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Potential antioxidant properties and hepatoprotective effects of *Juniperus phoenicea* berries against CCl_4 induced hepatic damage in rats

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ABSTRACT

Objective: To investigate the antioxidant and hepatoprotective properties of *Juniperus phoenicea* (*J. phoenicea*) berries against CCl_4-induced oxidative damage in rats.
Methods: Hepatotoxicity was induced in albino Wistar rats by single dose of CCl_4 dissolved in olive oil (1 mL/kg BW, 1/1 in olive oil, *i.p.*). Aqueous extract of *J. phoenicea* berries (AEJP) was administered at the dose of 250 mg/kg/day by gavage for 12 days.
Results: Obtained results revealed that administration of CCl_4 caused a significant increase in plasma ASAT, ALAT, ALP and LDH activities and total bilirubin concentration, compared to the control group. While, albumin and total protein concentration were significantly lower. Additionally, a significant decrease in the level of hepatic GSH, GPx and GST activities associated with a significant increase of MDA content in CCl_4 group than those of the control. However, the treatment of experimental rats with AEJP prevented these alterations and maintained the antioxidant status. The histopathological observations supported the biochemical evidences of hepatoprotection.
Conclusions: The results of the present investigation indicate that *J. Phoenicea* possesses hepatoprotective activity and this effect was may be due to its antioxidant properties.

1. Introduction

The liver as a vital organ has a wide range of functions in the body, including detoxification, plasma protein synthesis, and glycogen storage. Oxidative stress is considered as the imbalance between reactive oxygen species (ROS) production and antioxidant protective mechanism. It is principal cause of the development of hepatic disorders [1]. Various types of liver disorders are characterized by cirrhosis, jaundice, tumors, metabolic and degenerative lesions and liver cell necrosis *etc* [2]. The management of liver disorders is still a challenge to the modern medicine [3]. Conventional or synthetic drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects [4]. Carbon tetrachloride (CCl_4) is a highly toxic chemical and a well known hepatotoxin used extensively to investigate the hepatotoxicity in animal models [5]. CCl_4 by itself does not have cytotoxic effects on the liver but its metabolic products such as generated trichloromethyl free radicals are responsible for the toxicity and the production of lipid peroxidation [6]. It has

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been reported that antioxidants appear to act against disease processes by increasing the levels of endogenous antioxidant enzymes and decreasing lipid peroxidation [7]. Lots of studies indicate that natural substances from edible and medicinal plants exhibited strong antioxidant activity that could act against CCl_4-induced liver damage, because they contain lots of free radical scavenger such as phenolic acids and flavonoid compounds [8].

The genus *Juniperus* belongs to the Cupressaceae family, comprising about 67 species all over the world [9]. Plants from the *Juniperus* genus have found application in different European cuisines as a spice, flavouring for alcoholic drinks, as well as in cosmetics [10]. Furthermore, these plants have an extensively history of use in global folk medicine for various disorders, such as common colds, urinary and kidney infections and dermatological disorders [11]. Among their species, *Juniperus phoenicea* (*J. phoenicea*) is an evergreen tree indigenous to the North Africa. In Algeria, *J. phoenicea* grows on the steppes, commonly known as 'Arar'. This plant is considered as an important medicinal plant largely used in the Algerian folk medicine as a diuretic, stimulative, stomach tonic, pulmonary and depurative disinfectant. A decoction of the leaves and berries has been used to treat diarrhea, rheumatism, and diabetes. Previous phytochemical studies reveal that *J. phoenicea* contains a large variety of compounds, mainly diterpenoids, biflavonoids, lignans, phenyl propane glucosides, two furanone glucosides, norterpene and sesquiterpene glucosides [9]. There are many papers report on the chemical composition of leaves and berries essential oils of *J. phoenicea* grown in north Mediterranean basin [12]. However, little attention has been given to the phenolic contents, antioxidant and biological activities of *J. phoenicea* berries. Therefore; the aim of the present study was to evaluate the antioxidant and hepatoprotective properties of *J. phoenicea* berries against CCl_4-induced oxidative damage in rats.

2. Materials and methods

2.1. Chemicals

Carbon tetrachloride and all other chemicals and reagents used in the study were obtained from Sigma-Aldrich Corporation (St. Louis, Missouri, USA).

