ISSN: 0974 - 0376

The Ecoscan : Special issue, Vol. IX: 257-265: 2016
AN INTERNATIONAL QUARTERLY JOURNAL OF ENVIRONMENTAL SCIENCES
www.theecoscan.com

IMPACT OF CADMIUM ON SOME BIOCHEMICAL PROFILES OF THE FRESH WATER CAT FISH CLARIAS GARIEPINUS (BURCHELL, 1822)

Sanghamitra Sahu *et al.*,

KEYWORDS

Hypercholesterolemia
Gluconeogenesis
Glycogenolysis
Glutamate oxaloacetate transaminase (GOT) and glutamate Pyruvate transaminase (GPT)

Proceedings of National Conference on
Harmony with Nature in Context of
Resource Conservation and Climate Change
(HARMONY - 2016)
October 22 - 24, 2016, Hazaribag,
organized by
Department of Zoology, Botany, Biotechnology & Geology
Vinoba Bhave University,
Hazaribag (Jharkhand) 825301
in association with
National Environmentalists Association, India
www.neaindia.org

The Ecoscan
AN INTERNATIONAL QUARTERLY JOURNAL OF ENVIRONMENTAL SCIENCES

SANGHAMITRA SAHU, SUBHASHREE DEBASMITA AND ANIMESH KUMAR MOHAPATRA
Department of Life Science Education,
Regional Institute of Education, Bhubaneswar - 751 022, Odisha, INDIA
e-mail: sanghamitra813@gmail.com

ABSTRACT

The present investigation was aimed to observe the impacts of cadmium (Cd) in vivo on fresh water cat fish, *Clarias gariepinus* exposed to sub-lethal concentration of 0.4 mg/L and 0.8 mg/L for 15, 30, 45 and 60 days. LC50 value was calculated using Finney's probit analysis. Serum biochemical parameters (protein, glucose, cholesterol) and hepatic and renal tissue biochemical parameters (protein, GPT and GOT) were measured both in control and experimental fish after exposure period. The declining trends were observed in the protein levels of liver tissue with an increase in the concentration of Cd and duration of exposure for exp 1 (lower dose) to exp 2 (higher dose) i.e. 18.7435 ±1.33 and 14.1081 ± 0.79 μg/g wt tissue respectively with respect to control 25.6898 ± 1.33 μg/g wt tissue for 60 days. Similar declining trend was also observed for hepatic and renal tissue protein content. The upsurge in mean total serum glucose was observed in treated fish *i.e.* 110.1075 ± 6.37 and 141.02 ± 8.10 mg/dl in comparison to control fish i.e. 65.1575 ± 4.16 mg/dl. There was enhanced level of GOT and GPT in hepatic and renal tissues. In conclusion, the magnitude of alterations in biochemical parameters was influenced by both concentration and exposure duration.

***Corresponding author**

INTRODUCTION

Heavy metals constitute a core groups of aquatic pollutant because of their bio-accumulative and non-biodegradable properties (Vagas *et al.,* 2001; Deepali and Madhu, 2007) and enter into aquatic environment through industrial effluents, agricultural wastes, sewage disposal, soil leaching and rainfall (Prasath and Arivoli, 2008; Hassan *et al.,* 2009). The life sustaining quality of water deteriorates when heavy metals reach the aquatic bodies and cause damage to both flora and fauna (Kotsanis and Georgudaki, 1999; Zyadah and Abdel-Bakey, 2000; Samanta *et al.,* 2005). Several researchers have reported alterations in tissue carbohydrate, protein and lipid content as well as serum glucose, cholesterol and protein level in response to heavy metal exposure in fishes (Almeida *et al.*, 2001; Shukla *et al.,* 2002; Yang and Chen, 2003; Bedii and Kenan, 2005; Sobha *et al,* 2007; Anandhan and Hemalatha, 2009; Ozgur and Kargam, 2010; Rajput *et al.,* 2012; Mohanty et al, 2013 and Arya, 2014). Deleterious effect of heavy metals on enzymatic activity has also been reported in different fishes (Karan *et al.,* 1998; De Smet and Blust, 2000; Rao, 2006 and Naveed *et al.,* 2010).

Among heavy metals cadmium is a non-corrosive and highly toxic metal listed in "Black list" of European community (Manson, 1996). It is a nonessential element with no biological function. It is used in battery, plastics, metal alloys, dye and metal plating industries. Effluents from such activities are sources of cadmium in aquatic environment. Cadmium builds up in soft tissues like liver, kidney, and brain over its life span which is termed as bioaccumulation. Literature on biochemical study pertaining to catfishes is limited in Indian context. Therefore the present study aims to investigate the effect of sub-lethal concentrations of cadmium on serum biochemical parameters (glucose, protein and cholesterol) and tissue biochemical parameters (protein, GOT and GPT) in fresh water catfish *Clarias gariepinus* during different exposure periods (15, 30, 45 and 60 days).

MATERIALS AND METHODS

The present study was carried out in the department of Life Science, Regional Institute of Education Bhubaneswar, Odisha. Live and healthy freshwater cat fish, *Clarias gariepinus* were collected from the State Fishery Development Corporation having length 24-26cm and weight 100g. The fish were treated with 0.05% $KMnO_4$ solution for 2 minutes to avoid dermal infection and were maintained in large tanks filled with de-chlorinated tap water. These fishes were provided cat fish feed (4% of their body weight) once daily. They were left for 4 weeks for acclimatization prior to experiment and water was changed regularly at interval of two days with fresh de-chlorinated water. The median lethal concentration of $CdCl_2$ for *Clarias gariepinus* was calculated by following the Probit analysis method (Finney, 1971). After acclimation, the fishes were randomly separated into three groups (group of 25 fish) and transferred into three tanks (300 liters capacity each). One of the groups served as control while the other two groups are experimental groups exposed to two different sub-lethal concentration of $CdCl_2$ *i.e.* 0.4 mg/l and 0.8 mg/l till the end of experiment. The fish were maintained in the tanks with water replaced every alternate day with fixed concentration of $CdCl_2$ in the experimental tanks till the end of experiment. Biochemical studies were made by collecting blood and tissues (liver and kidney) after 15, 30, 45 and 60 days of exposure to $CdCl_2$ using five fishes each from control and both experimental groups every time.

