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Use Of C-Phycocyanin To Reduce The Toxicity Of Arsenic On Rats

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Abstract

Introduction: Arsenicis one of the heavy metals found in drinking water andone of the free radical generators. In comparison, the blue protein C-phycocyanin occupies a unique role among the many bioactive components of Spirulina due to its applications in the food industry and the treatment of some diseases.

Methods: Twenty male rats were used in the experiment, have been divided into four groups, one group of five rats represented the control group. five rats were administered only sodium arsenite 6.3 mg/kg orally, while another five rats administered both C-phycocyanin300 mg/kg and sodium arsenite at the same time; meanwhile, the remaining group of five rats were fed C-phycocyanin only; after that, their hematological and biochemical parameters were examined.

Results: Our results have shown that C-phycocyanin at a dosage of 300 mg/kg could decrease arsenic toxicity by reducing elevated liver enzyme levels AST and ALT and reduction in endogenous antioxidant enzymes SOD, CAT, and MDA. Furthermore, the hematological results of rats fed with C-phycocyanin plus sodium arsenite investigateda staistiticalimprovement in plateletsand elevated WBC levels,whilethe levels ofhematocrit,hemoglobin and erythrocytesreveals asignificant improvement compared with groups that treated with only sodium arsenite.

Conclusion: It was concluded that arsenic-induced oxidative stress has been attenuated by C-phycocyanin. As a result, C-phycocyanin may be protecting the liver and the entire body from arsenic toxicity.

Keywords: C-phycocyanin; antioxidant enzymes, arsenic toxicity.

Introduction

Spirulinaplatensisis a microscopic and filamentous cyanobacteriumthat has been proposed as natural and environmentally safe microalgae as a candidate for toxicant elimination, such as heavy metals which can chelate and remove them (Fang et al., 2011). Two phycobiliproteins are formed by Spirulina: C-phycocyanine as the main pigment and alloC-phycocyanine which is present in much lesser amounts at a ratio of approximately

10:1(Sotiroudis and Sotiroudis, 2013).C-phycocyanin is an algae-isolated, light-harvest pigment binding protein (Eriksen, 2008). C-phycocyaninwhich shows beautiful ocean blue is mostly extracted from Arthrospira (Kumar et al., 2014). C-phycocyanin is a natural, non-toxic pigment with high solubility in water is used as a natural blue dye in a commercial application such as food coloring agents and the cosmetic industry, and its price strongly depends on its purity (Stanic-Vucinicet al., 2018). It's used in ice cream, desserts, chewing gum, powdered drinks, cold drinks, cake, candy, sweets, pudding, milkshakes, chocolates, snacks, jelly, pasta, tablets with a sugar coating, capsules, etc.

A technique of extraction was a prerequisite for maximizing algae recovery from C-phycocyanine. The extraction of C-phycocyanine requires a cell breakdown and release of the cell's protein (Niuet al., 2006). The alternative combination of gel adsorption with ion-exchange chromatography can be used to extract and purify this pigment (Herrera et al. 1989). C-PC extraction from wet cyanobacterium biomass is also recorded in some papers (Bermejo et al., 2006; Soniet al., 2008). Nonetheless, for large-scale operations, mechanical cell decay methods (Gacesa and Hubble, 1990) are generally favored because the biomass should be completely decomposed with high product and activity yields.

The methods used in the extraction of C-phycocyaninare: first, freezing and thawing method (Zhang et al., 2012), S. platensis powder is dissolved to a certain degree in water,. Secondly, direct osmosis (Herrera et al., 1989), S. platensis powder is dissolved for a night in distilled water, low-salt or buffer solution, causing breakage of the cell wall of S. platensis; this approach is easier but time-consuming. Third, in the ultrasonic method (Li et al., 2007), Ultrasound treatment of the S. platensis solution accelerates the rupture of cell walls by direct osmosis. This approach greatly decreases the length of treatment. Fourthly, in the chemical reagent method, active surface anion, for example, has been used to smash cell membranes and protein dissolving within moderate circumstances (Lin et al., 1997). This approach maintains the cell wall intact and enhances the purity of C-phycocyanine. Fifth, enzyme digestion, in this method KCL lysozyme used to destroy the cell wall of S. platensis, increasing the yielding of extraction. This approach would take a limited period so it is an appropriate process for a lot of samples, nevertheless, it has specific potential hydrogen, temperature, and other circumstances (Li et al., 1999).

