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Comparative Evaluation of Antioxidant and Hepatoprotective Effects of Three Olive Leave Species Cultivated in Aljouf Region, Saudi Arabia

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ABSTRACT

Aim of the work: the purpose of this study was to evaluate the antioxidant, anti-inflammatory and hepatoprotective effects of extract from 3 olive leave cultivars; K18, Nibali and Sorani growing in Aljouf region, Saudi Arabia.

Methods: all plants were extracted with 80% methanol and the extracts were used to determine the total phenolic content, the total flavonoid content, the DPPH radical and anti-lipid peroxidation scavenging activities. *In vivo* hepatoprotective effect of these olive leaves extracts (OLEs) was evaluated in rabbit intoxicated with CCl4. Expression of iNOS, caspase-3, TNF-α and IL-1β along with the histopathological findings of the examined liver were recorded to evaluate the extent of hepatoprotective of each extract.

Results: methanolic extracts of all plants exhibited DPPH and peroxyl radicals scavenging activity that was well correlated with their total phenolic and flavonoid contents. The phenolic, flavonoid contents, DPPH, and peroxyl scavenging activities were in the following orders: Nibali> K18 > Sorani. Pretreatment with OLE (400 mg/kg) along with CCl_4 was significantly alleviated liver damage as indicated by improvement of the microscopic picture of liver and reduced hepatic iNOS, caspase-3, TNF-αand IL-1β expression compared to CCl_4 intoxicated group. However, the hepatoprotective effects of Nibali and K18 OLEs were more prominent compared to those exerted by Sorani OLE.

Conclusion: all OLEs had potent anti-inflammatory, antioxidant and antiapoptotic activities that preserved hepatic cells against CCl4-induced hepatic damage through attenuation of pro-inflammatory cytokines, oxidative stress and apoptotic pathways, however, these activities were probably species dependent.

Keywords: olive leave, antioxidant, hepatoprotective**,** anti-inflammatory**,** histopathology, immunohistochemistry**.**

INTRODUCTION

Liver is a critical body organ that has many biological functions. Removal and inactivation of toxic substances and drugs is the primary function of the liver. Hepatotoxicity is liver damage exerted by a direct toxic effect of some chemicals and overloads of drugs, a reactive metabolite or an immunologically-mediated response [1-3]**.** The hepatic toxicity also attributed to the changes in oxidative stress and alteration in acute phase proteins [4]**.** Up until now, drug development and research still unable to establish a reliable hepatoprotective drug. So, many studies were carried out on different natural sources including medicinal plants to evaluate their hepatic protective efficacies [4-6]**.** Medicinal plants are considered as a low-cost source of unique constituents, which could be used broadly for the development of new drugs for treatment of different hepatic disorders. Among these medicinal plants, olive leaves which are a valuable one which has been widely used in traditional remedies in European and Mediterranean countries [7]**.** Several studies reported that extra-virgin olive oil has higher phenolic content provided potential anti-inflammatory and antioxidant effects than olive oil [8,9]**.** Olive tree leaves contain similar polyphenols as those found in extra-virgin olive oil or the fruit itself, but at a much higher concentration [7]**.** Therefore, olive leaves are considered as a rich source for biologically active phenolic compounds and have superior antioxidant activity, anti-inflammatory power and radical scavenging effects [10-13]**.** These bioactive properties of the olive leaves have formed a base for their beneficial health effects such as anti-hypertensive [14]**,** cardioprotective [15]**,** hypoglycemic [16] and hypo-cholesterolemic agents [17]**,** In addition, several flavonoids, namely luteolin and apigenin, which have revealed anticancer activities were also detected only in olive leaves extract and were not detected in the oil from the fruit [18-20]**.**

Therefore, the current study was designed to compare the potential antioxidant, anti-inflammatory and hepatoprotective effects of methanol extracts obtained from three species of olive leaves (Nibali, K18, and Sorani) cultivated in Saudi Arabia. This comparison was based on *in vitro* properties of these extracts as well as the

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histopathological and immunohistochemical evaluations of their effects.

MATERIALS AND METHODS

Chemicals

All chemicals were used in this study were of high analytical grade and obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Rabbit antibodies against iNOS, caspase-3, TNF-α, and IL-1β were purchased from Proteintech Biotechnology (Wuhan, China).

Plant materials

Fresh olive leaves of 3 cultivars (K18, Nibali, and Sorani) were collected from a local olive farms in Aljouf region, Saudi Arabia. Botanical identification and authentication were performed by Biology Department, College of Science, Aljouf University, Saudi Arabia.

Preparation of plant methanol extracts

Twenty grams of the dry powder from each plant materials were macerated in methanol (150 mL), at room temperature for 48 hours. The extraction solvent was collected and concentrated using rotary evaporator then the dried extract was kept frozen at -20 ° C.

Estimation of total phenolic and flavonoids contents

Total phenolic content of extracts was estimated using Folin-Ciocalteu reagent. A calibration curve was performed in parallel under the same operating conditions using gallic acid as a positive control. The results were expressed as mg gallic acid equivalent per gram of dry extract (mg GAE/g extract) [21]. Total flavonoids content was determined according to the method of **Eberhardt *et al*.** [22]. The total flavonoids content of each extract was determined from the standard curve based on rutin; the content was expressed in mg of rutin equivalents (RE) per gram of dry extract (mg RE/g extract).

