Original Research Article

In vitro study the impact of algae extracts as anti-proliferative against cancer cell line

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:** Aqueous and methanol extracts of *Dictyota ciliolate* (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, 1mg/ml and positive control 0.3% H2O2 at doses 0.5 mg/ml for 24,48 and 72h (for two cell lines). **Results**: AEDC showed the most effective antitumor activityagianst HCT116 and HePG2, the IC50 dose for HCT116 cells was 0.05 mg/ml at 72 hrs, while for the HePG2 it was 0.01 mg/ml at 72 hrs. 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. **Conclusıon:** the antitumor effect of AEDC or MEDC is promising for development of chemotherapeutic agent as effective with no site effects.

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

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 is a broad term used for identifying a large number of diseases. A normal cell suddenly turn 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 [7].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 [8].

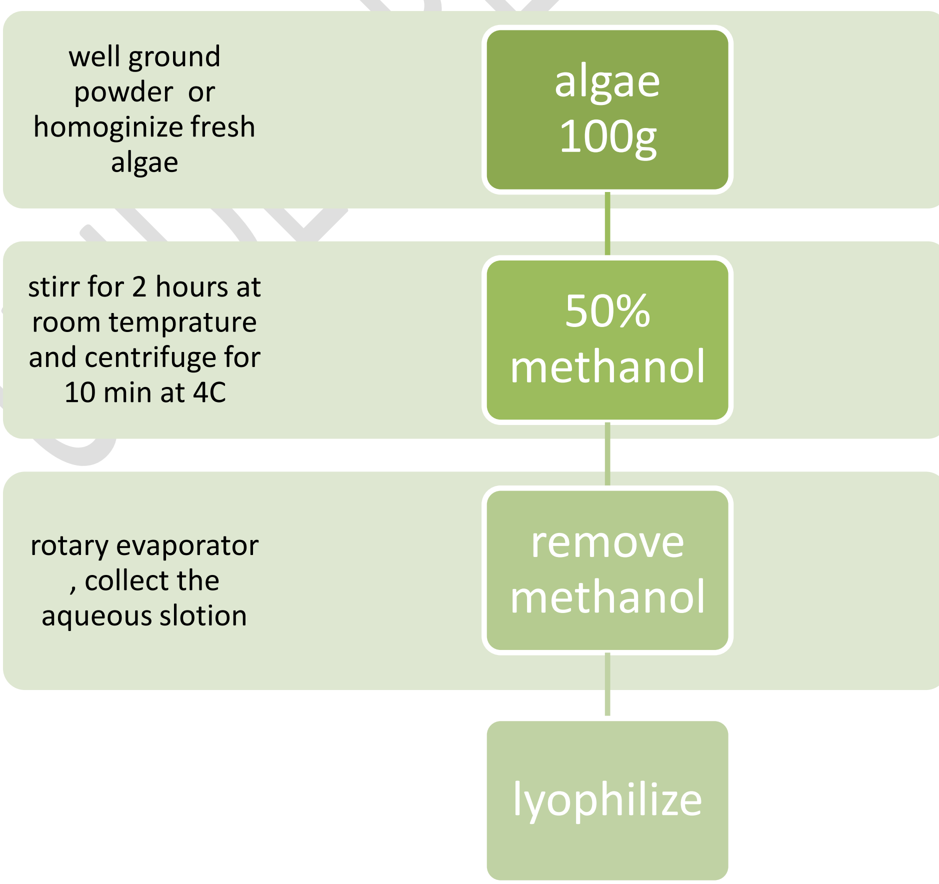
Natural products from marine sources offer abundance for drug development to treat cancer. Marine algae have provided a rich source of functional active compounds for applications in medical, cosmetic and fields [9]. Molecules made from algae show different bioactivities, like immunomodulation, antibacterial, anticancer and are well documented for their use in an ethno-pharmacological context. Red Sea shore is rich with marine microorganisms such as green and blue algae 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 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 [10].

