Antioxidant, anti-inflammatory and hepatoprotective effects of olive fruit pulp extract: in vivo and in vitro study

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ABSTRACT
The current study aimed to investigate the antioxidant, anti-inflammatory and hepatoprotective activity of olive fruit pulp extract (OFPE) through in vivo- and in vitro-based studies. Oral administration of OFPE demonstrated a significant reduction in liver biomarker enzymes, inflammatory marker (IL-6, TNF-α, and C-reactive protein (CRP)) level and recovered the antioxidant enzymes and total antioxidant capacity levels when compared to CCl4 treated group. The histopathological alterations were seen in CCl4 treated mice. The hepatocyte architecture was maintained in OFPE treated group as compared to the CCl4 treated group. The anti-inflammatory activity of the OFPE was evaluated through in vitro and was found to be similar with a reference drug, ibuprofen. Current findings revealed that OFPE displays hepatoprotective effect through enhancement of antioxidant enzyme level, reduction of anti-inflammatory and maintenance of hepatocyte architecture. Thus, olive fruit extract might be utilized as a therapeutic alternative in the treatment of hepatic complications.

1. Introduction
The liver is one of the most significant organs which assumes a fundamental job in metabolic homeostasis [1] and hence the principle place where numerous toxicants cause intense injury [2]. Various types of medications have been accounted for to be engaged in liver-related pathogenesis and damage to the architecture of hepatocytes. Medications and chemicals instigate lipid peroxidation and other oxidative injuries resulting in liver mutilation. Liver mutilation is regularly associated with alteration of a few capacities that cause extreme lethal ailments. Present yet maybe unrecognized, overall evaluations show that 844 million individuals have chronic liver diseases, with a death pace of 2 million passings per year [3]. Moreover, cirrhosis, the final product for chronic liver maladies [4] and passing by cirrhosis is supposed to significantly increase by 2030 [5]. According to the Centre for Disease Control and Prevention report, chronic liver mutilation and cirrhosis were the 12th chief reason for death in the USA, accounting for more than 36,000 demises [6], and Europe has the greatest load of liver disorders in the world [7]. The liver cirrhosis is the end-stage of several chronic liver diseases and account for more than one million deaths every year world-wide. It is also one of the key risk factors of hepato-cellular carcinoma [7–8]. Moreover, the high prevalence of obesity and diabetes are the main causes of prevalence of non-alcoholic fatty liver diseases (NAFLD) [8]. It is noteworthy that non-alcoholic fatty liver disorders include a wide range of fatty liver changes from simple steatosis to non-alcoholic steatohepatitis and cirrhosis [9]. As per Saudi Arabia record, its prevalence has been stated to be around 7–10% [9–11].

Carbon tetrachloride (CCl4) is ordinarily utilized as a solvent for diverse polarity lacking compounds and has been reported to possess hepatotoxic property. Therefore, CCl4 has been widely used as a hepatotoxin to evaluate the potential of drugs and plant extracts to prevent or cure liver damage for long times [12]. CCl4 toxicity occurs mostly because of the production of several sorts of reactive oxygen species (ROS). ROS have the potential to bind with polyunsaturated fatty acids (PUFAs), to form aloxy and peroxyl radicals, to damage the cell membrane and to change the activity of enzymes [13]. Henceforth, CCl4 possesses a strong ability to trigger liver injury [14], and oxidative stress is among the most important mechanisms engaged with parleying the procedure of liver fibrosis [15]. In addition, exposure of CCl4 causes changes such as centrilobular steatosis, inflammation, apoptosis and necrosis [16–18]. Fibrosis and cirrhosis are known to be triggered when damage surpasses the repairing limit of the liver [16,17].

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Treatment based on allopathic drugs has been found to be very effective but have been reported to possess severe adverse effects. The medicinal plants or its active compound are rich sources of antioxidants and such antioxidant property of herbs may be involved in the management of diseases including liver diseases [19]. In this regard, olive fruits as a whole and its oil have been confirmed to have a beneficial role in curing disease through its anti-inflammatory, antioxidant and other therapeutic implications including hepatic dysfunctions. Olive tree (Olea europea L.) is an agriculturally important plant belonging to family Oleaceae. The plant is cultivated in all the countries of Mediterranean countries because of its palatable fruits and oil [20].

