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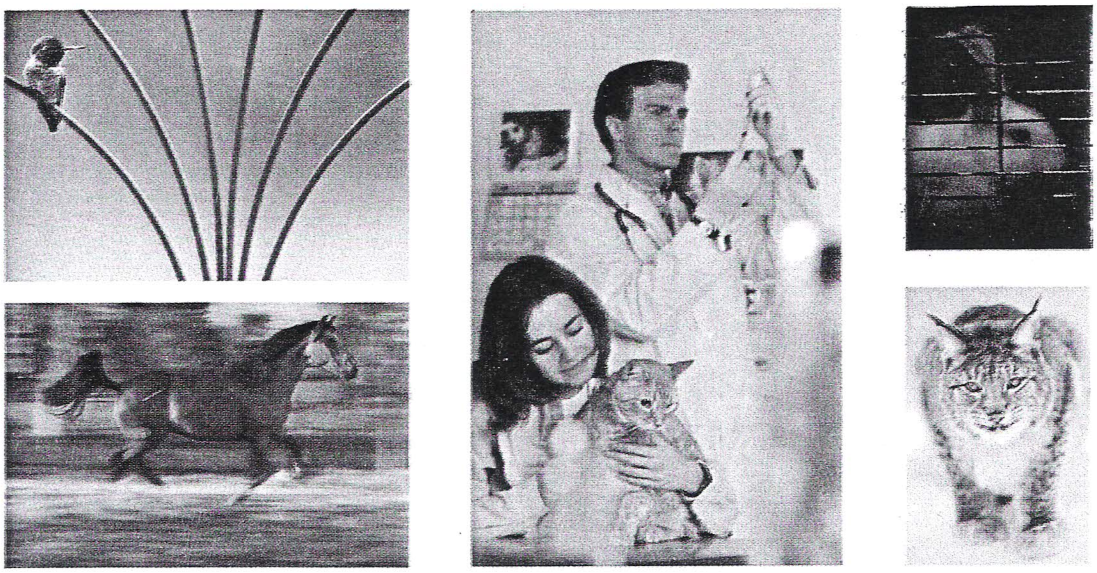

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Effects of Cadmium on Sera Glucose and Cortisol Levels in *Clarias gariepinus* (Burchell, 1822)

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Abstract: The effects of cadmium on sera glucose and cortisol levels of *Clarias gariepinus* was studied after exposing the animals to 0.25, 0.50 and 1.00 ppm Cd over 30 days. An autoanalyser was used in determining sera parameters. Sera glucose levels showed no significant difference between the control and 0.25 and 0.50 ppm Cd exposed fish whereas 1.00 ppm cadmium decreased sera glucose significantly ($p<0.05$). An inverse relationship was found in sera cortisol levels which showed significant increase at 0.25 and 0.50 ppm Cd compared with the control fish ($p<0.05$).

Key words: *Clarias gariepinus*, cadmium, sera, glucose, cortisol, fish

INTRODUCTION

Discharge of heavy metals together with industrial and rural wastes increase their levels in soil and surface waters which in turn have negative effects on aquatic organisms (Simone *et al*., 2006; Van Dyk *et al*., 2007). Cadmium is known as the most dangerous environmental and industrial pollutant (Mendez-Armenta and Rios, 2007). It is extracted as raw material during the production of zinc, lead and copper and used in batteries, plastics, metal alloys, dye and metal plating industries (Agency for Toxic Substances and Disease Registry, 1998). It has no biological function and even at low concentrations it accumulates mainly at metabolically active tissues which in turn cause tissue damages, vertebral abnormalities, respiratory disturbances and finally death (De Smet and Blust, 2001). Studies carried out with various fish species has shown that cadmium upsets osmoregulation, reproduction and development (Sorensen, 1991; Lemaire-Gony and Lemaire, 1992; Soengas *et al*., 1996) and cause hypocalemia, hyperglisemia and hypocalcemia by increasing membrane permeability (Grose *et al*., 1987; Sorensen, 1991).

