RESEARCH ARTICLE

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Selective Toxicity Effect of *Chrysaora quinquecirrha* Crude Venom on Human Colorectal Tumor Cells by Directly Targeting Mitochondria

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Abstract

Objective: Compounds isolated from marine animals have different pharmacological effects. In this study, we investigated the effects of sea nettle (Chrysaora quinquecirrha) crude venom on human colon cancer mitochondria. **Methods:** First, mitochondria were isolated from healthy colon tissue and cancerous colon tissue, and then mitochondrial function (SDH activity), reactive oxygen species (ROS) level, mitochondrial membrane potential (MMP) collapse, mitochondrial swelling, and cytochrome c release were measured. **Results:** The results showed that crude venom of Chrysaora quinquecirrha (180, 360 and 720 µg/ml) can significantly impair mitochondrial function (**P<0.01 and ***P<0.001) and consequently increase the level of ROS (*P<0.05 and ****P<0.0001), collapse in MMP (*P<0.05 and ****P<0.0001), mitochondrial swelling (**** P<0.0001) and release of cytochrome c (* P<0.05 and *** P<0.001) only in mitochondria isolated from human colon cancer tissue. **Conclusion:** The results concluded that crude venom of Chrysaora quinquecirrha (180, 360 and 720 µg/ml) has no side effects on normal mitochondria and only selectively affects cancerous mitochondria. It seems that after further research, Chrysaora quinquecirrha can be considered as a drug candidate for the treatment of patients with colon cancer.

Keywords: Marine animal- reactive oxygen species- oxidative stress- cancer

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Introduction

Colon cancer is known as one of the preventable cancers. Worldwide, this type of cancer is known as one of the most common malignant tumors and is also often diagnosed in advanced stages (Ahmed, 2020; Hu et al., 2021). In 2020, it was shown that the number of new cases of cancer is 19.3 million and the number of deaths is 10 million (Cao et al., 2021). In 2016, research has shown that the incidence of colorectal cancer in Iran is 8.1-8.3 per 100,000 in men and 6.5 to 7.5 per 100,000 in women (Rafiemanesh et al., 2016). In recent years, the use of chemotherapy in the treatment of cancer has been discussed for reasons such as drug resistance and also tumor relapse (Alfarouk et al., 2015). Research has shown that apoptotic signaling is impaired in colon cancer (Abraha and Ketema, 2016). Also, a positive association has been reported between compounds that induce apoptosis and a reduction in the spread of cancer. Accordingly, the induction of apoptosis can be considered as a simple approach in the treatment of various cancers (Yang et al., 2021). Mitochondrial organics play an important role in signaling apoptosis (Jiang et al., 2019; Abate et al., 2020).

Mitochondria are recognized as a target in the treatment of cancer (Jeena et al., 2019; Grasso et al., 2020). Evidence suggests that mitochondria are involved in several physiological processes, including the production of reactive oxygen species (ROS) and the activation of apoptotic signaling (Zhang et al., 2011; Eckert et al., 2013; Jaworski et al., 2019). In cancer cells, the level of oxidative stress is higher than the level of antioxidants. In addition, cancer cells are vulnerable to overproduction of ROS. Thus, mitochondrial dysfunction (as one of the most important sources of ROS production) in cancer cells by exposure to various compounds can be associated with an increase in the level of ROS and death of these cells (Sak, 2014).

In recent years, targeting mitochondria has attracted the attention of medical researchers. Anti-cancer agents or

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"mitocans" can contribute to cancer treatment by targeting mitochondria. The target of different mitochondrial parts can be helpful in various types of cancers. The target of mitochondria with "mitocans" is associated with damage to the mitochondrial membrane and an increase in the level of ROS and cell death. Therefore, targeting mitochondria in cancer cells with anti-cancer compounds can help cancer treatment (Battogtokh et al., 2018; Pustylnikov et al., 2018). Research shows that apoptotic signaling (mitochondrial pathway) in colon cancer is disturbed and the level of anti-apoptotic proteins is higher than pro- apoptotic proteins (Abraha and Ketema, 2016). The mitochondrial content in human colorectal cancer tissues is higher than normal colon mucosa. In this cancer, the number of mitochondrial DNA (mtDNA) copy is high (Sun et al., 2018).

Cancer is known to be the deadliest disease in the world. Therefore, the discovery of new drugs with novel mechanisms of action is essential for the treatment of cancer (Khalifa et al., 2019; Wei et al., 2020; Zhang et al., 2021). Compounds of natural origin have been used in the treatment of any type of cancer due to their various pharmacological activities (Kim and Kim, 2018; Subramaniam et al., 2019). In recent years, isolated compounds from marine animals have attracted the attention of cancer researchers (Khalifa et al., 2019; Wei et al., 2020; Zhang et al., 2021). These compounds have been able to induce apoptosis in cancerous tissues and cells through an increase the production of ROS and impairing mitochondrial function (Mirshamsi et al., 2017; Zangeneh et al., 2018). Marine compounds have been used in the treatment of colon cancer in various studies (Taira et al., 2018; Van Blarigan et al., 2018; Ruiz-Torres et al., 2019). In this study, we investigated the effects of sea nettle (Chrysaora quinquecirrha) crude venom on human colon cancer mitochondria.

