Effectiveness of aqueous extract of marjoram leaves in the treatment of aspartame liver toxicity

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Background and objectives
Although there are several toxicological studies on aspartame (ASP), its histopathological effects on the liver have received little attention. Natural marjoram [*Origanum majorana* (OM)] is extensively studied for its ability to protect cells from damages. The present study was to evaluate the chronic effects of ASP, and marjoram as well as the protective effect of aqueous extract of marjoram leaves (OM) against ASP-induced liver toxicity.
Materials and methods
Seventy-two female albino rats (180±20 g) were divided into nine groups: G1 as a control, G2 and G3 received ASP, G4 and G5 were administrated water extract OM, and G6, G7, G8, and G9 were treated with ASP+OM. Serum glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, vascular endothelial growth factor levels, and histological examination of the liver tissues were detected for all groups.
Results and conclusion
ASP caused a significant increase in glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, and vascular endothelial growth factor levels. A combination of OM (100 or 300 mg/rat) with ASP (50 or 100 mg/rat) improved most of the previous parameters. Histological examination of the liver showed some abnormality, but no significant morphological changes in G6, G7, G8, and G9. The treated rats (G2 and G3) showed hepatic fibrosis, nuclear changes, hepatocyte degeneration disruption hepatic cords, and cytoplasmic vacillations. ASP groups treated with OM showed improvement of many hepatic changes. Thus, OM may have a protective effect on ASP hepatotoxicity.

Keywords:
aspartame, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, liver, marjoram, vascular endothelial growth factor

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Introduction

Aspartame (ASP) is the most used artificial sweetener in the world. Each year more than 34 000 000 pounds of ASP are produced and are used in more than 6000 products including more than 500 pharmaceuticals. Hundreds of millions of people consume ASP worldwide [1,2].

ASP was first approved by the Food and Drug Administration in 1981 as a sweetener tablet, and for use in chewing gum, breakfast cereal, and as a general-purpose sweetener in all foods and drinks [3]. By far, ASP has been the most controversial artificial sweetener because of its potential toxicity. It is suggested that serious further testing and research be undertaken to eliminate all controversies surrounding this product. Despite numerous toxicological studies on ASP, its histopathological effects on the liver and the kidneys have received little attention [4,5]. Certainly, the effect of ASP on liver cancer is not clear until now. Several studies provide no evidence to support an association between ASP and cancer [6,7].

Liver function tests are a helpful screening tool, which are an effective modality to detect hepatic dysfunction and give information about the state of a patient's liver [8]. These tests include liver transaminases [aspartate aminotransferase (AST) and alanine aminotransferase (ALT)] [9,10]. Experimental studies demonstrated that vascular endothelial growth factor (VEGF) promotes tumor growth, angiogenesis, and metastasis formation [11–14]. Serum high VEGF level indicates a poor prognosis for patients with hepatocellular carcinoma [15].

Marjoram is one of the kitchen herbs. Marjoram [*Origanum majorana* (OM)] is found in many world countries such as Egypt, Arabia, and Morocco [16–18]. It is cultivated for its aromatic leaves for flavoring and other culinary purposes. It is also excellent in salads. Many of the folklore medicinal claims about marjoram were confirmed in different experimental models [17]. It is a well-liked home remedy for chest infection, cough, sore throat, rheumatic pain, nervous disorders, cardiovascular diseases, epilepsy, insomnia, skin care, flatulence, and stomach disorders in Egypt and Arabia [18–20]. Marjoram has a high antioxidant capacity due to its high polyphenolic content [20]. The medicinal effects of marjoram include the stimulation of gastrointestinal tract, hypoglycemic, diuretic, and antibacterial activities [21].

Hence, despite the increasing popularity of ASP, its various clinical effects with particular regard to their safety require further investigations. The present work was carried out to study the chronic effect of ASP and/or OM on some physiological aspects of the female albino rats as well as the protective effect of the aqueous extract of marjoram leaves against hepatotoxicity of ASP.