Arsenic is a naturally occurring element that is ubiquitous in the environment. Arsenic is a gray metallic element, it is classified at the Periodic Table in group 15; it is toxicologically known as heavy metal, in general, it is present in the air,soil andwater from naturalsources (Mandal and Suzuki, 2002). Worldwide there are millions of people who expose to arsenic at

levels that are considered more than safe. Many of these people are exposed to the consumption of contaminated drinking water. Chronic arsenic exposure was related to a wide variety of diseases and human health issues, there are reports about arsenic Poisoned by oral consumption from contaminated food and drinking water (Hu et al., 2020).

As the overproduction of reactive oxygen species is well known to involveof arsenic toxicity and associated diseases, the use of plants antioxidant, has been extensively used as a therapeutic agent against arsenic- toxicity.The antioxidants materialscan reduce the toxicity of arsenic by chelating and scavenging free radicals (Rana, 2007). The arsenic ingested in inorganic forms has been converted into monomethyl-and dimethyl-metabolites in varying amounts and does seem to be toxic by action at various sites, probably contributing to fetal mortality (Vahter, 2000).

The aim of this current work was to estimate the protective activity of purified C-phycocyanin extracted from Spirulinaplatensis alga against the adverse effect and the oxidative damage induced by arsenic. The biochemical and hematological changes in male albino rats are the focus of our research.

Methods

Spirulinaplatensis was obtained from the Environmental Laboratory at the College of Science, University of Baghdad, Iraq. Culture modified Zarrouk media (Al-Yasiri, 2018) using special tools to achieve optimal conditions (direct sunlight, aquarium heaters, calibration of pH was done at 10 to 12, aquarium air pump and adding a glass cover can decrease the amount of evaporation and keeping the heat at night, and also prevent contamination from any airborne organisms).

Extraction of C-phycocyanin

About 125 g of S.platensis powder was mixed with 1 litter deionized waterand continuously shaken at 30 °C for 24 hours to decompose algae cell wall. Mixed substances were centrifuged for 10 minutes at 5000 rpm and filtered to remove the cell debris using Whatman filter paper No.1 and the obtained extract was considered as crude extract (Chu et al., 2010).

Purification of C-phycocyanin

Ammonium sulfate$(NH_4)_2SO_4$ 65% (Bio Xtra, >99 %; Sigma-Aldrich) was added in 100 ml extract to achieve 25% and 50% saturation with continuous stirring for one hours, the product solution was stored overnight in dark condition at 4°C , after that the precipitation was collected by centrifugation at 15,000 g for 30 min. the blue precipitate was dissolved in

0.1M PBS pH 7 and stored at 4°C until the examination time while the colorless, clear supernatant was discarded (Kumar et al., 2013; Prabakaran and Ravindran, 2013; Kumar et al., 2014),by using dialyzes membrane,ten ml of ammonium sulfate extract was dialyzed against the extraction buffer,the resultant extract was removed from filtered through 0.45 µm filter, after that the absorption spectra of C-phycocyanin was measured by using UV–vis spectrophotometer (Boussiba and Richmond, 1979).

Calculation of C-phycocyanin concentration: The concentration ofC-phycocyanin(mg/ml) was calculated according to the following equation (Anteloet al., 2010):C-phycocyanin mg/ml= (A620 − 0.474 × A652) / 5.34

Where5.34 is a constant factor, A620 is absorbance at 620 nm, A652 is absorbance at 652 nm

C-phycocyanin extract purity: C-phycocyanin extractpurity wasmeasured according to the A^{620}/A^{280} ratio.Theconcentration of proteins in the solution calculated at 280nm whilethe maximum absorption of the C-phycocyanin calculated at 620 nm .purity=A^{620}/A^{280}(Bennett and Bogard, 1973).

Extraction yield: was calculated according to following equation (Silveiraet al., 2007): Yield = (C-phycocyanin − V)/ DB

Where Yield is the C-phycocyaninextraction yield(mg) / dry biomass (g), V is the solvent volume (ml) and DB is the dry biomass (g).

Determination of antioxidativeactivity ofDPPH: The antioxidativeaction of C-phycocyanin were measured by using the DPPH assay (Kumar et al., 2008), were used (10, 25, 50, 75, 100, 150, 200, 250 and 300 mg/ml). DPPHantioxidative activity was calculated according to the following equation:

$$\textbf{Scavenging activity}\ (\%) = \frac{\mathbf{A}\ \text{blank} - \mathbf{A}\ \text{sample}}{\mathbf{A}\ \text{blank}} * \mathbf{100}\%$$

A Blank is the C-phycocyanin and ethanol solutionsabsorbance(without DPPH), where A Sample is the C-phycocyanin and DPPH absorbance at 517 nm.

Experimental protocol

Five months of 180–200g of male albino rats were housed with a temperature of 25±1 °C.

