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# SCREENING OF SOME MARINE ALGAE FOR ANTITUMOR ACTIVITIES

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**ABSTRACT:** *The methanol extract of five marine macroalgae belonging to the divisions, (Rhodophyta, Chlorophta, and Phaeophyta) was evaluated for its anti-tumor activity against Ehrlich Ascites Carcinoma (EAC) bearing Swiss albino mice. short-term toxicity studies were performed initially in order to determine the extract with high antitumor potential invitro,then the Acute toxicity studies were performed to ascertain the safety of methanolic extract of Enteromorpha compressa.The protocol started with tumor inoculation of 0.1ml of($1x10^6$ EAC cells /ml) subcutaneously into the right thigh of the lower limb of the mice. After 24 hrs of tumor inoculation, methanolic crude extracts were administered intraperitoneally at a dose of 250 and 500mg/kg body weight of extracts and 1ml/kg body weight of Dimethyl sulfoxide 5% (DMSO). After treatment of 12 consecutive doses three days a week and after 24-hours of last dose and 18-hours fasting, the animals in each group were weighed and sacrificed. The effect of methanolic crude extract of Enteromorpha compressa on the growth of transplantable Ehrlich solid tumor was assessed, also the alterations in the hematological profile, Further, the effect of methanolic crude extract of Enteromorpha compressa on lipid peroxidation (LPO),glutathione peroxidase (GPx), antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) activities were measured from hepatic tissues., Treatment with methanolic extract of Enteromorpha compressa resulted in a significant reduction in the solid tumor volume percent (62.4% & 65% )for the lower and higher dose respectively,decreasing the levels of lipid peroxidation and increasing the levels of glutathione, superoxide dismutase and catalase. Therefore, from the present study it can be concluded that Enteromorpha compressa crude extract exhibited remarkable antitumor activity against Ehrlich Ascites Carcinoma in Swiss albino mice.*

***Key words:*** *Seaweeds; Crude extract; Antitumor activity; Ehrlich Ascites Carcinoma; Enteromorpha compressa.*

## INTRODUCTION

Seaweeds are floating and submerged plants of shallow marine meadows. They have salt tolerance because the osmolarity of cytoplasm is adjusted to match the osmolarity of the seawater so that desiccation does not occur. They lack true stems, roots and leaves; however, they possess a blade that is leaf like, a stripe that is stem like, and a holdfast that resembles roots like terrestrial plants. Seaweeds contain photosynthetic pigments and use sunlight to produce food and oxygen from carbon dioxide, and the water (Babuselvam and Ravikumar., 2011) Marine algae or seaweeds are excellent source of bioactive natural products so it has been studied as potential biocidal and pharmaceutical agents (Seenivasan *et al.,* 2012). Commercially available varieties of marine macro algae are commonly referred to as seaweeds. Macro algae can be classified as red algae (rhodophyta), brown algae (phaeophyta) or green algae (chlorophyta) depending on their nutrient and chemical composition. Red and brown algae are mainly used as human food sources.

Most of the bioactive substances isolated from marine algae are chemically classified as brominated, aromatics, nitrogen-heterocyclic, nitrosulphuric-heterocyclic, sterols, dibutanoids, proteins, peptides and sulphated polysaccharides (Kolanjinathan *et al.,* 2009).

Research on the natural products chemistry and chemical defenses of algae

over the past 40 years has resulted in the isolation of over 15,000 novel compounds, many of which have been shown to have bioactive properties (Blunt *et al.,*2011) and (Cardozo *et al.,*2007).Seaweeds have recently received significant attention for their potential as natural antioxidants, aiming to the discovery of compounds and/or extracts that can counteract free Radical-induced and other oxidative stress processes, and so doing decrease the incidence of human diseases directly related to these processes

(Vijayavel and Martinez, 2010).Currently, algae represent about 9% of biomedical compounds obtained from the sea (Jha and Zi-rong, 2004).Compounds with cytostatic, antiviral, antihelmintic, antifungal and antibacterial activities have been detected in green, brown and red algae .(Newman *et al.,*2003). From this viewpoint the present study was carried out to evaluate the antitumor activity and antioxidant status of 70% methanol extract of *Enteromorpha compressa* against Ehrlich ascites carcinoma (EAC) in Swiss albino mice.

## MATERIALS AND METHODS:

### 1. Plant material and extract

The algae "seaweeds" were harvested in the spring of 2010 from an exposed rocky side near the western edge of Abu-Qir Bay, Alexandria, Egypt. Botanical identification was done using standard literature and taxonomic keys, all seaweeds were identified according to Abbot and Hollenberg (1976) ,Taylor (1985) and Aleem (1993).as shown in table (1)

Algal samples were cleaned of epiphytes and extraneous matter, and necrotic parts were removed. Algal samples were washed successively with tap water; distilled water and air dried under shade for 2 weeks.The air dried samples were cut into small pieces and powdered in a mixer grinder. The extraction was carried out using methanol (70%) (1:15, w/v) on a rotary shaker at 150 rpm at room temperature (25 - 30°C) for 72 h.The extracts from three consecutive soakings were pooled and filtered using filter paper (Whatman No 4). The obtained filtrate was freed from solvent by evaporation under reduced pressure. The residues (crude extracts) obtained were then weighed and kept at –20 °C in airtight bottle.

### 2. Preparation of suspensions and solutions

The algal extracts was suspended in distilled water using dimethyl sulfoxide (DMSO, 5%) in different concentrations; (500, 1000, 1500, 2000and4000µg/ml) for *in vitro* cytotoxicity assay.

### 3. *In vitro* antitumor activity:

Short term cytotoxicity assay of the algal methanolic crude extracts was determined by using trypan blue dye exclusion method (Sunila and Kuttan,2004) Ehrlich Ascites Carcinoma (EAC) was inculated in peritoneal cavity of healthy albino mice by injecting a suspension of tumor cells (1 x $10^6$ cells/ml) intraperitoneally. The cells were aspirated aseptically from the peritoneal cavity of the mice on day 15, washed with normal saline and centrifuged for 15 min at 1500 rpm in a cooling centrifuge.

