RESEARCH ARTICLE

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Toxicity Effect of Holothuria Lessoni Sea Cucumber on Cancerous Mitochondria Obtained from Rat Model of Hepatocellular Carcinoma

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Abstract

Objective: Hepatocellular carcinoma (HCC) is a significant type of liver cancer. In spite of many treatment approaches, its treatment is still associated with challenges. Therefore, new approaches with minimal side effects are necessary for its treatment. Sea cucumbers are marine animals that have many biologically active compounds. They are rich in useful compounds and have high nutritional value. It has been reported to have many pharmacological effects, including anticancer. This research was designed to investigate the effects of *Holothuria lessoni* (*H. lessoni*) sea cucumber toxicity in HCC model rats. **Methods:** Cancer was induced in rats using diethyl nitrosamine (200 mg/kg DEN/single dose) + 2-acetylaminofluorene (2-AAF/ dietary/ 0.02% w/w for two weeks). After 15 weeks, hepatocytes and mitochondria were isolated to evaluate toxicity tests. **Result:** The results of the study showed that *H. lessoni* (62.5, 125, and 250 µg/ml) were able to cause toxicity only in cancerous mitochondria by increasing the level of free radicals, disrupting the permeability of the mitochondrial membrane, and initiating cell death signaling ($p<0.05$). **Conclusion:** It was suggested that *H. lessoni* sea cucumber may be beneficial in the treatment of HCC along with selected drugs. However, more studies are needed.

Keywords: Hepatocellular Carcinoma- sea cucumber- holothuria lessoni- mitochondria- animal model.

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Introduction

Cancer is regarded as one of the most crucial public health issues worldwide and its treatment is a challenge [1, 2]. The GLOBOCAN report shows that there is an increase in both the incidence of cancer and its mortality [3]. Hepatocellular carcinoma (HCC) is a cancer that is both common and aggressive, and has a high mortality rate. The most common and significant type of liver cancer is HCC. Similar to other cancers, there are numerous therapeutic approaches to treat HCC, but most of them are ineffective and have side effects. Therefore, scientists are searching for compounds that are effective for the treatment of various cancers and have minimal side effects [4-6]. Recent research has focused on natural compounds that can prevent and treatment of cancer with minimal side effects. Marine compounds have been investigated as one of the most significant compounds [7, 8].

Sea cucumbers belong to the category of marine products. These Marine compounds are nutritious and have diverse nutritional profiles, which include lipids, proteins, vitamins, and minerals. Compared to other organisms, sea cucumbers have more high-protein levels, such as arginine, tryptophan, and lysine, and lower levels of lipids [9-11, 7]. The bioactive compounds phenolics, saponins, peptides, fatty acids (omega-3/6), sulfated polysaccharides, lectins, and chondroitin sulfate in sea cucumbers are responsible for many pharmacological effects, such as anti-cancer activity [12, 13]. Cytotoxic activity, tumor growth reduction, drug resistance reduction, cell cycle arrest, and apoptosis induction are mechanisms by which these compounds can exert their anti-cancer effect. Many studies have been conducted on sea cucumber because of its anti-cancer effects and low side effects

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[14, 13, 15]. Additionally, it has been demonstrated that sea cucumber can be used as a supplement in preclinical studies due to its anti-cancer properties [16].

In previous studies, we have shown that compounds of natural origin have the ability to target intracellular organelles, including mitochondria [17-19]. In recent years, mitochondria have been studied by scientists as a target in cancer treatment. Targeted therapy in cancer treatment has been studied by researchers as an attractive research field [20-22]. Differences between mitochondria in cancer cells and normal cells can help kill cancer mitochondria. In addition, mitochondria are sensitive and vulnerable in liver tumors [23, 24]. Therefore, these differences can help kill mitochondria in cancer cells. Also, the use of compounds that target cancer mitochondria and cause toxicity in them has become an attractive target in cancer treatment [25-27]. Therefore, we investigated the toxicity of *Holothuria lessoni* (*H. lessoni*) sea cucumber on cancer mitochondria in the hepatocellular rat model. The novelty of this research was to investigate the effects of *H. lessoni* on succinate dehydrogenase (SDH) activity, oxidative stress through the measurement of ROS level, membrane damage of organelles such as mitochondria, mitochondrial swelling and cell death by assessing cytochrome c release in HCC rat.

Materials and Methods

Chemicals

In this study, rhodamine 123 (Rh 123) and 2,7-dicloroflurescein diacetate (DCHF-DA) were purchased from Sigma Aldrich Company (St. Louis, MO, USA). Cytochrome-c release was evaluated using a Quantikine Rat/Mouse Cytochrome c Immunoassay kit (R&D Systems, Inc., Minneapolis, MN, USA). In addition, all chemicals were of the highest commercial grade available.

Animals

After purchase, the animals (Male Wistar rats) were kept under standard conditions of temperature, humidity, and lighting cycle. All the experiments were performed according to the guidelines of the ethics and animal supervision committee (ID: IR.ZUMS.AEC.1401.033). In this study, an effort was made to cause minimal suffering to the animals.

