Egypt. J. Chem. **Vol. 65,** No. 2 pp. 181 - 190 (2022)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

The ameliorative effects of *Eremina desertorum* snail mucin in combination with Silymarin against experimentally induced liver fibrosis

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Abstract

Mucin is known by its biological and pharmacological activities. The aim of current study is to evaluate the hepatoprotective and the antioxidant activities of mucin extracted from *Eremina desertorum* snail as well as chemical profiling using GC-MS/MS analysis. This investigation confirmed the hepatoprotective and the antioxidant effects of mucin either alone or in combination with Silymarin on carbon tetrachloride (CCl_4) liver damage. Male albino mice were injected intraperitoneally with 0.5ml /kg b.wt of 40% CCl_4, twice/week for 8 weeks. The treated groups treated orally with Mucin (after 8 weeks of CCl_4 intoxication, twice a week for four weeks) or Silymarin (from the first day of the experiment with a dose of 140 mg/kg, three times/week until the sacrifice day). After 12 weeks, all animals were sacrificed by cervical dislocation. Results showed that CCl_4 induced a hepatotoxic effect represented in increasing liver enzymes, MDA and IL-2 while decreasing total protein, albumin, CAT, SOD and GSH. Amelioration in these parameters occurred after treatment of CCl_4 intoxicated mice with either Silymarin or *E. desertorum* mucin where they caused increases in the activity of the antioxidant parameters while, reduced the level of MDA and liver enzymes activity. Also, the histopathological alterations that caused by CCl_4 were improved after the treatment with this extract. GC-MS/MS analyses led to the identification of ten compounds were categorized as monoterpenes, sesquiterpenes, quinolines, and fatty acid esters. Conclusively, *E. desertorum* mucin enhanced antioxidant activity and ameliorated the CCl_4-induced liver damage and it could be used as a hepatoprotective agent.

Keywords: *Eremina desertorum;* Mucin; Antioxidant; Hepatoprotective activity; GC-MS/MS

1. Introduction

Liver is responsible for detoxification of many toxins and regulation of many of the physiological functions in our bodies. Therefore, liver can be damaged from these agents when they were in excess [1]. Recently, Liver fibrosis incidence increased rapidly as it occurred when the liver was injured chronically [2]. The main cause of liver fibrosis was the over production of extracellular matrix components (ECM) from the hepatic stellate cells (HSC) [3]. The activation of HSC is a key issue in the pathogenesis of hepatic fibrosis [4]. Therefore, the improvements of the activated HSC were crucial goals in treating the hepatic fibrogenesis cascade [5]. Carbon tetrachloride (CCl_4) is a hepatic toxin for induction of experimental liver fibrosis in the laboratory [4]. The hepatotoxicity of CCl_4 is due to reductive dehalogenation products that removed the hydrogen atom from the unsaturated fatty acids leading to lipid peroxidation and liver injury [1].

Reactive oxygen species (ROS) were recognized as toxic by-products of aerobic metabolism. These free radicals or reactive species are highly energetic molecules that have the ability to interact with the cells of the body, which leads to major changes like mutations. The accumulation of these molecules inside the body leads to what is known as the phenomenon of oxidative stress due to the imbalance between the amount of such species and endogenous antioxidants, inside the living body. Naturally

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Receive Date: 04 July 2021, Revise Date: 15 July 2021, Accept Date: 08 August 2021
DOI: 10.21608/EJCHEM.2021.84043.4114

occulting antioxidant compounds can attenuate the destructive effects of these species through their free radical scavenging activities [6-12]. These natural antioxidants like Phenolic antioxidants, Fat-soluble vitamin E (α-tocopherol) and water-soluble vitamin C (L-ascorbic acid) and plant extracts can inhibit the free radical formation [12].

Silymarin is *Silybum marianum* plant extract that has antioxidant, anti-inflammatory and antifibrotic power properties [13]. Navarro et al stated that Silymarin might relieve the lipid peroxidation, the excessive free radicals production and restore normal levels of superoxide dismutase and it might alleviate the CCl_4-induced liver fibrosis in rats [14]. The effect of Silymarin could be potentiated by the combination with other plants that have antioxidant properties like Ginger [4] or Moringa [1]. It promoted the hepatocyte regeneration, reduced the inflammatory reaction, and inhibited the fibrogenesis in the liver [13].

Bodies of desert snails like *Eremina desertorum* have rich mucus that secreted from their pedal gland [15] to keep their bodies moisture [16]. This mucus consists of mucopolysaccharides and glycoproteins [17]. Nowadays, there are a great interest about using snail mucus- derivate drugs in wounds, superficial healing and muco adhesive formulations [18]. Its biological importance was due to its protein content that alleviated wounds and lessened the inflammatory process [19]. Also, the extracted mucin from snail mucous could be used in muco adhesive formulations for ocular, nasal, gastro-intestinal, buccal and vaginal drug administration as it had wonderful therapeutic activities [20].

