Hepatoprotective and antioxidant effects of *Octopus vulgaris* extract against Depakine®-induced hepatotoxicity in adult rats

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Background and objective
Antiepileptic drug Depakine® is often used, although it can cause birth defects in both human and animals. This study's goal was to assess the *Octopus vulgaris* extract's (OE) ability to protect against the hepatotoxicity caused by Depakine in an effort to advance its clinical application.
Patients and methods
Four groups of adult male Wistar rats (150–180 g b.w.) have been designed at random (10 rats each) as: 1) healthy control group; 2) healthy rats treated orally with OE (50 mg/kg/day); 3) rats administrated orally with Depakine® (500 mg/kg/day); 4) rats treated with OE in combination with Depakine.
Results and conclusion
After 6 weeks of treatment, the results demonstrated that OE was effective in lowering Depakine®-induced hepatotoxicity. This was shown by a significant rise in liver glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) values as well as albumin and total protein levels. Additionally, there was a considerable drop in the serum levels of tumor necrosis alpha (TNF-α), interlukin-1beta (IL-1β), interlukin-4 (IL-4), interlukin-6 (IL-6), and interlukin-10 (IL-10), which exacerbated the structural recovery of the liver's histological image. Conclusion: OE was highly effective in reducing the oxidative stress caused by Depakine® and protecting the liver from its toxic effects. OE is a viable supplement candidate for liver protection against the negative effects of that antiepileptic medication.

Keywords:
Depakine ®, hepatotoxicity, Immunomodulation, *Octopus vulgaris*, Rat

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Introduction

Oxidative damage can enhance the susceptibility to seizure events by affecting cell excitability. A number of conditions, including liver fibrosis, stroke, diabetes mellitus, and atherosclerosis, have been associated to oxidative stress, mitochondrial dysfunction, excessive levels of free radical generation in the organs, and an unbalanced relationship between free radical concentrations and antioxidant defenses. These conditions are in addition to epileptogenesis pathology [1].

Anti-epileptic medications (AEDs) come in many different forms, yet epilepsy still hasn't completely improved. In addition to various restrictions in some AED regimens, more than 30% of patients have drug-resistant epilepsy [2,3]. Additionally, certain AEDs include toxic byproducts that might increase the signs of oxidative stress and mitochondrial dysfunction in other organs, such as liver toxicity linked to prolonged use of Depakine®; Depakine® metabolites result in oxidative stress due to higher ROS production [4]. The limitations of conventional AEDs underscore the need for research into other therapies that can strengthen conventional anti-epileptics and mitigate their side effects.

Patients with complex partial seizures that happen either alone or in conjunction with other types of seizures can be treated with Depakine® (valproic acid) as a monotherapy or supplementary medication [5]. One of the most widely used antiepileptic drugs in the world, Depakine® (valproic acid), is a first-line treatment for generalized and focal epilepsies, including special epilepsies [6]. It is an antiepileptic drug that has a variety of side effects but is typically well tolerated. Serious side effects, including hepatotoxicity, caused by Depakine® (valproic acid), hemorrhagic pancreatitis, coagulopathies, and bone marrow suppression, can occur in some people [7], however, the frequency and occurrence of certain uncommon adverse effects, such as encephalopathy, are still unknown [8]. The central nervous system disorder

epilepsy is characterized by uncontrolled nerve cell activity and, occasionally, convulsive convulsions with or without loss of consciousness. A popular antiepileptic drug called Depakine® (valproic acid) is particularly toxic to the liver [4].

