International Journal of Clinical Biology and Biochemistry
www.biochemistryjournal.net
Online ISSN: 2664-6196; Print ISSN: 2664-6188
Received: 01-11-2018; Accepted: 02-12-2018; Published: 01-01-2019
Volume 1; Issue 1; 2019; Page No. 01-04

Redox activity of algae extract from red sea, jeddah

Morog R Maddah[1], Etimad H Huwait[2], Soonham S Yaghmoor[3], Tahani M Alghamdi[4], Taha A Kumosani[5], Said S Moselhy[6]
[1, 2, 5] Biochemistry Department, Faculty of Science, King Abdulaziz University (KAU), Jeddah, Kingdome, Saudi Arabia
[2, 3, 5] Experimental Biochemistry Unit, King Fahd Medical Research Center, KAU, Saudi Arabia
[3, 5] Production of Bio products for Industrial Applications Research Group, KAU, Saudi Arabia
[4] Collage of Pharmacy, Ibin Sina Medical Collage, Jeddah, Saudi Arabia
[6] Biochemistry Department, Faculty of Science, Ain Shams University, Cairo, Egypt

Abstract
Molecules made from algae show different bioactivities, like immunomodulation, antibacterial, anticancer and are well documented for their use in an ethno pharmacological context. This study was designed to explore the in *vitro* antioxidant and anti-proliferative activity of chloroform extract of *Gracilaria dendroides (CEGD)* algae extract obtained from Red sea at Jeddah. *Gracilaria dendroides (GD)*, was washed by distilled water to remove salts and stored at -20°C until extraction. The GSH level we found dose-dependent increase in GSH levels in MCF7 cells following exposure to CEGD after 72 hr, indicating the activity as antioxidant. GSH is an important sulfur-containing antioxidant, which maintains the intracellular redox status by efficiently regulating the cellular defenses protecting against the development of oxidative stress by directly scavenging ROS. It was concluded that the application of CEGD to MCF7 breast cancer cells has significantly retarded the antioxidative potential and caused loss of membrane integrity of cancer cells which resulted in cell death through apoptosis. Therefore, could be used for further anticancer therapy.

Keywords: *Gracilaria dendroides*- antioxidants-anticancer

1. Introduction

Reactive oxygen species (ROS) can be divided into 2 groups: free radicals and nonradicals [1]. These ROS play an important role in degenerative or pathological processes, such as cancer, aging, neurodegenerative disorders, Alzheimer's disease, atherosclerosis, diabetes and inflammation [2]. As a result of normal cellular metabolism and environmental factors (such as cigarette smoke or air pollutants) in the living organisms, ROS are produced. Reactive oxygen species can harm cell structure, such as nucleic acids, proteins, carbohydrates and changes their functions. Regulation of reducing and oxidizing (redox) state is important for cell viability, proliferation, activation and organ function [3]. Nonenzymatic antioxidants include tocopherol, alkaloids, carotenoids and flavonoids, as well as the major cellular redox buffers ascorbate and glutathione (GSH) [4]. Human possess antioxidant system to protect against free radicals. These systems include (a) endogenous, some antioxidans produced in the body and (b) exogenous, obtained from diet. The first includes (a) enzymatic defenses, such as catalase, superoxide dismutase, glutathione, which metabolize hydrogen peroxide lipid peroxides and superoxide, thus preventing most of the formation of the toxic $OH^{\bullet}$ and (b)nonenzymatic defenses, like glutathione, the iron binding proteins (ferritin and transferrin), protein thiols, urate, histidine peptides, melatonin and dihydrolipoic acid [5].

