Hydroclathrus clathratus as anti-damaging agent against lung injury in male albino rats

Reem Mohammed Alharbi

Abstract

Background: The present investigation is designed to evaluate the antioxidant and protective efficacy of the brown alga, Hydroclathrus clathratus (C.Agardh) M. Howe, against copper-induced lung injury in male albino rats. The present study was carried out on 24 adult male albino rats, they were randomly divided into four groups (n = 6) (A group, control rats; B group, rats received 100 mg/kg body weight of H. clathratus ethanolic extract; C group, rats augmented with 100 mg/kg body weight of CuSO4; and D group, rats were supplemented with 100 mg/kg of CuSO4 and 100 mg/kg of H. clathratus ethanolic extract). All the experimental treatments were given orally and daily for 28 days.

Results: It was showing that Cu treatment was found to induce lung toxicity, histopathologically, Cu revealed severe degenerative and necrotic lesions in the lung. Also, Cu caused a significant decrease in glutathione-S-transferase (GST) count and glutathione (GSH); meanwhile, malondialdehyde (MDA) content was increased. Consistently, mRNA and protein expression levels of proapoptotic (caspase-3 and Bax) marker showed a significant upregulation, whereas the anti-apoptotic (Bcl-2) level was significantly downregulated in lung tissues of CuSO4-intubated groups. Moreover, H. clathratus plus CuSO4-treated group showed improvement in the histopathological changes of lung injury. The bronchi and bronchioles appeared like those of the control, where the alveoli showed thin septa in some parts and thickened septa in other parts.

Conclusion: Findings revealed that the natural antioxidant activity of H. clathratus could protect the lung tissue from the damage produced by CuSO4.

Keywords: Hydroclathrus clathratus, Apoptosis, Lung injury, Albino rats

1 Background

Copper is an ingredient of a number of metalloenzymes such as catalase, peroxides, and cytochrome oxidase [1] and is a perfect factor, highly occurrence in human and animal tissues [2]. It is required for ceruloplasmin which supports to compensate reactive oxygen species [3] and has the efficiency to oxidize Fe (II) and regulates iron efflux from certain cells [4]. Among the medical implementation of Cu is its exploitation in certain types of dental amalgam and intrauterine contraceptive devices (IUCD). It seems in various enzymes, encourages the absorption of iron, and expands to transfer electrical signals in the body. Although Cu is very important for normal physiological and biological functions in humans and animals, the excessive exposure of Cu increased induces adverse toxic effects such as hemolysis, gastrointestinal distress, and hepatorenal damage [5]. Copper is a catalyst or stimulus that causes oxidative process during the formation of reactive oxygen species and fatal injury for lipid peroxidation [6]. It can be imbibed into the systemic current from the skin, gastrointestinal tract, and lungs. It restricts to amino acids and plasma albumin in the portal blood, then imparted to the liver where it is combined to ceruloplasmin and progressive to the plasma. Kidney and liver are the main target organs of

Correspondence: r_0660@hotmail.com
Biology Department, Science College, University of Hafer Al-Baten, Hafer Al-Baten, Saudi Arabia

© The Author(s). 2020 Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.
CuSO₄ leading to hepatorenal toxicity [7]. Therefore, studies on the copper toxicity are of especial interest.

Apoptosis assay can be induced by a wide variety of stimuli such as glucocorticoids, radiation, and pesticides, and it plays an important role in many fundamental biological processes such as embryogenesis, metamorphosis, tissue homeostasis, development, and regulation of the immune system [8]. The most biological activities of different species of the marine algae attribute to its natural polymer [sulfated polysaccharides (SPs)] that can be isolated from it [9]. The chemical composition and structure of the sulfated polysaccharides were varied according to whether it is isolated from green, brown, or red algae [10]. These frequencies, including the quantitative and qualitative constituents of protein, carbohydrate, sulfates, and its amount of sulfation, can income diverse pharmacologic usage that has been examined in vitro and in vivo in animal models with committed results. These results may be of advantage incurring different illness and can be supposedly used as anticancer, anti-allergy, antiviral, antioxidant, and anti-coagulating agents [11]. In recent decades, there has been an increasing interest from researchers and the pharmaceutical industry in marine algae [12–15]. This fact is due to their enormous potential as a source of molecules and bioactive substances, which may be used in the development of new drugs. Today, in addition to the conventional wisdom, it is known undoubtedly that many of the resulting compounds of algae from secondary metabolism act as stimulators of the primary metabolism of the person who ingests it, stimulating the activity of certain endocrine glands, blood circulation, exchanges of mineral elements, and the physiological elimination of toxins. Due to recent studies in this area of knowledge, several of these compounds were characterized, and their respective pharmacological properties were determined—cholesterol reducers, anticoagulants (important in the prevention of stroke), antimicrobials, antitumor, antiviral, anthelmintics, anti-inflammatory, antacids, growth regulators, immunoregulatory, etc [16–18].