Harti et al found that *Achatina fulica* snails slime could rapidly heal wounds and reasoned this effect to its content of glycosaminoglycan [21]. Also, the slime of *Helix aspersa* snails ameliorated the experimentally induced colitis as it contained natural anti-inflammatory and antioxidant molecules that offered protection against colon inflammation [18]. Therefore, the purpose of the present research is to confirm the ameliorating activities of the mucin extracted from *Ereminia desertorum* snails´ mucus alone or in combination with Silymarin on experimental liver fibrosis induced by CCl_4, using different biochemical, histopathological parameters and its relationship with the oxidative stress.

2. Materials and Methods

2.1. Animals

White male albino mice of CD1, 6–8 weeks (wk) old (18-20 g), were obtained from the Animal House from the Schistosome Biological Supply Centre, Theodore Bilharz Research institute, Giza-Egypt (SBSC, TBRI). Mice were maintained for 8 wk in plastic cages in an animal room, at temperature ranging between 20–25ºC and were fed Purina chaw (20% protein) and given tap water. The study was approved by The Animal Ethics Committee, TBRI.

2.2. Test materials

Silymarin (milk thistle powder containing 80% Silymarin) was purchased from Sigma (St. Louis, Missouri, USA). It was orally administered starting from the first day of the experiment at a dose of 140 mg / kg, three times / week until the day of sacrifice. CCl_4 was purchased from Adwic Chemicals Co. (Cairo, Egypt).

2.3. Preparation of the "*E. desertorum* slime and mucin"

To extract the slime, the simplest means in heliciculture were used to get a pure fresh slime, i.e. with a sterile wooden rod stimulate the snail by rubbing this rod on its muscular foot, this stimulate the snail to secret more slime; the slime was collected from one hundred snail and kept in a sterile container which was then preserved at (- 30°C) until use. The slime was macerated in water for 24 hours in 40°C by mixing water with the slime (2:1ml). The supernatant was received as WSF (Water soluble fraction). The fraction of slime (mucin fraction) of the WSF was gained by using ethanol precipitation by mixing supernatant resulted from the water maceration with absolute ethanol ratio of 1: 3, and then it was centrifuged at 2900 r.p.m., for 30 minutes. The precipitation was re-dissolved with Tris-Cl and finally mucin fraction was obtained [21].

2.4. GC-MS/MS analysis of extracted mucin

Mucin was analyzed by GC-MS/MS which was performed with an Agilent 6890 gas chromatograph equipped with a mass spectrometric detector (MSD) model Agilent 5973. A fused silica capillary column (HP-5MS), 5% phenyl polysiloxane as non-polar stationary phase (30 m60.25 mm6i.d) and 0.25 mm film thickness was used. Operating conditions were as follows: injector port temperature, 2508C. Helium was used as a carrier gas at a flow rate of 1.0 ml/min pulsed split less mode programmed at 88C/min to 2608C, and held for 18 min. The total analysis time was 41 min. A 1 ml volume was injected split less. The mass spectrometric detector (MSD) was operated in electron impact ionization mode with an ionizing energy of 70 eV, scanning from m/z 50–500. The ion source temperature was 2308C and the quadruple temperature was 1508C. The electron multiplier voltage (EM voltage) was maintained at 1100 V above auto tune, and a solvent delay of 3 min was employed. The instrument was manually tuned using perfluorotributylamine (PFTBA). Identification was based on comparison with the MS computer library (NIST Software Package, Finnigan) and on the respective retention indices. The separated

components were identified by matching data with those of the data published by Wiley (Wiley7n.1) library of mass spectra and literature comparison.

2.5. Experimental design Protocols

- Treatment of the animals before induction of inflammation:

The guidelines for use and care of all experimental animals were faithfully respected (according to the guide for the care and use of laboratory animals).

2.6. Experimental groups

- Normal negative control: 20 Mice.
- Positive control group: 20 mice were injected intraperitoneally with 0.5ml /kg b.wt of 40% CCl_4 (a mixture of pure CCl_4 and sterile olive oil v/v), twice a week for 8 weeks.
- 20 mice for CCl_4+Mucin group: (orally, after 8weeks, 20 ml of mucin/ Kg, twice a week for four weeks).
- 20 mice for CCl_4+ mucin +Silymarin group. Mice were injected intraperitoneally with 0.5ml /kg b.wt of 40% CCl_4, twice a week for 8 weeks and orally administered with Silymarin starting from the first day of the experiment (140 mg / kg, three times / week until the day of sacrifice), then they were administered orally with 20 ml of mucin/ Kg after 8weeks, twice a week for four weeks.

Samples: After the end of the experimental period (12 weeks) all animals were euthanized by cervical dislocation.

2.7. Serum preparation and biochemical investigation

Mice were sacrificed by cervical dislocation and the blood was collected in plastic tubes. Blood was allowed to stand at 37ºC for 1 hr, then over night at 4ºC, and centrifuged at 300 G for 30 minutes (min). Sera were separated and heat-inactivated at 56ºC for 30 min and stored in aliquots at -20ºC, until use.