Different kinds of bioactive molecules like monounsaturated fatty acids and phenolic compounds such as phenolic acids, phenolic alcohols, and flavonoids have been reported to be found in olives [21]. The high concentration of these monounsaturated fatty acids including oleic acid, phenolic compounds like hydroxytyrosol (HT) and oleuropein activate various signalling pathways in the hepatocytes. Activation of these signalling pathways results in the prevention of inflammation, oxidative stress, endoplasmic reticulum stress, mitochondrial dysfunction, and insulin resistance leading to prevention or resolution of liver damage [22].

Olive fruits and oil are a blend of diverse phenolic compounds including oleuropein, oleocanthal, oil, sugar, cellulose, protein, vitamins and minerals. Past investigation result proves that a combination of olive vegetable water and glucosamine can be a potential solution for various inflammatory conditions, like rheumatoid and osteoarthritis [23]. Free radical scavenging properties of HT, which are abundant in olives, have been convincingly verified in studies on rats with alloxan-prompted diabetes mellitus [24]. The aims of this study included to investigate whether olive fruit pulp could forestall liver injury actuated by CCl4 in mice by means of assessing the antioxidant status, histopathology, and the expressional pattern of tumour suppressor protein and to explore its antioxidant and anti-inflammatory potential through in vitro studies.

2. Materials and methods

2.1. Chemicals

Both analytical and purified grade chemicals have been acquired from Sigma–Aldrich Company (St. Louis, MO, USA). Antioxidant, inflammatory kits and antibody used in this study were purchased from Abcam Company, Cambridge, UK.

2.2. Olive fruits pulp extract

Green olive fruit (Olea europea L.) was bought from the neighbourhood advertise in Qassim, Saudi Arabia. The fruit pulp extract was prepared with little alteration as depicted in the previous work [25]. Briefly, 400 g of the olive fruit was ground in a blender and defatted thrice with three volumes of 70% ethanol. Absolute ethanol was used to extract the fruit pulp at 1:10 ratio (w/v) for 2 h at 80°C using a heating mantle. The supernatant was filtered by Whatman filter paper no. 1 (Whatman Ltd., Maidstone, UK). The filtrate was concentrated at 50°C under reduced pressure on the rotary evaporator and further, it was diluted in water:EtOH mixture (9:1, v/v). The final extract of ethanol was stored in closed and dark containers at 4°C until the time of the experiment.

It has been reported that along with other flavonoids, oleuropein is a major component of ethanolic extracts of olives. However, hydroxytyrosol glucoside and phenolic acids become more prevalent in water extracts [26].

2.3. In vitro study

2.3.1. Evaluation of antioxidant activity of olive extract by 1,1-diphenyl-2-picryl-hydrazyl assay

The free radical scavenging ability of olive fruit pulp extract (OFPE) was assessed by utilizing 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay. In short, a series of different concentrations (600–50 µg/ml) was prepared by sequentially diluting stock solution (10 mg/ml) of the olive pulp extract in water. Afterwards, 1 ml of 0.3 mM DPPH working solution prepared in absolute methanol was applied in duplicates to 2.5 ml of various aqueous dilutions of the extract. The mixtures were incubated for half-an-hour in the dark at room temperature. Then, the absorbance of incubation mixtures was estimated at 517 nm with a spectrophotometer against absolute methanol. To prepare the control, the extract was replaced with methanol. All the experiments were run in triplicates. The percentage of free radical scavenging activity was calculated in line with the accompanying equation.

\[
\% \text{ of free radical scavenging activity} = \left( \frac{(AOC - AOS)}{AOC} \right) \times 100, 
\]

where AOC is the absorbance of control and AOS is the absorbance in the presence of extract.

