Antimutagenic activity of Chloroformic and Methanolic extracts of muscle, liver and cartilage of *Sphyrna lewini* with the Ames test

Samadi, S. [*1]; Emtyazjoo, M.[1]; Khanipour, A. A.[2]; Moghadasi, Z.[1]; Nazarhaghighi, F.[3]

Received: August 2009 Accepted: May 2010

Abstract

For this study one species of Shyrnidae family caught along Persian Gulf in Bandarabbas city. *Sphyrna lewini* were transferred to the laboratory on spring 2007. The antimutagenic activity of the Methanolic and Chloroforamic extracts of muscle, liver and cartilage of *Sphyrna lewini* with the Ames test was investigated. The use of antimutagens and anticarcinogens in everyday life is the most effective procedure to prevent human cancer and genetic diseases. Since angiogenenesis is a key factor in tumor growth, inhibiting this process is one way to treat cancer. In this study the antimutagenic effect of the Chloroformic and Methanolic extracts of muscle, liver and cartilage on the damage induced by two mutagens was studied. The results driven from this study were inhibitory effect of two extracts. The highest antimutagenic effect was determined in the Potassium Permanganate and Sodium Azide as a mutagen was Methanolic extracts of cartilage. There is a general correlation between mutagenesis and the initiation stage of carcinogenesis. Mutagens appear to initiate the process by inducing the primary DNA lesion. These are called initiators and the damage they cause is generally irreversible.

Keywords*: Sphyrna lewini*, Antimutagenesis, Ames test

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[DOR: 20.1001.1.15622916.2011.10.1.12.1]

1-Faculty of marine science and technology, Azad Islamic University North Tehran Branch, P.O.BOX: 19735-181, Tehran, Iran.
2-National Fish Processing Research Center, P.O.BOX: 43145-1655 Bandar Anzali, Iran.
3-Faculty of Marine Science and Technology, Gilan University, Gilan.
[*]Corresponding author's email: setareh.s@hotmail.com

Introduction

Some environmental substances can damage DNA causing a series of disabilities in different organisms, human being particularly. Most of cancers have been related to industries development and deposition of mutagens in the body leading to various degenerative disorders and genetic defects in the offspring (Cuzzocrea et al., 2001; Migliore and Coppede, 2002). Recently, some studies have been done to find natural chemo-inhibiting substances capable of inhibiting, decelerating or reversing the carcinogenesis usually initiated by mutation (Surh, 1999).Today; however, there are various methods to collect samples testing carcinogenic and mutagenic activity of substances. Many of them have been as indicators. Ames plate incorporation test has been used by many scientists, which are valid indicators of mutagenicity and genotoxicity of some substances present in the natural environments (Rosenkraz, 2003).

Since angiogenesis is a key character of tumor growth, restraining blood vessel formation is one of the treating cancer methods. Cartilage is one of the natural resources rich in strong antiangiogenic activity. Purified antiangiogenic factors from shark cartilage, such as U-995 and Neovastat (AE-941), also showed antiangiogenic and antitumor activity. (Cho and Kim, 2004).Shark cartilage contains a substance that strongly inhibits the increasing and growth of new blood vessels toward solid tumors which results in restricts tumor growth (lee and Langer, 1983) .The abundance of this factor in shark cartilage, in contrast to cartilage from mammalian sources, may mark sharks an ideal source as an inhibitor and may help to explain the rarity of neoplasm in these animals (Lee and Langer, 1983).

An anti-inflammatory and analgesic property of water-soluble substances from shark cartilage has been studied using some conventional microbial tests such as electrophoretical assays, bacterial survival, transformation and the Salmonella/mammalian-microsome assay. The effects of shark cartilage are inducing target cells to protect themselves against DNA damage and mutagenesis. It is believed that shark cartilage roles, including preparation play a scavenger role in most reactive oxygen species and protect their cells against inactivation and mutagenesis (Felzenszwalb et al., 1998). Hammerhead shark (Sphyrnidae family) is identified as a highly derived, monophyletic group in the order of Carcharhiniformes, characterized by the presence of a ventrodorsally compressed and laterally widened pre-branchial head, known as the cephalofoil (Cavalcanti, 2007). The scalloped hammerhead, *Sphyrna lewini* is a globally exploited species of shark (Piercy et al., 2007).

