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Original Article

Holoturia arenicola extract modulates bile duct ligation-induced oxidative stress in rat kidney

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Abstract: Background*:* Acute Renal Failure (ARF) in patients with cirrhosis is one of the most frequently encountered complications of obstructive jaundice. Marine organisms from the Mediterranean Coast of Egypt are considered potential sources of bioactive molecules. The present study was undertaken to explore the curative effects of *Holothuria arenicola* extract (HaE) against renal injury induced by bile duct ligation in male albino rats. Methods: Fifty four male Wistar albino rats were assigned into two main groups, the Sham-operated control (received distilled water only for 28 days) and bile duct ligated (BDL) group, which divided into 2 subgroups, animals of these subgroups treated for 28 consecutive days as follow: Subgroup I (BDL), rats of this subgroup administered distilled water orally. Subgroup II, animals of this subgroup treated orally with HaE (200 mg/kg body weight). Results: BDL induced marked alteration on renal functions as manifested by a significant increase in the kidney function markers, serum creatinine, urea and uric acid. In addition, BDL caused significant increase in MDA level and significant decrease in GSH level as well as antioxidant enzymes activities (GST, SOD and CAT). However, administration of HaE for consecutive 28 days significantly reversed these changes, suggesting that the renal curative effect of HaE against oxidative stress- induced injury might be involved in decreasing lipid peroxide generation and stimulating antioxidant status. Conclusion: The present study revealed that HaE had a profound effect against BDL-induced oxidative stress in the kidney tissues which is the common feature of choestasis in the liver.

Keywords: *Holothuria arenicola,* antioxidant, bile duct ligation, kidney function

Introduction

Chronic liver disease (CLD) is an important cause of morbidity and mortality and represents a major health problem worldwide. Liver cirrhosis is a common disease in Egypt as Egypt has the highest prevalence of hepatitis C virus (HCV) in the world [1]. Obstructive jaundice, a frequently observed condition caused by obstruction of the common bile duct or its flow and seen in many clinical situations, may end up with serious complications like hepatic and renal failures [2]. Cholestasis is a reduction in bile flow that leads to the intrahepatic accumulation of bile acids and other toxic compounds with progression of liver pathology, including hepatocellular injury and fibrosis [3]. Acute Renal Failure (ARF) in patients with cirrhosis is one of the most frequently encountered complications of obstructive jaundice [4, 1]. Patients with obstructive jaundice may have a higher incidence of renal dysfunction and approximately 6%-8% of patients suffer from acute renal injury, with a mortality of over 68% [5]. Tubular epithelial injury represents an underestimated, but important cause of renal dysfunction in patients with cholestasis and advanced liver disease, but the underlying mechanisms are unclear [6].

Intrahepatic accumulation of reactive oxygen species is thought to be an important cause for the possible mechanisms of the pathogenesis of cholestatic tissue injury from jaundice [2]. Cholestatic liver fibrosis, characterized by excessive accumulation of extracellular matrix (ECM) proteins, is associated with bile acid-induced oxidative stress and lipid peroxidation [7]. Prolonged cholestasis, characterized by retention of bile compound, may cause renal damage which sometimes leads to renal failure [8]. In a situation of cholestasis, there is increasing renal excretion of products usually eliminated in the bile. This renal overload, with accumu-

lation of harmful substances to the glomeruli, may be responsible for functional disorders of the kidney, which may progress to renal failure. An increase in the levels of free oxygen radicals and in the levels of endogenous antioxidant enzyme plays an important role in renal malfunctions observed in obstructive jaundice [9].