2.3.2. Evaluation of in vitro anti-inflammatory activity by protein denaturation inhibition

The in vitro anti-denaturation of bovine serum albumin (BSA) by natural products can be considered to be a screening method for the development of new drug. It is an assay which does not involve living animal model in the drug developmental process. Grant et al. have reported that non-steroidal anti-inflammatory drugs like ibuprofen have the ability to prevent heat-induced denaturation of BSA at pathological pH [pH 6.2–6.5] [27]. Therefore, albumin denaturation inhibition was
utilized to assess in vitro anti-inflammatory action of olive fruit extract [28]. One per cent aqueous solution of BSA was prepared and a small amount of 1 N HCl was adjusted to the pH of the reaction mixture. Five hundred microlitres of BSA (1%) was added independently to 100 µl of different concentrations (50, 100–600 µg/ml) of olive fruit extract and also to 100 and 200 µg/ml of ibuprofen. The mixtures were allowed to remain at 37°C for 20 min in water bath, after that the temperature of reaction mixtures was increased to 51°C at which reaction mixtures were maintained for 20 min. The tests were cooled and turbidity was evaluated at 660 nm. Ibuprofen was used as a reference drug and was crushed into fine powder. About 200 mg of this powder was weighed out utilizing a digital analytical balance and we mixed this powder into 20.0 ml of double distilled water. The solutions had been mixed well utilizing a vortex. A serial dilution of ibuprofen was carried out for required concentration in the experiment. The samples were run in triplicates. The control contained an aqueous solution of BSA only. The percentage inhibition of protein denaturation was calculated utilizing the subsequent equation:

\[
\text{Percentage inhibition of protein denaturation} = \left( \frac{\text{AOC} - \text{AOS}}{\text{AOC}} \right) \times 100,
\]

where AOC is the absorbance of control and AOS is the absorbance in the presence of extract/ibuprofen.

2.4. Study based on an animal model

2.4.1. Animals

Swiss male albino mice had been collected from King Saud University, Saudi Arabia, and were kept at an ambient temperature of 23 ± 2°C at the animal house, with a relative humidity of 45–55% at 12-h dark as well as light period. A standard diet and water ad libitum were provided to rodents. The animals had been acclimatized for 1 week before they were utilized in experiments. All procedures relating to current analysis had been approved by the institutional ethical committee and performed in compliance with the ethical guidelines for the Employment of Animals in Laboratory studies of the Faculty of College of Applied Medical Sciences, Qassim University, Saudi Arabia.

2.4.2. Experimental design

Thirty-two mice (20–25 g each) were arbitrarily partitioned into four groups of eight mice in each group \((n = 8)\) 1 week after adjustment to research facility contexts. Group I frequently received typical saline and mice chow and this group was taken into account as a control group. Group II animals were treated with oral alimentation of CCl₄ (0.04 ml of 40% CCl₄ solution in olive oil) four times per week [29]. Animals in groups III had been orally supplied with OFPE at 150 mg/kg body weight [25] with minor dosage modification, and CCl₄ (0.04 ml of 40% CCl₄ solution in olive oil) [29] four days every week. At 150 mg/kg body weight, animals in group IV were treated orally with olive pulp extract only just four days a week. The experimental duration with different animal groups was continued for 8 weeks and were sacrificed. At the end of the experiment, all the animals were sacrificed and the blood was collected in plain vials. The blood was allowed to clot for 30 min at room temperature. The clotted blood was centrifuged at 4000 rpm for 10 min to get the serum. The serum was used to check the liver function enzyme activities, inflammatory marker level and antioxidant enzymes.

2.4.3. Liver enzymes activities

For the assessment of liver function, alanine amino transaminase (ALT), aspartate amino transaminase (AST) and alkaline phosphatase (ALP) had been spectrophotometrically measured utilizing a commercial kit (Crescent Diagnostic kits, Jeddah, Saudi Arabia) consistent with the manufacturer’s protocol and the result was deciphered accordingly.

