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Influence of EDTA against mercuric chloride induced hepatic enzymological changes in the fingerlings of *Labeo rohita* (Ham.)

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Abstract

We investigated the effect of median-lethal concentration of mercuric chloride (0.13 ppm for 96 hrs) on the activities of acid and alkaline phosphatases, glutamate pyruvate and glutamate oxalo acetate transaminase, glucose-6-phosphatase, glucose and also glycogen and their recovery in the fingerlings of the fish *Labeo rohita* (Ham.). A significant reduction in acid and alkaline phosphatases, glucose-6-phosphatase and glucose activities and concomitant increase in glutamate pyruvate and glutamate oxalo acetate transaminase and glycogen activities have been observed in liver tissue when exposed to mercuric chloride. During the recovery, due to EDTA treatment to the mercury treated fish all the above parameters reached the near normal levels. The results suggested that the exposure of EDTA competitively reduced the mercuric chloride toxicity in the fingerlings of the fish *Labeo rohita* (Ham).

Keywords : EDTA, *Labeo rohita*, liver damage, liver enzymes, mercuric chloride

INTRODUCTION

Pollution is one of the challenging problems to the environmental biologists (Kavitha and Jagadeesan, 2006). The global environment is polluted with heavy metals arising out of different industrial processes (Ramalingam *et al.,* 2002). Heavy metal pollution is a major problem in water environment (Rajasubramaniam, 2006). Mercury is used on large scale in industries, agriculture, medicines and dentistry and is considered to be the most toxic heavy metal (Masud *et al.,* 2005). Mercury in any chemical from has the capability to denature proteins, inactivate the enzymes and cause severe disruption of physiological processes (Masud *et al.,* 2001 & 2003).

Enzymes are essential factors, which enable many bio-chemical reactions that constitute life to proceed in the cells of body and changes in enzyme concentration in tissue cells therefore reflect the state of health. The liver is a major target organ for toxic compounds (Guillonzo *et al.,* 1995). The measurement of phosphatase (ACP and ALP) activity is an useful indicator of liver function (Nair *et al.,* 1998). Amino transfereases (GOT and GPT) are the reliable marker enzymes of liver and they are the first enzymes of liver in diagnostic enzymology when liver damage has occurred (Kuchel and Ralston, 1988). Transaminases play a major role in mobilizing L-amino acids for gluconeogenesis, since they function as links between carbohydrate and protein metabolism, especially under various physiological, pathological and environmental stress conditions that drain the energy produced continuously in order to cope up with the needs of compensatory adjustments (Knox and Greengard, 1965). Due to this unique property, GOT and GPT, the most important aminotransfereases are used for assessing the metabolic injury during the toxic stress and also to throw light on the extent of involvement of gluconeogenics pathway. For the latter purposes analysis of the enzyme, glucose-6-phophatase, a potent terminal key enzyme of gluconeogenesis in which glucose-6-phosphate is formed from pyruvic acid by reversal of glycolysis is essential (Verma, 1981; Rana and Sharma, 1982; Shaffi and Dubey, 1989).

Ethylene diamine tetra acetic acid (EDTA) is an effective chelatig agent of heavy metals (Licop, 1988) as it has a strong chelating ability for different heavy metals (Norvell, 1991; Kedziorek and Bourg, 2000). It has another advantage *i.e.,* bio-degradability in ground water system (Nowack, 1996). We report changes in liver enzymes such as ACP, ALP, Glucose-6-phosphatase, GOT and GPT and glucose and glycogen levels in the fingerlings of *Labeo rohita* treated with median-lethal concentration of mercuric chloride and recovery in their levels due to EDTA treatment.

MATERIALS AND METHODS

Fingerlings of *Labeo rohita* (7 to 9 cm length and 20 to 22 g) were collected locally and acclimated to the laboratory conditions for 30 days during which they were regularly fed with oil less groundnut cakes. They were kept in clear and unchlorinated water with the water temperature maintained at 28 ± 0° C, salinity from 0.4 to 0.5 ppm p^H from 7.4 to 7.8 and dissolved oxygen content from 7.2 to 7.4 ppm. *L. rohita* fingerlings were divided into 3 groups of 10 each for bioenzymological studies. Fingerlings of Group I was reared in metal free water and maintained as control. Group II and III were exposed to median lethal concentration of mercuric chloride (0.13

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ppm) for 96 hours. After 96 hours, Group III was again treated with EDTA at required concentration (5 ppm) (recovery period) for another 96 hours.

ACP and ALP activities were estimated by the method of Tenniswood *et al.,* (1976) and GOT and GPT activities by the method of King (1965). Glucose-6-phosphatase activity was determined by the modified method of Harper given by Bergmeyer (1965).Glycogen and Glucose were estimated by the method of Kemp and Heijhnigen (1954).

RESULTS AND DISCUSSION

In the liver of *L. rohita* fingerlings treated with median-lethal concentration of mercuric chloride the levels of glucose, glucose-6-phosphatase, ACP and ALP were lower when compared to control while the level of GOT, GPT and glycogen activities were higher (Table 1). ACP, ALP, glucose-6-phosphatase and glucose levels and reduction in the levels of GOT, GPT and glycogen to near normal level. Treatment with EDTA had resulted in the increase of Table 1.