Marine invertebrates constitute one of the major groups of marine organisms from which a wide range of medicinal benefits have been devised in addition to the large numbers of marine natural products that have been discovered till date [10]. Marine organisms having the highest chances for the identification of compounds with higher potency and novel biological activities [11]. However, there is increasing interest in the bioactivity of echinoderms extracts and secondary metabolites. The sea cucumber (Holothuria) is a marine invertebrate of the phylum Echinoderm and the class Holothuroidea found on the sea floor worldwide [12]. Esmat et al. [13] demonstrated the hepatoprotective activity of *Holothuria* extract against thioacetamide induced liver injury in a rat model. Moreover, data from our previous study (unpublished data) revealed the antifibrotic effect of the *Holothuria arenicola* extract against bile duct ligation in rats.

Marine organisms from the Mediterranean Coast of Egypt are considered potential sources of bioactive molecules, this study was undertaken to explore the curative effects of *Holothuria arenicola* extract (HaE) against renal injury induced by bile duct ligation in male albino rats through prohibition of oxidative stress.

Materials and methods

Sample collection and preparation

Sea cucumbers (*Holothuria arenicola)* were collected from Abu-Qir Bay in the Egyptian Mediterranean coast at the eastern Alexandrian coast (May-June 2012). The animals were transported to our laboratory in an ice box containing ice cubes and a few pinches of table salt. The animals were immediately washed under running tap water and cut open, and all visceral organs were removed and then the body walls of the animals were stored at -20°C until processing. The phosphate buffer extract was prepared according to the method of Yasumoto et al. [14]. The body wall of the animals was cut into small parts and blended in phosphate buffer (in a volume = 4 ml × tissue weight) and extracted at room-temperature (25°C) with pH 7.2 for 5 hours, the filtered was collected. The collected, filtered of the Sea cucumbers concentrated and lyophilized using a lyophilizer (LABCONCO, shell freeze system, USA).

Free radical scavenging activity

The free radical scavenging activities of the extract and ascorbic acid were analyzed by the DPPH assay [15]. A 1.0 ml of the test extract, at gradient final concentrations of 10-80 mg/ml, was mixed with 2 ml of 0.3 mM DPPH solution in MeOH in a cuvette. The absorbance was taken at 517 nm after 20 minutes of incubation in the dark at room temperature. The experiment was done in triplicates. The percentage antioxidant activity was calculated as follows:

% Antioxidant Activity [AA] = 100 - [{(Abs_{sample} - Abs_{blank}) × 100}/$Abs_{control}$]. Where Abs_{sample} was the absorbance of sample solution (2.0 ml) + DPPH solution (1.0 ml, 0.3 mM), Abs_{blank} was the absorbance of Methanol (1.0 ml) + sample solution (2.0 ml), $Abs_{control}$ was the absorbance of DPPH solution (1.0 ml, 0.3 mM) + methanol (2.0 ml).

High performance liquid chromatographic analysis

The phenolic components of sea cucumber extract were separated by high performance liquid chromatography using an Agilent 1100 device (Waldborn, Germany) equipped with a Zorbax reversed-phase 300SB C18 column (250-4.6 mm) with 5-mm particle size (Lawrence, KS, USA) and ultraviolet detector (G1314A) adjusted at 280 nm. Sample and authentic standards (50 mL; chlorogenic acid, coumaric acid, catechin, ascorbic acid, pyrogallol, and rutin) dissolved in dimethyl sulfoxide and acidified with a drop of acetic acid; then they injected onto the column. The mobile phase was 0.4% formic acid and acetonitrile (60:40, v/v) with a constant flow rate of 1 ml/min. The isolated peaks of the phenolic compounds in the sample were identified by comparing their relative retention times with those of the standards, and then the concentration (percentage) of each compound was calculated as peak area integration.

Ethical consideration

Experimental protocols and procedures used in this study were approved by the Cairo University, Faculty of Science Institutional Animal Care and Use Committee (IACUC) (Egypt), (CUFS/F/06/13). All the experimental procedures were carried out in accordance with international guidelines for the care and use of laboratory animals.