**Table (1). The collected and identified species of seaweeds are:**

| No | Organism | Division |
|---|---|---|
| 1 | *Enteromorpha compressa* | *Chlorophyta* |
| 2 | *Ulva lactuca* | *Chlorophyta* |
| 3 | *Sargassum vulgare* | *Phaeophyta* |
| 4 | *corallina elongata* | *Rhodophyta* |
| 5 | *jania rubens* | *Rhodophyta* |

The pellet was resuspended with normal saline and the process was repeated until to get a clear supernatant. The EAC cells were suspended in a known quantity of normal saline and the cell count was adjusted to 1 x $10^6$ cells/ml. Then, 0.1 ml of this cell suspension was distributed in to Eppendorf tubes and exposed to 0.1 ml of various concentrations of the crude extracts (500, 1000, 1500, 2000and4000µg/ml) and incubated at 37 ºC for 3 h. After 3 h, the trypan blue dye exclusion test was performed to determine the percentage cytotoxicity then the most effective extract was chosen.

## 4. Phytochemical analysis

The crude methanolic extract of *Enteromorpha compressa* was screened for the presence of various phytochemical constituents employing standard screening tests (Wagner *et al.,*1984).conventional protocol for detecting the presence of alkaloids, steroids, tannins, flavonoids, glycosides..,etc was used.

## 5. *In vivo* cytotoxicity activity

The acute toxicity study was conducted using the method of Lorke ,(1983) to determine the median lethal dose value (LD50) of the methanolic extract of *Enteromorpha compressa* in swiss albino mice. This method was carried out by a single intraperitoneal injection in twenty five animals (4 in each group) at different doses (1000, 1500, 2000, 2250 and 2500 mg/kg body weight). The LD50 was evaluated by recording mortality after 24 hours, and about $1/10^{th}$&$1/5^{th}$ of the LD50 dose has been considered for the anticancer activity.

## 6. *In vivo* antitumor activity

### 6.1. Animals

Forty female mice obtained from National Research Center, EL-Doky, Cairo, Egypt were used. With all experiments all mice were between 8 and 12 weeks old, weighing about 20-25 g and these animals were maintained at the animal house at the Genetic Engineering and Biotechnology Institute, Minufiya University. Mice were housed at 23 ± 2°C and in daily dark/light cycle. They were maintained under standard condition and fed standard chow and water ad libitum (free access to water and food).

### 6.2.Cell line:

Cell line of Ehrlich's Ascites Carcinoma (EAC) obtained from National Cancer Institute, Cairo University, Egypt. The EAC cell line was maintained in the peritoneal cavity by weekly serial intraperitoneal transplantation of $1.0x10^6$ cells in adult female mice weighing 18 to 20 gm.

### 6.3.Design of experimental animals

The Ehrlich ascites tumor (EAC) cells (1 x$10^6$ cells/mouse) were subcutaneously implanted into the right thigh of the lower limb of the mice as described by *Sugiura et al*, (1955) mice were divided into the respective groups and treatment started after 24 hours of tumor cells implantation. Each group was treated differently according to Mazumder *et al,* (1997) as the following;

### Group I (Normal control):

Six mice served as normal controls and were given only standard pellet diet and tap water.

### Group II (negative control):

Six mice received 5% DMSO dissolved in saline solution 0.9%, (5ml/kg/body weight, intraperitoneal.),

### Group III (Positive control):

Six mice inoculated with EAC ($1x10^6$ cells/mouse subcutaneously) +5% DMSO (5ml/kg/body weight, intraperitoneal.),

### Group IV:

Six mice inoculated with EAC ($1x10^6$ cells/mouse subcutaneously) and treated with the algal crude extract (250mg/kg/body weight, intraperitoneal.),

### Group V:

Six mice inoculated with EAC ($1x10^6$ cells/mouse subcutaneously) and treated with the algal crude extract (500mg/kg /body weight, intraperitoneal.).

The treatment started after 24hrs of tumor inoculation and animals were treated during 30 days, Tumor mass was measured from the 15$^{th}$ day of tumor induction and measured every 5 days using Vanier caliper.Tumor mass volume was calculated according to the formula $V=4/3\pi r^2$, where r is the mean of $r_1$, $r_2$ which are two independent radii of tumor mass (Kuttan *et al.,* 1990). At the end of the experimental period, mice were over night fasted, anaesthetized under diethyl ether (Sigma Co. USA) and blood was collected by cardiac puncture where blood was collected in dry clean tubes, the serum was separated, and tumors ,livers and kidneys were removed for further analysis.

## 7. Biochemical parameters

Blood was collected by cardiac puncture; blood serum was separated and used for the estimation of serum enzymes, Serum Glutamic Pyruvic Transaminase (SGPT) & serum glutamic oxaloacetic transaminase (SGOT) using commercial kits. Red blood cells"RBC", White blood cells"WBC" counts and hemoglobin levels were determined by routine clinical laboratory techniques. Differential leukocyte count"DLC" was carried out from leishman stained blood smears (Dacie and Lewis,1958)

## 8. Liver oxidative stress and antioxidants

Immediately after sacrificing the animals, Liver was isolated then, washed in ice cold normal saline to remove blood, bottled dry and stored at -20ºc for further analysis. Liver was crushed in tissue homogenizer and 10% w/v.Liver homogenate was prepared in 0.05M phosphate buffer "pH 7.4"and was used for the estimation of super oxide dismutase "SOD"(Nishikimi *et al.,*1972), catalase"CAT"(Aebi, 1984), ( Fossati, 1980), glutathione peroxidase"GPx" ( Paglia and Valentine, 1967) , and malon dialdehyde "MDA" ( Satoh, 1978). using a commercial kit,(Bio-diagnostic Company, Egypt).

## 9. Histopathological examination

After the dissection, tumors, livers, and kidneys were fixed in formaldehyde 10% and examined grossly for size, color and hemorrhage. A portion of tumor, liver and kidneys were cut into small pieces for histological analysis. Sections "5µm" were prepared and stained with hematoxylin and eosin. Histological analysis was performed under light microscopy (Culling, 1974).

## RESUITS

In the present study, tumor was induced in female mice by using the cell line of Ehrlich's Ascites tumor. The effect of *Enteromorpha compressa* crude extract was tested by its subcutaneous injection for a month. Biochemical and histological parameters were used to assess the protective and treatment effect of the crude extract.