Regarding their antioxidant activity, many genera of algae have been reported. These antioxidant substances include the following: mycosporine-like amino acids, sulfated polysaccharides, scytominen, phlorotannins, carotenoids, phycobiliproteins, porphyran, fucoidan, carrageenans, and ulvan. Through all the marine algae, the highest phytochemical constituents have been reported from brown marine algae [19]. H. clathratus, a brown marine alga, has been used for centuries in traditional cuisine and medicine of island countries such as Hawaii. H. clathratus is known to possess anticancer, anti-herpetic, anti-inflammatory, and anticoagulant properties and is now used as a mineral supplement in cosmetics and as soil additive (fertilizer) for its high concentration of micronutrients [20]. Extracts of H. clathratus have antiviral [21], antitumour [22], and antimicrobial [23] activity. The present study was aimed to explore the natural antioxidant activity of H. clathratus against copper-induced lung injury in male white albino rats.

2 Methods
2.1 Experimental alga
Fresh, matured, and healthy marine alga was collected from Red Sea coast. The algal specimen was selected, cleaned completely with sea water to remove all epiphytes, the uncleanness, and sand particles. The specimen was imperturbable in sterilized polyethylene bags and put in an icebox, then transferred to the laboratory immediately till the applied test work was done. The collected algal specimen was identified as H. clathratus (C. Agardh) M. Howe (Fig. 1). The specimen has been submitted to the phycological lab for keeping preservation H. clathratus belongs used in this study to brown algae.

2.2 Specimen planning
The specimen was gently rinsed with sterile distilled HOH to drive out sand, salt and epiphytes, and other solder substance. The algal body was then dried in shade conditions in open-air temperature. The completely dried algal materials were weighed, powdered, and then stored at −20 °C for further analysis.

2.3 Ethanolic extract
One hundred grams of powdered marine algal sample was soaked in the organic solvent ethanol (1:4 w/v), and extracted for 4 days at room temperature and the extracts were collected and concentrated. Finally, the sample was macerated with water for 24 h to obtain the aqueous extract. The extract was stored in airtight glass container at 4–8 °C for further analysis.
2.4 Physicochemical evaluations
The physicochemical analysis like ash content, weight loss while drying was calculated according to the methods prescribed in Indian Pharmacopoeia [24].

2.5 Preliminary phycochemical screening
Preliminary phycochemical screening of ethanolic extract of H. clathratus (C. Agardh) M. Howe was completed to check the phy-co-constituents by using principle familiar organizer according to Harborne [25].

2.6 Chemicals
Copper sulfate (CuSO₄), was purchased from Sigma Chemicals Company for Pharmaceutical Industries, St Louis, Missouri, USA. All other chemicals were of analytical grade and obtained from standard commercial supplies.

2.7 Experimental animals
Forty-five male Western rats with average weight of 150–180 ± 5.17 g were procured from the animal house unit of College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. The animals were staying in stainless-steel coop under favorable conditions and steady at standard laboratory conditions. The animals were adapted to laboratory conditions for 1 week before the start of the test. All animal procedures are in accordance with the general guidelines of animal care and the recommendations of the Canadian Committee for Care and use of animals [26]. The use of rat models in the present study was subject to approval by the ethical committee responsible for the laboratory principles of handling and care of experimental animals in accordance with the Canadian Committee for Care and use of animals [26].

2.8 Experimental design
Twenty-four adult male albino rats were randomly assigned to four groups (n = 6): the 1st group was kept as normal control group: received saline orally, daily for 28 days. The 2nd group received 100 mg/kg body weight of H. clathratus extracts suspended in 1% carboxymethyl-cellulose (CMC) for 28 days. The 3rd group rats were supplemented with 100 mg/kg/day (CuSO₄) orally for 28 days prior to sacrifice. The 4th group rats were administered orally by 100 mg/kg/day of CuSO₄ for 28 days and 100 mg/kg of H. clathratus extracts simultaneously. All rats were sacrificed at the day 28, specimen of lung tissues and blood were collected for more tests.

2.9 Preparation of samples for analysis
At the end of the experiment, the mice are slaughtered (the method of euthanasia used in rats is the used-acceptable physical method which causes a rapid loss of consciousness by disrupting the central nervous system), and immediately the lung was quickly excised, cleaned, and washed in cold saline. A portion of the lung was homogenized (10% w/v in cold phosphate-buffered saline [PBS]) by employing a Teflon homogenizer (Glas-Col, Terre Haute, IN, USA). The homogenate was centrifuged at 1000 rpm for 10 min at 4 °C, and the explicit supernatant was kept at −20 °C. The other piece was divided into two portions, one was kept frozen at −80 °C for DNA isolation and western blotting and the other was steady in 10% neutral buffered formalin for histological study.

