Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 8, 2015

Original Article

COMBINED EFFECTS OF CADMIUM AND MECURY ON SOME BIOCHEMICAL AND HISTOCHEMICAL CHANGES IN LIVER, KIDNEY AND GILLS OF *CHANNA PUNCTATUS* (BLOCH)

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Received: 06 Feb 2015 Revised and Accepted: 15 Jun 2015

ABSTRACT

Objective: The present study was conducted to investigate the effects of cadmium (Cd) and mercury (Hg) in combination at sub-lethal concentrations for 32 days on histochemical localization of heavy metals and on serum biochemical parameters such as glucose, triglyceride, cholesterol and total protein concentrations in *Channa punctatus*.

Methods: Biochemical estimations of serum glutamic-pyruvic transaminase (SGPT), glucose and different lipids were done using the standard protocols provided in the commercial kits purchased from Reckon diagnostics Pvt. Ltd., India. Histochemical analyses of liver, kidney and gills were determined by sulphide–silver method and heavy metal (cadmium and mercury) concentrations in gills, kidneys and liver were analyzed by Atomic Absorption Spectroscopy.

Results: Glucose, lipid, total protein and SGPT levels were significantly altered in fish exposed to Cd or Hg salt alone. However, combined exposure of Cd and Hg normalized all the aforesaid biochemical indices. The accumulation of heavy metals was in following order: cadmium content was more in kidney>liver>gills; whereas, Hg content was more in liver>kidney>gills. Histochemical analyses revealed that mercury accumulation was comparatively more than that of cadmium in all the tissues. Several histological abnormalities were noted in the liver, kidney and gills of the Cd or Hg treated animals. However, Cd in combination with Hg caused alleviation in the toxic effect of Hg on histochemical and biochemical parameters. Results of our study showed that Cd may have a protective effect against Hg toxicity.

Conclusion: Our findings indicate that cadmium may protect mercury-induced toxicity.

Keywords: Cadmium, Mercury, *C. punctatus*, Liver, Kidney, Blood glucose, Atomic absorption spectroscopy.

INTRODUCTION

Contamination of the marine environment by metals has increased in recent years, primarily due to the increase in global population and industrial development. Heavy metals are introduced into the environment through various routes such as industrial effluents and wastes, agricultural pesticide runoff, domestic garbage dumps and mining activities [1]. Mercury (Hg) is a well known global contaminant to aquatic species, more specifically to fishes and marine mammals [2]. Various aspects of mercury toxicity in fish species have been reported in the past [3, 4].

Cadmium is another toxic metal, which is widely used in mining and metallurgical operations; electroplating, manufacturing and vinyl plastic industries. In fact, Hg and Cd are the best known among the environmental contaminants [5].

Blood parameters are being increasingly used as indicators of the physiological or sub-lethal stress responses in fishes subjected to exogenous factors [6]. Measurement of serum biochemical parameters are useful as they help to identify the target organs toxicity as well as to provide early warning of potentially damaging changes in stressed organisms [7]. Therefore, the primary objective of this study was to evaluate individual and combined effects of Cd and Hg in a fish model, *C. punctatus*.

MATERIALS AND METHODS

C. punctatus (CP) fishes were procured from Khan River of Indore, M. P, India and were transferred to the laboratory. Fishes were acclimatized to laboratory conditions in glass aquaria for 20 days with a natural photoperiod (12 h light-12 h dark). The fishes were fed twice daily with prawn powder and were washed with 0·1% KMNO4 solution to avoid dermal infection. Water in the tank was changed daily and aerated to ensure sufficient oxygen supply. The tap water was analyzed for physico-chemical characteristics by the method described by APHA [8].

The analytical results of tap water showed a temperature of 23.6±2.0ºC; pH of 8.1±0.1; dissolved oxygen as 7.4±0.02 mg/l, total alkalinity of 180.0±10.00 mg/l CaCO3, and total hardness of 276.5±7.5 mg/l CaCO3.