Seaweeds identified as a sources 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 [11]. This study tested in *vitro* anticancer activity of algae extract (seaweeds) as natural products replaceable treatment to avoid the side effects of traditional drugs.

Materials and methods

Preparation of aqueous and methanol extracts of *Dictyota ciliolate*

Nontoxic algae samples collected from the Red sea in Jeddah *Dictyota ciliolate* (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 1L 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 were 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.



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 37C in a humidified incubator having 5% CO2.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 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 resuspended in 5 ml of complete DMEM.

Determination the antiproliverative 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 made 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_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 microplate reader (ELISA). In plate (48,72h) the media with treatments were removed and replaced with fresh media and daily added the doses.

Results and Discussion

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 [6].

Free radicals were considered as 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 .It is believed that oxidative stress is an important contributor to cancer and inflammation development. Micro-algal anti-inflammatory activity has ability to block the release of tumor necrosis factor α (TNFα), (one of the main effect 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, 1mg/ml and positive control 0.3% H2O2 at doses 0.5 mg/ml for 24,48 and 24h was 90, 72.4, 61.27, 62.89 and 88.63%, respectively. While after 48 hrs were 103.72, 78.77, 71.23, 65.12 and 76.15%, respectively. After 72 hrs 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% H2O2 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 Rhodophytae), brown algae (Pheaophytae) and green algae (Chlorophytae) 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 represents a renewable resources that remain unexplored in drug discovery [16].

Today, above 60% of commercially available anticancer drugs are natural origin. Doxorubicin, vincristine, mytomicin 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 micronutereints [21-25].

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

Table (1). Effect of aqueous extract (AEDC) on HCT116 after 24H 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

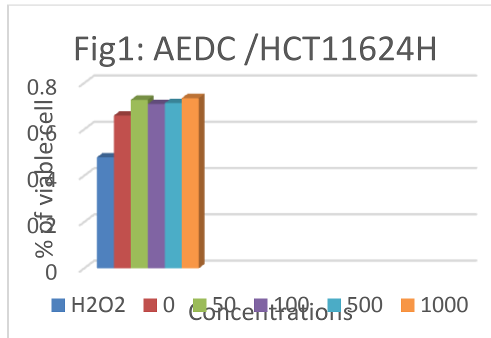


Table (2): Table (2). Effect of AEDC on HCT116   after 48 Hrs by WST-1 test.

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

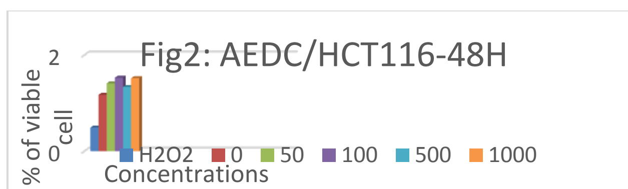


Table (3). Effect of AEDC on HCT116   after 72Hrs by WST-1 test.

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

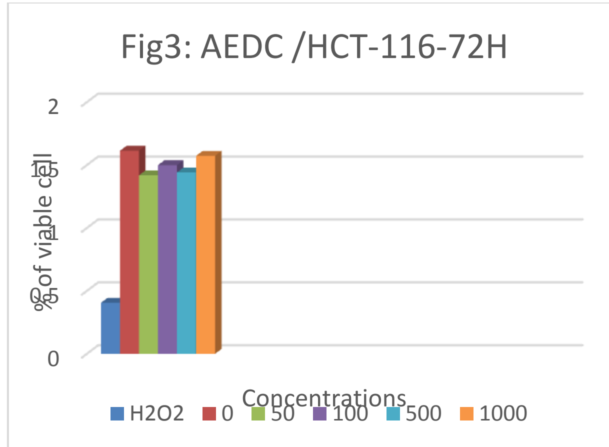


Table (4). Effect of methanol extarct (MEDC) on HCT116 after 24H by WST-1 test.

