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In vitro Study Anti-proliferative Potential of Algae Extract against Cancer Cell Line

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Oxidative stress is defined as imbalance between oxidant and antioxidant ratio, that lead to oxidative damage of biologically active molecules, finally lead to different disease and initiate carcinogenesis. The drug discovery using natural products as medicinal plants or marine organism still an important target for recent research. This study investigated anticancer activity of algae extract obtained from Red sea at Jeddah.
Materials and Methods: Aqueousand methanol extracts of*Dictyotaciliolata*(DC) were tested on HCT-116 and HepG2 cell lines using WST-1. Aqueous extract (AEDC) and methanol extract (MEDC) at doses 0.05, 0.1, 0.5, 1 mg/ml and positive control 0.3% H2O2 at doses 0.5 mg/ml for 24,48 and 72 h (for two cell lines).

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Results: AEDC showed the mosteffective antitumor activity against HCT116 and HePG2, the IC50 dose for HCT116 cells was 0.05 mg/ml at 72 h, while for the HePG2 it was 0.01 mg/ml at 72 h. These results showed that HePG2cells was more sensitive to the AEDC. However, IC50 for MEDC were 0.01 mg/ml for HCT116 and 0.05 for HepG2 at 48 Hrs. The algae extracts contain sulfated polysaccharides and different pigments as chlorophylls, carotenoids and phycobiliproteins. These pigments were approved as biological active biomolecules that exert different biological activities as antioxidants, antitumor and rich source of micronutrients. In addition, AEDC or MEDC exert apoptotic activity by increase activity of caspase 3 and 9 in HepG2.
Conclusıon: The antitumor effect of AEDC or MEDC is promising for development of chemotherapeutic agent as effective with no side effects.

Keywords: Algae extract; bioactive; anticancer; cell lines.

1. INTRODUCTION

Oxidative stress is termed as the shift in balance between oxidant/antioxidant in favor of oxidants [1]. Oxidative stress has harmful effect because oxygen free radicals hits biological molecules such as proteins, lipids, and DNA. An atom contains a central nucleus with pairs of electrons around it. Free radicals are highly reactive and unstable because the unpaired electrons tend to form pairs with other electrons [2]. It is usually under case of oxidative stress, reactive oxygen species were generated, such as hydroxyl ($OH^{\bullet-}$), peroxyl (OOH, $ROO^{\bullet}$) and superoxide ($O_2^{\bullet-}$) radicals [3]. Reactive oxygen species (ROS) can be divided into 2 groups: free radicals and nonradicals [4]. These ROS play an important role in degenerative or pathological processes, such as cancer, aging, neurodegenerative disorders, Alzheimer's disease, atherosclerosis, diabetes and inflammation. 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. Nonenzymatic antioxidants include tocopherol, alkaloids, carotenoids and flavonoids, as well as the major cellular redox buffers ascorbate and glutathione (GSH) [5].The second fatal diseases in the industrialized countries is cancer, next to cardiovascular diseases, and third fatal disease in India. 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 [6]. 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 [7].

Apoptosis is an energy dependent biochemical process of programmed cell death. It is a genetically programmed process of deliberate suicide by an unwanted cell in a multicellular organism during embryogenesis, metamorphosis and tissue turnover. But it can be trigerred by several stimuli like thermal stress, withdrawl of essential growth factors, treatment with synthetic compounds and several other factors [8].

Natural products from marine sources, offer abundance for drug development to treat cancer. Marine algae have provided a rich source of functional active compounds for applications in medical, cosmetic and fields [9]. Molecules made from algae show different bioactivities, like immunomodulation, antibacterial, anticancer and are well documented for their use in an ethno-pharmacological context.Red Sea shore is rich with marine microorganisms such as green (Chlorophyta) and blue algae (Cyanobacteria) that rich sources of biologically active compounds. Antioxidants come from algae important against different of diseases by protect the cells from oxidative damage.Algae exists 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 macroalgae (seaweeds) found in littoral zone, which included brown, green and red alga. Macroalgae 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 [9]. Seaweeds identified as asource of vitamins, protein, minerals, iodine and possess chemo preventive potentials. 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 [10]. This study tested *in vitro* anticancer activity of algae extract (seaweeds) and apoptotic activity treatment to avoid the side effects of traditional drugs.