Prior to experimentation, toxicity tests were conducted to determine the LC_{50} and safe concentration values of cadmium sulphate ($CdSO_4$) and mercuric chloride ($HgCl_2$) for 48 and 96 hours in the test fishes. The LC_{50} values of $HgCl_2$ and $CdSO_4$ (for 48 and 96 h) in *C. punctatus* were found to be 4.0 mg/l and 2.5 mg/l for $HgCl_2$ and 22 mg/l and 18 mg/l for $CdSO_4$ respectively.

The preliminary experiment in *C. punctatus* showed that $CdSO_4$ at 1.0 mg/l and $HgCl_2$ at 0.025 mg/l were less toxic (data not shown). Therefore, in this study, these sub-lethal doses of cadmium and mercury (1.0 mg/1 and 0.025 mg/l respectively) were considered.

In the next experiment, healthy adult fishes (40 in number) of nearly equal weight were divided in to four groups of 10 each and were transferred to glass aquaria in the laboratory. They were fed every day with prawn powder. The fishes were treated with sub-lethal concentrations of $CdSO_4$ and $HgCl_2$, i.e.1.0 & 0.025 mg/l respectively. They were exposed to test metals in alone as well as in combination for a period of 32 days.

Group I fishes without getting exposed to test metal served as the control, while the fishes of groups II and III were exposed to 1 mg/l of $CdSO_4$ and 0.025 mg/l $HgCl_2$ solutions, respectively. Fishes of group IV were exposed to a mixture of equivalent amount of $HgCl_2$ (0.025 mg/l) and $CdSO_4$ (1.0 mg/l). After experimental period of 32 days, the fishes were kept on fasting for 24 h, blood samples were collected, and serum was separated by centrifugation (10 min, at 4000 g, 4 °C) and stored at −20 °C until the biochemical assays were performed. The levels of serum glutamic pyruvic acid, glucose, triglyceride and cholesterol were measured using the protocols provided in commercial kits purchased from Reckon diagnostics Pvt. Ltd., India.

Total protein in serum was estimated following the method of Lowry *et al.* [9]. The fishes from each group were dissected. Liver kidney and gill tissues were removed and fixed in different fixatives. For the determination of heavy metals the modified Timm sulfide-silver method was followed [10] that were confirmed by the appearance of the brownish black color. A comparison of control and experimental sections was made to assess metal deposition in tissue sections.

In the fishes, the concentration of heavy metals in selected organs such as liver, gill and kidney was estimated by atomic absorption spectroscopy following the method of Liang *et al.* [11]. In brief tissues were dried at 105 °C for 48 h and then digested with concentrated nitric acid and perchloric acid (2:1 v/v) at 120 ºC for 3 h. After dilution the volume was made up to 5 ml with double distilled water. Cd and Hg concentrations of the tissues were then measured by atomic absorption spectrophotometer (Unicam 969, Analytical Technology Inc., Cambridge, United Kingdom).

Data analysis

Data have been presented as mean±S. E. M. and were analyzed by the analysis of variance (ANOVA) followed by Post hoc Newman-Keuls Multiple Comparison test, using trial version of Prism 4 software.

RESULTS

The data on the concentrations of heavy metals in liver, gill and kidney of the *Channa punctatus,* as estimated by atomic absorption spectroscopy have been presented in fig. 1.

Metal concentrations in the fish were expressed in mg/kg.

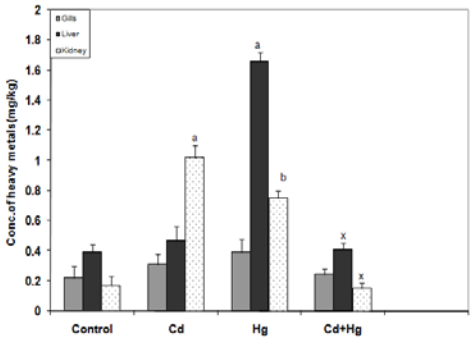

Fig. 1: **It shows the concentration (mg/kg dry weight) of test heavy metals following their individual or combined treatment, in different organs of freshwater fish, *Channa punctatus*. Each bar represents the mean±SEM (n=10). a, *P*<0.001 and b, *P*<0.01, as compared to the respective control value. x, *P*<0.001, as compared to the value of Cd and Hg treatment alone**

Heavy metal accumulation in the test fish was in the following order: Cadmium content was more in kidney>liver>gills; Hg content was more in liver>kidney>gills.