Blood sample was collected from the caudal vein of live fish of both control and experimental groups by using non-heparinized tubes for serum biochemical analysis. Blood was collected in the morning hours to avoid diurnal variation and was kept undisturbed for 24 hrs at 4° to 5°C for clotting. After 24hrs, the supernatant was pipetted out from coagulated blood and centrifuged at 2500 rpm for 20 min. Serum was then removed and stored at 4°C. The biochemical parameters were evaluated by following various techniques: Protein by Bradford protein assay (Bradford, 1976), Glucose by GOD method (Trinder, 1969), Cholesterol by CHOD-PAP method (Allain *et al.,* 1974). After the collection of blood samples, fishes were carefully dissected out and hepatic and renal tissues were used for biochemical analysis. The tissues were homogenized in buffer solution. The homogenates were centrifuged in microcentrifuge at 6000 rpm for eight minutes. The supernatant was pipetted out and used to estimate the protein by Bradford protein assay (Bradford, 1976) and GOT, GPT in these tissues by following IFCC method (Huang *et al.,* 2006). The significance of differences between control and experimental groups was statistically analyzed using student's t-test $p<0.05$ was taken as the level of significance.

RESULTS

The biochemical response of cadmium in fresh water cat fish *Clarias gariepinus* was studied by exposure to sub lethal concentrations of 0.4mg/l and 0.8 mg/l for 15, 30, 45 and 60 days. The mean values for the biochemical parameters for the fishes of the control tank and those of both treated tanks (lower and higher sub-lethal doses) on 15th, 30th, 45th and 60th day of exposure were observed. Results were expressed as mean ± SEM of five replicates and differences between means were considered to be significant when $p < 0.05$.

When the hepatic tissues were subjected for estimation of total proteins (μg protein/g wet tissue) at various sub-lethal concentrations for different exposure periods a gradual decrease (control > lower dose > higher dose) was noticed (Fig. 1 and Table 1) which was statistically significant ($P<0.05$). A similar significant decrease in mean total protein in renal tissues (μg protein/g wet tissue) was observed (Figure 2 and Table 2) in the treated fish in comparison to controlled fish (control > lower dose > higher dose) of 15th, 30th, 45th and 60th day exposure which was statistically significant ($P<0.05$). Like tissues protein a significant reduction in serum protein content (μg protein/ml serum) was also observed in cadmium treated fish in comparison to controlled fish for different exposure period (Fig. 3 and Table 3) and it was statistically

Table 1: Changes in protein in liver (μg protein/g wet tissue) of *Clarias gariepinus* exposed to sub-lethal concentration of Cadmium for a period of 60 days

	Group	N	Mean	SEM	SD	Mean difference		df	t	Significance
15th Day	Control	5	25.9085	.39306	.78613					
	Exp1(LD)	5	24.4966	.99661	1.99322	Control & Exp1	1.41192	4	1.257	.298
	Exp2(HD)	5	21.2843	.47691	.95381	Control & Exp2	4.62426	4	6.165	.009
30th Day	Control	5	24.7130	1.04212	2.08424					
	Exp1(LD)	5	22.1223	.99137	1.98275	Control & Exp1	2.59074	4	4.461	.021
	Exp2(HD)	5	20.2309	.71113	1.42226	Control & Exp2	4.48218	4	2.928	.061
45th Day	Control	5	25.8630	.48773	.97547					
	Exp1(LD)	5	20.3063	.84667	1.69334	Control& Exp1	5.55666	4	4.469	.021
	Exp2(HD)	5	17.9676	.30039	.60078	Control & Exp2	7.89543	4	11.002	.002
60th Day	Control	5	25.6898	.66924	1.33848					
	Exp1(LD)	5	18.7435	.66512	1.33025	Control & Exp1	6.94638	4	5.337	.013
	Exp2(HD)	5	14.1081	.79565	1.59131	Control & Exp2	11.58174	4	13.358	.001

Table 2: Changes in protein in renal tissues (μg protein/g wet tissue) of *Clarias gariepinus* exposed to sub-lethal concentration of Cadmium for a period of 60 days

	Group	N	Mean	SEM	SD	Mean difference		df	t	Significance
15th Day	Control	5	55.7280	1.67302	3.34605					
	Exp1(LD)	5	47.7840	1.96581	3.93162	Control & Exp1	7.94400	4	2.709	.073
	Exp2(HD)	5	44.7450	.71672	1.43343	Control& Exp2	10.98300	4	6.303	.008
30th Day	Control	5	61.2150	2.01619	4.03237					
	Exp1(LD)	5	46.1700	2.20733	4.41467	Control & Exp1	15.04500	4	5.220	.014
	Exp2(HD)	5	40.7460	1.35706	2.71411	Control& Exp2	20.46900	4	6.164	.009
45th Day	Control	5	66.7980	2.72234	5.44468					
	Exp1(LD)	5	44.3280	2.07611	4.15222	Control& Exp1	22.47000	4	5.123	.014
	Exp2(HD)	5	35.2050	1.39511	2.79022	Control& Exp2	31.59300	4	10.715	.002
60th Day	Control	5	75.2280	2.46106	4.92211					
	Exp1(LD)	5	39.6420	1.68822	3.37644	Control& Exp1	35.58601	4	9.982	.002
	Exp2(HD)	5	24.2670	.91877	1.83754	Control& Exp2	50.96100	4	23.672	.000

Table 3: Changes in serum protein content (µg protein/ml serum) of *Clarias gariepinus* exposed to sub-lethal concentration of Cadmium for a period of 60 days

	Group	N	Mean	SEM	SD	Mean difference		df	t	Significance
15th Day	Control	5	47.5200	.77101	1.54201					
	Exp1(LD)	5	44.9900	.89981	1.79961	Control & Exp1	2.53000	4	2.022	.136
	Exp2(HD)	5	36.9450	.74561	1.49123	Control & Exp2	10.57500	4	12.568	.001
30th Day	Control	5	47.2975	.95686	1.91371					
	Exp1(LD)	5	40.4975	.71388	1.42776	Control & Exp1	6.80000	4	5.635	.011
	Exp2(HD)	5	35.3450	.40699	.81398	Control & Exp2	11.95250	4	17.712	.000
45th Day	Control	5	43.7825	.79882	1.59763					
	Exp1(LD)	5	38.3100	1.16874	2.33748	Control& Exp1	5.47250	4	6.996	.006
	Exp2(HD)	5	32.0875	.79423	1.58847	Control & Exp2	11.69500	4	24.204	.000
60th Day	Control	5	46.3275	.93502	1.87003					
	Exp1(LD)	5	34.9400	1.15588	2.31177	Control & Exp1	11.38750	4	6.851	.006
	Exp2(HD)	5	25.5775	1.84977	3.69954	Control & Exp2	20.75000	4	7.461	.005