2.4.4. Antioxidant enzymes/total antioxidant capacity activity

After carrying out the manufacturer’s protocol (Abcam, Cambridge, UK), the catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and antioxidant enzymes were measured using standard kits and the result was deciphered in like way. The total antioxidant capacity (TAC) was measured using a trolox kit concurring to the manufacturer’s information (Abcam, Cambridge, UK). In short, both standard and sample wells were supplemented with 100 µl Cu²⁺ working solution and mixed. Later, the plates were incubated on an orbital shaker for 90 min at room temperature away from light. Estimation was performed at optical density (OD) 570 nm utilizing a microplate reader.

2.4.5. Determination of the serum IL-6, TNF-α and C-reactive protein levels

The blood samples were collected from each experimental group and after clot formation, it was centrifuged at 4000 rpm for 10 min and the serum was separated. The concentrations of serum cytokine Interleukin (IL)-6, tumor necrosis factor (TNF)-α and C-reactive protein (CRP) levels were determined by utilizing the kits obtained from Abcam (Cambridge, UK).

2.4.6. Pathological examination

At the time of sacrifice, the liver tissues of all animals were excised and fixed in 10% formalin solution and sections were cut into 5–10 µm sections and stained with H&E stain (haematoxylin–eosin stain) [30]. In addition, CCl₄-induced alterations were assessed by examining the pathological changes in liver tissue beneath a
light microscope and the result was deciphered in like manner.

2.4.7. Immunohistochemistry
The expression of phosphatase and tensin homologue protein (PTEN) was assessed by an earlier mentioned method of immunohistochemistry staining [31,32]. In short, segments were deparaffinized with xylene treatment and endogenous peroxidase activity was blocked utilizing 0.3% hydrogen peroxide. Additionally, the pressure cooker was used for antigen recuperation to reveal the location of the antigen. Then, the blocking step was performed with the blocking agent. Monoclonal PTEN antibodies were used on tissue followed by incubation in a humid chamber at 4°C overnight. Then, incubation with secondary antibody for 2 h was accompanied by incubation with the streptavidin–biotin enzyme complex for 45 min. Eventually, diaminobenzidine chromogen was utilized, and then counterstaining of sections was done with haematoxylin.

2.4.8. Statistical analysis
All the data were expressed as mean ± standard deviation. Differences among means were evaluated by one-way analysis of variance using statistical package for social science (SPSS). The \( p \) value, \( p < 0.05 \), was considered as statistically significant.

3. Result
3.1. Evaluation of antioxidant capacity of olive fruit extract by DPPH assay
DPPH free radical assay is an exceptionally well-known strategy to measure the antioxidant capacity of diverse antioxidant substances. In the DPPH assay, DPPH becomes reduced in the presence of hydrogen donating antioxidant substances and produces yellow-colored diphenyl-picryl hydrazine. Hence, the assessment of the decrease in absorbance at 517 nm can be used to indicate the reduction of DPPH by antioxidant substances. Within the current consideration, olive fruit extract was found to have an excellent DPPH free radical scavenging activity and this capacity was found to be most noteworthy at 600 µg/ml. The strong DPPH free radical scavenging activity recommends that olive fruit pulp would be a wealthy source of antioxidant polyphenolic compounds. The results of the DPPH radical scavenging test are shown in Figure 1.

3.2. Evaluation of in vitro anti-inflammatory activity by protein denaturation inhibition
Denaturation is a process during which certain external factors cause the disruption of native structures of protein. The denaturation of BSA brings about the expression of antigens related to type-III hypersensitivity reaction and therefore the commencement of an inflammatory response. In this way, we explored the potential of olive fruit extract to protect from protein denaturation to verify its anti-inflammatory activity. Our outcome indicates that OFPE is significantly powerful against heat-induced albumin denaturation and percent inhibition of denaturation increments with increment in concentration of the extract. The excellent anti-inflammatory activity was exhibited by the extract at 600 µg/ml. The protecting capacity of olive fruit extract against heat-induced protein denaturation suggests the presence of antioxidant polyphenolic compounds. Ibuprofen, a regular anti-inflammatory medicinal drug, exhibited the greatest inhibition at 200 µg/ml (Figure 2).
3.3. Body weight of mice
The body weight of mice was estimated in every single experimental group and it was seen that there were no significant difference in weights among all the test groups (p > 0.05). However, we definitely found an increase in body weight of all the animal groups during the 8 weeks of experimental design.