Materials and methods

Preparation of materials

ASP was purchased from a pharmacy in the form of tablets (20 mg/tablet). The human acceptable daily intake of ASP is 40 mg/kg [22]. The acceptable daily intake doses were converted to rat doses according to Ghosh [23]. ASP was dissolved in distilled water before administration by dose to each rat (50 and 100 mg/ml/rat) daily.

OM, a whole plant, was purchased from the local market in Madinah, Saudi Arabia. The dried leaves were ground and extracted by maceration in distilled water (200 g/1000 ml) for 1 day at 37–40°C [24]. The extract was then filtered and evaporated to dryness in a rotary evaporator The dried extract was dissolved in deionized distilled water to a final stock concentration of 100 and 300 mg/ml/rat for further use and the procedure was repeated weekly [25].

Animals

Seventy-two healthy female Wistar albino rats, body weight (180±20 g), were obtained from the Animal House at the National Research Centre. The animals were maintained under standard laboratory conditions and were allowed to have food and water. All rats were housed in clean plastic cages under controlled temperature (23±2°C), humidity (60±5%), and 12 h light/dark cycle. All animal methods are in accordance with the recommendations stated by ethics committee of the National Research Centre approval on animal care [26].

Experimental design

Groups

The rats were randomly and equally divided into nine groups: (n=8). G1 (control) was administrated distilled water daily. G2 and G3 (ASP) were administrated 50 and 150 mg ASP/rat daily, respectively. G4 and G5 (OM) were administrated 100 and 300 mg OM/rat daily, respectively. The cotreated groups (ASP+OM), G6 and G7, were administrated 50 mg ASP+100 or/and 300 mg OM daily, respectively and G8 and G9 were administrated 150 mg ASP or/and 100 or 300 mg OM daily, respectively.

Sample collections

At the end of the experimental period (6 months), all the animals were exposed to mild anesthesia. The blood was collected from the orbital sinus, left for 15 min at room temperature, and centrifuged at 3000 rpm for 15 min. The separated sera were kept at −20°C until biochemical analyses.

Biochemical determinations

Liver function measurements

Serum glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) were determined using the reagent kits purchased from Biodiagnostic Co. (Giza, Cairo, Egypt). ALT and AST were measured by spectrophotometer (Shimadzu UV Recording 2401 PC, Japan) as described by Reitman and Frankel [27], and were expressed in terms of U/l.

Tumor marker measurements

VEGF was measured by using a commercially available enzyme-linked immunoassay [28] (ELISA, Quantikine; R&D Systems Europe, Abingdon, UK). Optical density was measured at 450 nm using a microtiter plate reader (MR 5000, R&D Systems Europe, Abingdon, UK). All samples were assayed in duplicate, and expressed in terms of ng/l.

Histopathology

The control and treated rats were killed after being fasted about 12 h. Liver from different groups were removed and fixed by immersion in formaldehyde solution (10%). Eosin and hematoxylin stain were used to study the general histological structure of the liver [29]. The Periodic Acid Schiff (PAS) reaction technique and Masson's trichrome stain were used to

define the mucopolysaccharides, polysaccharides, and connective tissue elements (collagen) in the liver [30,31]. All slides were viewed under electronic microscope with camera attachment (Zeiss, Germany).

Statistical analysis

The data were statistically analyzed by statistical package for the social sciences, version 18. Quantitative data were represented as mean±SE. Comparisons were done through one-way analysis of variance. The difference was considered significant at *P* value less than or equal to 0.05 levels.

Results

Biochemical determination

The results of the current study (Table 1) showed that oral ASP (G2 and G3) administration caused marked liver dysfunction as evidenced by the significant increase in serum ALT and AST levels ($P<0.001$). Animals that received OM (G4 and G5) showed no significant differences in the level of GOT and GPT compared with the control group (G1). On the other hand, a significant decrease ($P<0.001$) was recorded in groups (G6–G9) comparable to G2 and G3. However, there was marked increase in the level of AST and ALT of treated rats with ASP+OM comparable to G1; this increase was more marked in G6 and G7 (50 and/or 150 mg ASP+100 mg OM) than G8 and G9 (50 and/or 150 mg ASP+300 mg OM).