Experimental design

After 1 week of habituation, the animals were separated into 2 groups for HCC induction. 1 group was placed as control and the other group was exposed to DEN (200 mg/kg, ip) and 2-AAF (dietary, 0.02% w/w) to induce HCC cancer. The duration of the study was 15 weeks. After the 15th week, biochemical and pathological tests were performed to prove HCC (data not reported) [24]. In the next step, mitochondria were incubated with different concentrations of *H. lessoni* sea cucumber extracts (methanolic, ethyl acetate and n-hexane) and toxicity tests were evaluated.

Collection of H. lessoni

H. lessoni samples were collected from the Qeshm Island Persian Gulf coastal waters (from 1-3 meters depth). The collection and identification of *H. lessoni* samples was done by Dr. Melika Nazemi in Bandar Abbas, Iran. To remove microscopic organisms, the samples were washed with distilled water and freshwater. The *H. lessoni* were washed twice with distillated water and stored at −20°C until use.

Preparation of H. lessoni extracts

H. lessoni samples (200 g dry powder weight) combined with three different solvents: n- hexane, ethyl acetate and methanol. In order to collect n-hexane (non-polar extract), ethyl acetate (semi-polar extract) and methanol (polar extract) extracts, *H. lessoni* samples were placed in n-hexane, ethyl acetate and methanol for 24 hours, 48 hours and 72 hours, respectively. Then, each of the extracts was filtered and evaporated using Rota vapor under low pressure for n-hexane, ethyl acetate and methanolic extracts at 35-40 °C, 38-40 °C and 40-45 °C, respectively.

Isolation of Mitochondria from Rat Hepatocytes

At the end of week 15, the animals were anesthetized with ketamine (intraperitoneal) and xylazine (intraperitoneal) in doses of 80 mg/kg, and 5 mg/kg, respectively. At first, hepatocytes were isolated according to standard protocol [28, 29]. Then, the hepatocytes were centrifuged in two steps to isolate the mitochondria. First step: 760 × g for 5 min, and two step: 8000 × g for 20 min [30, 31]. In order to evaluate toxicity parameters, mitochondria were exposed to different concentrations of *H. lessoni* extracts.

Evaluation of SDH activity

MTT reagent/test was used to measure mitochondrial SDH activity. 1 mg protein/ml of mitochondria was used for each test. To measure the activity of this parameter, mitochondria were suspended in corresponding assay buffer and incubated to *H. lessoni* extracts (methanolic, ethyl acetate and n-hexane) for 1hour. Finally, absorbance at a wavelength of 570 nm was evaluated to measure the activity of mitochondria SDH in both groups [32].

Evaluation of ROS assay

In order to measure the level of ROS using DCFH-DA probe, mitochondria were suspended in respiration buffer assay. Then, mitochondria incubated with *H. lessoni* extracts (62.5, 125 and 250 µg/ml). Finally, the measured fluorescence intensity level (EXλ = 488 nm / EMλ = 527 nm) indicates the generation level of mitochondria ROS. The ROS level in both groups was measured at 15, 30 and 60 min after incubated to different concentrations of *H. lessoni* extracts.

Evaluation of mitochondrial membrane potential (MMP) assay

Rh123 probe was used to measure MMP collapse. In order to measure this parameter, mitochondria were suspended in corresponding assay buffer, and then

incubated with different concentrations of *H. lessoni* extracts (62.5, 125 and 250 µg/ml). Finally, fluorescence intensity (EXλ = 490 nm/EMλ = 535 nm) was assayed to measure the MMP collapse in both groups. The MMP collapse in both groups was measured at 15, 30 and 60 min after incubated to different concentrations of *H. lessoni* extracts.

Evaluation of mitochondrial swelling

First, mitochondria were suspended in corresponding assay buffer. In the next step, mitochondria were incubated with different concentrations of *H. lessoni* extracts (62.5, 125 and 250 µg/ml). The absorbance of the samples was recorded at a wavelength of 540 nm. This test was measured at 15, 30 and 60 min after incubated to different concentrations of *H. lessoni* extracts.

Measurement of cytochrome c release

Cytochrome c release was monitored sequentially following mitochondria incubation with *H. lessoni* extracts, and the effects of inhibitory compounds were evaluated as per the manufacturer's kit instructions. All the samples were placed in the micro-plate wells according to the instructions of the kit. Optical density at a wavelength of 540 nm was measured to assay the release of cytochrome c in all samples.

Statistical analysis

Results are reported as mean ± S.D. GraphPad Prism software (version 8) was used for data analysis. The significance level was $p < 0.05$. Statistical significance was evaluated using the one-way ANOVA test (for assessment of SDH activity, and cytochrome c release) followed by the post hoc Tukey, and the two-way ANOVA test (for assessment of mitochondrial ROS level, MMP collapse, and mitochondrial swelling) followed by the post hoc Bonferroni test.