2.2. Plant material

The plant material consisted of mature 'berries' of *J. phoenicea*, collected in month of March 2013, from Aouinet province of Tebessa (Algeria). The identity of plant was confirmed by Professor A. Chefrour in the botany laboratory, Department of Pharmacy, Faculty of Medicine, Badji Mokhtar University, Annaba, Algeria. The berries were cleaned, dried in shade and powdered then stored in air tight container.

2.3. Preparation of plant extracts

2.3.1. Preparation of aqueous extract

Five grams of the dried berries powder of *J. phoenicea* were boiled in 50 mL of distilled water and heated for 15 min. The extract was then filtered through Whatman filter paper and directly administered orally by gavage to the animals at a volume of 250 mg/kg body weight (BW).

2.3.2. Preparation of methanol extract

Twenty grams of the dried berries powder of *J. phoenicea* were extracted at room temperature for 72 h, in 100 mL methanol (85%) three times. The hydro-alcoholic extract was filtered through Whatman filter paper, and stored at 4 °C. The filtrate was concentrated under reduced pressure at 60 °C. The extract was weighed and stored at 4 °C in storage vials.

2.4. Chemical characterization

2.4.1. Total phenolic contents

The total phenolic content was determined by the Folin-Ciocalteu method of Waterman and Mole [13]. A volume of 10 μL of the AEJP and MEJP was mixed with 50 μL of Folin-Ciocalteu reagent. After 5 min, 150 μL of 20% Na_2CO_3 was added and the mixture was shaken once again for 1 min. Finally, the solution was brought up to 790 μL by adding distilled water. After 90 min, the absorbance was measured at 760 nm and the total phenolic content was calculated from the calibration curve using gallic acid as a standard. The results were expressed as mg of gallic acid equivalent per g (mg GAE/g) of dry weight DW of extract.

2.4.2. Total flavonoid contents

Total flavonoid contents were measured by a colorimetric assay according to the method of Zhishen *et al* [14], and quercetin was used as a standard to construct the calibration curve. Briefly 250 μL of extracts were mixed with 1.25 mL of distilled water and 75 μL of 5% $NaNa_2$ solution. After 6 min, 150 μL of 10% $AlCl_3$ solution were added. 6 min later, 0.5 mL of 1M NaOH solution were added and then the final volume was adjusted to 2.5 mL with distilled water and mixed thoroughly. The absorbance was measured at 510 nm versus a blank prepared without extract and data were expressed in mg quercetin equivalent per g (mg QE/g) of DW of extract.

2.4.3. Total condensed tannin contents

Condensed tannins were determined according to the method of Julkunen-Tiitto [15]. A volume of 50 μL of extracts was added to 1.5 mL of 4% vanillin solution in methanol, and then 750 μL of HCl were added. The mixture was allowed to stand in the dark for 20 min, and absorbance was measured at 500 nm against methanol as a blank. Catechin was used to make the standard curve and the amount of total condensed tannins is expressed as mg catechin equivalent per g (mg CE/g) of DW of extract.

2.5. In vivo study

2.5.1. Animals and treatments

Twenty-four male albino Wistar rats (aged between 8 and 9 weeks) with an average weighing (180–200) g were provided from Pasteur Institute (Algiers, Algeria). They were housed in cages at room temperature of (22 ± 2) °C and kept under standard conditions of a 12 h light/dark cycle and minimum relative humidity of 40%. The rats were fed with a standard food supplied by (ONAB, El harrouch, Algeria) and water was offered *ad*

libitum. After acclimation for 2 weeks, the rats were divided into four groups each containing six rats.

Group I (control group): animals served as normal control.

Group II (CCl_4-treated group): animals served as toxic control, received intraperitoneal administration (*i.p.*) of CCl_4 (1 mL/kg BW, 1/1 in olive oil).

Groupe III (CCl_4+ AEJP-treated group): animals treated with AEJP at a dose of 250 mg/kg BW via oral gavage.

Groupe IV (AEJP-treated group): animals received AEJP alone (250 mg/kg BW).

