www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 11: 485-489 (2011) DOI: 10.4194/1303-2712-v11_3_21

SHORT PAPER

Biochemical Alterations in *Hoplobatrachus occipitalis* Exposed to Sub Lethal Concentrations of Cadmium

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Received 18 August 2010
Accepted 2 March 2011

Abstract

The adult crowned bullfrog, *Hoplobatrachus occipitalis* was exposed to 0.25, 0.50, 1.00 and 2.00 mg/L cadmium for 28 days. The effect of cadmium on selected biochemical parameters- superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and thiobarbituric acid reactive substances (TBARS) in the liver were tested. Biochemical observations revealed significant ($P<0.05$) dose-dependent increase in the specific activity of superoxide dismutase (SOD) and catalase (CAT) relative to controls. This could be due to increased production of these antioxidants to counteract oxidative stress and lipid peroxidation induced by cadmium. Glutathione (GSH) level decreased with increase in the concentration of heavy metal. Thiobarbituric acid reactive substances (TBARS) which is an index of lipid peroxidation increased as concentration of cadmium increased. The increased level of TBARS in the liver of cadmium exposed frogs is an indication of increased membrane lipid peroxidation which could lead to cell damage.

Keywords: Cadmium, *Hoplobatrachus occipitalis*, liver, biochemical parameters

Yarı Öldürücü Konsantrasyonlarda Kadminyum Muamelesi Yapılan *Hoplobatrachus occipitalis*'da Biyokimyasal Değişimler

Özet

Yetişkin kurbağalara 28 gün boyunca 0.25, 0.50, 1.00 ve 2.00 mg/L kadmiyum muamelesi yapılmıştır. Karaciğerde kadmiyumun etkileri seçilen parametreler doğrultusunda (biyokimyasal parametreler, süperoksit dismutaz (SOD), katalaz (CAT), glutat (GSH) ve tiyobarbitürük asit reaktif maddeleri (TBARS) değerlendirilmiştir. Süperoksit dismutaz (SOD) ve katalaz (CAT)'ın spesifik aktivitesinde kontrole kıyasla doza bağlı önemli derecede ($P<0,05$) artış olmuştur. Bunun nedeni oksidatif stresi ve lipit peroksidasyonunu önlemek için bu antioksidanların artan üretimi olabilir. Glutat ise ağır metal konsantrasyonunun artışı ile düşmüştür. Kadmiyum seviyesi arttıkça TBARS'da yükselmiştir. Kadmiyum muamelesi yapılan kurbağalarda TBARS'ın yükselen seviyesi, hücre hasarına öncülük edecek olan membrandaki lipit oksidasyonunun artışının bir indikatörüdür.

Anahtar Kelimeler: Kadmiyum, *Hoplobatrachus occipitalis*, karaciğer, biyokimyasal parametreler.

Introduction

Cadmium (Cd) is a heavy metal with no known biological function. It is very toxic to aquatic and terrestrial biota. Cadmium is introduced into the aquatic environment primarily by human activities including mining, fertilizer application and industrial discharges (James and Little, 2003; Ezemonye and Enuneku, 2005; Volgiatzis and Loumbourdis, 1997).

Adult frogs can acquire cadmium through their skin or orally by consumption and respiration. Once absorbed, it can be found in numerous amphibian tissues especially the liver, kidney, gonads, placenta, brain and bones (Sobha, 2007). Sources of human exposure to cadmium include food, cigarette smoke and alcoholic beverages (Jarrup *et al*., 1998). Cadmium has been shown to stimulate free radical production, deplete antioxidant levels resulting in oxidative deterioration of lipids, proteins and DNA and initiating various pathological conditions in animals and humans (Sarkar *et al*., 1997; Shaikh *et al*., 1999). It promotes an early oxidative stress and afterward contributes to the development of serious pathological conditions because of its long retention in some tissues (Bagchi *et al*., 2000). Cadmium may cause the deterioration of cell membranes by binding

to metallothionein or glutathione and consequently interfere with the ability of these proteins to avoid oxidative stress. Cadmium can also replace essential metals such as copper and zinc in several metalloproteins, altering the protein conformation and affecting their activity because this element interacts ubiquitously with sulphydryl groups of amino acids, proteins and enzymes (Serafim *et al*., 2007). Thus, the toxic effects of cadmium are related to changes in natural physiological and biochemical processes in organisms.