The aim of our study was to evaluate the antimutagenic properties of Methanolic and Chloroforemic extractions of liver, cartilage and muscle of *Sphyrna lewini* with the Ames test.

Material and methods

Samples of *Sphyrna lewini* were caught on 15 February, 2007 from Bandarabbas city in the Persian Gulf in, frozen at -80^{o}C were transferred from Bandarabbas to the laboratory, the muscle, cartilage and liver separated in sterile situation separately

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were extracted with chloroform-methanol according to Blight and Dyer's (1959) method as has been described by Ribeiro et al. (2001). Sodium Azide, Potassium Permanganate, Histidine and Biotine and Arachlor-1254 were prepared with the mark of Merck (Frankfurt, Germany).

Bacterial tester strains

The bacterial strains used in this study were kindly provided by Dr. B. N. Ames (California University at Berkley). The tester strains genotype should be confirmed, because of this, fresh overnight Nutrient broth cultures were used. Strains of *Salmonella typhimurium* have defense in dark repair of mutations (UVRB) and are unable to synthesize a protein of the cell wall (rfa). The strains were tested for presence of the ampicillin resistance factor; that is a convenient marker and makes it possible to test the presence of the R-factor plasmid.

Preparation of mutagens

All of the chemical mutagens were dissolved in OMSO 1.5 μgml^{-1} Sodium Azide and Potassium Permanganate.

Metabolic activation system (S_9 mixture) (short for "9000 g supernatant")

Many mutagen need to be metabolized by the cytochrome p-450 dependent mono oxygenase system before they elicit mutagenic activity mammalian hepatic microsomes or 9000xg supernatant (S_9), which contain this system, are commonly used for the activation of promutagens to mutagenic metabolites. The S_9 mixture was prepared according to Maron and Ames (1983).

Sprague Dawley male rats were pretreated with Arochlor 1254.

The treated rats were starved 24h before they were sacrificed, then their livers were removed aseptically, minced, and homogenate natant (S_9 fraction) was stored as aliquots at -80^oC.

Antimutagenicity test

Methanolic and Chloroformic extracts of cartilage, liver and muscle of *Sphyrna lewini*, in the first stage; the plate incorporation assay as outlined by Maron and Ames (1983) was used. 2ml of a top agar containing 0.5 mM histidine/biotin, 0.05 ml of a fresh overnight grown Salmonella culture of the tester strains TA 100, 0.1 ml of Sodium Azide or Potassium Permanganate and 0.5 ml of samples were added. After pouring the soft agar on minimal agar plate, the plates were incubated in 37^oC for 48h.The extracts were tested against the mutation induced by various agents on *S. typhimurium*. The positive control plates contained Sodium Azide or Potassium Permanganate was considered as extracts. Without mutagens and test samples and 0/5 ml DMSO water considered as negative control. The revertant colonies were counted manually to determine the inhibitory effects, expressed as an inhibition rate. In stage 2, the antimutagenic potential of the extract (1mg/plate) that expressed over 90% inhibition was evaluated against mutagens adding 0.05 ml of S_9 using plate incorporation assay (Maron and Ames, 1983) and its revertants were counted after incubation of the plates at 37^oC for 48h. Tester strains were checked routinely to confirm genetic features using the procedure described by Maron and Ames (1983). Experiments were performed in triplicate. Moreover, the genotypes of the tester strains (TA100) should be tested

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more thoroughly, as contamination or absence of certain mutations in the strain may decrease the sensitivity of the bacteria to some mutagens (Maron and Ames, 1983). In Salmonella mutants, the rfa mutation allows larger molecules to pass through the cell wall thereby increasing its ability to detect mutants (Maron and Ames, 1983). In addition, the uvrB mutation allows an increase in detection capability, as it deletes the gene that codes for the DNA excision repair system (Maron and Ames, 1983).