Arsenic ($NaAsO_2$) was chosen as a free radical generator in rat bodies at a dose of 6.3 mg/kg body weight.WHO have decreased the acceptable threshold level from 50 to 10 µg/L concerning health effects of drinking arsenic-contaminated well water(WHO, 2016)

Rats were divided at random into 4 groups of five rats each,

I: animals were served as control.

II: animals were administered orally 300 mg/kg of C-phycocyanin.

III: animals were administered arsenic as sodium arseniteat 6.3 mg/kg orally.

IV: animals were administered 300 mg/kg of C-phycocyanin followed by 6.3 mg/kg of arsenic as sodium arsenite.

All these treatments are performed daily for 8 weeks to assess their effectiveness (Sengupta, 2011). All rats were exposed to ether anesthesia by implies of a sliding upper chamber for sampling.

Parameters measurement

Liver enzymes: AST and ALT were measured using the serum colorimetric determination technique offered by Biomerieux-France for the kits.

Antioxidant enzymes measurement (SOD, MDA, CAT): Cell Technology SOD colorimetric Detection Kit-USA, utilizes a highly water-soluble tetrazolium salt, absorbance can be measured at 440 nm. Therefore, inhibition activity (SOD) can be determined (Brown and Godstein, 1983). Malondialdehyde (MDA) was determined in serum using the procedure of Lipid Peroxidation (MDA) Assay Kit from BioVision, USA, by procedure described by (Guidet and Shah, 1989). catalase was determined according of the Catalase Assay kit from BioVision, USA.

Complete blood counts (CBC) were performed by anautomated digital counter.

Results

. The results ofalgae growth curve showed log and lag stationary phases. However, there was no discernible lag phase, but the exponential growth phase was longer and more obvious at 10 -14 days and above; while the stationary phase shorter and the culture did not reach the death phase.

The purification results showed effective method for purifying C-phycocyanin from the cyanobacterial strain S. platensis.

After ammonium sulphate precipitation, the purity of C-phycocyanin was 1.2 and after dialyses the purity was 1.8, this revealed the purity increased many times through purification steps. The absorption spectra of the purified C-phycocyanin reported a prominence at 620 nm.

Antioxidative capacity (In vitro free radical scavenging ability): The anti-oxidant action of C-phycocyanin extract results investigated that color turns from purple to yellow at all nine concentrations. It was observed that 300 mg/ml was better than the other concentrations by

achieving the higher antiradical activity 94.284%;

However, rats treated with sodium arsenite and C-phycocyanin extract for 8 weeks.The blood parameters such as WBC, RBC, Hb, PCV, Platelets; liver function enzymes AST and ALT; enzymatic antioxidants SOD, CAT, and MDA showed significant alterations when compared to control groups

As shown in table 1, rats treated with only sodium arsenite reported a significant increase in ALT andAST level as compared with the control and only C-phycocyanin group; while rats which given C-phycocyaninand sodium arseniterevealed a significant decrease of ALT andAST levels .

Table 1: Effect of C-phycocyaninon liver enzymes in rats treated with sodium arsenite.

Group	Mean ± SE	
	GOT	GPT
Control	76.00 ±0.57 b	28.67 ±0.88 b
C-Phycocyanin 300 mg\kg	74.67 ±2.02 b	23.67 ±0.88 c
Sodium arsenite, 6.3 mg/kg	82.67 ±1.45 a	38.67 ±0.88 a
300 mg/Kg of C-Phycocyanin + sodium arsenite 6.3 mg/Kg	76.00 ±2.51 b	30.00 ±0.57 b
LSD value	5.854 *	2.662 **
The different letters in the same column differed significantly.		

The results of antioxidant enzymesobtained a significant increase in three types of endogenous antioxidant enzymes SOD, CAT, and MDA in rats treated with only sodium arsenite, in comparison with C-phycocyanin plus sodium arsenite group and rats that was fed on C-Phycocyanin extract only, which showed a reduction in SOD, CAT, and MDA. Interestingly, this is confirming the results in table.1 of liver functions which provoked remarkable hepatoprotective activity by a significant reduction of MDA level, SOD, and CAT activity of sodium arsenitetreated rats.

Table 2: Effect of C-phycocyanin extract on the (SOD, MDA, and CAT) in rats treated with sodium arsenite.

