International Journal of Scientific Research in Science and Technology
© 2018 IJSRST | Volume 4 | Issue 11 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X

Evaluation of Biochemical and Histochemical Changes Following the Combined Treatment of Mercury and Cadmium in a Fresh Water Cat Fish, *Clarias Batrachus* (Linn)

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ABSTRACT

Objective: The main objective of this study was to determine the combined effects of cadmium (Cd) and mercury (Hg) at sub-lethal concentrations for 32 days on histochemical localization of heavy metals and on serum biochemical parameters including serum glutamic-pyruvic transaminase (SGPT) enzyme activity; glucose, triglyceride, cholesterol and total protein concentrations in *Clarias batrachus*.
Methods: Histochemical demonstration of Hg and Cd salts in liver and kidney was determined by sulphide–silver method and SGPT, glucose, triglyceride and cholesterol in the serum were measured using the standard protocols provided in the commercial kits purchased from Reckon diagnostics Pvt. Ltd., India.
Results: Serum SGPT, glucose, triglyceride, cholesterol and total protein levels were significantly altered in fish exposed to Cd or Hg salt alone. However, combined exposure of Cd and Hg normalized all the above mentioned biochemical parameters. Histochemical analysis demonstrated enormous amount of metals in the liver and kidney tissues exposed to Hg and Cd alone. Mercury accumulation in *C. batracus* was comparatively more than that of cadmium in both the tissues.
Conclusion: While exposure Hg or Cd adversely altered the biochemical parameters in the test catfish, following the combined exposure of both the metals, the concentrations of metal accumulation were decreased in both the tissues of *C. batracus*.
Keywords: Cadmium, Mercury, *Clarias batrachus*, Liver, Kidney, Blood glucose.

INTRODUCTION

Heavy metals are considered as highly dangerous contaminants of aquatic biota. They cause stress response in fish . As an indicator of environmental livability, fish seems to be very important since it is affected by its habitat (namely water), which is very often polluted by effluents from industries, pesticides washed out from agricultural lands and detergents from household drains etc. In fact, the release of heavy metals into the aquatic environment causes water pollution problems because of their toxicity, persistence and bio-accumulation. In recent years, Cd has become a problem of higher magnitude because of the toxic nature of the pollutant in sea, river and estuary waters.

Fish absorbs metal directly from contaminated water or indirectly from feeding on living organisms of the contaminated water . Another toxic metal is mercury. In fact, one of the most dangerous pollutants that can be found in waters is mercury. The hazards of these two heavy metal pollution have received much attention all over the world in recent years, because both Cd and Hg concentration are increasing every year , although antagonistic and synergistic effects of heavy metals are well documented in a variety of aquatic species.

Liver and kidneys are pivotal organs of the body responsible to maintain the homeostasis of the body, as liver is center of metabolism and detoxification; while kidneys are involved in an elimination of the wasteful chemicals from the body and selective reabsorpotion . Accumulations of the chemical pollutants are known to adversely affect the histology and functioning of liver, kidney, muscle and other organs of fish .

Studies on the harmful effects of environmental stressors such as metals are well documented. However, the literature on the combined effects of mercury and cadmium is very scanty. Hence the present study was aimed to determine the combined effects of mercury and cadmium on the histochemcal localization of heavy metals and on the changes in serum biochemical parameters in a fish model, *Clarias batrachus* (C. B.)

MATERIALS AND METHODS

From the Khan river of Indore, M. P., India, the living and healthy specimens of *Clarias batrachus* were collected and were kept in glass aquaria. Fishes were acclimatized for 15 days in stored tap water and were treated with 0.1% $KMnO_4$ solution to avoid any dermal infection. Some of these fishes were used for the determination of LC_{50} values.

The preliminary experiment showed that 96 h-LC_{50} of $CdSO_4$ and $HgCl_2$was found to be 10 mg/l and 1.0 mg/l for $HgCl_2$ respectively. Therefore, in this study, the sub-lethal doses of cadmium and mercury (1.0 mg/1 and 0.01 mg/l) were considered, i. e. 1/10th of the 96h-LC_{50}.