The marine environment is defined by unique features, such as low steady temperatures, high salinity and pressure, absence of sunlight and oxygen, etc [9,10]. This pinpoints the particular biochemistry of marine life, which frequently leads to the synthesis of distinctive metabolites by marine micro- and macro-organisms; these compounds frequently have encouraging biological properties; however, some of them can be employed to treat and/or prevent human diseases [11,12] in addition to food additives and cosmetic components [13]. As a result, 16 medications that were discovered and produced using natural marine-derived compounds, are currently approved for use by patients. Eleven of these medications, out of the total of 16, are used to treat various forms of human cancer [9,11]. Additionally, a staggering array of dietary supplements made from the extracts and semi-purified fraction of edible marine species that were said to be good for human health are available [14,15]. Many of them were thought to have anti-inflammatory, immune-boosting, and cancer-preventing qualities [16]. A major source of many biological compounds with antioxidant, anticancer, antileukemic, antibacterial, and antiviral properties is marine invertebrates, particularly cephalopods [17]. Since extracts with chemopreventive activity have already been isolated, *O. vulgaris* has been described in particular as a source of bioactive compounds [18]. As a result, the bioactive substances found in Octopus' salivary glands are crucial for the development of new drugs and useful as indicators in neurophysiological research. Thus, we conducted the current study because there is an urgent need to determine the toxicity of Octopuses from the genus *Octopus Lamrck*, 1798 (Cephalopoda: Octopoda: *Octopodidae*) in Egypt. As salivary gland poisons in cephalopods, numerous physiologically active compounds have been isolated from the salivary glands [19], However, the objective of this study was to assess the protective capacity *O. vulgaris* extract's (OE) against hepatotoxicity brought on by Depakine®.

Materials and methods

Chemicals

Valproic acid (Depakine®) was purchased from the United Company for Drugs, Assiut, Egypt.

Specimen collection and venom extraction

Specimens of the cephalopod *O. vulgaris* were collected using a small type of landing net from tide area in the Egyptian Red Sea. According to [20–22], then they were accurately examined to identify their species level. With reference to earlier reports, a pair of posterior salivary glands was subsequently identified based on their anatomical location [19,23]. The posterior salivary glands were carefully dissected and cut into small pieces, suspended in acetic acid (2%), and spun at 500 g for 5 min at 4°C. The particles were extracted three more times. Prior to usage, the supernatant was lyophilized and placed in a freezer at −80°C.

Animals and experimental design

Forty adult male albino rats (weighing between 150 and 180 g) were obtained from the Animal Colony, National Research Center, Egypt. Before beginning the experiment, the animals were kept under temperature control (25°C±1) and light control (12/12 h light/dark cycle) with free access to food and water for a week, for acclimatization. The Al-Azhar University Faculty of Science's Ethics Committee, Assiut, Egypt, accepted the proposal, and the animals have got human care in accordance with the standard institution's guidelines. Following acclimation to the conditions in the experimental room, the animals were randomly separated into four groups, each with ten rats: group 1 healthy control rats orally received water (0.5 ml/kg/day) for consecutive 6 weeks, group 2 healthy rats orally ingested with OE (50 mg/kg/day) for consecutive 6 weeks [24], group 3 healthy rats intoxicated orally with Depakine® (500 mg/kg/day) (dissolved in distilled water) [25] for 6 weeks, and group 4 rats intoxicated with Depakine® oral administration combined with ingestion of OE for 6 weeks at the same mentioned doses.

Blood and tissue sampling

All animals were weighed at the conclusion of the treatment session, fasted for the night, and blood samples were obtained using heparinized, sterile glass capillaries from the retro-orbital plexus. The sera were separated from the blood samples by centrifuging them at a cold temperature for 10 min. The sera were then divided into aliquots and kept at −80°C until biochemical tests were run. After blood was obtained, the animals were swiftly beheaded and a part of each liver was extracted, cleaned in saline, dried, and wrapped in aluminum foil before being kept at −80°C for biochemical evaluations and DNA fragmentation determination. For histological processing and microscopic analysis, a second part of each liver was soaked in a formalin-saline (10%) buffer.

Tissue homogenization

The liver sample was homogenized to a 10% homogenate (w/v) in an ice-cold phosphate buffer (50 mM, pH 7.4). The homogenate was then centrifuged at 5000 rpm for 20 min to separate the nuclear and mitochondrial fractions. The supernatant was then divided into aliquots and kept at −80°C until the biochemical tests were carried out.

Biochemical determinations

The activities of serum alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), aspartate aminotransferase (ASAT), and alanine aminotransferase (ALAT) were determined spectrophotometrically using reagent kits purchased from Human Gesell Schaft für Biochemical und Diagnostic mbH, Germany. Reagent kits obtained from DiaSys Diagnostic System GmbH, Germany were used to measure serum total protein, albumin and bilirubin levels. Using reagent kits purchased from Biodiagnostic, Giza, Egypt, hepatic levels of reduced glutathione (GSH) and nitric oxide (NO), as well as the activities of superoxide dismutase (SOD) and catalase (CAT) were determined. However, the level of malondialdehyde (MDA) was determined chemically as described by Ruiz-Larnea *et al.* [26].

