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Full Length Research Paper

Evaluation of anticarcinogenicity effect of *Artemia urmiana* by *Salmonella typhimurium* TA 100 strain

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Free radicals are produced during oxidative metabolism in body and they can attack all components of cells and cause cellular changes and early aging. Antioxidant materials role for balance maintenance in cells has currently attracted more attention. The aim of this study is investing of antimutation effect of *Artemia*, cyst and decapsulated egg extract by Ames test and *Salmonella typhimurium* TA 100 strain. These extract prevent reverse mutation caused by carcinogenic materials. In comparison with positive controls (sodium azid, $KMno_4$, cresol) and negative control (distilled water) antimutagenicity properties of *Artemia* was evaluated and confirmed. In addition, the value of astaxanthin in those extracts was assayed. For quality analysis and separation of astaxanthin, we used thin-layer chromatography (TLC) chromatography and column chromatography, respectively. Then, the presense of astaxanthin in these extracts was confirmed by spectrometer IR test. The results showed that antimutagenicity properties of *Artemia urmiana* are the same with other salty water *Artemia*. Antimutagenicity and anticarcinogenicity properties in decapsulated egg, dried *Artemia* and cyst are 85, 70 and 100%, respectively.

Key words: Ames test, anticarcinogenicity, antioxidant, *Artemia urmiana*, *Salmonella typhimurium* TA 100.

INTRODUCTION

Investigation of anticancer features of *Artemia urmiana* by using the *Salmonella typhimurium* TA100 strain has been done. However, human has discovered the presence of *Artemia* in salty rivers long a time ago. The first report about *Artemia* was registered by Schlosser in 1755 A.D. He observed *Artemia* in the samples of the water that he has provided from salty rivers in a place near Lymington. He called it *Artemia linneaus* by the scientific name of the crab of salt water and in 1819 a person whose name was Litch recalled it *Artemia salina*. At last Artemia in Urmia sea was called *Artemia urmiana* by Clark and Bawn (1976), and by the time being, many researches and investigations have being done about different subjects of *Artemia* by ISA (International Study of *Artemia*) in different countries (Lorenz and Cysewski, 2000). Now a day *Artemia* (that is the famous shrimp of salty water) is a good source of protein for human, a food for marines and is used in drugs (Magbool and Suresh, 2003). Because of it contains Caretonoids such as Astaxantin, Kantanxantin and Autooxicuein, *Artemia* is able to do different biological performances such as protection against light, removing the injuries caused by oxygen, immune adjusting in body, and antioxidant activities. Also reactive oxygen species (ROS) may play a major role as endogenous initiators of degenerative processes such as deoxyribonucceic acid (DNA) damage and mutation that may be related to cancer (Nair and Risch, 2000) cancer (Nair and Risch, 2000). Test of carcinogens on rats, is expensive and time consuming. Recently, bacteria have been using for assessment of the carcinogenicity of suspected materials which are not only cost- effective but also give prompt and excellent results.

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Abbreviations: ISA, International study of *Artemia*; **TLC,** thin-layer chromatography; **ROS,** reactive oxygen species; **FT-IR,** fourier transform infrared.

Table 1. Survey of genotype results of *Salmonella typhimurium TA 100* strain.

Strain	rfa mutation	UVRB mutation	R-Factor
Salmonella typhimurium TA100	+	+	+

One of the methods of assaying carcinogenic effects via bacteria is Ames test by use of *Salmonella* strains which because of mutation have lost the histidine synthesis potential and in the medium without histidine will face a reverse mutation which leads to histidine synthesis. By measuring the developed colonies in the medium, the carcinogenicity effect will be identified (Motorelmansk zeger, 2000). This article discusses different steps of growth of *Artemia* that has antioxidant and more anticancer activities so that in proper step we can use it as foodstuff and drug for human, poultries and marines.

MATERIALS AND METHODS

Artemia was caught from Urmia Lake and transferred to the laboratory on summer 2005. The dried *Artemia*, cyst, and decapsulated egg gathered and froze at -10°C until utilization. *Salmonella typhimurium* TA 100 strain was received directly from Professor Ames (University of Beverly, CA).

Confirming genotypes of tester strains

The tester strains genotype should be confirmed, so fresh overnight nutrient broth cultures were used for this purpose. *Salmonella typhimurium* strain has deficient in dark repair of mutations (UVrB), and unable to synthesize a portion of the cell wall (rfa).

rfa mutation

TA 100 strain was tested for crystal violet sensitivity. For this test, nutrient agar plates were seeded with cultures of the motional strain and sterile filter paper disc soaked in crystal violet was placed on the surface of each cultured plate. After 12 h incubation the clear zone of inhibition (approximately 14 mm) was appeared around the disc indicating the presence of the rfa mutation which permits large molecules such as crystal violet to enter and kill the bacteria.