Table 4: Changes in serum glucose (in mg/dl) of *Clarias gariepinus* exposed to sub-lethal concentration of Cadmium for a period of 60 days

	Group	N	Mean	SEM	SD	Mean difference		df	t	Significance
15th Day	Control	5	56.6800	2.27536	4.55073					
	Exp1(LD)	5	63.7525	1.99051	3.98102	Control & Exp1	-7.07250	4	-2.660	.076
	Exp2(HD)	5	82.1675	1.73477	3.46955	Control& Exp2	-25.4875	4	-7.477	.005
30th Day	Control	5	60.9125	1.73619	3.47238					
	Exp1(LD)	5	81.0075	3.13926	6.27853	Control & Exp1	-20.0950	4	-9.658	.002
	Exp2(HD)	5	94.5200	1.48558	2.97116	Control& Exp2	-33.6075	4	-11.796	.001
45th Day	Control	5	61.1375	2.10094	4.20188					
	Exp1(LD)	5	92.8175	1.40022	2.80044	Control & Exp1	-31.6800	4	-16.151	.001
	Exp2(HD)	5	119.2150	2.23148	4.46295	Control& Exp2	-58.0775	4	-50.973	.000
60th Day	Control	5	65.1575	2.08221	4.16442					
	Exp1(LD)	5	110.1075	3.18798	6.37596	Control& Exp1	-44.9500	4	-8.893	.003
	Exp2(HD)	5	141.0200	4.05138	8.10277	Control& Exp2	-75.8625	4	-14.715	.001

significant ($p<0.05$). In both the tissues and the serum the decrease was more significant with increasing dosage concentration and length of exposure.

The result of the present study showed a significant upsurge in mean total serum glucose (in mg/dl) was observed in case of cadmium treated fish in comparison to controlled fish of 15th, 30th, 45th and 60th day exposure (Figure 4 and Table 4). Following treatment with sub-lethal concentrations of cadmium chloride the fishes showed rise in serum cholesterol (Figure 5 and Table 5) with both increasing exposure duration as well as concentration of cadmium. The increase in case of both serum glucose and cholesterol was directly proportional to both dosage and duration dependent decrease (control < lower dose < higher dose) and was statistically significant ($p<0.05$).

From this experiment, it was observed that there was increase the level of mean GOT activity in the tissues of liver (in U/L) when the test species were exposed to different sub-lethal concentrations of cadmium in different time courses (Figure 6 and Table 6) in comparison to GOT activity of control fish (control < lower dose < higher dose). Further, it was observed that there was increase in the level of GOT activity (in U/L) in renal tissues (Fig. 7 and Table 7), when *Clarias gariepinus* were exposed to different sub-lethal concentrations of cadmium in different exposure period (control < lower dose < higher dose). In both hepatic and renal tissues, the GOT activity was statistically significant ($p<0.05$) and the increase was more significant with increasing days of exposure.

Following the exposure of $Cdcl_2$ to the fishes, a significant rise in GPT activity in hepatic tissues (U/L) was observed as compared to control (Figure 8 and Table 8). The results of the present study also demonstrated that the exposure of cadmium caused significant increase in the activities of GPT activity in renal tissues (U/L) in treated fish than controlled fish of 15th, 30th, 45th and 60th day exposures (Figure 9 and Table 9). In both hepatic and renal tissues, the GPT activity was statistically significant ($P<0.05$) and the increase was more significant with increasing days of exposure (control < lower dose < higher dose).

DISCUSSION

Heavy metals, one of the major environmental stressors are known to alter biochemical parameters in fishes. The tissue and serum biochemical indices could be used as sensitive and major biomarkers in eco-toxicological studies related to the effects of metal contamination and fish health (Oner *et al.*, 2008 and Arya, 2014). In the present study, exposure to sub-lethal concentrations of cadmium chloride caused significant alterations in biochemical parameters of the fish *Clarias garipienus*.

Protein is the most abundant biochemical constituent present in the animal body and involved in major physiological events. In fish, proteins are one of the main energy sources which play an important role in the maintenance of blood glucose. Changes in tissue and serum protein is one of the important

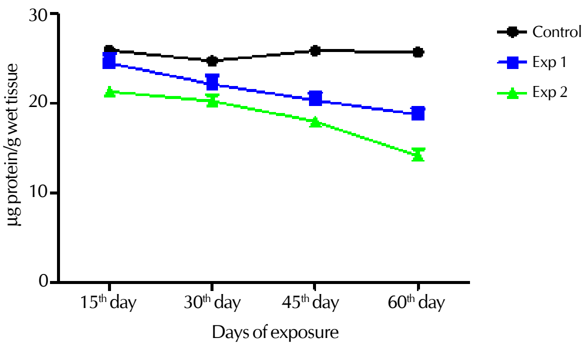

Figure 1: Protein in liver tissues of *Clarias gariepinus* exposed to sub-lethal concentration of Cadmium

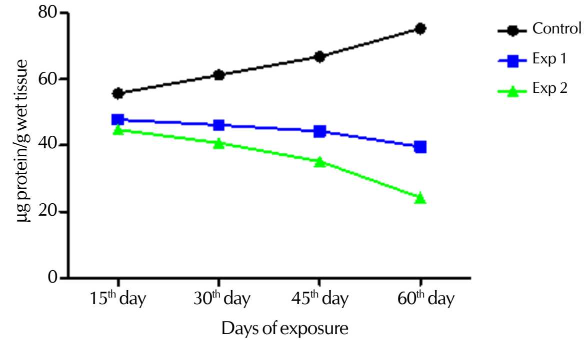

Figure 2: Protein in renal tissues of *Clarias gariepinus* exposed to sub-lethal concentration of Cadmium

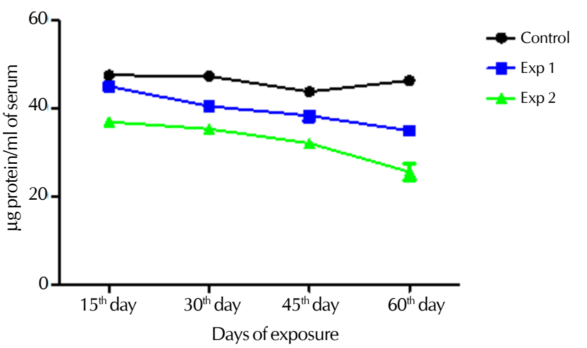

Figure 3: Serum Protein content of *Clarias gariepinus* exposed to sub-lethal concentration of Cadmium.