Results

Effects of H. lessoni on mitochondrial SDH activity

As shown in Figure 1A-C, all 3 *H. lessoni* extracts (methanolic, ethyl acetate and n-hexane) at concentrations of 62.5, 125 and 250 µg/ml has been able to decrease the activity of mitochondrial SDH in the cancer (HCC) group (Figure 1A-C). This event was not reported in the normal group (results not shown). SDH is part of complex II in the mitochondrial respiratory chain (MRC). Disturbance in it is associated with dysfunction of mitochondria and increase in the level of ROS.

Effects of H. lessoni on mitochondrial ROS

In the HCC rat model group, the results showed that 15, 30 and 60 min after incubation of mitochondria with different concentrations of *H. lessoni* extracts (62.5, 125 and 250 µg/ml), the level of ROS increased significantly (Figure 2A-C). An increase in the level of ROS may be associated with subsequent consequences such as disruption of MMP and activation of cell death signaling.

Effects of H. lessoni on MMP collapse

The results of our study showed that all extracts of *H. lessoni* (methanolic, ethyl acetate and n-hexane) at concentrations of 62.5, 125, and 250 µg/ml have significantly induced the collapse in MMP in the HCC group (Figure 3 A-C). But, this effect of *H. lessoni* extracts was not reported in the normal group. A collapse in MMP has been observed at 15, 30, and 60 min after incubation of mitochondria with different concentrations of *H. lessoni* extracts (62.5, 125, and 250 µg/ml). These results may indicate a selective effect of *H. lessoni* only on HCC mitochondria.

Effects of H. lessoni on mitochondrial swelling

In the HCC rat model group, the results showed that 15, 30 and 60 min after incubation of mitochondria with different concentrations of *H. lessoni* extracts (62.5, 125 and 250 µg/ml), the mitochondrial swelling increased significantly (Figure 4A-C).

Effects of H. lessoni on cytochrome release

As shown in Figure 5A-C, all 3 *H. lessoni* extracts (methanolic, ethyl acetate and n-hexane) at concentration

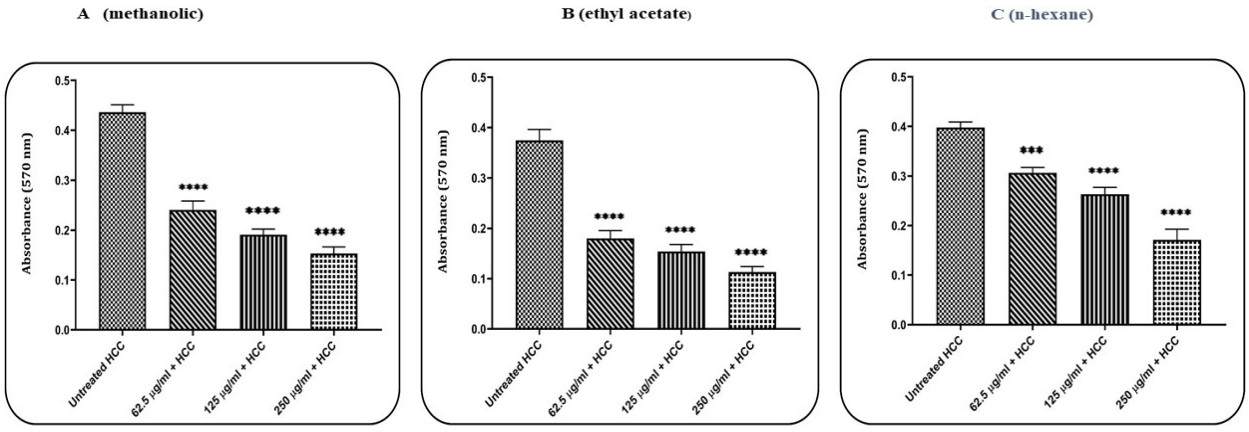

Figure 1. SDH activity. The effect of *H. lessoni* (62.5, 125, and 250 µg/ml) on Mitochondrial SDH Activity. Data were represented as the mean ± SD (n = 3). *** ($p<0.001$) and **** ($p<0.0001$) significant difference with untreated HCC group.

