Including Effectiveness of the blue crab (*portunus pelagicus*) antioxidants inhibit oxidative stress

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Abstract: today, the role and importance of the human body antioxidant defense system in prevention of many diseases has been proved completely on the other hand the need to strengthen the immune system is inevitable that the consumption of synthetic antioxidants in numerous adverse effects Experts are always following the use of natural antioxidants in this regard must recommend extensive research to find natural resources and the needs of modern human antioxidant is ongoing results of many studies have shown that marine resources are rich in antioxidants are the blue swimmer crab research in this respect were fractions antioxidant extracted from muscle tissue extracts such, antioxidant capacity showed significant antioxidant addition fractions such significant effects in inhibiting oxidative stress, including inhibition hemolysis of red blood cells and protection of Thiol groups of blood showed in a laboratory.

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1. Introduction

The human body in a state health system, a balance between free radicals and anti oxidant rate each factor that causes interference can be above the equilibrium incidence of some diseases cause. Including atherosclerotic cancer and premature aging recent research necessary to strengthen the body's antioxidant system through diet or supplements nutritional shows, although the late twentieth century as the only plant sources of natural antioxidant sources are identified in the recent decade extensive research on fish showed that some species of marine plants and animals significant amounts of anti-tumor compounds, anti-viral and contain antioxidants, blue crab research in the Gulf of antioxidant capacity consideration Therefore, the species was muscle tissue above the isolation and the environment was then Buffering homogenize blue crab muscle tissue for extraction process Sequestration antioxidants ammonium sulfate and dialysis, and then it was sad, extract the above route by the end of gel from 150, then the maximum for the fraction of total antioxidants was selected in 5 different concentrations Measurement for efficacy in inhibiting oxidative damage was used in this section of the human red blood cell model used and the effectiveness of the blue crab fractions antioxidant in human erythrocyte hemolysis inhibition and the amount of blood Thiol protecting groups was Calculated reviews and tests show that the blue crab at the level of antioxidants can significantly inhibit human erythrocyte hemolysis and blood Thiol protecting groups are the results of this study blue crab as a rich source of natural antioxidants that can be defined as the a source of marine biotechnology in food production complexes and compounds for drug prevention and treatment of disease pathogenesis that free radicals are used.

2. Material and Methods

Crab fishing by trawler were incubated with samples of ice and immediately transferred to the laboratory, the samples described tissue, muscle separation and buffer Tracy (5%, 5.7PH =) transferred along with muscle tissue was then centrifuged buffer homogenize iceman temperature for 10 minutes c 40 and g × 6000, was centrifuged, sedimentary layer was discarded, for the separation layer supernatant Sequestration method was used proteins thus under the terms of the first magnetic mixer adding the necessary amounts of solid ammonium sulfate saturation level was 50 percent, after centrifugation (g × 6000, c40) for 30 min, sedimentation at this stage was maintained and sup layer according to the previous stage saturation of 70 percent required rate of solid ammonium sulfate was added after this stage centrifugal sedimentation with sediment before step combined amount of the buffer size after mixing Tracy added, for 12-hour dialysis, the dialysis bag was transferred (input buffer during the dialysis, buffer was Tracy was replaced every 2 hours) after dialysis Inundation by sucrose for 10 hours was the end result of solution outside the dialysis bags to human transmission found and the

potential for antioxidants extraction chromatography was used in the first chromatography column containing seeds Pharmacia diameter 5.2 cm and 60 cm in height, Tracy salt buffer mobile phase, flow rate 40 ml per hour was elected , fractions obtained by the end of the device fraction collector column, were collected (170 fraction) then all the above fractions light absorption wavelength 280 nm and 540 nm was read and help protein and Bioret kit by Randox rate Each antioxidant was calculated fractions (122-117 and 97-93 and 58-52 and 26-20), which had the maximum amount of antioxidants to second column purification transferred to the second phase chromatography column containing DEAE cellulose column diameter 5.1 cm and 60 cm height Tracy salt buffer mobile phase, flow rate was 40 ml per hour by the end of the column device 150 fraction collector raised before the rate matching step, the amount of total protein and antioxidants, all calculated fractions was then fractions (89-84 and 71-68 and 48-42 and 27-25 and 16-12), which had the maximum amount of antioxidants microbial filter method, is sterile in order to review the effectiveness of the inhibition of oxidative damage were used.