ACP is a lysosomal enzyme found in endoplasmic reticulum, while ALP is known to be a membrane bound enzyme found in hepatocytes (Shakoori *et al.,* 1992). Loss of ACP and ALP activities in liver tissue of mercuric chloride intoxicated fish may be due to consequent changes in the permeability of plasma membrane in addition to change in the balance between synthesis and degradation of enzyme proteins (Jagadeesan and Kavitha 2006). Sastry and Gupta (2005) reported that $HgCl_2$ intoxication significantly decreased the ACP and ALP activities in the fresh water teleost fish *Channa punctatus*. Humtsoe *et al.* (2007) stated that the decreased level of ACP and ALP indicated disturbance in the structure and integrity of cell organelles like endoplasmic reticulum and membrane transport system.

In the present study the level of GOT, GPT and Glycogen activities were increased and simultaneously the Glucose and Glucose-6-phosphatase activities were decreased in liver tissue of fish treated with $HgCl_2$ for 4 days, which indicated the impact of mercury toxicity. The increase in GOT and GPT may be due to the hepatocellular necrosis which caused increase in permeability of cell membrane resulting in the release of transaminase activity (Vandenberghe, 1995). Similar result was obtained by several investigators on the liver tissue of mice, *Mus musculus* treated with $HgCl_2$ (Jagadeesan and Kavitha, 2006; Sharma *et al.* 2002 and Margarat, 2001).

The reduction in glucose-6-phosphatase activity, in the present study was quite significant as it was similar to the effect caused by toxic compounds on the two aminotransferases (Rajamannar and Manohar, 2000). Reduced glucose-6-phosphatase level was also reported by Rana and Sharma (1982) when the fish *Channa punctatus* was exposed to mercurial toxicity. Similar results was obtained by several investigators on various fishes treated with various toxic compounds (Verma, 1981; Shaffi and Dubey, 1989).

Glucose are the most important fraction of carbohydrate as glucose supplies the immediate energy needed by tissues. A marked decrease in glucose content may be due to glucose utilization by the liver tissues to meet excess amount of energy demand imposed by severe anaerobic stress of mercury intoxication. Another reason for the decline in the glucose content in the respective tissues may be due to enhancement of glycogen synthesis (Sankar Samipillai, 2003). Havu (1969) had reported that, in lower vertebrates, heavy metal induced liver toxicity that results in the release the hormones that promote glyconeogenesis, which may in turn enhance the secretion of insulin causing an enhancement of glycogen synthesis.

During the recovery period, due to EDTA treatment decrease in the levels of ACP, ALP, glucose-6-phosphatase and glucose and increase in the levels of GOT, GOT and glycogen to near normal levels were observed in mercuric chloride intoxicated fish. Since EDTA is a chelating agent which could involve the removal of heavy metal toxicity (Burrough and Kastner, 1993), it is concluded that EDTA could be used as an antagonist against the toxic effects of mercury.

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Table 1. Changes (Mean ± *S.D.*) in the levels of ACP, ALP, GOT, GPT, Glucose-6-phosphatase, Glucose and Glycogen in the liver tissue of the fingerlings of *Labeo rohita* treated with 4 days of median – lethal dose of mercuric chloride followed by 4 days of EDTA treatment

Variables	Control	$HgCl_2$ Treatment	$HgCl_2$ + EDTA Treatment	*F*	Sig. *P*
ACP (Values expressed as µ moles of phenol librated / minure / 100mg protein)	2.61 ± 0.31	1.36 ± 0.42	1.86 ± 0.12	29.639	<0.001
ALP (Values expressed as µ moles of phenol librated / minure / 100mg protein)	28.26 ± 1.35	22.82 ± 0.87	31.80 ± 0.89	98.681	<0.001
GOT (Values expressed as IU / L)	8.20 ± 0.57	9.06 ± 0.61	7.81 ± 0.68	50954	<0.012
GPT (Values expressed as IU / L)	6.17 ± 0.70	6.64 ± 0.83	4.70 ± 0.36	11.773	<0.001
Glucose-6-phosphatase(Values expressed as Micromole / mg protein / hour)	0.145±0.007	0.111±0.007	0.125±0.004	36.224	<0.001
Glucose(Values expressed as mg / g)	7.656±0.412	3.638±0.239	5.332±0.684	105.202	<0.001
Glycogen(Values expressed as mg / g	155.47±3.163	168.94±2.430	157.56±2.133	46.204	<0.001

Table 2. Mean percentage over control in the levels of ACP, ALP, GOT, GPT, Glucose-6-phosphatase, Glucose and Glycogen in the liver tissue of the fingerlings of *Labeo rohita* treated with 4 days of median – lethal dose of mercuric chloride followed by 4 days of EDTA treatment

Variables	$HgCl_2$ Treatment	$HgCl_2$ + EDTA Treatment
% COUTC[a]	-47.89*	- 28.73*
% COHgT[b]		+ 36.76*
% COUTC[a]	-19.25*	+12.53*
% COHgT[b]		+39.35*
% COUTC[a]	+10.48*	- 4.76*
% COHgT[b]		+ 13.76*
% COUTC[a]	+ 07.62*	-23.83*
% COHgT[b]		-29.22*
% COUTC[a]	-23.448*	-13.793*
% COHgT[b]		+12.612*
% COUTC[a]	-52.481*	-30.355*
% COHgT[b]		+46.564*
% COUTC[a]	8.664*	1.344*
% COHgT[b]		-6.736*

[a] Percentage change over untreated control

[b] Percentage change over mercury treated.

* Significantly different over control at 5% level ('*t*' test)

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