DPPH free radical scavenging assay

DPPH free radical scavenging activity of each extract was estimated according to the method described by **Cheung *et al.*** [23]. Briefly, 160 µl of 0.2 mM DPPH in ethanol was mixed with 40 µl of each extract (0.01–1 mg/ml) in 96 microplates. Ascorbic acid was used as a reference compound. The percent scavenging of DPPH was calculated using the formula:

$$\% \text{ scavenging activity} = A_{control} - A_{sample} / A_{control} \times 100$$

where $A_{control}$ and A_{sample} were the absorbance values at 517 nm of the control and the sample extract, respectively.

Anti-lipid peroxidation assay

Lipid peroxidation was carried out as described in a previous study [24]. Briefly, phosphatidylcholine micelles were prepared by sonicated on ice 300 mg phosphatidylcholine in 30 mL 10 mM phosphate buffer at pH 7.4 for 2 hours. To a total volume (1 ml) containing potassium phosphate buffer at pH 7.4 (10 mM), the liposome (250 µl), and extract, or extraction solvent (450 µl), was added to $FeCl_2$, H_2O_2 and ascorbic acid each in a final concentration of 125 µM. After incubating the mixture at 30°C for 4 h, 250 µl of the final mixture was added to 500 µl TCA-TBA-HCl reagent (15% w/v, TCA; 0.375% w/v, TBA; 0.25 M HCl) and heated at 100°C on a boiling water bath for 15 min. After centrifugation at 3,000 g for 5 min the absorbance was monitored at 532 nm against blank. Trolox was used as a reference antioxidant. The percent lipid peroxidation inhibitory activity was calculated using the formula:

$$\% \text{ inhibitory activity} = A_{control} - A_{sample} / A_{control} \times 100$$

Where $A_{control}$ and A_{sample} are the absorbance values at 532 nm of the control and the sample extract, respectively.

Animals

Forty apparently healthy adult male rabbits weighing 1.5 to 2 kg were obtained from local markets and kept in animal house, College of Applied Medical Sciences, Aljouf University. These animals were housed individually in stainless steel wire cages with controlled environment at 21 ± 2 ^{o} C and relative humidity at 50 ± 5 under a 12 h dark and light cycle for one week of acclimatization before experimentation. The animals were fed standard rabbit's diet and provided water *ad libitum*. All the experimental procedures were carried out in accordance with international guidelines for care and use of laboratory animals and they were approved by the Bioethical Committee of the Aljouf University.

Experimental design

Rabbits were randomly categorized into eight equal groups. In the first and second groups rabbits were given *ad libitum* water and access of food and served as non-intoxicated control group. In the third and fourth groups rabbits were received a daily oral dose of Nibali OLE (400 mg/kg) for 7 days. In the fifth and sixth groups rabbits were given orally K18 OLE at a dose of 400mg /kg/day for 7 days. In the eighth and seventh groups rabbits were received a daily oral dose of Sorani OLE (400mg /kg). At the 8th day of the experimental period, hepatotoxicity was induced in all the experimental groups expect the first control group, with a single intraperitoneal injection of CCl_4 at a dose of 0.75 ml/kg. All animals were examined daily for any clinical signs and mortality rate. Twenty-four hours post-injection of CCl_4, rabbits in all groups were

anesthetized with chloroform and then sacrificed by decapitation.

Histopathological study:

Small tissue specimens were collected from liver of rabbits in all the groups and immediately fixed in 10% neutral buffered formalin. After proper fixation, the fixed specimens were dehydrated in ascending grades of ethyl alcohol, cleared in xylol and embedded in paraffin wax. Then, 5 mm tissue paraffin sections were prepared and stained with H&E stain for the microscopic examination [25].

Immunohistochemical study:

Immunostaining was performed on four randomly chosen tissue paraffin sections from each group. The assays of the hepatic levels of iNOS , caspase-3 , TNF-alpha and IL-1β were performed according to the manufacturer's instructions. Brown color in the cytoplasm of the cells was considered a positive reaction.

For quantitative analysis, five non-overlapping fields in slides of four chosen tissue paraffin sections from each group were examined for morphometric measurement of the area percentage of iNOS, caspase 3, TNF-α and IL-1β reaction at a magnification of 400X. immunohistochemical images were assessed by densitometry using Image-Pro Plus (Version 6.0; Media Cybernetics; Silver Spring, Maryland, USA) on Olympus DP2-BSW Image computer system (Olympus Imaging System Tokyo, Japan).

The study was done after approval of ethical board of Al Jouf university.

Statistical analysis:

The recorded data were analyzed using Statistical Package for the Social Sciences (SPSS) software version 20 (SPSS Inc. Chicago, USA). One-way analysis of variance (one-way NOVA) test was used to compare between control and other groups. Values in the text were expressed as means ±standard deviation (SD) and differences with $P \leq 0.05$ were considered to be statistically significant.

RESULTS

Antioxidant studies analysis

Antioxidant activity of the extracts from 3 cultivars was assessed through two different methods: DPPH assay and anti-lipid peroxidation assay. Total phenolic and total flavonoids contents of three extracts were also evaluated (**Table 1**).

Total phenolic and total flavonoids content

Total phenolic contents of Nibali cultivar was higher (21.77 mg GAE/g extract) than the other 2 cultivars [K18 (17.20 mg GAE/g extract), Sorani (12.77 mg GAE/g extract)]. Also, Nibali cultivar had the highest total flavonoids content (2.65 mg RE/g extract), followed by K18 (2.24 mg RE/g extract) and Sorani cultivars (1.45 mg RE/g extract).

DPPH radical scavenging activity

The obtained results showed that extracts of all 3 cultivars possess activity against DPPH free radicals. The extract of Nibali cultivar had the highest DPPH scavenging activity (41%), followed by K18 cultivar (34%) and Sorani cultivar (27%). In comparison with that produced by vitamin C (79 %) the activities of these extracts were weak.