Experimental animals

The experimental animals used in this study were male Wistar rats (*Rattus norvegicus*) weighing 150-160 ± 5 g. The animals were obtained from the National Research Center (NRC, Dokki, Giza). Animals were grouped and housed in polyacrylic cages (six animals per cage) in the well-ventilated animal house of the Department of Zoology, Faculty of Science, Cairo University. Animals were given food and water *ad libitum*. Rats were maintained in a friendly environment with a 12 h/12 h light-dark cycle at room temperature (22°C-25°C). Rats were acclimatized to laboratory conditions for 7 days before commencement of the experiment.

Toxicity study (OECD 420)

Wistar rats weighing (150-160 g) were used for acute toxicity study. The animals (12 rats) were divided into control and test groups containing six animals each. The rats were administered orally with sea cucumbers *Holothuria arenicola* extract (HaE) at dose levels of 5 g/kg (high dose) and 2 g/kg (low dose). Normal control rats received the same amount of vehicle (distilled water) only. Animals were observed carefully for 24 hours after extract administration and then for the next 14 days. At the end of this experimental period, the rats were observed for signs of toxicity, morphological behavior, and mortality. Acute toxicity was evaluated based on the number of deaths (if any). Acute toxicity was calculated as OECD guidelines 420 (Fixed dose method) [16, 17].

Bile duct ligation induced liver damage

Bile duct ligation performed according to Vogel and Vogel [19]. Rats were anesthetized with ketamine and chlorpromazine (100 mg/kg ketamine and 0.75 mg/kg chlorpromazine; ip). Laparotomy was performed under antiseptic conditions. A mid-line incision in the abdomen was made, exposing the muscle layers and the line alba, which was then incised over a length corresponding to the skin incision. The edge of the liver was then raised and the duodenum pulled down to expose the common bile duct, which pursues an almost straight course of about 3 cm from the hilum of the liver to its opening into the duodenum. There was no gall bladder, and the duct was embedded for the greater part of its length in the pancreas, which opens into it by numerous small ducts. A blunt aneurysm needle was passed under the part of the duct selected, stripping the pancreas away with care, and the duct was divided between double ligatures of cotton thread. The peritoneum and the muscle layers as well as the skin wound were closed with cotton stitches. In sham-operated rats, abdominal incision was made without a bile duct ligation.

Experimental design

Fifty four male Wistar rats were assigned into two main groups, the Sham-operated control (18 rats/group) and bile duct ligated (BDL) group (36 rats/group). The bile ducts of animals of Group II were ligated for 14 days. After 14 days of surgery, the animals of Group I received only distilled water for 28 days. Second group was divided into 2 subgroups (18 rats/subgroup), animals of these subgroups treated for 28 consecutive days as follow:

Subgroup I (BDL). Rats of this subgroup administered distilled water orally.

Subgroup II (HaE). Animals of this subgroup treated orally with HaE (200 mg/kg body weight).

Animal handling

Animals were euthanized on the 8^{th}, 15^{th} and 29^{th} days of treatment after being fasted overnight under deep anesthesia with ketamine and chlorpromazine. Blood collected by cardiac puncture. Blood was collected in centrifuge tubes. Kidney was removed and immediately blotted using filter paper to remove traces of blood stored at -80°C for biochemical analysis.

Sample preparation

Serum preparation: Blood samples collected in centrifuge tubes were centrifuged at 3000 rpm

Table 1. Effect of *Holothuria arenicola* extract (HaE) on the serum creatinine, urea and uric acid concentrations (mg/dl) in bile duct ligated (BDL) rats