Materials and Methods

Chemicals and Reagents

2,7-dicloroflurescein diacetate (DCHF-DA) and rhodamine 123 (Rh 123) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Furthermore, Cytochrome c release assay kit were purchased from R and D Systems (Inc., Minneapolis, MN, USA). All other chemicals were of the highest commercial grade available.

Crude Venom Preparation

Venom preparation was performed based on our previous study. Centrifugation was performed at 12,000 ×g for 20 min to separate the crude venom. Then, crude venom was stored at -20°C until the experiments. Finally, the protein concentration of the Chrysaora quinquecirrha venom was determined using Bradford assay (Bradford, 1976; Mirshamsi et al., 2017).

Tissue samples

All tissues (healthy and cancerous) used in this study were provided by the surgical center of Imam Khomeini hospital, Iran. The pathology and histology of each sample were confirmed by the pathologist. All patients selected in the study were in the age range of 46 to 69 years. Prior to the study, selected patients did not receive other treatments, including chemotherapy or radiotherapy. Cancerous samples were collected from the core of the colon tumor tissue, while healthy samples were collected from sites distant of the tumor and examined by a pathologist for the absence of tumor cells (Fakhri et al., 2017). All processes were conducted according to the ethical standards and protocols approved by the Experimentation Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran, and informed consent was obtained from all the patients.

Mitochondria isolation

Mitochondrial isolation in both groups (healthy and colon cancer groups) was performed based on our previous study. Initially, the samples collected from both groups were washed with ice-cold buffer and then cut into smaller pieces and homogenized. In the following, Mitochondria were isolated after two steps of centrifugation (centrifuge at 1,500 × g for 10 min at 4°C and centrifuge at 15,000 × g for 15 min at 4°C) (Fakhri et al., 2017). Eventually, all experiments were performed on mitochondria.

Succinate dehydrogenase activity (SDH) assay

In this study, according to our pilot study with MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide) assay we used a concentration range of 25 to 500 µg/ml of the three compound. The IC_{50} concentration was 360 µg/ml. Subsequently, like many other cytotoxicity studies protocol we used concentrations of 180 (1/2 IC_{50}), 360 (IC_{50}) and 720 µg/ml (2 IC_{50}) for our further experiments. At first, isolated mitochondria from healthy and cancerous groups were suspended in Tris buffer. In the following, the mitochondria were incubated with various concentrations of Chrysaora quinquecirrha crude venom (180, 360 and 720 µg/ml) for 1 hour at 37 °C. Then, MTT dye (0.4%) and dimethyl sulfoxide (DMSO) were added, respectively. At the end of this experiment, absorbance was measured at 570 nm (ELISA reader) (Zhao et al., 2010; Salimi et al., 2016).

Reactive Oxygen Species (ROS) level assay

In the first step, mitochondria isolated from healthy and cancerous groups suspended in the respiratory assay buffer and then incubated with various concentrations Chrysaora quinquecirrha crude venom (180, 360 and 720 µg/ml) for 5, 30 and 60 min at 37°C. Then, DCHF-DA probe (10 µM) was added to the mitochondrial suspension. Following, the ROS generation was measured using a fluorescence spectrophotometer at EX = 488 nm and EM = 527 nm (Eskandari et al., 2015; Ghanbari et al., 2017).

Mitochondrial swelling assay

At the beginning of this experiment, isolated mitochondria from healthy and cancerous groups were suspended in mitochondrial swelling assay buffer. In the following, the mitochondria were incubated with various concentrations of Chrysaora quinquecirrha crude venom (180, 360 and 720 µg/ml) for 5, 30 and 60 min at 37°C.

At the end of this experiment, absorbance was measured at 540 nm (ELISA reader) (Ghanbari et al., 2017).

Mitochondrial Membrane Potential (MMP) collapse assay

In the first step, isolated from healthy and cancerous groups suspended in the mitochondrial membrane potential (MMP) assay buffer and then incubated with various concentrations Chrysaora quinquecirrha crude venom (180, 360 and 720 µg/ml) for 5, 30 and 60 min at 37°C. Then, Rh 123 probe (10 µM) was added to the mitochondrial suspension. Following, the MMP collapse was measured using a fluorescence spectrophotometer at EX = 490 nm and EM = 535 nm (Baracca et al., 2003).

Cytochrome c release assay

The Quantikine Rat/Mouse Cytochrome c Immunoassay kit (R and D Systems, Inc., Minneapolis, MN, USA) was used for the determination of cytochrome c release.