Fish are widely used to determine the stress conditions caused by various pollutants in evaluating diseases, determining physiological changes and in hematological studies (Wedemeyer and Yasutake, 1977; Duthie and Tort, 1985; Cyriac *et al*., 1989; Wepener *et al*., 1992). Glucose is the main high energy compound in fish needed for biological functions which is stored in muscle and liver in the form of glycogen and its level in sera is controlled by the endocrine system (Dange, 1986). Since its level in sera changes rapidly under the effect of heavy metals, it is widely measured in toxicological studies (Heath, 1995).

Cortisol is a stress hormone which increases gluconeogenic enzyme activity to compensate the increased demand of energy under the effect of metals and initiate the synthesis of glucose from sources other than carbohydrates (Vijayan *et al*., 1997). The release of cortisol hormone in fish is dependent on stress factors such as hunger, stocking density, reproduction, physical and chemical properties of water and also the effect of pollutants (Mommsen *et al*., 1999; Pottinger *et al*., 2000; Chen *et al*., 2003). *Clarias gariepinus* is widely distributed in streams and drainage channels of Turkey especially in East Mediterranean region. Their habitats are influenced directly by rural, industrial and agricultural activities. The species is tolerant to extreme changes in environmental conditions and consumed as a protein source. Since toxic compounds effect metabolic and physiologic events in fish which is an important link in aquatic food chain and consumed as a protein source, present study was undertaken to determine the effect of 0.25, 0.50 and 1.00 ppm cadmium on sera glucose and cortisol levels of *C. gariepinus* after 30 days of exposure to the metal.

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MATERIALS AND METHODS

C. gariepinus was obtained from a private cultivation facility near Silifke, Mersin. Experiments were carried out under controlled laboratory conditions set at 25±1°C and illuminated for 12 h. Similar size of fish, 20±2 cm in length and 65±5 g in weight were used in the experiments since metabolic activity and the parameters studied depended upon size. Fish were placed in 8 glass aquaria having a size of 40×100×40 cm and filled with 120 L tap water and were adapted to laboratory conditions for 15 days. The same sizes of 4 glass aquaria were used in the experiments. About 120 L of 0.25, 0.50 and 1.00 ppm Cd solutions were added in the first three aquaria and the fourth one was filled with the same amount of tap water and used as control. Experiments were run in triplicate each containing two fish and hence 6 fish were placed in each aquarium. Some physical and chemical parameters of the experimental media were as follows; temperature: 24±1°C; total alkalinity: 342±0.57 ppm $CaCO_3$ Dissolved oxygen: 6.82±0.39 ppm O_2; total hardness: 257.8±3.67 ppm $CaCO_3$ pH: 8.18±0.07.

Experimental tanks were aerated using a central aeration system. Fish were fed once a day with readymade fish feed (Pinar pellet, No. 2) at amounts of 2% of the total biomass. Water in experimental and control tanks were replaced once in two days to avoid changes in concentration due to adsorbtion, precipitation and evaporation. Cadmium solutions were prepared using cadmium chloride mono hydrate ($CdCl_2.H_2O$, Merck) with tri-sodium citrate ($C_6H_5Na_3O_7$. 5,5 H_2O, Merck) in order to prevent precipitation of cadmium (Brown and Ahsanullah, 1971; Kargin and Erdem, 1992). Fish were removed from the aquaria and were anaesthetized with Ethylene Glycol Monophenyl Ether ($C_8H_{10}O_2$, Merck) since the parameters studied change under stress. Fish were washed with tap water to remove metal residues on their body and dried with filter paper.

Fresh blood samples were collected by caudal puncture and aliquots were immediately transferred into centrifuge tubes having no coagulants in them. Sera obtained by centrifuging (Hettich, Universal-1000) these samples at 3500 rpm for 5 min were used to determine glucose and cortisol levels using a Cobas-Integra 400/700/800 Autoanalyser. The data was statistically evaluated by Analysis of Variance using SPSS 11.0 statistical package.

RESULTS AND DISCUSSION

Some morphological and behavioral changes were observed in *C. gariepinus* at the beginning of cadmium exposure such as erythema and rupture in fins due to uncontrolled swimming, feed avoidance, increased respiration, movement towards the surface, sudden reactions against outer stimulants and fade in coloration. Sera glucose and cortisol levels of *C. gariepinus* exposed to 0.25, 0.50 and 1.00 ppm Cd over 30 days are shown in Fig. 1 and 2, respectively.