The experimental procedures were carried out according to the National Institute of Health Guidelines for Animal Care and approved by the Ethics Committee of the University. At the end of the 12 days experiments, the rats were sacrificed by cervical decapitation. Blood was collected and centrifuged at 2 200 g for 15 min. Plasma samples were stored at 20 °C for biochemical analysis of ASAT, ALAT, ALP, LDH, total bilirubin, albumin and total protein.

2.5.2. Tissue preparation

Liver samples were dissected out and washed immediately in 0.9% NaCl solution, and then were homogenized on ice in buffer solution of phosphate-buffered saline 1: 3 (w/v; 1 g tissue with 3 mL PBS, pH 7.4) and centrifuged at 10 000 ×*g* for 15 min at 4 °C. The resulting supernatants were used to determine antioxidant enzyme activities, reduced glutathione (GSH) and malondialdehyde (MDA) contents.

2.5.3. Plasma biochemical parameters

Biochemical parameters were assayed according to standard methods. The levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), total bilirubin, albumin and total proteins were estimated using commercial kits from Spinreact laboratories, Spain (AST, ref. 1001160 and 1001161; ALT, ref. 1001170 and 1002171; ALP, ref. 1001130 and 1001131; LDH, ref. 1001260; total bilirubin, ref. 1001044; albumin, ref. 1001020–1001023; total proteins, ref. 1001291).

2.5.4. Tissue biochemical parameters

2.5.4.1. Estimation of lipid peroxidation levels

Lipid peroxidation process is determined in supernatant of homogenate liver tissue by the thiobarbituric acid (TBA) method which estimates the malondialdehyde formation (MDA) according to Buege and Aust [16]. Absorbance of TBA-MDA complex was determined at 530 nm and the level of hepatic MDA was expressed as nmoL/mg protein.

2.5.4.2. Estimation of reduced glutathione levels

Reduced glutathione (GSH) was estimated by the method of Ellman [17] modified by Jollow *et al* [18]. Determination of GSH is based on the reaction of DTNB [5,50- dithiobis-(2-nitrobenzoic acid)] with GSH and yield a yellow color with a maximum absorbance at 412 nm. Reduced glutathione concentration was expressed as nmoL/mg protein.

2.5.4.3. Estimation of antioxidant enzymes activities

Glutathione peroxidase (GPx) activity was assessed by a modification of the colorimetric method of Flohe and Günzler [19] using hydrogen peroxide as a substrate in the presence of GSH. The absorbance was recorded at 420 nm and specific activity of this enzyme is expressed as µmoL GSH/mg protein.

Glutathione-S-transferase (GST) activity was assayed by following the conjugation of glutathione with 1-chloro-2,4-dinitrobenzene (CDNB) at 340 nm as described by Habig *et al* [20]. The enzyme activity was expressed as nmol CDNB conjugate/min/mg protein.

2.5.4.4. Protein determination

Protein content of liver homogenates was determined according to Braford [21], using bovine serum albumin as a standard.

2.5.5. Histopathological examination

A portion of the liver was fixed in 10% formalin for at least 24 hand then embedded in paraffin. Sections of 5 µm in thickness were cut, deparaffinized, dehydrated, and stained with hematoxylin and eosin (H&E) according to method of Hould [22]. The histopathological changes in the liver were observed under a light microscope.

2.6. Statistical analysis

The data were expressed as mean ± SEM. Differences between groups were analyzed by one-way analysis of variance (ANOVA), followed by Tukey's *t*-test. The results were considered significant if $P \leq 0.05$.

3. Results

3.1. Phenolic contents

Phytochemical analysis of the AEJP and MEJP showed high content of total phenolics (38.86 ± 0.23) and (49.43 ± 0.88) mg of GAE/g of DW of extracts respectively. Flavonoids are a group of polyphenolic compounds with various chemical structures and characteristics. The total flavonoid content of the AEJP was (2.09 ± 0.37) mg of QE/g, while MEJP contained (2.88 ± 0.10) mg of QE/g. Finally, values obtained for the condensed tannins contents of AEJP and MEJP were (15.12 ± 0.70) and (23.25 ± 0.96) mg of CE/g, respectively.