Parameters	Group	Experimental period (days)		
		7	14	28
Creatinine (mg/dl)	Sham	1.5 ± 0.07[a]	1.2 ± 0.12[a]	1.53 ± 0.2[a]
	BDL	2.88 ± 0.11[c]	2.95 ± 0.07[c]	2.93 ± 0.05[c]
	BDL + HaE	1.93 ± 0.15[a,b]	1.73 ± 0.71[b]	1.17 ± 0.3[a,b]
% of improvement		63	101.6	115.03
Urea (mg/dl)	Sham	21.54 ± 1.03[a,b]	21.43 ± 1[a,b]	23.33 ± 1.54[a,b]
	BDL	30.31 ± 1.17[b]	26.88 ± 2.63[a]	28.7 ± 6.32[b]
	BDL + HaE	21.58 ± 5.44[a,b]	21.05 ± 0.69[a]	17.47 ± 1.1[a]
% of improvement		40.53	27.20	48.14
Uric acid (mg/dl)	Sham	4 ± 0.09[a]	3.99 ± 0.07[a]	3.95 ± 0.08[a]
	BDL	4.29 ± 0.14[a]	5.9 ± 0.38[b]	6.84 ± 0.26[c]
	BDL + HaE	3.79 ± 0.4[a]	4.33 ± 0.68[a]	4.53 ± 0.54[a]
% of improvement		12.5	39.34	58.48

Values are given as mean ± SE for 6 rats in each group. Unshared letters between groups are the significance values at $p<0.05$.

for 20 minutes. Serum stored at -20°C until used for biochemical assays.

Kidney homogenate preparation: Kidney tissue was homogenized (10% w/v) in ice-cold 0.1 M Tris-HCl buffer (pH 7.4). The homogenate was centrifuged at 3000 rpm for 15 min. at 4°C and the resultant supernatant was used for biochemical analysis.

Biochemical assessment of kidney function

The appropriate kits (Bio-Diagnostic, Dokki, Giza, Egypt) were used for the determination of serum creatinine [19], urea and uric acid [20].

Oxidative stress markers assessment

Oxidative stress markers were detected in the resultant supernatant of kidney homogenate. The appropriate kits (Biodiagnostic kits, Biodiagnostic Dokki, Giza, Egypt) were used for the determination of malondialdehyde (MDA) [21], glutathione reduced (GSH) [22], catalase (CAT) [23], glutathione-S-Transferase (GST) [24] and superoxide dismutase (SOD) [25].

Statistical analysis

Values were expressed as means ± SE. To evaluate differences between the groups studied, one way analysis of variance (ANOVA) with Duncan post hoc test was used to compare the group means and $P<0.05$ was considered statistically significant. SPSS for Windows (version 15.0) was used for the statistical analysis.

% improvement = treated mean - injured mean/ control mean × 100.

Results

Effects of Holothuria arenicola extract (HaE) on serum creatinine, urea and uric acid

The levels of the serum creatinine, urea, and uric acid in the Sham, BDL and HaE treated groups showed in **Table 1**. BDL group showed a significant increase ($P<0.05$) in creatinine, urea and uric acid levels following the three tested periods as compared to the Sham group (**Table 1**). However, treatment with HaE significantly decreased ($P<0.05$) the serum creatinine, urea, and uric acid levels after the three tested periods. The observed changes in the kidney function markers showed that 28 days of treatment recorded the most improvement percentages than the other two tested periods. Serum creatinine, urea, and uric acid levels were ameliorated by 115.03%, 48.14% and 58.48%, respectively.

Effect of Holothuria arenicola (HaE) extract in improving the oxidative status of the kidney

MDA levels were assessed as an indicator of lipid peroxidation. The kidney MDA was found to be higher in the BDL group compared to their

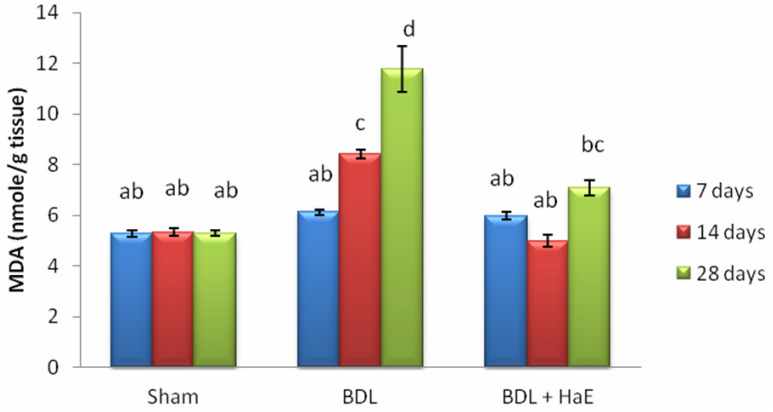

Figure 1. Effect of *Holothuria arenicola* (HaE) extract on the kidney Malondialdehyde (MDA) level of BDL rats. *Data are means ± SEM of six rats in each group. *Unshared letters between groups are the significance values at P<0.05.

