HEAVY METAL CONTENT IN CRABS OBTAINED FROM BODO CITY AND ITS EFFECT ON LIVER FUNCTION MARKERS OF ALBINO RATS

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Abstract
Crabs are aquatic food which is widely consumed in the Niger-Delta region of Nigeria. They are particularly sensitive to heavy metals and xenobiotics. Hence, the aim of this study was to determine the heavy metal concentration and its effect on the liver following the consumption of heavy metals contaminated crabs from Bodo City, Nigeria. In this experimental study, 16 albino rats were randomly divided into 4 groups of 4 rats each, including the Control (given normal saline), Low Dose (0.075ml of stock), High Dose (0.13ml of stock) and Very High Dose (0.7ml of stock) groups. The test doses were selected based on serial dilution of the stock solution, where 100g of fresh crab was homogenized, dissolved in 100ml of saline and centrifuged. The supernatant was filtered, forming the stock solution(1g/ml). The doses were administered orally for 5 weeks. Blood samples of the animals was collected via cardiac puncture for the determination of the various liver biomarkers. Data were compared statistically between groups using ANOVA at $p \leq 0.05$, indicating significant difference. The order of average heavy metal concentrations in crab samples was Fe>Cr>As>Pb>Cd. From the obtained results, the levels of some metals such as Fe, As, Pb, Cr were higher than the acceptable values for human consumption designated by World Health Organization (WHO). The comparison of the result with WHO standard showed that crab have been contaminated with heavy metals. The results from the liver function test, showed a significant increase in AST, ALT and ALP level in all the groups when compared to the control. Total protein, Albumin and total bilirubin concentrations showed no significant difference between the control and the experimental rats. The occurrence of high levels of Fe, As, Pb and Cr in the aquatic environment is thought to be a result of industrial and maritime traffic in Bodo City. The high level of heavy metals would have contributed to the increase of the liver function enzymes and could have some negative effect on the liver.

Keywords: Crabs, Heavy Metal, Effect, Liver, Markers, Albino Rats

Introduction

Crabs belong to a group of animals known as decapod crustaceans. Their common characteristics include two pairs of jointed antennae, segmented body, a calcareous hard shell and paired jointed limbs. They are common source of food consumed either wholly or partially (Baird and Ulanowics, 1999). Crabs have contact with water bodies, most of which have received wastes which are capable of harboring toxicants like heavy metals (Olusegun *et al*., 2008).

Heavy metals are non-biodegradable and have the tendency to bio-accumulate in organisms and thus are considered as serious pollutants of the aquatic environment because of their toxicity (Cogun *et al*., 2017). Heavy metals can be accumulated by marine organisms through respiration, adsorption and ingestion (Mbeh *et al.,* 2019).

Heavy metals pollution of the aquatic environment is a global concern, contributed by rapid and disorganized urban and industrial development (Mbeh *et al*., 2019); discharge chemicals from mining and manufacturing industries and from city wastes (Cogun *et al*., 2017). Agricultural wastes also contribute to the pollution, as they find their way into the water bodies through runoff (Neji *et al*., 2019).

A number of incidental discharge or spills of crude oil into the environment in the Niger Delta of Nigeria, have been due to the rising exploration and exploitation activities of oil production and the successful drilling and piping of the crude oil to their refining/storage depots in that area. Most of the aquatic and terrestrial ecosystems and shorelines in the oil producing communities encompass important agriculture lands and are under continuous cultivation. When oil spills on-shore or near-shore, it inevitably affects soil ecosystem, thus affecting fishing productivity and fishermen that depend on it for livelihood (Osuji and Adesiyan, 2005).

Therefore, proper and effective clean up measures are needed in these polluted areas due to the rapid disappearance and death of aquatic organism around the oil spill polluted water, as well as retarded mangrove growth, chlorosis of leaves and the attendant low fishing productivity that still manifest many years after recorded incidence of spill (UNEP, 2011).It is on this background, that this study was aimed at assessing the current levels of heavy metals in crab from Bodo City and the effects on the liver following the consumption of heavy metals contaminated crabs (*Callinectes sapidus*).

Material and Methods

Study Area

Bodo city is located in Gokana Local Government Area of Rivers State, Nigeria. It has geographical co-ordinates: 4^{o} 37' 0" North, 7^{o} 16' 0" East.