2.8. Liver function parameters

Measurement of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined in serum according to the method described by Reitman and Frankel [22]. Serum albumin concentration: Formation of albumin/brom-cresol-green complex at pH 4.2 and photometric measurement of the absorbance were detected by the Biodiagnostic kits. Total protein concentration was determined in liver homogenate according to the method of Doumas [23].

2.9. Investigation of the biomarkers of liver oxidative stress

The oxidative stress enzymes are detected in supernatant of the tissue homogenate for each group. Bio diagnostic kits (Biodiagnostic Dokki, Giza, Egypt) were used for the determination SOD and catalase (CAT) [24]. In addition, tissue malondialdhyde (lipid peroxide) was done according to Ohkawa et al [25], and reduced glutathione (GSH) was done according to method of Beutler [26].

2.10. Enzyme Linked Immunosorbent Assay (ELISA)

Serum murine IL-2 levels were measured using ELISA kit (Koma Biotech.; SinoGeneClon Biotech Co., Ltd respectively) according to Engvall and Perlmann [27]. The cytokine concentration was obtained from a regression curve prepared with the help of Microplate Manger software (Bio-Rad).

2.11. Histopathological studies

Fixed liver tissue samples were dehydrated by passing in ascending series of alcohol then cleaned with xylene and embedded in paraffin wax. Sections of the tissues 5–6 µm thickness were prepared by using a rotary microtone and stained with hematoxylin and eosin (H&E) dye [28].

2.12. Statistical analysis

The data were expressed as mean ± S.D. and the comparison between two means was done using student's t-test [29]. The (P) value less than 0.05 was considered as statistically significant. The data analysis was done with SPSS version 20.

3. Results and Discussion

GC-MS/MS examination of the mucin extracted from *E. desertorum* snails led to the identification of ten compounds. The total peak areas of the identified compounds constitute 73.22% (Table 1, Figures 1, 2). The detected compounds were identified as Benzo[f]quinoline, Tricyclo[3.1.0.0(2,4)]hex-3-ene-3-carbonitrile, Limonene, Glycerol 1, 2-diacetate, Dodecane, 4-methyl-, Phenol, 2,5-bis(1,1-dimethylethyl), Tridecane, 2-methyl-, Dodecane, 2,6,10-trimethyl-, Hexadecanoic acid, ethyl ester, and Phenol, 2,2'-methylenebis[6-(1,1-d imethylethyl)-4-methyl (Figure 3). The identification was achieved via using computer search user-generated reference libraries, incorporating mass spectra [30-32]**.** The identified compounds were categorized as monoterpenes, sesquiterpenoids, alcohol esters, fatty acid esters, fatty acids, phenol derivatives, quinolines, and branched alkanes. Several biological activities like antimicrobial [33, 34], antioxidants [34, 35], antibiofilm [36], and cytotoxic [36] were attributed to the presence of such classes of naturally occurring compounds. Sallam et al (2009) stated that the major identified compounds in the mucin of three common land snails, *Eobania vermiculata*, *Theba pisana* and *Monacha obstructa* are Oxime, methoxy-phenyl and cyclotrisiloxane, hexamethyl, whilst variation in the chemical compostion could be attributed to certain external factors such as temperature, humidity, light intensity, soil conditions and food supply [15]. Moreover, the extracted mucin contained glycosaminoglycan showed anti-inflammatory and antioxidant actions that cured the colon inflammations [16, 18].

Table 1. Chemical composition of mucus extracted from *E. desertorum* snails

No.	R_t (min)	Area%	M.wt	M.F.	Main Fragments	Identified compounds	Class/Category
1	3.716	0.60	179	$C_{13}H_9N$	77, 133, 151, 179	Benzo[f]quinoline	Benzoquinolines
2	4.804	18.87	103	C_7H_5N	51, 76, 103	Tricyclo[3.1.0.0(2,4)]hex-3-ene-3-carbonitrile	Cyclic alkene derivatives
3	6.835	19.61	136	$C_{10}H_{16}$	55, 71, 80, 93, 107, 121, 136	Limonene	Monoterpenes
4	11.390	6.12	176	$C_7H_{12}O_5$	61, 74, 86, 103, 116, 145, 157	Glycerol 1, 2-diacetate	Glycerol derivatives
5	13.741	0.86	184	$C_{13}H_{28}$	57, 71, 85, 99, 113, 127, 155, 169	Dodecane, 4-methyl-	Branched alkanes
6	14.056	2.75	206	$C_{14}H_{22}O$	57, 77, 91, 105, 121, 142, 191, 206	Phenol, 2,5-bis(1,1-dimethylethyl)	Phenol derivatives
7	16.883	0.83	198	$C_{14}H_{30}$	57, 71, 85, 99, 113, 127, 141, 155, 169	Tridecane, 2-methyl-	Branched alkanes
8	19.686	0.73	212	$C_{15}H_{32}$	57, 71, 85, 99, 113, 141, 155, 169, 183	Dodecane, 2,6,10-trimethyl-	Sesquiterpenes
9	20.545	2.07	284	$C_{18}H_{36}O_2$	57, 88, 105, 143, 157, 241	Hexadecanoic acid, ethyl ester	A long-chain fatty acid ethyl ester
10	25.323	20.78	340	$C_{23}H_{32}O_2$	57, 91, 121, 149, 177, 214, 283, 340	Phenol, 2,2'-methylenebis[6-(1,1-d imethylethyl)-4-methyl-	Diphenyl methane derivatives
		Total area: 73.22%					

Rt: Retention time; M.W.: Molecular weight; M.F.: Molecular formula.