Statistical analysis

Data are presented as means ± SD. All statistical analyses were done using the GraphPad Prism software (version 6). All experiments were analyzed using one-way and two-way analysis of variance (ANOVA) followed by the Tukey and Bonferroni post hoc test, respectively. Statistical significance was set at $P < 0.05$.

Results

Chrysaora quinquecirrha crude venom and mitochondria SDH activity

The results in Figure 1A-B showed that there was only a positive correlation between decrease in SDH activity in the mitochondria from cancer group after incubation with crude venom of Chrysaora quinquecirrha at concentrations of 180, 360 and 720 µg/ml (Figure 1A-B).

Chrysaora quinquecirrha crude venom and mitochondria ROS level

In mitochondria from colon cancer group, the results showed that crude venom of Chrysaora quinquecirrha in all applied concentrations (180, 360 and 720 µg/ml) was able to increase the level of ROS (Figure 2B). However, this effect of this crude venom has not been reported in mitochondria isolated from healthy colon tissue (Figure 2A). These results suggest that Chrysaora quinquecirrha crude venom can cause oxidative stress only in the mitochondria of the cancer group.

Chrysaora quinquecirrha crude venom and MMP collapse

The results showed that there was only a positive correlation between collapse in MMP in the mitochondria from cancer group at 5, 30 and 60 min after incubation with crude venom of Chrysaora quinquecirrha at concentrations of 180, 360 and 720 µg/ml (Figure 3A-B). Furthermore, collapse in the MMP can lead to irreversible events such as apoptosis.

Chrysaora quinquecirrha crude venom and mitochondria swelling

In the following, we evaluated the mitochondrial swelling in the mitochondria obtained from healthy and cancerous groups. The results showed that Chrysaora quinquecirrha at concentrations of 180, 360 and 720 µg/ml and during incubation times of 5, 30 and 60 was able to significantly increase mitochondrial swelling only in the cancer group (Figure 4A-B).

Chrysaora quinquecirrha crude venom and cytochrome c release

In mitochondria from colon cancer group, the results showed that crude venom of Chrysaora quinquecirrha in all applied concentrations 180, 360 and 720 µg/ml was able to increase the release of cytochrome c (Figure 5B). However, this effect of this crude venom has not been reported in mitochondria isolated from healthy colon

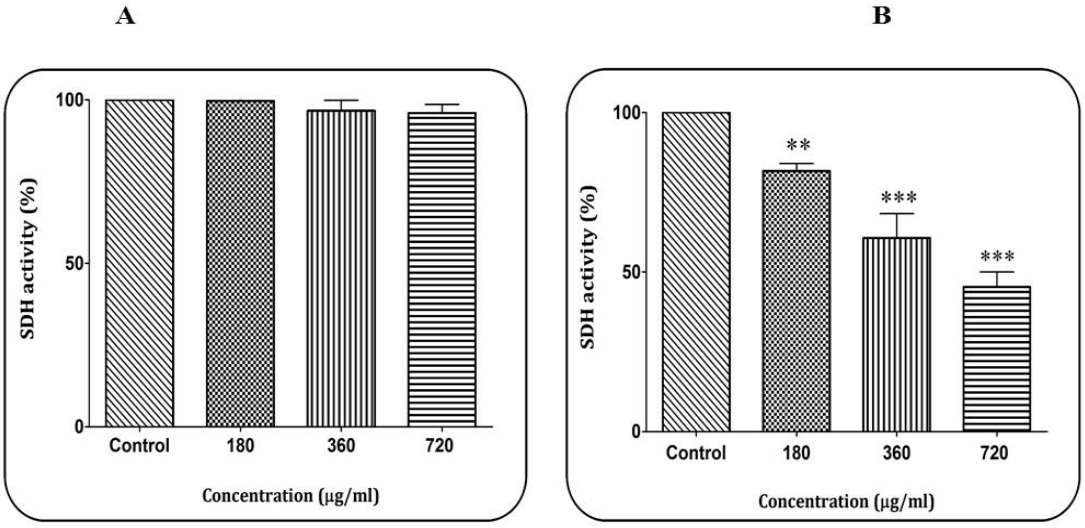

Figure 1. SDH Activity Assay. A, Healthy colon group; B, Colon cancer group. Data are shown as mean ± SD (n=3). ** P<0.01 and *** P<0.001 versus control group.