2. MATERIALS AND METHODS

2.1 Preparation of Aqueous and Methanol Extracts of *Dictyotaciliolata* (Phaeophyceae)

Nontoxic algae samples collected from the Red sea in Jeddah*Dictyotaciliolata* (DC) 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 1 L 50% methanol by mixing with a magnetic stirrer. Extract were centrifuged for removal of alga particles. After centrifugation at 4°C for 10 min, the supernatant was collected and extraction solutions were dried by vacuum-evaporator (MEDC). Then the aqueous fractions (AEDC) 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.

2.2 Human Cancer Cell Line (HCT116 and HepG2)

The colon cancer (HCT116) and hepatocellular carcinoma (HepG2) human cancer cell line were 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 37°C in a humidified incubator having 5% CO_2.To collect the cells, HCT116 and HepG2 cells were removed and discarded the old media from flask and washed the cells by 5ml PBS, and treated with 2 ml of trypsin-EDTA for 5min. 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 resuspended in 5 ml of complete DMEM.

2.3 Determination the Anti-proliferative Effect of Extract on HCT116 and HepG2 Cells by WST-1 Assay

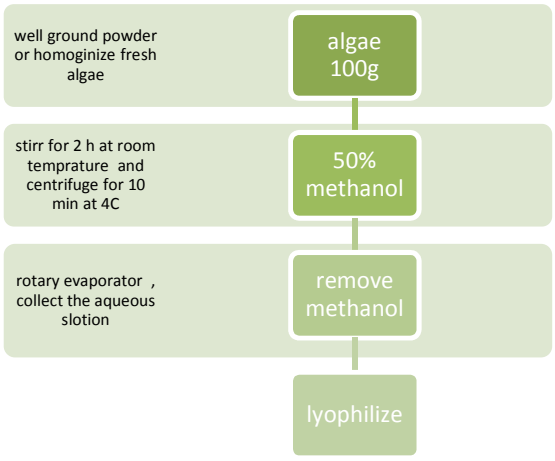
Aqueous and methanol extracts were dissolved in distilled water and added to media to make the working standard for each. Cell proliferation was determined by the WST-1 kit. Cells (HCT-116 and HepG2) were Seeded in 96-well plate at a density of $5x10^3$ cells/well and incubated at 37°C in 5% CO_2overnight. In next day cells were treated with aqueous and methanol extracts at doses 0.05, 0.1, 0.5, 1 mg/ml and positive control 0.3% H_2O_2 at doses 0.5 mg/ml for 24,48 and 72 h (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 microplate reader (ELISA). In plate (48,72 h) the media with treatments were removed and replaced with fresh media and daily added the doses.

2.4 Caspase-3 and 9 Assay

This colorimetric assay is based on the concept that a hydrolysis of the substrate acetyl-Asp-Glu-Val-Asp p-nitroanilide (Ac-DEVD-pNA) which is a peptide is done by caspase 3, resulting in the release of p-nitroaniline (pNA) moiety having a high absorbance at 405 nm (εmM = 10.5). The concentration of the pNA released from the substrate is calculated from the absorbance values at 405 nm or from a calibration curve prepared with defined pNA solutions. A suitable kit will be used for this assay.

2.5 Data Analysis

Results obtained were processed using online softwares such as SPSS version 20. P value <0.05 was considered as significant.



3. RESULTS AND DISCUSSION

Human possess antioxidant system to protect against free radicals. These systems include (a) endogenous, some antioxidants 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 [11].

Free radicals were considered as 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 existing 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.It is believed that oxidative stress is an important contributor to cancer and inflammation development. Micro-algal anti-inflammatory activity have ability to block the release of tumor necrosis factor α (TNFα), (one of the main effects of inflammation in lipopolysaccharide (LPS)-stimulated monocytic leukemia cells [12].