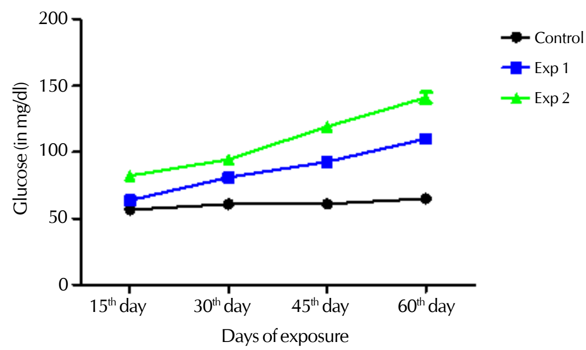

Figure 4: Serum glucose content of *Clarias gariepinus* exposed to sub-lethal concentration of Cadmium.

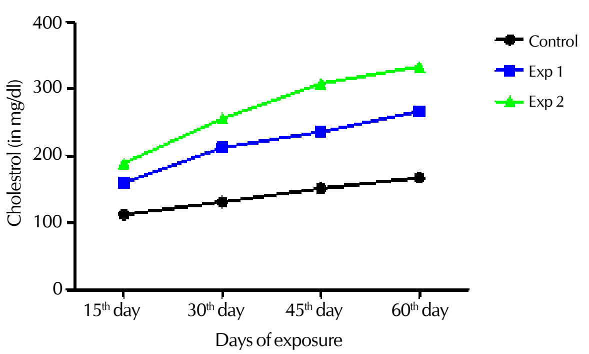

Figure 5: Serum cholesterol content of *Clarias gariepinus* exposed to sub-lethal concentration of Cadmium

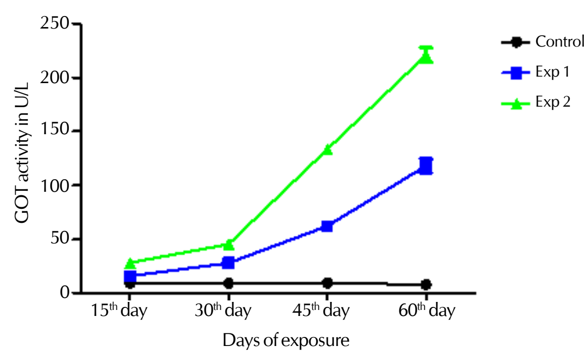

Figure 6: GOT activity in liver of *Clarias gariepinus* exposed to sub-lethal concentration of Cadmium

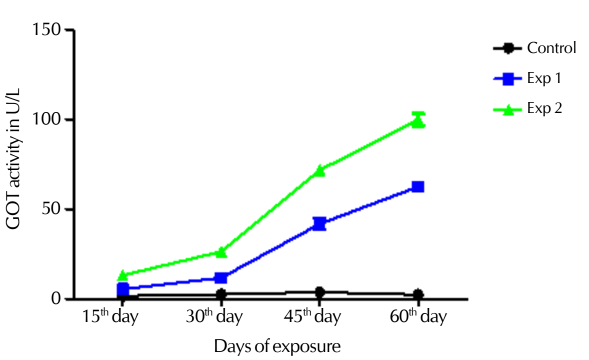

Figure 7: GOT activity in renal tissues of *Clarias gariepinus* exposed to sub-lethal concentration of Cadmium

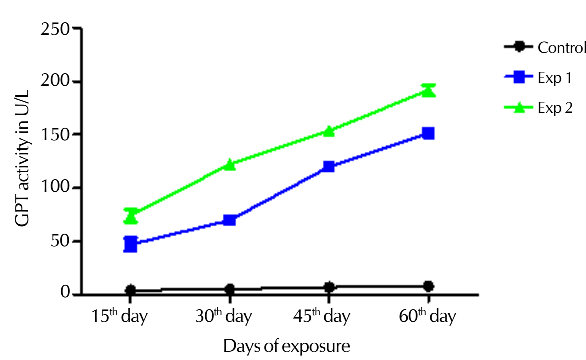

Figure 8: GPT activity in liver tissues of *Clarias gariepinus* exposed to sub-lethal concentration of Cadmium

Table 5: Changes in serum cholesterol level (in mg/dl) of *Clarias gariepinus* exposed to sub-lethal concentration of Cadmium for a period of 60 days

	Group	N	Mean	SEM	SD	Mean difference		df	t	Significance
15th Day	Control	5	112.2900	7.24178	14.48356					
	Exp1(LD)	5	159.3725	5.64963	11.29925	Control & Exp1	-47.08250	4	-7.238	.005
	Exp2(HD)	5	188.7600	6.08523	12.17046	Control & Exp2	-76.47000	4	-6.110	.009
30th Day	Control	5	130.7900	3.50951	7.01903					
	Exp1(LD)	5	212.1525	5.10388	10.20777	Control & Exp1	-81.36250	4	-12.547	.001
	Exp2(HD)	5	255.4325	3.30778	6.61557	Control & Exp2	-124.64250	4	-18.910	.000
45th Day	Control	5	151.1775	2.42922	4.85845					
	Exp1(LD)	5	235.4725	3.51394	7.02788	Control & Exp1	-84.29500	4	-26.476	.000
	Exp2(HD)	5	307.6875	4.42936	8.85873	Control& Exp2	-156.51000	4	-34.391	.000
60th Day	Control	5	166.9275	3.75009	7.50019					
	Exp1(LD)	5	266.2400	2.87352	5.74703	Control& Exp1	-99.31250	4	-17.999	.000
	Exp2(HD)	5	333.1575	3.91788	7.83575	Control& Exp2	-166.23000	4	-45.717	.000