This suggests that cadmium accumulation was Maximum in the kidney and least in the gills; whereas mercury content was highest in liver and least in gills.

Cadmium decreased the glucose and total cholesterol levels significantly (*P*<0.001 or *P*<0.01, as compared to the respective control value), without any significant change in SGPT, protein and triglyceride levels. However, fishes exposed to Hg increased the glucose, triglyceride and SGPT levels, but decreased the protein and cholesterol levels significantly (*P*<0.001 for all, as compared to the respective control values). Following the combined treatment all the alterations observed by the treatment of individual metal were normalized and values were almost near to that of control (fig. 2).

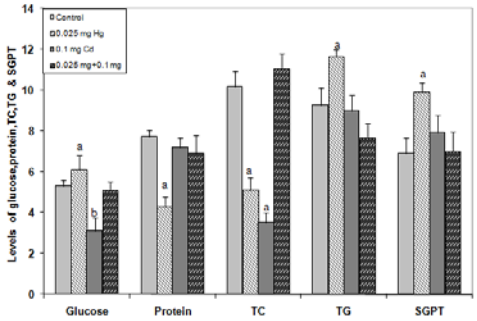

Fig. 2: It shows the levels of serum glucose (mg/dl), triglyceride (mg/dl), cholesterol (mg/dl), total protein (mg/dl) and serum glutamic-pyruvic transaminase activity (IU/l) following the individual or combined exposure of cadmium and mercury(last dark shaded bar) for 32 days in *Channa punctatus*. Each bar represents the mean±SEM (n=10). a, *P*<0.001 b, *P*<0.01 as compared to the respective control values

Histochemical localization of Hg and Cd

Liver (fig. 3)

Liver of control fish did not show the presence of mercury or cadmium. However, in mercury (0.025 mg/l) exposed animals, the metal accumulation was more in the hepatocytes and the distribution was uneven. When exposed to Cd (0.1 mg/l), it was also deposited unevenly in hepatocytes, although in lesser amount as compared to mercury. In liver sections of Hg+Cd treated fish, metal deposition was lesser than that of Hg or Cd alone exposed animal.

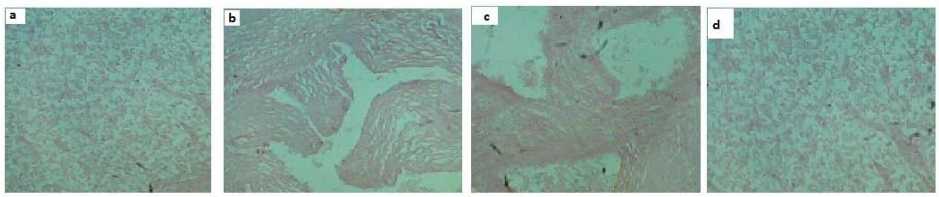

Fig. 3: It shows the histochemical localization of Hg and Cd in the liver of *C. punctatus*. a) Control liver shows no trace of metal. b) Liver exposed to Cd shows less distribution of metal throughout the central hepatocytes. c) Hg exposed liver shows distribution of metal throughout the central hepatocytes. d) Section of liver exposed to Cd+Hg shows faint metal deposition throughout the hepatocytes.

Kidney (fig. 4)

While no metal deposition was detected in the kidney sections of the control fish, in the low dose of Hg exposed animals, deposition of metal was noticed in both proximal and distal tubules of the kidney and it was more on higher dosed animals. However, in the kidney exposed to both the metals, lesser accumulation of metals was observed in proximal tubules.