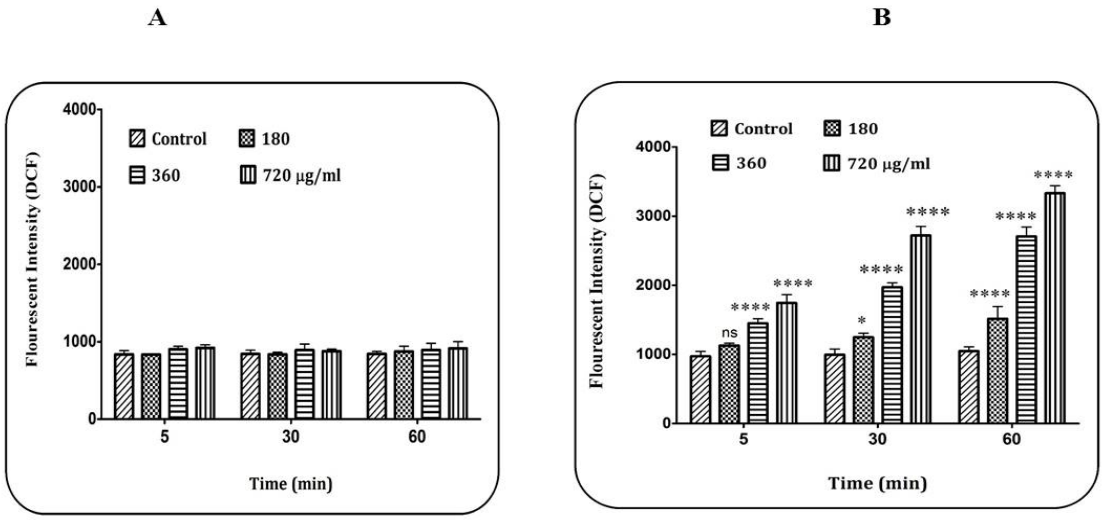

Figure 2. ROS Level Assay. A, Healthy colon group; B, Colon cancer group. Data are shown as mean ± SD (n=3). * P<0.05 and **** P<0.0001 versus control group.

tissue (Figure 5A).

Discussion

In this study, we investigated the effects of Chrysaora quinquecirrha crude venom on mitochondrial parameters isolated from human colon cancer tissue. In fact, the study was designed to investigate the association between ROS and induction of apoptosis in human colon cancer mitochondria exposed to Chrysaora quinquecirrha crude venom. Natural compounds extracted from marine metabolites have antitumor effects through various mechanisms (Yang et al., 2020). In unstressed cells, mitochondria consume approximately 95% of cellular O2. In oxidative conditions, it is known as the main sites for the production of ROS (Abele et al., 2007). Targeting mitochondria and the consequent disruption of mitochondrial membranes, production of ROS and induction apoptosis is one of the mechanisms by which marine compounds can kill cancer cells (Kluza et al., 2006; Dyshlovoy et al., 2020; Varela et al., 2020). Extracts/compounds of natural origin have been accepted by the scientific community for the prevention/treatment of cancer due to their properties such as low toxicity, cheap costs and easy availability (DEMİR et al., 2020).

At the beginning of the study, the in vitro effects of Chrysaora quinquecirrha crude venom on mitochondrial function (SDH activity) was investigated in the healthy and colon cancer mitochondria. Exposure with 180, 360 and 720 µg/ml of Chrysaora quinquecirrha crude venom significantly decreased the SDH activity only in colon cancer mitochondria. In previous studies, we have shown that compounds isolated from marine animals such as Persian Gulf Snail (Conus textile) and Persian Gulf Marine Mollusk (Turbo Coronatus) have the ability to impair mitochondrial function by acting on the respiratory chain. These results are agreement with the study that this crude venom was able to impair mitochondrial function

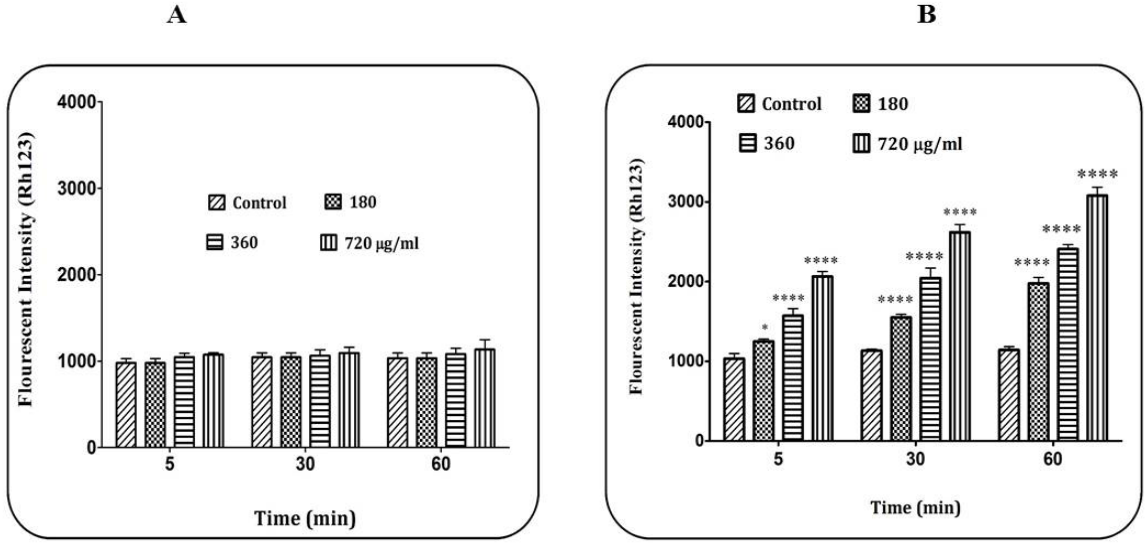

Figure 3. MMP Collapse Assay. A, Healthy colon group; B, Colon cancer group. Data are shown as mean ± SD (n=3). * P<0.05 and **** P<0.0001 versus control group.