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

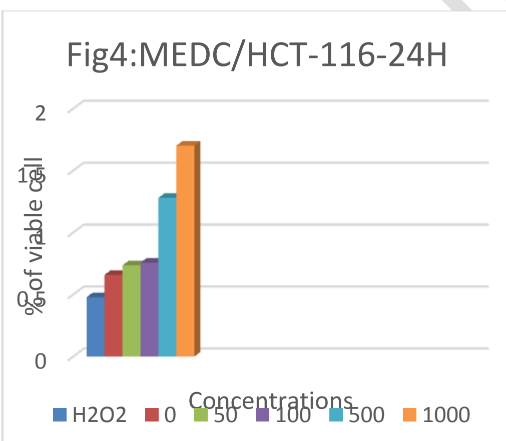


Table (5). Effect of methanol extarct (MEDC) on HCT116 after 48H by WST-1 test.

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

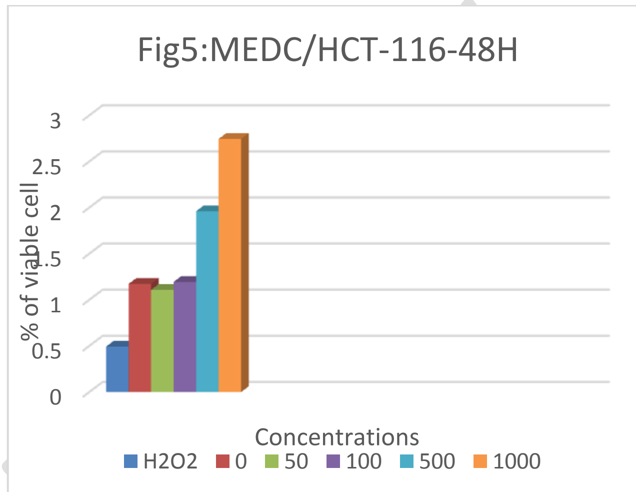


Table (6). Effect of methanol extarct (MEDC) on HCT116 after 72H by WST-1 test.

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

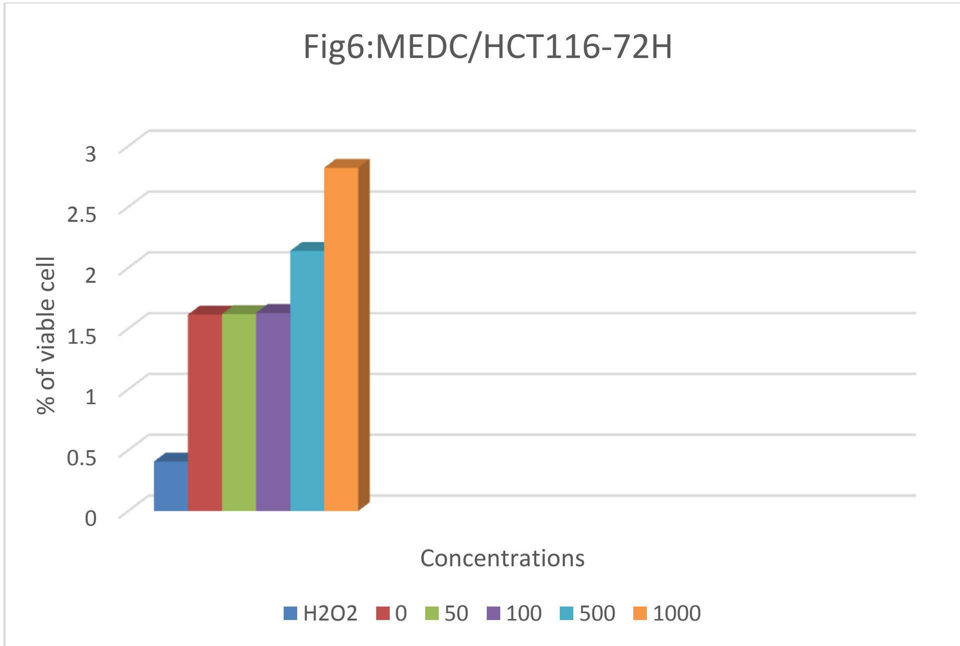


Fig6:MEDC/HCT116-72H

Table (7). Effect of aqueous extract (AEDC) on HepG2 after 24H 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