2.10 Biochemical assays

2.11 Assay of oxidative stress
Glutathione-S-transferase (GST) test was performed according to the method of Habig et al. (1974). Lipid peroxidation was evaluated in homogenate lung tissue by regulating malondialdehyde (MDA) according to the method described by Preuss et al. [27]. Reduced glutathione (GSH) constituents were performed according to the method described by Beutler et al. [28].

2.12 Quantitative reverse transcription PCR analysis of gene expression (qRT-PCR)
Total RNA was segregated from the frozen specimen using Invitrogen reagent and patronize by RNase-free DNase InVitrogen. Clarified RNA was quantified at 260 nm and for reverse transcription, RNA specimen with A260/A280 ratios ≥ 1.7 was chosen. Additionally, RNA probity was doubtless by formaldehyde-containing agarose gel electrophoresis. Reverse transcription was carried out with 5 μg RNA using RevertAidTM First Strand cDNA Synthesis Kit (Fermentas, USA).

The PCR reaction inclusive primary denaturation at 95 °C for 3 min, forty rounds of denaturation at 95 °C for 15 s, safeguard at 5 °C for 30 s, and expansion at 72 °C for 40 s, and the last stride at 60 °C increased about half °C each 10 s up to 95 °C. Melting curve dissection was outright to review the specificity of the used primers. Each test includes a distilled water control. The verbosity data were analyzed and approved to Livak and Schmittgen [29] and the values were normalized to β-actin.

2.13 Western blot analysis
Western blotting for ovarian caspase-3, Bax, and Bcl-2 was conducted by using the criterion mode. The frosted lung tissues were homogenized in ice-cold lysis buffer and the homogenates were centrifuged at 10,000×g for 10 min. Protein concentration was decided according to Bradford (1976). Equivalent value of proteins was electrophoresed using 10% SDS/PAGE and electrotransferred to PVDF membranes. The membranes were plugged in 5% w/v
skimmed milk powder in PBS/tween 20 (PBST) for 1 h at open-air temperature. The membranes were mixed with the antibodies for caspase-3, Bax, Bcl-2 and, β-actin (Santa Cruz Biotechnology, USA) diluted 1:1000 in blocking buffer. Then, the membranes were mixed with the corresponding secondary antibodies for 1 h at open-air temperature, then washed, and developed. The optical density was quantify with Image J and normalized to β-actin.

2.14 Microscopic examination
The lung tissue was routinely processed and sectioned at 4–5 μm thickness with a microtome then stained with hematoxylin and eosin (H&E) according [30].

2.15 Statistical analysis
Data were statistically analyzed using the Statistical Analysis System software (SAS) [31]. Effect of treatments on biochemical was performed by the analysis of variance. Means were compared using Duncan’s multiple range test at a significance level of p ≤ 0.05. Values are represented as means ± standard errors.

3 Results
3.1 Physicochemical evaluations of H.clathratus
The results of the physicochemical analysis are shown in Table 1.

3.2 Preliminary phycochemical screening of the algal extracts
Preliminary phycochemical screening of the studied alga extract of H. clathratus (C. Agardh) M. Howe was carried out to detect the phyco-constituents. The obtained data indicate the presence of phytosteroids, tannin, saponin, alkaloid, phenol, terpenoids, coumarins, cardiac glycoside, phlobatannins, flavonoids, anthraquinones, and steroids (Table 2).

3.3 Detection of lung MDA, GSH, and GST content
LPO (lung lipid peroxidation) was assayed as nmol malondialdehyde (MDA)/100 mg tissue. It was shown a significant elevation (p < 0.001) in copper sulfate-injected rats as compared to the control group. On the other hand, Co treatment with Hydroclathrus clathratus extract has shown a significantly ameliorated in the MDA level. Regarding to GSH and GST content, copper produced a significant depletion of GSH and GST (p < 0.001) as compared to the control group. However, Hydroclathrus clathratus co-administration induced a significant increasing lung GSH and GST content (p < 0.01) when compared to rats injected with copper sulfate only (see Table 3).

3.4 Quantitative reverse transcription PCR analysis of gene expression
Quantitative reverse transcription PCR test of gene expression clarifies a significant upregulation of caspase-3 and Bax tissues (p < 0.001) of copper sulfate-injected rats as compared with the control group (see Fig. 1a, b). However, co-administration by H. clathratus ethanolic extract was significantly downregulate caspase-3 and Bax mRNA expression levels (p < 0.001) compared to rats received H. clathratus only. Western blotting data clarify significant high level of protein expression of caspase-3 and Bax tissues of copper sulfate (p < 0.001) in treated rats. On the other hand, oral gavage of H.clathratus concomitant with copper sulfate generated a significant reduction in protein expression scale (p < 0.001) of all investigated parameters as contrast to copper sulfate-treated mice (see Fig. 2a, b).

