



Full Length Research Paper

Effects of shrimp contaminated with cyanobacterial toxins on hepatic cytoplasmic proteins and mitochondrial redox state in albino rats

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ABSTRACT

The dose-response effects of extracts of fresh and dry shrimps contaminated with cyanobacterial toxins on total hepatic proteins and redox state were determined in the livers male of albino rats. Redox state was determined based on mitochondrial $NAD^+/NADH$ ratios which was measured at 260nm and 340nm, respectively. Total protein was determined in liver cytoplasmic fraction by the biuret method. The rats were grouped and fed with various doses of either fresh or dry shrimp extracts, while control rats were not fed either shrimp extract. Results show classical dose-response decreases of over 50% in cytoplasmic total protein content in rats fed with 2.5ml/kg bw of fresh shrimp extract and 5.0ml/kg bw of dry shrimp extract respectively when compared to the control value. Similarly, there were decreases in mitochondrial $NAD^+/NADH$ ratios in rats fed with 2.5ml/kg bw and 5.0ml/kg bw of fresh and dry shrimp extracts over control, although the percent decreases were below 25% of control. These results suggest the probable interference of the toxin with redox reaction pathway in the mitochondria and inhibition of cytoplasmic protein "pool", and thus potential hepato-toxicity to humans in the Niger Delta who consume these shrimps on a daily basis, and consistent with other reports by others

Keywords: Albino rats, shrimp, cyanobacterial toxins and cytoplasmic.

INTRODUCTION

The Rivers State axis of the Niger Delta region of Nigeria water-ways are major commercial sources of seafoods including shellfish, crustaceans and different species of fish; hence there is a very high consumption of seafoods by the population. Indeed, it is their staple diet.

The environment is saline and cyanobacterial (blue-green algal) blooms have become common occurrences. Cyanobacteria are present in all marine waters and are photosynthetic blue-green algae classified with bacteria in the kingdom monera (Vanora et al, 2012).

Consumption of seafoods contaminated with cyanobacterial toxin has been reported to have hepatotoxic effect (Shaw G,R et al, 2009). Cyanobacterial blooms are toxic and have harmful effect on humans whether through drinking water or through consumption of seafoods and the toxins from the blooms were reported to inhibit protein phosphatase (Falconer et.al, 2011). Exposure to high levels of species of cyanobacteria producing toxins causes amyotrophic lateral sclerosis. (Day P. et al, 2009) Cyanobacteria toxins accumulate in seafoods during cyanobacterial bloom

(Eaglesham G. K, 2004) and have hepatic and renal toxicity and in many cases the toxicity is sublethal to the aquatic species (Gross Y, 2002) and is also associated with carcinogenicity (Bynder P.G, 2001).

In this paper, we describe the probable molecular mechanism or pathway of the hepatotoxic effect of shrimp contaminated with cyanobacterial toxins in albino rats.

MATERIALS AND METHODS

Collection of cyanobacterial contaminated shrimp

Fresh shrimp and water were collected from St. Bartholomew River in Rivers State, Niger-Delta, Nigeria and subjected to microbial tests which confirmed the presence of cyanobacteria. This was consistent with report by the International Journal of Environmental Research and Public Health in 2012 which reports that cyanobacteria are abundant in fresh, brackish and marine waters worldwide.

Table 1. Total Protein Content of Liver Cytoplasmic Fraction in Rats fed with 2.5ml/kg bw and 5.0ml/kgbw of Fresh and Dry Shrimp Extract

| Treatment | Average Total Protein Concentration (mg/ml) | Percentage decrease over control |
|---|---|----------------------------------|
| Control (Not fed) group | 90.3 | - |
| Fed with 2.5ml/kg bw of fresh shrimp solution | 67.8 | 24.91 |
| Fed with 5.0ml/kg bw of fresh shrimp solution | 52.5 | 41.86 |
| Fed with 2.5ml/kg bw of dry shrimp solution | 57.4 | 36.43 |
| Fed with 5.0ml/kg bw of dry shrimp solution | 41.6 | 53.93 |

Preparation of 10% (w/v) Each of Fresh and Dry Shrimp Extracts

100g each of fresh and oven-dried shrimps were separately blended in 100ml distilled water and the solution was made- up to 1000ml with more distilled water.

The solutions were thoroughly shaken and allowed to stand at room temperature for four (4) hours in order to dissolve the cyanobacteria toxins (blooms) after which, they were thoroughly shaken again and filtered through cheese cloth to remove the shaft. The filtered solutions, now extracts, were stored at 4°C and used to feed the animals by gavage for fourteen (14) days.

