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Full Length Research Paper

Toxic effects of heavy metals (cadmium plus mercury) on haematological parameters and DNA damage in *Lates calcarifer*

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The present study was carried out to evaluate the effects of toxic metals (cadmium plus mercury) on the haematology and DNA damage of fish, *Lates calcarifer* for every 24 h up to 96 h. A significant gradual decrease of haemoglobin (-35.01 to -24.11) and hematocrit (-35.01 to -31.78) was noted at acute concentration of cadmium plus mercury (3.0 ppm) as compared to that of control. The comet assay of fish blood cells exhibited a significant higher DNA damage and the highest OTM was observed in 96 h of acute mixed metals concentration. From this investigation, the results proved that the combined metal toxicity induced haematological and DNA damage of fish *Lates calcarifer* than individual metal toxicity.

Key words: DNA damage, haemoglobin, hematocrit, heavy metals, *Lates calcarifer*.

INTRODUCTION

Heavy metal pollution is one of the most important environmental problems today. Modern industries are to a large extent, responsible for contamination of the environment. Nriagu and Pacyna (1988) reported that industrial wastes contain various types of toxic chemicals. Three kinds of toxic heavy metals are of concern including toxic heavy metals such as Hg, Cr, Pb, Zn, Cu, Ni, Cd, As, Co, Sn, etc. Heavy metals are dangerous because they tend to bioaccumulate.

Toxic metal pollutants in marine water tend to increase steadily thereby representing the greatest hazard to human consumers of fish. Investigations on the toxic effect of metals upon fish is accompanied by the analysis of changes in some haematological and biochemical blood indices (Hoyle et al., 2007). Haematological variables remain as veritable tools in determining the sub-lethal concentration of pollutants such as heavy metals in fish (Witeska, 2003). Haematological abnormalities have also been studied in various toxicant- Haematological variables remain as veritable tools in determining the sub-lethal concentration of pollutants such as heavy exposed fish: *Chana punctatus* to cadmium (Karuppasamy et al., 2005); *Cyprinus carpio* to carbofuran (Chandra et al., 2001); changes in the haematological profile of the fish exposed to mercury have been observed in *Hoplias malabaricus* (Oliveira-Ribeiro et al., 2006), *Tilapia mossambica* (Menezes and Quasim, 1984), *Oreochromis aureus* (Allen, 1994), *Ctenopharyngodon idella* (Shakoori et al., 1994), *Aphanius dispar* (Hilmy et al., 1980).

In view of the above, it was felt that it would be worthwhile to study the changes in blood haemoglobin, hematocrit level and DNA damage of fish to mixed metals which would throw a clear light on the extent of effect that it causes. Hence in the present work, we studied the toxic

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effects of cadmium plus mercury on haematology and DNA damage in fish *Lates calcarifer.*

MATERIALS AND METHODS

Test species

Specimens of *L. calcarifer* were collected from Rajiv Gandhi Centre for Aquaculture (RGCA), Thirumullaivasal, Sirkali, Tamil Nadu, India. Specimens were acclimatized to laboratory conditions at Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai for 15 days.

Water was changed daily and fish were fed *adlibitum* with flour pellets and ground dried shrimp twice a day. For experimental studies, fish ranging from 8 to 10 cm in length and weighing 6 to 8 g were selected.

Chemicals

Cadmium and mercury chlorides were obtained from Merck [Merck Company, Darmstadt, Germany, Glasxo India Limited, Bombay, India (No. 17584)] and used without further purification. All chemicals used were of analytical grade.

Experimental design

The qualities of the water was determined according to the method of Apha et al. (1976) and were as follows: dissolved oxygen 5.4 ± 0.02 mg/L; pH 8.6 ± 0.2; water temperature 28.0 ± 2.0°C; salinity 38 ± 0.07 ppt; total hardness 8.2 ± 2.0 mg/L; calcium 5.0 ± 0.1 mg/L; magnesium 3.0 ± 2.0 and total alkalinity 16.0 ± 06 mg/L. Preliminary studies were carried out to find out the median lethal concentration (LC_{50}) of cadmium plus mercury for 96 h by Probit analysis method of Finnery (1978).