3.5 Histopathological studies
During the histopathological studies and at stained sections of the lung of the control group (A), the data showed normal alveoli with thin alveolar septa, clear alveolar sacs, and the bronchioles are lined with columnar epithelial cells and the pulmonary blood vessels are normal in appearance (see Fig. 3a). Also, in group B, lung tissues treated with H. clathratus showed a normal histological

Table 1 Physicochemical analysis of H.clathratus

| Parameter          | Values (% w/w) |
|--------------------|----------------|
| Extractive values  | 1.4 ± 0.06     |
| Total ash          | 4.21 ± 0.07    |
| Acid insoluble ash | 2.31 ± 0.12    |
| Water soluble ash  | 3.04 ± 0.01    |

Table 2 Phycochemical screening of ethanolic extract of H.clathratus

| Phytochemical tests | Ethanolic extract |
|---------------------|-------------------|
| Phytosteroids       | +                 |
| Coumarins           | +                 |
| Saponins            | +                 |
| Alkaloids           | +                 |
| Phenols             | +                 |
| Terpenoids          | +                 |
| Tannins             | +                 |
| Cardiac glycosides  | +                 |
| Phlobatannins       | +                 |
| Flavonoids          | +                 |
| Anthraquinones      | +                 |
| Steroids            | +                 |
| Glycosides          | -                 |
| Quinones            | -                 |
structure similar to the lung tissues of the A group (see Fig. 3b). However, in group C the data revealed the proliferation of alveolar cells, dilated pulmonary vessels surrounded by inflammatory cellular infiltrations, and marked hyperplasia of the walls of dilated bronchioles. At the same time, the lung of rats showed many pathological changes. The lungs had dilated bronchioles with corrugated walls and thickened epithelial lining and loss of normal architecture in some areas which resulted from the proliferation of alveolar cells which appeared closely packed with pyknotic nuclei (see Fig. 3c). Dilated pulmonary vessel surrounded with inflammatory cellular infiltrations were also observed. Other parts showed thickened inter-alveolar septa, with shedding of cellular debris in lumen. Collapsed alveoli with compensatory dilatation of neighboring ones separated by thickened interalveolar septa. Aggregations of inflammatory cell were observed. Fibrosis of the surrounding bronchial muscle layer together with partial shedding of the mucosal lining, also cellular debris with extravasation of red blood cells in the bronchiole lumen was observed. Moreover, numerous areas of cellular infiltrations in connective tissue surrounding the bronchioles were also seen.

Lung tissue of male albino rats received CuSO₄ in addition to H. clathratus showed obvious recovery from the damage produced by the exposure to Cu such as normal architecture and normal appearance of many alveoli and bronchioles, also the bronchi and bronchioles appeared like those of the control (Fig. 3d). However, some blood vessels were still congested and hemolysis blood cells appeared in their lumens and few inflammatory cells were also seen.

Table 3 Effect of H. clathratus on pulmonary oxidative stress and antioxidant defense system parameter (data expressed as mean ± SD)

| Parameter                  | Group A (n = 6) | Group B (n = 6) | Group C (n = 6) | Group D (n = 6) |
|----------------------------|----------------|----------------|----------------|----------------|
| MDA (nmol/mg protein)      | 1.86 ± 0.55    | 1.89 ± 0.23    | 8.24 ± 0.98*   | 2.87 ± 0.33*   |
| GSH (μg/mg protein)        | 6.4 ± 0.31     | 7.13 ± 0.24    | 5.13 ± 0.16    | 6.14 ± 0.04*   |
| GST (U/min/mg protein)     | 1.76 ± 0.04    | 1.80 ± 0.20    | 1.02 ± 0.05*   | 1.57 ± 0.14*   |

*Significant to control group at p < 0.05. *Significant to Cu group at p < 0.05. Values were considered significantly different Pa < 0.001 versus control group and Pb < 0.001 versus group C.

Fig. 2 Gene expression levels were assessed in the lung of control and experimental rats.
4 Discussion

4.1 Physicochemical evaluations of *H. clathratus*

Standardization is an important tool in order to establish their identity, purity, safety, and quality. Physicochemical analysis will be helpful in identification and authentication of the alga material. The physicochemical evaluation of algal ethanolic extract can provide a valuable provenance of acquaintance and provide suitable criterions to find the goodness of this marine alga in relish search or implementations [32].

4.2 Preliminary phytochemical screening of the algal extracts

Marine algae are opulent in secondary metabolites which contain flavonoid, alkaloid, tannin, steroid, saponin, glycoside, and correlating active substances, which are of large medication prominence in the drug manufacture. Some of the antioxidant substances of the marine algae such as phenols and flavonoids play a vital role in the bioactivity which includes anti-inflammatory [33, 34].

