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Research Article

ANTICANCER ACTIVITY OF METHANOLIC AND AQUEOUS EXTRACT OF *ULVA FASCIATA* IN ALBINO MICE

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ABSTRACT

Seaweeds are primitive non flowering aquatic plants, which have been a source of food, feed and medicine since ancient times. The selected green seaweed *U. fasciata* collected from Gulf of Mannar is a unique marine habitat that belongs to the diverse class of seaweeds. Hence the present study was conducted with the specific objectives to explore the anticancer activity of green seaweed, *Ulva fasciata.* The anticancer activity was tested against Dalton's ascitic lymphoma (DAL) in Swiss albino mice at the dose of 200 mg/kg, body weight. In this study, drug treatment was given after the 24 hrs of inoculation, once daily for 14 days. 5 -fluorouracil (20 mg/kg) was used as standard drug. The albino mice were evaluated for Median Survival Time (MST), and cancer cell count, packed cell volume, hematological parameters was compared with the standard by collecting blood from retro orbital blood vessel of mice. The parameters were reduced in tumor induced mice. These observations suggest that both the extract MEUF and AEUF possess anticancer effect against Dalton's Ascitic Lymphoma (DAL)

Keywords: *Ulva Fasciata,* Anticancer activity.

INTRODUCTION

Marine algae are one of the natural resources in the marine ecosystem. They contain various biologically active compounds which serve as a component for nutraceutical and pharmaceutical industry. Until now, more than 2400 marine natural products have been isolated from seaweeds of subtropical and tropical populations[1]. Recent findings evidenced that seaweeds possess antiviral[2], antibacterial[3], antifungal[4] and antitumor[5] potentials, among numerous others. Seaweeds have recently received significant attention for their potential as natural antioxidants. Antioxidant activity of marine algae may arise from carotenoids, tocopherols and polyphenols. These compounds directly or indirectly contribute to inhibition or suppression of free radical generation. The epidemiological data are supported by rodent model studies demonstrating protective effects of dietary kelps and other red and green algae against mammary, intestinal and skin carcinogenesis[6].

Recently, much attention has been paid to the anticancer activity of seaweed constituents. Several investigators have reported that crude seaweeds or their organic extracts have anti-proliferative activity in human cancer cell lines *in vitro*, as well as inhibiting activity in tumors growing in mice *in vivo*[7], and antigenotoxic effect in human lymphocyte cultures *in vitro*. During the past decade, the search of novel substances with considerable potential for chemosensitization was advantageous in revealing the compounds of natural origin possessing the ability of enhancing the cytotoxic activity of chemotherapeutic. For example, phytochemicals such as flavonoids, polyphenol-rich extracts and isolated phlorotannin components have been shown to inhibit proliferation of cancer cells and to influence anti-inflammatory responses[8, 9].

Recently, the polysaccharides and peptides, isolated from seaweeds have become a matter of great interest for cancer therapy. The mechanisms of their anticancer activity are related to their ability to suppress the growth of cancer cells. Cytotoxic or cytostatic effects, to enhance the immune responses, and to inhibit tumor angiogenesis [10]. Several marine algal polysaccharides, fucoidans, ulvan in particular have been found to induce apoptosis in cancer cells[11].Hence the present study was planned to evaluate the effect of methanolic and aqueous extract of green seaweed *Ulva fasciata* against Dalton's Ascitic lymphoma (DAL).

MATERIALS AND METHODS

Selection of Seaweed material

Ulva fasciata belongs used in this study to Green seaweeds. The seaweeds were collected freshly from Gulf of Mannar coast (9^{0}16'47" N to 79^{0}7'12" E) of India and authenticated by CSMCRI. The collected sample was washed immediately in seawater and then washed thoroughly with fresh water, transported to the laboratory in an iced condition. Initially, the seaweed was shade dried at room temperature for 48-72 hours. The shade dried seaweed was powdered and used for further experiments.

Preparation of seaweed extract

Methanolic Extract

The dried seaweeds were coarsely powdered and 250g of this seaweed powder was packed in soxhlet extractor of one litre capacity. The solvent methanol was added into the flask and heated. The temperature was maintained at 60^{0}C to 70^{0}C throughout the extraction. The soluble active constituents of the extract remained in the flask and the process was repeated until the compounds were completely extracted. The liquid extract was then cooled and concentrated by using a rotary evaporator at 30–45^{0}C. The extract was stored in labeled sterile screw-capped bottles at 4^{0}C until it was used[12].