Gills (fig. 5)

In control fishes, gill sections did not show any metal deposition. But in fishes following Hg exposure, major portion of the gill was observed black due to the greater accumulation of Hg. Cd exposure led to lesser accumulation, as compared to only mercury exposed animals. Cd was mainly localized at the free tips of the secondary gill filaments. In Cd+Hg treated fishes metal accumulation in the gills

was localized very less in pilaster cells. Overall, concentrations of metals were less as compared to Cd or Hg alone exposed animals.

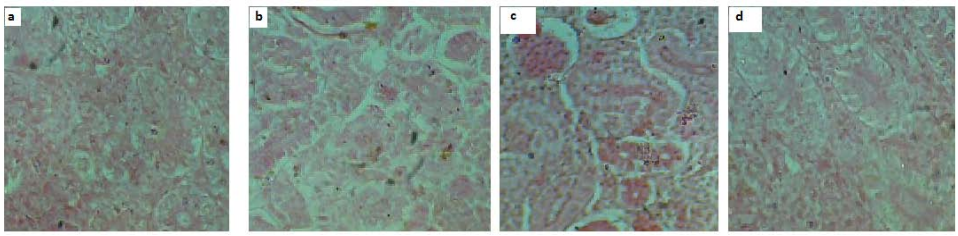

Fig. 4: **Shows the histochemical localization of Hg and Cd in the kidney of *C. punctatus*. a) Control kidney shows no trace of metal. b) Kidney exposed to Cd shows less distribution of metal. c) Section of kidney exposed to Hg shows less distribution of metal. d) Section of liver exposed to Cd+Hg shows faint metal deposition**

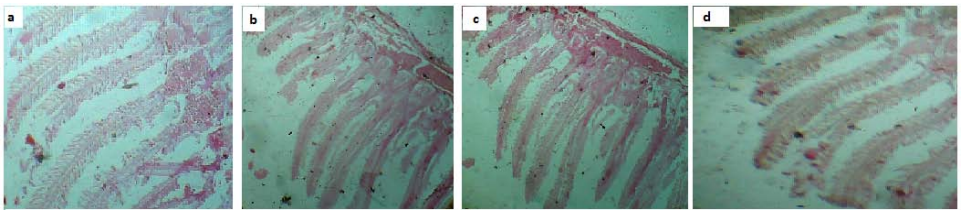

Fig. 5: **Shows the histochemical localization of Hg and Cd in the gill of *C. punctatus*. a) Control gill showing no deposition of metal. b) Gill exposed to Cd shows less distribution of metal. c) Section of gill exposed to Hg shows more distribution of metal. d) Section of gill exposed to Cd+Hg shows faint metal deposition**

DISCUSSION

Environmental pollution and subsequent contamination of land, water and soil are sometimes considered as causative factors for growing health problems. Very often, fishes and other aquatic animals are also subjected to environmental stressors, because of their permanent exposure to dissolved toxic substances through their gills, skin, and digestive tract [12]. This was evident in our study on estimating the concentrations of heavy metals by atomic absorption spectroscopy in some organs such as liver, gill and kidney of the fresh water fish *C. punctatus,* collected from Khan river, Indore, India. The findings revealed that cadmium was accumulated mostly in the kidney and least in the gills, whereas mercury content was high in liver and least in gills in the test fish. Majority of previous studies reported higher amounts of accumulated mercury in liver tissue because of its higher metabolic activities and the presence of more metallothionein (MT) which acts as absorbent of mercury [13-15]. This might be a reason in the present study also. Cadmium content in the kidney was found to be higher in both the fishes. This finding is in agreement with the commonly accepted view that Cd is primarily absorbed by the liver, where it is bound by MTs and is transported to the kidney. At higher Cd concentrations, the kidney itself also absorbs Cd directly from the blood [16].