4.3 Detection of lung MDA, GSH, and GST content

The quantity of lipid peroxides was increased significantly in Cu toxic rats compared with those of the control group. Many technicalities have been suggested to illustrate Cu-induced cellular poisoning. Cu can occur in the oxidized Cu$^{2+}$, or in the reduced Cu$^{+}$ case. In active cells, Cu serve as promoting catalyst in the production of hydroxyl or superoxide radicals, and hydrogen peroxide via the Haber-Weiss reaction [35], which can give rise oxidative deterioration and prompt opposite efficiency [36]. Rise contents of copper may induce high oxidative disrupt to DNA, proteins, or lipids. Recently, it has been suggested that copper-oxide nanoparticles (CuONPs) are poisonous to skin cells and that extracellular signal-regulated kinase, and so the p53 may be the key factors regulating the cytotoxicity [37]. CuONPs also induced oxidative exertion and apoptosis in HaCaT human keratinocytes [38]. In further investigations, human MCF-7 cells were managed with copper (Cu$^{2+}$) in a dose-echo way and used macerate total reflection (FTIR) concerted with computational analysis to determine cellular changes. Cupric ions induced bimodal dose-response efficiency on cells, while proteins and lipids seemed to be the main cell targets [39].

In the present data, the lipid peroxidation levels were increased and the GSH and GST activity were reduced. On the other hand, there are damages in lipids and so decreasing in lipid peroxidation obstetrics with the considerable in the liver and the nephritic GSH in the Cu
administer set. It is accordance with earlier work by [40]. Treatment by *Hydroclathrus clathratus* extracts markedly decreased MDA levels and improves the antioxidant defenses. It is due to the antioxidantative properties of the marine algae which are wealthy parent of proteins, minerals, vitamins, and dietary fiber [41, 42].

### 4.4 Quantitative reverse transcription PCR analysis of gene expression

The present data on the mRNA expression level of Bcl-2 was done in Fig. 2c. There is a significant downregulation of mRNA expression (*p* < 0.05) in copper sulfate-treated group as compared to the control group. The administration with copper sulfate and *H.clathratus* induces a significant upregulation of both Bcl-2 mRNA (*p* < 0.05) expression levels.

The present data revealed a significant increase in the lung pro-apoptotic (caspase-3 and Bax) genes expression and significant decrease levels of anti-apoptotic Bcl-2 expression in the copper sulfate-administered rats as compared to the control group. It suggested an upregulation of caspase-3 and Bax in addition to downregulation of Bcl-2 genes and proteins, and may participate in that copper prompt the activation of apoptosis through caspase-dependent and independent pathways as reported by Santos et al. [43]. The Bcl-2 protein family encourages programmed cell death by the mitochondrial pathway of apoptosis which is also recognized as intrinsic. In response to different cytotoxic exertions, pro-apoptotic proteins start to redaction apoptogenic agents such as cytochrome c into the cytosol and prompt caspase activation in the cytosol which is known in both initiation and execution of apoptosis [44]. It has been clarified that over-expression of Bcl-2 can save cells from apoptosis mediated by ROS [45]. Bcl-2 itself does not have antioxidant efficacy, but it may act indirectly to increase the levels and/or activities of endogenous antioxidants within cells [46]. On the other hand, changes in the Bax and Bcl-2 expression also prompt caspase-3 and activate apoptotic processes [47]. My data clarify that the caspase-3 protein expression was found to be elevated in the exposed mice. These changes resulted in elevation number of apoptotic in pulmonary tissue of the exposed rats.

The recorded data has demonstrated that *H. clathratus*-treated group has reduce caspase-3, Bax while increase Bcl-2 expression when compared to copper group. It may be due to the antioxidant effect of the studied alga. It is in accordance with [48] who found that, chemical analysis of *H.clathratus* algal extract was detected the presence of high amount of certain ingredients such as flavonoids (acacetin, Luteo.6-arabinosel-glucose, naringin, hesperetin, rosmarinic, and naringenin) and phenols (benzoic, e-vanillic, pyrogallol, catechin, salycilic, and ellagic). The antioxidant effect of alga extract may be due to the presence of these flavonoid and phenol compounds which have ability to scavenge free radical produce by copper sulfate.

### 4.5 Histopathological studies

The result is in agreement with Kim et al. [49] who reported various histopathological changes in the lung tissue as a result of copper treatment. In this respect, Hatch et al. [50] announce that the capability of rats to respiratory hitting increased significantly following inhalational or installation exposure to Cu and that the increased tendency might be due to a decrease in functions of alveolar macrophages and cilia cells.