Sample Collection

Callinectes sapidus (crabs), were collected from Bodo City in Gokana Local Government Area in Rivers State, Nigeria. The sampling period was during the wet season. Wet season was chosen for this study because of high consumption rate of crabs and high surface runoff from contaminated coastal water in the wet season.

Sample Processing and heavy metal determination

The fleshy part of the crab sample was carefully removed from its shell and oven dried thoroughly using Memmert drying oven (U27, Germany) for 3 days at a temperature of 50-60^{o}C after which it was ground to powder using silinic mortar (pyrex). The powdered form of the sample was weighed into a crucible container, and then introduced into a furnace to derive the ash at 450-500^{o}C for 6 hours. A crucible thug was used to bring down the crucible from the furnace and placed in a desiccator where it was allowed to cool. After cooling, 5ml of 10% hydrochloric acid was used to dissolve ash content to near dryness. After that, it was filtered and made up to 20ml with distilled water before used for the metal analysis using atomic absorption spectroscopy (Nkpaa *et al.,* 2013).

Preparation of sample (Aqueous)

The preparation of the crab was done according to the method described by Archibong *et al* (2014). Fresh crab was obtained from the market and was rinsed in water to remove debris, after which the non-edible parts where removed. One hundred grams of the fresh crab was weighed out and homogenized for 5 minutes using tissue blender. The homogenate was then dissolved in 100ml of saline (0.9% NAC). After dissolving the homogenate, it was then centrifuged for 10min using 10,000rmp. The supernatant was then poured into a clean container via filter paper, fitted functional and this formed the stock solution of 1g/ml.

EXPERIMENTAL DESIGN

Sixteen albino rats of both male and female sex weighing between (25-35g) were used for this study. The animals were obtained from the animal house of the Department of Biochemistry, University of Port Harcourt, Nigeria. They were kept in a well-ventilated plastic cages and left

under laboratory conditions for one week for acclimatization. The animals were randomly selected and assigned to four groups; the control, Low Dose (LD), High Dose (HD) and Very High Dose (VHO) groups of four rats each. The test doses were selected based on pre-determined LD50 values (Archibong *et al*., 2014) and on serial dilution of the stock solution. The extract was administered orally based on the weight of each rat. The low dose groups received 0.075ml of the extracts daily, while the high dose group received 0.13ml and the very high dose groups received 0.7ml of the extracts daily. The control group received 0.6ml of normal saline daily.

The animals were weighed weekly. All animals had access to food and water adlibitum. The feeding period lasted for 5 weeks, the animals were sacrificed at the end of experimental period and blood samples of the rats were collected by cardiac puncture. In the handling of the animals all ethical standards laid down in 1964 declaration of Helsinki were strictly adhered to.

Animal Schedule

Group one serve as control and the animals were administered with 0.6ml of normal saline daily with normal rat feeds, group two animals were given low dose (L.D) – 0.075ml of stock with normal rat feeds, group three animals were given high dose (HD) – 0.13ml of stock with normal rat feeds and group four animals were given very high dose (VHD) – 0.7ml of stock with normal rat feeds.

Statistical analysis

Statistical analysis of data was represented as Mean ± standard deviation and subjected to one-way analysis of variance (ANOVA) using statistical software SPSS. A level of $p \leq 0.05$ was considered statistically significant.

Results

Heavy Metal Concentration in Crab

The result of the Heavy Metal concentration in Crab is shown on Table 1. Results showed that the concentration of Iron (Fe), Arsenic (As), Lead (Pb) and Cadmium (Cd) in crab were higher than the WHO recommended values.

Table 1. Mean ± SD of Heavy Metal Concentration in Crab

Heavy Metals Site	Fe(mg/kg)	As(mg/kg)	Pb(mg/kg)	Cd(mg/kg)	Cr(mg/kg)
USEP/WHO	0.5	0.02	0.01	0.001	0.05
Bodo City	5016.5 ± 0.41*	3.56 ± 0.04*	3.11 ± 0.49*	0.71 ± 0.01*	4.05 ± 0.45

Liver markers of Albino Rats Fed with Crab from Bodo city

Table 2, shows the liver markers of albino rats fed with crab from Bodo city**.** The result showed that Aspartate transaminase (AST) level significantly (≤ p-value) increase in group 2 (28.25 ± 8.65) and group 3 (31.0 ± 6.05) when compared with control (17.75 ± 4.42). Alanine transaminase (ALT) showed significant increase in group 2 (16.12 ± 1.65) and group 3 (16.52 ± 3.07) when compared with control (12.32 ± 1.71). Alkaline phosphatase (ALP) showed significant increase in group 1 (20.65 ± 2.90), group 2 (22.70 ± 4.37) and group 3 (30.25 ± 2.59) when compared with control (13.70 ± 0.16).