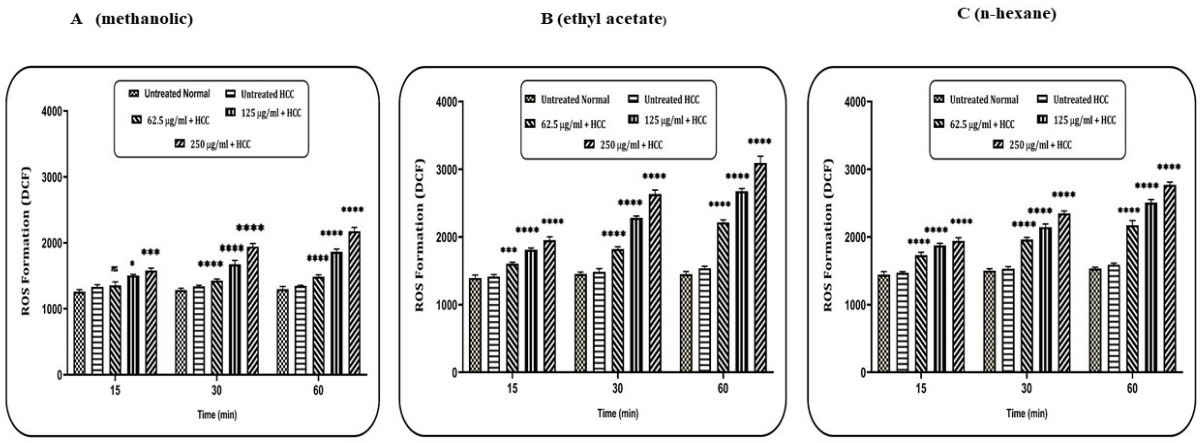

Figure 2. ROS Assay. The effect of *H. lessoni* (62.5, 125, and 250 µg/ml) on mitochondrial ROS. Data were represented as the mean ± SD (n = 3). * (p<0.05), *** (p<0.001), and **** (p<0.0001) significant difference with untreated HCC group.

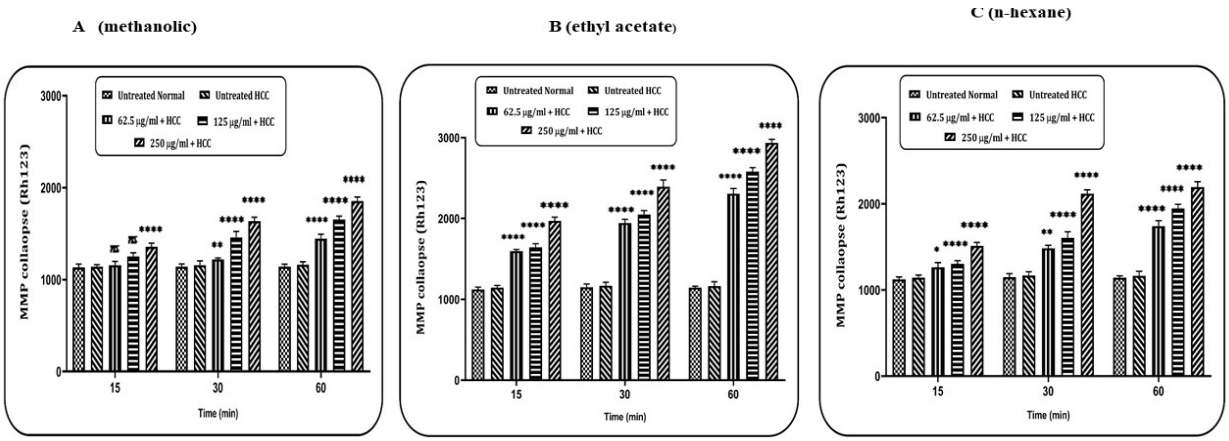

Figure 3. MMP Collapse Assay. The effect of *H. lessoni* (62.5, 125, and 250 µg/ml) on MMP collapse. Data were represented as the mean ± SD (n = 3). * (p<0.05), ** (p<0.01), and **** (p<0.0001) significant difference with untreated HCC group.

of 125 µg/ml has been able to increase the release of cytochrome c in the cancer group (Figure 5A-C). The highest concentration of *H. lessoni* extracts (250 µg/ml) extracts had no effect on the release of cytochrome c from normal mitochondria. Furthermore, the results showed that CsA and BHT reduce the effect of *H. lessoni* extracts (125 µg/ml) extracts on the release of cytochrome c from cancerous mitochondria (Figure 5A-C).

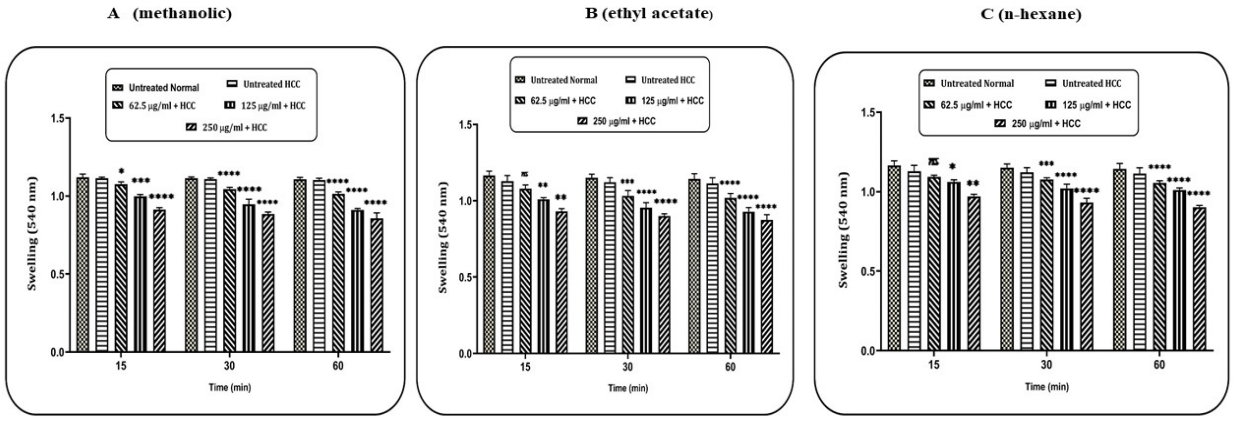

Figure 4. Mitochondrial Swelling Assay. The effect of *H. lessoni* (62.5, 125, and 250 µg/ml) on mitochondrial swelling. Data were represented as the mean ± SD (n = 3). * (p<0.05), ** (p<0.01), *** (p<0.001), and **** (p<0.0001) significant difference with untreated HCC group.