The observed protective effect of the investigated alga may be attributed to its rich content of active compounds such as phenolics, sulfated polysaccharides, glycosides, or carotenoids. In this respect, sulfated polysaccharides and carotenoids from some brown algae have shown in vitro antitumoral metastatic and anticancer effect in mice [51].

Therefore, anti-inflammatory effects presented in the current study suggested that the properties of anti-inflammatory components in these species may be interfering with some of the mediators of inflammation, by either control their production or antagonizing their activities [52]. Indeed, it was reported that marine algal extracts decrease the production of inflammatory prostaglandins and leukotrienes [53].

### 5 Conclusion

In conclusion, the observed anti-inflammatory activity in this study may be attributed to several anti-inflammatory compounds have been reported from macroalgal species, which include polyunsaturated fatty acids, alkaloids, and carotenoids. So, the current study imparts new information on the protective nature of brown alga *H. clathratus* against Cu-induced lung injury through apoptosis in it. The present findings suggest that *H. clathratus* algal extract is able to decline the lung injury through the down-regulation of caspase-3 and Bax expression and upregulation of Bcl2 which may be attributed to the antioxidant nature of the active contents of the studied alga *H. clathratus*.

**Abbreviations**

- GST: Glutathione-S-transferase; GSH: Glutathione; MDA: Malondialdehyde; CuSO₄: Copper sulfate; Cu: Copper; H. clathratus: *Hydroclathrus clathratus*; Fe: Iron; SPs: Sulfated polysaccharides; HOH: Water; °C: Temperature; CMC: Carboxymethylcellulose; PBS: Phosphate-buffered saline; PCR: Polymerase chain reaction; RNA: Ribonucleic acid; qRT: Quantitative reverse transcription; PVDF: Polyvinylidene difluoride membrane; PBS: Pharmaceutical Benefits Scheme; PBST: Phosphate-buffered saline; LBST: Phosphate-buffered saline with Tween detergent; Bcl-2: B cell lymphoma 2; H&E: Hematoxylin and eosin; SAS: Statistical Analysis System software; LPO: Lung lipid peroxidation.
Acknowledgements
Not applicable.

Author's contributions
The author read and approved the final manuscript.

Funding
Not applicable.

Availability of data and materials
All data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate
This study was conducted with the approval of the Animal Ethics Committee of Hafer Al-Baten University, Hafer Al-Baten, Saudi Arabia. The Guide of Care and Use of Animals in Research and Teaching as outlined by the committee was used to manage the animals (no. committee’s reference number).

Consent for publication
Not applicable

Competing interests
The author declares that he has no competing interests.

Received: 16 December 2019 Accepted: 12 March 2020
Published online: 14 April 2020