The results in Table 1 also showed that there is a significant increase ($P<0.015$) in the serum level of VEGF in G2 and G3 treated with ASP as compared with G1. The rats treated with ASP+OM (G8 and G9) showed a slight increase in VEGF level compared with the control (G1). In addition, the rats treated with $_{50\text{ and/or }150\text{ mg}}$ ASP+300 OM (G8 and G9) recorded a significant decrease in ALT and VEGF than that treated with 50 and/or 150 mg ASP+100 OM (G6 and G7).

Our study observed that the treatment with OM successfully ameliorated the elevated of ALT, AST, and VEGF; however, a high dose of OM (300 mg) is more effective.

Histopathology

Figures 1–5 showed the transverse sections of the liver following various treatment regimens described earlier.

The negative (G1) and Origanum majorana control (G3 and G4)

The liver of control rats (G1) and OM (G4 and G5) showed no detectable differences in the histological structure of their liver, so all of them were pooled together. The liver showed a normal structure where the hepatocytes are arranged in branching, interconnecting cords of hepatocytes. The hepatic cords were radiating from the central vein and interposed with hepatic sinusoids. Also the hepatocytes contain rounded or oval, central, vesicular nuclei, and prominent central nucleoli. Areas of portal tracts appeared to be arranged on the periphery of the hepatic lobule (Fig. 1a). Masson's trichrome stain showed the normal distribution of the collagen fibers in the liver (Fig. 1b). PAS reaction showed normal distribution of PAS-positive material in the hepatocytes (Fig. 1c).

The chronic effect of aspartame

Examination of serial transverse section of the liver tissue of G2 treated with ASP (50 mg) showed

Table 1 Effect of oral administration of aspartame, aqueous extract of marjoram (*Origanum majorana*), and ASP+*Origanum majorana* on some liver function tests (alanine aminotransferase and aspartate aminotransferase) and serum vascular endothelial growth factor, each value (mean±SE)

Groups	Treatment	GOT (U/l)	GPT (U/l)	VEGF (ng/l)
G1	Control	39.6±7.93	15.7±1.97	232.6±33.72
G2	ASP (50 mg/kg body weight)	83.1±3.98[a]	32.3±0.76[a]	374.5±9.69[a]
G3	ASP (150 mg/kg body weight)	73.0±3.21[a]	39.4±5.35[a]	450.2±42.4[a]
G4	OM (100 mg/kg body weight)	41.4±5.22	19.3±0.76	245.1±16.04
G5	OM (300 mg/kg body weight)	40.0±3.74	18.0±0.01	267.6±29.98
G6	ASP+OM (50 mg+100 mg/kg body weight)	57.9±2.61[a,b,c]	26.1±1.22[a,c]	318.1±3.79[a,b,c]
G7	ASP+OM (150 mg+100 mg/kg body weight)	63.3±29[a,b,c]	29.86±0.90[a,c]	348.4±10.80[a,c]
G8	ASP+OM (50 mg+300 mg/kg body weight)	49.0±1.53[b,c]	18.4±2.25[b,c,d]	297.9±2.28[a,b,c]
G9	ASP+OM (150 mg+300 mg/kg body weight)	53.0±1.29[a,b,c]	24.1±0.69[a,b,c,e]	267.6±29.98[b,c,d,e]
P		<0.001	<0.001	<0.015