Aqueous Extract

Powdered seaweed (100 g) was dipped into 20 volumes of distilled water and kept at room temperature for 2 h, then homogenized and refluxed at 100^{0}C for two hours. After cooling, the resulting material was centrifuged 10,000 g for 15 min. The supernatant was collected and centrifuged again at 8000rpm for 15min to obtain a clarified mixture. The supernatant so obtained was lyophilized (Alpha 1-2 LD Plus, Martin Christ, Germany) and stored at 4 ºC in a refrigerator for further use as crude aqueous extract[13].

Selection of Animals

Healthy adult male Swiss albino mice of commonly used laboratory strains were employed, weighing 20-25g. Each animal at the commencement of its dosing was between 8 and 12 weeks old and its weight was in an interval within ± 20% of the mean weight of any previously dosed animals. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC). They were allowed to acclimatize in the departmental animal facility for one week before the start of the experiment. They were fed a standard diet and water *ad libitium*. The study room was maintained at approximately 25 ± 2 ^{0}C in a 12-hours light/dark cycle and were housed in individual standard isolation cages (45×35×25 cm). After a sufficient period of acclimatization, they were used to evaluate anticancer activity.

Induction of cancer using DAL cells

Dalton's Ascites Lymphoma (DAL) cells were supplied by Amala Cancer Research Centre, Trissur - Kerala, India. The cells were maintained *in vivo* in Swiss albino mice by intraperitonial transplantation. While transforming the cancer cells to the grouped animal the DAL cells were aspirated from the peritoneal cavity of the mice using saline. The cell count was done and further dilutions were made so that total cell should be 1 x 10^6, this dilution was given intraperitoneally (i.p) and the cancer cells were allowed to grow in the mice for of a minimum of seven days before starting the study. Ascitic fluid was drawn from cancer-bearing mice at the log phase (days 7-8 of cancer bearing) of the cancer cells. Each animal received 0.2 ml of cancer cell suspension containing 1 × 10^6 cancer cells i.p.

Grouping of animals

Swiss Albino mice were divided into five groups of six each. All the animals in four groups were injected with DAL cells (1 x 10^6 cells/mouse) intraperitoneally, and the remaining one group from the normal control group. Group I served as the normal control. Group II served as the cancer control. Group I and II receive normal diet and water. Group III served as the positive control, was treated with injection flurouracil at 20 mg/kg body weight, oral route. Group IV served as the treatment control, which was treated with methanolic extract of *Ulva fasciata* (MEUF) at 200 mg/kg body weight, in i.p, (As per LD_{50} Value). Group V served as treatment control which was treated with aqueous extract of *Ulva fasciata* (AEUF) at 200 mg/kg of body weight, in i.p (As per LD_{50} Value). In this study, drug treatment was given after the 24 hrs of inoculation, once daily for 14 days. After the last dose, all mice from each group were sacrificed; the blood was withdrawn from each mice by retro orbital plexus method and the following parameters were analyzed.

1. Hematological parameters- WBC count, RBC count, Hb content, Platelet count, packed cell volume. (pentra-120 Automated Hematology Analyzer)

2. Lipid profile- Cholesterol, Triglycerides-CHOD PAP method

3. Liver function tests- AST, ALP, ALT- Modified IFCC/UV kinetic method,

4. Derived parameter- Body weight, Life span (%), Cancer Cell Count.

Percentage increase in life span (ILS)

The mortality was recorded by monitor the effect of MEUF and AEUF on cancer growth and percentage increase in life span (ILS %) was calculated[14].

% ILS was calculated by the following formulae

$$\%ILS = \frac{\text{Life span of treated group}}{\text{Life span of control group}} - 1 \times 100$$

Assessment of Cancer cell count

The fluid (0.1ml) from the peritoneal cavity of each mouse was withdrawn by sterile syringe and diluted with 0.8 ml of ice cold Normal saline or sterile Phosphate Buffer Solution(PBS) and 0.1 ml of tryphan blue (0.1 mg/ml) and total numbers of the living cells were counted using haemocytometer (improved Neubauer chamber).

Total number of cells per ml = average no of cells × dilution factor 2 ×10^4

Statistical Analysis

The experimental results were expressed as the mean ± S.E.M. The haematological and biochemical parameters were subjected to statistical analysis by one way Analysis of Variance to determine the significant difference between the groups. ANOVA was done with Graphpad Prism software. All Pairwise Multiple Comparison Procedures by Student-Newman-Keuls Method. Data were accepted as statistically significant difference was obtained at p <0.05.