Treatment of Animals

Fifty adult male albino rats (150gms each) were obtained from the animal house in the University of Port Harcourt Rivers State, Nigeria. The animals were brought to the biochemistry unit in the Rivers State University of Science and Technology, Port Harcourt, Nigeria and were allowed to acclimatize for four days. They were subsequently divided into five groups A, B, C, D, and E of ten animals per group. Group A served as control while groups B, C, D and E were fed with fresh or dry shrimp solutions by oral gavage at doses of 2.5 ml/kg body weight and 5.0ml/kg body weight.

The treatment was done every 24 hours (once a day) for fourteen days, the control (untreated) group was not treated with either of the shrimp extracts but were sacrificed on the fifteenth day with the treated animals. The livers of all were excised and stored at 4°C in 0.05M potassium phosphate buffer of p^H. 7.5.

Extraction of Mitochondrial and Cytoplasmic Fractions

Mitochondrial and cytoplasmic fractions were extracted from the liver homogenates of both treated and control

rats as described in detail elsewhere (Oruambo, I. F., 2006; Oruambo, I. F. et al, 2007).

Total Protein Estimation of the Cytoplasmic Fraction

Total protein concentration of the cytoplasmic fraction was done by the Biuret reagent method as described elsewhere (Oruambo I. F. et al, 1987).

Determination of NAD⁺/NADH Ratio of the Cytoplasmic and Mitochondrial Fractions

The NAD⁺/NADH ratio for both the cytoplasmic fraction and mitochondrial fraction was done by ultraviolet and visible spectrophotometry at 260nm (NAD⁺) and 340nm (NADH).

RESULTS

DISCUSSION

Table 1 shows that there are decreases in absorbance and hence in concentrations of cytoplasmic protein in the groups fed with both fresh and dry shrimp extracts, compared to control, and greater extent of decreases in the groups fed with dry shrimps extract than fresh shrimp extract.

The percent decreases are near-linear in both groups suggesting that there is interaction of the toxins with protein synthetic machinery, such as detoxification enzymes. Of relevance is the near-dose response decreases in the cytoplasmic total protein concentration. This may be consistent with the finding by Falconer, et al in 2011 of inhibition of an enzyme protein, protein phosphatase by these toxins.

However, it is interesting that the dry shrimp extract induced the greater decrease in cytoplasmic protein content than the fresh shrimp extract, thus it is probable that the toxins may be heat-resistant/stable.

In Table 2, the dose-response decreases, in liver mitochondrial NAD⁺/NADH ratios between shrimp-fed rats and control rats shows a similar pattern as that in Table

Table 2. NAD⁺/NADH Ratio of Liver Mitochondrial Fraction of Albino Rats Fed with 2.5ml/kg bw and 5.0ml/kg bw of Fresh and Dry Shrimp Extract

| Treatment | Average Total NAD ⁺ /NADH Ratio | Percentage Decrease Over Control |
|---|--|----------------------------------|
| Control (Not fed) group | 0.589 | - |
| Fed with 2.5ml/kg bw of fresh shrimp solution | 0.563 | 4.41 |
| Fed with 5.0ml/kg bw of fresh shrimp solution | 0.543 | 7.87 |
| Fed with 2.5ml/kg bw of dry shrimp solution | 0.483 | 18.00 |
| Fed with 5.0ml/kg bw of dry shrimp solution | 0.455 | 22.75 |

1. Again, the extent of decrease in the dry shrimp is greater than that for fresh shrimps. In both cases, the decreases in NAD⁺/NADH ratio suggest increased oxidation reactions in the mitochondria because as NADH levels increase, NAD⁺ content depletes. This is suggestive of increased oxidation in the mitochondria in response to toxin-induction.

This may have implication in increased ATP-energy production in the mitochondria again perhaps in response to shrimp toxin induction, or increased oxidation-driven detoxification.

Once again, as in the results for cytoplasmic proteins in table 1, it seems the toxins are heat-stable/resistant because the dry shrimp extract induced a greater extent of decreases in NAD⁺/NADH ratio, and invariably, increases in NADH levels, ie. accelerated NAD⁺ linked oxidation whereby NAD⁺ level is depleted.

It is plausible therefore to suggest that there is a probable hepatotoxicity of the dry and fresh shrimp extract, the dry shrimp being greater, because as indicators, detoxification enzymes that are NAD⁺ and/or NADP⁺ dependent may have been significantly induced, and/or increases in ATP – energy production may have resulted.

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