The mutagenicity of Sodium Azide and Potassium Permanganate in the absence of test samples was defined as 100% or 0% Inhibition.

The calculation of percent inhibition was done according to the formula below:

$$\% \text{ INHIBITION} = [1 - T/M\ ] \times 100$$

Where, T is the number of revertant per plate in the presence of mutagen and the test sample and M is the number of revertants per plate in the positive control. The number of spontaneous revertants was subtracted from the numerator and the denominator (Negi et al., 2003; Ames, 1983). Data was reported as mean ± SD.

Table 1: Antimutagenic activity of Methanolic extracts of muscle, liver and cartilage of *Sphyrna lewini* against Potassium Permanganate in Salmonella (TA100)

Potassium Permanganate				
Average number of revertants				
Positive control	Negative control	Liver	Muscle	Cartilage
1256	489	1042	642	688
1256	488	920	1101	892
2521	619	848	656	451
		Liver	Muscle	Cartilage
	Inhibition٪	27.9	80.1	74.1
		43.8	20.2	47.4
		88.0	98.1	108.8

Table 2: Antimutagenic activity of Methanolic extracts of muscle, liver and cartilage of *Sphyrna lewini* against Sodium Azide (NaN_3) in Salmonella (TA100)

Sodium Azide				
Average number of revertants				
Positive control	Negative control	Liver	Muscle	Cartilage
1591	416	1329	418	448
2842	511	1421	675	789
2504	678	995	1780	679
		Liver	Muscle	Cartilage
	Inhibition	22.3	99.8	97.3
		61.0	93.0	88.1
		82.6	39.6	99.9

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Table 3: Antimutagenic activity of Chloroformic extracts of muscle, liver and cartilage of *Sphyrna lewini* against Potassium Permanganate in Salmonella (TA100)

Potassium Permanganate				
Average number of revertants				
Positive control	Negative control	Liver	Muscle	Cartilage
1945	389	1280	458	385
1735	405	1200	879	680
2427	549	640	1420	1360
		Liver	Muscle	Cartilage
	Inhibition	42.7	95.6	100.3
		40.2	64.4	79.3
		95.2	53.6	56.8

Table 4: Antimutagenic activity of Chloroformic extracts of muscle, liver and cartilage of *Sphyrna lewini* against Sodium Azide (NaN_3) in Salmonella (TA100)

Sodium Azide				
Average number of revertants				
positive control	Negative control	Liver	Muscle	Cartilage
2022	469	1424	477	1128
1964	426	1540	433	730
1485	697	1499	701	479
		Liver	Muscle	Cartilage
	Inhibition	38.5	99.5	57.6
		27.6	99.5	80.2
		-1.8	99.5	127.7

Table **5: Antimutagenic activity of Methanolic extracts of muscle and cartilage and Chloroformic extract of muscle of *Sphyrna lewini* against Potassium Permanganate in Salmonella (TA100) in the presence of S_9.**

Potassium Permanganate				
Average number of revertants				
Positive control	Negative control	Methanolic extract of muscle	Chloroformic extract of muscle	Methanolic extract of cartilage
1551	556	580	957	558
1864	472	519	881	501
1610	509	530	913	521
		Methanolic extract of muscle	Chloroformic extract of muscle	Methanolic extract of cartilage
Inhibition		97.6	59.7	99.80
		96.6	70.6	97.92
		98.1	63.3	98.91
	Mean	97.4	64.5	98.9
	SD.	0.7	5.6	0.9

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Table 6: Antimutagenic activity of Methanolic extract of muscle and cartilage and Chloroformic extract of muscle of *Sphyrna lewini* against Sodium Azide (NaN_3) in salmonella (TA100) in presence of S_9.