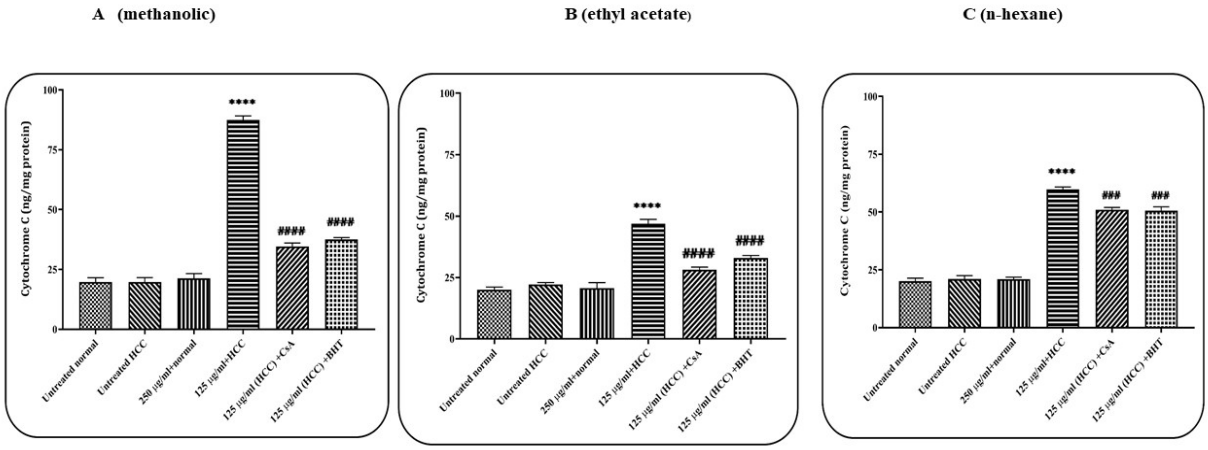

Figure 5. Cytochrome c Release Assay. The effect of *H. lessoni* (62.5, 125, and 250 µg/ml) on cytochrome c release. Data were represented as the mean ± SD (n = 3). **** ($p<0.0001$) significant difference with untreated HCC group. ### ($p<0.001$), and #### ($p<0.0001$) significant difference with 125 µg/ml + HCC group.

Discussion

Conventional approaches in the treatment of HCC have been associated with side effects. Accordingly, researchers are searching for drugs with high efficiency and low side effects in the treatment of liver cancer. Sea cucumbers are marine animals that have both high nutritional value and different pharmacological effects, including anti-cancer activity. The pharmacological effects of sea cucumber are due to the presence of bioactive compounds in their structure. Thus, we decided to study the toxic effects of *H. lessoni* sea cucumber as a treatment for HCC. Today, the focus of many scientists has been on targeted therapy for cancer treatment. Researchers have investigated mitochondria as a potential cancer treatment target [33-35]. Furthermore, compounds that target mitochondria in cancer cells have been examined, and these compounds have the potential to help in the treatment of different cancers. In this study, the objective is to investigate the effects of sea cucumber extracts on HCC mitochondria [33-35].

We have previously demonstrated that the natural compounds found in sea cucumbers and sponges can cause selective toxicity on cancer mitochondria. The results of Nursid et al., the study showed that H. atra sea cucumber has good cytotoxicity on T47D, MFC7, WiDr, and HeLa cell lines and can be considered as an anti-cancer candidate [36]. In this research, the toxic effect of the *H. lessoni* extracts on SDH activity has been studied, and results in HCC mitochondria revealed that *H. lessoni* extracts have potent toxicity on SDH activity. The effect of sea cucumber has been documented in our previous studies and is in accordance with the findings of this study [18, 19].

In the current research, *H. lessoni* extract induced an increase in the ROS level of the HCC mitochondria that is dependent on both concentration and time. The report is in accordance with the findings that HCC and colorectal cancer cells respond favorably to the effects of marine compound such as H. oculata and S. japonicas (methanolic extracts) on ROS generation [37, 19]. ROS is enhanced by inhibiting various electron transport chain (ETC) complexes. The antioxidant system's disruption and targeting of Bcl-2 anti-apoptotic family and ion channels in the mitochondrial membrane are linked with this increase. These events lead to mitochondrial dysfunction and cell death [38, 39] . Previous research has demonstrated that compounds with an increase in ROS levels can cause cytotoxicity in cancer cell lines [40, 41]. Our previous studies have documented the effects of sea cucumbers, which are consistent with the findings of this study. [18, 19]. *H. lessoni* can cause toxicity on HCC mitochondria by raising the level of ROS, but it has no adverse effects on normal mitochondria. It is possible that the generation of ROS caused by *H. lessoni* in HCC mitochondria is one of the most important mechanisms of this sea cucumber in causing toxicity in HCC mitochondria.