Group	Mean ± SE		
	SOD	CAT	MDA
Control	2.77 ±0.08 b	2.16 ±0.08 c	2.10 ±0.05 c

C-Phycocyanin 300 mg\kg	2.03 ±0.08 c	1.80 ±0.05 d	1.33 ±0.03 d
Sodium arsenite, 6.3 mg/kg	3.16 ±0.14 a	2.87 ±0.08 a	2.83 ±0.06 a
300 mg/Kg of C-Phycocyanin + sodium arsenite 6.3 mg/Kg	2.20 ±0.05 c	2.46 ±0.03 b	2.43 ±0.03 b
LSD value	0.326 **	0.230 **	0.163 **

While hematological parameters in the table.3 show a significant increment of WBC count in groups were injected with 6.3 mg/Kg sodium arsenite compared with control and C-phycocyanin group, while rats were given C-phycocyanin plus sodium arsenite exposed a significant improvement of elevated WBC. Also, there was a significant reduction of PCV,Hband RBC count, in arsenic groups compared with the control andC-phycocyanin fed group.

As for platelet count, the results of platelets concluded increment in the mean values of platelets in groups were given sodium arsenite,

Table 3: Effect ofC-phycocyanin extract on WBC, RBC, Hb, PCV, and Platelets in grouptreated with sodium arsenate.

Group	Mean ± SE				
	WBC	RBC	Hb	PCV	Platelets
Control	10.56 ±0.23 c	8.63 ±0.72 b	12.12 ±0.06 a	37.36 ±0.20 a	809.67 ±3.38 c
C-Phycocyanin 300 mg\kg	7.86 ±2.98 c	8.36 ±0.37 a	12.05 ±0.06 a	37.06 ±5.20 a	802.67 ±5.20 c
Sodium arsenite, 6.3 mg/kg	21.33 ±0.61 a	5.48 ±0.56 c	10.04 ±0.19 c	31.13 ±0.57 c	1586.67 ±32.12 a
300 mg/Kg of C-Phycocyanin + sodium arsenite 6.3 mg/Kg	16.26 ±0.43 b	8.76 ±0.24 ab	10.94 ±0.14 b	33.83 ±0.42 b	1156.33 ±37.69 b
LSD value	5.034 **	1.657 **	0.420 **	1.260 **	80.343 **

Discussion

The water extraction method was adopted because C-phycocyanin is water-soluble and alcohol- and ester-insoluble (Liu et al., 2016). It is considered a modification for the direct osmosis extraction method (Herrera et al., 1989). The ammonium sulfate which used as precipitatingfactor to produce ahigh C-phycocyanin in the extract (Benedetti et al., 2006).

Arsenic causes elevation of ROS in circulation (Hernández-Aquino and Muriel, 2017).However, the elevated serum AST and ALT levels could be indicative of liver damage (Islam et al., 2011). One of the markers of hepatotoxicity is the activity of ALT, so elevated levels of ALT suggest liver dysfunction (Kashyapet al., 2009). ALT is just cytoplasmic, but AST is located in the cell cytoplasm and mitochondria. But C-phycocyanin can efficiently scavenge free radicals and contribute to stability of cellular and basal membranes (Safari et al., 2020).

The concept of an antioxidant is any substance present at low concentrations significantlyprevents or slows the substrate oxidation (Halliwell and Gutteridge, 1989).

The SOD and CAT, as antioxidant enzymes that scavenge harmful ROS; while MDA was elevated when cells face environmental toxicants (Mohamed et al., 2016). This explains the significant increases of MDA levels in rats treated with sodium arsenite; and it has been noted that the antioxidant potential of the protein C-phycocyanin reduced the level of lipid peroxidation through provided significant recovery in depleted SOD activity (Farooq et al., 2014).

The WBC is protects the body fromdifferent infection (Erlingeret al., 2004).Shortening the lifespan of erythrocytes that lead to anemia is a typical sequel in arsenic-exposed populations, reported environmental exposure to arsenic was associated with anemia (Biswas et al., 2008; Mahmud, 2009).

this rapid elevation of platelets could be returned to the release of stored platelets from the spleen (Gammulleet al., 2012). Platelets are increasingly considered as critical players in immune responses, their immunoregulatory effects are in part conjugated with platelet interactions with innate immune cells such as neutrophils and monocytes (Duerschmiedet al., 2014). Decreased of WBC, platelet in rats which given C-phycocyanin are criteria for immune system activation. It seems that C-phycocyanin extract may prevent hemolysis of RBC and maintain WBC and platelets from negative effects of ROS, because of their antioxidant properties. Recent studies have demonstrated platelets contribute to atherosclerosis, sepsis, hepatitis (Smyth et al., 2009)

Conclusion: It was concluded that C-phycocyanin at a dosage of 300 mg/kg has a proven antioxidant action through decreased arsenic toxicity by reducing elevated liver enzyme levels AST and ALT as well enhances the body defense system by a reduction in SOD, CAT, and MDA; therefore C-phycocyanin can efficiently scavenge free radicals and be a therapeutic option for shielding the liver from arsenic poisoning.

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