

In vitro anticancer activity of Astaxanthin

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Abstract

This study was designed to determine the *in vitro* anticancer potential of the Astaxanthin isolated from shrimp shell waste (ETC) against Ehrlich Ascites Carcinoma (EAC) induced cancer in swiss albino mice. The anticancer activity was assessed using *in vitro* cytotoxicity, mean survival time, tumor volume and hematological studies. The reliable criteria for evaluating the potential of any anticancer agent is the prolongation of lifespan of the animal and decrease in WBC count of blood. The high dose of ETC (200 mg/kg, orally) significantly reduced the tumor growth which was demonstrated by increased lifespan of the mice and restoration of hematological parameters. ETC was also found to be cytotoxic in the *in vitro* parameter which shows that ETC possesses significant anticancer potential.

Keywords: Cancer; Astaxanthin; Cytotoxicity; Shrimp

1. Introduction

Lung cancer causes 28% of global cancer-related deaths and 15% of all diagnosed cancers [1]. It can be subdivided into two broad categories, small-cell lung cancer (SCLC) and non-small-cell lung cancer (NSCLC), the latter being the most common type [2]. The treatment of NSCLC includes surgery, radiotherapy, tyrosine kinase inhibitors, immunotherapy, and platinum-based chemotherapy [3]. With respect to chemotherapy, it appears that natural plant products play an important role, making a considerable contribution to approximately 60% of available chemotherapeutic cancer drugs [4].

The development and progression of cancer involve abnormal changes in DNA methylation which lead to the activation of certain protooncogenes, such as c-Myc, as well as to the inactivation of certain tumour suppressors, such as p16 [5]. It seems that certain dietary components affect the process of carcinogenesis, through DNA methylation. In fact, it has been shown that folate, as methyl group donors, and riboflavin, vitamin B6, and vitamin B12, as cofactors in one-carbon metabolism, are associated with DNA synthesis and methylation and, hence, may play a role in carcinogenesis and cancer risk; DNA global hypomethylation is associated with lung cancer [6, 7].

Astaxanthin, as various carotenoids, can go about as a quencher of singlet oxygen and other free radicals by holding the stimulated imperativeness of singlet oxygen onto the polyene electron-rich chain, turning out first in the excitation of the carotenoid to a triplet state, and a while later in the dispersal of the additional vitality as warmth by unwinding back to the ground state. Thusly, it keeps cell sections or tissues from being hurt. The carotenoid structure remains unaltered, and arranged to go about as a radical quencher. Astaxanthin can act similarly as chain-breaking hostile to oxidant, and

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along these lines shield lipid-rich cell films from degradative oxidation through course of action of lipid hydro peroxides. Common Astaxanthin is a dietary supplement with incredibly successful cell fortification advantages for human applications. Astaxanthin traps more free radicals than some different cell reinforcements Astaxanthin has been exhibited to cross the human blood cerebrum hindrance, thusly can clearly go about as a great cancer prevention agent in the mind and the eyes.

Astaxanthin, as different carotenoids, can go about as a quencher of singlet oxygen and other free radicals by retaining the energized vitality of singlet oxygen onto the polyene electron-rich chain, coming out first in the excitation of the carotenoid to a triplet state, and afterwards in the dissipation of the extra energy as heat by relaxation back to the ground state. Along these lines, it prevents cell segments or tissues from being harmed. The carotenoid structure stays unaltered, and prepared to go about as a radical quencher. Astaxanthin can act likewise as chain-breaking anti-oxidant, and in this way shield lipid-rich cell membranes from degradative oxidation through arrangement of lipid hydro peroxides. Natural Astaxanthin is a dietary supplement with amazingly effective cell reinforcement benefits for human applications. Astaxanthin traps more free radicals than some other antioxidants Astaxanthin has been demonstrated to cross the human blood brain barrier, in this way can straightforwardly go about as a heavenly antioxidant in the brain and the eyes.

On account of having a hydrophobic, or "oil-adoring" polyene chain from one point of view and a "water-cherishing" keto-hydroxyl-substituted ionone ring on the other, Astaxanthin is fit for crossing the phone film bilayer, thusly showing cancer prevention agent protection and fortifying the entire cell layer. Astaxanthin enhances the diverse cell reinforcements like vitamin E and C.

Astaxanthin ensures nucleic acid components of DNA, staying away from transformation to hereditary material due to oxidative stress.

2. Material and methods

Shell waste as of the remote ocean shrimp *Aristeus alcockii*. The example be amassed commencing the place Cochin, Kerala, India. The shrimp squander was elated to the examination office in a sterile compartment stacked with ice. The living thing was identified with the styles advanced under the trade name "Red Shrimp". The wastes made from the shrimp are cephalothorax, stomach shell and tail isolate. Following meat from the cephalothorax was cleared and the waste was washed under running water and dried under shade. They were assembled in polyethylene sacks and set away at - 20 °C until use. A minute arrangement of test was secured wet, ensuing to removing the accompanying meat, was full in polythene covers and set away at - 20 °C.

Short term cytotoxicity was assessed by Trypan blue exclusion method and Lactate dehydrogenase (LDH) leakage assay.¹²

2.1. Trypan blue exclusion method

Trypan blue dye assay method was carried out to evaluate the *in vitro* cytotoxicity potentials of Astaxanthin. Different concentrations (5, 10, 50, 100, 150 and 250mcg/ml) of extracts were prepared. In a test tube, 100µl of extract was mixed with 800µl of phosphate buffer saline and 100µl (1X10⁶ in 1ml) of Dalton's Ascitic Lymphoma (DAL) was added. Similar method was followed with Ehrlich Ascitic Carcinoma (EAC) cell line also. Each concentration of the extracts was tested in triplicate. All the samples were incubated at 37°C in an incubator for 30min. About 100µl of trypan blue dye was added to all the test tubes and the number of dead cells was counted in a haemocytometer under a compound microscope. Percentage of cytotoxicity was calculated by the following formula.