Anti-lipid peroxidation activity

In the present study, peroxyl radical scavenging activity of 3 OLEs cultivars was measured using a liposome oxidation system and the results were expressed as % inhibition. Based on the obtained results, it was cleared that, the extract of Nibali variety had better peroxyl scavenging activity (54%) as compared to other 2 varieties [K18 (39%), Sorani (35%)]. Compared to Trolox, all the three varieties of OLEs exhibited a weak radical scavenging activity.

Histopathological findings:

Microscopic examination of liver of rabbits in the first (Control), the third (Nibali OLE), fifth (K18 OLE) and seventh groups (Sorani OLE) groups showed normal cytoarchitecture of the hepatic lobules (**Fig, 1A**). In contrast, the histopathological findings of the examined liver in the second (CCl4-intoxicated) group were evidenced by marked hepatocellular damage in the form of diffused fatty and ballooning degeneration of hepatocytes with pyknosis in their nuclei (**Fig. 1B).** Apoptosis in numerous hepatocytes were scattered throughout these degenerated cells (**Fig. 1C).** Severe congestion with focal hepatic hemorrhages was observed (**Fig. 1D).** Multiple areas of hepatic necrosis infiltrated with inflammatory cells mainly lymphocytes were prevalent (**Fig. 1E**). Moreover, severe bile ductal hyperplasia and formation of newly formed bile ducts were also detected. (**Fig.1F).** Compared to CCl4 induced hepatotoxicity group, the examined liver of rabbits pretreated with OLEs showed well detected improvement in the histological appearance of the liver. In the fourth group (Nibali OLE + CCl4), scattered areas of mild vacuolar degeneration in some hepatocytes and preriportal aggregation of some inflammatory cells were the main detectable microscopic hepatic lesions (**Fig.1G**). In addition, congestion and aggregation of few mononuclear inflammatory cells with mild bile ducts hyperplasia were also found in one of the examined liver in this group (**Fig. 1H).** In the sixth group (K18 OLE + CCl4), congestion of hepatic sinusoids with mild hepatic degenerative changes were seen in most of the examined liver

(**Fig. 1I).** However, in the other examined liver of the same group, ballooning, hydropic and vacuolar degeneration of some hepatocytes and pyknosis in their nuclei were recorded (**Fig. 1J).** In the eighth group (Sorani OLE + CCl4), moderate degenerative changes in the form of ballooning and hydropic degeneration of hepatocytes and pyknosis in their nuclei with bile ductal hyperplasia and periductal inflammatory cellular aggregation were prevalent in the most of the examined liver **(Fig. 1K).** Moreover, perivascular lymphocytic cellular aggregation was also noticed in liver tissue of this group **(Fig. 1L).**

