Cadmium Nanoparticle (CdNP) Induced Anatomical and Biochemical Changes in Mud Crab Scylla olivacea (Herbst, 1796)

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ABSTRACT

Cadmium has been found to produce wide ranges of biochemical and physiological dysfunctions in humans and animals. In the present study, toxic effects of Cadmium nanoparticle (CdNP) in the vasdeference of mud crab Scylla olivacea was evaluated. Crabs were exposed to different concentrations of CdNP (from 0 to 120 ppm/kg) for 8 days. The toxicity study revealed that LD₅₀ value was found to be 40 ppm/kg of crab on day 6. CdNP (20 ppm/kg) induced abnormal structural changes in the vasdeference such as disintegration of principle cells, vacuolar degeneration, distortion of stereocilia and seminiferous epithelia. In addition, CdNP also induced antioxidant enzymes such as Catalase (CAT), Glutathione Peroxidase (GPx) and Superoxide dismutase (SOD). CdNP also modulates the activity of mitochondrial enzymes such as Lactate dehydrogenase (LDH), Succinate dehydrogenase (SDH) and Malate dehydrogenase (MDH).

Keyword: Cadmium nanoparticle, S. olivacea, Vas deference, antioxidant enzymes.

INTRODUCTION

Cadmium (Cd) is one of the most toxic heavy metals for humans. The main source of non-occupational exposure to Cd includes smoking, air, and food and water contaminated by Cd [1]. Cd is a common inorganic contaminant of coastal sediments and waters due to anthropogenic pollution and natural sources [2,3]. It can be accumulated in aquatic animals (e.g. crabs, shrimps, oysters and mussels) after entering through different way such as respiratory tract, digestive tract, surface penetration etc. [4]. It is seriously harmful to the growth of aquatic life and survival, resulting in decline of their populations. At the same time, as aquatic food products, these animals exposed to Cd might threaten human health. Cd in waters can be absorbed by aquatic organisms via respiratory system, digestive system and body surface without significant excretion [5].

Rivers and lake shores are the areas primarily affected by diluted cadmium waste from industrial facilities in big cities [6]. Cadmium is a highly toxic heavy metal, since it causes deleterious effects in organisms at low levels of exposure [7]. Several studies showed that toxic agents may affect behavioral parameters [8,9]. Behavioral changes are good indicators of damage to the central nervous system, as a consequence of exposure to toxic agents [10]. Accumulation of cadmium in living organisms is a major ecological concern, especially because of its ability to accumulate very quickly. By contrast, the excretion of cadmium from living organisms is a slow process. Various toxic effects of cadmium have been reported, as well as its accumulation in liver, kidney and testes [11]. 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 sulphhydryl groups of amino acids, proteins and enzymes [12]. Thus, the toxic effects of cadmium are related to changes in natural physiological and biochemical processes in organisms. The organisms developed a protective defense against the deleterious effects of essential and inessential heavy metals and other xenobiotics that produce degenerative changes like oxidative stress in the body [13]. In general, they are not biodegraded and therefore, their bioaccumulation in fish, oyster, mussels, sediments and other components of aquatic ecosystems have been reported from all over the world. It appears that problem of heavy metals accumulation in aquatic organisms needs continuous monitoring and surveillance owing to biomagnifying potential of toxic metals in human food chain [14,15]. Hence, the we made an attempt to study the CdNP induced anatomical and biochemical changes in vasdeference of S. olivacea.

MATERIALS AND METHODS

Experimental animals
Fresh samples of male species of *Scylla olivacea* was collected from Pulicate Lake, Pulicate, Tamil Nadu, India. The male crabs were maintained separately in tanks with aerator which was (capacity of 1000 L) filled with filtered (0.45 mm pore) sea water. The sea water was changed periodically and crabs were fed with commercial fish feed. The morphological identification and authentication of species was done by a Scientist from Central Institute of Brackishwater Aquaculture (CIBA), Santhome, Chennai, India.

**Toxicity tests**

The acute semistatic toxicity test was carried out according to the standard methodology described by Food and Agriculture Organization (FAO) [16] and the American Public Health Association [17]. Semistatic toxicological bioassays were carried out for 120 h. Different concentrations such as 20, 40, 60, 80, 100 and 120 ppm of CdNP suspension of 100 nm in size (Sigma and Co., Bangalore, India) was injected intraperitoneally per kg of crab weight. Three replicates of at least 10 animals were exposed to the above stated concentrations. One group without CdNP treatment was maintained as control. The criteria to determine death was the complete absence of movement once the animals were gently touched with a glass rod. Mortality was recorded every 24 h, a period of time after which dead crabs were removed. A probit analysis was used to estimate the concentration and 95% confidence limits of CdNP that kills 50% of the exposed crab (LD50).