Pro-inflammatory cytokines, apoptotic biomarkers, and tumor markers

Using a Dynatech Microplate Reader Model MR 5000 (country Canada) and reagent kits purchased from SinoGeneClon Biotech Co., Hang Zhou, China, the concentrations of serum tumor necrosis alpha (TNF-α), interlukin-1beta (IL-1β), interlukin-4 (IL-4), interlukin-6 (IL-6), interlukin-10 (IL-10), and alpha fetoprotein (AFP) were measured.

DNA fragmentation percentage

The percentage of DNA fragmentation was determined as described earlier [27]; by centrifuging the cleaved DNA samples and quantifying the DNA in the supernatant and pellet, using the diphenylamine testto grade the DNA damage. The DNA ratio in the supernatant to the total DNA in the supernatant and pellet was used to determine the degree of DNA fragmentation. The proportion of fragmented DNA was calculated from the absorbance reading at 578 nm using the following equation:

$$\text{DNA fragmentation \%} = \frac{\text{A supernatant}}{\text{A supernatant+A pellet}} \times 100$$

Histopathology

In order to conduct a histopathological investigation, 5 μ thick paraffin sections were prepared and stained with hematoxylin and eosin stain [28], afterward, the stained slides were inspected using a light microscopy (Olympus CX31, Japan) and photomicrographs were obtained using a digital camera (Olympus Camera C –5060, Japan) in the photomicrograph.

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Statistical analysis

According to Steel and Torrie [29], one way analysis of variance (ANOVA) was used to compare multiple pairs of means, and then post hock (Tukey) test was applied with a P-value ≤ 0.05. This was done with the help of the SAS program; copyright (c) 1998 by SAS Institute Inc., Cary, North Carolina, USA.

Results

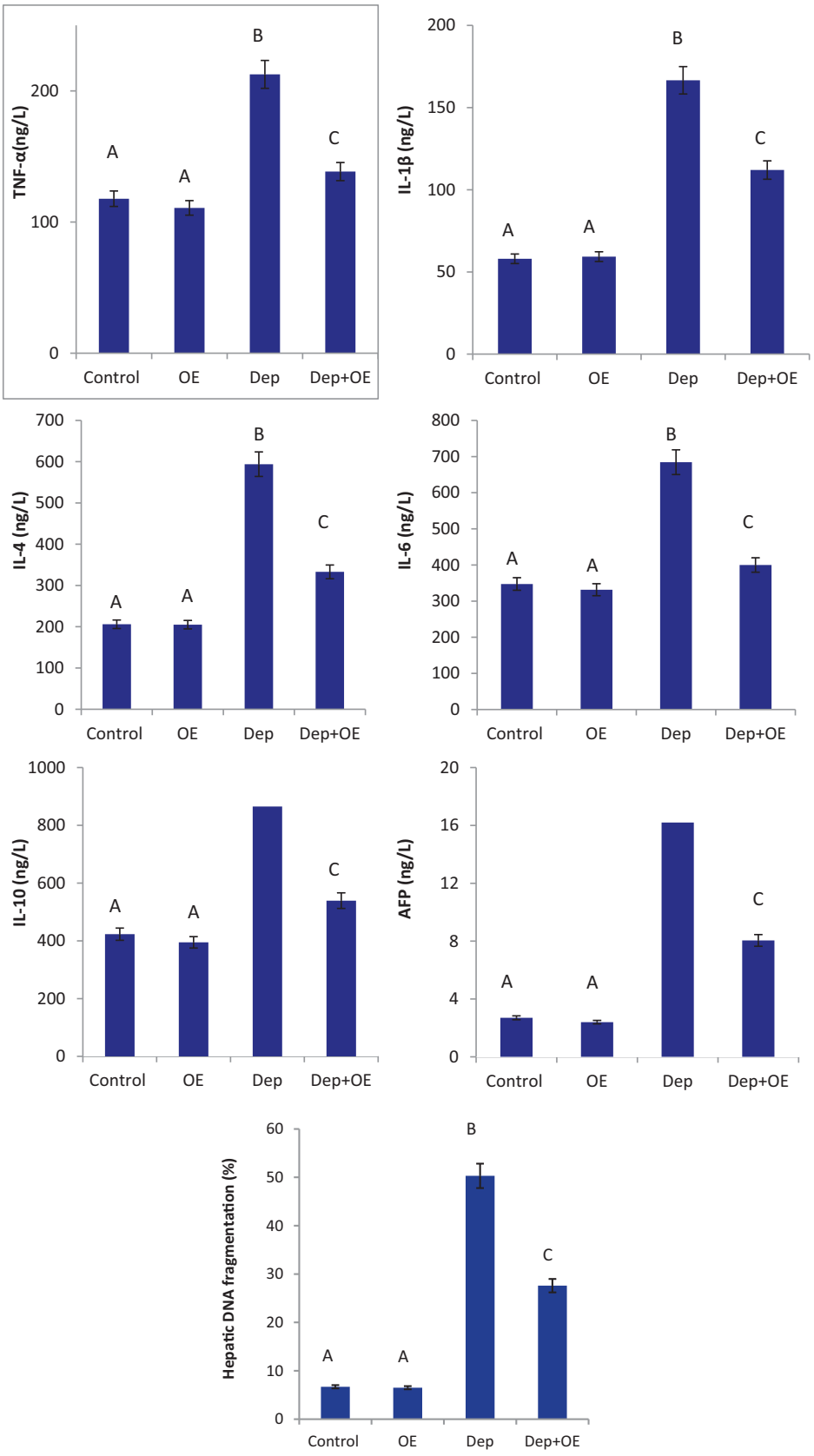
In comparison to control group, the obtained results showed a significant increase in TNF-α, IL1β, IL-4, IL-6, IL-10, AFP, and hepatic DNA fragmentation level post Depakine®-intoxication. Interestingly, administration of rats with OE besides Depakine®-intoxication led to a marked reduction in the measured inflammatory cytokines (TNF-α, IL1β, IL-4, IL-6, and IL-10); tumor marker (AFP) and hepatic DNA fragmentation to values close to those of normal control group when compared with Depakine®-intoxicated animals (Fig. 1).