Global declines and extinctions in amphibian populations have been previously reported (Houlahan *et al*., 2000; Thompson, 2004). Potential causes have been linked to industrial and agricultural chemicals, climatic changes, bacterial and fungal infections, changes in amount and quality of habitat (Carr *et al*., 2003). Akani and Luiselli (2002) in a study of amphibian faunal diversity and conservation status in the Niger Delta basin, southern Nigeria, reported that amphibian populations were declining due to chemical contaminants, habitat destruction and exploitation. While some information exists on the toxicity of cadmium to amphibians, very little work has been done on local African species especially adults. Frogs occupy a special position in the food web due to their biphasic life cycle. Practically, there are greater chances of transferring cadmium accumulated to higher organisms particularly to man.

Hoplobatrachus occipitalis is a frog native to southern Nigeria and other African countries. This work studies the biochemical effects of cadmium on *H. occipitalis* as a contribution to understanding and further attenuating the phenomenon of declining amphibian populations.

Materials and Methods

Adults of *Hoplobatrachus occipitalis* were collected from unpolluted spawning ponds in Oghara Community in the Niger Delta ecological zone of Nigeria. They were collected using hand nets to prevent injury to animals during capture since they are active animals. Acclimation to laboratory conditions was done for two weeks prior to experiments (Goulet and Hontella, 2003) in plastic tanks measuring 49 cm in length x 29 cm in width x 24 cm in height with dechlorinated tap water (2 litres at a slant). The frogs were fed *ad libitum* daily with termites. They experienced a natural photoperiod of approximately 10: 14, light/dark period at a laboratory temperature range of 27-28°C.The mean values for the test water quality were as follows; temperature 26±1°C; pH 5.7±0.4; dissolved oxygen 4.7±0.7 ppm and hardness 36±1.24 ppm.

The initial mean weight of frogs was 55.23±0.53 g. There was no significant difference ($P>0.05$) between the mean weights of frogs used in the experiments. Since metabolic activity changes with size and affects the parameters to be measured (Canli and Furness, 1993), individuals of similar weights were used.

Cadmium as $CdCl_2.H_20$ was used for the sub lethal tests. Stock solutions of the toxicant ($CdCl_2$) were prepared by dissolving the toxicant in distilled water to a final volume of 1.0 L. Each treatment solution was prepared after a range-finding test by diluting the stock solution with water to achieve the appropriate exposure concentrations (Ezemonye and Enuneku, 2006). Four sublethal concentrations (0.25, 0.50, 1.00 and 2.00 mg/L) cadmium were dosed to frogs for 28 days. There were three replicate tanks per treatment and five individuals per tank including controls. The amphibians were fed with termites.

On the 28^{th} day one individual from each tank was sacrificed for the determination of hepatic superoxide dismutase, catalase, glutathione and thiobarbituric acid reactive substances. Each frog was decapitated. The liver was quickly excised and placed on ice until required for homogenization.

The levels of total superoxide dismutase (SOD) activity in liver homogenate was determined by the method of Misra and Fridovich (1972).The ability of superoxide dismutase to inhibit the auto-oxidation of adrenalin at pH 10.2 makes this reaction a basis for the SOD assay.

Catalase activity was determined according to the method of Sinha (1971) by measuring the rate of decomposition of hydrogen peroxide (H_2O_2).