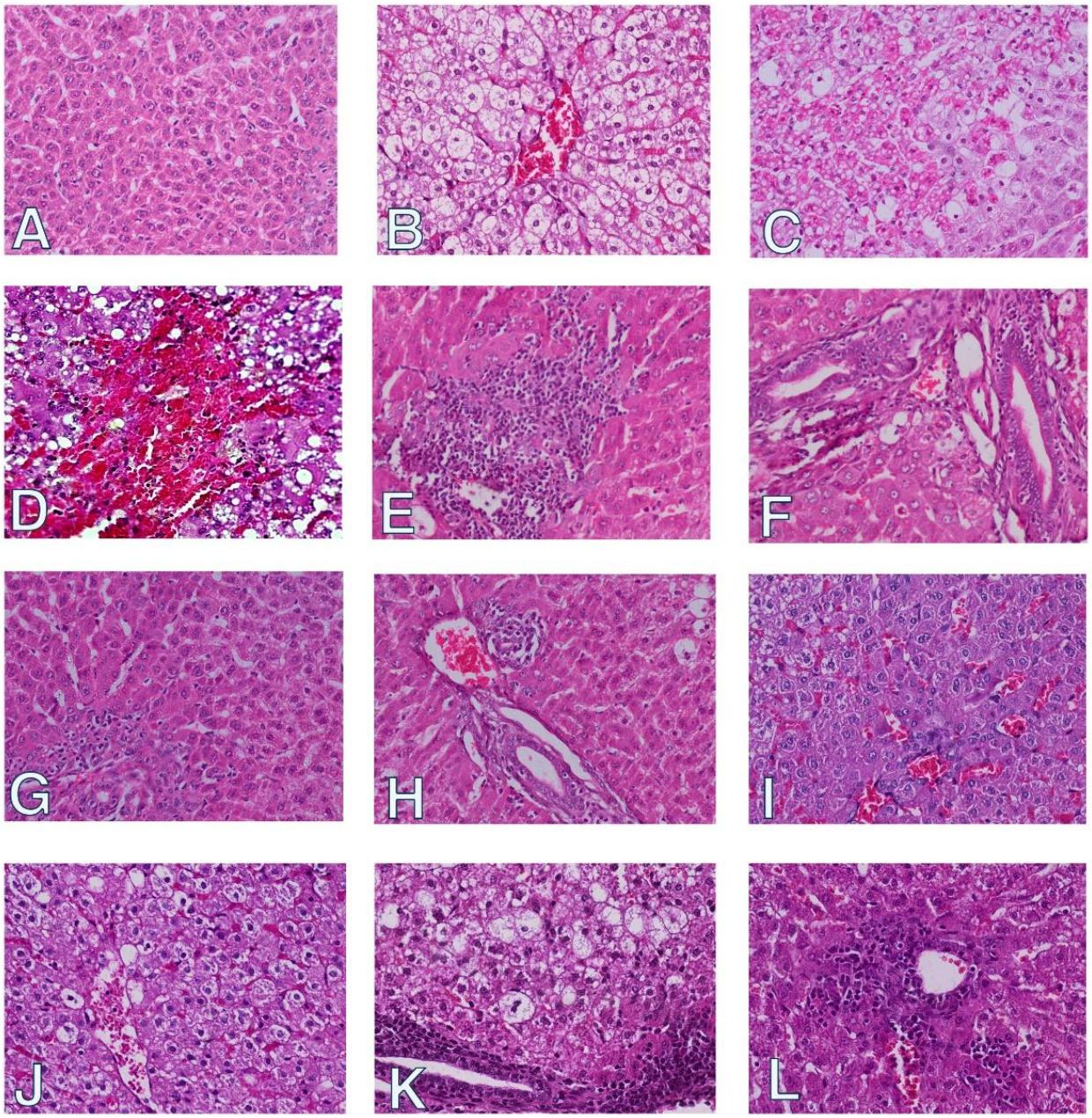

Fig 1: liver of rabbit of the control group showing normal cytoarchitecture of liver tissue (A); CCl4 intoxicated group (B-F) showing congested central vein with ballooning degeneration of hepatocytes and pyknosis of nuclei (B), numerous apoptotic hepatic cells scattered in-between degenerated hepatocytes (C), focal hemorrhage and hepatic steatosis (D), hepatic necrosis infiltrated with inflammatory cells (E) and bile ductal hyperplasia with formation of newly bile ducts and aggregation of few mononuclear inflammatory cells (F); Nibali OLE + CCl4 group (G, H) showing mild vacuolar degeneration of hepatocytes and preriportal aggregation of inflammatory cells (G) and congestion with aggregation of mononuclear inflammatory cells and mild bile ductal hyperplasia (H); K18 OLE + CCl4 group (I, J) showing congested sinusoids with mild hydropic degeneration of hepatic cells (I) and ballooning of hepatocytes with pyknosis of their nuclei (J); Sorani OLE + CCl4 group (K, L) showing ballooning and hydropic degeneration of hepatocytes, pyknosis of their nuclei with bile ductal hyperplasia and periductal inflammatory cellular aggregation (K) and perivascular lymphocytic cellular aggregation (L) (H & E X 200.)

Immunohistochemistry analysis and quantitative assessment of hepatic iNOS, Caspase-3 and pro-inflammatory cytokines (TNF-α and IL-1β) expressions

Immunohistochemical analysis of the liver tissue sections of rabbits in the first, third, fifth and seventh groups, revealed weak iNOS expression on walls of the hepatic sinusoids (**Fig 2A).** In the second group, IP injection of CCl4 at a single dose of 0.75 ml/kg was associated with marked expression of iNOS throughout the hepatic tissues, suggesting that hepatocytes exhibited an increase in oxidative stress after induction of CCl4 hepatotoxicity (**Fig. 2B).** As shown in **table 1**, this expression of iNOS-positive cells was significantly increased in the CCl4-intoxicated group compared to the first control group (32.234±2.332 vs 0.461±0.091). Conversely, pre-administration of OLEs was apparently deceased this expression of hepatic iNOS (**Fig.2C-E).** In addition, the morphometric analysis of immunohistochemical images from these groups revealed that these decreases in iNOS expression were in species dependent manner, where the suppressing effects of Nibali and K18 OLEs (0.415±0.051, 0.461±0.091) were more prominent compared to those exerted by Sorani OLE (3.052±0.447). However, in comparison with the first control group (0.242±0.060) iNOS expression in all pretreated groups remained high.

Regarding to caspase-3 immunoreactivity, weak caspase-3 expression which mostly found on walls of the blood vessels and sinusoids was detected in the control group (**Fig. 3A).**

In contrast, the hepatocytes of rabbits intoxicated with CCl4 showed marked expression of caspase-3 with abundant caspase-3-positive cells in the examined liver (**Fig 3B).** Compared to CCL4 hepatotoxicity model group, pretreatment of OLEs was greatly reduced the hepatic caspase-3 expression (**Fig. 3 C-E).** Also, the morphometric analysis revealed a significant decrease in the area percentage of caspase3 immunoreactivity in the liver of rabbits in these pretreated groups compared with those in CCl4 group. Moreover, the morphometric analysis of immunohistochemical images revealed that the hepatoprotective effects of all extracts against apoptosis induced by CCl4 were nearly similar (**Table 1).**

For determination of the role of OLEs on proinflammatory cytokines in CCl4 hepatotoxicity, immunohistochemical analysis and quantitative assessment of TNF-α and IL-1β expression were carried out. In the control group the hepatocytes showed no or only weak TNF-α immunoreactivity that restricted in the sinusoidal cells (**Fig. 4A).** While, marked increase in expression of TNF-α was recorded throughout the hepatic tissues in CCl4 intoxicated group (**Fig.4B**). The morphometric analysis of immunohistochemical images of liver in CCl4 group revealed significantly increase in hepatic TNF-α expression compared to those in the control group (27.969± 1.100 vs 0.231±0.047). In comparison with CCl4 group, the examined liver of rabbits pretreated with OLEs particularly in rabbits pretreated Nibali OLE showed diminished number of TNF-α-stained cells (**Fig 4 C-E**) with significant a decrease in TNF-α immunoreactivity (**Table 1).**

Results of immunostaining for IL-1β in the liver sections from the control group showed that the IL-1β immunoreactivity was also predominantly localized in the sinusoidal cells (**Fig. 5 A**).