3.2. Effects of AEJP on blood biochemical parameters

The results of blood biochemical parameters are presented in Table 1. Administration of CCl_4 induced significant increase in the enzymatic activities of AST, ALT, ALP, LDH and total bilirubin levels when compared with those of the normal control group. Conversely, animals treated with AEJP exhibited significant decrease in the activities of the enzymes AST, ALT, ALP, LDH and total bilirubin as compared to CCl_4 group. Albumin and total protein concentration were decreased in CCl_4-treated group compared to the control and also to the CCl_4+ AEJP group. Moreover, no significant differences in these biochemical parameters were found between control group and AEJP-treated group.

3.3. Effects of AEJP on lipid peroxidation

Lipid peroxidation of biomembranes is one of the principal degenerative effects of free radicals. As shown in Table 2, the

Table 1

Changes in biochemical parameters of control and treated rats.

Parameters	Control	CCL_4	CCL_4+ AEJP	AEJP
AST (U/L)	179.04 ± 12.70	$255.12 \pm 11.45^{***}$	$200.53 \pm 15.66^{\#\#\#*}$	190.20 ± 12.74
ALT (U/L)	56.27 ± 4.93	$77.05 \pm 9.13^{***}$	$61.41 \pm 5.51^{\#\#}$	58.45 ± 7.71
ALP (U/L)	190.39 ± 12.36	$260.61 \pm 10.84^{***}$	$202.65 \pm 7.67^{\#\#\#}$	184.54 ± 9.97
LDH (U/L)	618.16 ± 18.60	$768.50 \pm 12.54^{***}$	$661.50 \pm 22.47^{\#\#\#**}$	631.83 ± 14.4
Total protein (g/L)	81.09 ± 5.81	$68.18 \pm 4.25^{***}$	$76.87 \pm 2.70^{\#\#}$	78.89 ± 3.42
Albumin (g/L)	41.22 ± 7.48	$29.83 \pm 2.06^{**}$	$36.40 \pm 7.23^{\#}$	40.73 ± 2.85
Total bilirubin(mg/L)	1.37 ± 0.33	$2.33 \pm 0.44^{**}$	$1.92 \pm 0.13^{\#**}$	1.52 ± 0.19

Values are given as mean ± SEM for groups of six animals each. $^*P \leq 0.05$ compared with control group; $^{**}P \leq 0.01$ compared with control group; $^{***}P \leq 0.001$ compared with control group. $\#P \leq 0.05$ compared with CCL_4 group; $\#\#P \leq 0.01$ compared with CCL_4 group; $\#\#\#P \leq 0.001$ compared with CCL_4 group.

Table 2

Effect of AEJP on liver tissue MDA, GSH levels and antioxidant enzyme activities of control and treated rats.

Parameters	Control	CCl_4	CCl_4+ AEJP	AEJP
GSH△	120.870 ± 15.380	85.700 ± 14.420**	$113.660 \pm 11.360^{\#\#}$	123.700 ± 12.540
MDA△	0.390 ± 0.100	0.560 ± 0.090**	$0.440 \pm 0.070^{\#}$	0.380 ± 0.030
GPx▲	2.006 ± 0.070	1.700 ± 0.128***	$1.930 \pm 0.040^{\#\#*}$	2.010 ± 0.060
GST△	0.031 ± 0.003	0.017 ± 0.003***	$0.027 \pm 0.003^{\#\#\#*}$	0.029 ± 0.001

Values are given as mean ± SEM for groups of six animals each. △: nmol/mg protein; ▲: μmol GSH/mg protein. $^*P \leq 0.05$ compared with control group; $^{**}P \leq 0.01$ compared with control group; $^{***}P \leq 0.001$ compared with control group. $\#P \leq 0.05$ compared with CCL_4 group; $\#\#P \leq 0.01$ compared with CCL_4 group; $\#\#\#P \leq 0.001$ compared with CCL_4 group.