UvrB (UVrB) mutation

The UVrB mutation was confirmed by demonstrating UV sensitivity in TA100 strain which showed this mutation.

R- factor

The R- factor of TA 100 strain was tested for the presence of the ampicillin resistance factor. It is a convenient marker that makes it possible to test for the presence of the R- factor plasmid. The specific regions of the R-factor plasmid PKM 101 DNA that are essential for enhancement of UV, chemical mutagens, replication and ampicillin resistance have been identified.

Astaxanthin exploitation

10 g of each dried *Artemia*, cyst, and decapsulated egg separately were placed into glass flasks. Petroleum ether, acetone and water were added at a ratio of 1.5:7.5:1 to the samples and left under cover for 2 h. Then the solvents were evaporated in rotary evaporator until sample dryness. The resulting pigments were redissolved in an adequate volume of diethyl ether. These solutions were analyzed by thin-layer chromatography (TLC) analysis and Fourier transform infrared (FT-IR) spectroscopy. In TLC, mixture 25% acetone in n- hexane was used as developers (Kobayashi and Katsuraji, 2001). In addition, the values of Astaxantin in those extracts were assayed. To quality analyze and separate Astaxantin, we used TLC chromatography and column chromatography respectively. Then we proved this test IR in these extracts.

Ames test

In antimutagenicity test, the inhibitions of mutagenic activity of sodium azide and permanganate potassium by the test samples were determined. 1 ml of solution of the tested compounds and 0.1 ml of an overnight bacterial culture suspension cultivation for 16 h at 37°C, approximate cell density $(2\text{-}5)\times10^8$ cell/ml and 0.1 ml of solution of the positive mutagens were carefully mixed with 3 ml of melted top agar containing 50 µmol/l of histidine- biotin, and poured onto minimal glucose agar plates. Positive and negative controls were also included in each assay. Sodium azide and potassium permanganate were used as diagnostic mutagens (0.1 ml) in the positive control and plates without mutagens and test samples were considered as negative control. Their revertants were counted after incubation of the plates at 37°C for 48 h. Tester strains were checked routinely to confirm genetic features by using the procedure described by Maron and Ames (Negi et al., 2003).

RESULTS

Salmonella typhimurium TA 100 strain has special mutation in its operon histidine that makes it dependent to outer histidine source. In relation to mutagenic material this mutation is reversible, so independent bacteria to histidine will come out. According to Tables 2, 3 and 4, the antimutation properties of dry *Artemia* extract, decapsulated egg and cyst extract in the Presence of Positive mutations were confirmed. By using of *Salmonella typhimurium* TA 100 strain was shown that the positive control containing the mutating materials like azid sodium, Permanganat Potasium and cresol causes reverted mutation in operon histidine (His+). According to Tables 2, 3 and 4, in presence of anti-mutagenic material comparing with control sample, the number of reverted colonies reduced. Indeed, rate of mutation suppression was evaluated higher than 40% that was indicating strong antimutagenicity effect of comparison with three

Table 2. The results of investigation of preventing and moderating of mutagenesis effects of Azide sodium by dried *Artemia*, cyst and decapsulated egg by using *Salmonella typhimurium* TA 100.

Examined material	Number and percent of reverted colonies of bacterium *Salmonella typhimurium* TA 100					
	1		2		3	
Positive control (Azid sodium)	1600	-	1500	-	1440	-
Negative control	440	-	512	-	524	-
Dried *Artemia* extract	600	86%	720	78%	800	69%
Decapsulated egg extract	500	94%	724	78%	632	88%
Cyst extract	480	96%	360	100%	400	100%

Table 3. The results of investigation of preventing and moderating of mutagenesis effects of permangeant potasium by dried *Artemia*, cyst and decapsulated egg by using *Salmonella typhimurium* TA 100.

Examined material	Number and percent of reverted colonies of bacterium *Salmonella typhimurium* TA 100					
	1		2		3	
Positive control (Permangeant potasium)	1680	-	1760	-	2000	-
Negative control	440	-	512	-	524	-
Dried *Artemia* extract	724	77%	884	70%	1016	66%
Decapsulated egg extract	600	87%	724	83%	800	81%
Cyst extract	500	87%	480	100%	360	100%

Table 4. The results of investigation of preventing and moderating of mutagenesis effects of cresol by dried *Artemia*, cyst and decapsulated egg by using *Salmonella typhimurium* TA 100.