The short term cytotoxicity assay that was carried out on the algal methanolic crude extracts using trypan blue dye exclusion method showed that the most active antitumor crude extract in vitro is that of *Enteromorpha compressa*. Another short term cytotoxicity assay that was carried out on the *Enteromorpha compressa* methanolic crude extract using trypan blue dye exclusion method in different dose concentration (500, 1000, 1500, 2000 and 4000µg/ml) showed that the activity of the extract as antitumor agent on EAC cells in vitro is a dose concentration dependant as shown in table(2) & Fig (1).

## The effect of *Enteromorpha compressa* methanolic extract on hematological parameters

As shown in table 3a and 3b the hemoglobin content in the tumor control mice was significantly decreased to 9.4±0.9 (g%) in comparison with normal mice 12.88±1.5 (g%).Treatment of EAC bearing mice with Enteromorpha methanolic extract at the dose of 250 and 500 mg/Kg increased the hemoglobin content to 11.8±1.0 and 12.4±1.0 respectively.

Moderate change in the RBC count was observed for the extract treated mice. The total WBC count was significantly higher in the treatment groups as compared to that of normal. In differential leukocytes, the percentage of neutrophils was increased while the lymphocyte count was decreased in the in the extract treated group.

## The effect of *Enteromorpha compressa* methanolic extract on different biochemical parameters

As shown in table 3a, the inoculation of Ehrlich Ascites Carcinoma (EAC) caused significant increase in the levels of SGOT, SGPT in the serum of tumor control animals when compared to the normal group.The treatment with *Enteromorpha* methanolic extract at doses of 250&500 mg/Kg reversed these changes towards normal levels.

## The effect of *Enteromorpha compressa* methanolic extract on liver lipid peroxidation and antioxidants

As shown in Table 4, the levels of lipid peroxidation in liver tissue were significantly increased in EAC control group (2.92±0.04 n.moles of MDA/gm tissue) as compared to the normal group (1.9±0.03 n.moles of MDA/gm tissue), where the Glutathione peroxidase levels in the liver tissue were significantly decreased in tumor control group (2.9±0.12 U/gm tissue) as compared to the normal group (3.42±0.03 U/gm tissue).

**Table (2). In vitro cytotoxic activity of the *Enteromorpha compressa* methanolic crude extract**

| EAC cells | Concentration (µg/ml) | %Death |
|---|---|---|
| ($1\times10^6$cells/ml) | Control | 1% |
| ($1\times10^6$cells/ml) | 500 | 10% |
| ($1\times10^6$cells/ml) | 1000 | 50% |
| ($1\times10^6$cells/ml) | 1500 | 85% |
| ($1\times10^6$cells/ml) | 2000 | 94% |
| ($1\times10^6$cells/ml) | 4000 | 99% |

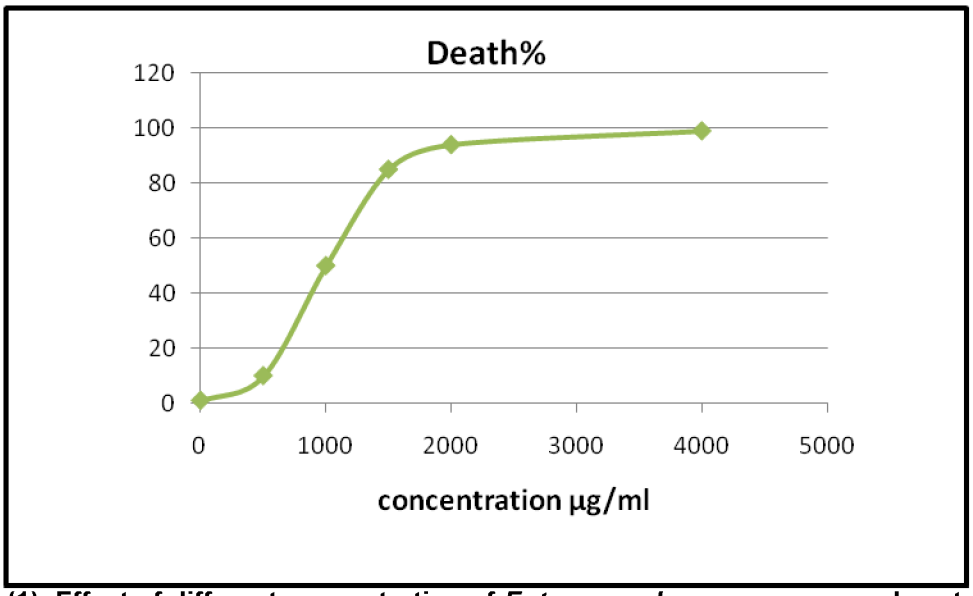



**Figure (1). Effect of different concentration of *Enteromorpha compressa* crude extract on EAC cell line invitro.**

**Table (3a). Effects of methanolic crude extract of *Enteromorpha compressa* on hematological parameters of EAC treated mice.**

| Prameters | Normal control | DMSO (5ml/kg) | EAC ($1\times10^6$cells) +5%DMSO | EAC ($1\times10^6$cells)+ 250 mg dose. | EAC ($1\times10^6$cells)+ 500 mg dose. |
|---|---|---|---|---|---|
| Hemoglobin g% | $12.88\pm1.5^a$ | 13.2±1.4 | $9.4\pm0.9^b$ | $11.8\pm1.0^{a,b}$ | $12.4\pm1.0^{a,b}$ |
| Total RBCs (Cells/ml $\times10^9$) | $5.4\pm0.5^a$ | 5.88±0.6 | $3.65\pm0.4^b$ | $4.32\pm0.2^{a,b}$ | $4.78\pm0.4^{a,b}$ |
| Total WBCs (Cells/ml $\times10^6$) | $9.8\pm2.8^a$ | $11.2\pm3.0^b$ | $23\pm3.1^b$ | $21.2\pm6.6^{a,b}$ | $19.0\pm0.4^{a,b}$ |
| SGOT(U/L) | $93.33\pm1.5^a$ | $94.6\pm1.7^b$ | $121.33\pm2.3^b$ | $112.0\pm1.2^{a,b}$ | $102.0\pm0.9^{a,b}$ |
| SGPT(U/L) | $111.66\pm2.7^a$ | $115.9\pm3.0^b$ | $210.83\pm1.9^b$ | $169.0\pm2.3^{a,b}$ | $146.30\pm1.9^{a,b}$ |