Table 6: Changes in GOT activity in liver (in U/L) of *Clarias gariepinus* exposed to sub-lethal concentration of Cadmium for a period of 60 days

	Group	N	Mean	SEM	SD	Mean difference		df	t	Significance
15th Day	Control	5	9.3325	.37961	.75923					
	Exp1(LD)	5	15.9875	.89854	1.79708	Control & Exp1	-6.65500	4	-7.473	.005
	Exp2(HD)	5	28.2225	1.57572	3.15145	Control & Exp2	-18.8900	4	-10.954	.002
30th Day	Control	5	9.1950	.66795	1.33590					
	Exp1(LD)	5	27.9500	1.97625	3.95249	Control & Exp1	-18.7550	4	-7.407	.005
	Exp2(HD)	5	45.4350	1.51025	3.02049	Control & Exp2	-36.2400	4	-22.747	.000
45th Day	Control	5	9.5400	.73622	1.47244					
	Exp1(LD)	5	62.3350	2.56021	5.12042	Control& Exp1	-52.7950	4	-16.568	.000
	Exp2(HD)	5	133.6025	4.20169	8.40339	Control & Exp2	-124.0625	4	-28.628	.000
60th Day	Control	5	8.1025	.82600	1.65200					
	Exp1(LD)	5	118.2025	6.54502	13.09003	Control & Exp1	-110.1000	4	-16.729	.000
	Exp2(HD)	5	220.9675	6.74504	13.49007	Control & Exp2	-212.8650	4	-28.417	.000

Table 7: Changes in GOT activity in renal tissues (in U/L) of *Clarias gariepinus* exposed to sub-lethal concentration of Cadmium for a period of 60 days

	Group	N	Mean	SEM	SD	Mean difference		df	t	Significance
15th Day	Control	5	1.8375	.21868	.43737					
	Exp1(LD)	5	5.6625	.52509	1.05019	Control & Exp1	-3.82500	4	-6.568	.007
	Exp2(HD)	5	13.3425	1.10342	2.20684	Control & Exp2	-11.50500	4	-11.76	.001
30th Day	Control	5	2.8000	.14669	.29337					
	Exp1(LD)	5	11.9925	1.03266	2.06532	Control & Exp1	-9.19250	4	-8.245	.004
	Exp2(HD)	5	26.5425	1.81800	3.63599	Control & Exp2	-23.7425	4	-13.581	.001
45th Day	Control	5	3.8875	.20139	.40277					
	Exp1(LD)	5	41.9250	3.03335	6.06670	Control& Exp1	-38.0375	4	-12.099	.001
	Exp2(HD)	5	71.9900	1.45028	2.90056	Control & Exp2	-68.1025	4	-47.208	.000
60th Day	Control	5	2.6200	.30735	.61471					
	Exp1(LD)	5	62.6350	2.15994	4.31988	Control & Exp1	-60.0150	4	-31.703	.000
	Exp2(HD)	5	100.0225	3.27512	6.55023	Control & Exp2	-97.4025	4	-28.046	.000

indicators of heavy metal poisoning and for determining the physiological state of the fish, assessment of the protein content can be considered as a diagnostic tool (Kakkar and Jaffery, 2005). In the present investigation, a significant gradual decline in protein content in liver and renal tissues was observed in *Clarias gariepinus* on exposure to sub-lethal concentrations of cadmium. After 15, 30, 45 and 60 days of exposure the liver and renal tissues showed constant depletion in the amount of protein in comparison to control fishes. These observations revealed that the decline in the protein level was directly proportional to the dosage of cadmium. Significant fall in total protein content of tissues in fishes exposed to heavy metals has also been reported in *Channa punctatus* (Shukla *et al.*, 2002; Agrahari and Gopal, 2007; Kawade and Khillare, 2015), *Catla catla* (Sobha *et al.*, 2007; Prasanth and Arivoli, 2008), *Cirrhinus mrigala* (Kumar and Dahiya, 2013), *Clarias batrachus* (Arya, 2014), *Labeo rohita* (Mohanty *et al.*, 2013), *Mystus cavasius* (Palanisamy *et al.*, 2011) and *Tilapia mossambica* (Kulkarni *et al.*, 2005). The decrease in protein content in $CdCl_2$ exposed fish as observed in the present study may be due to reduced protein synthesis or due to enhanced proteolysis caused by nephrosis, by cirrhosis or due to metabolic utilization of the keto-acids to gluconeogenesis pathway for the synthesis of glucose or used in TCA cycle for energy production (Sobha *et al.*, 2007; Mohanty *et al.*, 2013; Rajput *et al.*, 2012 and Arya, 2014). Attar (2005) and

Table 8: Changes in GPT activity in liver (U/L) of *Clarias gariepinus* exposed to sub-lethal concentration of Cadmium for a period of 60 days

	Group	N	Mean	SEM	SD	Mean difference		df	t	Significance
15th Day	Control	5	4.3650	.21481	.42961					
	Exp1(LD)	5	47.1750	6.00028	12.00056	Control & Exp1	-42.8100	4	-7.136	.006
	Exp2(HD)	5	74.2925	5.82924	11.65848	Control & Exp2	-69.9275	4	-12.046	.001
30th Day	Control	5	5.2775	.46809	.93618					
	Exp1(LD)	5	69.9550	2.86230	5.72459	Control & Exp1	-64.6775	4	-20.096	.000
	Exp2(HD)	5	127.4175	7.13330	14.26659	Control & Exp2	-122.1400	4	-17.206	.000
45th Day	Control	5	7.1475	.66643	1.33285					
	Exp1(LD)	5	120.1600	1.75451	3.50903	Control& Exp1	-113.0125	4	-56.126	.000
	Exp2(HD)	5	153.8925	2.30701	4.61401	Control & Exp2	-146.7450	4	-63.217	.000
60th Day	Control	5	8.3025	.47867	.95734					
	Exp1(LD)	5	151.5475	2.62563	5.25127	Control & Exp1	-143.2450	4	-65.962	.000
	Exp2(HD)	5	191.7575	4.92499	9.84998	Control & Exp2	-183.4550	4	-36.210	.000

