acute liver damage in the rat by inhibiting NF- B, proinflammatory cytokines production and oxidative stress. Biochim Biophys Acta 2007; 1770: 989-996.

29) Giner E, Andujar I, Recio MC, Rios JL, Cerda-Nicolas JM, Giner RM. Oleuropein ameliorates acute colitis in mice. J Agric Food Chem 2011; 59: 12882-12892.

30) Luedde T, Schwabe RF. NF-kB in the liver-linking injury, fibrosis and hepatocellular carcinoma. Nat Rev Gastroenterol Hepatol 2011; 8: 108-118.

31) Jemai H, Mahmoudi A, Feryeni A, Fki I, Boullagui Z, Choura S, Chamkha M, Sayadi S. Hepatoprotective effect of oleuropein-rich extract from olive leaves against cadmium-induced toxicity in mice. BioMed Res Int 2020; 2020: 4398924.

32) Alirezaei M, Dezfoulian O, Kheradmand A, Neamati S, Khonsari A, Pirzadeh A. Hepatoprotective effects of purified oleuropein from olive leaf extract against ethanol-induced damages in the rat. Iran J Vet Res 2012; 13: 218-226.

33) Fki I, Sayadi S, Mahmoudi A, Daoued I, Marrekchi R, Ghorbel H. Comparative study on beneficial effects of hydroxytyrosol-and oleuropein-rich olive leaf extracts on high-fat diet-induced lipid metabolism disturbance and liver injury in rats. BioMed Res Int 2020; 2020: 1-15.

34) Vidičević S, Tošić J, Stanojević Ž, Isaković A, Mitić D, Ristić D, Dekanski D. Standardized Olea europaea L. leaf extract exhibits protective activity in carbon tetrachloride-induced acute liver injury in rats: The insight into potential mechanisms. Arch Physiol Biochem 2020; 126: 399-407.

35) Elgebaly HA, Mosa NM, Allach M, El-Massry KF, El-Ghorab AH, Al Hroob AM, Mahmoud AM. Olive oil and leaf extract prevent fluoxetine-induced hepatotoxicity by attenuating oxidative stress, inflammation and apoptosis. Biomed Pharmacother 2018; 98: 446-453.

36) Slama K, Boumendjel M, Taibi F, Boumendjel A, Messarah M. Atriplex halimus aqueous extract abrogates carbon tetrachloride-induced hepatotoxicity by modulating biochemical and histological changes in rats. Arch Physiol Biochem 2020; 126: 49-60.

37) Alsharif KF, Almalki AA, Al-Amer O, Mufti AH, Theyab A, Lokman MS, Ramadan SS, Almeer RS, Hafez MM, Kassab RB, Abdel Moneim AE. Oleuropein protects against lipopolysaccharide-induced sepsis and alleviates inflammatory responses in mice. IUBMB life 2020; 72: 2121-2132.

38) Qnais E, Alqudah A, Bseiso Y, Gammoh O, Wedyan M, Alotaibi B. Exploring the therapeutic potential of fisetin: a comprehensive study on its anti-nociceptive and anti-inflammatory effects. Europ Rev Med Pharmacol Sci 2023; 27: 10773-10784.

39) Qin Y, Tian YP. Protective effects of total glucosides of paeony and the underlying mechanisms in carbon tetrachloride-induced experimental liver injury. Arch Med Sci 2011; 7: 604-612.

40) Boll M, Lutz WD, Becker E, Stampfl A. Mechanism of carbon tetrachloride-induced hepatotoxicity. Hepatocellular damage by reactive carbon tetrachloride metabolites. Z Naturforsch C 2001; 56: 649-659.

41) Sun L, Zhang Y, Wen S, Li Q, Chen R, Lai X, Zhang Z, Zhou Z, Xie Y, Zheng X, Zhang K. Extract of Jasminum grandiflorum L. alleviates CCl_4-induced liver injury by decreasing inflammation, oxidative stress and hepatic CYP2E1 expression in mice. Biomed Pharmacother 2022; 152: 113255.