The levels of reduced glutathione in liver homogenates were determined by the method of Jollow *et al.* (1974). The reduced form of glutathione (GSH) in most instances is the bulk of cellular non-protein sulphydryl groups. This method is based upon the development of relatively stable yellow colour when Ellman's reagent (5^1, 5^1-dithoibis-2-nitrobenzoic acid) is added to sulphydryl compounds. The chromophoric product, 2-nitro-5-hiobenzoic acid, resulting from the reaction of Ellman's reagent with reduced glutathione possesses a molar absorption at 412 nm. The absorbance at 412 nm is proportional to the reduced glutathione content.

A breakdown product of lipid peroxidation, thiobarbituric acid reactive substances (TBARS) was determined by the method of Buege and Aust (1978).

Results

Changes in biochemical parameters in *H. occipitalis* exposed to cadmium are presented in Figures 1-4. There was a significant increase ($P<0.05$) in the specific activity of SOD and CAT in the liver of *H. occipitalis* exposed to cadmium relative to controls. Hepatic SOD and catalase levels in the highest concentration (2.00 mg/L) increased by 92.23% and 96.67% respectively relative to controls. The increase was concentration dependent. There was a 76.17% decrease in reduced glutathione levels

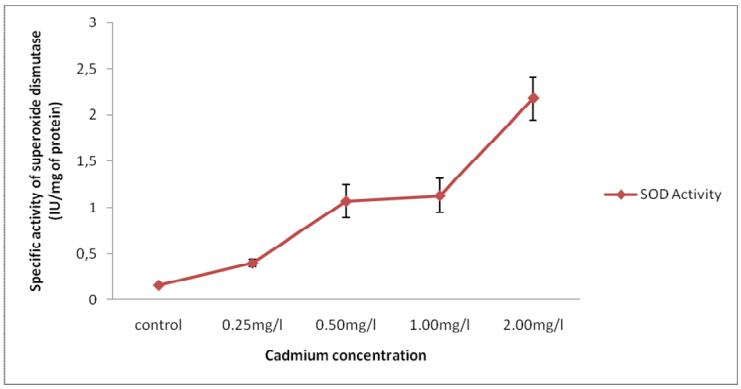

Figure 1. Specific activity of SOD in *H. occipitalis* exposed to cadmium

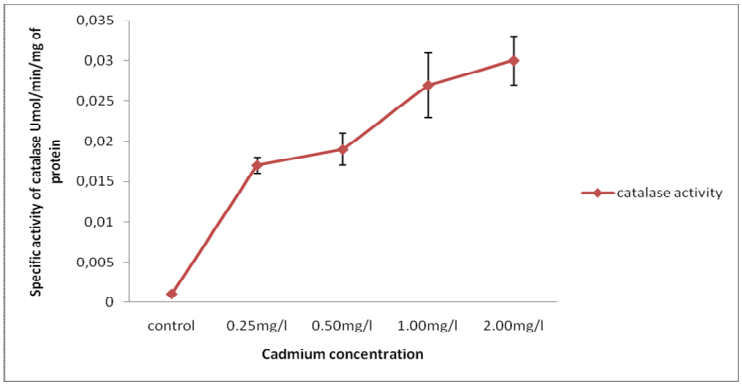

Figure 2. Specific activity of catalase in *H. occipitalis* exposed to cadmium

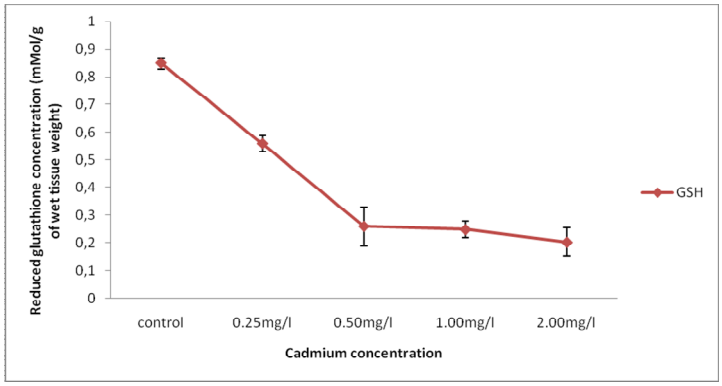

Figure 3. Reduced glutathione concentration in *H. occipitalis* exposed to cadmium.