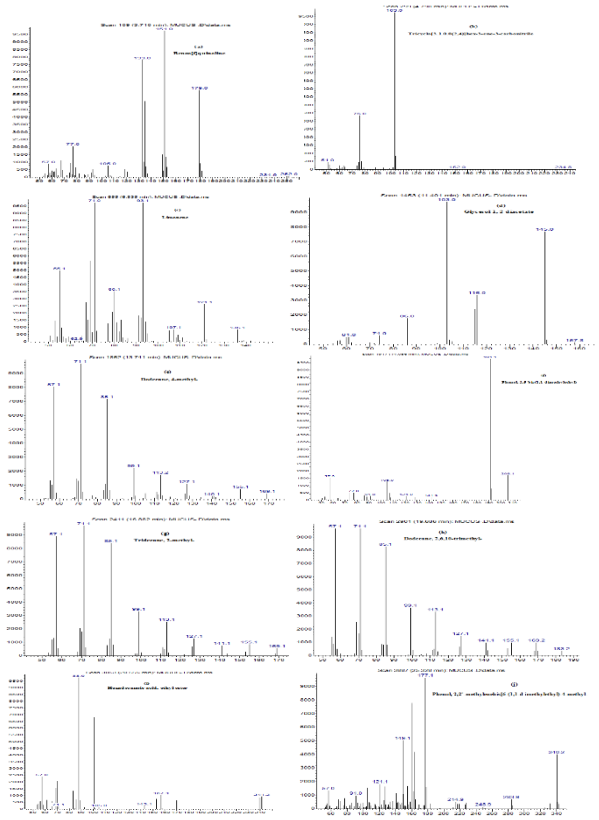


Fig. 1. Mass spectrometry spectra and postulated fragmentation pattern of some identified compounds detected in the mucus extracted from *E. desertorum* snails. (a) Benzo[f]quinoline; (b) Tricyclo[3.1.0.0(2,4)]hex-3-ene-3-carbonitrile; (c) Limonene; (d) Glycerol 1, 2-diacetate; (e) Dodecane, 4-methyl-; (f) Phenol, 2,5-bis(1,1-dimethylethyl); (g) Tridecane, 2-methyl-; (h) Dodecane, 2,6,10-trimethyl-; (i) Hexadecanoic acid, ethyl ester; and (j) Phenol, 2,2'-methylenebis[6-(1,1-d imethylethyl)-4-methyl-.

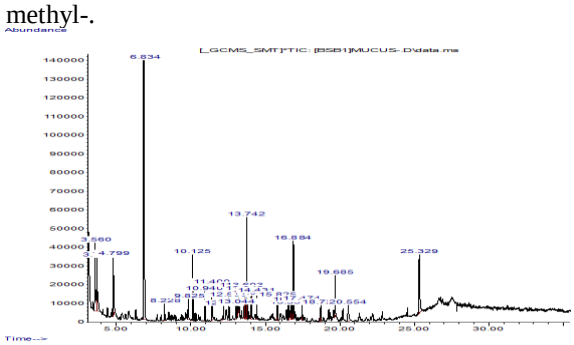


Fig. 2. GC-MS chromatogram of mucin extracted from mucus of *E. desertorum*.

On the other hand, the results in table 2 showed that there are significant increases (P<0.01) in serum ALT, AST and ALP activities accompanied with a significant reduction in serum albumin and total proteins levels in CCl_4 intoxicated group compared with those of the normal group. On the other hand, mice received CCl_4 plus Silymarin and/ or *E. desertorum* snail mucin showed significant improvements in all of these liver function tests compared with those of animals received CCl_4 alone. Also, the results showed that intoxication of mice with CCl_4 induced significant (P<0.01) increase in MDA level, significant (P<0.05) decreases in the activities of SOD and CAT and GSH content compared with those of normal group **(**Table 3). On contrast, the administration of Silymarin and/ or *E. desertorum* snail mucin resulted in significant improvement (P<0.05) in all of these parameters compared with CCl_4 intoxicated group. The present result showed that intoxication of mice with CCl_4 induce significant (P<0.05) increase in IL-2 level

compared with normal control group. On contrast, the administration of Silymarin and/ or snail mucin resulted in significant improvement ($P<0.05$) in IL-2 level compared with those of mice administered CCl_4 alone (Figure 4).