The data in Table 1 show that administration of rats with OE alone did not disturb the activity of serum ASAT, ALAT, ALP and GGT, or the level of bilirubin; while Depakine®- intoxication led to a significant elevation in the activity of these parameters when both groups were compared with the corresponding values of the control group. Fortunately, co-ingestion of OE together with oral administration of Depakine® greatly reduced the deteriorations in the aforesaid parameters caused by Depakine®.

Similarly, Table 1 demonstrates that after Depakine® intoxication, there was a significant drop in serum total protein and albumin levels as compared with the control group. Intriguingly, OE co-treatment of rats together with oral administration of Depakine® significantly increased serum total proteins and albumin levels. The treated of OE after intoxication with Depakine® significantly ameliorated the deteriorations in the parameters.

In comparison to the control group, Table 2 demonstrates that Depakine® intoxication resulted in a considerable increase in the levels of hepatic MDA

Figure 1



TNF-α, IL1β, IL4, IL6, IL10, α-FP and histogram DNA fragmentation of control, Depakine®-intoxicated and OE-treated male albino rats. Within each column, means with different superscript letters are significantly different ($P \leq 0.05$) using one way (Tukey tests) ANOVA test.

Table 1 Markers of liver function of control, Depakine®-intoxicated and *Octopus vulgaris* extract –treated male albino rats

	Control	*Octopus vulgaris* extract	Depakine®	Depakine®+ *Octopus vulgaris* extract
ALAT (U/L)	34.4 ± 8.1^{A}	36.6 ± 4.1^{A}	83.6 ± 4.9^{B}	54.6 ± 3.07^{C}
ASAT (U/L)	39.9 ± 7.4^{A}	41.6 ± 2.4^{A}	91.3 ± 3.8^{B}	63.7 ± 3.1^{C}
GGT (U/L)	49.2 ± 5.8^{A}	48.02 ± 2.4^{A}	97.05 ± 9.4^{B}	75.1 ± 7.5^{C}
ALP (U/L)	91.5 ± 12.5^{A}	101.6 ± 5.07^{A}	290.7 ± 4.02^{B}	156.2 ± 25.6^{C}
Albumin (g/dl)	4.1 ± 0.17^{A}	3.96 ± 0.09^{A}	2.9 ± 0.10^{B}	3.49 ± 0.08^{C}
Total protein (g/dl)	8.3 ± 0.34^{A}	8.01 ± 0.09^{A}	5.9 ± 0.06^{B}	6.59 ± 0.18^{C}
Bilirubin total (mg/dl)	0.32 ± 0.02^{A}	0.28 ± 0.04^{A}	1.04 ± 0.03^{B}	0.63 ± 0.03^{C}
Bilirubin direct (mg/dl)	0.059 ± 0.003^{A}	0.048 ± 0.002^{A}	0.217 ± 0.008^{B}	0.13 ± 0.008^{C}