The control group for HepG2 was 100% and the viability with 0.05, 0.1, 0.5, 1 mg/ml and positive control 0.3% H_2O_2 at doses 0.5 mg/ml for 24,48 and 24 h was 90, 72.4, 61.27, 62.89 and 88.63%, respectively. While after 48 h were 103.72, 78.77, 71.23, 65.12 and 76.15%, respectively. After 72 h 46.33, 27.34, 25.79, 26.39 and 27.1% respectively.Assuming The control group for HCT116 was 100% and the viability with 0.05, 0.1, 0.5, 1mg/ml and positive control 0.3% H_2O_2 at doses 0.5 mg/ml for 24,48 and 72h was 80, 82.4, 71.27, 72.89 and 78.63%, respectively. According to these results the antiproliferative effects of the AEDC or MEDC increased depend on the concentration of extract.

Long time ago, in many Asian countries like China and Japan, the seaweeds have been used as medicinal herbs and functional foods because of their abundance in vitamins, minerals, lipids, and bioactive substances such as polysaccharide, polyphenol, and protein. Many crude extract and compounds derived from different algae have been tested for their antitumor activities because some algae species were used to treat cancer [13].

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 (Bae et al. 2015). Marine algae are non-flowering plants without root, stem and leaves. They are divided into three groups: red algae Rhodophyta), brown algae (Pheaophyceae) and green algae (Chlorophyta) based on their nutritional components and chemical composition [14].

Several investigations have shown that a high food intake of natural phenols with the presence of many types of antioxidants like flavonoids which are commonly exist in macro algal and plants strongly associated with longer life expectancy, lower risk of some diseases, and different types of cancer.The number of compounds isolated from marine organisms is now 28,000 with hundreds of new compounds discovered each year [15].

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 representing a renewable resource that remain unexplored in drug discovery [16].

Today, above 60% of commercially available anticancer drugs are natural origin. Doxorubicin, vincristine, mitomycin C and vinblastine are ant proliferative drugs that play an important role in cancer chemotherapy in a number of solid tumors and hematological malignancies [17-20].

The algae extracts contain sulfated polysaccharides and different pigments as chlorophylls, carotenoids and phycobiliproteins. These pigments were approved as biological active biomolecules that exert different biological activities as antioxidants, antitumor and rich source of micronutrients [21-25].

Table 1. Effect of aqueous extract (AEDC) on HCT116 after 24 h by WST-1 test

Extract conc. cell no		0	50 µg	100 µg	500 µg	1000 µg
	0.500	0.663	0.726	0.743	0.800	0.638
	0.462	0.660	0.637	0.637	0.746	0.864
	0.476	0.656	0.820	0.749	0.594	0.703

Table 2. Effect of AEDC on HCT116 after 48 h by WST-1 test.

Extract conc. cell no	0	50 µg	100 µg	500 µg	1000 µg
0.514	1.174	1.215	1.361	1.353	1.659
0.470	1.180	1.408	1.716	1.513	1.232
0.495	1.165	1.614	1.513	1.145	1.667

Table 3. Effect of AEDC on HCT116 after 72 h by WST-1 test

Extract conc. cell no	0	50 µg	100 µg	500 µg	1000 µg
0.449	1.896	1.798	1.777	1.683	1.742
0.363	1.482	1.647	1.388	1.509	1.763
0.407	1.466	0.813	1.336	1.134	1.215

Table 4. Effect of methanol extract (MEDC) on HCT116 after 24 h by WST-1 test

Extract conc. cell no	0	50 µg	100 µg	500 µg	1000 µg
0.500	0.663	0.650	0.772	1.267	1.620
0.462	0.660	0.812	0.662	1.278	1.715
0.476	0.656	0.747	0.838	1.297	1.770

Table 5. Effect of methanol extract (MEDC) on HCT116 after 48 h by WST-1 test

Extract conc. cell no	0	50 µg	100 µg	500 µg	1000 µg
0.514	1.174	1.130	1.098	1.764	2.722
0.470	1.180	1.031	1.322	2.240	2.752
0.495	1.165	1.165	1.164	1.879	2.761

Table 6. Effect of methanol extract (MEDC) on HCT116 after 72 h by WST-1 test

Extract conc. cell no	0	50 µg	100 µg	500 µg	1000 µg
0.449	1.896	1.697	1.561	2.181	2.990
0.363	1.482	1.449	1.782	2.139	2.661
0.407	1.466	1.711	1.545	2.095	2.816