Healthy adult fishes (40 in number) of nearly equal weight were divided in to four groups and were transferred to glass aquaria in the laboratory. They were fed every day with prawn powder. Physicochemical characteristics of experimental water were as follows: temperature 29±1°C, pH 7.6±0.2, dissolved oxygen concentration, 7.8-8.3 mg/l, total alkalinity: 22.0 ±4.00 mg/l, and total hardness: 11.0 ± 0.05 mg/l.

Fishes were treated with sub-lethal concentrations of cadmium sulphate ($CdSO_4$) and mercuric chloride ($HgCl_2$) i. e.1.0 & 0.01mg/l respectively. They were exposed to each metal alone as well as in combination for a period of 32 days as mentioned below.

Group I served as the control, while the fish of groups II and III were exposed to 1 mg/l of $CdSO_4$ and 0.01 mg/l $HgCl_2$ solutions, respectively. Fishes of group IV were exposed to a mixture of $HgCl_2$ (0.01 mg/l) and $CdSO_4$ (1.0 mg/l). Experimental period was 32 days. On completion of the experimental period, the fishes were kept on fasting for 24 h and then blood samples were collected. Serum was separated by centrifugation of whole blood (10 min, 4000 g, 4°C) and stored at –20°C until the experimental assays were performed. The levels of serum glutamic pyruvic acid (SGPT), 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.*[10]. The fishes from each group were dissected, liver and kidney tissues were removed and fixed in different fixatives. For the determination of heavy metals, the modified method of Timm sulfide -silver method was followed.

RESULTS

Results are presented on Fig.1, 2 and 3. Following the exposure of $CdSO_4$ to the fishes, a significant decrease in glucose and cholesterol levels was observed (P<0.001or P<0.01) as compared to control. Although no significant changes in triglyceride levels were observed, there was a significant reduction in protein levels (P<0.001) as compared to control value. SGPT activity was also increased significantly (P<0.01).

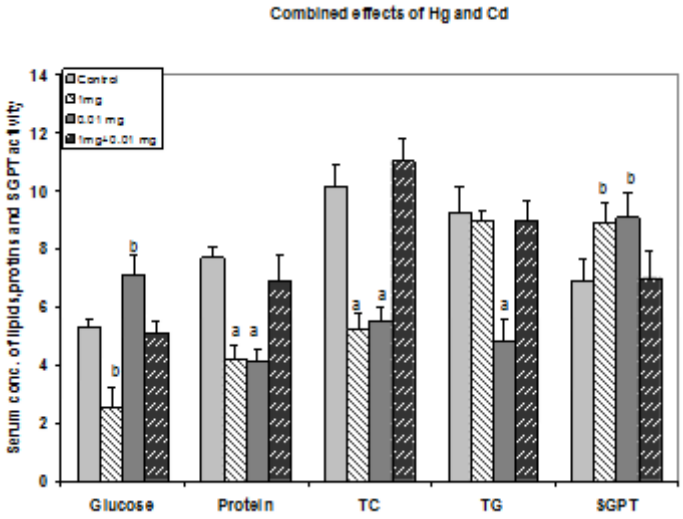

Fig. 1 : Changes in the levels of serum glucose (mg/dl); triglyceride (mg/dl); cholesterol (mg/dl); total protein (mg/dl) and glutamic-pyruvic transaminase (SGPT) activity (IU/L) following the combined exposure of cadmium and mercury for 32 days in ***Clarias batrachus***. Each bar represents the Mean ± SEM (n=10). [a], P<0.001 [b], P< 0.01 as compared to the respective control value.