The concentration at which 50% survival/mortality occurred was taken as a median lethal concentration (LC_{50}) for 96 h, which was 3.0 ppm for cadmium plus mercury. Acute toxicity studies were conducted for 96 h with a sampling interval of 24 h with four replicates of one treatment. All the tanks were filled with 20 L of water and 3.0 ppm/L of cadmium plus mercury was added to each tank. Twenty fish were introduced into each tank. A common control was also maintained.

The experimental set up had 5 replicates. Toxicant was renewed daily in all the experimental tanks. At the end of every 24 h, live fishes were taken from the experimental medium and blood was drawn from the heart region by cardiac puncture, with heparin as an anticoagulant and transferred into small vials kept in ice-cold condition.

Blood samples were collected for the analysis of haemoglobin content and hematocrit level. The haemoglobin content was estimated by the cyanmethemoglobin method using diagnostic reagent kit and hematocrit or packed cell volume was analysed by microhematocrit (capillary) method. Student's t test was used to study the significance.

Comet assay

The DNA damage was assessed by comet assay method. The comet assay or alkaline single cell gel electrophoresis was basically performed according to the original protocols of Singh et al., (1988). Briefly, the standard slides were immersed vertically in 1% Normal melting agarose (NMA) at 55°C and left vertically to allow the agarose to solidify. The slides were then kept at 4°C until use.

RESULTS

Table 1 and Figure 1 shows the changes in the haemoglobin (Hb) content and haematocrit (Hct) level of fish exposed to cadmium plus mercury. The Hb content decreased throughout the exposure period showing minimum percent decrease of -24.03 at the end of 72 h whereas the maximum percent decrease of -53.62 was observed at the end of 48 h. Similarly, Hct level also decreased throughout the exposure period showing minimum percent decrease of -31.64 at the end of 72 h and maximum percent decrease of -50.66 at the end of 48 h.

DNA damage

The cadmium plus mercury exposed fish exhibited a significantly ($p < 0.01$) higher DNA damage in the blood cell as compared to the control. In general, DNA damage was observed to be concentration and time dependent (Figure 2). The highest OTM was observed at 96 h. The distribution of comet cells exhibited different OTM in control and exposed specimens. In control, the cells with higher OTM were predominantly low. However in cadmium plus mercury treated fish, there was a clear shift with predominance of large number of cells. There was pointed increase in DNA damage in treated fish than that of the control which subsequently increased with increase of the exposure period.

DISCUSSION

The haematological characteristics of various cultivated fish species have been studied to characterize their haematological profile (Ranzani-Paiva et al., 2004). Oshode et al., (2008) reported that the observation of haematological parameters allows the most rapid detection of changes in fish. According to Nussey et al., (1995), the study of the haematological picture is frequently utilized for the detection of physiopathological changes in different stress conditions such as exposure to heavy metals. A decrease in the erythrocyte count or haematocrit indicates the worsening of an organism state and it is developing anaemia. Similar findings were observed in *Puntius conchonius* after mercury toxic exposure of 2 to 3 weeks duration by Gill and Pant (1987).

The decreases in haemoglobin concentration signifies that the fish's ability to provide sufficient oxygen to the tissues is restricted considerably and this will result in decrease of physical activity (Nussey et al., 1995). Reddy and Bashanihideen (1989) reported that the significant decrease in the haemoglobin concentrations may also be due to either an increase in the rate at which the haemoglobin is destroyed or to a decrease in the rate of haemoglobin synthesis. Panigrahi and Misra (1987) observed reductions in haemoglobin content and red blood cell count

Table 1. Haemoglobin and hematocrit content in the blood of *Lates calcarifer* during the short term metals (Cd plus Hg) exposure for varying periods.