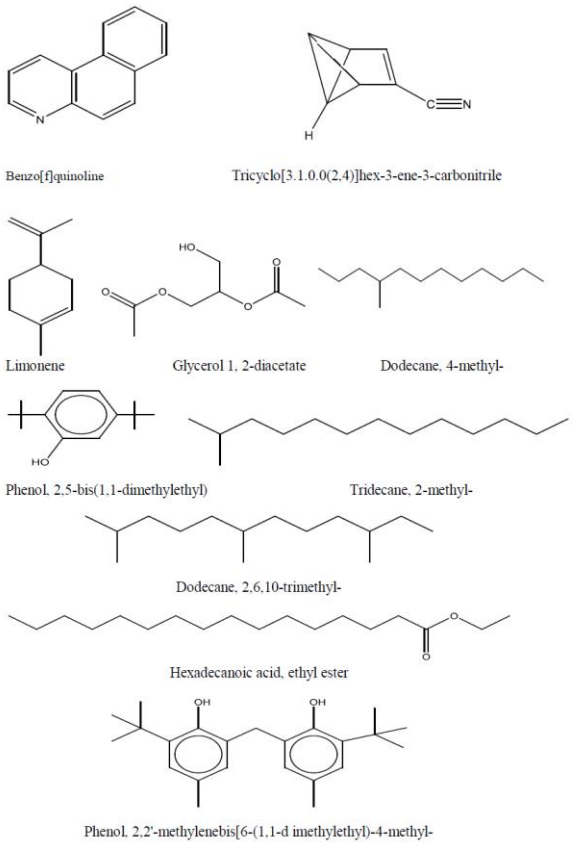


Fig. 3. Chemical structures of identified compound in *E. desertorum* snail mucin.

Histopathogical results showed that there is no sign of toxicity or mortality was recorded during the experiment as well as the weights and temperatures of the mice were stable. The present results showed that CCl_4 intoxicated group has hepatic tissue with degenerated hepatocytes arranged in thick plates, scattered lymphocytes and dilated congested sinusoids (Fig.5. b). While after treatment either with mucin and/or Silymarin, the histopathological sections showed hepatocytes with mild vacuolation with almost normal structure and architecture, hepatocytes arranged in thin plates and aggregation of lymphocytes. Also, liver sections from mucin and Silymarin treated group showed hepatocytes with mild degeneration, almost normal structure and architecture, hepatocytes arranged in thin plates, scattered hepatic cells necrosis and hepatocytes with binucleated nuclei (Fig.6. c, d, e).

Health supplement of natural origin could reduce the risk of several oxidative damages with minimal side effects [37]. Carbon tetrachloride (CCl_4) is a hepatotoxin that is used in the experimental studies [4] and associated with oxidative stress and free radicals [1]. The chronic liver diseases that were caused by chemical-induced oxidative stress [13] could be improved via the antioxidant medicines such as Silymarin [4]. Silymarin is a herbal remedy that has a hepatoprotective and anti-inflammatory activities [13]. The efficacy of any hepatoprotective drug focused on how it alleviated the oxidative stress and inhibited the inflammatory responses that had been caused by the hepatotoxicants [38]. The use of natural products was a good option for the treatment of liver fibrosis [39]. In order to try this paradigm the present investigation studied the effect of mucin either alone or in combination with Silymarin on

Table 2. Effect of CCl_4, Silymarin and *E. desertorum* snail mucin treatment on the mean activities of alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) enzymes, serum albumin and total protein of treated mice

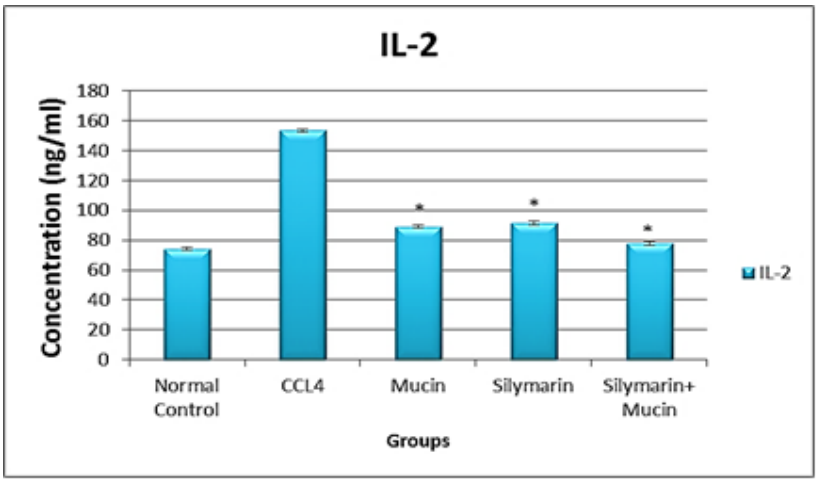
Animal groups	AST (GOT) U/L	ALT(GPT) U/L	Alkaline phosphatase IU/L (ALP)	Total protein (g/dl)	Albumin g/100 ml
Normal	76.94±2.24	34.77±2.75	94.53±2.18	8.19±0.48	5.87±0.23
CCl_4 intoxicated	113.88±2.27[a]	64.41±3.03 [a]	121.36±1.29 [a]	4.07±0.18 [a]	0.463.32±0.1 [a]
CCl_4+ mucin	64.79±1.25 [b]	45.11±2.22 [b]	84.96±1.93 [b]	6.95±0.23 [b]	3.8±0.2 [b]
CCl_4+ Silymarin	71.09±1.73 [b]	47.59±1.39 [b]	73.38±1.44 [b]	4.89 ±0.23 [b]	4.23±0.09 [b]
CCl_4+Silymarin+mucin	62.89±1.73 [b]	42.23±1.39 [b]	78.69±2.01 [b]	6.92±0.47 [b]	5.16±0.18 [b]