**Table (3b). Differential**

| Parameters | Normal control | DMSO (5ml/kg) | EAC ($1\times10^6$cells) +5%DMSO | EAC ($1\times10^6$cells)+ 250 mg dose. | EAC ($1\times10^6$cells)+ 500 mg dose. |
|---|---|---|---|---|---|
| Lymphocytes % | $73.6\pm4.9^a$ | 71.4±4.8 | $33.5\pm5.0^a$ | $43.6\pm5.6^{a,b}$ | $59.8\pm5.2^{a,b}$ |
| Monocytes % | 1.83±1.0 | 2.1±1.2 | 1.4±1.0 | 1.1±0.6 | 1.2±0.8 |
| Neutrophil % | $24.8\pm4.0^a$ | 26.6±4.2 | $63.3\pm5.4^b$ | $41.4\pm5.9^{a,b}$ | $36.9\pm4.9^{a,b}$ |
| Eosinophil % | 0.66±1.0 | 0.6±0.9 | 1.6±1.2 | 0.6±0.8 | 0.6±0.8 |

*Experimental groups were compared with EAC control group.
Values are expressed as Mean±SD of 6 mice per group.
(a)-significant as compared to Ehrlich ascites (EAC) group at ($P < 0.01$).
(b)-significant as compared to normal control group at ($P < 0.05$)

**Table (4). Effects of different doses of methanolic crude extract of *Enteromorpha compressa* on liver lipid peroxidation and antioxidants of EAC treated mice**

| Parameters | Normal control | DMSO (5ml/kg) | EAC ($1\times10^6$cells) +5%DMSO | EAC ($1\times10^6$cells)+250 mg dose. | EAC ($1\times10^6$cells)+ 500 mg dose. |
|---|---|---|---|---|---|
| MDA (n.moles /gm tissue) | $1.9\pm0.03^a$ | 1.62±0.03 | $2.92\pm0.04^b$ | $1.83\pm0.01^{a,\square}$ | $1.98\pm0.01^{a,b}$ |
| Glutathione Peroxidase (U/gm tissue) | $3.42\pm0.03^a$ | 3.2±0.02 | $2.9\pm0.12^b$ | $3.89\pm0.21^{a,b}$ | $3.12\pm0.03^{a,b}$ |
| SOD (U/gm tissue) | $5.32\pm0.43^a$ | 4.49±0.40 | $3.82\pm4.49^a$ | $4.01\pm0.33^{a,b}$ | $4.85\pm0.01^{a,b}$ |
| CAT (U/gm tissue) | $28.46\pm1.91^a$ | 26.4±1.90 | $10.8\pm0.11^a$ | $19.89\pm1.17^{a,b}$ | $24.98\pm0.01^{a,b}$ |

*Experimental groups were compared with EAC control group.
Values are expressed as Mean±SD of 6 mice per group.
(a)-significant as compared to Ehrlich ascites (EAC) group at ($P < 0.01$).
(b)-significant as compared to normal control group at ($P < 0.05$).

The levels of lipid peroxidation in liver tissue were decreased (1.83±0.01 &1.98±0.01 n.moles/gm tissue), where the Glutathione peroxidase levels were increased (3.89±0.21 &3.12±0.03 U/gm tissue) by the administration of *Enteromorpha* methanolic extract at doses of 250&500 mg/Kg respectively as compared to tumor control mice.

SOD levels in tumor bearing mice were significantly decreased to 3.82±4.49 U/gm tissue, in comparison with the normal mice 5.32±0.43 U/gm tissue. The administration of *Enteromorpha* methanolic extract significantly increased the levels of SOD to 4.01±0.33 U/gm tissue and 4.58±0.01 U/gm tissue at the doses of 250&500 mg/Kg respectively.

The CAT levels in tumor bearing mice were significantly decreased (10.8±0.11 U/gm tissue) in comparison with normal mice (28.46±1.91 U/gm tissue).

Where with the administration of *Enteromorpha* methanolic extract the CAT levels were significantly increased to (19.89±1.17 U/gm tissue) & (24.98±0.01 U/gm tissue) at the doses of 250&500 mg/Kg respectively.

## The effect of Enteromorpha methanolic extract on EAC induced solid tumor

In EAC control animals, the solid tumor volume was found to be drastically increased from day 0 to day 30, however with the administration of *Eenteromorpha* methanolic extract at doses of 250 & 500 mg/Kg, the solid tumor volume of treated animals was found to have lower growth rate when compared to EAC control mice as shown in table No5. About 62.4% & 75% reduction in solid tumor volume was observed for the lower and higher dose treatment of the extract respectively, on day 30 of the experiment. No toxic symptoms or deaths were observed in animals for both doses of the extract treatment.

## The effect of Enteromorpha methanolic extract on histpathology of internal organs

The liver histological sections (Fig.2),showed the branched anastomosed cords of hepatocytes radiating from the central vien (CV) and the cells are separated by sinusoids (S) lined by flat endothelial cells and Von kupffer cells (arrows) in normal control mice.

No prominent histological changes in DMSO mice group, Hypertrophy of liver cells, necrosis and hepatocytes with vacuolated cytoplasm with deeply stained pyknotic nuclei (arrows) in tumor control group , focal areas of necrosis (N), congested central vien (CV) and hepatocytes with vacuolated cytoplasm with deeply stained pyknotic nuclei (arrows) in 250mg dose mice group ,and a normal cellular architecture of hepatic lobules with weakly congested central vein in 500mg dose mice group.