While, in CCl4 intoxicated group, there was intensive immunostaining of hepatic cells distributed throughout the hepatic tissue particularly in the vicinity of inflammatory cellular aggregation and around the central veins **(Fig. 5 B & C).** Compared to CCl4 group, only faintly stained Il-1β immunoreactivity was detected with few number of positive cells in the centrilobular areas in OLEs pretreated groups (**Fig. 5 D-F**). Moreover, significant reduction in the area percentage was observed in the liver of rabbits pretreated with OLE groups (**Table 1**).

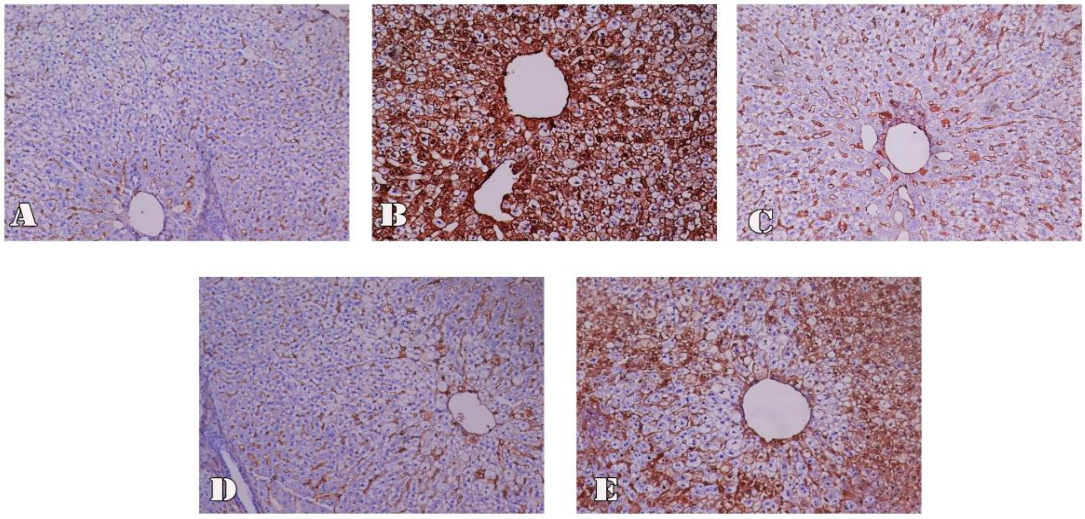

Fig. 2. Immunostained liver sections showing immunohistochemical distribution of iNOS in the control group (A), CCl4 intoxicated group (B), pretreated Nibali OLE +CCl4 group (C), pretreated K18 OLE +CCl4 group (C) and pretreated Sorani OLE +CCl4 group (E)(X200).

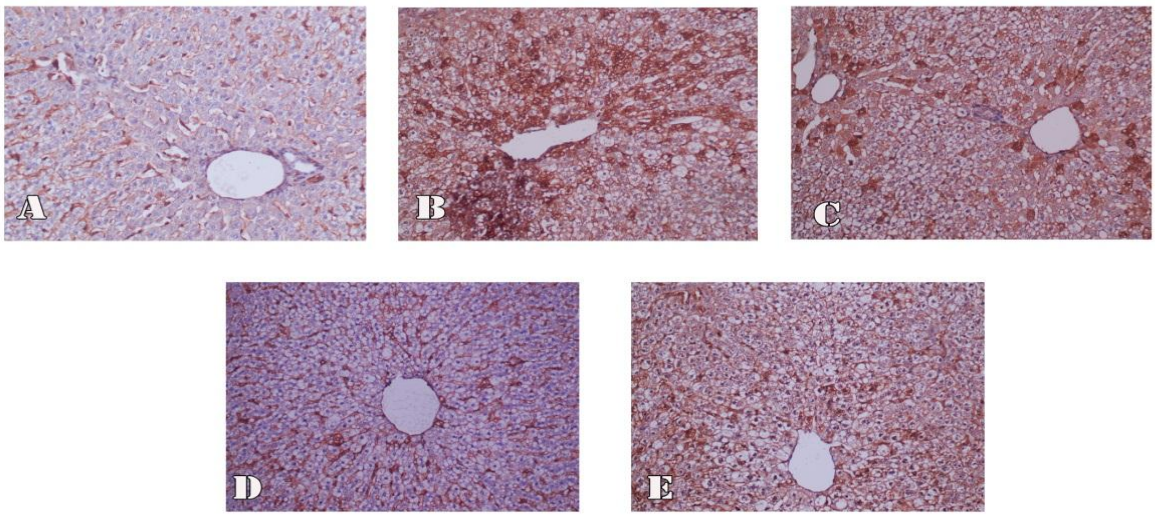

Fig. 3. Immunostained liver sections showing immunohistochemical expression of caspase-3 in control group (A), CCl4 intoxicated group (B), pretreated Nibali OLE +CCl4 group (C), pretreated K18 OLE +CCl4 group (C) and pretreated Sorani OLE +CCl4 group (E). X200