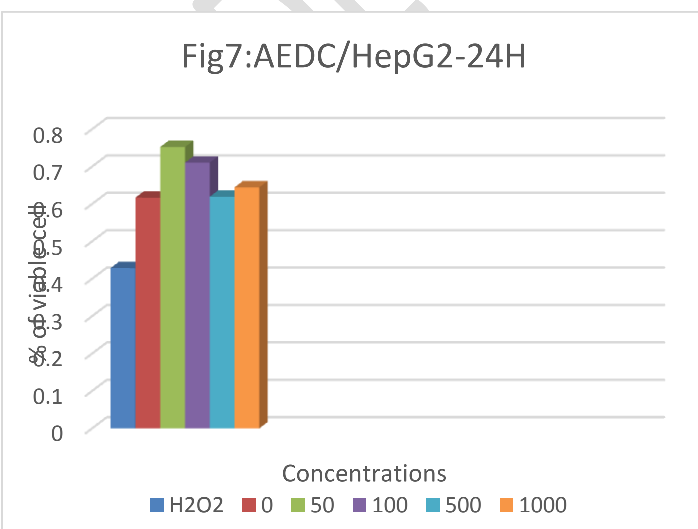


Fig7:AEDC/HepG2-24H

Table (8). Effect of aqueous extract (AEDC) on HepG2 after 48H 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

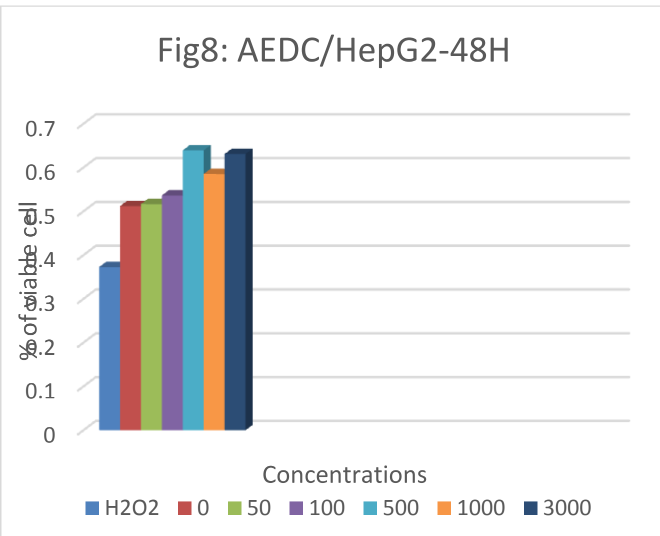


Table (9). Effect of aqueous extract (AEDC) on HepG2 after 48H by WST-1 test.

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

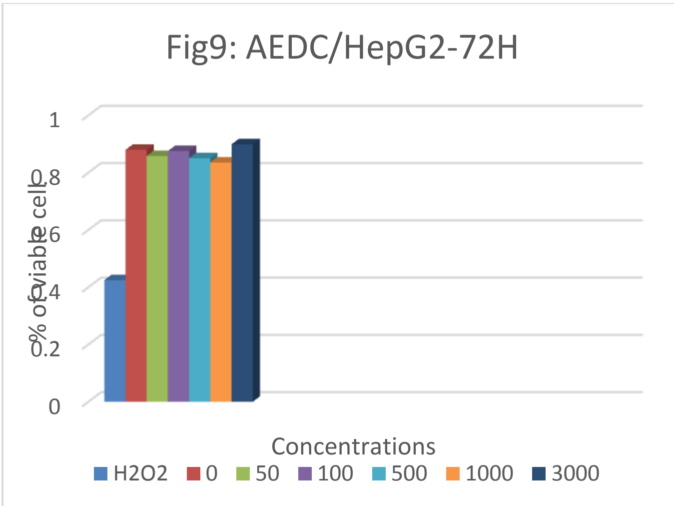


Table (9). Effect of aqueous extract (AEDC) on HepG2 after 72H by WST-1 test.