**Table (5). Effect of *Enteromorpha compressa* methanolic crude extract on solid tumor volume.**

| Design of treatment | Solid tumor volume (ml) | | | | |
|---|---|---|---|---|---|
| | 15th day | 20th day | 25th day | 30th day | %Inhibition |
| Tumor control | $7.5 \pm 1.0^{b}$ | $9.0 \pm 1.0^{b}$ | $11.88 \pm 1.4^{b}$ | $13.66 \pm 1.5^{b}$ | - |
| 250 mg/kg. dose | $4.50 \pm 1.2^{a}$ | $4.53 \pm 1.3^{a}$ | $4.91 \pm 1.2^{a}$ | $5.01 \pm 1.0^{a}$ | 62.4 |
| 500 mg/kg. dose | $3.33 \pm 1.0^{a}$ | $3.38 \pm 1.0^{a}$ | $3.39 \pm 0.8^{a}$ | $3.42 \pm 0.9^{a}$ | 75 |

*Experimental groups were compared with EAC control group.
Values are expressed as Mean±SD of 6 mice per group.
(a)-significant as compared to Ehrlich ascites (EAC) group at ($P < 0.01$) .
(b)-significant as compared to the treatment. at ($P < 0.001$).

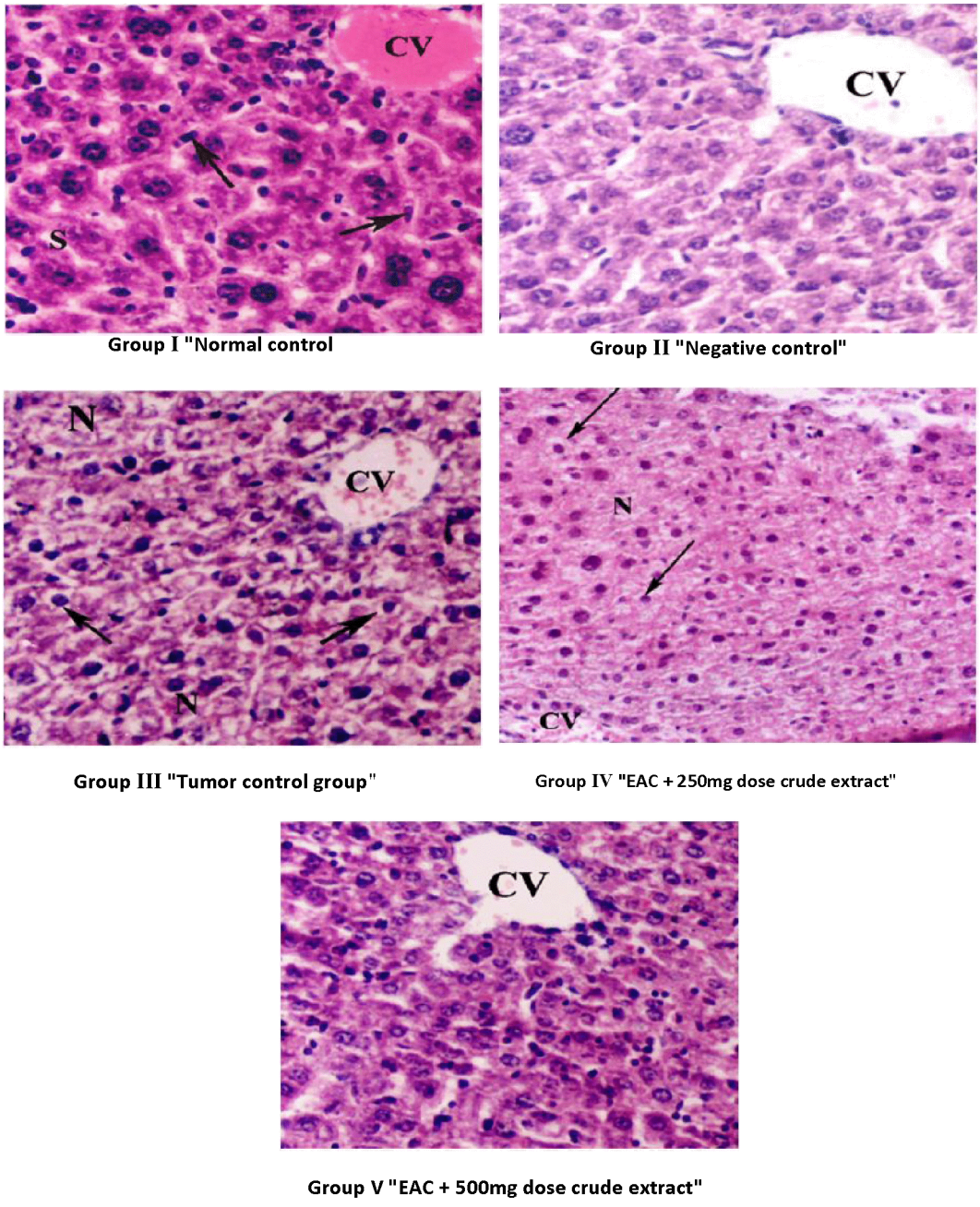



**Fig.2: Histological study of liver in different experimental groups.**

Where, the renal cortex histological sections (Fig.3), showed a normal architecture of renal capsule (C), proximal (P), and distal convoluted tubules in normal control mice group ,nearly the same histological profile as in normal control group with a mild interstitial congestion (arrow) in DMSO mice group , a large area of interstitial hemorrhage (h) and aglumerular atrophy (G) in tumor control mice group , aglumerular debris with ruptured Bowman's capsule (Bc) and congestion of blood vessels (arrows) in 250mg dose mice group , and nearly the same histological profile as in normal control group in 500mg dose mice group.

## Discussion

In recent years, marine resources have attracted attention in the search for bioactive compounds to develop new drugs and healthy foods In particular, seaweeds are a very important and commercially valuable resource for food, fodder, soil conditioners and pharmaceuticals. ( Kelman *et al.*,2012 ).

Cancer is a pathological state involving uncontrolled proliferation. The present work represents the antitumor activity of algal crude extract on mice transplanted with Ehrlich Ascites Carcinoma (EAC) which is a very rapidly growing carcinoma with very aggressive behavior (Ito *et al .,* 1987) and (Lee *et al.,* 2003). This model is mouse-originated tumors frequently used in antitumor related research in vivo (Segura *et al.,* 2000). In cancer chemotherapy, the major problems are myelosupression and anemia (Islam *et al.,* 2013). The anemia encountered in tumor bearing mice is mainly due to reduction in RBC or hemoglobin percentage and this may be due to iron deficiency or due to hemolytic or myelopathic conditions (Hirsch , 2006). Treatment with *Enteromorpha* methanolic crude extract reverted the hemoglobin content, RBC and WBC cell count near to normal values and also brought back the serum biochemical parameters towards normal "table 3a".this indicates that the extract possesses protective action on the haemopoietic system.