$\% \text{ dead cells} = \text{Number of dead cells} / \text{Sum of dead cells and living cell} \times 100.$

2.2. Lactate Dehydrogenase (LDH) leakage assay

LDH leakage assay was carried out using LDH cytotoxicity detection kit by Sigma Aldrich Inc., USA, according to protocol in the user's manual. To determine IC₅₀, different concentrations of Astaxanthin extracts were incubated with 100 µl of DAL and EAC cell suspensions having 1x 10⁶ cell /ml in 96 well plates and incubated at 37°C for 4 hrs in 5% CO₂ atmosphere. All the control and test substances were tested in triplicates and mean ± SEM of the absorbance values were recorded to calculate the cytotoxicity.

LDH leakage (%) related to control wells containing cell culture medium without extracts was calculated by $[A]_{\text{test}} / [A]_{\text{control}} \times 100$. Where $[A]_{\text{test}}$ is the absorbance of the test sample and $[A]_{\text{control}}$ is the absorbance of the control sample.

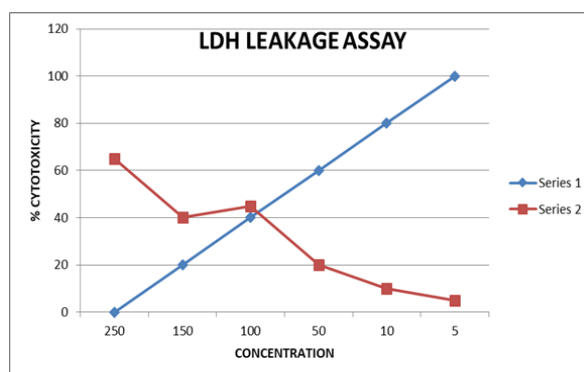
2.3. Statistical Analysis

All the assays coming under *in vitro* anticancer assay were performed in triplicate and the results were expressed as mean \pm standard deviation.

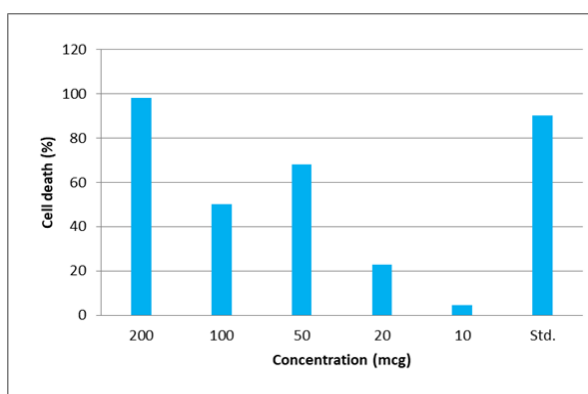
3. Results

Anticancer activity of EEDI and AEDI against the test cells DAL and EAC by trypan blue exclusion and LDH leakage assay methods. In trypan blue exclusion method, 250, 150, 100 mcg/ml of AST showed more significant effect against DAL towards both the cell lines. The inhibition concentration was compared with that of control. A dose dependent increase in the % of LDH leakage was observed. A maximum leakage of LDH was observed at a concentration of 250mcg/ml. From figure 2, the % of LDH release was increased with increasing concentration of EEDI which is in direct proportion to the cell death. Maximum cell death after incubation was observed at 250 mcg/ml concentration.

Drug concentration ($\mu\text{g/ml}$)	Percentage cell death(DLA or EAC)			
	1	2	3	4
20	96	100	98	100
5	82	100	88	91
2.5	64	70	70	75



LDH leakage Assay



Drug concentration v/s % cell death

3.1. Trypan blue assay

All these point to the possibility of developing Astaxanthin isolated from shrimp shell waste as a novel, potential agent in the area of cancer chemotherapy. The present study highlighted the antitumor activated of tumour cells were aspirated from the peritoneal cavity of the tumour bearing mice.

4. Discussion

Now-a-days the side effects of synthetic drugs are increasing. Many incidents such as incompatibility, drug interactions such as synergism, cumulative toxicity, teratogenicity, idiosyncrasy, habituation, addiction etc are more, in herbals as well in synthetic drugs. Here marine drugs tend to achieve more significance. Marine drugs produce less drug toxicities when compared to drugs from other sources. Marine products used as drugs are mostly substances that are produced by the marine species for their self-protection. These products have less chance to develop drug interactions and drug toxicities. These variables have accentuated the squeezing requirement for new, successful and safe marine medications, which thus has opened up another region of research for the researchers. The branch of marine medication inquire about has quick advanced with its high magnitudinal exercises, which is exemplified by the countless research publications and by the proliferation of monograph and reviews on varied subjects like pharmacology, therapeutics, photochemistry, drug formulations, medicinal chemistry etc. The enormous cost of marine products and availability has hampered the prospects of development of new marine drugs by using the common available edible species; it also reduces the risk of toxicity from the species as it is an already edible species. The isolation of drugs from species such as shrimps has resulted in the diminished cost of sample collection.

5. Conclusion

The cytotoxic study was carried out. The test compounds were studied for short term *in vitro* cytotoxic studies. Dalton's lymphoma ascites cells were used. Maximum activity were obtained, future work should be carried out in this area.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors declare that article does not bear any type of conflicts of interest.

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