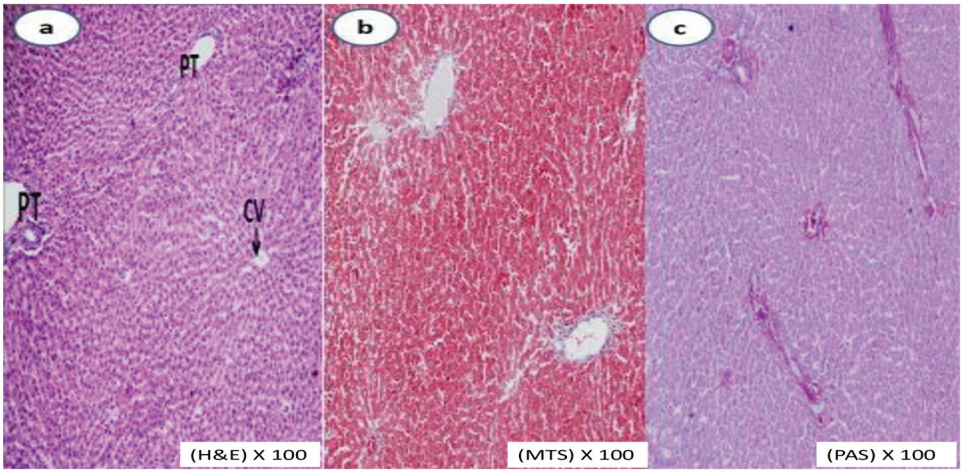
GOT, glutamate oxaloacetate transaminase; GPT, glutamate pyruvate transaminase; VEGF, vascular endothelial growth factor. Values differ significantly at *P* value less than or equal to 0.05, highly significantly at *P* value less than or equal to 0.001. [a]Differ significantly versus control (G1). [b]Differ significantly versus G2 (50 mg ASP/kg body weight). [c]Differ significantly versus G3 (150 mg ASP/kg body weight). [d]Differ significantly versus G6 (50 mg ASP+100 mg OM/kg body weight). [e]Differ significantly versus G7 (150 mg ASP+100 OM mg/kg body weight).

moderate degenerative and necrotic changes in hepatocytes. The cell boundaries of some hepatocytes became ill defined or ruptured as shown in Fig. 2a. Most of the hepatocytes lost their characteristic polygonal shape and became rounded, oval, or ballooned and the cytoplasm filled with numerous vacuole-like spaces. The nuclei of some hepatocytes showed variable degrees of degeneration (hyperchromatism, pyknosis, and the nuclei showed necrotic changes in the form of fragmentation and complete disappearance) (Fig. 2a). Masson's trichrome stain and PAS showed a moderate increase in collagen fiber deposition (Fig. 2b) and moderate reduction of the distribution of the PAS-positive material (Fig. 2c) as compared with G1. However, in G3 (150 mg ASP) the hepatic parenchyma lost its normal architecture. Most of the hepatic cords became disorganized and lost its radial arrangement around the central vein. Disruption of the hepatic cords and blood sinusoids by circumscribed cellular masses were detected (Fig. 3a). The nuclei of the majority of the hepatocytes showed variable degrees of degeneration and necrosis ranging from hyperchromatism, pyknosis fragmentation to complete disappearance (Fig. 3a). Masson's trichrome stain showed a massive increase in collagen fiber deposition in the liver of G3 rat (Fig. 3b). Figure 3c showed marked reduction in the distribution of PAS-positive material in the hepatocytes of G3 as compared with G1.

The protective effect of aqueous extract of marjoram leaves (Origanum majorana)

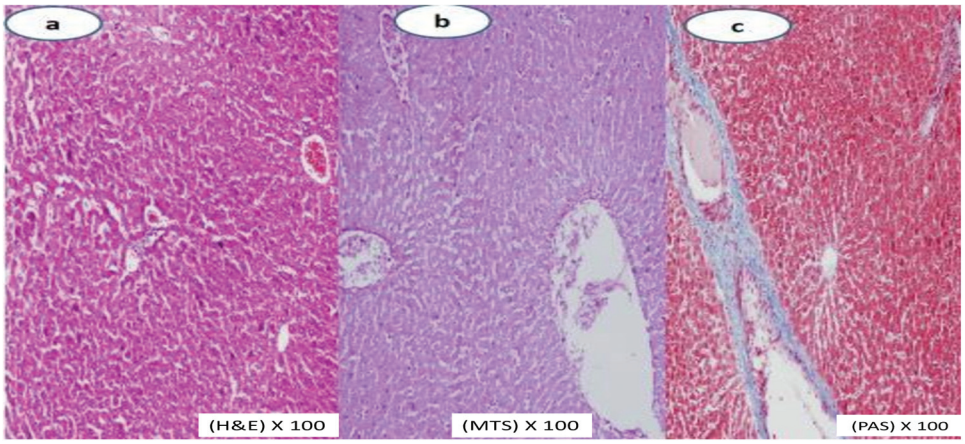
The female of G6 (50 mg ASP+100 mg OM) showed that most of the hepatocytes, hepatic cords, and nuclei appeared to be of nearly the same architecture as their control (G1). However, some hepatocytes showed variable sized vacuoles in their cytoplasm. Few nuclei