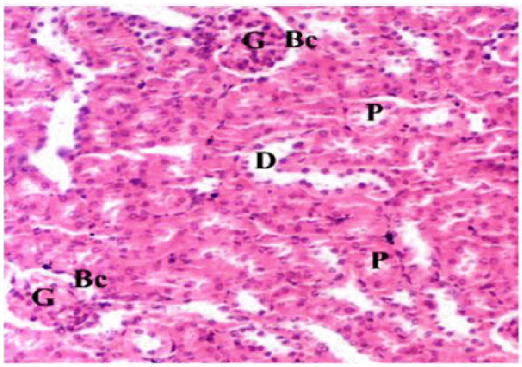
The free radical scavenging system, SOD and CAT are present in all oxygen metabolizing cells and their function is to provide a defense mechanism against the potentially damaging reactions of superoxide and hydrogen peroxide. High levels (up to 0.05µmol/h per $10^4$cells) of $H_2O_2$ are constitutively released from wide variety of human tumors (Devi *et al.,*2011).Excessive production of free radicals will result in oxidative stress, leading to damage of macromolecules such as lipids and can induce lipid peroxidation Increased lipid peroxidation causes degeneration of tissues and lipid peroxide formed in the primary site are transferred through circulation and can provoke further damage to the cells. (Gurunagarajan and Pemaiah, 2010).

SOD and CAT are involved in the clearance of superoxide and $H_2O_2$.

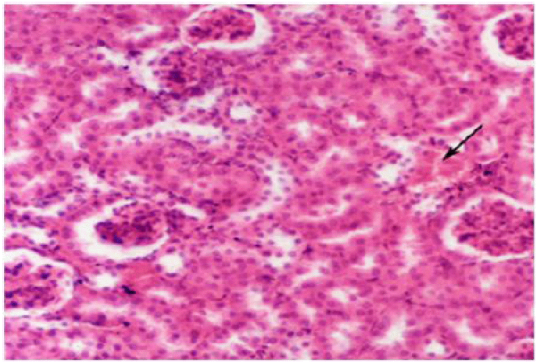
Decrease in SOD and CAT activities described in tumors is regarded as markers of malignant transformation. Lowered activities of SOD and CAT were reported in several cancers ( Kavitha *et al.,* 2006). The significant elevation of SOD and CAT by the crude extract treatment confirms the potent antioxidant activity of *Enteromorpha compressa* methanolic extract.The elevation of lipid peroxidation is known to be associated with cancer.

Malondialdehyde,(MDA),the end product of lipid peroxidation, was reported to be higher in cancer tissue than in normal tissues ( Muthuraman *et al.,* 2008). This indicates the reduction in free radical yield and subsequent decrease in harm and damage to the cell membrane and decrease in MDA production. In the present study, the histological examination of removed organs of Ehrlich Ascites Carcinoma inoculated animals showed marked changes indicating the toxic effect of this tumor.

The normalization of these effects observed in the tissues treated with *Enteromorpha* methanolic extract supports the potent hepatoprotective and antioxidants effects of the extract.




Group I "Normal control"



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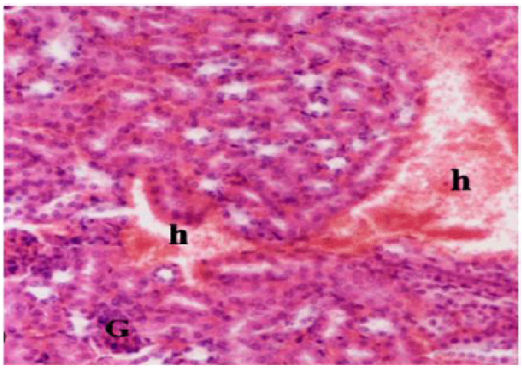
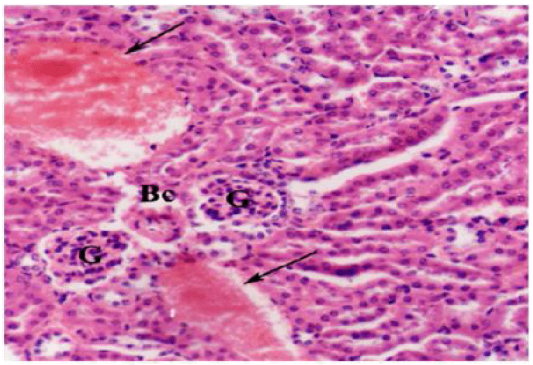
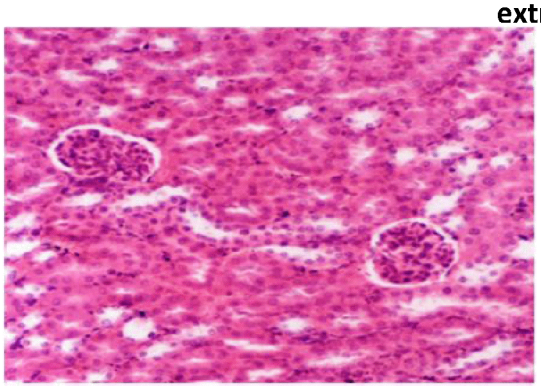



Group III "Tumor control"




Group IV "EAC + 250mg dose crude extract"



Group V "EAC + 500mg dose crude extract"

**Fig.3: Histological study of renal cortex in the different experimental groups:**

Preliminary phytochemical studies indicated the presence of flavonoids, alkaloids, saponins, steroids, and triterpenoids in *Enteromorpha compressa* methanolic crude extract . Many ~~such~~ compounds are known to possess potent antitumor properties. (Kintzios, 2006)

The extract of *Enteromorpha compressa* is rich in flavonoids and saponins. Flavonoids have been found to possess antimutagenic and antimalignant effect Moreover, they have a chemo preventive role in cancer through their effects on signal transduction in cell proliferation and

inhibition of neovascularization (Kumar *et al.,* 2011), Saponins have been found beneficial on inhibition of tumor angiogenesis by suppressing its inducer in the epithelial cells of blood vessels and then on adhering, invasion and metastasis of tumor cells. They also exhibit the antitumor effect by cell cycle arrest and apoptosis (Man *et al.,* 2010).The anti tumor properties of the extract may be due to the presence of these compounds.