Data are presented as mean±SEM. Data were subjected to one way ANOVA followed by post hoc (Tukey) test at $P\leq0.05$. Within each column, means with different superscript letters are significantly; OE (*Octopus vulgaris* extract).

Table 2 Hepatic values of malondialdehyde (MDA), nitric oxide (NO), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) of control, Depakine®-intoxicated and *Octopus vulgaris* extract –treated male albino rats

	Control	*Octopus vulgaris* extract	Depakine®	Depakine®+ *Octopus vulgaris* extract
MDA (μmol/g tissue)	9.7 ± 2.1^{A}	9.4 ± 1.0^{A}	21.4 ± 3.6^{B}	14.3 ± 0.74^{C}
NO (μmol/g tissue)	22.2 ± 4.0^{A}	20.8 ± 1.05^{A}	39.4 ± 4.6^{B}	32.8 ± 3.9^{C}
GSH (nmol/g tissue)	136.8 ± 3.8^{A}	136.8 ± 4.2^{A}	92.7 ± 1.6^{B}	129.2 ± 7.6^{C}
SOD (U/g tissue)	773.8 ± 250^{A}	741 ± 91^{A}	430.8 ± 51.3^{B}	528.6 ± 110^{C}
CAT (U/g tissue)	26.7 ± 5.5^{A}	25.1 ± 5.3^{A}	15.3 ± 1.5^{B}	22.8 ± 5.1^{C}

Data are presented as mean±SEM. Data were subjected to one way ANOVA followed by post hoc (Tukey) test at $P\leq0.05$. Within each column, means with different superscript letters are significantly; OE (*Octopus vulgaris* extract).

and NO together with a noticeable decrease in GSH, SOD, and CAT values. In contrast to the Depakine® group, therapy of OE in addition to Depakine® oral administration resulted in a considerable decline in hepatic MDA and NO levels as well as a noticeable improvement in GSH, SOD, and CAT values.

Histopathological findings

Examination of liver sections of control rats and rats given OE revealed normal lobular histomorphological architecture. Hepatocyte plates that radiate from the central vein make up the normal liver lobules. Blood sinusoids with Kupffer and endothelial cell linings separated the cell plates. The hepatocytes were polyhedral, had vesicular, centrally located nuclei, and homogenous acidophilic cytoplasm (Fig. 2a, b).

Careful microscopic examination of liver sections of rats of the Depakine®-treated group displayed marked changes in almost entire lobules. These changes expressed by mild distortion of the lobular architecture along with diffuse hepatocytes vacuolar degeneration (Fig. 3a) and multifocal areas of coagulative necrosis (Fig. 3b). Diffuse congestion of sinusoids, central veins (Fig. 3c) and portal blood vessels associated with endothelial damage, vacuolation of tunica media and portal hemorrhages were also detected (Fig. 3d). In addition, dilatation of bile ductules and appearance of newly formed ones as well as periductular fibrosis infiltrated with mononuclear cells were noticed (Fig. 3e).