The second fatal diseases in the industrialized countries is cancer, next to cardiovascular diseases, and third fatal disease in India. Cancer is a broad term used for identifying a large number of diseases. A normal cell suddenly turns into malignant cell and start dividing without check, leading to the growth of tumors or abnormally rise in the number of dispersed cells like the blood corpuscles. Cancer can take place in any part of the body and in any organ or tissue. About 50% of all cancers are attributed to life style, e.g. alcohol consumption, tobacco habits, diet and exposure to industrial toxins (Devi 2004). Colon cancer is one of the most common forms of cancer. Mortality and incidence of colon cancer is the third among the other cancer types [6]. Natural products from marine sources, offer an abundance for drug development to treat cancer [7]. Marine algae have provide a rich source of functional active compounds for applications in medical, cosmotic and fields [8]. Molecules made from algae show different bioactivities, like immunomodulation, antibacterial, anticancer and are well documented for their use in an ethno pharmacological context [9]. The drug discovery using natural products as medicinal plants or marine organism still an important target for recent research. Marine microorganisms such as green and blue algae were identified as rich sources of biologically active compounds. Antioxidants, come from algae important against different of diseases by protect the cells from oxidative damage [10]. Algae are exist where there is a light to do photosynthesis: in the lakes, sea and rivers, on walls and soil, in plants and animals. There are two major types of algae: the macro algae (sea weeds) found in littoral zone, which included brown, green and red alga. Macro algae are found in shallow area of oceans (around the shores), because they need light to survive. While the micro algae exist in littoral and benthic habitats and also throughout the ocean as phytoplankton [11]. Seaweeds identified as a sources of Vitamins, protein, minerals, iodine and possess chemo preventive potentials [12]. They offer food and

home for different marine animals, provide beauty to the underwater landscape and are valuable to human as a food and industrial raw materials [13].

This study was designed to explore the in *vitro* antioxidant and anti-proliferative activity of chloroform extract of *Gracilaria dendroides (CEGD)* algae extract obtained from Red sea at Jeddah.

2. Materials and methods

Different nontoxic algae samples collected from the Red sea in Jeddah: *Gracilaria dendroides (GD),* was washed by distilled water to remove salts and stored at -20°C until extraction. Dried Algae powders (100 g) were extracted for 2 h at room temperature in 1L 50% choloroform by mixing with a magnetic stirrer. Extract were centrifuged for removal of alga particles. After centrifugation at 4 °C for 10 min, the supernatant were collected and extraction solutions were dried by vacuum-evaporator. Then the aqueous fractions containing bioactive compound lyophilized and the dried residues weighed and calculated based on the weight of dry alga powder and used as dry Algae extracts.

3.4.3 Hct116 and HepG2 cell lines processing and maintenance

3.4.3.1 Cell culture

Human breast cancer cell line (MCF-7) was cultured in DMEM (Dulbecco's Modification of Eagles Medium) and supplemented with 10% FBS, 100 units/ml penicillin and 0.1 mg/ml streptomycin to form complete DMEM at 37C in a humidified incubator having 5% CO2.To collect the cells, it was removed and discarded the old media from flask and washed the cells by 5ml PBS, and treated with 2 ml of trypsin-EDTA for 5minutes. The reaction of trypsin was stopped by adding 3ml of complete DMEM. Then the mixture was transferred into a tube and centrifuged at 4000 rpm for 5 minutes. The supernatant was removed, then cell pellet was suspended in 5 ml of complete DMEM.

3.4.3.4 Assessment of algea extract on MCF-7 cells by WST-1 Assay

Aqueous and methanol extracts were dissolved in distilled water and added to media to made the working standard for each. Cell proliferation was determined by the WST-1 kit. Cells MCF-7 was Seeded in 96-well plate at a density of $5x10^3$ cells/well and incubated at 37°C in 5% Co_2 overnight. In next day cells were treated with aqueous and methanol extracts at doses 0.05, 0.1, 0.5, 1mg/ml and positive control 0.3% H2O2 at doses 0.5 mg/ml for 24, 48 and 72h (for two cell lines). After incubation 10 µl of WST-1 proliferation reagent was added to each well and continued to incubate the cells for 2-4h. And the absorbance was measured at 450 nm using a micro plate reader (ELISA). In plate (24, 48,72h) the media with treatments were removed and replaced with fresh media and daily added the doses.

Membrane Integrity and LDH Measurement

Cell membrane integrity of was evaluated by determining the activity of lactate dehydrogenase (LDH) leaking out of the cell according as The LDH assay is based on the release of the cytosolic enzyme, LDH, from cells with damaged cellular membranes**.** Lactate dehydrogenase (LDH) activity in the cell medium was determined using LDH Kit [Bio vision, England].

Assay of reduced glutathione (GSH) level

The GSH level in cell lysates was estimated by the method of (Ellman, 1959).

Caspase-3 Assay

The activity of caspase-3 was determined from the cleavage of the caspase-3 substrate (N-acetyl-DEVD-p-nitro anilin). Caspase-3 activity was measured by using Caspase-3 activity Colorimetric Assay Kit (Bio vision, England) according the procedure supplied by the manufacturer.