Fig. 1



(a–c) Transverse sections of the liver of female rats of G1 (Control), and G4 and G5 (100 and 300 mg OM/kg body weight/day). OM, *Origanum majorana*.

Fig. 2



(a–c) Transverse sections of the liver of female rats of G2 (50 mg ASP/kg body weight/day).

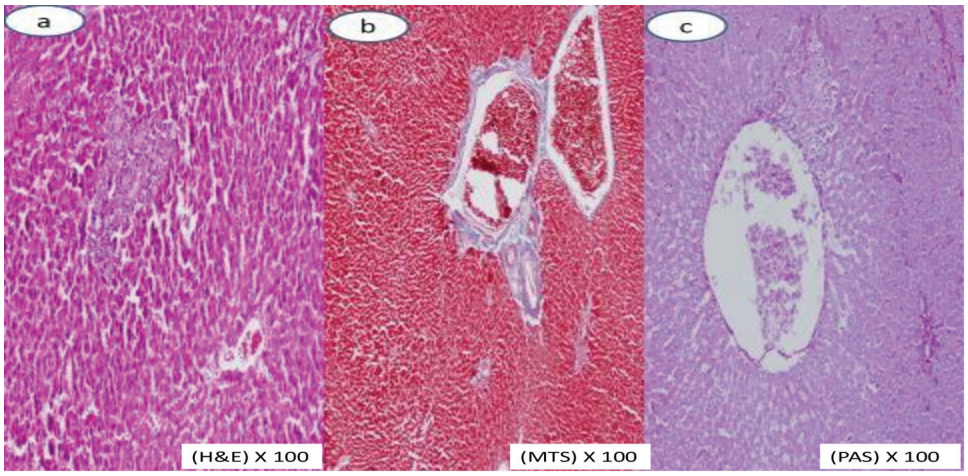
showed variable degrees of degeneration ranging from hyperchromatism, pyknosis to fragmentation (Fig. 4a). Figure 4b showed a decrease in the deposition of collagen fibers in G6 as compared with G2 (50 ASP), but it appeared moderately increased when compared with G1 (control). Figure 4c shows the restoration of most of the distribution of PAS-positive material in the hepatocytes in G6 as compared with G1. Examination of the serial transverse section of the liver of G7 (150 mg ASP +100 mg OM) showed that few of the hepatic parenchyma were formed of normal architecture. Some hepatocytes showed variable sized vacuoles in their cytoplasm. The nuclei of some hepatocytes were rounded or oval, vesicular with prominent nucleoli. The nuclei of other hepatocytes showed variable degrees of degeneration ranging from hyperchromatism, pyknosis to fragmentation (Fig. 5a). Masson's trichrome stain showed a decrease in the deposition of collagen fibers in G8 (Fig. 5b) as compared with G4, but it appeared moderately increased when compared with G1. PAS reaction showed restoration of most of the distribution of PAS positive in G8 (Fig. 5c) as compared with G1.

Examination of the serial transverse section of the liver of G7 and G9 (50 or 150 mg ASP+300 mg OM) showed nearly similar results that were observed in G1, G4, and G5.