References
1. Lutsenko S (2014) Modifying factors and phenotypic diversity in Wilson’s disease. Ann. NY. Acad. Sci 1315:56–63
2. Pal A, Scotto M, Prasad R, Squitti R (2015) Towards a unified vision of copper involvement in Alzheimer’s disease: a review connecting basic Experimental and clinical research. J. Alzheimers Dis 44:342–354
3. Linder MC (2010) Nutritional biochemistry of copper with emphasis on the perinatal period. In: Avignano L, Rossi L (eds) Biochemical Aspects of Human Nutrition. Research Signpost, Trivandrum, Kerala, India, pp 143–179
4. Vashchenko G, MacGillivray RTA (2013) Multicopper oxidases and iron metabolism. Nutrients. 5(7):2289–2313
5. Roychoudhury S, Massanyi P (2014) Introduction to male reproduction and clinical research. J Alzheimers Dis 44:342–354
6. Abuba PM, Albertini R (2001) Methods for monitoring oxidative stress, lipid peroxidation and oxidation resistance of lipoproteins. Clin Chim Acta. 306(1–2):1–17
7. Hashish EA, Elgaml SA (2016) Hepatoprotective and nephroprotective effect of hydrochloric acid on rat liver and kidney. Int. J. Basic. Med. 6:318–321
8. Roychoudhury S, Massany P, Stawarz R, Kacaniova M, Kolesarova A (2013) Cytotoxicity and genotoxicity of copper oxide nanoparticles in human skin keratinocytes. Int J Toxicol 32:296–324
9. woodsland AS (2011) Statistical Analysis System. Version 9.30. User’s Guide, SAS Institute Inc., Cary, NC, USA.
10. Anonymous Indian Pharmacopoeia (1996) Controller of publications, Government of India. 4th Ed. II. New Delhi.
11. Roychoudhury S, Nath S, Massany P, Stawarz R, Kacaniova M, Kolesarova A (2013) Cytotoxicity and genotoxicity of copper oxide nanoparticles in human skin keratinocytes. Int J Toxicol 32:296–324
12. Abdel-rahaf N, Al-Enazi NM, Almahayden AA, Ameen F, Ibraheem IBM (2018) Potential use of green algae as a biosorbent for hexavalent chromium removal from aqueous solutions. Saudi J Biol Sci 25:1733–1738
13. Abdel-rahaf N, Mohamed HM, Mostafa S, Ibraheem IBM (2017) Controlling of microbial growth by using Cystoseira barbata extract. Egyptian J Botany. 57(3):469–477
14. Ibraheem IBM, Abdel-rahaf N, Mohamed HM, Fassihy R, Hamed S (2017) Impact of the microbial suppression by using the brown alga Dictyota dichotoma extract. Egyptian J Botany. 57(7):205–214
15. Alarifi S, Ali D, Verma A, Alakhtani S, Ali BA (2013) Cytotoxicity and genotoxicity of copper oxide nanoparticles in human skin keratinocytes. Int J Toxicol 32:296–324
16. Al-Homaidan AA, Al-Qantani HS, Al-Ghanayem AA, Ameen F, Ibraheem IBM (2018) Potential use of green algae as a biosorbent for hexavalent chromium removal from aqueous solutions. Saudi J Biol Sci 25:1733–1738
17. Abdel-rahaf N, Mohamed HM, Mostafa S, Ibraheem IBM (2017) Controlling of microbial growth by using Cystoseira barbata extract. Egyptian J Botany. 57(3):469–477
18. Ibraheem IBM, Abdel-rahaf N, Mohamed HM, Fassihy RY, Hamed S (2017) Impact of the microbial suppression by using the brown alga Dictyota dichotoma extract. Egyptian J Botany. 57(7):205–214
19. Foodplusplus (2008) www.foodplusplus.org/fileadmin/files/news/2004-01-22SFRTD1_launchBrussels.pdf. Accessed 25.03.10.
20. Seaweed Industry Association (2011) Hydroclathrus clathratus (SA, Seattle, 2011), Available at: https://seaweedindustry.com/seaweed/type/ hydroclathrus -clathratus.
21. Wang H, Enchoong VO, Put OA (2007) Antiviral polysaccharides isolated from Hong Kong brown seaweed Hydroclathrus clathratus. Sci China C Life Sci 50(5):611–618
22. Jayasooriya RGPT, Moon DO, Choi YH, Yoon CH, Kim GY (2011) Methanol extract of Hydroclathrus clathratus inhibits production of nitric oxide, prostaglandin E2 and tumor necrosis factor-α in lipopolysaccharide stimulated BV2 microglial cells via inhibition of NF-κB activity. Tropical J Pharmaceutical Res 10(6):723–730
23. Arunkumar K, Sivakumar SR (2012) Seasonal influence on bioactivity of seaweeds against plant pathogenic bacteria Xanthomonas axonopodis pv. citri (Hass) Vauterin et al., Afr.J.Microbiol.Res. 6(20), 4324-4331.
24. Anonymous Indian Pharmacopoeia (1996) Controller of publications, Government of India. 4th Ed. II. New Delhi.
25. Harborne AJ (1998) Phytochemical methods a guide to modern techniques of plant analysis. Springer Science & Business Media. ISBN 0 412 57260 5 (HB) and 0 412 57270 2 (PB).
26. Canadian Council on Animal Care (1993) Guide to the care and use of experimental animals. Ottawa, Ontario, Canada, CCAC.
27. Reuss CV, Wood TC, Szumianski CL, Rafqogians RB, Ottermen DS, Girard B, Scott MC, Weinsibhoum RM (1998) Human histamine N-methyltransferase pharmacogenetics: common genetic polymorphisms that alter activity. Molec Pharmac. 53:708–717
28. Beutler E, Duron O, Kelly BM (1963) Improved method for the determination of blood glutathione. J Lab Clin Med 61:882–888
29. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-delta delta C(T)) method. Methods 25(4):402–408