2.1. The impact on blue crab antioxidants inhibits oxidative damage in a laboratory

2.1.1. Liz inhibition of red blood cells
A - **Suspension the blood**:

The blood in healthy individuals heparin pipe and then collected by centrifugation, plasma was separated from red blood cells, Rbc above three times by saline washing, and ultimately pure red blood cells were prepared from pure erythrocyte above dilution method in phosphate buffer salt, 2 percent suspension (equivalent hematocrit 12) was prepared.

B - Hemolysis of red blood cells

Of antioxidant fractions sterile, the pipe with concentrations (100%, 75%, 50%, 25%) was produced (with dilution buffer were PBC), then pipe that includes Prepare the mixture for 2 hours the temperature was then incubated with 370 centrifuges (g × 1000) of 10 minutes and the upper layer of light absorption wavelength 415 nm was read (represents the amount of hemolysis) and the control tube (No extract fractions) the rate of hemolysis were compared with values based on percent inhibition of hemolysis in the vicinity with fractions, compared to the control rate (percentage of hemolysis showed percent) was calculated.

2.2. Protection of Thiol groups

After the suspension of red blood cells, 50 micro litter of the suspension Rbc 1000 Tracy and 50th micro litter buffer fraction antioxidant (concentrations of four) the composition and light absorption wavelength of 4 micro litter 12 nm was read then pipe above 20 micro litter reagent DTNB added and 30 minutes and then incubated in the laboratory temperature optical absorption was read at 412 nm, pipe controls included buffer suspension cells and introduced Tracy DTNB prepared and its optical absorption 413 nm was read , then read the values in light absorption were the following formula according to the amount of computation protected Thiol groups were obtained Thiol protecting groups for the amount of each of the five branches fractions antioxidant concentrations separately tested and was measured at the end of percentage protection for each concentration fractions Thiol groups compared with control was calculated.

3. Results

Determine the antioxidant capacity of a blue crab: After chromatography, fractions from each stage (gel filtration, ion exchange) levels of total protein and total antioxidant were analyzed (Tables 1 and 2).

3.1. effect of antioxidant fractions blue crab in the inhibition of hemolysis of red blood cells

Fractions group of the A_1 the most total antioxidants were also dilution, fractions with total antioxidant levels (12, 9, 6 and 3 and 2 / 1) mmol liter in the equivalent concentrations (100, 75, 50, 25 and 10) respectively were prepared, then each fraction cells were in the vicinity, after adding a certain amount of AAPH for 2 hours incubation period temperature was 37 degrees then created hemolysis rate for all pipes and pipe control (No fraction antioxidant) spectrophotometric method at 415 nm wavelength was read and the percentage hemolysis inhibition was calculated (compared to control samples), all the numbers obtained in 0.05> p was considered significant (Table 3).

As is clear in Table 3 fractions antioxidant concentration of 100 percent blue crab (the amount of antioxidants in 12 mmol L) no effect on hemolysis of red blood cells did not fraction concentration of 75 percent (total antioxidant levels in 9 mmol L) maximum effectiveness in inhibiting the rate of hemolysis showed 95 percent plus extract dilution effects of the above reduction in hemolysis fraction the lowest effect concentration and the rate of 10 percent antioxidant 2 / 1 mmol l showed that (a rate 7 / 19 percent).

Table 1: Results of total protein and total measured antioxidant derived from gel filtration chromatography blue crab muscle tissue

step purification	fraction	Total protein(mg/l)	Total antioxidant (m mol / Lit)
(sephadex G100)	crude tissue extract	1360	42.5
	group A (26-20)	580.5	21.2
	group B (58-52)	204.9	6.8
	Group C (97-93)	405	14.7
	Group D (122-117)	167	5.3

Table 2: Results of measurement of protein and total antioxidant fractions A group from ion exchange chromatography

stage purification	fraction	Total protein (mg/l)	Total antioxidant
DEAE cellulose	group A (26-20)	580/5	21/2
	A_1	199	12
	A_2	105.4	7.3
	A_3	174	10.2
	A_4	59.5	5.1
	A_5	29	4.6

3.2. The effectiveness of the blue crab fractions antioxidant protection Thiol groups (-SH) blood

In this section the concentrations of five triple antioxidant fractions blue crab was prepared the same step, then any effects in protecting blood Thiol groups were determined according to procedures based on mg protein vs control calculation, all numbers obtained in 05/0p <are considered significant. (Table4).