Sodium Azide				
Average number of revertants				
Positive control	Negative control	Methanolic extract of muscle	Chloroformic extract of muscle	Methanolic extract of cartilage
1881	594	649	598	595
1921	625	721	695	677
1894	566	670	630	640
		Methanolic extract of muscle	Chloroformic extract of muscle	Methanolic extract of cartilage
Inhibition				
		95.7	99.7	99.9
		92.6	94.6	96.0
		92.2	95.2	94.4
	Mean	93.5	96.5	96.8
	SD.	1.9	2.8	2.8

Results

The antimutagenic effect was considered moderate when the inhibitory effect was 25-40% and strong when it was more than 40%.Inhibitory effect of less than 25% was considered weak and was not recognized as a positive result. Tables.1-1 to 1-4 show the results obtained with the plate incorporation method in the *S. typhimurium* strain (TA 100) without S9 mix. Consequently the results shows the percentage of protective effect of the Methanolic and Chlorformic extracts of muscle, liver and cartilage of *Sphyrna lewini* on the reversion potential of the mutagens tested. All extracts were effective in reducing the number of frame shift mutation induced by Sodium Azide and Potassium Permanganate. As shown in table 5,6 the major result indicates that Choloroformic extracts and Methanolic extracts of cartilage and muscle extract are able to induce on evident decrease on the mutagenicity of the indirectly acting mutagen Sodium Azide or Potassium Permanganate, which both act as genotoxic compounds through a liver S_9 Fraction.

Discussion

The *S. typhimurium* reverse mutation assay is the most commonly used method to assess the mutagenic potential of test chemicals and natural substances which may cause base-pair and form shift mutation in the genome of this bacteria (Maron and Ames, 1983).The present study is a reverse mutation assay where the reduction in Histidine+revertant colonies in the Standard Mutagen induced plates by the addition of sample indicates the antimutagenicity of the sample. It has been suggested that regularly consuming anticarcinogens and antimutagens in the diet may be the most effective way of preventing human cancer. It was of interest to verify whether shark tissue was capable of antimutagenic action against known mutagens (Sodium Azide or Potassium Permanganate).Preliminary researches have revealed that shark cartilage has

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possible antimutagenic, antioxidant, anti-inflammatory, and analgesic activities, antianiogenesis (Fontenele et al., 1996; Fontenele et al., 1997). Natural products from flora and fauna are frequently used as nutritional supplements and medicaments. Evidence for shark-cartilage containing preparation functioning as an antimutagen was detected. The putative role of shark-cartilage containing preparation in protecting cells against lesions induced by hydrogen peroxide in normal and low iron level conditions was investigated. As the same in my case, these data suggest that shark-cartilage containing preparation can play a scavenger role for reactive oxygen species and protect against DNA lesions in cells (Gomes et al., 1995).As we know there is a close correlation between mutagenesis and carcinogenesis (Moron and Ames, 1983). Ruan et al. (1989) reported that antimutagenic substances may prevent cancer because they can destroy mutagens both inside and outside body cells, and block mutagens that damage DNA and cause mutation in cells therefore we suggest use of shark cartilage to prevent cancer. If your studies about fish processing (Hasanzati Rostami et al., 2010) and biotechnology (Rostamzad et al, 2010) were carries out in Iran in last decade. The mutagenicity of Sodium Azide or Potassium Permanganate in all cases was reduced by more than 80% in stage 2. The highest antimutagenic effect determined in the Potassium Permanganate and Sodium Azide as a mutagen was Methanolic extracts of cartilage. This suggests that cartilage extracts may help protect against free radical and reduce mutagen. The results of the present investigations demonstrate the significant antimutagenic activities of cartilage extracts. The findings suggest the potential of the extracts of *Sphyrna lewini* cartilage as a chemo preventive agent. Hence, the consumption of this shark or extract of cartilage or cartilage powder may actually be giving protection to the human body against mutation of cells and cancer inducing processed food substances we consume daily.

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