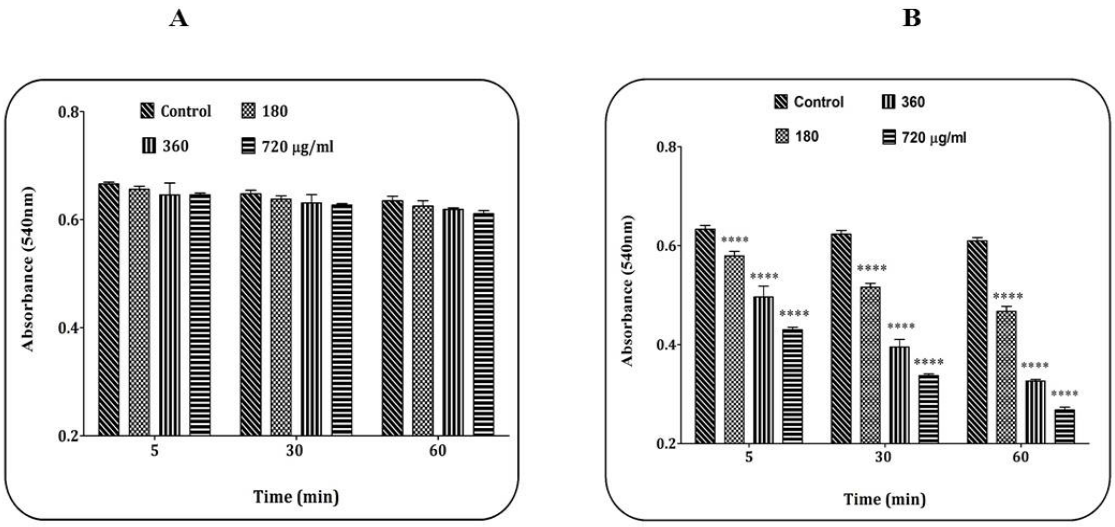

Figure 4. Mitochondrial Swelling Assay. A, Healthy colon group; B, Colon cancer group. Data are shown as mean ± SD (n=3). **** P<0.0001 versus control group.

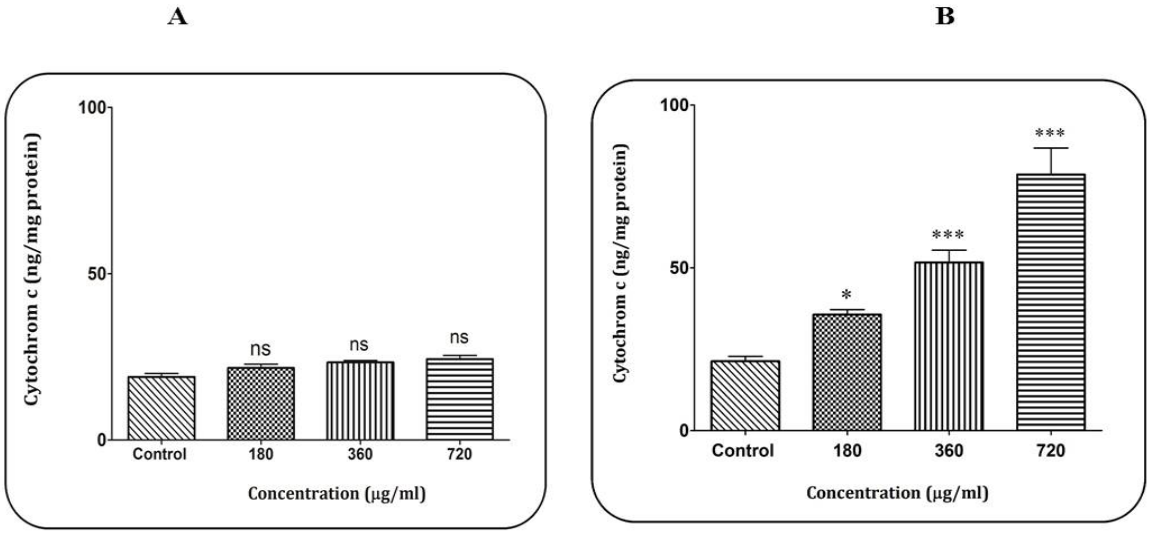

Figure 5. Cytochrome c Relese Assay. A, Healthy colon group; B, Colon cancer group. Data are shown as mean ± SD (n=3). * P<0.05 and *** P<0.001 versus control group.

(Zangeneh et al., 2019; Salimi et al., 2021).