Haemoglobin			
Exposure h	**Control**	**Cadmium plus mercury**	**t test**
24	6.134±0.021	3.986±0.040 (-35.01)	107.4
48	6.212±0.011	4.123±0.110 (-53.62)	104.4
72	6.012±0.014	4.567±0.011 (-24.03)	144.5
96	6.763±0.014	5.132±0.015 (-24.11)	163.1
Hematocrit			
Exposure h	**Control**	**Cadmium plus mercury**	**t test**
24	18.402±0.063	11.958±0.120 (-35.01)	92.05
48	18.636±0.033	12.369±0.330 (-50.66)	44.76
72	18.036±0.042	13.701±0.033 (-31.64)	108.37
96	20.289±0.042	15.396±0.045 (-31.78)	122.32

Values are mean ± S.E. of five individual observations. Values in parentheses are percent change over control; +: denotes percent increase over control; -: denotes percents decrease over control; *values are significant at 5% level; degree of freedom at 8t = 2.236.

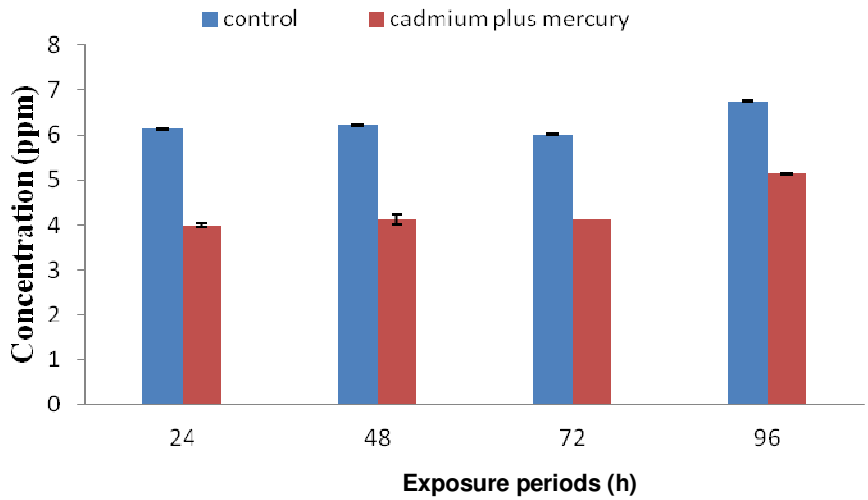
of the fish *Anabas scandens* treated with mercury. Decrease in haemoglobin, Red blood cell (RBC) count and Hct was observed in fish *Tinca tinca* exposed to mercuric chloride and lead (Shah and Altindag, 2004).

Decline in haemoglobin and hematocrit was observed in *Channa Punctatus* exposed to mercury by Sastry and Sharma (1980). According to Clarkson et al., (1961), about half of the mercury in blood is associated with red blood cells and the remaining half forms a complex with serum albumin by combination with sulfhydryl groups. Haematocrit decreased significantly in the mercuric chloride treated fish when compared with the control fish. The disturbed haemoglobin synthesis due to an effect of lead on aminolevulinate dehydratase (ALA-D) may result in anaemia (Santos and Hall, 1990). Similar findings were observed in the *O. mossambicus* treated with TIE showed reduction in the Hb content and Hct level. High concentrations of heavy metals or long-term exposure of fish to their sublethal concentrations usually decrease the above-mentioned indices.