## Conclusion

It is concluded that the methanol extract of the alga *Enteromorpha compresaa* at the doses of 250&500 mg/Kg was effective in inhibiting the growth of Ehrlich carcinoma, solid tumor model.The biochemical and histological studies supported it's antioxidant and hepatoprotective properties.The alga crude extract needs further investigation to elucidate it's mechanism of action and isolation of it's active constituents.

## REFERENCES


Abbott, I.A. and J. Hollenberg (1976). *Marine Algae of California*. Stanford,University Press. 827 pp.

Aebi, H., (1984). Catalase. In: L. Packer(Ed) methods in enzymology, Academic pres, Orlando, 105: 121-126.

Aleem, A.A. (1993). *The marine algae of Alexandria, Egypt.* Ed.Univ. of Alex.,Egypt , 138-155 .

Babuselvam, M. and S. Ravikumar (2011). Screening of Male Anti-Fertility Compounds from Marine Seaweed Macro Algae. Division of Marine Microbiology and Medicine, Manonmaniam Sundaranar University: Rajakkamangalam, India; pp. 1–14

Blunt, J.W., B.R. Copp, M.H. Munro, P.T. Northcote and M.R. Prinsep (2011). Marine natural products. *Nat. Prod. Rep.*,*28*, 196–268.

Cardozo, K.H.M., T. Guaratini, M.P. Barros, V.R. Falcão, A.P. Tonon, N.P. Lopes, S. Campos, M.A. Torres, A.O. Souza, P. Colepicolo and E. Pinto (2007). Metabolites from algae with economical impact. *Comp Biochem Physiol C-Toxicol Pharmacol 146*: 60-78.

Culling, C. F. A. (1974). Hand book of Histopathological and Histochemical Techniques (including museum techniques) 3rd ed. Pp.361.

Dacie, J.V. and S.M. Lewis (1958). Practical Hematology. 2nd ed. London: J and AChurchill; 38–48.

Devi, G. K., K. Manivannan, G. Thirumaran, F. A. A. Rajathi and P. Anantharaman (2011). In vitro antioxidant activities of selected seaweeds from Southeast coast of India, *Asian Pac J Trop Biomed*; 3(1): 205-211.

Faulkner, D.J. (2002). Marine natural products. *Nat. Prod. Rep.*, *19*, 1–49.

Fossati, P., L. Prencipe and G. Berti (1980). Use of 3, 5-dichloro-2-hydroxy-benzenesulfonic acid/4-aminophenazone chromogenic system in direct enzyme assay of uric acid in serum and urine. Clin. Chem., 26: 227-231.

Guidance document on acute oral toxicity testing" (2001). series on testing and assessment No. 24, organization for economic co-operation and development, OECD Environment, health and safety publications, Paris (www.oecd.org/ehs).

Gurunagarajan, S. and B. Pemaiah (2010). Antitumor and antioxidant potentials of ethanolic extract of *Leonotis nepetaefolia R.Br*. against Ehrlich Ascites carcinoma cell lines, *J of Pharmacy Res.*3(12) :p26-78.

Hirsch, M.D. (2006). An anniversary for cancer chemotherapy. Journal of American Medical Association 296: 1518-1520.

Islam, F., S. M. M. Ali and J. A.a Khanam (2013). Hepatoprotective effect of acetone semicarbazone on Ehrlich ascites carcinoma induced carcinogenesis in experimental mice,*Asian Pac J Trop Biomed*; 3(2): 105-110.

Ito H., K. Shimura, H. Itoh and M. Kawade (1997). Antitumor effects of a new polysaccharide protein complex (ATOM) prepared from *Agaricus blazei* (Iwade strain 101) Himematsutake and its mechanisms in tumor-bearing mice, *Anticancer Res* , 17:277-284.

Jha, R.K. and X. Zi-rong (2004). Biomedical compounds from marine organisms. *Mar. Drugs*, *2*,123–146.

Kavitha, K. and S. Manoharan (2006). Anticarcinogenic and antilipid peroxidative effects of Tephrosia purpurea (Linn) pers. in 7, 12- dimethyl Benz (a) anthracene (DMBA) induced hamster buccal pouch carcinoma. Ind J Pharmacol.; 38:185–189.

Kelman, D., E. K. Posner, K. J. McDermid , K. Nicole, T. Patrick, R. Wright and A. D. Wright (2012). Antioxidant Activity of Hawaiian Marine Algae, *Mar. Drugs*, *10*, 403-416.

Kintzios, S.E. (2006). Terrestrial plant derived anticancer agents and plants used in anticancer research. *Critical Rev Plant Sci*, 25: 79-113.

Kolanjinathan, K., P. Ganesh and M. Govindarajan (2009). Antibacterial activity of ethanol extracts of seaweeds against fish bacterial pathogens, European Rev. Med. Pharmacol. Sci., 13: 173- 177.

Kumar, R. S., B. Rajkapoor and P. Perumal (2011). Antitumor and Cytotoxic Activities of Methanol Extract of *Indigofera linnaei* Ali, *Asian Pacific J Cancer Prev,* 12, 613-618.

Kuttan, G., D.M. Vasudevan and R. Kuttan (1990). Effect of a preparation from Viscum album on tumor development in vitro and in mice, J. Ethnopharmacol., , 29, 35-41.

Lee, Y.L., H.J. Kim, M.S. Lee, J.M. Kim, J.S. Han, E.K. Hong, M.S. Kwon and M.J. Lee (2003). Oral administration of *Agaricus blazei* (H1 strain) inhibited tumor growth in a Sarcoma 180 inoculation model. *Exp. Anim.* 52, 371-375.

Lorke, D.A. (1983). A new approach to practical acute toxicity testing. Arch toxicol; 54:275-287.