MMP collapse has a crucial role in the apoptosis signaling. The release of cytochrome c into the cytosol can cause cell death (apoptosis/necrosis) when mitochondrial membrane damage causes opening of the MPT pore [42]. Our findings revealed that addition of *H. lessoni* extracts resulted in the collapse of the MMP in HCC mitochondria. The effect of sea cucumber has been documented in our previous studies and is in accordance with the findings of this study [18, 19]. The collapse in MMP is associated with mitochondrial swelling and the release of cytochrome c. Furthermore, mitochondrial swelling plays a role in the cell survival and cell death. Mitochondrial swelling can be dangerous for mitochondrial function [43, 44]. Our findings show that the incubation of HCC mitochondria with *H. lessoni* extracts leads to mitochondrial swelling in HCC mitochondria. Thus, *H. lessoni* has the potential to cause mitochondrial dysfunction in HCC mitochondria. Our findings indicated that *H. lessoni* is responsible for releasing cytochrome c from HCC mitochondria. The consequence of this event may be associated with the activation of cellular death signaling. Sea cucumber has been proven to have anticancer activity, and this is due to its bioactive compounds including saponins, chondroitin sulfate, phenolic compounds, sulfated polysaccharides,

glycosaminoglycans, fucoidan, peptides, lectins, sterols, and cerebrosides [9, 10]. The reported effects of *H. lessoni* could be attributed to these bioactive compounds.

In conclusion, the in vitro results in this research shown that the Persian Gulf *H. lessoni* could elicited anti-cancer effect via production of ROS, disruption of mitochondrial membrane permeability, apoptosis signaling induction that may be helpful in the treatment of HCC along with selected drugs. Accordingly, detection of its chemical ingredients is highly suggested.

Author Contribution Statement

Nazanin Shahbazi, and Mahsa Barzegar contributed to this research in carrying out the experiments, analyzing the data and writing the paper. Melika Nazemi contributed to this research in sample collection and Identification. Zhaleh Mohsenifar contributed to this research in the investigation of biochemical and histological parameters. Jalal Pourahmad, Mohammad Reza Eskandari and Enayatollah Seydi contributed to this research in formulating the research question (s), designing the study, carrying it out as thesis supervisor, analyzing the data, and writing paper.

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The data provided in this manuscript were extracted from the PharmD thesis of Dr. Nazanin Shahbazi.

Ethics Committee Approval

All the experiments were performed according to the guidelines of the ethics and animal supervision committee (ID: IR.ZUMS.AEC.1401.033).