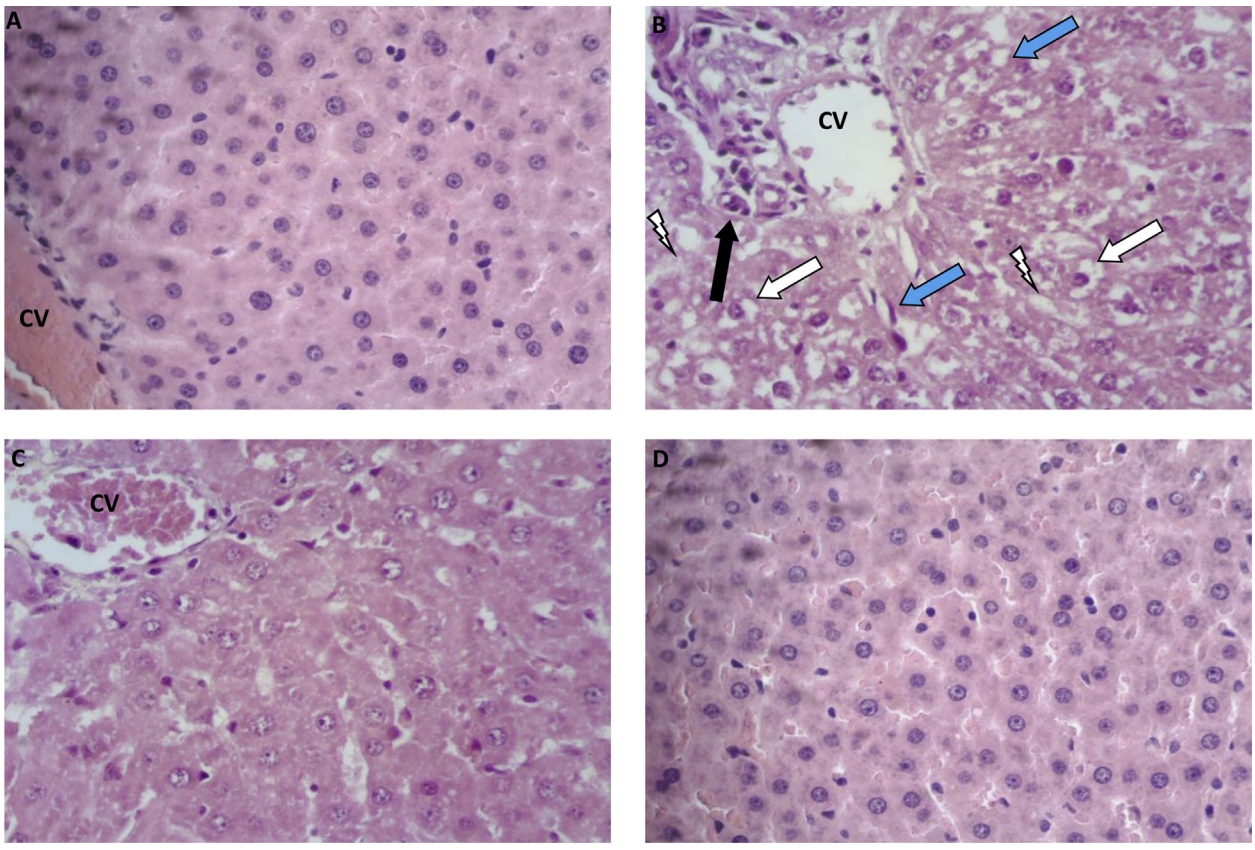

Figure 1. Photomicrograph of H&E stained sections of liver from a control rat showing normal histological structure (Figure 1A, ×400). CCl_4-treated rats liver showing necrosis around the central vein (blue arrow), inflammatory cell infiltration (black arrow), ballooning degeneration (white arrow) and sinusoidal dilatation (bolt) (Figure 1B, ×400). Liver sections of the animals administered AEJP showed moderate degree of liver damage and inflammatory cell, protection from hepatocyte degradation and centrilobular necrosis (Figure 1C, ×400). AEJP-treated rat liver showing normal appearance of hepatocytes (Figure 1D, ×400).

MDA level was significantly increased in the CCl_4-treated animals compared to the normal. AEJP significantly caused diminution of CCl_4- elevated MDA level compared to those of CCl_4 group.

3.4. Effects of AEJP on GSH contents

GSH constitutes the first line of defense against free radicals. The toxicity of CCl_4 significantly decreased the hepatic GSH levels in the CCl_4-treated animals compared to the normal control group (Table 2). Administration of AEJP ameliorated the GSH levels compared to that of CCl_4-treated group.