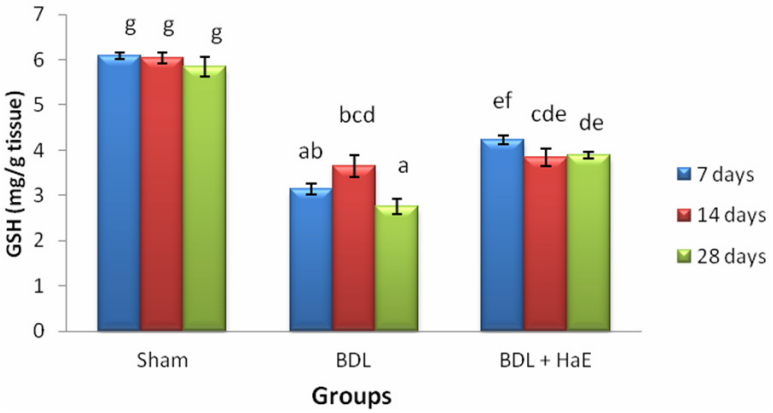

Figure 2. Effect of *Holothuria arenicola* (HaE) extract on the kidney reduced glutathione (GSH) level of BDL rats. *Data are means ± SEM of six rats in each group. *Unshared letters between groups are the significance values at P<0.05.

corresponding sham-operated control group following all tested periods, but this increase was significant ($P<0.05$) after 14 and 28 days of bile duct ligation. Treatment with HaE significantly decreased ($P<0.05$) the MDA levels following 14 and 28 days of treatment (**Figure 1**). However, the maximum improvement percentage was recorded following 28 days of HaE treatment.

The hepatic GSH level showed a significant reduction in the BDL group ($P<0.05$) compared to their corresponding sham-operated control group following all tested periods. Administration of HaE for 7 and 28 days significantly increased the level of GSH as compared to their corresponding BDL group (**Figure 2**). The highest improvement percentage was recorded following 28 days of HaE treatment.

Bile duct ligation significantly ($P<0.05$) decreased the level of CAT in the kidney tissues in all tested groups as compared to their corresponding controls (**Figure 3**). However, treatment with HaE at 7, 14 and 28 days significantly ($P<0.05$) increased levels of CAT as compared to the time matched BDL groups.

Concerning the effect of bile duct ligation on the SOD activity, bile duct ligation significantly ($P<0.05$) decreased the level of SOD in the kidney tissues in all tested groups as compared to their corresponding controls (**Figure 4**). However, treatment with HaE after 28 days significantly ($P<0.05$) decreased SOD level as compared to their time matched BDL groups. The highest improvement percentage was recorded following 28 days of HaE treatment.

Discussion

Kidneys are dynamic organs and represent the major control system maintaining the body haemostasis. Changes in renal function are one of the most common manifestations of severe illness. Induction of kidney dysfunction in experimental animals is important for studying new therapeutic agents, including nutraceuticals that may possess therapeutic or protective effect towards kidney dysfunction. Antioxidant and anti-inflammatory agents play a critical role in body protection by scavenging active oxygen and free radicals and neutralizing lipid peroxides [26]. The antioxidant potential, and ameliorative activities of the sea cucumbers, *Holothuria atra* and *Holothuria arenicola* against hepatic injury were investigated recently [13, 27]. Phenolic-rich materials are the main sources of food for the sea cucumbers, that can account for the presence of the active phe-