**Histology**

Experimental crabs were sacrificed and vasdeference tissue samples were taken after 2, 4, 6 and 8 d of exposure of 20 ppm/kg of CdNP. The vasdeference was carefully dissected out and fixed in 4% buffered formalin, embedded in paraffin, sectioned (8 mm thickness) on a microtome (Microm, HM330, Heidelberg, Germany), stained with hematoxylin and eosin (H and E) and examined with an Olympus microscope (Tokyo, Japan).

**Protein extraction and quantification**

A known quantity of vasdeference of male *S. olivacea* was ground in a pre-chilled mortar and pestle in 50 mM phosphate buffer (pH 7.2) amended with 0.01% polyvinyl poly pyrrolidone and 0.001% ascorbic acid in a ratio of 1:3, filtered and centrifuged (6000 x g) to obtain a clear supernatant. The cell-free supernatant was used as a protein source. Protein content was determined by the method of [18] using bovine serum albumin fraction V (Sigma Chemical Co., Bangalore) as a standard.

**Catalase (CAT) activity**

Catalase activity was determined in vasdeference tissue colorimetrically according to [19]. The rate of disappearance of H2O2 is followed by observing the rate of decrease in the absorbance at 240 nm. The CAT activity was calculated as μM of H2O2 consumed/min/mg protein and the result were expressed as Units/mg protein.

**Superoxide dismutase (SOD) activity**

The SOD activity was estimated by the method of McCord and Fridovich [20]. Cyt-c reduction was measured for 3 min in a 1.5 mL assay mix containing SOD buffer 1 (50 mM KH2PO4 and 0.1 mM EDTA at pH 7.8), 10 μM Cyt-c (Sigma), 50 mM xanthine (Sigma, Steinheim, Germany) at 550 nm on a Cary 3E UV/Vis double beam spectrophotometer (Varian, Middelburg, Netherlands) equipped with a temperature controlled cell attached to a water bath. The SOD activity was expressed as Units/mg. protein.

**Glutathione Peroxidase (GPx) activity**

GPx activity was assayed by method of [21]. The reaction mixture containing of 0.2 mL of EDTA, 0.1 mL sodium azide, 0.1 mL of H2O2, 0.2 mL of GSH, 0.4 mL of phosphate buffer and 0.5 mL of homogenate was incubated at 37°C for 10 min, the reaction was arrested by the addition of 0.5 mL of TCA and the tubes were centrifuged at 1500 x g. To the 0.2 mL of supernatant, 3 mL of disodium hydrogen phosphate and 1.0 mL of DTNB were added and the color was read at 412 nm immediately. The activity of GPx was calculated as μM of glutathione oxidize/min/mg protein and the result expressed as Units/mg. protein.

**Lactate Dehydrogenase (LDH) activity**

LDH activity was assayed by the method of [22]. A reaction mixture contains 0.1M Potassium phosphate (pH 7.0), 0.01M NADH, 0.1M Pyruvate (Sodium pyruvate) and 0.2 ml diluted LDH stock solution (10mg/ml). The decrease in absorption at 340nm was followed at 25°C.

**Malate Dehydrogenase (MDH) activity**

MDH activity was assayed by the method of [23]. An assay mixture contains 9.1ml of 0.1M of potassium phosphate, 0.2ml of 0.01M NADH and 0.5ml of 0.1M oxaloacetic acid. The decrease in absorption at 340nm was followed at 25°C.

**Succinate Dehydrogenase (SDH) activity**

SDH activity was assayed by the method of [24]. A reaction mixture contains 0.4ml of the tissue extract, 5ml of Phosphate buffer, 0.2ml of sodium succinate, 0.2ml of sodium azide and 0.2ml of DCPIP solution. Absorbance was measured immediately at 610nm.