3.4. Effects of olive fruit extract on serum ALT, ALP and AST activities
The levels of serum biomarkers of liver injury were analysed to evaluate the liver damage (Figure 3). Liver damage triggered by CCl4 was noticed by a noteworthy increase in the liver enzymes level. The levels of serum ALT (155.6 U/L), AST (170.2 U/L) and ALP (170.3 U/L) were significantly increased in serum of group treated with CCl4 when compared to control group (ALT = 60.2 U/L, AST = 78.3 U/L, ALP = 75.4 U/L) (p < 0.05). However, an increase in these serum biomarker levels was considerably decreased in group that received OFPE plus CCl4 (ALT = 92.7 U/L, AST = 105.3 U/L, ALP = 115.5 U/L) (p < 0.05). This outcome indicates that olive fruits are beneficial in the protection from liver damage by decreasing the level of liver function enzymes.

3.5. Effects of olive fruit extract on CAT, GPx and SOD activities
The activities of antioxidant enzymes like CAT, SOD and GPx were estimated in all test groups (Figure 4). It was revealed that CAT, SOD and GPx meaningfully reduced in the CCl4 treated group as compared to the control group (p < 0.05). CCl4-induced decrease in CAT (8.5 U/mg protein), SOD (42.3 U/mg protein) and GPx (6.5 U/mg protein) activities was found to be recovered after treatment with OFPE. Whereas antioxidant enzyme levels were high in OFPE group only (CAT = 37.3 U/mg protein, GPx = 30.2 U/mg protein, SOD = 105.3 U/mg protein). The difference in antioxidant enzyme level in the group dealt with CCl4 and the group treated with both CCl4 plus OFPE (CAT = 24.3 U/mg protein, GPx = 18.3 U/mg protein, SOD = 68.5 U/mg protein) was statistically significant (p < 0.05).

3.6. Effects of olive fruit extract on TAC
TAC was measured in all experimental groups and it was noticed that TAC was considerably reduced in the group that received only CCl4 (20.3 nM) as compared to the control group (65.3 nM) (p < 0.05) (Figure 5). The decrease in TAC induced by CCl4 treatment was recovered after treatment with OFPE (48.3 nM).

Figure 3. The levels of serum biomarkers of liver injury were significantly increased in the group treated with CCl4 when contrasted to the control group and the group that received OFPE only. Increment in the levels was significantly decreased in serum of group received both OFPE and CCl4. The values represent mean ± scanning electron microscope (SEM), with n = 8 animals in each group. Statistically significant differences are indicated as # significance at p < 0.05 compared to the control group and * significance at p < 0.05 compared to the disease control.
Figure 4. CAT, SOD and GPx were considerably reduced in the group treated with CCl4 only in comparison to the control group. CCl4 induced decrease in CAT, SOD and GPx activities recovered after treatment with OFPE and difference in the antioxidant enzymes level in the group treated with CCl4 and the group treated with CCl4 plus OFPE was statistically significant. The values represent mean ± SEM, with n = 8 animals in each group. Statistically significant differences are indicated as # significance at p < 0.05 compared to the control group and * significance at p < 0.05 compared to the disease control.

Figure 5. Levels of TAC were measured. TAC was extensively increased in the OFPE plus CCl4 received group as compared to the CCl4 treated group only. The values represent mean ± SEM, with n = 8 animals in each group. Statistically significant differences are indicated as # significance at p < 0.05 compared to the control group and * significance at p < 0.05 compared to the disease control.

3.7. Measurement of the inflammatory marker

The level of inflammatory markers including IL-6, TNFα and CRP was measured in all groups using respective enzyme linked immunosorbent assay (ELISA) kits (Figure 6). It was noted that the level of these markers was high in the CCl4 treated group. However, olive extract reduced the levels of these markers, and the difference in the level of IL-6 in the extract plus CCl4 treated group and the CCl4 treated group only was statically relevant (p < 0.05).