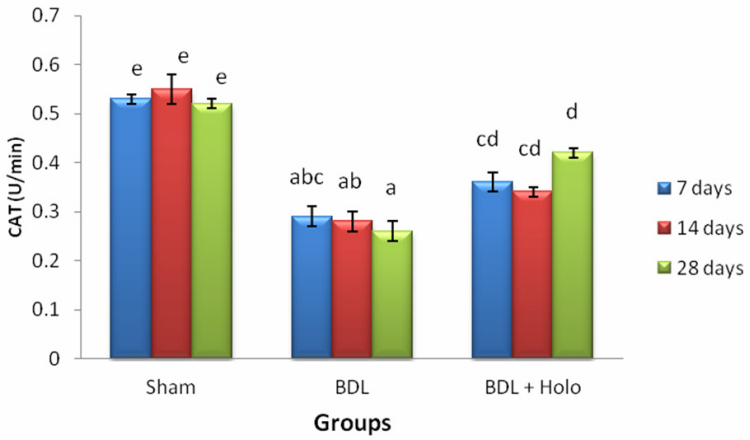

Figure 3. Effect of *Holothuria arenicola* (HaE) extract on the kidney catalase (CAT) activity of BDL rats. *Data are means ± SEM of six rats in each group. *Unshared letters between groups are the significance values at P<0.05.

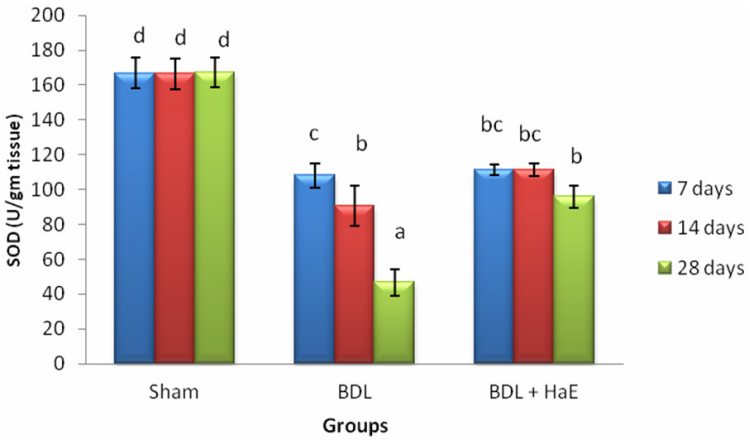

Figure 4. Effect of *Holothuria arenicola* (HaE) extract on the kidney super oxide dismutase (SOD) activity of BDL rats. *Data are means ± SEM of six rats in each group. *Unshared letters between groups are the significance values at P<0.05.

nolic antioxidant compounds in the body wall of sea cucumbers [27].

Experimental impairment of kidney function is induced through treatment by specific chemical or drugs or through surgical means. In the present study, kidney dysfunction induced through surgical means by bile duct ligation in Wistar rats. Bile duct ligation induces a kind of liver fibrosis, that etiologically and pathogenitically resembles the biliary fibrosis in the human beings and is shown to induce cholestasis-related liver function impairments [28]. Acute biliary obstruction is associated with the development of renal impairment and oxidative stress [29]. The oxidative stress known to occur as a systemic response to cholestasis could give rise to the involvement of organs other than liver, such as the kidney [30].

Acute renal failure (ARF) is a common complication in cirrhotic patients [31]. These complications include water-balance abnormalities, sodium retention and a predominant observation is reversible renal vasoconstriction that can lead to hepatorenal syndrome and renal failure [32]. Urea and creatinine are bio-indicators of the renal function [33] and the underlying presence of component(s) of the metabolic syndrome [34]. Hence, imbalance in their physiological homeostasis could evoke pathological conditions. Viewed in conjunction of the reports of Mahmoud et al. [35] and Costa et al. [36], data from the present investigation reflect that BDL induced marked alteration on renal functions as manifested by a significant increase in the kidney function markers, serum creatinine, urea and uric acid. The elevation of the serum urea and creatinine concentrations following BDL appear to suggest the possible up-regulation of protein catabolism and concomitant rise in the synthesis of creatinine that needs to be excreted with urine (formed via the reactions of the urea cycle). Moreover, Pereira et al. [37] showed that, rats at 6 wk of BDL showed features of hepatorenal syndrome, including a significant increase in the serum creatinine and reductions in creatinine clearance, water excretion and urinary sodium concentration.