DNA fragmentation analysis

The MCF7 cells were seeded (0.5*106cells/well) in 6-well plates and 24 h, after which the medium was replaced with fresh medium containing CEDG (60,80ng/µl) and incubated for 72 h, after which cells were scraped off and DNA extraction from MCF7 cells were carried using (Quick-gDNA™ Mini Prep) the extracted DNA was separated by 1.0% agarose gel electrophoresis and visualized by ethidium bromide staining under UV lamb.

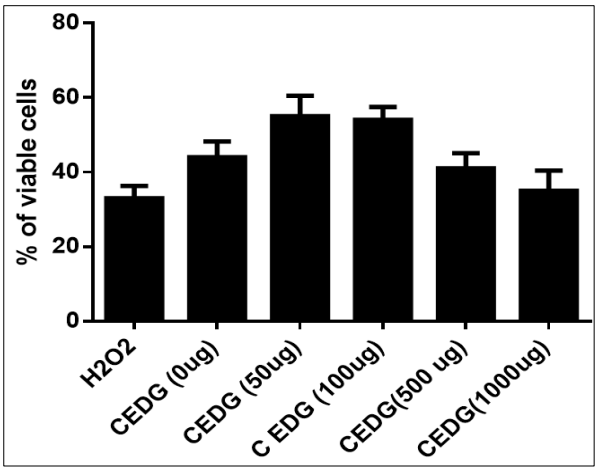

Fig 1: CEDG/MCF-7- 24hours

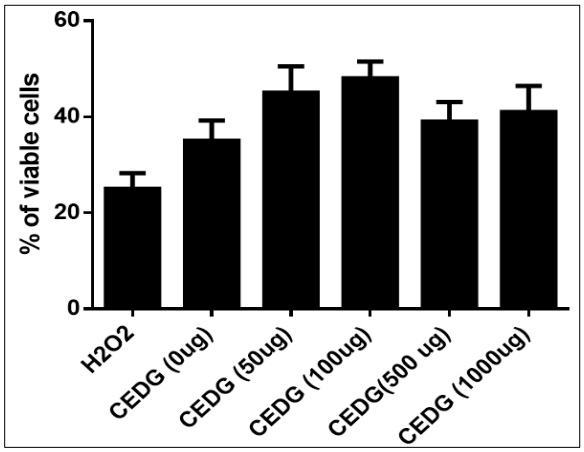

Fig 2: CEDG/MCF-7- 48hours

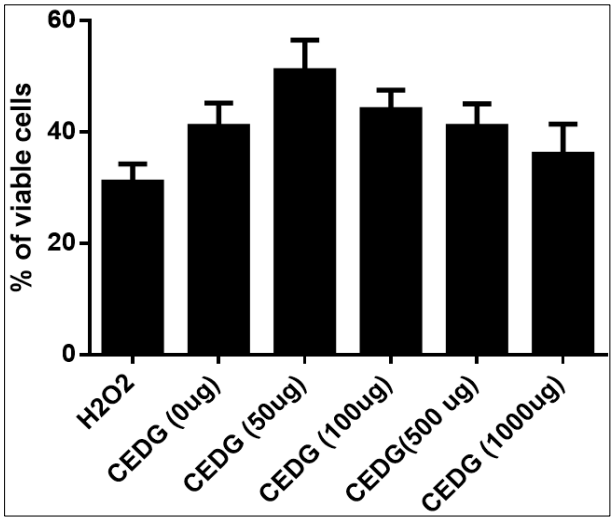

Fig 3: CEDG/MCF-7- 72hours

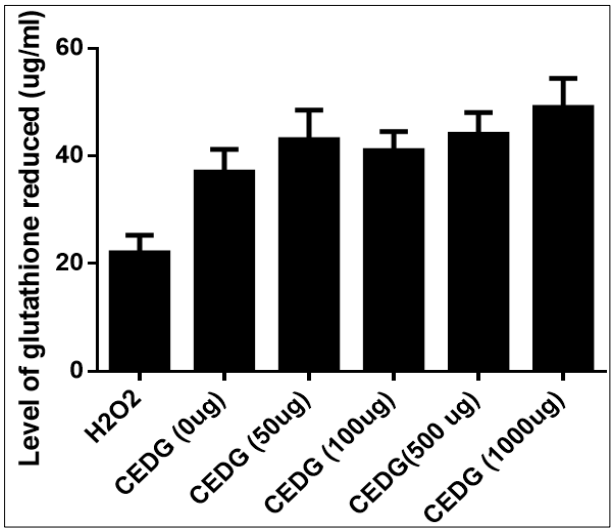

Fig 4: Concentration of CEDG

4. Results and Discussion

Oxidative stress is a main cause of inflammations involved in a variety of diseases such as neurodegenerative. Free radicals (e.g., hydroxyl, nitric oxide radicals) and reactive intermediates are the main oxidative stress effector. Free radicals can be very harmful to RNA, DNA, and proteins when they are exist in high amount, but in small amount they are quickly converted into less interacting forms. We used two assays to evaluate the antioxidant potential, the Cellular Antioxidant Activity and the Cellular Lipid Peroxidation Antioxidant Activity Assays [14].

We performed a series of tests to determine the cytotoxic potential of CEGD in in vitro systems (MCF7). As demonstrated in the result the viability of MCF7 cells decrease by increasing the concentration of CEGD and time exposure CEGD induced cytotoxicity in a dose- and time-dependent manner this is in agreement with study of [15].

Our results also showed that LDH leakage was significantly increased by increasing the dose of CEGD, LDH leakage from cells is also an evidence of cell membrane damage. Studies have shown that LDH level was elevated in cells culture medium after exposure of cells to CEGD [16].

The GSH level we found dose-dependent increase in GSH levels in MCF7 cells following exposure to CEGD after 72 hr, indicating the activity as antioxidant.

GSH is an important sulfur-containing antioxidant, which maintains the intracellular redox status by efficiently regulating the cellular defenses protecting against the development of oxidative stress by directly scavenging ROS [17].