3.5. Effects of AEJP on antioxidant enzyme activities

The activities of GST and GPx of the liver tissue are shown in Table 2. Injection of CCl_4 led to a lower GST and GPx activities compared to the normal control group. However, animals treated with AEJP showed significant increase in GPx and GST as compared to CCl_4 group.

3.6. Histopathological studies

The liver section in normal control animals indicated the presence of normal hepatic cells with well preserved cytoplasm prominent nucleus and visible central veins (Figure 1A). In contrast, the CCl_4-treated rats showed liver sections with severe structural damage characterized by necrosis around the central vein, inflammatory cell infiltration, ballooning degeneration and sinusoidal dilatation (Figure 1B). However, the liver sections of the animals administered AEJP at the dose of 250 mg/kg (BW) showed moderate degree of liver damage and inflammatory cell, protection from hepatocyte degradation and centrilobular necrosis (Figure 1C). Whereas, the histopathological examination of the liver AEJP-treated group showed normal hepatocytes comparable to the normal control group (Figure 1D).

4. Discussion

Plants rich in secondary metabolites, including phenolic compounds, have antioxidant activity due to their redox properties and chemical structures. The results indicate that our extracts of *J. Phoenicea* berries contained high levels of total phenolic compounds, tannins and flavonoids. However, the results of phenolic contents (38.86 ± 0.23) and (49.43 ± 0.88) mg of GAE/g of DW of AEJP and MEJP respectively, were lower than that reported by Hayouni *et al* [23] for *J. phoenicea* from Tunisia, which ranged from (66.10 ± 1.20) to (202.00 ± 0.43) mg GAE/g of dry material. In another recent work, Amalich *et al* [24] recorded 5.35 mg GAE/g of total phenolic compounds of *J. phoenicea* berries from Morocco. Such variation in composition can be attributed to the diversity of geographical environments (soil, sunlight, temperature, precipitation, *etc*). Many studies suggest that antioxidant substances block the action of free radicals which have been implicated in the pathogenesis of many diseases including atherosclerosis, Alzheimer, cancer and liver disorders [25]. It has been reported that the antioxidant activity of flavonoids and condensed tannins depends on the presence of free OH groups, especially 3-OH [26].

Results of our phytochemical analysis suggest that *J. phoenicea* berries contain phytochemical constituents that are capable of donating hydrogen to a free radical to scavenge the potential damage. Many extracts from plants have been investigated for hepatoprotective and antioxidant effects against hepatotoxin-induced liver damage [27]. Carbon tetrachloride (CCl_4) is one of the most commonly used hepatotoxins in experimental study of liver diseases. Among the various mechanisms involved in the hepatotoxic effect of CCl_4, one is oxidative damage through free radical generation. CCl_4 is biotransformed by cytochrome P450 to the trichloromethyl free radical (CCl_3) that induces membrane lipid peroxidation and disturbs Ca^{2+} homeostasis to produce hepatocellular injury [28]. In our study *in vivo*, we examined the hepatoprotective activity of AEJP against CCl_4-induced hepatotoxicity in rats. Results showed that administration of CCl_4 developed significant hepatic damage and oxidative stress as evidenced by substantial increases in the plasma activities of AST, ALT, ALP, LDH, and total bilirubin. These findings are in agreement with those reported by [4,5,29]. Administration of AEJP significantly prevented hepatocyte injury induced by CCl_4. Reduction in the level of AST, ALT and LDH is an indication of the hepatic cell regeneration process. While, reduction in the level of ALP with the concurrent depletion with the raised bilirubin level suggests the stability of the biliary function [4]. In addition, the level of total protein and albumin were significantly reduced in CCl_4 treated rats compared to normal rats. AEJP has increased the levels of plasma total protein and albumin, which indicates hepatoprotective activity. Stimulation of protein synthesis has been advanced as a contributory hepatoprotective mechanism which accelerates the regeneration process and the production of liver cells [30].

Among the many secondary products during lipid peroxidation, MDA is a commonly used biomarker for the assessment of lipid peroxidation [4,31]. Its elevated levels could reflect the degrees of lipid peroxidation injury in hepatocytes [32–34]. In the present study, we found that the level of MDA was increased in the liver of CCl_4 treated rats compared to normal rats. Treatment with AEJP significantly reversed these changes. Hence it may be possible that the mechanism of hepatoprotection of extract is due to its antioxidant effect.