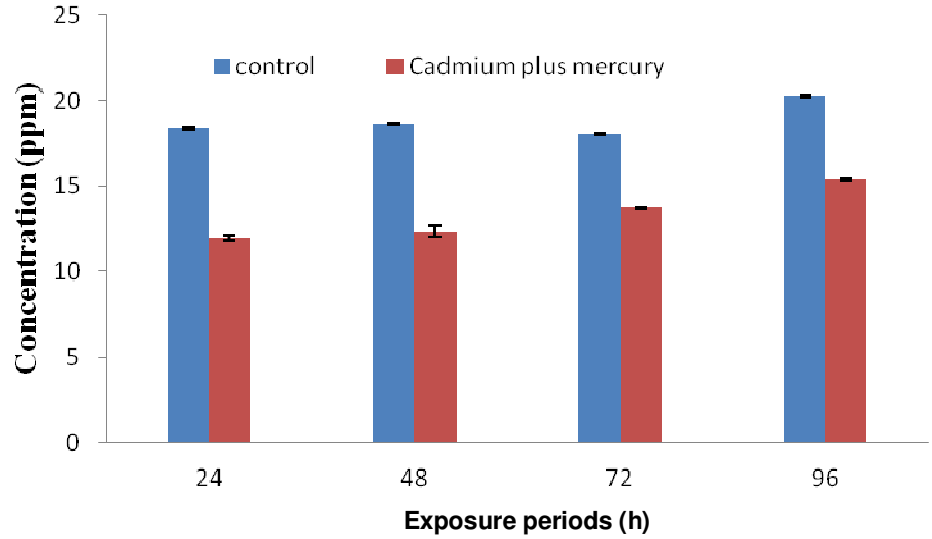
Similar results with significant reduction of RBC and Hb% content in fishes exposed to different heavy metals have been reported previously by Goel et al., (1985) and Goel and Sharma (1987). Karuppasamy et al., (2005) found a significant decreased in total erythrocyte count, haemoglobin content, hematocrit value and mean corpuscular haemoglobin concentration in air breathing fish, *C. punctatus* after exposure to sublethal dose of Cd (29 mg Cd/L). A long-term exposure of perch (*Perca fluviatilis*) (12 and 27 days) to the model heavy metal mixture (zinc, copper, lead, cadmium, and mercury) caused a decrease of the haematocrit level, haemoglobin concentration, mean erythrocyte volume and an increase in glucose depending on the concentration of the mixture. In the present study, the decrease levels of haemoglobin and heamatocrit may be due to anaemia effect or increased destruction of hemoglobin or a decrease in the rate of haemoglobin synthesis. It should be noted that this mixture of heavy metals had no effect on the count of white blood cells, while lower concentrations of lymphocytes were determined in the blood of perch dwelling in natural water bodies polluted with mixtures of heavy metals of similar composition (Larsson et al., 1984) but in our study, the mixture of metals reduced the Hb and Hct levelling of fish *L. calcarifer.*

DNA strand breakage is a sensitive indicator of genetic damage and a useful tool for monitoring the genotoxic effects of a wide range of pollutants on aquatic organisms (Shugert, 1988). The comet assay, a simple and sensitive technique for analyzing and quantifying DNA strand breakage is widely applied to the cells of aquatic organisms, including fish, to detect the genotoxicity of a wide range of chemical agents (Lee and Steinert, 2003). In the present investigation, during acute mixed metals treatment, very high DNA damage was observed in fish *L. calcarifer*. Similar observation was made by several authors.

Shi et al., (2005) reported that Cd (5 mg/L) could cause DNA damage in liver of goldfish (*Carassius auratus*); DNA damage was also found in trout liver cells exposed to Cd at the concentrations of 0.11 to 1.12 mg/L (de Faverney et al., 2001). These data also suggests that fish are sensitive to very low Cd concentrations. Bucio et al., (1999) reported that comet-assay studies carried out on mercury compounds indicate that low concentrations (5 M) of mercury chloride produce DNA damage in a human hepatic cell line and that this compound produces DNA damage by a non-apoptotic mechanism in a human cell line with monocytic characteristics (Ben-Ozer et al., 2000). Previous studies have shown that in response to



(A)



(B)

Figure 1. (A) Haemoglobin and (B) hematocrit content in the blood of *Lates calcarifer* during the short term metals (Cd plus Hg) exposure for varying periods.

2000). Previous studies have shown that in response to metal exposure, there is an increase in the formation of oxygen free radicals or reactive oxygen species (ROS) in rats, rainbow trout and *M. rosenbergii* (Stohs and Bagchi, 1995; Bopp et al., 2008); this can result in widespread damage to cells because of lipid peroxidation and genotoxicity. Respiratory burst, the release of reactive oxygen species (ROS) by haemocytes, is a critical step in the innate immune response. Parasites are eliminated following phagocytosis. Under normal conditions, the production and destruction of ROS is well regulated, but under environmental oxidative stress, the balance between pro-oxidative and antioxidative reactions is shifted in favour of the former (Achard-Joris et al., 2006).