There was no significant difference in sera glucose levels of fish exposed to 0.25 and 0.50 ppm Cd compared with control while a significant decrease was observed at 1.00 ppm Cd ($p<0.05$) (Fig. 1). In contrast to glucose levels, sera cortisol levels increased significantly in fish exposed to 0.25 and 0.50 ppm Cd compared with the control ($p<0.05$) (Fig. 2).

No mortality was observed in *C. gariepinus* exposed to 0.25, 0.50 and 1.00 ppm Cd over 30 days. The early mentioned behavioral and morphological changes under the effect of cadmium such as swimming abnormalities and swimming towards surface due to respiratory disturbances were also observed in *Ctenopharyngodon idellus* exposed to cadmium (Yorulmazlar and Gul, 2003).

It was reported that in addition to hypoxic conditions, dense stocking and starvation, exposure to heavy metals also result in stress conditions in fish (Gill *et al.*, 1993; Vaglio and Landriscana, 1999). The increased energy need under stress conditions are compensated from the carbohydrates stored as glycogen in tissues such as muscle and liver (Wendelaar, 1997) and also from non carbohydrate sources such as proteins and lipids through gluconeogenic enzymes (Levesque *et al.*, 2002).

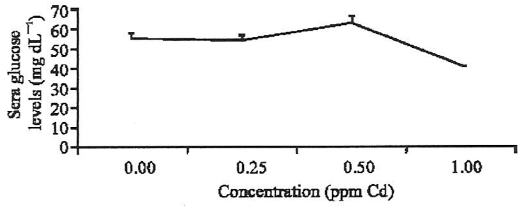

Fig. 1: Effects of cadmium on sera glucose levels (mg dL^{-1}) of *C. gariepinus*

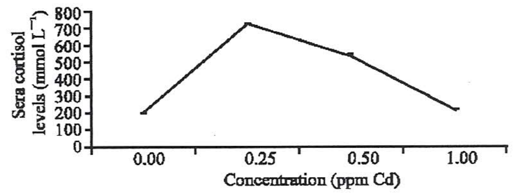

Fig. 2: Effects of cadmium on sera cortisol levels (nmol L^{-1}) of *C. gariepinus*

Cadmium increased glucose levels in *Sebastes schlegeli* compared with controls on long term exposure (Kim *et al.*, 2004) while in *Salmo salar* (Soengas *et al.*, 1996) and *Tilapia zillii* (Ghazaly, 1992) metal increased glucose levels on short term exposures. The metal did not affect sera glucose levels in *Anguilla rostrata* (Gill *et al.*, 1993) and decreased in *Puntius conchonius* (Gill and Pant, 1983). Exposure to 0.25 and 0.50 ppm cadmium had no effect and 1.00 ppm Cd decreased sera glucose levels in *C. gariepinus*. Energetic adaptation might explain the stability of sera glucose at lower concentrations while the decrease at highest concentration might be due to increased usage of glucose as metabolic energy. Homeostasis against varying environmental conditions is maintained by hormonal mechanisms in animals. Cortisol, a glucocorticoid plays an important role in maintaining homeostasis. Stress factors such as presence of heavy metals affect cortisol levels in fish (Gill *et al.*, 1993; Ricard *et al.*, 1998). Exposure to sublethal levels of cadmium over 30 days increased cortisol levels in adult *Oncorhynchus mykiss* while it had no effect on juveniles (Ricard *et al.*, 1998). Short term exposure to cadmium did not affect cortisol levels in various fish species (Dang *et al.*, 2001; Drastichova *et al.*, 2004). Changes in sera cortisol levels under the effect of cadmium in *C. gariepinus* might show that the increased energy need under the effect of metal was compensated through gluconeogenic pathways.

CONCLUSION

It was concluded that exposure to the tested concentrations of cadmium over 30 days caused changes in carbohydrate metabolism of *C. gariepinus*.

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