In the mitochondrial respiratory chain, SDH is the same as complex II, and its disruption can be accompanied by an increase in the level of ROS. Thus, a decrease in the activity of complex II in the mitochondrial respiratory chain by this marine venom can be associated with an increase in the level of ROS only in the colon cancer mitochondria. The results showed that there was a positive correlation between the increases in the level of ROS only in the mitochondria of colon cancer tissue after exposure to different concentrations of Chrysaora quinquecirrha crude venom. Previous studies have shown that marine animals induce oxidative stress and apoptosis in colon cancer and human neuroblastoma SH-SY5Y cell lines by increase in the level of intracellular ROS, collapse in MMP, and activation of caspase-3. The results of our study showed that Chrysaora quinquecirrha crude venom was able to increase the level of ROS in the mitochondria of colon cancer and also caused the collapse of the MMP. The results of our study are agreement with the results of previous studies (Morabito et al., 2012; Ruiz-Torres et al., 2019). ROS has a dual physiological role. They are involved in low concentrations in cell viability and proliferation and conversely in high concentrations in cell killing. Research has shown that various compounds have been able to reduce proliferation and survival in cancer cells by increasing the level of ROS (Gill et al., 2016; Yang et al., 2019; Jin et al., 2020).

Next, we assessed MMP collapse and mitochondrial swelling. The results indicate collapse in MMP and mitochondrial swelling in mitochondria of colon cancer after exposure to different concentrations of Chrysaora quinquecirrha crude venom. In fact, these two events are irreversible and can lead to the release of pro-apoptotic proteins from the mitochondria and the activation of apoptosis. Research has shown that venoms derived from compounds of natural origin, such as sea anemone (*H. magnifica*) and Naja ashei, can cause release of

cytochrome c from the mitochondria into the cytosol, which is associated with the induction of cell death signaling (Ramezanpour et al., 2014; Rozman Antolikova et al., 2019). The results of our study showed that Chrysaora quinquecirrha crude venom was able to release of cytochrome c from the mitochondria of colon cancer. These results are in agreement with our previous study (Zangeneh et al., 2019; Salimi et al., 2021). Finally, our results showed that Chrysaora quinquecirrha crude venom was able to release of cytochrome c from cancerous mitochondria. Cytochrome c is known as one of the key factors in the regulation of apoptosis. Also, its release from mitochondria is associated with apoptosis (Kalpage et al., 2020; Yadav et al., 2020). It seems that Chrysaora quinquecirrha crude venom with release of cytochrome c can induce apoptosis in colon cancer mitochondria.

Our results in this research recommend that Chrysaora quinquecirrha crude venom has been able to increase the level of ROS in colon cancer by acting on mitochondria. Subsequently, ROS was able to release of cytochrome c by acting on MMP. Therefore, Chrysaora quinquecirrha crude venom can be a promising source for colon cancer drug candidates.

Author Contribution Statement

Enayatollah Seydi contributed to this research in carrying out the experiments, analyzing the data and writing the paper. Nahid Jafarzadeh contributed to this research in carrying out the experiments and performing statistical analysis as the thesis student. Parvaneh Naserzadeh Amir Vazirizadeh, and Mohammadreza Mirshamsi contributed in this research in carrying out some experiments. Jalal Pourahmad 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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Ethics approval

All investigations were done according to the standard protocols approved by the ethic of Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Availability of data

All data generated or analyzed during this study are included in this published article.

Conflict of Interests

The authors declare that they have no conflict of interest.

References

Abate M, Festa A, Falco M, et al (2020). Mitochondria as playmakers of apoptosis, autophagy and senescence. *Semin Cell Dev Biol*, **98**, 139-53.

Abele D, Philipp E, Gonzalez P, et al (2007). Marine invertebrate mitochondria and oxidative stress. *Front Bioscience*, **12**, 933-46.

Abraha AM, Ketema EB (2016). Apoptotic pathways as a therapeutic target for colorectal cancer treatment. *World J Gastrointest Oncol*, **8**, 583-91.

Ahmed M (2020). Colon Cancer: A Clinician's Perspective in 2019. *Gastroenterol Res*, **13**, 1-10.

Alfarouk KO, Stock CM, Taylor S, et al (2015). Resistance to cancer chemotherapy: failure in drug response from ADME to P-gp. *Cancer Cell Int*, **15**, 71.

Baracca A, Sgarbi G, Solaini G, et al (2003). Rhodamine 123 as a probe of mitochondrial membrane potential: evaluation of proton flux through F(0) during ATP synthesis. *Biochim Biophys Acta*, **1606**, 137-46.

Battogtokh G, Cho YY, Lee JY, et al (2018). Mitochondrial-targeting anticancer agent conjugates and nanocarrier systems for cancer Treatment. *Front Pharmacol*, **9**, 922.

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical biochemistry, **72**, 248-54.

Cao W, Chen HD, Yu YW, et al (2021). Changing profiles of cancer burden worldwide and in China: a secondary analysis of the global cancer statistics 2020. *Chin Med J (Engl)*, **134**, 783-91.