Table 3: The rate the effectiveness of the blue crab fractions antioxidant in inhibiting hemolysis of red blood cells

Fraction	Total antioxidant (mmol/l)	Inhibition percent
control	0	0
concentration 100 percent (Group A)	12	0
concentration of 75 percent	9	98.5
concentration of 50 percent	6	89.2
concentration of 25 percent	3	75.9
concentration of 10 percent	1/2	46

Table 4: Effect of blue crab fractions antioxidant protection Thiol groups fraction type and amount of antioxidant

fraction type and amount of antioxidant	Protein (mol/mg)	Percent to control
control	930	100
12	920.7	99
9	890	95.7
6	821.1	88.3
3	605.4	65.1
1.2	404.5	43.5

4. Discussion

The antioxidant capacity measurement showed that blue crab fractions muscle tissue from this species contain considerable quantities of antioxidants average 5 / 10 mmol were lit. Toresin and Kerstin 1998 (8) also reviews some fish, including salmon successful extraction of synthetic antioxidants were Astaxantin name. Clark and colleagues (1998), while reviews of gel were able to jack in the antioxidant properties of these prove. Smith and Bell 1996 (9), while crab carcinus menas review declared that Super oxide enzyme extracted from a laboratory in such a strong antioxidant properties shows. According to World Health Organization (WHO 2007) standard rate of serum antioxidant capacity in normal individuals, 1.03 - 1.77 mmol l that this value depends on the age, sex,

nutrition and the geographical conditions of the Hang the tea and colleagues Watch 2007 (16) antioxidant capacity in such a way the average shrimp Penaeus monodon have announced 4 compared with values above antioxidant capacity blue crab is significantly more.

For measuring the effectiveness of antioxidants blue crab model of red blood cells were used. Cruz Silva and colleagues (2000) (17) red blood cell model ideal model to evaluate stress Oxidative laboratory environments experience and introduced them announced that free radicals attack cells, causing lipid per oxidation and membrane proteins and eventually to hemolysis are, in this study results showed that fractions A1 (100 percent concentration and antioxidant levels in 12 mmol L) not only to prevent blood cell hemolysis, but also has had the effect per oxidant.

1999 Partasaraty and colleagues announced that the antioxidants in different concentrations can be different effects of antioxidant vitamin E Per oxidant even have the same feature to update itself, State hawthorn 1998 (18) showed that concentrations of vitamin C in 60-100 property antioxidant concentrations, but not more than this amount will Per oxidant properties, Frag and Partners 1999 (19) announced that its antioxidant properties and chemical components of each combination of concentration is related. Other fractions blue crab concentrations 75, 50 and 25 percent respectively, the values 9, 6, 3 and 1.2 mmol l antioxidants were all showed inhibition of addition, as Table 3 shows the reduced dose antioxidant effect Atpase considerably reduced. Burton and colleagues 2009 declared that what proteins Thiol groups who are building cell membranes and how those buildings hemoglobin (especially glutathione) exist, a very important factor in membrane stability and are soluble oxidative stress during this groups to the oxide and formation of disulfide bond to protect the cells against free radicals are combined, so if Thiol groups against oxidation in the cell's ability to protect against oxidative stress is taken up.

This study showed that blue crab fractions antioxidant to protect the good Thiol groups have a protective effect above fraction dilution and reduced antioxidant capacity, it declined (Table 4).

Considering the importance of antioxidants and their role in prevention of the pathogenesis and treatment of disease free radicals (13) The results of this study showed that blue crab having significant amounts of antioxidants in this area is of great import can be a natural source of antioxidant should be exploited.

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