**RESULTS AND DISCUSSION**

The effect of CdNP (20ppm/kg) on the anatomy of vasdeferens of *S. olivacea* were presented in Fig. 1. The control crabs showed normal architecture of the epithelium was proximally squamous, distally and columnar in the intermediate region, forming a deep blind (Fig. 1A). The intermediate region of the vasdeferens was lined internally with simple cubic epithelium. On day 2 of CdNP exposure, disintegration of principle cells and epithelium were evident (Fig. 1B). On day4, further vacuolar degeneration appeared in the spermatogenic epithelium (Fig.1D). On day 6, the number of distortion of stereocilia and the seminiferous epithelia were atrophied as marked in basal cell (Fig.1F). As the day progresses (day 8), the epithelium was condensed to form a dense pseudo coulmnar epithelium and exhibited extensive necrosis, complete deterioration of laminar properia was observed in vasdeference (Fig.1H).

Lipid peroxidation is one of the main indicators of oxidative damage, which plays a crucial role in the toxicity of many xenobiotics [25]. If the concentrations of prooxidant agents exceed those of antioxidant agents, oxidative damage should be manifested. This might imply that some of the antioxidant enzymes, such as SOD, CAT and GPx, play important roles in clearing away the peroxidation product. The results of CAT and GPx are in agreement with our histopathological findings that CdNP caused injury to vasdeference. Cadmium nanoparticle (20ppm) exposure resulted in increased Catalase (CAT)
activity in the vas deference of *S. olivacea* than in control crabs. The CAT activity started increasing on day 2 compared to control and reached peak on day 10 of exposure (Fig. 2). CdNP exposure recorded increased Glutathione peroxidase (GPx) activity in the vas deference of *S. olivacea* than in control crabs. The GPx activity started increasing right on day 2 compared to its control and reached maximum on day 10 of exposure (Fig. 3). Similarly, an increased SOD activity in the vas deference of *S. olivacea* than in control crabs was also recorded upon exposure to CdNP. The SOD activity started increasing on day 2 and recorded maximum on day 10 of exposure compared to their respective controls (Fig. 4).

![Fig.1. Effects of CdNP (20 ppm) on the anatomical structure of vas deference in *Scylla olivacea* by light microscope. Scale bar, 10µm. Pc- Principle cells; Ep- Epithelium; Lu- lumen; St- Stereocilia; Sm- Smooth muscle; Bc- Basal cells; Lp- Lamina propria; Pce- Pseudo columnar epithelium. A) Control (Day 2) B) CdNP treated (Day2) C) Control (Day 4) D) CdNP treated (Day4) E) Control (Day 6) F) CdNP treated (Day 6); G) Control (Day 8) H) CdNP treated (Day 8) ](image1)

![Fig.2. Catalase activity (CAT) activity in vas deference of *S. olivacea* after exposure of CdNP.](image2)

![Fig.3. Glutathione peroxidase (GPx) activity in vas deference of *S. olivacea* after exposure of CdNP.](image3)

![Fig.4. Superoxide dismutase (SOD) activity in vas deference of *S. olivacea* after exposure of CdNP.](image4)

![Fig.5. Lactate Dehydrogenase activity in vas deference of *S. olivacea* after exposure of CdNP.](image5)
Cd exerts adverse effects on structures and functions of reproductive organs directly by creating hormonal imbalance thereby modulates sperm progress motility and/or function (viability), all of which may culminate in hypogonadism and infertility [26]. CdNP also modulated the levels of mitochondrial enzymes in vas deference of S. olivacea. In general, CdNP resulted in increased LDH activity than in control crabs. The LDH activity started increasing on day 2 compared to control and reached maximum on day 8 of CdNP exposure (Fig.5). Results of MDH activity in vas deference of S. olivacea exposed to Cadmium nanoparticle (20ppm) were presented in Figure 6. CdNP resulted in increased MDH activity than in control crabs. The MDH activity started increasing on day 2 and maximum activity was recorded on day 8 of CdNP exposure. Activity of SDH also regulated in vas deference of S. olivacea exposed to CdNP. CdNP resulted in increased SDH activity in vas deference than in control crabs. SDH activity started increasing in exposed crabs on day 2 compared to control and reached maximum on day 6 of exposure (Fig.7).

The present study clearly demonstrated that acute exposure to CdNP led to deterioration of vas deference of mud crab Scylla olivacea, which may lend strong support to the conclusion that acute exposure to CdNP results in a cumulative and/or progressive vas deference injury and induce antioxidant defence.

CONCLUSION

In the present study, we made an attempt to study CdNP induced structural and biochemical changes in vas deference of mud crab Scylla olivacea. Based on the results obtained, we can conclude that CdNP alters both structural integrity and biochemical defence in vas deference of the mud crab.

REFERENCES

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