Table 7. Effect of aqueous extract (AEDC) on HepG2 after 24 h by WST-1 test

Extract conc. cell no	0	50 µg	100 µg	500 µg	1000 µg	3000 µg
0.321	0.422	0.525	0.624	0.652	0.637	0.731
0.429	0.591	0.521	0.509	0.727	0.544	0.619
0.368	0.523	0.504	0.476	0.539	0.575	0.543

Table 8. Effect of aqueous extract (AEDC) on HepG2 after 48 h by WST-1 test

Extract conc. cell no	0	50 µg	100 µg	500 µg	1000 µg	3000 µg
0.321	0.422	0.525	0.624	0.652	0.637	0.731
0.429	0.591	0.521	0.509	0.727	0.544	0.619
0.368	0.523	0.504	0.476	0.539	0.575	0.543

Table 9. Effect of aqueous extract (AEDC) on HepG2 after 72 h by WST-1 test

Extract Conc. Cell No	0	50 µg	100 µg	500 µg	1000 µg	3000 µg
0.429	0.782	0.837	1.118	0.893	1.008	0.847
0.423	1.037	0.792	0.809	0.920	0.735	0.814
0.422	0.820	0.946	0.700	0.739	0.764	1.036

Table 10. Effect of methanol extract (MEDC) on HepG2 after 24 h by WST-1 test

Extract Conc. Cell No	0	50 µg	100 µg	500 µg	1000 µg	3000 µg
0.373	1.513	1.255	1.308	1.241	1.190	1.419
0.395	1.102	0.949	1.225	1.147	1.174	1.215
0.402	1.001	1.028	1.269	1.186	0.708	1.139

Table 11. Effect of methanol extract (MEDC) on HepG2 after 48 h by WST-1 test

Extract Conc. Cell No	0	50 µg	100 µg	500 µg	1000 µg	3000 µg
0.321	0.422	0.675	0.899	1.101	1.717	Out
0.429	0.591	0.688	0.620	1.100	1.648	Out
0.368	0.523	0.719	0.712	1.206	1.800	Out

Table 12. Effect of methanol extract (MEDC) on HepG2 after 27 h by WST-1 test

Extract Conc. Cell No	0	50 µg	100 µg	500 µg	1000 µg	3000 µg
0.429	0.782	0.913	0.986	1.427	1.794	Out
0.423	1.037	1.004	1.029	1.427	1.957	Out
0.422	0.820	1.146	1.055	1.342	1.819	Out