RESULTS AND DISCUSSION

Effect on tumor Growth

In the DAL tumor control group, the average life span of the animal was found to be 46% MEUF and AEUF at the dose of 200 mg/kg body weight increase the life span to 76% and 74% respectively. These values changed significantly after the treatment of *Ulva fasciata* extract. However, the average life span of 5- FU treatment was found to be 92%, indicating its potent anticancer nature. The anticancer nature of MEUF and AEUF was evidenced by the significant reduction in percent increase in body weight of animal treated with MEUF and AEUF at the dose of 200 mg/kg body weight when compared to DAL cancer bearing mice.

Many studies have reported the useful effects of seaweed extracts against DAL. When DAL is induced in animals, the cancer cell count in the peritoneal fluid has been used as the marker to confirm the proliferation of cells. For a similar observation, in this study a cancer control group was used. The increased cell count after 14 days confirmed the proliferation of cells in this group. A decrease in cancer cell count as a confirmatory evidence for protection against DAL has been reported[15]. In this study also, a similar decrease was observed following the administration of the extracts. Consequently increased life span was observed in extract treated mice.

Organic components have been identified in algae of *Ulva* genus. Simple bromophenols, especially 2,4,6-tribromophenol, lipid components, dimethylsulfoniopropionate (DMSP) have been largely described for this genus[16]. Another study reported that a sulfated heteropolysaccharide from *Ulva rigida*, which belongs to the same family as Enteromorpha, could markedly activate macrophages[17]. It was also found that oversulfated fucoidan inhibited the growth of Lewis lung carcinoma and B16 melanoma in mice, and reported that increasing numbers of sulfate groups in the fucoidan contributes to the effectiveness of its anti-angiogenic and antitumor activities[18].

Table 1: Effect of MEUF and AEUF on the life span, and body weight and cancer cell count

Treatment	Number of animals	% ILS life span	Increase in body weight grams	Cancer cell count ml x 10^6
G_1	6	>>30 days	1.30±0.009	-
G_2	6	46%	8.60±0.18**	2.38±0.02**
G_3	6	92%	1.83±0.03***	1.01±0.03***
G_4	6	76%	7.45±0.06***	2.06±0.03***
G_5	6	72%	7.45±0.04***	1.97±0.02***

G_1 – Normal Control, G_2 – Cancer Control, G_3 – Standard, G_4 – MEUF, G5 – AEUF

All values are expressed as mean SEM for ± 6 animal in each group.

** – Values are significantly different from control (G_1) at p <0.01

*** – Values are significantly different from cancer control (G_2) p < 0.001

MEUF– methanolic extract of *Ulva fasciata*

AEUF – Aqueous extract of *Ulva fasciata*.

Effect on Hematological Parameters

As shown in Table.2 RBC, Hb, Platelets were decreased and WBC counts significantly increased in the DAL control group as compared to the normal control group. Treatment with MEUF and AEUF at the dose (200 mg/kg) significantly increases the Hb content, RBC, Platelets and significantly decreased the WBC count to about normal level. All these results suggest the anticancer nature of both extracts. However, the standard 5-FU at the dose of 20 mg/kg body weight produced better result in all these parameters.

Hematological parameters of tumor bearing mice in 14 days were found to be significantly altered compared to the normal group. The total WBC count of cancer control Group II was 8.29Cells /mlx10^3 which was found to be increased with a reduction of the Hb content of the RBC. At the same time, total WBC count of Group III, IV and V seems to be equivalent without any alteration in their values. Thus, the seaweed extract MEUF and AEUF are active against DAL cells which maintain hematological parameters.

The total number of RBC showed modest changes in treated groups. At the same time interval on MEUF and AEUF extract of at a dose of 200mg/kg restored all the altered hematological parameters to almost near normal. This may be due attributed to the iron content of the seaweeds and also increase the bioavailability of iron in cancer treated mice.

A decrease in RBC count and increase in WBC and PCV count following cancer cell proliferation. As reported earlier by Rajkapoor *et al*[19], the present study also similar results were seen after treatment with the extracts. However from the above observations on other parameters, it was concluded that the aqueous extract of *Ulva fasciata* possesses activity against DAL.