Deposition of mercury was also found to be more in liver of fish; *C. punctatus*. The reason could be that the mercury is naturally delivered to the liver through portal system, because liver acts as an important target organ. A large volume of blood flow to renal tissues causes the kidney to be exposed to high levels of circulating compounds and may lead to xenobiotic accumulation in the kidneys [17]. The results of mercury localization in liver corroborated the earlier finding showing a higher incidence of metal deposits in hepatocytes [18]. Regarding gills, similar accumulation pattern of mercury and cadmium were observed in the fish confirming the ability of this tissue to reflect metal bioavailability. In fact, similar to the present finding, in another study, gills were found to be one of the least contaminated tissues, probably because of the consequence of the frequent renewal through exfoliation and erosion to which it is subjected [19].

Measurement of serum biochemical parameter are especially useful to help identify target organs of toxicity as well as the general health status of animals, and is advocated to provide early warning of potentially damaging changes in stressed organisms [20].

Changes of blood glucose are a good indicator of metal stress in fish [21] and alterations in the glucose level is related to renal injury, liver damage, and lack of nutrition [22]. Exposure of Hg for 32 days enhanced the glucose levels in the test fish, similar to the earlier findings [23, 24]. This increase in serum glucose level might be due to metal stress. However, simultaneous cadmium exposure for similar duration decreased the glucose levels. The reduced level of glucose probably reflected the exhaustion of the energy reserves of the organism and an impaired capacity of fish to restore them to acclimatized condition [25].

It is generally accepted that increased activity of SGPT in extra cellular fluid or plasma is a sensitive indicator of even minor cellular damage [26]. The test fishes, exposed to 0.1 mg/l Cd for 32-days showed nearly normal SGPT activity (may be due to lower concentration of Cd used in this study). Similar observation was also reported earlier [27]. However, in mercury exposed fishes, SGPT level was enhanced significantly probably because of increased transaminase activity, associated with rapid breakdown of carbohydrate and proteins to compensate the increased energy crisis, as suggested in another finding [28]. There was also a reduction in cholesterol concentration after treating the fishes with cadmium sulphate for 32-days in CP. This decreased cholesterol could be due to the possible inhibition of the conversion of esterified cholesterol to free cholesterol by accumulated cadmium.

With respect to changes in triglyceride concentration an increase in triglyceride level was noticed in Hg treated group indicating its adverse effects in the liver. However, there was no significant alteration in triglyceride level in cadmium alone treated animals, which could be due to acclimation to the low concentration of toxicant [29]. A depletion of total protein content was also noticed in Hg exposed groups. This depletion by Hg might be due to reduced protein synthesis or a protein breakdown caused by cirrhosis or due to an increase in the rate of protein binding to the plasma toxicants. This may be a general adaptation syndrome [30]. The adverse effect of Hg could be attributed to the destruction or necrosis of cellular structures and consequent impairment in protein synthesis by the heavy metal or due to the arrested metabolism in the liver [31].

The interesting finding in the present study is that almost all metal-induced changes in biochemical parameters were nearly normalized following their combined exposure.

Possible mechanism of counteracting the effects of mercury by cadmium may be related to the beneficial effects of Cd when administered in low concentration. Moreover, Cd is known to enhance some enzyme activities in animals [32]. Interestingly, with respect to cadmium the adverse effects are moderate and less than that of mercury suggesting that mercury is more toxic than cadmium, as reported earlier [33]. None the less this observation suggests that cadmium might be counteracting the effects of mercury in the test fish. Somewhat similar type of observation was also made earlier by cadmium and chromium [34].

Higher accumulations of metals in different organs were observed by individual exposure of metals. Interestingly, the accumulation of metals significantly reduced following the combined exposure, indicating that Cd plays the protective role against mercury-induced histochemical alterations in *Channa punctatus.*

The present study involving the combined effects of mercury and cadmium in the test fish appears to be the first report indicating the ameliorating effects of Cd in mercury toxicity of a fish.

CONFLICT OF INTERESTS

Declared None

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