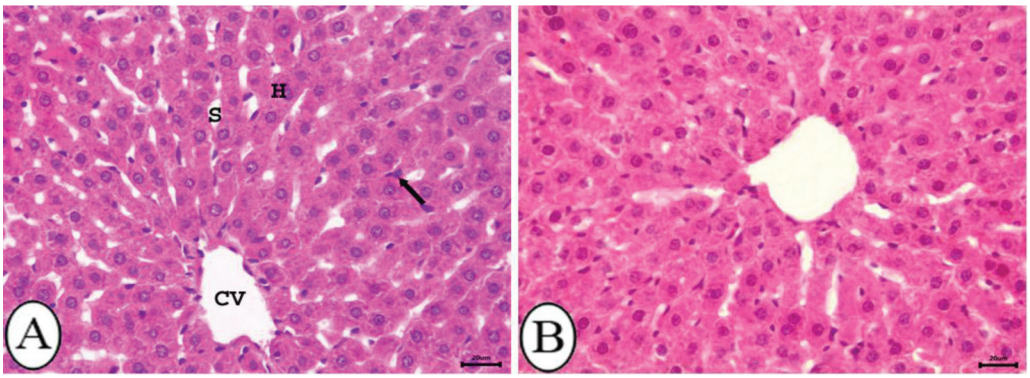
Treatment with OE abrogated the histopathological hepatic lesions induced by Depakine® and displayed a pronounced improvement in the parenchymal structure. Mild histomorphological changes were observed in some hepatic lobules and expressed by mild vacuolation of hepatocytes (Fig. 4a). Coagulative necrosis of sporadic hepatocytes, mild congestion of sinusoids (Fig. 4b) central veins and portal blood vessels were also noticed (Fig. 4c).

Discussion

Since the liver is the main organ involved in the metabolism and breakdown of many anticonvulsants, drug toxicity could result. Hepatotoxic reactions that cause damage to the liver include a variety of symptoms, from minor to transient damage to deadly liver failure [30]. It's common knowledge that 20% of valproate users may see a rise in their liver enzyme levels [31].

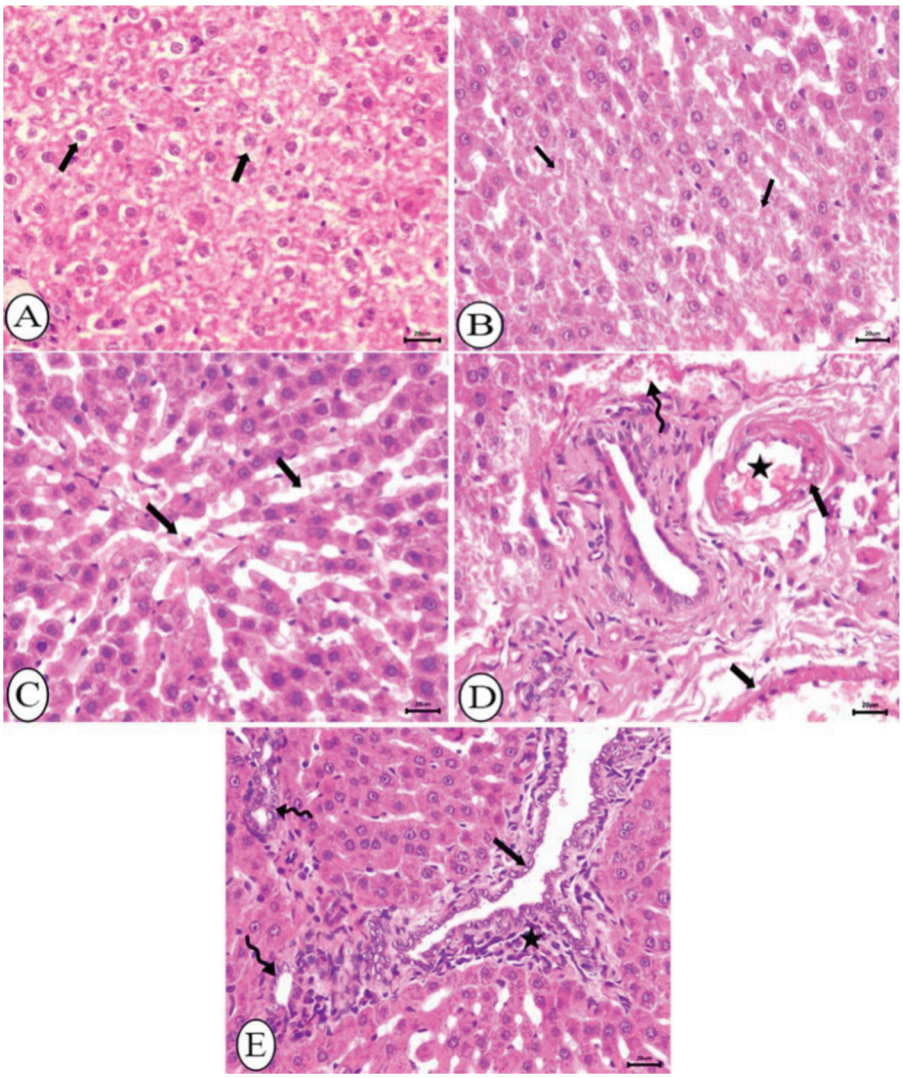
In the current investigation, Depakine®-induced hepatotoxicity is demonstrated by significantly elevated serum ALAT, ASAT, ALP, and GGT activity, as well as bilirubin level, coupled with decreased albumin level. These results are consistent with other previous studies [32,33]. The fact that the Depakine®-treatment for a month increased lipid peroxidation (the process of unsaturated fatty acid degradation in cell membranes by the chain reactions of free radical self-stimulation) in the