Anemia/ myetosuppression have been frequently observed in ascites lymphoma. Anemia encountered in ascites lymphoma is mainly due to iron deficiency, either by haemolytic or myelopathic conditions which finally lead to reduced RBC number[20]. Treatment with crude extract of *Ulva fasicata* brought back the hemoglobin content, RBC and WBC count more or less to normal levels, thus supporting its hematopoietic protecting activity without inducing myelotoxicity the most common side effects of cancer chemotherapy.

Table 2: Effect of MEUF and AEUF on Hematological parameters

TREATMENT	Total WBC Cells /mlx10^3	RBC Count Mill/cumm	Hb Gm/dl	PCV	Platelets Lakhs/cumm
G1	5.23 ±0.07	7.62±0.03	12.32 ±0.07	37.65±0.08	2.57±0.24
G2	8.29±0.17**	4.06±0.09**	5.67±2.06**	47.92±0.14**	1.85±0.04**
G3	5.68 ±0.14***	6.86±0.04***	10.23±3.08***	38.78±0.16***	2.41±0.03***
G4	5.12±0.05***	6.12±0.09***	10.96±1.06***	39.67±0.15***	2.09±0.09***
G5	5.96±0.03***	5.67±0.08***	9.38 ±0.03***	38.34±0.08***	2.06 ±0.04***

G_1 – Normal Control, G_2 – Cancer Control, G_3 – Standard, G_4 – MEUF, G5 – AEUF

All values are expressed as mean SEM for ± 6 animal in each group.

** – Values are significantly different from control (G_1) at p <0.01

*** – Values are significantly different from cancer control (G_2) p < 0.001

MEUF– methanolic extract of *Ulva fasciata*

AEUF – Aqueous extract of *Ulva fasciata*.

Effect on Biochemical Parameters

The inoculation of DAL cells caused a significant increase in the level of Total Cholesterol, Aspartate transaminase, Alanine transaminase, Alkaline Phosphatase in the cancer control animals (G_2), when compared to the normal group. The treatment with MEUF and AEUF at the dose of 200 mg/kg body weight reversed these changes towards the normal level. All the value was found to be significant. The treatment with standard 5- FU also gave similar results. The enzyme activity rises in the serum more than the normal level, which indicates serious tissue damage; these results coincide with similar study reported by Soujanya et al[21].

It was reported that the presence of cancer in the human body or in the experimental animal is known to affect many functions of the liver. The significantly elevated level of total cholesterol, TG, AST, ALT, ALP in the serum of cancer inoculated animal indicated liver damage and loss of functional integrity of cell membrane[21]. The significant reversal of these changes towards the normal was observed by MEUF and AEUF treatments.

Markedly elevated serum ALP, hyperalakline-phosphatasemia, is seen predominantly with more specific disorders; including malignant biliary cirrhosis, hepatic lymphoma and sarcoidosis[22].

Table 3: Effect of MEUF and AEUF on serum Enzymes and lipid proteins

Treatment	Cholesterol (mg/dl)	TGL (mg /dl)	AST (U/L)	ALT (U/L)	ALP (U/L)
G_1	72.30±0.32	118.25±0.30	40.46 ±0.50	28.49 ±0.09	30.42 ±1.80
G_2	142.44±0.07**	170.45±0.65**	85.65±0.14**	55.81±0.38**	78.23±0.61**
G_3	98.49±0.72***	130.91±0.38***	53.58±0.13***	38.28±0.45***	40.12±0.50***
G_4	110.59±0.25***	140.45±0.21***	72.51±0.33**	54.47±0.28***	46.12±3.50***
G_5	114.43±0.25***	148.97±0.19***	74.36±0.33***	50.25±1.27***	44.46±3.12***

G_1 – Normal Control, G_2 – Cancer Control, G_3 – Standard, G_4 – MEUF, G5 – AEUF

All values are expressed as mean SEM for ± 6 animal in each group.

** – Values are significantly different from control (G_1) at p <0.01

*** – Values are significantly different from cancer control (G_2) p < 0.001

MEUF– methanolic extract of *Ulva fasciata*

AEUF – Aqueous extract of *Ulva fasciata*.

CONCLUSION

Therefore in conclusion, the present study showed a decrease in cancer cell count as a confirmatory evidence for protection against DAL. Consequently increased life span was observed with extract treated mice. The haematological and biochemical alterations observed in cancer bearing animals in the present study may be due to the reduction of antioxidant level following cancer proliferation. However, administration of *Ulva fasciata* extracts significantly alters this level in cancer-bearing animals. Thus, from the above observations on other parameters it was concluded that the seaweed *Ulva fasciata* possesses anticancer activity against DAL.

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