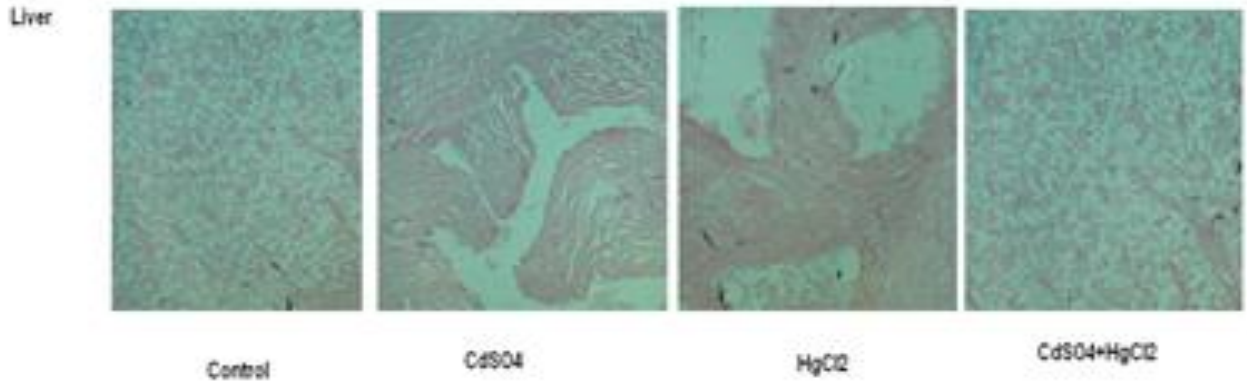

Fig. 2 : Histochemical localization of mercury and cadmium in ***C. batrachus*** in Liver: Control liver showing faint deposition of metal. Section of liver of 0.01 mg/l $HgCl_2$ exposed fish showing faint deposition in the peripheral region and heavy accumulation in the central zone of the liver. Cd exposed liver showing uneven distribution of metal in the hepatocytes. Liver exposed to mercury and cadmium Hg + Cd showing faint metal deposition throughout the hepatocytes.

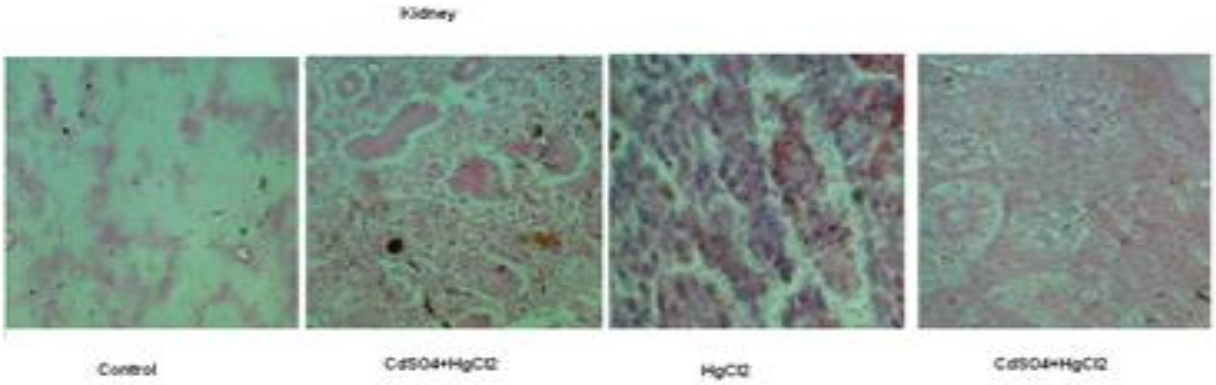

Fig. 3 : Histochemical localization of mercury and cadmium in *C. batrachus* in kidney Control kidney shows no traces of metal. Kidney treated with $HgCl_2$ exhibits metal deposition in kidney tubules. Kidney exposed to $CdSO_4$ shows metal accumulation in proximal and distal kidney tubules. Kidney exposed to mixture of Hg + Cd shows less accumulation of metals throughout the kidney section.

In $HgCl_2$ alone exposed groups (Fig.1), glucose and SGPT levels were increased significantly ($P<0.01$). But, the levels of protein, cholesterol and triglyceride decreased significantly ($P<0.001$ or $P<0.01$, as compared to control values). However, following the combined treatment of Hg and Cd and exposed to similar duration, no significant changes were observed in any of the aforesaid parameters.