Availability of data

Data will be made available on request.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Saqban LH, Abdul Alamir Mezher Z, Hussain Ali I. Cytotoxic effect of the crude alcoholic extract of the fruits of citrullus colocynthis on human hepatocyte carcinoma (hep-g2). Arch Razi Inst. 2022;77(4):1389-95. https://doi.org/10.22092/ari.2022.357807.2104.
2. Wargasetia TL, Ratnawati H, Widodo N. Sea cucumber compounds targeting nf-κb in cancer treatment. Bioinform Biol Insights. 2022;16:11779322221091740. https://doi.org/10.1177/11779322221091740.
3. Liu G, Zhang S, Lin R, Cao X, Yuan L. Anti-tumor target screening of sea cucumber saponin frondoside a: A bioinformatics and molecular docking analysis. Front Oncol. 2023;13:1307838. https://doi.org/10.3389/fonc.2023.1307838.
4. Kim EA, Lee JH, Heo SJ, Jeon YJ. Saringosterol acetate isolated from hizikia fusiforme, an edible brown alga, suppressed hepatocellular carcinoma growth and metastasis in a zebrafish xenograft model. Chem Biol Interact. 2021;335:109362. https://doi.org/10.1016/j.cbi.2020.109362.
5. Luparello C, Branni R, Abruscato G, Lazzara V, Drahos L, Arizza V, et al. Cytotoxic capability and the associated proteomic profile of cell-free coelomic fluid extracts from the edible sea cucumber holothuria tubulosa on hepg2 liver cancer cells. Excli J. 2022;21:722-43. https://doi.org/10.17179/excli2022-4825.
6. Ren X, Xie X, Chen B, Liu L, Jiang C, Qian Q. Marine natural products: A potential source of anti-hepatocellular carcinoma drugs. J Med Chem. 2021;64(12):7879-99. https://doi.org/10.1021/acs.jmedchem.0c02026.
7. Wei W, Fan XM, Jia SH, Zhang XP, Zhang Z, Zhang XJ, et al. Sea cucumber intestinal peptide induces the apoptosis of mcf-7 cells by inhibiting pi3k/akt pathway. Front Nutr. 2021;8:763692. https://doi.org/10.3389/fnut.2021.763692.
8. Yu-Lin D, Dong-Yue Z, Jiang Y-F, Zheng F, Hao Y, You-Jin J. 6-bromohypaphorine isolated from red sea cucumbers apostichopus japonicus exhibits potent anticancer activity in a549 cancer cell line. Chinese J Anal Chem. 2021;49(12):37-42. https://doi.org/10.1016/j.cjac.2021.05.001.
9. Fagbohun OF, Joseph JS, Oriyomi OV, Rupasinghe HPV. Saponins of north atlantic sea cucumber: Chemistry, health benefits, and future prospectives. Mar Drugs. 2023;21(5). https://doi.org/10.3390/md21050262.
10. Hossain A, Dave D, Shahidi F. Northern sea cucumber (cucumaria frondosa): A potential candidate for functional food, nutraceutical, and pharmaceutical sector. Mar Drugs. 2020;18(5). https://doi.org/10.3390/md18050274.
11. Liang Q, Ahmed F, Zhang M, Sperou N, Franco CM, Feng Q, et al. In vivo and clinical studies of sea cucumber-derived bioactives for human health and nutrition from 2012-2021. Front Mar Sci. 2022;9:917857. https://doi.org/10.3389/fmars.2022.917857.
12. Santhanam R, Azam NSM, Khadar ASA, Louise A, Dominic G, Sofian NSA, et al. Anticancer potential of three sea cucumber species extracts on human breast cancer cell line. Squalen. 2022;17(2):85-94. https://doi.org/10.15578/squalen.669.
13. Wargasetia TL, Ratnawati H, Widodo N. Anticancer potential of holothurin A, holothurin B, and holothurin B3 from the sea cucumber Holothuria scabra. InAIP Conference Proceedings 2020 Apr 21 (Vol. 2231, No. 1). AIP Publishing.
14. Ujianti I, Semara Lakshmi B, Nurusshofa Z, Sukarya W, Indriyanti L. Network pharmacology analysis reveals bioactive compounds and potential targets of sea cucumber for cervical cancer therapy. F1000Res. 2023;12:1358. https://doi.org/org/10.12688/f1000research.138298.1.
15. Wargasetia TL, Widodo. Mechanisms of cancer cell killing by sea cucumber-derived compounds. Invest New Drugs. 2017;35(6):820-6. https://doi.org/10.1007/s10637-017-0505-5.
16. Baharara J, Amini E, Afzali M, Nikdel N, Mostafapour A, Kerachian MA. Apoptosis inducing capacity of holothuria arenicola in ct26 colon carcinoma cells in vitro and in vivo. Iran J Basic Med Sci. 2016;19(4):358-65.
17. Amidi S, Hashemi Z, Motallebi A, Nazemi M, Farrokhpayam H, Seydi E, et al. Identification of (z)-2, 3-diphenylacrylonitrile as anti-cancer molecule in persian gulf sea cucumber holothuria parva. Mar Drugs. 2017;15(10):314. https://doi.org/10.3390/md15100314.
18. Arast Y, Seyed Razi N, Nazemi M, Seydi E, Pourahmad J. Non-polar compounds of persian gulf sea cucumber holothuria parva selectively induce toxicity on skin mitochondria isolated from animal model of melanoma. Cutan Ocul Toxicol. 2018;37(3):218-27. https://doi.org/1080/15569527.2017.1414227.