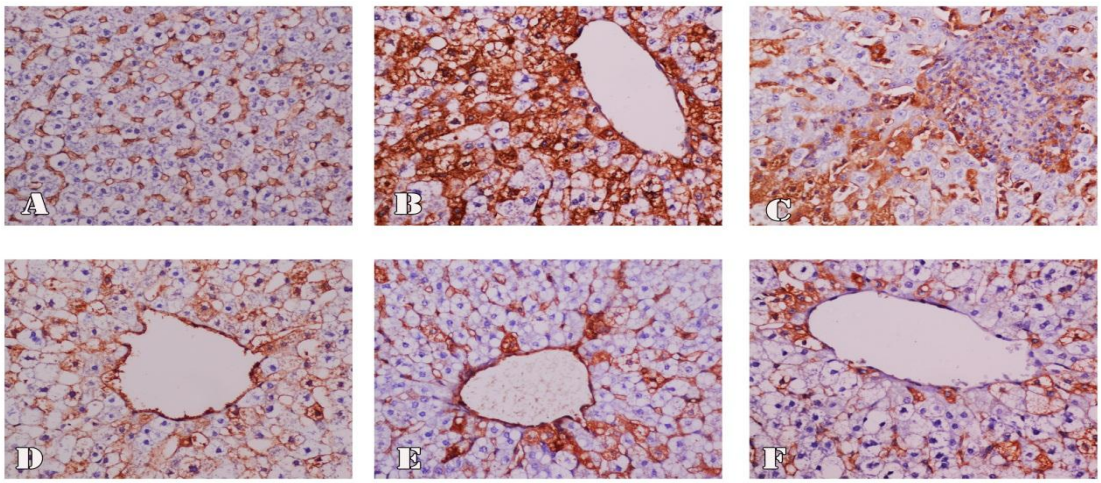

Fig. 4. Immunostained liver sections showing immunohistochemical distribution of TNF-α in control group (A), CCl4 intoxicated group (B), pretreated Nibali OLE +CCl4 group (C), pretreated K18 OLE +CCl4 group (C) and pretreated Sorani OLE +CCl4 group (E)(X200).

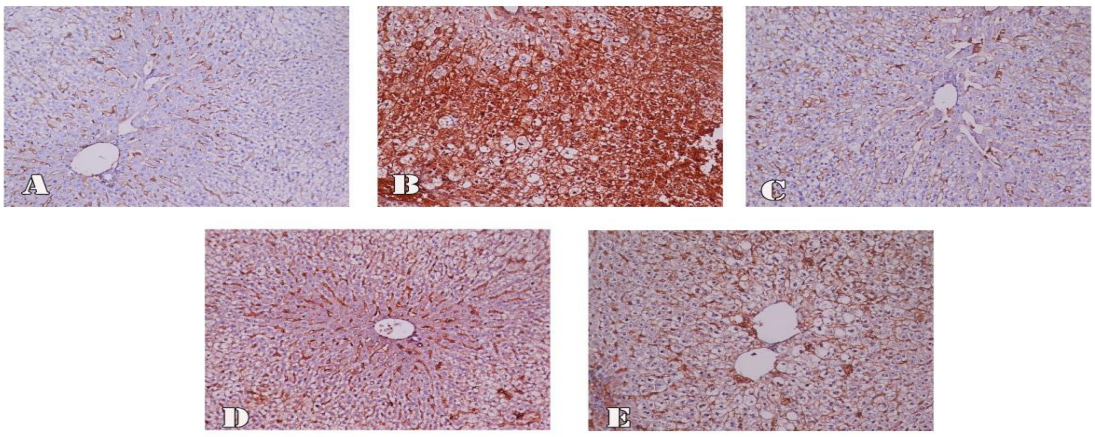

Fig. 5: Immunostained liver sections showing immunohistochemical distribution of IL-1β in control group (A), CCl4 intoxicated group (B), pretreated Nibali OLE +CCl4 group (C &D), and pretreated Sorani OLE +CCl4 group (E)(X400).

Table 1. Comparison of levels of total phenolics, total flavonoids, DPPH and peroxyl radical scavenging activities of the three OLE varieties. Values presented are mean ± SD; n = 3.

	TFC (mg GAE/g dw)	TFC (mg RE/g dw)	DPPH Scavenging %	Peroxyl Scavenging %
K18	17.20 ± 0.34	2.24 ± 0.353	34.01 ± 0.21/500µg/ml	39.43 ± 0.54/500µg/ml
Nibali	21.63 ± 0.69	2.64 ± 0.310	41.42 ± 0.32/500µg/ml	54.71 ± 0.68/500µg/ml
Sorani	12.77 ± 1.37	1.45 ± 0.092	27.22 ± 0.11/500µg/ml	35.23 ± 0.24/500µg/ml
Vitamin C	-	-	79 ± 0.11/ 125µg/ml	-
Trolox	-	-	-	75 ± 0.14/250µg/ml

Table 2. area percentage (A%) of hepatic iNOS, Caspase-3 and pro-inflammatory cytokines (TNF-α and IL-1β) expressions

Groups	iNOS	Caspase-3	TNF-α	IL-1β (A%)
Control	0.242±0.060	0.223±0.048	0.231±0.047	0.407±0.064
CCl_4	32.234±2.332*	39.421±2.161*	27.969± 1.100*	26.307±1.504*
CCl_4 + Nibali OLE	0.415±0.051**	1.021±0.054**	3.138±0.341**	3.114±0.455**
CCl_4+ K18 OLE	0.461±0.091**	0.931±0.201**	5.004±0.718**	3.769±0.814**
CCl_4+ Sorani OLE	3.052±0.447**	1.190±0.061**	9.254±1.152**	4.477±0.716**

Data is expressed as mean ± standard deviation, P. value = probability of chance, P< 0.05 is significant
* indicate a significant differences (*P<0.001 vs control group. ** P<0.001 vs CCl4 group, ANOVA test).