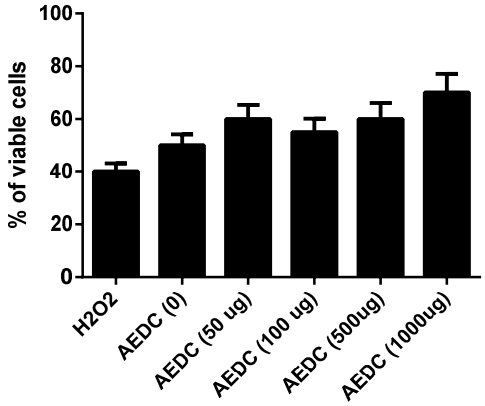


Fig. 1. AEDC /HCT11624H

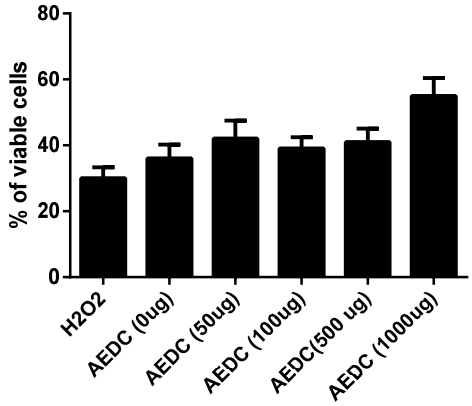


Fig. 3. AEDC/HCT116- 72hours

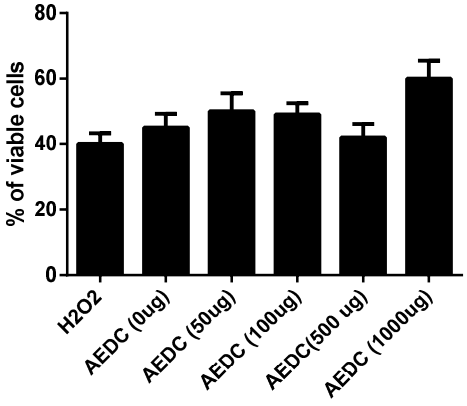


Fig. 2. AEDC/HCT116- 48hours

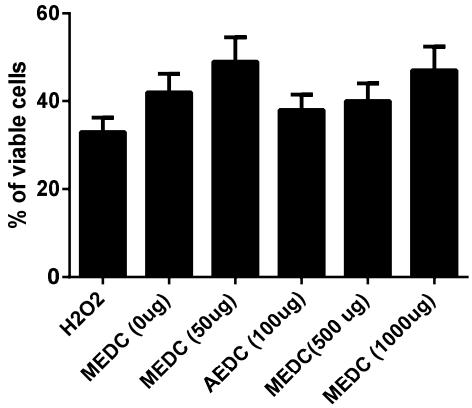


Fig. 4. MEDC/HCT116- 24hours

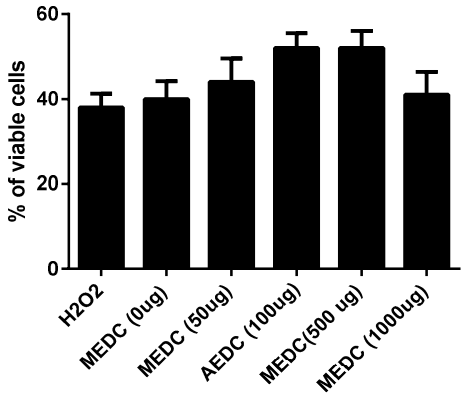


Fig. 5. MEDC/HCT116- 48hours

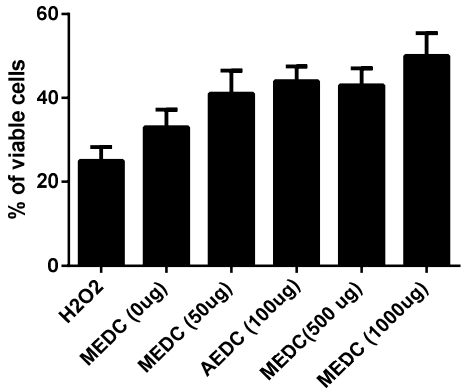


Fig. 6. MEDC/HCT116- 72hours

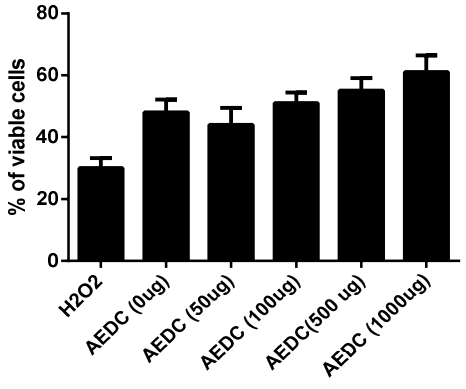


Fig. 7. AEDC/HepG2- 24hours

Apoptotic cells can be distinguished from the normal cells by the distinguished modifications and features they exhibit like DNA fragmentation. Caspases are a group of proteins which play a major role in the initiation and execution of apoptotic pathways. They contain a key cysteine residue in the catalytic site and selectively cleave proteins at sites just C-terminal of aspartate residues.There are nearly 13 caspases known to be discovered and widely studied and can be detected easily by simple assays. Generally monoclonal or polyclonal antibodies are used against caspases and pro-caspases. Results obatained in Table 13 showed apoptotic activity of AEDC or MEDC that showed increases activity of caspase 3 and 9 which included in apoptotic activity via release of cytochrome C from mitochondria and increased programmed cell death.