- a: significant from Normal group b: significant from CCl_4 intoxicated group

Table 3. Effect of CCl_4, Silymarin and *E. desertorum* snail mucin treatment on the mean activities of superoxide dismutase (SOD) and catalase enzymes and the mean levels of reduced glutathione (GSH), and malondialdehyde (MDA) of treated mice

Animal groups	Catalase	SOD	GSH	MDA
Normal	23.32±1.5	117.29±2.5	10.88±0.18	4.21
CCl_4 intoxicated	12.65±0.71[a]	55.99±2.6 [a]	6.99±0.18 [a]	5.4 [a]
CCl_4+ mucin	17.99±1.0[b]	82.1±2.4 [b]	8.01±0.16 [b]	4.3 [b]
CCl_4+ Silymarin	17.21±0.8[b]	73.58±5.9 [b]	7.33±0.27 [b]	4.2 [b]
CCl_4+Silymarin+mucin	19.61±2.0[b]	75.88±3.2 [b]	7.84±0.15 [b]	4.1 [b]

- a: significant from Normal group b: significant from CCl_4 intoxicated group



- **Fig. 4.** Histogram shows the effect of CCl_4, Silymarin and *E. desertorum* snail mucin treatment on IL-2 level**.**

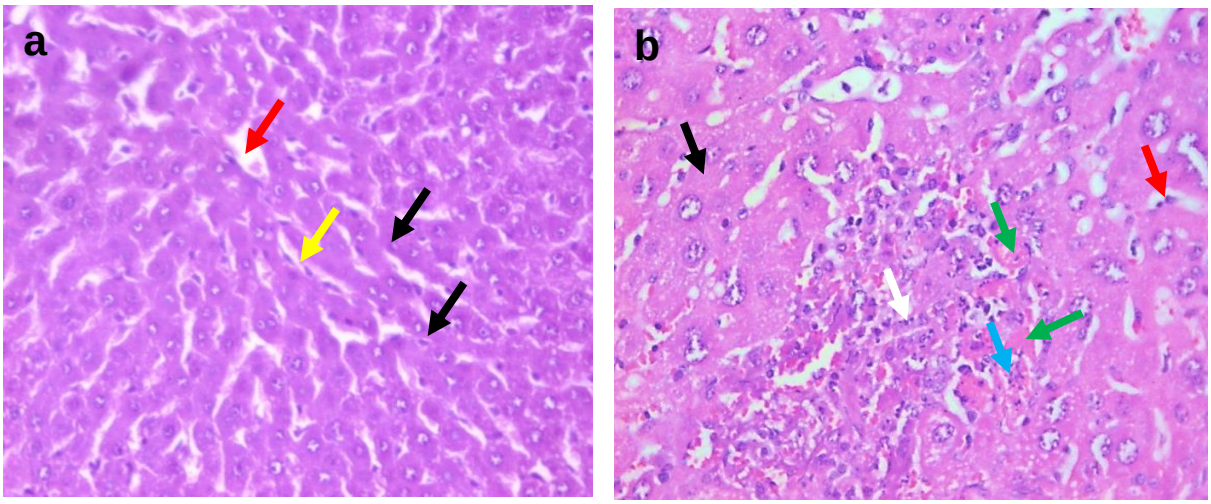


Fig. 5. Liver section from **a) normal control group:** showed hepatic tissue with normal structure and architecture, hepatocytes arranged in thin plates (black arrow) and sinusoids (yellow arrow), central vein (red arrow). **b) Positive CCl_4 group:** showed hepatic tissue with hepatocytes arranged in thick plates (black arrow) and dilated congested sinusoids with (red arrow), many hepatic cell necrosis (green arrow), degenerated hepatocytes (blue arrow), scattered lymphocytes (white arrow) (H&E, x400).