3.8. Histopathological examination

Control group mice liver tissue showed normal architecture of hepatocytes and normal hepatic cells. However, significant alteration of liver tissues was noticed in CCl4-injured mice, characterized by infiltration of lymphocytes, haemorrhages, oedema, hepatocellular damage and blood vessel dilation. Whereas treating the mice with olive extract showed potentiality to ameliorate such changes and histological appearance look pretty much like that of the control group, and hepatocellular damage was lesser in the olive extract group than the group that received CCl4 only (Figure 7).

3.9. Expression of PTEN

The expression of PTEN protein was investigated in all the groups via immunohistochemistry staining. PTEN
Figure 6. The levels of different inflammatory markers were found to be increased in the group treated with CCl₄. However, OFPE reduced the levels of these markers. The values represent mean ± SEM, with n = 8 animals in each group. Statistically significant differences are indicated as # significance at p < 0.05 compared to the control group and * significance at p < 0.05 compared to the disease control.

Figure 7. Photomicrographs of sections of liver of mice (H&E 40 ×) showing (a) the normal architecture of hepatocytes of the control group (n = 8). (b) and (c) CCl₄ treated mice (n = 8) showed loss of architecture, haemorrhage, congestion, oedema, infiltration of lymphocytes and remarkable blood vessel dilations. (d) Treatment with OFPE plus CCl₄ (n = 8) showing significantly less liver tissue alteration as compared to the CCl₄ treated group (n = 8). (e) Olive treatment showing normal architecture of hepatocytes.

protein expression was detected in the control group. Furthermore, the group dealt with CCl₄ (disease control) as well as the group treated with both CCl₄ and extract also showed expression of PTEN protein, and expression pattern among the different groups was statistically irrelevant (Figure 8).

4. Discussion

The liver is an important organ which plays a significant role in metabolism, detoxification and protein synthesis. In the current study, we investigated the antioxidant activity, in vitro anti-inflammatory potential of OFPE,
OFPE administration significantly reduced serum levels of those parameters in the group of mice treated with CCl₄.

These results are consistent with the findings of previous researchers, Kang and Koppula, who found that the administration of OFPE decreased the raised levels of serum ALT, ALP and AST remarkably in mice treated with CCl₄ and olive pulp extract when compared to CCl₄ treated mice (p < 0.05) [35].

Similarly, administration of extra virgin olive oil and its methanol extract exhibited the similar hepatoprotective effect and was found to prevent the elevated levels of serum glutamate pyruvate transaminase, glutamate oxaloacetate transaminase and ALP activity. Also, the administration of olive oil was reported to improve principal parameters of oxidative stress, histopathological alterations and enzymatic indicators of hepatic injury [36]. These results are in accordance with the previous studies which reported the aqueous extract of A. halimus and J. phoenicea abrogates the CCl₄-induced hepatotoxicity by modulating biochemical, antioxidant and histological changes [37,38].

CAT, SOD and GPx constitute an antioxidant defense system of our body that detoxify ROS and, thus, prevent their damaging effects. In our present investigation, the significant reduction in activities of CAT, SOD and GPx had been noticed in the animal group treated with CCl₄. Moreover, OFPE administration intoxicated with CCl₄ significantly restored antioxidant enzyme levels relative to mice treated with CCl₄ only. The restoration of antioxidant activity as evidenced by increased levels of SOD, CAT and GPx suggests that OFPE enhances detoxification of ROS and recovers activity of antioxidant enzymes. Many researchers have reported similar findings suggesting that the hepatoprotective effects of various medicinal plant extract or active compounds occur through enhancement of the antioxidant level. In 2016, Al Seeni et al. reported that treatment of hepatotoxic rats with olive oil and N. sativa oil demonstrated a critical improvement in every single biochemical parameter compared with the positive CCl₄ control group [36]. In parallel, other natural compounds like baicalein has been reported to prevent the hepatic damage though the modulation of antioxidant enzymes, and oxidative stress level [39].