Metal accumulation

With respect to histochemical localization of metals it was observed that mercury deposition in liver and kidney(Figs 2 and 3 respectively) was to a greater extent as compared to CdSO4. On the other hand exposure to Hg + Cd (in combination), accumulation of metals were less both in kidney and in liver and the values were somewhat similar to control fishes.

DISCUSSION

Heavy metals, as environmental stressors are known to alter serum biochemical parameters in fishes, which suggest that serum biochemical indices could be used as important and sensitive biomarkers in ecotoxicological studies concerning the effects of metal contamination and fish health. Results of the present study clearly indicated that $HgCl_2$ exhibited deleterious effects in *Clarias batrachus* as evidenced by an increase in glucose and SGPT levels. The blood glucose level has been used as an indicator of stress in fishes. Findings of some earlier studies indicated an increase in plasma cortisol, glucose and lactate levels in different fish species exposed to stressors like heavy metals. In our study also, an increase in glucose and SGPT levels was observed as compared to the control values in the serum of the fishes exposed to mercury. Heath (1995) reported that the effects are cortisol mediated that is released from the adrenal cortex, that in turn helps in glycogen storage in liver by reducing the use of glucose by tissues and it keeps blood glucose at high concentrations. Similar report is also available indicating high glucose concentrations following exposure of mercury in fishes. Exposure of mercury for 32 days significantly lowered the cholesterol levels which may be due to the bioaccumulation of metals which might have inhibited the conversion of esterified cholesterol to free cholesterol. Significant

decrease in triglycerides content in blood plasma of *C. batarachus* by Mercury indicated its adverse effect on the liver. A similar effect of sublethal concentration of malathion has also been reported earlier in *C. batrachus*. Changes in protein is one of the earliest indicators of heavy metal poisoning. A significant decrease in protein levels was also observed following Hg exposure. The depletion of protein content during mercury toxicity may be due to reduced protein synthesis and/or due to enhanced proteolysis in the various organs of fish. Earlier also depletion of protein content had been observed in all the organs of *C. batrachus following the* exposure of mercury.

Although fishes exposed to cadmium showed the significant decrease in glucose, cholesterol and protein levels, triglyceride level remained unchanged. This decrease in the glucose levels might have resulted from a inhibition in glycogenolysis in the liver or a reduction in the rate of absorption of glucose through the intestine. The reduced level of glucose found at the end of exposure of Cd could be a reflection of the exhaustion of the energy reserves of the organism and an impaired capacity of fish to restore them.

The decrease in protein content by Cd may be either due to reduced protein synthesis or a protein breakdown caused by nephrosis, by cirrhosis or by an increase in the rate of protein utilization as a general adaptation for the excess toxicant (cadmium) in the plasma. Exposure to cadmium as well as mercury resulted in significantly elevated levels of SGPT which may either due to leakage of these enzymes from hepatic cells and thus raising levels in blood or due to increased synthesis as suggested in earlier studies. These adverse effects may be related to the observed accumulation of Hg in liver and kidney which are considered to be major target organs of toxicity. Interestingly, with respect to cadmium the adverse effects are moderate and less than that of mercury suggesting that mercury is more toxic than cadmium. These findings corroborate with an earlier report.

When both the heavy metals were added in the water and fishes were exposed to a similar period, toxic effects were minimized and normal levels in the observed indices were obtained. Surprisingly, combined exposure of Hg and Cd had lesser adverse effects in catfish, in terms of metal accumulation, thus exhibited antagonistic effects to individual treatment. This observation suggests that cadmium might be counteracting the effects of mercury in fish. Somewhat similar type of observation was also made earlier by cadmium and chromium.

Possible mechanism of counteracting the effects of mercury by cadmium may be related to the beneficial effects of Cd when administered in low dose. Moreover Cd is known to enhance some enzyme activities in animals. This might have been the cause in this study also. The present study involving the combined effects of mercury and cadmium in a catfish appears to be the first report indicating the ameliorating effects of Cd in mercury toxicity of a fish.

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