DISCUSSION

Liver is the major target organ of toxicity where several compounds are metabolized and eventually excreted. Hepatotoxicity remains a major reason for drug withdrawal from pharmaceutical development and clinical use. The mechanisms of hepatotoxicity are still being explored and include both hepatocellular and extracellular mechanisms [(26)]. In the present work, animal model of liver injury induced by CCl_4 was used to screen hepatoprotective effect of OLE from K18, Nibali, and Sorani cultivars on histopathological and immunohistochemical evaluation. In this study, CCl_4-induced hepatotoxicity was characterized microscopically by hepatic steatosis, apoptosis, and necrosis. These histopathological changes were supported by the results of immunohistochemical study where expressions of iNOS and caspase-3 were significantly increased in CCl4 intoxicated group compared to the control group. These results are supported by the previous hypothesis of **Ritesh *et al.*** [(27)] who mentioned that CCl_4 can activate the endoplasmic stress pathway and the generation of reactive oxygen species with induction of oxidative stress and lipid peroxidation. In addition, CCl_4 sensitize hepatocytes to certain receptors and these

activated receptor complexes cause caspase apoptotic cascade and induce apoptosis [28].

In the present work, oral administration of rabbits with prior to their exposure to CCl_4 protected hepatocytes from oxidative stress and apoptosis induced by CCl_4, where only degeneration of some hepatic cells was recorded in these groups OLEs particularly those of Nibali species. These findings were confirmed by the results of immunohistochemical study, where expression of iNOS-positive cells, and caspase-3 positive cells were significantly decreased in these pretreated groups comparing to CCl_4 intoxicated rabbits. These results are supported by those reported in previous studies [29,30].

According to the results of quantitative assessment of hepatic iNOS and caspase-3 expression, it was obvious that the anti-apoptotic effect of three extracts was nearly the same. While, their antioxidant activities were different, where the inhibitory effect of Nibali OLE against nitric oxide production was more than those exerted by OLEs of K18 and Sorani. This finding was supported by the *in vitro* antioxidant activity of the OLE varieties, where Nibali cultivar had better peroxyl scavenging with the highest total flavonoid content and DPPH scavenging activity, followed by K18 and then Sorani cultivars.

Concerning to the anti-inflammatory effect of OLEs, few previous studies demonstrated the potential of OLE as an anti-inflammatory agent [7,31,327]. In the present investigation, CCl4 hepatotoxicity induced inflammatory reaction evidenced by the presence of multiple areas of inflammatory cellular infiltration particularly around hyperplastic bile ducts with intensive TNF-α and IL-1β immunoreactivity in the examined liver. While, pretreatment with OLEs before induction of CCl4 hepatotoxicity substantially diminished the microscopic inflammatory changes induced by CCl_4 and significantly reduced TNF-α and IL-1β expression. These results are in a harmony with those mentioned in previous investigations [31,327].However in the present work, quantitative assessment of these hepatic proinflammatory cytokines revealed that Nibali OLE had potent anti-inflammatory effects than OLEs of K18 and Sorani. Finely, the present study concluded that OLEs of Nibali, K18 and Sorani cultivars had potent anti-inflammatory, antioxidant and anti-apoptotic activities that preserved hepatic cells against CCl_4-induced hepatic damage through attenuation of inflammatory markers, oxidative stress and apoptotic pathways. More, these activities of OLEs were probably species dependent.

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REFERENCES

1. **Navarro VJ and Senior JR (2006):** Drug-related hepatotoxicity. N. Engl. J. Med. 354: 731-739.
2. **Saukkonen JJ, Cohn DL, Jasmer RM, Schenker S, Jereb JA *et al.* (2006):** An official ATS statement: hepatotoxicity of antituberculosis therapy. Am. J. Respir. Crit. Care Med., 174: 935-952.
3. **Deng X, Luyendyk JP, Ganey PE and Roth RA (2009)**: Inflammatory stress and idiosyncratic hepatotoxicity: hints from animal models. Pharmacol. Rev., 61: 262- 282.
4. **Oshima Y, Namao K, Kamijou A, Matsuoka S, Nakano M, Terao K and Ohizumi Y (1995)**: Powerful hepatoprotective and hepatotoxic plant oligostilbenes, isolated from the oriental medicinal plant *Vitis coignetiae* (Vitaceae). Experientia, 51: 63–66.
5. **Abbasi AM, Khan MA, Ahmad M, Zafar M, Khan H, Muhammad M and Sultana S (2009)**: Medicinal plants for the treatment of jaundice and hepatitis based on socio-economic documentation. Afr. J. Biotechnol., 8:1643-1650
6. **Flora G, Gupta D and Tiwari A (2013)**: Preventive efficacy of bulk and nanocurcumin against lead-induced oxidative stress in mice. Biol. Trace Elem. Res., 152:31–40.
7. **Sedef N and Sibel K (2009)**: Olive tree (*Olea europaea*) leaves: potential beneficial effects on human health. Nutr. Rev., 67: 632–638.
8. **Farràs, M, Valls, R.M.; Fernández-Castillejo, S.; Giralt, M.; Solà, R.; Subirana, I.; Motilva M, Konstantinidou V, Covas M and Fitó M (2013)**: Olive oil polyphenols enhance the expression of cholesterol efflux related genes *in vivo* in humans. A randomized controlled trial. J. Nutr. Biochem., 24: 1334–1339.
9. **Castañer O, Corella D, Covas MI, Sorlí JV, Subirana I, Flores-Mateo G, Nonell L, Bulló M, de la Torre R, Portolés O *et al.* (2013)**: *In vivo* transcriptomic profile after a mediterranean diet in high-cardiovascular risk patients: a randomized controlled trial. Am. J. Clin. Nut., 98: 845–853.
10. **Omar SH (2010):** Cardio protective and neuroprotective roles of oleuropein in olive. Saudi Pharm. J.,18(3):111-121
11. **Lee OH, Lee BY, Lee J *et al.* (2009)**: Assessment of phenolics-enriched extract and fractions of olive leaves and their antioxidant activities. Bioresource Technol.,100(23):6107-6113.
12. **Benavente-García O, Castillo J, Lorente J, Ortuño A and Del Río J A (2000)** Antioxidant activity of phenolics extracted from Olea europea L. leaves. Food Chem., 68(4): 457-462.
13. **Khalatbary AR and Zarrinjoei GR (2012)**: Anti-inflammatory effect of oleuropein in experimental rat spinal cord trauma. Iran. Red Crescent Med. J. ,14(4): 229-234.