Table 9: Changes in GPT activity renal tissues (U/L) of *Clarias gariepinus* exposed to sub-lethal concentration of Cadmium for a period of 60 days

	Group	N	Mean	SEM	SD	Mean difference		df	t	Significance
15th Day	Control	5	36.2725	2.84645	5.69289					
	Exp1(LD)	5	45.0625	1.82819	3.65639	Control & Exp1	-8.79000	4	-5.069	.015
	Exp2(HD)	5	58.9275	1.79893	3.59785	Control & Exp2	-22.6550	4	-6.116	.009
30th Day	Control	5	40.9100	2.18419	4.36839					
	Exp1(LD)	5	70.7050	2.10683	4.21366	Control & Exp1	-29.7950	4	-9.463	.003
	Exp2(HD)	5	96.5875	1.61449	3.22899	Control & Exp2	-55.6775	4	-15.413	.001
45th Day	Control	5	49.0650	2.50211	5.00421					
	Exp1(LD)	5	104.1875	3.94486	7.88972	Control& Exp1	-55.1225	4	-9.172	.003
	Exp2(HD)	5	131.7625	4.70467	9.40933	Control & Exp2	-82.6975	4	-20.559	.000
60th Day	Control	5	45.3100	1.07348	2.14697					
	Exp1(LD)	5	123.2875	2.18238	4.36476	Control & Exp1	-77.9775	4	-31.501	.000
	Exp2(HD)	5	167.7875	2.40531	4.81062	Control & Exp2	-122.4775	4	-36.509	.000

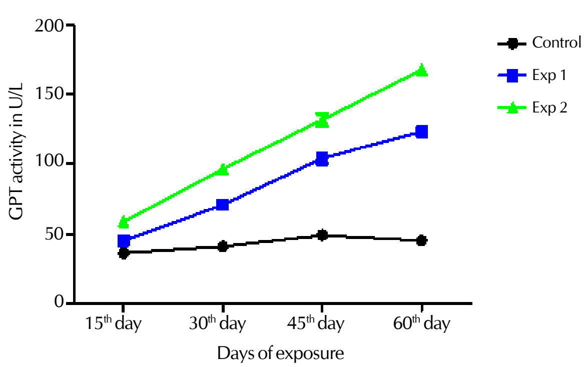

Figure 9: GPT activity in renal tissues of *Clarias gariepinus* exposed to sub-lethal concentration of Cadmium

Sethuraman *et al.* (2011) observed hyperproteinemia in blood of *Oreochromis niloticus* following cadmium administration and stated that it is possibly either due to water loss in the serum, the relative changes in the mobilization of blood protein or elevated *de novo* synthesis. However, a decrease in serum protein level has been observed in the present study. This is in agreement with the findings of Kumar *et al.* (2005), Maruthappan *et al.* (2005) and Kumar and Dahiya (2013) in fresh water fish *Cirrhinus mrigala* when exposed to lead and zinc. The reduced serum protein level can be attributed to proteolysis due to increased protease activity in tissues which form amino acids and used in TCA cycle for energy production during stress condition.

The blood glucose level has been used as one of the important indicator of stress in fishes. Arya (2014) observed a decrease in serum glucose level in *Clarias batrachus* when exposed to cadmium. He suggested that the decrease in serum glucose level might have resulted from an inhibition in glycogenolysis in the liver or due to reduction in the rate of absorption of glucose through the intestine. The present study demonstrated that the fresh water cat fish, *Clarias gariepinus* exposed to sub-lethal concentrations of $CdCl_2$ displayed a significant elevation in the level of serum glucose during all exposure periods. The elevated serum glucose level can be attributed to enhanced breakdown of glycogen to glucose through glycogenolysis in the tissues of fish to withstand the stress condition mediated by increased level of circulating glucocorticoids and catecholamines released from adrenal tissues (Gluszak *et al.*, 2007). Hyperglycemic condition may also be due to decrease in the activity of some enzymes like phosphofructokinase, lactate dehydrogenase and citrate kinase that reduces the rate of glycolysis in tissues (Almeida *et al.*, 2001) or due to increased rate of gluconeogenesis (Kumar *et al.*, 2011). Increase in serum glucose level under stress due to heavy metal toxicity has been reported in several species like *Oreochromis niloticus* (Almeida *et al.*, 2001), rainbow trout (Chowdhury *et al.*, 2004), *Cyprinus carpio* (Bedii and Kenan, 2005; Kumar *et al.*, 2011), *Catla catla* (Sobha *et al.*, 2007) and *Tilapia mossambica* (Sethuraman *et al.*, 2011). The increased serum glucose level in the present study is an evidence of stress due to cadmium exposure. Ozgur and

Kargam (2010) stated that coping with such stress is an energy demanding process that requires the fish to mobilize metabolically energy substrates through gluconeogenesis. To meet this challenge, glucose is mobilized through gluconeogenesis (Vosyliene, 1999). Serum glucose increase due to toxicants has been associated with hypothalamus-sympathetic chromaffin cells (Mc Donald and Milligan, 1997) instead of the hypothalamus-pituitary-interenal axis (Arends *et al*., 1999) that is known to have tremendous influence on carbohydrate metabolism.

The concentration of cholesterol is an essential structural component of membranes and the precursor of all steroid hormones. Heavy metals are known to have hazardous effects on cell structures, especially on the membranes. In the present investigation, serum cholesterol level increased significantly as compared to control fish. The magnitude of increase was influenced by both duration of exposure and concentration. The reports of many investigators (Yang and Chen, 2003; Kumar *et al.,* 2011; Sethuraman *et al.,* 2011; Kumar and Dahiya, 2013; Arya, 2014 and Oluah *et al.,* 2014) support the increase of serum cholesterol concentrations in the metal exposed fishes. Reduced serum cholesterol level has been reported in *Oreochromis niloticus* (Ozgur and Kargam, 2010) and in *Clarias batrachus* (Arya, 2014) exposed to heavy metals. Arya (2014) stated that the decreased serum cholesterol level may be due to the bioaccumulation of metals which might have inhibited the conversion of esterified cholesterol to free cholesterol. The observed hypercholesterolemia in the present study could have resulted in part to the adverse effect of cadmium on the liver leading to altered cholesterol metabolism causing the release of cholesterol into blood (Oner *et al*., 2008).