Man, S., W. Gao, Y. Zhang, L. Huang and C. Liu (2010). Chemical study and medical applications of saponins as anticancer agents. *Fitoterapia*, 81, 703-14.

Mazumder, U.K., M. Gupta, S. Maiti and M. Mukherjee (1997). Antitumor activity of *Gygrophila spinosa* on Ehrlich ascites carcinoma and sarcoma-180 induced mice. Indian Journal of Experimental Biology, 35, 473-477.

Meenakshi, S., S. Umayaparvathi, M. Arumugam and T. Balasubramanian (2012). In vitro antioxidant properties and FTIR analysis of two seaweeds of Gulf of Mannar, *Asian Pac J Trop Biomed*; 1(1):S66-S70.

Newman, D.J., G.M. Cragg and K.M. Snader (2003). Natural products as sources of new drugs over the period 1981–2002. *J. Nat. Prod.*, *66*, 1022–1037.

Nishikimi, M., N.A. Roa and K. Yogi (1972). Measurement of superoxide dismutase. *Biochem.Biophys. Res. Common*, 46:849-854.

Ohkawa, H, N. Ohishi and K. Yagi (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* 95: 351–358.

Paglia, D.E. and W.N. Valentine (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab Clin Med. 70:158-169.

Satoh, K. (1978). Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. *Clinica Chimica Acta*, 90, 37-43.

Seenivasan, R., M. Rekha, H. Indu and S. Geetha (2012). Antibacterial Activity and Phytochemical Analysis of Selected Seaweeds from Mandapam Coast, India, Journal of Applied Pharmaceutical Science 2 (10),159-169.

Segura, J.A., L.G. Barbero and J. Marquez (2000). Ehrlich as-cites tumour unbalances splenic cell population and reduces responsiveness of T cells to *Staphylococcus aureus* enterotoxin B stimulation. Immunology Letter 74: 111–115

Sugiura, K., C. C. Stock and M. M. Sugi ura (1955). “The effect of phosphoramides on the growth of a variety of mouse and rats tumors,” Cancer Research, 2(1): 38 – 50.

Sunila, E.S. and G. Kuttan (2004). Immunomodulatory and antitumor activity of Piper longum Linn and piperin. *J Ethnopharmacol*, 90, 339-46.

Taylor, W.S. (1985). Marine algae of the eastern tropical and subtropical coasts of

the Americas,University of Michigan Press, Ann Arbor, pp 870.

Vijayavel, K. and J.A. Martinez (2010). *In vitro* antioxidant and antimicrobial activities of two Hawaiian marine *limu*: *Ulva fasciata* (Chlorophyta) and *Gracilaria salicornia* (Rhodophyta). *J. Med. Food*, *13*, 1494–1499.

Wagner, H., S. Bladt and E.M. Zgainski (1984). Alkaloid drugs. Plant drug analysis.Berlin: Springer-Verlag, Berlin Heidel Berg,51:298-334.

# دراسة لنشاط بعض الطحالب البحرية كمضادات للسرطان

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## الملخص العربى:

تهدف هذه الدراسة لبحث نشاط المستخلصات الخام لخمسة أنواع من الطحالب البحرية تنتمى الى مجموعات مختلفة (الطحالب الحمراء،الطحالب الخضراء،الطحالب البنية) كمضادات للسرطان. (***Ehrlich Ascites Carcinoma***)

بعد اجراء دراسة لسمية المستخلصات الخام وذلك بتحضينها على مدى قصير مع الخلايا السرطانية بتركيزات مختلفة ثم تعيين نسبة الخلايا الميتة وذلك باستخدام شريحة عد الدم (*Haemacytometer*) تم اختيار أكثر المستخلصات فاعلية.وتم اختيار المستخلص الخام لطحلب ال ***Enteromorpha compressa***

ثم تم اجراء تجربة لتعيين نصف الجرعة المميته(LD50 ) وذلك عن طريق الحقن فى تجويف البطن حيث كانت ٢٥٠٠ مجم/كجم من وزن الفئران البيضاء. ولدراسة تأثير المستخلص الخام لطحلب ال ***Enteromorpha compressa*** على الفئران البيضاء من خلال حقن جرعة(١x ١٠$^{٦}$ /مل)من الخلايا السرطانية ***(Ehrlich Ascites Carcinoma)*** عن طريق الحقن تحت الجلد على الفخذ الأيمن لتكوين الورم الصلب (خمسة مجموعات لكل مجموعة ستة من الفئران البيضاء). ثم تم بدء الجرعات العلاجية بعد ٢٤ ساعة من حقن الخلايا السرطانية حيث تم حقن جرعتين من المستخلص الخام لطحلب ال ***Enteromorpha compressa*** (٢٥٠و٥٠٠ مجم/كجم من وزن الفأر)كما تم حقن مجموعة أخرى من المجموعات بثنائى الميثيل سلفوكسيد( ٥ %) (DMSO) ١ مل /كجم من وزن الفأر.وبعد اثنتى عشرة جرعة متتالية ثلاثة جرعات لكل أسبوع وبعد ٢٤ ساعة من أخر جرعة تم وزن الفئران بكل مجموعة وتشريحها.

ولبيان تأثير المستخلص الخام لطحلب ال ***Enteromorpha compressa*** على الفئران تم أجراء تحاليل على الدم لتعيين الهيموجلوبين وعد كرات الدم الحمراء والبيضاء وكذلك بعض العوامل البيوكميائية كبعض وظائف الكبد (SGPT & SGOT) بالاضافة لتعيين انزيمات مضادات الاكسدة كما تم استخراج الورم الصلب وقياس حجمه حيث تم اختزاله بنسبة ٦٢.٤ و٦٥% للجرعة المنخفضة ثم الجرعة العالية بالترتيب. كما تم دراسة تأثير المستخلص بجرعتيه على بعض الاعضاء الداخلية للفئران فى المجموعات من خلال شرائح ال Histopathology. وقد وجد من خلال النتائج السابقة أن المستخلص الخام لطحلب ***Enteromorpha compressa*** له فاعلية عالية كمضاد للسرطان (***Ehrlich Ascites Carcinoma***) كما أثبت المستخلص فاعليته كمضاد للأكسدة فى الفئران البيضاء.

## 7. Hematological Parameters