Demir S, Demir EA, Aliyazicioğlu Y (2020). Selective cytotoxic effect of Astaxanthin on human lung and colon cancer cells. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*, **23**, 1489-94.

Dyshlovoy SA, Kudryashova EK, Kaune M, et al (2020). Urupocidin C: a new marine guanidine alkaloid which selectively kills prostate cancer cells via mitochondria targeting. *Sci Rep*, **10**, 9764.

Eckert GP, Lipka U, Muller WE (2013). Omega-3 fatty acids in neurodegenerative diseases: focus on mitochondria. P*rostaglandins Leukot Essent Fatty Acids*, **88**, 105-14.

Eskandari MR, Moghaddam F, Shahraki J, et al (2015). A comparison of cardiomyocyte cytotoxic mechanisms for 5-fluorouracil and its pro-drug capecitabine. *Xenobiotica*, **45**, 79-87.

Fakhri A, Omranipour R, Fakhri S, et al (2017). Naja Naja Oxiana Venom Fraction Selectively Induces ROS-Mediated Apoptosis in Human Colorectal Tumor Cells by Directly Targeting Mitochondria. *Asian Pac J Cancer Prev*, **18**, 2201-8.

Ghanbari F, Nasarzadeh P, Seydi E, et al (2017). Mitochondrial oxidative stress and dysfunction induced by single- and multiwall carbon nanotubes: A comparative study. Journal of Biomedical Materials Research - Part A, 105, 2047-55.

Gill JG, Piskounova E, Morrison SJ (2016). Cancer, Oxidative Stress, and Metastasis. Cold Spring Harb Symp Quant Biol, 81, 163-75.

Grasso D, Zampieri LX, Capelôa T, et al (2020). Mitochondria in cancer. *Cell Stress*, **4**, 114-46.

Hu Y, Zhang K, Zhu X, et al (2021). Synergistic Inhibition of Drug-Resistant Colon Cancer Growth with PI3K/mTOR Dual Inhibitor BEZ235 and Nano-Emulsioned Paclitaxel via