Oxidative stress, an imbalance between the generation of reactive oxygen species (ROS) and antioxidant defense capacity of the body, is closely associated with the majority of chronic diseases [38]. Oxidative stress mediates a wide range of renal impairments, ranging from acute renal failure, obstructive nephropathy

and glomerular damage to chronic renal failure associated with inflammation [39, 40]. The possibly enhanced production of the reactive oxygen species (ROS) could be renotoxic consequently impairing the functional capacity of the kidney. The renin-angiotensin system (RAS) plays an important role in controlling liver fibrosis [41]. Accumulating evidence suggests that angiotensin-II stimulates intracellular formation of ROS such as superoxide anion and hydrogen peroxide that leads to kidney damage [42]. In consonance with the report of Ara et al. [43], data from the present investigation showed a significant elevation in the MDA and a significant reduction in the GSH levels in the kidney tissue following BDL in rats as compared to Sham group. The increased MDA level suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals [44]. Treatment with HaE at the tested dosage (200 mg/kg) significantly reversed these changes, suggesting that the renal curative effect of HaE against oxidative stress- induced injury might be involved in decreasing lipid peroxide generation and stimulating antioxidant status. The present study confirms our previous studies [27], demonstrating that sea cucumber body wall extract significantly decreased MDA levels in injured kidney tissues, suggesting that the antifibrotic mechanism of HaE may be attributable to its phenolic antioxidant effect.

Antioxidant enzymes play an important role in the protection of the kidney against oxidative stress [45]. Superoxide dismutase (SOD), one of the important intracellular antioxidant enzymes, present in all aerobic cells and may play an important role in the pathophysiology of cholestatic liver injury and acute renal failure [46]. Catalase protects cells from the accumulation of H_2O_2 by dismutating it to form H_2O and O_2 or by using it as an oxidant in which it works as a peroxidase [47]. Viewed in conjunction the finding of Somi et al. [48], the present study demonstrated that bile duct ligation usually decrease antioxidant enzyme (GST, SOD and CAT) activities in hepatic tissue that may be attributable to mitochondrial toxicity induced by high concentration of biliary acids in chronic cholestasis. Moreover, micro-perfusion studies have shown that biliary acids decrease fluid absorption from the proximal tubules [49]. In accord with our results, Sanzgiri et al. [50], have reported that the enhanced free radical concentration resulting from the oxidative stress conditions can cause loss of enzymatic activity. Treatment with HaE in the present study restored the activity of the studied antioxidant enzymes following the three tested periods.

The enhancement of the antioxidant enzymes showed that the HaE could be possesses not only the capacity to scavenge the ROS, but also the capacity to block the BDL-induced massive ROS production. In addition, treatment with HaE normalized the antioxidant levels through their rich of polyphenolic compound especially chlorogenic acid that has the ability to scavenge free radicals [27].

In conclusion, the present study revealed that HaE had a profound effect against BDL-induced oxidative stress in the kidney tissues which is the common feature of choestasis in the liver, as it alleviates the alterations in urea, creatinine and uric acid levels as well as the oxidative stress markers in the kidney (MDA, GSH, SOD and CAT). However, further studies on this Egyptian freshwater clam extract from a Bivalve *Coelatura aegyptiaca* must be carried out to provide the opportunity to develop a new food adducts from this clam for the prevention and treatment of oxidative stress-induced injuries.

Disclosure of conflict of interest

None.

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