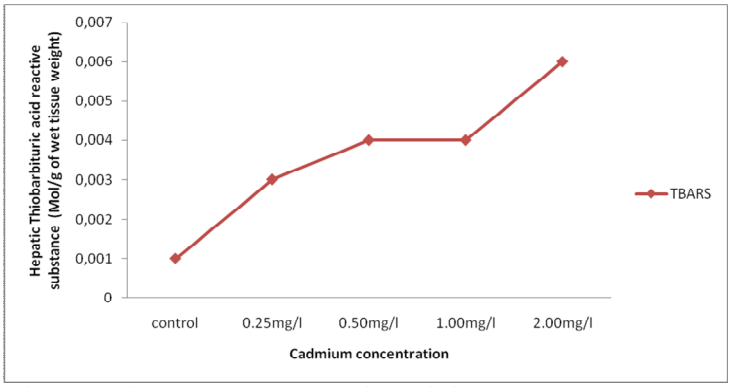

Figure 4. Changes in hepatic TBARS in *H. occipitalis* exposed to cadmium.

relative to control group. Reduced glutathione concentration in *H. occipitalis* exposed to cadmium decreased ($P<0.05$) with increase in concentration of cadmium. TBARS which is an index of lipid peroxidation increased (83.83%) relative to controls in the liver of frogs exposed to cadmium. TBARS increased with increase in concentration of cadmium at $P<0.05$ level of significance.

Discussion

Biochemical parameters are the best indicators of stress situations caused by heavy metals. The results of the present study showed that cadmium significantly altered the antioxidant levels of *H. occipitalis* after 28 days. Our findings show that there was an increase in hepatic levels of superoxide dismutase and catalase. Cadmium is known to alter antioxidant levels and induce oxidative stress in living systems (Suru, 2008; El-Demerdash *et al*., 2004). Superoxide dismutase and catalase are important antioxidants and play a crucial role in counteracting oxidative stress. The increase in SOD and catalase observed in the study of Gupta *et al.* (1991) who reported that cells increase the production of antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidise in order to circumvent oxidative stress.

Our findings also show that cadmium exposure caused a decrease in glutathione levels in the liver of *H. occipitalis*. Several authors have reported similar trends (Sarker *et al*., 1997; Park *et al*., 2001). Cellular GSH is very sensitive to oxidative stress. It acts as the first line of defence in cadmium toxicity (Singhal *et al*., 1987. The decrease in hepatic level of GSH may be the consequence of enhanced GSH utilization to conjugate cadmium, counteract reactive oxygen species and lipid peroxidation products (Sen, 1997). Furthermore, cadmium has been reported to inhibit a variety of thiol-containing enzymes which include γ-glutamyl cysteine, the rate limiting enzyme in the biosynthesis of GSH (Jinna *et al*., 1989). *Rana ridibunda* exposed to cadmium (534 ppm) for 4, 10 and 30 days showed a decrease in GSH concentration following a time- and Cd concentration dependent pattern (Volgiatzis and Loumbourdis, 1997).

The increased level of TBARS in the liver of cadmium exposed frogs is an indication of increased membrane lipid peroxidation which is in agreement with earlier findings (Bagchi *et al*., 1996; Sarkar *et al*., 1995). Cadmium has been reported to induce lipid peroxidation in membranes leading to cell damage (Casalino, 1997). Asagba *et al.* (2007) reported that there is a direct relationship between the degree of tissue damage and the level of TBARS.

Acknowledgement

The authors are grateful to the Vice Chancellor and Principal Officers of Western Delta University, Oghara, Delta State, Nigeria for the colossal contributions in cash and equipment in making this scientific study a success.

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