19. Seydi E, Motallebi A, Dastbaz M, Dehghan S, Salimi A, Nazemi M, et al. Selective toxicity of persian gulf sea cucumber (holothuria parva) and sponge (haliclona oculata) methanolic extracts on liver mitochondria isolated from an animal model of hepatocellular carcinoma. Hepat Mon. 2015;15(12). https://doi.org/10.5812/hepatmon.33073.
20. Cheng X, Feng D, Lv J, Cui X, Wang Y, Wang Q, et al. Application prospects of triphenylphosphine-based mitochondria-targeted cancer therapy. Cancers (Basel). 2023;15(3). https://doi.org/10.3390/cancers15030666.
21. Musicco C, Signorile A, Pesce V, Loguercio Polosa P, Cormio A. Mitochondria deregulations in cancer offer several potential targets of therapeutic interventions. Int J Mol Sci. 2023;24(13). https://doi.org/10.3390/ijms241310420.
22. Sun Y, Zhang H, Lu G, Wang H, Lu Y, Fan L. Mitochondria-targeted cancer therapy based on functional peptides. Chin Chem Lett. 2023;34(5):107817. https://doi.org/org/10.1016/j.cclet.2022.107817.
23. Armstrong JS. Mitochondria: A target for cancer therapy. Br J Pharmacol. 2006;147(3):239-48.
24. Seydi E, Rasekh HR, Salimi A, Mohsenifar Z, Pourahmad J. Myricetin selectively induces apoptosis on cancerous hepatocytes by directly targeting their mitochondria. Basic Clin Pharmacol Toxicol. 2016;119(3):249-58. https://doi.org/10.1111/bcpt.12572.
25. Dong L, Gopalan V, Holland O, Neuzil J. Mitocans revisited: Mitochondrial targeting as efficient anti-cancer therapy. Int J Mol Sci. 2020;21(21). https://doi.org/10.3390/ijms21217941.
26. Fialova JL, Raudenska M, Jakubek M, Kejik Z, Martasek P, Babula P, et al. Novel mitochondria-targeted drugs for cancer therapy. Mini Rev Med Chem. 2021;21(7):816-32. https://doi.org/10.2174/1389557520666201118153242.
27. Mani S, Swargiary G, Singh KK. Natural agents targeting mitochondria in cancer. Int J Mol Sci. 2020;21(19). https://doi.org/10.3390/ijms21196992.
28. Pourahmad J, O'Brien PJ. Biological reactive intermediates that mediate chromium (vi) toxicity. Adv Exp Med Biol. 2001;500:203-7. https://doi.org/10.1007/978-1-4615-0667-6_27.
29. Severgnini M, Sherman J, Sehgal A, Jayaprakash NK, Aubin J, Wang G, et al. A rapid two-step method for isolation of functional primary mouse hepatocytes: Cell characterization and asialoglycoprotein receptor based assay development. Cytotechnology. 2012;64:187-95. https://doi.org/10.1007/s10616-011-9407-0.
30. Barogi S, Baracca A, Castelli GP, Bovina C, Formiggini G, Marchetti M, et al. Lack of major changes in atpase activity in mitochondria from liver, heart, and skeletal muscle of rats upon ageing. Mech Ageing Dev. 1995;84(2):139-50. https://doi.org/10.1016/0047-6374(95)01640-6.
31. Pourahmad J, O'Brien PJ. Contrasting role of na(+) ions in modulating cu(+2) or cd(+2) induced hepatocyte toxicity. Chem Biol Interact. 2000;126(2):159-69. https://doi.org/10.1016/s0009-2797(00)00162-9.
32. Zhao Y, Ye L, Liu H, Xia Q, Zhang Y, Yang X, et al. Vanadium compounds induced mitochondria permeability transition pore (ptp) opening related to oxidative stress. J Inorg Biochem. 2010;104(4):371-8. https://doi.org/10.1016/j.jinorgbio.2009.11.007.
33. Ghosh P, Vidal C, Dey S, Zhang L. Mitochondria targeting as an effective strategy for cancer therapy. Int J Mol Sci. 2020;21(9). https://doi.org/10.3390/ijms21093363.
34. Guo X, Yang N, Ji W, Zhang H, Dong X, Zhou Z, et al. Mito-bomb: Targeting mitochondria for cancer therapy. Adv Mater. 2021;33(43):e2007778. https://doi.org/10.1002/adma.202007778.
35. Jeena MT, Kim S, Jin S, Ryu JH. Recent progress in mitochondria-targeted drug and drug-free agents for cancer therapy. Cancers (Basel). 2019;12(1). https://doi.org/10.3390/cancers12010004.
36. Nursid M, Marraskuranto E, Chasanah E. Cytotoxicity and apoptosis induction of sea cucumber holothuria atra extracts. Pharmacognosy Res. 2019;11(1). https://doi.org/10.4103/pr.pr_3_18.
37. Kim JL, Park SH, Jeong S, Kim BR, Na YJ, Jo MJ, et al. Sea cucumber (stichopus japonicas) f2 enhanced trail-induced apoptosis via xiap ubiquitination and er stress in colorectal cancer cells. Nutrients. 2019;11(5). https://doi.org/10.3390/nu11051061.
38. Liu Y, Fiskum G, Schubert D. Generation of reactive oxygen species by the mitochondrial electron transport chain. J Neurochem. 2002;80(5):780-7. https://doi.org/10.1046/j.0022-3042.2002.00744.x.
39. Orrenius S. Reactive oxygen species in mitochondria-mediated cell death. Drug Metab Rev. 2007;39(2-3):443-55. https://doi.org/10.1080/03602530701468516.
40. Kostrzewa T, Wołosewicz K, Jamrozik M, Drzeżdżon J, Siemińska J, Jacewicz D, et al. Curcumin and its new derivatives: Correlation between cytotoxicity against breast cancer cell lines, degradation of ptp1b phosphatase and ros generation. Int J Mol Sci. 2021;22(19). https://doi.org/10.3390/ijms221910368.
41. Sharma P, Goyal D, Baranwal M, Chudasama B. Ros-induced cytotoxicity of colloidal copper nanoparticles in mcf-7 human breast cancer cell line: An in vitro study. J Nanopart Res. 2020;22:1-11. https://doi.org/10.1007/s11051-020-04976-7.
42. Robertson JD, Orrenius S. Molecular mechanisms of apoptosis induced by cytotoxic chemicals. Crit Rev Toxicol. 2000;30(5):609-27. https://doi.org/10.1080/10408440008951122.
43. Javadov S, Chapa-Dubocq X, Makarov V. Different approaches to modeling analysis of mitochondrial swelling. Mitochondrion. 2018;38:58-70. https://doi.org/10.1016/j.mito.2017.08.004.
44. Kluza J, Gallego MA, Loyens A, Beauvillain JC, Sousa-Faro JM, Cuevas C, et al. Cancer cell mitochondria are direct proapoptotic targets for the marine antitumor drug lamellarin d. Cancer Res. 2006;66(6):3177-87. https://doi.org/10.1158/0008-5472.can-05-1929.

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