Cadmium is known to displace Zn and Fe ions from metalloproteins, resulting in their inactivation as well as the release of free Fe that can then catalyze the generation of reactive oxygen species through the Fenton reaction (Stohs and Bagchi, 1995). Cd has been shown to induce ROS production in freshwater goldfish (*Carassius auratus*) after exposure for 24 h (Shi et al., 2005). Cd-promoted oxidative stress leads to DNA strand damage, especially mitochondrial DNA (Yakes and Van Huten, 1997).

Xiuying et al., (2010) reported that DNA damage and Tail length (TL) significantly increased in rat when the Cd level was higher than 0.41 mg/L. However, Tail moment (TM) significantly increased in all the experimental groups.

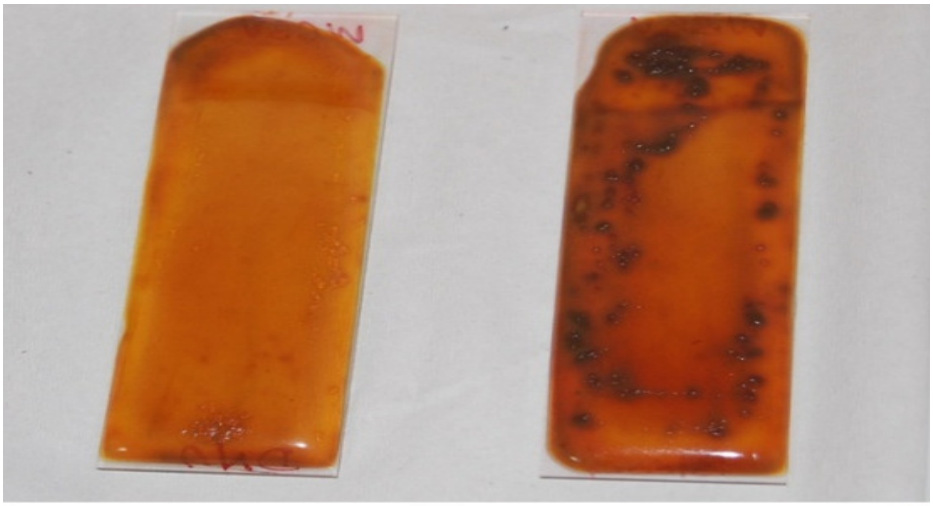


Figure 2. Photograph showing comet assay and blood DNA damage in fish *Lates calcarifer*.

The results suggested that Cd has the potential to induce DNA damage in the liver of fish at very low concentrations. Shi et al. (2005) reported that Cd (5 mg/L) could cause DNA damage in liver of goldfish *C. auratus*; DNA damage was also found in trout liver cells exposed to Cd at the concentrations of 0.11 to 1.12 mg/L (Risso De Faverney et al., 2001). These data also suggest that fish are sensitive to very low Cd concentrations. DNA damage can be directly caused by exposure to acute acid stress as a result of increased metal cations or indirectly through ROS production (Hengstler et al., 2003; Jolly et al., 2004). DNA Damage was also found in trout liver cells exposed to Cd at the concentrations of 0.11 to 1.12 mg/L (de Faverney et al., 2001).

Bucio et al., (1999) reported that comet assay studies carried out on mercury compounds indicate that low concentrations (5 M) of mercury chloride produce DNA damage in a human hepatic cell line and that this compound produces DNA damage by a non apoptotic mechanism in a human cell line with monocytic characteristic (Ben-Ozer et al., 2000). Mercury chloride confirms its genotoxic properties. Methyl mercury has produced concentration-dependent increases in DNA damage in isolated bottlenose dolphin leukocytes (Taddei et al., 2001). Copper exposure also causes an increase in the DNA damage as observed in erythrocytes from the gilthead bream, *Sparus aurata*, and in haemocytes of the bivalve *Scapharca inaequivalvis* (Gabbianelli et al., 2003). It is well known that the increased generation of ROS results in DNA damage and apoptosis (Kawanishi et al., 2002; Shi et al., 2004). In the present work, acute toxic effects of mixed metals have caused DNA damage in fish *L. calcarifer,* which might be due to the above reasons.

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