Figure 2



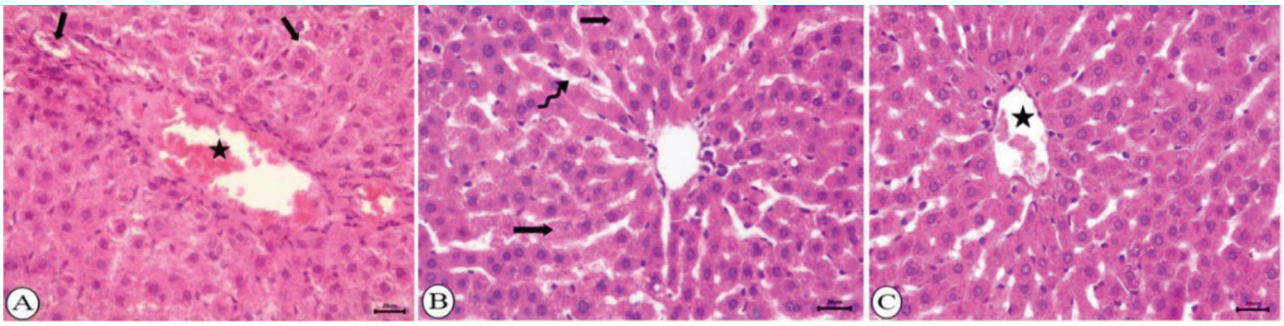
Photomicrograph of liver sections (H and E, bar=20 μm). a. Control rats showing normal architecture of hepatic lobule which formed of plates of hepatocytes (H), central vein (CV), blood sinusoids (S) and kupffer cells (arrow). b. *Octopus vulgaris* extract control group showing normal histological picture with no changes.

Figure 3



Photomicrograph of liver sections of Depakine®-treated group (H and E, bar=20 μm). a. Showing mild distortion of the lobular architecture and diffuse hepatocytes vacuolar degeneration (arrows). b. Showing focal areas of coagulative necrosis (arrows). c. Showing diffuse sinusoidal congestion (arrows). d. Showing congestion of portal blood vessel (star), endothelial damage and vacuolation of tunica media (arrows) as well as portal hemorrhage (notched arrow). e. Showing dilatation of bile ductule (arrow), multiple small newly formed bile ductules (notched arrows) and periductular fibrosis infiltrated with mononuclear cells (star).

Figure 4



Photomicrograph of liver sections of rats treated with *Octopus vulgaris* extract and Depakine® (H and E, bar=20 μm). a. Showing mild vacuolar degeneration of hepatocytes (arrows) and congestion of portal blood vessel (star). b. Showing coagulative necrosis of sporadic hepatocytes (arrows) and mild sinusoidal congestion (notched arrow). c. Showing congestion of central vein (star).

blood and tissues of animals may be explained by this large rise. As the process outputs, free radicals, penetrate cell membranes and attack the DNA directly resulting in the apoptosis phenomenon [34]. These enzymes are also more prevalent because of how excessive drug exposure damages and destroys liver tissue, which causes the drug to be discharged from the liver's cytosol into the bloodstream and raises concentrations of liver enzymes [35]. Bilirubin is used to measure the hepatocytes' capacity for conjugation and excretion, and it was discovered that in the group receiving Depakine®, these capacities were dramatically increased [36]. Depakine® causes mitochondrial malfunction, which reduces gluconeogenesis and protein synthesis due to ATP depletion [37]. Co-administration of OE had a hepatoprotective benefit against Depakine® when it greatly reduced the elevated ALAT, ASAT, ALP, and GGT activities to nearly the control level. In many hepatotoxic scenarios, the protective effect of OE on the liver enzymes has been demonstrated [38]. Both in vivo and in vitro cancer models demonstrated the anticancer effect of OE [39]. Typically, amines, proteins, and peptides are found in the chemical makeup of *Octopus vulgaris* [40]. These substances include octopamine and endecapeptideeledoisin [41], 5-hydroxytryptamine [42], amine substances [43]. In addition, proteolytic enzymes and a glycoprotein called as cephalotoxin were identified from the OE [44,45]. In addition, the *O. vulgaris* includes a variety of bioactive compounds, including tyramine and histamine [46].