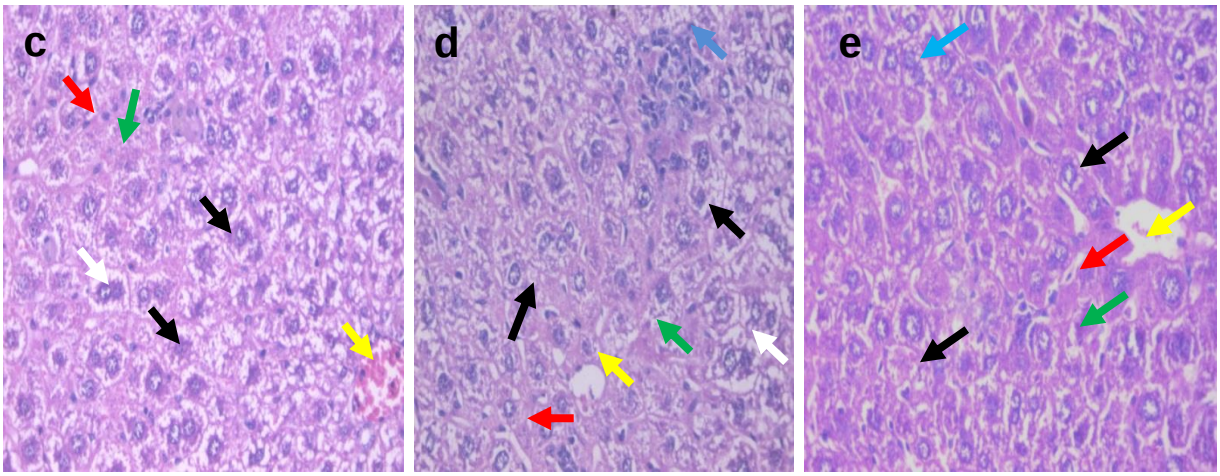


Fig. 6. Liver section from **c) Mucin treated group** showed hepatocytes with mild vacuolation with almost normal structure and architecture, hepatocytes arranged in thin plates (black arrow) and sinusoids (red arrow), central vein (yellow arrow), scattered hepatic cells necrosis (green arrow), binucleated nuclei (white arrow)**. d) Silymarin treated group** showed

hepatocytes with mild hydropic degeneration with almost normal structure and architecture, hepatocytes arranged in thin plates (black arrow) and sinusoids (red arrow), central vein (yellow arrow), scattered hepatic cells necrosis (green arrow), binucleated nuclei (white arrow), lymphocytes aggregates (blue arrow). **e) Mucin and Silymarin treated group** showed hepatocytes with mild degeneration, almost normal structure and architecture, hepatocytes arranged in thin plates (black arrow) and sinusoids (red arrow), central vein (yellow arrow), scattered hepatic cells necrosis (green arrow), hepatocytes with binucleated nuclei (blue arrow) (H&E, x400).

CCl_4 induced liver toxicity. On the other side, the present results showed that there were significant increases in serum ALT, AST and ALP activities accompanied with a significant reduction in serum albumin and total proteins levels in that CCl_4 intoxicated group compared with the normal group. On the other hand, the intoxicated CCl_4 mice that treated with either Silymarin and/ or snail mucin extract showed significant improvements in all of these liver function tests compared to mice received CCl_4 alone. These results in good accordance with El-bakry et al who found that CCl_4 induced significant increase in the serum activities of ALT, AST and ALP and reasoned these alterations to the oxidative stress mediation. While, the reduction in serum total protein and albumin concentrations after CCl_4 intoxication might be due to liver damage through induction of lipids peroxidation and cellular membrane inflammation. Moreover, treatment with *Moringa oleifera* administration causes significant amelioration in levels of ALT, AST, ALP, albumin and total protein after CCl_4-intoxication and concluded that *Moringa oleifera* leaves extract could improve these hepatotoxic effects **[1]**.

Silymarin is a hepatoprotective agent that can be used alone or in combination for treating liver fibrosis in humans **[4]**. In rat, the high dose of Silymarin could restore the hepatic fibrosis induced by CCl_4 **[**40**]**. Where, it decreased the elevation of aspartate aminotransferase (AST), alanine aminotransferase, and alkaline phosphatase in serum. The same results obtained from Wang et al who confirmed the hepatoprotective effect of the natural compound Zerumbone (ZER) which ameliorated the acute liver injury in CCl_4-induced mice models through amelioration of AST and ALT activities [41]. Results showed that intoxication of mice with CCl_4 induce significant increase in MDA level, a significant decrease in activities of CAT and SOD as well as GSH content compared with those of normal control group. On contrast, the administration of either Silymarin and/ or snail mucin extract resulted in significant improvement in all of these parameters compared with those of the intoxicated CCl_4 mice. These results were in consistence with previous study of Safhi (2018) who stated that CCl_4 treatment in Swiss albino mice has reduced the antioxidant enzyme such as GSH, GPx, GR, GST, CAT, and SOD compared to normal group and confirmed the protective effects of Zingerone against CCl_4 induced nephrotoxicity (CCl_4+ Zingerone) through increasing the antioxidant enzymes than CCl_4 treated group [42]. The present investigation showed that intoxication of mice with CCl_4 induced significant increase in IL-2 level compared with normal control group. On contrast, the administration of Silymarin and/ or snail mucin resulted in significant improvement compared with those of mice administered CCl_4 alone. Safhi observed that CCl_4 significantly increased the cytokines such as IL-1β, IL-2, and TNFα levels as compared to normal group, while after the treatment with Zingerone significantly attenuated the levels of IL-1β, IL-2, and TNFα in group 3 compared to CCl_4 group **[**42**]**. Also, he confirmed that the protective effects of Zingerone against CCl_4 induced nephrotoxicity via modulation of inflammatory cytokines, and apoptosis [42]. Wang et al (2019) showed that Zerumbone pre-treatment could inhibit the production of inflammatory cytokines TNF-α and IL-6 in CCl_4-intoxication mice and related its hepatoprotective effect of ZER to the down-regulating the inflammatory response [41]. The combination of Silymarin and ginger could reduce the severity and incidence of liver fibrosis through their anti-tumor and anti-inflammatory effects as they exerted synergistic effects [4].