Albumin and bilirubin concentrations showed no significant difference ($p \leq 0.05$) when control was compared with group 1, group 2 and group 3.

Table 2 Liver Markers of Wistar Albino Rats Fed with Crab contaminated with heavy metals from Bodo city

	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	TP (g/dl)	ALB (g/dl)	TB (mg/dl)
CRT	17.75±4.42^{a}	12.32±1.71^{a}	13.70±0.16^{a}	62.0±2.58^{a}	36.25±1.70^{a}	6.0±0.81^{a}
Group 1	23.0±2.94*^{b}	15.37±1.49^{a}	20.65±2.90*^{b}	57.50±2.08^{a}	35.0±2.16^{a}	8.45±0.68^{a}
group 2	28.25±8.65*^{c}	16.12±1.65*^{c}	22.70±4.37*^{b}	60.50±3.10^{a}	36.25±2.16^{a}	8.45±1.98^{a}
group 3	31.0 ± 6.05*^{c}	16.52±3.07*^{c}	30.25±2.59*^{c}	61.50±2.38*^{b}	37.0±2.16^{a}	8.10±2.27^{a}

Values are expressed as Mean± standard deviation. Values with same superscript show no significant difference while values with different superscript show significant difference.

Values with superscript (*) /a differ significantly at 0.05 when control was compared with group 1, group 2 and group 3.

Values with superscript (b) differ significantly when group 1 was compared with group 2, group 3 and control.

Values with superscript (c) differ significantly when group 2 was compared with group 1, group 3 and control.
Values with superscript (d) differ significantly when group 3 was compared with group 1, group 2 and control.

Discussion

The study looked at the heavy metals present in crab from Bodo city, Nigeria and how it affects the liver when consumed by albino rats. The concentrations of the heavy metals were in the order Fe>Cr>As>Pb>Cd and they were all above the permissible limits.

The concentration of Chromium (Cr), Cadmium (Cd), iron (Fe) and lead (Pb) were all higher than those reported by Neji *et al*. (2019). Pb and Fe concentrations were also higher than those of Alagoa and Yabefa, (2019). Similarly comparing the Cd value with the report of Olowu *et al*. (2010), Cd concentration in this study was higher than theirs; however, the concentration of Cr in their report was higher than the value for this study. The occurrence of high levels of Fe, As, Pb and Cr in the aquatic environment is thought to be as a result of industrial and maritime traffic in Bodo City.

Heavy metals have harmful effects on human, some of its complicated toxic effect include gastrointestinal and kidney dysfunction, nervous system disorder, skin lesions, vascular damage, immune system dysfunction, birth defects and cancers. Exposure to high Pb dosage may induce abdominal colic pain, bloody diarrhea and kidney disorder. Cadmium, arsenic and chromium are carcinogenic metals which disrupts DNA synthesis and repair (Balali-Mood *et al*., 2021). High concentration of Cadmium also could lead to chronic kidney dysfunction inducing cell injury and death (Ezemonye *et al*., 2019). Results of the liver markers of wistar rats fed with crab contaminated with heavy metals from Bodo city showed high level of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities. The total bilirubin, total protein and albumin concentration showed no significant difference when compared with the control. The results of liver function enzymes support the claims of Niture *et al*. (2021), which says that acute cadmium poisoning causes increased levels of liver damage markers such as alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase. Also, studies have shown increase levels of ALT and ALT (Kim *et al.,* 2021) and ALP associated with lead poisoning. Furthermore, arsenic has been correlated with liver disease through oxidative stress and it leads to increased ALT and AST activities (Li *et al*., 2015). Chromium has also been associated with increase ALT, AST and total bilirubin

concentration (Farag and El-Shetry, 2020). Thus, the increase of the liver enzyme activities present in this study would have been associated with the presence of these heavy metals found in the crab.

Conclusion

The heavy metal analysis in crab was higher when compared to the permissible limit of WHO, which indicated that crab has the ability of absorbing these heavy metals. The consumption of this crab could pose possible health hazard/liver damage. The high level of heavy metals could have contributed to the increase of the liver function enzymes and could have some negative effect on the liver.

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