The current study's findings demonstrated that chronic oxidative stress brought on by Depakine® can directly promote cell necrosis and activate the apoptotic pathway in the liver tissues of intoxicated rats. This was demonstrated by the significantly increased levels of hepatic MDA and NO and the antioxidant battery (GSH, SOD, and CAT) in the liver tissues [1,47].

Depakine's hepatotoxic metabolites are created by cytochrome P450 oxidation. As a protective detoxifying mechanism, these harmful metabolites then conjugate with reduced glutathione (GSH), causing GSH consumption. Furthermore, Depakine® effect on cytochrome P450 2E1 is linked to increased ROS generation and thus higher oxidative stress [37,48]. One of the pathways for Depakine®-induced hepatotoxicity has been strongly postulated as oxidative stress, where free radicals and reactive oxygen species (ROS) are generated and lipid peroxidation takes place [49] leading to damage of DNA [50] and apoptosis [37] ending up with liver necrosis and cell death [51,52]. Depakine®-related toxic metabolites have also been found to cause hepatotoxicity [50]. It's interesting that OE was able to defend against Depakine® since it significantly increased the activity of radical scavengers and slowed the advancement of oxidative stress. Restoration of GSH has a variety of roles in antioxidant defense, including direct free radical scavenging and serving as a co-substrate for glutathione peroxidases' peroxide detoxification. Activation of SOD and CAT by OE serves to shield the cell from oxidative stress in this way [53].

In the current investigation, administration of Depakine® led to a significant rise in the serum levels of AFP, TNF-α, IL-1β, IL-4, IL-6, and IL-10. These results echo those of earlier research that have been conducted [54]. ROS are associated with inflammation and are produced by neutrophils and macrophages in response to pro-inflammatory cytokines including TNF-α and IL-1β [55]. Depakine®-activated tubules, according to [56], produces MCP-1, which increases the penetration of

mononuclear cells and their maturation into macrophages, which then release pro-inflammatory cytokines including TNF-α and IL-1β. The current scenario is completely consistent with earlier findings [57]. Pro-inflammatory cytokine concentrations are a sign of continuing inflammation [58]. It has been demonstrated that blood levels of IL-6 are elevated in both acute and chronic liver disease [59]. The levels of these cytokines (TNF-α, IL-1β, IL-4, IL-6, and IL-10) were noticeably reduced when OE and Depakine® were administered together compared to Depakine® rats. As seen by the lowered levels of TNF-α, IL-1β, IL-4, IL-6, and IL-10 in blood serum, the supplementation of OE inhibited Depakine®-induced inflammation in this setting and supported biochemical and histological findings. These results demonstrated a key role for OE-mediated decrease of inflammation in the hepatoprotective effects of OE. *Octopus vulgaris* represent the majority of octopuses captured in Egypt [58]. Similarly to Indo-Pacific genera (including Hapalochlaena, the blue-ringed octopus), they possess digestive enzymes and neurotoxins in glands connected to their horny beak and may use this venom for defense or to subdue prey. Blue-ringed octopus venom contains mainly tetrodotoxin (like puffer fish venom), while common octopus venom is composed of cephalotoxin, a toxin less powerful than tetrodotoxin, but also capable of causing paralysis and other manifestations in humans [59,60]. The exact effects of the digestive enzymes are not known, but they can clearly provoke inflammatory reactions in victims' tissue.

Conclusion

Our findings show that Octopus vulgaris extract greatly reduced the liver damage caused by Depakine® through anti-inflammatory, hepatoprotective, and antioxidant mechanisms of its hereditary ingredients. OE may be useful in treating a variety of liver-related diseases and may aid in patient comfort and the long-term management of Depakine® use.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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