The CEGD in current study also activated caspase-3, and the activity was increased to about (93, 87 and 89) % of the level of the control group when the cells were treated for 24, 48 and 72 hr respectively. The activation of caspase-3 was seen in a dose manner. The activation signal of caspase-3 seemed to be through the induction of ROS, which is the general pathway of toxic chemicals to induce ROS generation. Many other chemicals, which have cytotoxicity through the apoptotic mechanism, showed increased ROS and caspase-3 activity then, finally, apoptosis in cultured cells [18]. The internucleosomal DNA cleavage on agarose gel electrophoresis of The DNA extracted from iron oxid nanoparticles treated MCF7cells showed fragmentation after 72 hr of treatment. Porter *et al*., (1991) had reported that caspase- 3 activation may cause chromosome condensation and also DNA fragmentation to trigger apoptosis of cells. It was reported that nanoparticles due to their small size are capable of reaching the nucleus and interacting with DNA. They may also exhibit an indirect effect on DNA through their ability to generate ROS

Although there are a significant advanced in the development of synthetic drug, natural products and natural-derived compounds are still of high interest because of different health beneficial properties with high potential that can be used as natural food products [19]. Marine algae are non-flowering plants without root, stem and leaves. They are divided into three groups: red algae (Rhodophytae), brown algae (Pheaophytae) and green algae (Chlorophytae) based on their nutritional components and chemical composition [20]. The number of compounds isolated from marine organisms is now 28,000 with hundreds of new compounds discovered each year [21]. Newly, there are considerable focus in explore the potential of biotechnology of micro-organisms (e.g., micro-algae) because they have a shorter generation periods, easier in planting, and they are represents a renewable resources that remain unexplored in drug discovery [22]. It was concluded that the application of CEGD to MCF7 breast cancer cells has significantly retarded the antioxidative potential and caused loss of membrane integrity of cancer cells which resulted in cell death through apoptosis. Therefore, could be used for further anticancer therapy.

5. References

1. Lahaye M, Inizan F, Vigouroux J. NMR analysis of the chemical structure of ulvan and of ulvan-boron complex formation. Carbohydr. Polymers. 1998; 36:239-249.
2. Latham H. Temperature stress-induced bleaching of the coralline alga Corallina officinalis: a role for the enzyme bromoperoxidase. Biosci. Horizon. 2008; 1(2):104-113.
3. Maron D, Ames B. Revised methods for the Salmonella mutagenicity test. Mutat. Res. 1983; 113:173-215.
4. Matanjun P, Mohamed S, Mustapha NM, Muhammad K, Ming CH. Antioxidant activities and phenolics content of eight species of seaweeds from north Borneo. J. Appl. Phycol. 2008; 20(4):367-373.