14. **Susalit E, Agus N, Effendi I, Tjandrawinata RR, Nofiarny D, Perrinjaquet-Moccetti T et al. (2011)**: Olive (*Olea europaea*) leaf extract effective in patients with stage-1 hypertension: Comparison with Captopril. Phytomedicine,18: 251-258.
15. **Nekooeian AA, Khalili A and Khosravi MB (2014)** Oleuropein offers cardioprotection in rats with simultaneous type 2 diabetes and renal hypertension. Indian J. Pharmacol., 46(4): 398-403.
16. **Kontogianni VG, Charisiadis P, Margianni E, Lamari FN, Gerothanassis IP, and Tzakos AG (2013)**: Olive leaf extracts are a natural source of advanced glycation end product inhibitors. J. Med. Food, 16 (9): 817–822.
17. **Jemai H, El Feki A and Sayadi S (2009)**: Antidiabetic and antioxidant effects of hydroxytyrosol and oleuropein from olive leaves in alloxan-diabetic rats. J. Agric. Food Chem., 57(19): 8798- 8804.
18. **Pratheeshkumar P, Son Y, Budhraja A, Wang X, Ding S, Wang L, Hitron A, Lee J, Kim D, Divya S *et al.* (2012)**: Luteolin inhibits human prostate tumor growth by suppressing vascular endothelial growth factor receptor 2-mediated angiogenesis. PLoS ONE,7:513-519.
19. **Barrajón-Catalán E, Taamalli A, Quirantes-Piné R, Roldan-Segura C, Arráez-Román D, Segura-Carretero A, Micol V and Zarrouk M (2015)**: Differential metabolomic analysis of the potential antiproliferative mechanism of olive leaf extract on the JIMT-1 breast cancer cell line. J. Pharm. Biomed. Anal.,105:156–162.
20. **Gonzalez-Menendez P, Hevia D, Rodriguez-Garcia A, Mayo J C and Sainz RM (2014)**: Regulation of GLUT transporters by flavonoids in androgen-sensitive and -insensitive prostate cancer cells. Endocrinology,155:3238–3250.
21. **Kim D O, Heo H J, Kim Y J, Yang H S and Lee C Y (2005)**: Sweet and sour cherry phenolics and their protective effects on neuronal cells. Journal of Agricultural and Food Chemistry, 53(26): 9921–9927.
22. **Eberhardt MV, Lee CY and Liu RH (2000)**: Antioxidant activity of fresh apples. Nature, 405: 903-904 .
23. **Cheung LM, Cheung PCK and Ooi VEC (2003):** Antioxidant activity and total phenolics of edible mushroom extracts. Food Chem., 7: 249-255 .
24. **Patro BS, Rele S, Chintalwar GJ, Chattopadhyay S, Adhikari S *et al.* (2002)**: Protective activities of some phenolic 1, 3- diketones against lipid peroxidation: possible involvement of the 1, 3-diketone moiety. Chembiochem., 3: 364-370.
25. **Bancroft JD, Stevens A and Turner DR (1996)** :Theory and Practice of Histological Technique. 4th ed., Churchill Livingston, N.Y., London, San Francisco, Tokyo.
26. **Andrade RJ, Lucena MI, Fernández MC, Pelaez G, Pachkoria K *et al.* (2005)**: Drug-induced liver injury: an analysis of 461 incidences submitted to the Spanish
registry over a 10-year period. Gastroenterology, 129: 512-521
27. **Ritesh A, Suganya HV, Dileepkumar Y, Rajashekar T and Shivanandappa A (2015):** Single acute hepatotoxic dose of CCl4 causes oxidative stress in the rat brain. Toxicology Reports, 2: 891-895
28. **Wang K (2014)**: Molecular mechanisms of hepatic apoptosis. Cell Death Dis.,5: 996-1003.
29. **Kaeidi A, Esmaeli-Mahani S, Sheibani V, Abbasnejad M, Rasoulian B, Hajializadeh Z *et al.* (2011):** Olive (*Olea europaea L*) leaf extract attenuates early diabetic neuropathic pain through prevention of high glucose-induced apoptosis: in vitro and *in vivo* studies. J. Ethnopharmacol.,136: 188-196.
30. **Boss A, Bishop K, Marlow G, Barnett M, and Ferguson L (2016):** Evidence to support the anti-ancer effect of olive leaf extract and future directions. Nutrients, 8: 513: 1-22.
31. **Chebbi Mahjoub R, Khemiss M, Dhidah M, Dellai A, Bouraoui A and Khemiss F (2011):** Chloroformic and methanolic extracts of Olea europaea L. leaves present anti-Inflammatory and analgesic activities. ISRN Pharmacol., 2011: 1-5.
32. **Khadija M A (2013)**: The effect of olive leaf extract in decreasing the expression of two proinflammatory cytokines in patients receiving chemotherapy for cancer. A randomized clinical trial. The Saudi Dental Journal, 25(4): 141–147.