Discussion

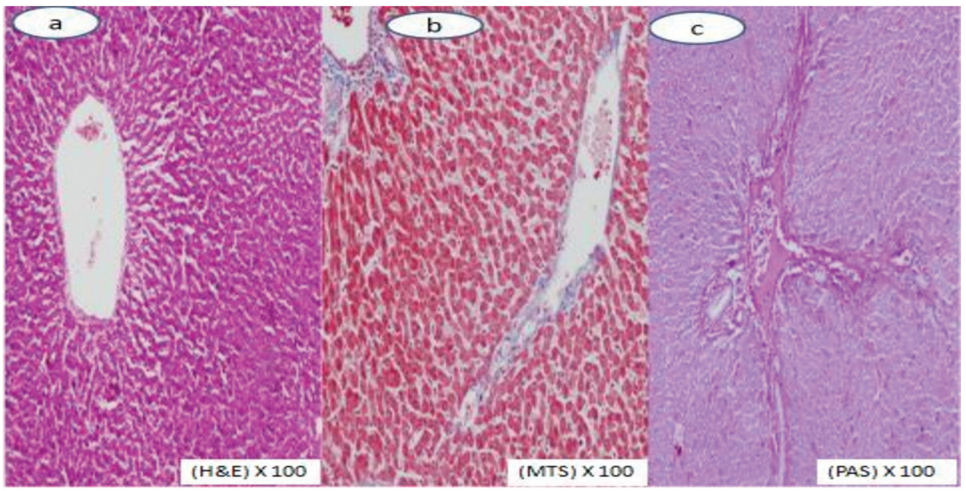
The liver is a target organ that plays a major role in detoxification and excretion of many endogenous and exogenous compounds. It plays an important role in the metabolism and biotransformation of the toxic compound [32]. Therefore, any type of injury or impairment of its function produces hepatotoxicity

Fig. 3



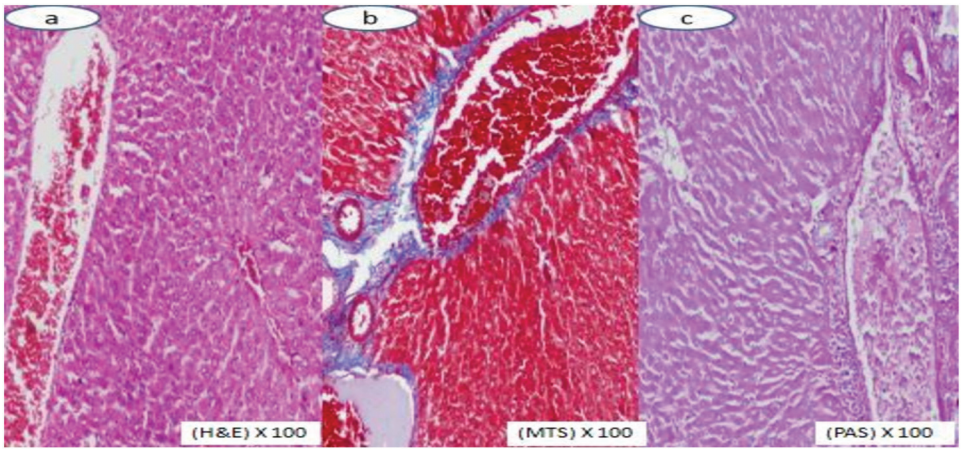
(a–c) Transverse sections of the liver of female rats of G3 (150 mg ASP/kg body weight/day).

Fig. 4



(a–c) Transverse sections of the liver of female rats of G6 (50 mg ASP+100 mg OM/kg body weight/day). OM, *Origanum majorana*.

Fig. 5



(a–c) Transverse sections of the liver of female rats of G7 (150 mg ASP+100 mg OM/kg body weight/day). OM, *Origanum majorana*.

and causes health complications. The present study showed that ASP induction in rats (G2 and G3) remarkably increased the level of GOT and GPT. This increase may be an indication of initial cell injury occurring in hepatic pathology. Liver biomarker enzymes, GOT, and GPT have been associated with liver dysfunction [33]. It causes enzymes to leak out as a result of hepatocyte injuries and altered membrane integrity [34]. Our results found that there was a significant dose-dependent increase in VEGF in both ASP-treated groups (G2 and G3) than the control. VEGF belongs to the growth factors believed to act predominantly on vascular endothelial cells. VEGF is a critical proangiogenic factor in cancer, which is known to stimulate the proliferation of hepatocytes through the VEGF-R1 pathway [35]. Serum VEGF levels in gastric patients were high compared with normal controls, and were linked with the tumor. Its level became low after radical resection of the primary tumor, suggesting that determination of serum VEGF levels may be clinically useful [36]. A number of studies have shown that serum VEGF concentrations may reflect several cancer progression such as pancreatic, lung, breast, gastric, and hepatic cancer and that their determination may be clinically useful [37,38].