30. Banchroft JD, Stevens A, Turner DR (1996) Theory and practice of histological techniques. Fourth Ed. Churchill Livingstone, New York, London, San Francisco, Tokyo.
31. SAS (2011) Statistical Analysis System. Version 9.30. User’s Guide, SAS Institute Inc., Cary, NC, USA.
32. Sampathkumar S, Ramakrishnan N (2011) Pharmacognostic and phytochemical investigation of Naringi crenulata (Roibx). Nicols. Stem. Anc Sci 25:1733
33. Beutler E, Duron O, Kelly BM (1963) Improved method for the determination of blood glutathione. J Lab Clin Med 61:882–888
34. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-delta delta C(T)) method. Methods 25(4):402–408
35. Banchroft JD, Stevens A, Turner DR (1996) Theory and practice of histological techniques. Fourth Ed. Churchill Livingstone, New York, London, San Francisco, Tokyo.
36. Scott MC, Weinshilboum RM (1998) Human histamine N-methyltransferase pharmacogenetics: common genetic polymorphisms that alter activity. Molec Pharmac. 53:708–717
37. Beutler E, Duron O, Kelly BM (1963) Improved method for the determination of blood glutathione. J Lab Clin Med 61:882–888
38. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-delta delta C(T)) method. Methods 25(4):402–408
39. Banchroft JD, Stevens A, Turner DR (1996) Theory and practice of histological techniques. Fourth Ed. Churchill Livingstone, New York, London, San Francisco, Tokyo.
40. Scott MC, Weinshilboum RM (1998) Human histamine N-methyltransferase pharmacogenetics: common genetic polymorphisms that alter activity. Molec Pharmac. 53:708–717
41. Beutler E, Duron O, Kelly BM (1963) Improved method for the determination of blood glutathione. J Lab Clin Med 61:882–888
42. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-delta delta C(T)) method. Methods 25(4):402–408
43. Banchroft JD, Stevens A, Turner DR (1996) Theory and practice of histological techniques. Fourth Ed. Churchill Livingstone, New York, London, San Francisco, Tokyo.
44. Scott MC, Weinshilboum RM (1998) Human histamine N-methyltransferase pharmacogenetics: common genetic polymorphisms that alter activity. Molec Pharmac. 53:708–717
45. Beutler E, Duron O, Kelly BM (1963) Improved method for the determination of blood glutathione. J Lab Clin Med 61:882–888
46. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-delta delta C(T)) method. Methods 25(4):402–408
47. Banchroft JD, Stevens A, Turner DR (1996) Theory and practice of histological techniques. Fourth Ed. Churchill Livingstone, New York, London, San Francisco, Tokyo.
41. Yen CY, Chiu CC, Haung RW, Yeh CC, Huang KJ, Chang KF, Hseu YC, Chang FR, Chang HW, Wu YC (2012) Antiproliferative effects of goniothalamin on Ca9-22 oral cancer cells through apoptosis; DNA damage and ROS induction. Mutat Res. 747(2):253–258
42. Ibraheem IBM, Abd Elaziz BE, Moawad A, Hassan HM, Mohamed WA, Abdel-Rao G (2017) Antimicrobial and anti-inflammatory effects of two different marine red algae species collected from Quseir, the Red Sea, Egypt. Asian J Biol 2(2):1–10
43. Santos S, Silva AM, Matos M, Monteiro SM, Alvaro AR (2016) Copper induced apoptosis in Caco-2 and Hep-G2 cells: expression of caspases 3, 8 and 9, AIF and p53. Comp Biochem Physiol C Toxicol Pharmacol. 185-186:138–146
44. Czubetor PE, Lessene G, Strasser A, Adams JM (2014) Control of apoptosis by the Bcl-2 protein family: implications for physiology and therapy. Nat Rev Mol Cell Biol 15(1):49–63
45. Hockenbery DM, Oltvai ZN, Yin XM, Milliman CL, Korsmeyer SJ (1993) Bcl-2 functions in an antioxidant pathway to prevent apoptosis. Cell 75(2):241–251
46. Lee M, Hyun DH, Marshall KA, Ellerby LM, Bredesen DE, Jenner P, Halliwell B (2001) Effect of overexpression of BCL-2 on cellular oxidative damage, nitric oxide production, antioxidant defenses, and the proteasome. Free Radic Biol Med 31(12):1550–1559
47. Rana SVS (2008) Metals and apoptosis: recent developments. J Trace Elem Med Bio 22:262–284
48. Vimala T, Poonghuzhali TV (2017) In vitro antimicrobial activity of solvent extracts of marine brown alga, Hydroclathrus clathratus (C. Agardh). M. Howe from Gulf of Mannar. J App Pharmaceutical Sci 7(04):157–162
49. Kim SK, Thomas NV, Li X (2011) Anticancer compounds from marine macroalgae and their application as medicinal foods. Adv Food Nutr Res 64:213–224
50. Hatch R, Rosenfield RL, Kim MH, Tredway D (1981) Hirsutism: implications, etiology, and management. Am J Obstet Gynecol. 140:815–830
51. Zhang Z, Teruya K, Yoshida T, Eto H, Shirahata S (2013) Fucoidan extract enhances the anti-cancer activity of chemotherapeutic agents in MDA-MB-231 and MCF-7 breast cancer cells. Mar Drugs 11:81–98
52. Olajide OA, Makinde MJ, Awe SO (1999) Effects of the aqueous extract of Bridelia ferruginea stem bark on carrageenan induced oedema and granuloma tissue formation in rats and mice. J Ethnopharmacol 66(1): 113–117
53. James MJ, Gibson RA, Clerand LG (2000) Dietary polyunsaturated fatty acids and inflammatory mediator production. Am J Clin Nutr 71:343–348

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.