5. Matoh T, Shoji IDA, Takahashi E. A rapid and sensitive assay for ferredoxin–glutamate synthase. Bull. Res. Inst. Food Sci. Kyoto Univ. 1980; 43:1-6.
6. Matsukawa R, Dubinsky Z, Kishimoto E, Takenchi M, Niki E, Karube I. A comparison of screening methods for antioxidant activity in seaweeds. J. Appl. Phycol. 1997; 9:29-35.
7. Melo MRS, Feitosa JPA, Freitas ALP, de Paula RCM. Isolation and characterization of soluble sulfated polysaccharide from the red seaweed Gracilaria cornea. Carbohydr. Polymers. 2002; 49:491-498.
8. Mori J, Matsunaga T, Takahashi S, Hsegaula C, Saito H. Inhibitory activity on lipid peroxidation of extracts from marine brown alga. Phytother. Res. 2003; 17(5):549-551.
9. Mundt S, Kretlow S, Jansen R. Fatty acids with antibacterial activity from the cyanobacterium Oscillatoria redekei HUB 051. J. Appl. Phycol. 2003; 15:263-267.
10. Mutawie HH. Studies on biological active compounds from marine algae in Jeddah cornice of Saudi Arabia. Ph.D. Thesis, Umm Al-Qura University, 2006.
11. Nascimento SC, Correalima RN, Diu MB, Koening ML. Cytotoxic and antitumoral effects of seaweed extracts from northeastern coast of Brazil. Mem. Soc. Brot. 1993; 29:79-84.
12. Pantakar MS, Oehninger S, Barnett T, Williams RL, Clark GF. A revised structure for fucoidan may explain some ofits biological activities. J Biol. Chem. 1993; 268:21770-21776.
13. Pengzhan Y, Zhang Q, Li N, Wang Y, Li Z. Polysaccharides from Ulva pertusa (Chlorophyta) and preliminary studies on their antihyperlipidemia activity. J Appl. Phycol. 2003; 15:21-27.
14. Ruperez P. Antioxidant activity of sulphated polysaccharides from the Spanish marine seaweed Nori. In Proceedings of the COST 916 European Conference on Bioactive Compounds in Plant Foods. Health Effects and Perspectives for the Food Industry, Tenerife, Canary Islands, Spain, April, 2001; p. 114.
15. Ruperez P, Ahrazem O, Leal JA. Potential antioxidantcapacity of sulfated polysaccharides from the edible marine brown seaweed Fucus vesiculosus. Agric. Food Chem. 2002; 50:840.
16. Vairappan C, Motonari D, Minoru S, Michio M. Antibacterial halogenated metabolites from the Malaysian Laurencia species. Phytochemistry. 2001; 58:291-297.
17. Yan X, Nagata T, Fan X. Antioxidative activities in some common seaweeds. Plant Foods Hum. Nutr. (Dordrecht, Netherlands). 1998; 52:253-262.
18. Antonisamy MJ, Raj EDS, UV–VIS, HPLC studies on *Amphiroaanceps* (Lamarck) Decaisne. Arabian J Chem, 2011. [E Pub ahead of Print]
19. Keyrouz R, Abasq ML, Le Bourvellec C. Total phenolic contents, radical scavenging and cyclic voltammetry of seaweeds from Brittany. *Food Chem*. 2011; 126:831-836.
20. Gupta S, Abu-Ghannam N. Recent developments in the applica-tion of seaweeds or seaweed extracts as a means for enhancing the safety and quality attributes of foods. *Innov. Food Sci. Emerg. Technol*. 2011, 12:600-609.
21. Gumul D, Ziobro R, Zięba T, Rój E. The influence of addition if defeated blackcurrant seeds on pro-health constituents and tex-ture of cereal extrudates, J Food Qual. 2011; 34:395-402.
22. Li YX, Wijesekaraa I, Li Y, Kima SK. Phlorotannins as bioactive agents from brown algae. *Process Biochem*. 2011; 46:2219-2224.