Examined material	Number and percent of reverted colonies of bacterium *Salmonella typhimurium TA100*					
	1		2		3	
Positive control (cresol)	1560	-	2400	-	2800	-
Negative control	664	-	520	-	480	-
Dried *Artemia* extract	1000	62%	920	78%	840	84%
Decapsulated egg extract	724	93%	648	92%	734	89%
Cyst extract	480	100%	800	85%	360	100%

promotagen materials (Azid sodium, Permanganat Potasium and cresol). The result coming out of chromatography of thin layer TLC has showed that the progressing of the samples produced from the extract of dried *Artemia*, cyst and decapsulated egg on the silica gel paper leads to the formation of a dark orange band with Rf=40%. This band from color and RF measurement point of view looks like astaxantin reference, and the main created stigma was in result of the separation of the primary samples. Five weak spots from dried *Artemia* and two weak spots from cyst and one weak stigma from decapsulated egg have been observed (Figure 1).

At first it was assumed that the light colored bands are related to the ester from astaxantin or even to the rest of the combinations in it, but after doing spectrographic with spectrophotometer and comparing them, it was observed that these spots are like to the spectrums in result of the primary bands and the light colored bands are related to astaxantin extract, because astaxantin can combine with oxygen in the air and change to other isomers.

Comparison of spectrums related to the main band of dried *Artemia* extract, cyst and decapsulated egg showed that the depth and intensity of created pigments are almost the same. It showed that the thickness of pigment in *Artemia* egg was the most and in dried *Artemia* and cyst were fewer than the expected sample.

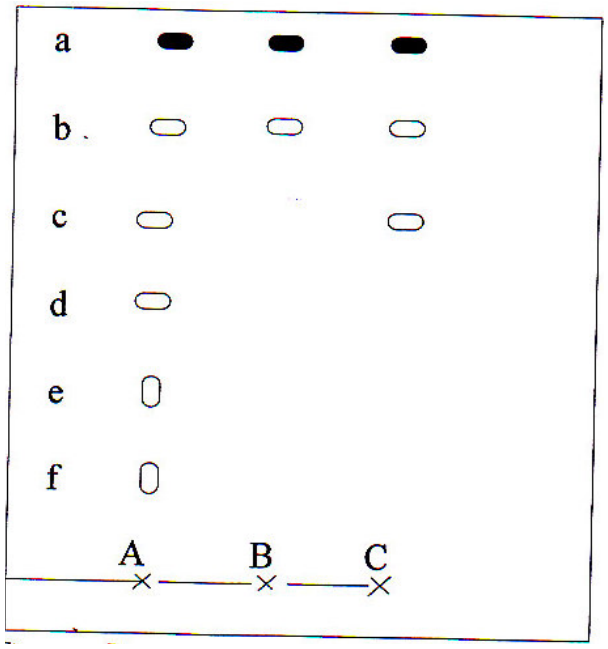

Figure 1. Chromatography of thin layer of dried *Artemia* extract (A), Decapsulated egg (B) and cyst (C) on Chromatographic paper of silica gel.

DISCUSSION

One of the main reasons of death in industrialized societies is cancer. In the last two decades, different kinds of mutating, cancer making and chemical materials in foods have been known (Sarmineto and Ochoa, 2002). Today scientists believe these genetic damages and changes caused by succession of mutating DNA or mutation in genes and other genetic changes in chromosomes structure have role in cancer. According to Ames theory 80% of mutating material cause cancer (Gerber et al., 2002) and most of these mutating and cancer makings show their damaging effects by producing the free radicals and active oxygen. Free radicals are related to various physiological and pathological events such as inflammation, immunization, aging and etc. It has been suggested that compounds which possess antioxidant activity, can inhibit mutation and cancer because they can scavenge free radicals or induce antioxidant enzymes (Mikula and Ikova, 2003). Thus, daily use of antioxidants has an important role in protecting body against the free radicals, even most of the antioxidants are known as anticancers (Rosenkraz, 2003). Antioxidant materials such as carotenoids, astaxantin, fixed vitamin C, autooxicuien, lipids, fish oil and etc has shown anticancer features of them. TLC analysis showed that astaxantin and its isomers seemed to be the dominant carotenoied in *Artemia* (Duarte and Lunec, 2005). Epidemiological studies indicate that there is a close relationship between diet, life style and human cancer (Nair and Risch, 2000; Mehrabian and Shirkhodaei, 2006). The result of this research is in accordant with other researches. According to Ong formula, antimutagenicity effect is higher than 40%, so recent examined materials are high antimutagenicity ability (Ong et al., 1980). Presence of carotenoid pigments such as β-caroten, kantaxanthin and astaxanthin have been found in *Artemia* structure that most of them are antioxidant properties (Duarte and Lunec, 2005). Compounds such as glutation in marine food materials play major role in elimination and limitation of free radicals (Trusheva, 2006).

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