Antioxidant enzymes can detoxify free radicals by converting them back to more stable molecules within the cell [30]. GPx belongs to a class of enzymes that catalyze the reduction of H_2O_2 and hydro peroxides to non-toxic products [35]. It has been reported that CCl_4 produces an decreased liver GPx activity [29,36,37]. Our results confirm this effect which showed that GPx activity was significantly decreased in the liver of CCl_4 treated animals compared with control group rats. CCl_4 administration also significantly decreased the activity of GST, which plays an important role in protecting cells against ROS mediated injury by detoxification of lipid hydro peroxides formed due to oxidative damage [38]. However, GPx and GST activity was significantly elevated by administration of AEJP to CCl_4-treated rats. The decrease in the activity of antioxidant enzymes suggested that the balance between the oxidant and pro-oxidant was disturbed by CCl_4, and treatment of experimental rats with *J. Phoenicea* effectively reverts this imbalance and restores the level of antioxidant enzymes. The effect of *J. Phoenicea* on GST activity can be explained by an increase in the transcription of the gene encoding GST, since it has been

reported that genes encoding phase II enzymes are highly induced by antioxidant compounds [39].

Regarding non-enzymic antioxidants, GSH is an intracellular reductant and protects cells against free radicals, peroxides and other toxic compounds. In addition, GSH is central to the cellular antioxidant defenses and acts as an essential cofactor for antioxidant enzymes including GPx and GST [40,41]. Depletion of hepatic GSH has been shown to be associated with an enhanced toxicity to chemicals, including CCl_4 [42]. In the present study, a decrease in hepatic tissue GSH level was observed in the CCl_4-treated group compared to normal group. It has been demonstrated that in free radical-mediated hepatic cell injury, consumption of hepatocellular GSH is associated with the initiation of cell injury with lipid peroxides formation [6]. The increase in hepatic GSH level in the rats treated with AEJP may be due to a novo GSH synthesis or GSH regeneration.

Furthermore, the histopathological findings of liver samples are in agreement with the results of biochemical studies. CCl_4 caused damage to the hepatic architecture and produced histological changes such as inflammatory cell infiltration, necrosis of hepatocytes and sinusoidal dilatation. These results are in accordance with those obtained by Ben Hsouna *et al* [29] and Kale *et al* [43] which indicate that CCl_4 cause histopathological liver changes in rats. Administration of AEJP improved the structure of hepatic cells, confirming the hepatoprotective effect of AEJP. In fact, the relatively high polyphenol content of the AEJP is indicative of antioxidative properties *in-vivo*.

Our results were in line with previous studies on hepatoprotective activity of *J. phoenicea* berries due to their folkloric use against liver diseases in some countries. In a study of Alqasoumi *et al* [44], the petroleum ether, chloroform and methanol fractions obtained from the aerial parts of *J. phoenicea* growing in Saudi Arabia showed significant hepatoprotective activity against carbon tetrachloride induced liver injury in rats. The authors showed that hinokiflavone isolated from the aerial parts of *J. phoenicea* exhibited significant hepatoprotective activity comparable with the standard drug silymarin in reducing the elevated liver enzymes and restoring normal appearance of hepatocytes. On the other hand, the AEJP berries growing in Libya was tested against thioacetamide-induced hepatocytotoxicity in rats. They found that the extract cause a significant antihepatotoxic effect as compared to silymarin used as reference drug [45]. The authors claimed that the antihepatotoxic activity of *J. phoenicea* berries against thioacetamide might be due to their polyhydroxylated flavonoids, quinic acid derivative and polyunsaturated fatty acids.

The results of this study suggest that *J. phoenicea* protected rats against CCl_4 induced hepatotoxicity. The hepatoprotective effects of *J. phoenicea* may be due to its antioxidant and free radical scavenging properties. However, further investigations are essential to elucidate the precise mechanism of active agents of *J. phoenicea* protection against CCl_4 induced hepatotoxicity.

Conflict of interest statement

We declare that we have no conflict of interest.

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