In the current study, examination of serial transverse sections of the liver of female albino rats which were administrated with ASP (G2 and G3) showed that the hepatic parenchyma became disorganized. The histopathological changes of the liver may be related to the hazardous effects of the metabolites of ASP on the cell [39]. The ASP-treated rats showed that the hepatic cords and blood sinusoids were disrupted by circumscribed cellular masses. These masses were formed of inflammatory infiltrates [40]. McCance and Grey [41] mentioned that the inflammatory cells cooperate to protect the host from tissue damage. The current work showed increased collagen fiber deposition in the liver tissue. These can be related to the effect of the metabolites of ASP on the cell proteins. This was confirmed by Mourad [42] who observed a significant elevation in lipid peroxidation level in the liver tissue of adult male rats after 4 and 6 weeks of treatment with ASP. Lipid peroxidation caused oxidative damage to proteins and nucleic acids and the results of these reactions increased the collagen [42]. The present study also showed reduction of the PAS-positive material in the liver tissue. This finding was in line with Hamoudah [40].

In the current work, it was observed that the degree of histopathological changes became more obvious in rats of G3 (150 mg of ASP) than in G2 (50 mg of ASP), which suggests that the degree of toxicological changes were dose related to ASP administration. This is in agreement with Soffritti *et al.* [2] who observed that there is a close association between dose level and liver cancer.

The chronic effect of OM on the liver of albino rat showed the normal distribution of collagen fibers and the distribution of PAS-positive material, indicating that this plant does not have toxic effects. This is in agreement with a previous study who observed that the animals treated with the water extract of OM (up to 8 g/kg body weight) did not manifest any significant clinical or macroscopic signs of toxicity or mortality [43].

The current study demonstrated that OM has protective effects against the hepatotoxicity induced by ASP in female rats. The activity of transaminases (GOT and GPT) was increased in rats after exposure to ASP; however, coadministration of OM with ASP

to rats decreased GOT and GPT levels These results are in agreement with the previous studies which reported that OM extract affords a significant hepatoprotective effect against liver injury [44,45]. Also, administration of OM with ASP ameliorates the VEGF level compared with ASP-treated rats. The antitumor activities of OM have been reported in various studies [46].The present results show that hepatic parenchyma and hepatic cords in cotreated rats (50 or 150 mg ASP+100 mg OM) were more or less similar to that of the control group. Few hepatocytes showed variable sized vacuoles in their cytoplasm and few nuclei showed variable degrees of degeneration. The OM had antihepatotoxic activity; rats treated with an extract of marjoram showed almost complete normalization of liver tissues, no fatty degeneration, and no necrosis [47]. The observed improvements may be due to the presence of many antioxidant components found in marjoram). El-Shafeey and Taha [48] showed that feeding on marjoram was able to reduce and sometimes completely remove the toxic effect of C-tetrachloride that induced liver injury in rats [48]. Another study suggested that OM possesses apparent protective and curative effect on Cd-induced hepatotoxicity [49].

The protective effect of OM on the toxicity of ASP may be attributed to the antioxidant activity of marjoram, and this in accordance with Badee *et al.* [50].

Conclusion

In conclusion, ASP induces probable liver tissue damage as evidenced by an increase in GOT and GPT enzyme activities as well as mild increase in serum VEGF levels and histopathological lesions of the liver. Moreover, the study suggests that OM is a useful herbal plant in protection against the hepatotoxic effect due to ASP. Further clinical studies are required to assess the safety and benefits of OM in human beings.

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Conflicts of interest

There are no conflicts of interest.

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