The prevention of oxidative damage by olive extract can be explained by a possible molecular mechanism suggested by previous researchers. The supplementation with docosahexaenoic acid and extra virgin olive oil possibly prevents liver steatosis by upregulation of peroxisome proliferator activated receptor (PPAR)-α and Nrf2 with concomitant downregulation of sterol regulatory element-binding protein (SREBP)-1c and necrosis factor (NF)-kB [40].

IL-6, TNF-α and CRP are inflammatory markers that increase at the sites of inflammation or infection. Our data have demonstrated that CCl₄ treatment caused...
the elevation of pro-inflammatory cytokines which is in accordance with earlier findings [41]. However, our results demonstrate that OFPE consumption leads to considerable reduction in IL-6.

Consequently, our data suggest that OFPE has a great role in alleviating liver damage triggered by CCl4. In accordance with these findings, a previous study has documented increased levels of various serum biomarkers of liver injury including TNF-α, IL-6, IL-1β and monocyte chemoattractant protein (MCP)-1 in liver tissues following treatment with CCl4 and inhibition of the increased levels of these factors by dose dependent pre-treatment with breviscapine [42].

Furthermore, our study showed that mice treated with CCl4 exhibited various alterations, characterized by inflammatory cell infiltration, congestion, oedema and blood vessel dilatation. However, OFPE significantly ameliorated the liver tissues alterations induced by CCl4. Such findings are in agreement with previous results, which suggested remarkable liver injuries in group dealt with CCl4 by means of mild to extreme hepatocellular deterioration and necrosis. CCl4 treated mice pre-treated with 200 mg/kg silymarin and olive fruit pulp extract-ethyl acetate (OFO-EA) extract at 200 mg/kg noticeably ameliorated the hepatic lesions [35].

PTEN acts as tumour suppressor gene and is situated within the 10q23 vicinity. It presents instructions for making a multifunctional protein with excellent phosphatase effects [43]. Many researchers have reported the lack of PTEN protein expression in diverse kinds of tumour [44].

There are many reports regarding the presence of phenolic compounds as major components in olive that includes hydroxytyrosol, tyrosol, oleuropein, 4-hydroxy phenyl acetic acid, protocatechuic acid, caffeic acid and p-coumeric acid [35]. However, olive fruit pulp methanolic extract has been documented to contain some other phenolic compounds including verbascoside, rutin, caffeoyl-quinic acid, luteolin-4-glucoside and 11-methyl-oleuside using reverse phase high performance liquid chromatography (RP-HPLC) and electrosprayionization-mass spectrometry (ESI-MS) techniques [45].

Hydroxytyrosol is one of the most important molecules with highest protecting capacity that have the ability to regulate several signalling pathways linked with inflammatory and antioxidant response. Therefore, hydroxytyrosol may be involved in anti-steosis [46]. Besides, the presence of hydroxytyrosol, oleic acid, tocopherols and/or PUFAs has been considered to be responsible for the hepatoprotective effect of olives because of their protective ability against oxidative stress-induced damage [34,47].

The expression pattern of PTEN protein was noticed in all experimental groups. The PTEN positivity among the different groups was statistically insignificant. Our finding was in accordance with a previous study which is based on Ajwa dates extract, which reported that the expression pattern of PTEN protein was insignificant in different groups of animals [48].

5. Conclusion

Our findings have suggested that olive fruit extract possesses notable antioxidant activity that can contribute in prevention of ROS mediated disorders such as inflammation and liver injury. What is more, our investigation showed the anti-hepatotoxic capability of olive fruit extract towards liver injury prompted by using CCl4 through decrease in liver enzymes, decrease in oxidative toxicity and reduced inflammatory responses and in vitro anti-protein denaturation activities. Moreover, present study documents that olive fruit extract exhibits the hepatoprotective effect through maintenance of hepatocytes architecture. Thus, olive fruit extract may be used as a therapeutic option to prevent hepatic complications because it provides a strong protection against liver injury.

Data availability

The data used to support the findings of the current study are included within the article.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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