In the tissues of fish and other organisms, transaminases play an important role in carbohydrate and amino acid metabolism (Atroshi *et al*., 2000). The data of present study showed that the exposure of cadmium caused significant elevation in the GOT (AST) and GPT (ALT) activity in hepatic and renal tissues. Enhanced activity of these enzymes in liver and kidney may be due to increased synthesis by induction because of cadmium. Similar findings were also observed by Naveed et al. (2010) in *Channa punctatus*. Increased activities of both aminotransfereses indicated amplified transamination processes. In order to cope with the energy crisis during toxicant-based stress an increase in transamination occurs due to amino acid input into the TCA cycle (Rao, 2006). Since the transaminases are considered as an index of gluconeogenesis, stimulation of its activity in the present study suggests increased mobilization of FAA (free amino acids) into tissue gluconeogenesis. Enhanced activity of transaminases also suggests increase in feeding of keto acids into the TCA cycle due to greater availability of amino acids (due to increased proteolysis) and participation for greater needs of energy synthesis during cadmium exposure (De Smet and Blust, 2000).

ACKNOWLEDGEMENT

The authors wish to thank Dr. Panchanan Mishra and Dr. Abhalaxmi Singh for valuable suggestions and Dr. Anoop Parida and Ms. Niharika Kanungo for providing laboratory facilities to carry out the experiments.

REFERENCES

Agrahari, S. and Gopal, K. 2007. Fate and toxicity of cadmium and lead accumulation in different tissues (gills, liver, kidney, brain) of fresh water fish, *Channa punctatus*. *J. Ecophysiol. Occup. Hlth.* **7:** 151-155.

Almeida, J. A., Novelli, E. L., Dal Pai Silva, M. and Junior, R. A. 2001. Environmental cadmium exposure and metabolic responses of the Nile tilapia, *Oreochromis niloticus*. *Environ. Pollut.* **114(2):** 169-75.

Allain, C. C., Poon, L. S., Chan, C. S. G., Richmond, W. and Fu, P. C. 1974. Enzymatic determination of total serum cholesterol. Clin. Chem. **20(4):** 470-475.

Anandhan, R. and Hemalatha, S. 2009. A study on the biochemical changes in selected tissues of the zebra fish, *brachydanio rerio* (Ham.) exposed to sub lethal aluminium chloride. *The Bioscan*. **4(2):** 315-319.

Arends, R. J., Mancers, J. M., Munoz, J. L., Wendelaar-Bonga, S. E. and Flik, G. 1999. The stress response of the gill of seabream (*Sparus aurata L.*) to air exposure and confinement. *J. Endocrinol.* **163:** 149-157.

Arya, A. 2014. Evaluation of biochemical and histochemical changes following the combined treatment of mercury and cadmium in a fresh water cat fish, *Clarias batrachus* (Linn.). *International J. Pharmacy and Pharmaceutical Sciences*. **6(10):** 356-358.

Atroshi, F., Rizzo, A., Sankari, S., Biese, I., Westermarck, P. and Veijalainen, P. 2000. Liver enzyme activates of rats exposed to Ochratoxin A and T-2 toxin with antioxidants. *Bull. Environ. Contam. Toxicol.* **64:** 586-592.

Attar, A. M. 2005. Biochemical effects of short term cadmium exposure on the freshwater fish, *Oreochromis niloticus*. *J. Biol. Sci.* **5:** 260-265.

Bedii, CiCi, K. and Kenan, E. 2005. The effect of Cadmium on levels of glucose in serum and glycogen reserves in the liver and muscle tissue of *Cyprinus Carpio* (L.,1758). *Turk. J. Vet. Anim. Sci.* **29:** 113-117.

Bradford, M. M. 1976. Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72:** 248-254.

Chowdhury, M. J., Pane, E. F. and Wood, C. M. 2004. Physiological effects of dietary cadmium acclimation and waterborne cadmium challenge in rainbow trout: respiratory, ionoregulatory and stress parameters. Comp. Biochem. *Physiol. Toxicol. Pharmacol.* **139(1-3):** 163-173.

Deepali, S. and Madhu, T. 2007. Hexavalent chromium induced biochemical alterations in air breathing fish, *Channa punctatus*. *J. Ecophysiol. Occu. Hlth.* **7:** 171-175.

De Smet and Blust, G. A. 2000. Stress response and changes in protein metobolism of in carp *Cyprinus carpio* during cadmium exposure, Ecotoxicol. *Environ. saf.* **48:** 255-258.

Finney, D.J. 1971. Probit analysis, 3rd edition, Cambridge University Press, Cambridge. p. 333.

Gluszak, L., Miron, Dios, S., Mores, B., Simoes, R., Schetinger, M., Morch, V. and Loro, V. 2007. Acute effect of glyphosate herbicide on metabolic and enzymatic parameters of Silver Catfish, Rhambia quelen.Comp. Biochem. *Physiol. Toxicol. Pharmacol.* **146:** 519-524.

Hassan, S.A., Fariduddin, Q., Ali, B., Hayat, S. and Ahmad, A. 2009. Cadmium: Toxicity and tolerance in plants. *J. Environ. Biol.* **30:**165-174.

Huang, X. J., Choi, Y. K., Im, H. S., Yarimaga, O., Yoon, E. and Kim,

H. S. 2006. Aspartate Aminotransferase (AST/GOT) and Alanine Aminotransferase (ALT/GPT) detection techniques. *Sensors (Basel)*. **6(7):** 756-782.

Kakkar, P. and Jaffery, F. N. 2005. Biological markers for metal toxicity. *Envion. Toxicol. Pharm.* **19:** 335-49.

Karan, V., Vitorovic, S., Tutundzic, V. and Poleksic, V. 1998. Functional enzyme activity and gill histology of carp after copper sulphate exposure and recovery. *Ecotoxicol. Environ. saf.* **40:** 49-55.

Kawade, S. and Khillare, Y. 2015. Impact of cadmium on the biochemical contents in certain tissues of freshwater fish, *Channa punctatus (Bloch).* **4(1):** 1430-1441.

Kotsanis, N. and Georgudaki, J. L. 1999. Arsenic induced liver hyperplasia and kidney fibrosis in rainbow trout (*oncorhynchus mykiss*) by microinjection technique: A sensitive animal bioassay for environmental metal toxicity. *Bull. Environ. Contam. Toxicol.* **62:** 169-178.