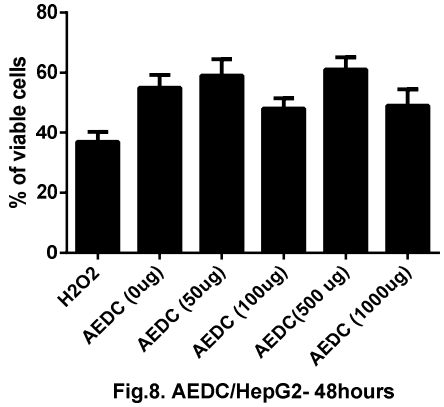


Fig.8. AEDC/HepG2- 48hours

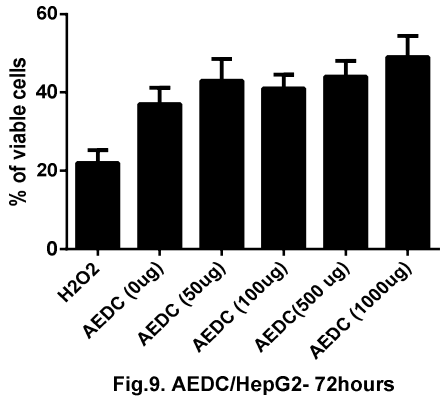


Fig.9. AEDC/HepG2- 72hours

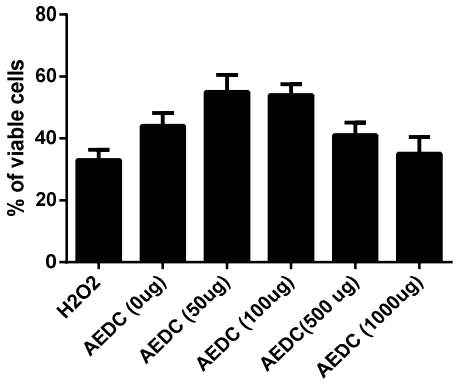


Fig.10. MEDC/HepG2- 24hours

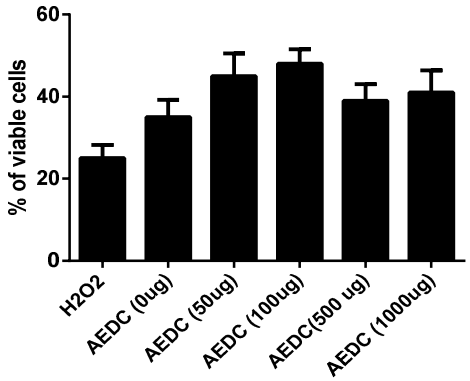


Fig.11. MEDC/HepG2- 48hours

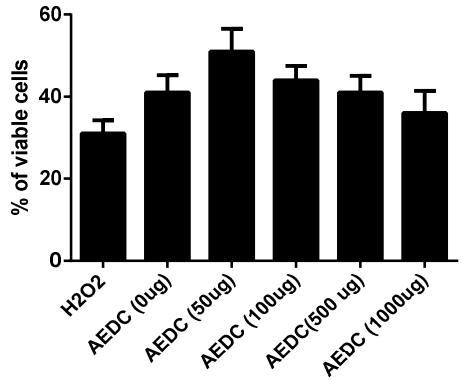


Fig.12. MEDC/HepG2- 72hours

Table 13. The activities of caspase-3 and caspase-9 (µU/ml) in HepG2 cell line .P ≤ 0.05 was considered significant (Mean ± SD)

Parameter	Control (untreated)	MEDC (0.05 mg/ml)	AEDC (1.0 mg/ml)
Caspase-3	0.15 ± 0.03	0.75 ± 0.09[a]	0.85 ± 0.06[a,b]
Caspase-9	0.27 ± 0.03	0.79 ± 0.09[a,b]	0.69 ± 0.06[a,b]

4. CONCLUSION

Data obtained revealed that, dose and duration of AEDC or MEDC for antitumor activity was dependent. They also provided information about the molecules responsible for activity of apoptosis and programmed cell death and their mechanisms of action, paving thus the way to develop new herbal medicines and forming the foundation for future studies.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard ethical approval has been collected and preserved by the authors.

ACKNOWLEDGMENT

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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