Histopathological results of the present study confirmed the hepatotoxic effect of CCl_4 on hepatic cells and the hepatic recovery after treatment of intoxicated mice with *Eremina desertorum* snail mucin either alone or in combination with Silymarin. CCl_4 intoxicated group has hepatic tissue with degenerated hepatocytes arranged in thick plates, scattered lymphocytes and dilated congested sinusoids. While after treatment either with mucin and/or Silymarin groups showed hepatocytes with mild vacuolation with almost normal structure and architecture, hepatocytes arranged in thin plates and aggregation of lymphocytes. Liver sections from mucin and Silymarin treated group showed hepatocytes with mild degeneration, almost normal structure and architecture, hepatocytes arranged in thin plates, scattered hepatic cells necrosis and hepatocytes with binucleated nuclei. El-bakry et al confirmed the hepatotoxic effect of CCl_4 on hepatic cells and observed the hepatic recovery after treatment of intoxicated rats with *Moringa oleifera* extract. This recovery is due to the improvement in hepatic architecture disorganization, severe hepatic fatty degeneration together with inflammatory cells infiltration after *Moringa oleifera* extract administration [1]. Dutta et al observed that the CCl_4 group has hepatocellular necrosis, bile duct proliferation, sinusoidal dialation, inflammation

(leukocyte infiltration), vascular congestion, loss of structure of hepatic nodules, fatty infiltration, vascular degeneration and calcification. This injury was down regulated by the administration of standard drug Silymarin and found that leaf extract of *Croton bonplandianus* Baill has better potentiality to protect hepatocellular damages than the standard drug Silymarin [37].

4. Conclusion

Conclusively, the present work revealed that *E. desertorum* mucin could be used as a potential hepatoprotective, antioxidant and anti-inflammatory agent for hepatic disorders against CCl_4 induced hepatotoxicity which confirmed by histopathogical study. Administration of *E. desertorum* mucin led to obvious reduction in the elevated level of liver enzymes (ALT, AST and ALP) as well as SOD, catalase and GSH compared to Silymarin as reference drug. Further studies were needed to develop it into a drug for treatment of liver fibrosis with safe and low cost.

5. Conflict of Interest

The authors declare no conflict of interest.

6. Acknowledgments

The authors would like to thank the financial support of the internal project "103 M", Theodor Bilharz Research institute, Giza, Egypt

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Funding

The financial support of the internal project "103 M", Theodor Bilharz Research institute, Giza, Egypt.

Availability of data and materials

All the data obtained during the study are presented in this manuscript. Any further enquiries for additional information are available upon request from the corresponding author.

Authors' contributions

AMI and MAG: Conceived and designed the study. AMI: Collected snails from the field, performed the experiments and analyzed the data. TMH: Reared mice, give them the treatment and sacrificed them. OAH: Perform the histopathological section. MAG: Analyzed GC-MS/MS data. AMI and MAG: Wrote the paper. OAH and TMH: Revised the paper. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Ethical approval had been granted approval by the Ethics Committee of Theodor Bilharz Research Institute (TBRI) number [PT (511)].

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interest.

Abbreviations

GC-MS/MS: Gas chromatography-mass spectrometry/mass spectrometry; MDA: Malondialdehyde; IL-2: Interleukin-2; CAT: Catalase; SOD: Superoxide dismutase; GSH: Glutathione; ECM: Extracellular matrix components; HSC: Hepatic stellate cells; SBSC: Schistosome Biological Supply Centre; r.p.m.: Rotation per minute; MSD: Mass spectrometric detector; PFTBA: Perfluorotributylamine; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ELISA: Enzyme-linked immunosorbent assay; S.D.: Standard deviation; SPSS: Statistical Package for the Social Sciences; ALP: Alkaline phosphatase; GPx: Glutathione peroxidase; GR: Glutathione reductase; GST: Glutathione S-transferase; IL-1β: Interleukin-1β; TNFα: Tumor necrosis factor alpha; IL-6: Interleukin 6.

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