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

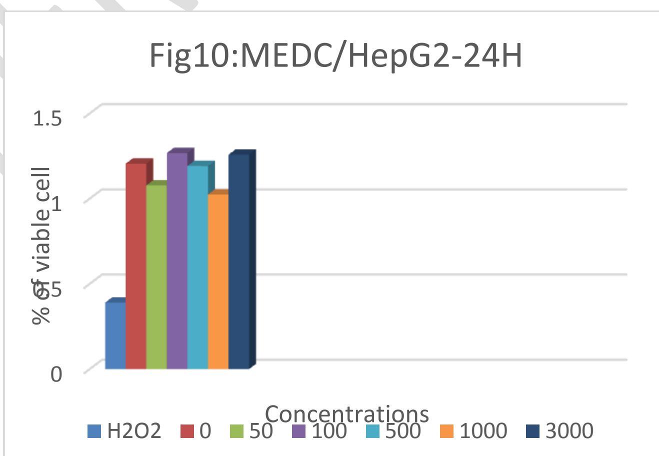


Table (10). Effect of methanol extract (MEDC) on HepG2 after 24H by WST-1 test.

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

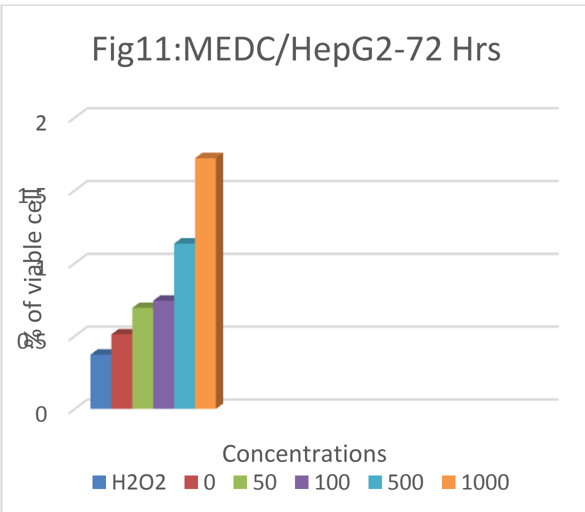
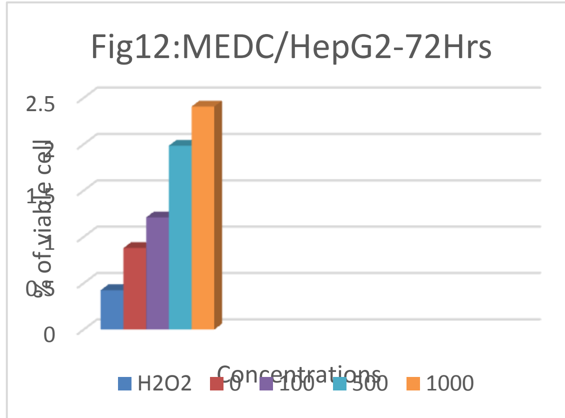


Table (11). Effect of methanol extract (MEDC) on HepG2 after 48H by WST-1 test.

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

Table (12). Effect of methanol extract (MEDC) on HepG2 after 48H by WST-1 test.

Extract Conc. Cell No	0	50 µg	100 µg	500 µg	1000 µg	3000 µg
0.429	0.782	1.267	1.131	2.300	2.429	Out
0.423	1.037	1.313	1.219	1.840	2.368	Out
0.422	0.820	1.407	1.279	1.811	2.422	Out



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