Reducing Multidrug Resistance and Promoting Apoptosis. *Int J Nanomedicine*, **16**, 2173-86.
Jaworski S, Strojny B, Sawosz E, et al (2019). Degradation of Mitochondria and Oxidative Stress as the Main Mechanism of Toxicity of Pristine Graphene on U87 Glioblastoma Cells and Tumors and HS-5 Cells. *Int J Mol Sci*, **20**.
Jeena MT, Kim S, Jin S, et al (2019). Recent Progress in Mitochondria-Targeted Drug and Drug-Free Agents for Cancer Therapy. *Cancers (Basel)*, **12**.
Jiang JH, Pi J, Jin H, et al (2019). Oridonin-induced mitochondria-dependent apoptosis in esophageal cancer cells by inhibiting PI3K/AKT/mTOR and Ras/Raf pathways. *J Cell Biochem*, **120**, 3736-46.
Jin W, Lu S, Wang X, et al (2020). Raddeanin A suppresses lung cancer cell proliferation via induction of apoptosis and increased production of ROS. *Cell Mol Biol (Noisy-le-grand)*, **66**, 174-9.
Kalpage HA, Wan J, Morse PT, et al (2020). Cytochrome c phosphorylation: Control of mitochondrial electron transport chain flux and apoptosis. *Int J Biochem Cell Biol*, **121**, 105704.
Khalifa SAM, Elias N, Farag MA, et al (2019). Marine Natural Products: A Source of Novel Anticancer Drugs. *Mar Drugs*, **17**.
Kim C, Kim B (2018). Anti-Cancer Natural Products and Their Bioactive Compounds Inducing ER Stress-Mediated Apoptosis: A Review. *Nutrients*, **10**.
Kluza J, Gallego MA, Loyens A, et al (2006). Cancer cell mitochondria are direct proapoptotic targets for the marine antitumor drug lamellarin D. *Cancer Res*, **66**, 3177-87.
Mirshamsi M, Vazirizadeh A, Omranipour R, et al (2017). Persian Gulf stonefish (Pseudosynanceia melanostigma) venom fractions selectively induce apoptosis on cancerous hepatocytes from hepatocellular carcinoma through ROS-mediated mitochondrial pathway. *Hepatitis Monthly*, **17**.
Morabito R, Condello S, Currò M, et al (2012). Oxidative stress induced by crude venom from the jellyfish Pelagia noctiluca in neuronal-like differentiated SH-SY5Y cells. *Toxicol In Vitro*, **26**, 694-9.
Pustylnikov S, Costabile F, Beghi S, et al (2018). Targeting mitochondria in cancer: current concepts and immunotherapy approaches. *Transl Res*, **202**, 35-51.
Rafiemanesh H, Pakzad R, Abedi M, et al (2016). Colorectal cancer in Iran: Epidemiology and morphology trends. *Excli J*, **15**, 738-44.
Ramezanpour M, da Silva KB, Sanderson BJ (2014). The effect of sea anemone (H. magnifica) venom on two human breast cancer lines: death by apoptosis. *Cytotechnology*, **66**, 845-52.
Rozman Antolikova N, Kello M, Zigova M, et al (2019). Naja ashei venom induces mitochondria-mediated apoptosis in human colorectal cancer cells. *Acta Biochim Pol*, **66**, 207-13.
Ruiz-Torres V, Rodríguez-Pérez C, Herranz-López M, et al (2019). Marine Invertebrate Extracts Induce Colon Cancer Cell Death via ROS-Mediated DNA Oxidative Damage and Mitochondrial Impairment. *Biomolecules*, **9**.
Sak K (2014). Cytotoxicity of dietary flavonoids on different human cancer types. *Pharmacogn Rev*, **8**, 122-46.
Salimi A, Eybagi S, Seydi E, et al (2016). Toxicity of macrolide antibiotics on isolated heart mitochondria: a justification for their cardiotoxic adverse effect. *Xenobiotica*, **46**, 82-93.
Salimi A, Rahimitabar N, Vazirizadeh A, et al (2021). Persian Gulf Snail Crude Venom (Conus textile): A Potential Source of Anti-Cancer Therapeutic Agents for Glioblastoma through Mitochondrial-Mediated Apoptosis. *Asian Pac J Cancer Prev*, **22**, 49-57.
Subramaniam S, Selvaduray KR, Radhakrishnan AK (2019). Bioactive Compounds: Natural Defense Against Cancer? *Biomolecules*, **9**.
Sun X, Zhan L, Chen Y, et al (2018). Increased mtDNA copy number promotes cancer progression by enhancing mitochondrial oxidative phosphorylation in microsatellite-stable colorectal cancer. *Signal Transduct Target Ther*, **3**, 8.
Taira J, Miyazato H, Ueda K (2018). Marine Peroxy Sesquiterpenoids Induce Apoptosis by Modulation of Nrf2-ARE Signaling in HCT116 Colon Cancer Cells. *Mar Drugs*, **16**.
Van Blarigan EL, Fuchs CS, Niedzwiecki D, et al (2018). Marine ω-3 Polyunsaturated Fatty Acid and Fish Intake after Colon Cancer Diagnosis and Survival: CALGB 89803 (Alliance). *Cancer Epidemiol Biomarkers Prev*, **27**, 438-45.
Varela AT, Neves RAF, Nascimento SM, et al (2020). Mitochondrial impairment and cytotoxicity effects induced by the marine epibenthic dinoflagellate Coolia malayensis. *Environ Toxicol Pharmacol*, **77**, 103379.
Wei J, Gou Z, Wen Y, et al (2020). Marine compounds targeting the PI3K/Akt signaling pathway in cancer therapy. *Biomed Pharmacother*, **129**, 110484.
Yadav N, Gogada R, O'Malley J, et al (2020). Molecular insights on cytochrome c and nucleotide regulation of apoptosome function and its implication in cancer. *Biochim Biophys Acta Mol Cell Res*, **1867**, 118573.
Yang HL, Liu HW, Shrestha S, et al (2021). Antrodia salmonea induces apoptosis and enhances cytoprotective autophagy in colon cancer cells. *Aging (Albany NY)*, **13**, 15964-89.
Yang JH, Yu K, Si XK, et al (2019). Liensinine inhibited gastric cancer cell growth through ROS generation and the PI3K/AKT pathway. *J Cancer*, **10**, 6431-8.
Yang Y, He PY, Zhang Y, et al (2020). Natural Products Targeting the Mitochondria in Cancers. *Molecules*, **26**.
Zangeneh F, Vazirizadeh A, Mirshamsi MR, et al (2018). Induction of Apoptosis by an Extract of Persian Gulf Marine Mollusc, Turbo Coronatus through the Production of Reactive Oxygen Species in Mouse Melanoma Cells. *Asian Pac J Cancer Prev*, **19**, 3479-88.
Zangeneh F, Vazirizadeh A, Mirshamsi MR, et al (2019). Induction of Apoptosis by Extract of Persian Gulf Marine Mollusk, Turbo Coronatus through the ROS-Mediated Mitochondrial Targeting on Human Epithelial Ovarian Cancer Cells. *Iran J Pharm Res*, **18**, 263-74.
Zhang QT, Liu ZD, Wang Z, et al (2021). Recent Advances in Small Peptides of Marine Origin in Cancer Therapy. *Mar Drugs*, **19**.
Zhang Y, Jiang L, Hu W, et al (2011). Mitochondrial dysfunction during in vitro hepatocyte steatosis is reversed by omega-3 fatty acid-induced up-regulation of mitofusin 2. *Metabolism*, **60**, 767-75.
Zhao Y, Ye L, Liu H, et al (2010). Vanadium compounds induced mitochondria permeability transition pore (PTP) opening related to oxidative stress. *J Inorg Biochem*, **104**, 371-8.