Kulkarni, A. S., Venkateshwaran, K. and Wavde, P.N. 2005. Toxic effects of arsenic and iron on fresh water chichlid fish *Tilapia mossambica*. Bioinfonet. **2(3):** 189-192.

Kumar, P., Palanivel, S., Mathan, R. and Sarasu. 2011. Sublethal effects of chromium on some biochemical profiles of the fresh water teleost, *cyprinus carpio.* **2(1):** 295-300.

Kumar, P., Gupta, Y. G., Tiwari, S. and Singh, A. 2005. Hematological and biochemical abnormalities in *Cirrhinus mrigala* (Ham.) induced by lead. J. Ecophysio. Occupl. Health. **5(3&4):** 213-216.

Kumar, A. and Dahiya, T. 2013. The impact of zinc on liver and serum proteins and cholesterol levels of fish (*Cirrhinus mrigala*). The Bioscan. **8(3):** 1057-1059.

Manson, C. F. 1996. Biology of fresh water pollution. 3rd Edition. Longman, U. K. pp. 1-4

McDonald, G. and Milligan, L. 1997. Ionic, osmotic and acid–base regulation in stress. In: Iwama GK, Pickering AD, Sumpter JP and Schreck CB (eds.). Fish stress and health in aquaculture. *Cambridge University Press, Cambridge*. pp. 5-118.

Mohanty, B. P., Mahananda, M. R. and Pradhan, S. 2013. Cadmium induced toxicity and antioxidant activities in *Labeo rohita* (Hamilton). *Environment and Ecology Research.* **1(2):** 41-47.

Maruthappan, V., Ramesh, M., Noortheen, A. and Saraswathi, K. 2005. Lead induced biochemical changes and recovery in a freshwater fish *Cyprinus corpio*. *Indian J. Environ. Toxicology.* **14(2):** 70-74.

Naveed, A., Janaiah, C. and Venkateshwaralu, P. 2010. The effect of lihocin toxicity on protein metabolism of fresh water edible fish, *Channa pucntatus* (Bloch). J. Toxicol. Environ. Health Sci. **3(1):** 18-23.

Oluah, N. S., Ulasi, A. M. O. and Nwani, C. D. 2014. Changes in the leucocyte and serum Biochemistry in *Clarias gariepinus* (Burchel) Exposed to Sublethal Lead Chloride. *J. Nat. Sci. Res.* **4(18):** 55-62.

Oner, M., Atli, G. and Canli, M. 2008. Changes in the serum biochemical parameters of the freshwater fish *Oreochromis niloticus* following prolonged metal (Ag, Cd, Cr, Cu and Zn) exposures. *Environ. Toxicol. Chem.* **27:** 360-366.

Ozgur, F. and Kargam, F. 2010. Individual and combined effects of heavy metals on serum biochemistry of nile Tilapia *Oreochromis niloticus*. *Arch. Environ. Contam. Toxicol.* **58:** 151-157.

Palanisamy, P., Sasikala, G., Mallikaraj, D., Bhuvaneshwari, N. and Natarajan, G. M. 2011. Changes in protein content in muscles of *Mystus cavacius* (Ham) exposed to electroplating industrial effluent. Environ. *J. Pharmaceutical and Biol. Archives.* **2(2):** 669-672.

Prasath, P. M. D. and Arivoli, S. 2008. Biochemical study on freshwater fish *Catla catla* with reference to mercury chloride. *Iran. J. Environ. Health. Sci. Eng.* **5(2):** 109-116.

Rajput, V., Singh, S. K., Arpita, Kirti and Abhishek. 2012. Comparative toxicity of Butachlor, Imidacloprid and Sodium fluoride on protein profile of the walking cat fish *Clarias batrachus*. *J. Applied Pharmaceutical Science.* **02(06):** 121-124.

Rao, V. J. 2006. Sublethal effects of an organophosphorus insecticide (RPR-II) on biochemical parameters of Tilapia, *Oreochromis mossambicus*. Comp. Biochem. *Physiol. Part C: Toxicol. Pharmacol.* **143(4):** 492-8.

Sethuraman, V., Haq, B., Chezhian, A., Shanker, S. and Selvan, D. S. 2011. Changes due to the effect of the Heavy Metals ($HgCl_2$ and $ZnSO_4$) concentration on the marine fish, *Tilapia mossambica* (Peters, 1852). **3(6):** 333-341.

Shukla, V., Rathi, P. and Shastry, K. V. 2002. Effect of Cadmium individually and in combination with other metals on the nutritive values of fresh water fish, *Channa punctatus*. *J. Environ. Biol.* **23(1):** 105-110.

Sobha, K., Poornima, A. and Veeraiah, K. 2007. A study on biochemical changes in the fresh water fish *Catla catla (Hamilton)* exposed to the heavy metal toxicant cadmium chloride, Kathmandu University, *J. Science, Engineering and Technology.* **1(4):** 112-117.

Samanta, S., Mitra, K., Chandra, K., Saha, K., Bandopadhyaya, S. and Ghosh, A. 2005. Heavy metals in water of the rivers Hoogley and Haldi and their impact on fish. *J. Environ. Biol.* **26(3):** 517-523.

Trinder, P. 1969. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Ann. Clin. Biochem.* **6:** 24-7.

Vagas, V. M. F., Migliavacca, S. B., Demelo, A. C., Horn, R. C., Guidobono, R. R., Ferreira, I. C. F. S. and Pestana, M. H. D. 2001. Genotoxicity assessment in aquatic environment under the influence of heavy metals and organic contaminants. *Mutat. Res.* **490:** 141-158.

Vosyliene, M. Z. 1999. The effect of Heavy metals on haematological indices of fish. *Act. Zool. Lith. Hydro.* **9:** 76-82.

Yang, J. and Chen, H. C. 2003. Effects of gallium on common carp *Cyprinus carpio* on serum biochemical contents. *J. Environ. Public* Health. **2(4):** 56-58.

Zyadah, M. A. and Abdel-Bakey, T. E. 2000. Toxicity and bioaccumulation of copper, zinc and cadmium in some aquatic organisms. *Bull. Environ. Contam. Toxicol.* **64:** 740-745.