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Original Article

Effect of Sublethal Concentration of Zinc Sulphate on the Serum Biochemical Parameters of Freshwater Cat Fish, *Clarias Batrachus* (LINN)

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Abstract

Zinc is a necessary trace element that contributes cumulative polluting effect to aquatic organism. The effect of sublethal concentration of zinc sulphate was investigated on the serum biochemical parameters of *Clarias batrachus.* This study was carried out to evaluate the sub chronic toxicity of zinc at three levels (10mg/L, 20mg/L and 30mg/L) for 15, 30 and 45 days. The biochemical changes in plasma showed significant increased in glucose, lipids (Triglycerides and cholesterol), serum phosphatases (acid and alkaline phosphatases) and serum transaminases (SGOT & SGPT) where as decreased in bilirubin and protein level. These results indicate that both concentration and period of exposure of zinc sulphate can alter the plasma's biochemical contents of *Clarias batrachus.* Thus it can be concluded that fishes can effectively used as monitors of water quality with respect to heavy metal, zinc as well as toxicant.

Keywords: Zinc; Chronic Toxicity; Biochemical Changes; *Clarias Batrachus*.

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Introduction

The rapid development of industry and especially chemical industry has created serious problems of water pollution. Human destructive influence on the aquatic environment is in the form of sublethal pollution, which results in chronic stress conditions that have negative effect on aquatic life (Mason, 1991). Heavy metals occur naturally in the environment and are found in varying levels in the ground and surface water. These elements are generally released in small amounts into the environment by processes like weathering of rocks, volcanic eruption etc. and their intake/exposure is necessary in trace amounts for good health. But, presently, due to anthropogenic activities there is a steady increase in their concentration in all the habitats due to discharge of these metals into natural aquatic ecosystems. Sometimes, aquatic organisms are exposed to unnaturally high levels of these metals. Heavy metals easily get absorbed in the body and get transported to various organs through blood. When in circulation, these metals not only affect the blood components but also elicit marked alterations in the histology as well as physiology of the target organs. Fish are relatively sensitive to changes in their surroundings environment. Fish health may therefore reflect and give a good indication of the health status of a specific aquatic ecosystem.

Heavy metal, Zinc is essential for proper functioning of the body. Zinc is a necessary trace element that contributes to the structure of more than 300 proteins which play a role in the growth, reproduction, development and immune system catalysts in fish (Watanabe et al., 1997). It is used in various industrial operations forms and excessive zinc finds its way into reservoirs, lakes and rivers. Excessive zinc enters the environment as a result of human activities such as mining, purification of zinc, lead and cadmium ores, burning of coal and burning of waste. Although, small quantities of zinc are required for normal development and metabolism of organism, but if levels exceed the normal physiological requirements, it can act as a toxicant. Exposure to excess zinc has been reported to bring about haematological as well as biochemical changes in various organs of fishes. This results in general enfeeblement, retardation of growth and may bring about metabolic and pathological changes in various organs in fish.

In recent years, biochemical variables were used more when clinical diagnosis of fish physiology was applied to determine the effects of external stressors and toxic substances. Hadi *et al.*, (2009) suggested that biochemical changes of fish be used in determining the toxicity of pollutants. Therefore, estimation of serum biochemical parameters thus, proves to be a diagnostic tool in toxicology to access the general health status of an organism and their impact on target organs.

Clarias batrachus is widely distributed fish in Asia. In these areas, it is extremely popular on account of its tasty flesh, its unparalleled hardness, its rapid growth and high fish market price. It inhibits tropical swamps, lakes and rivers. These are generally strong fishes and possess accessory respiratory organ for air breathing that enable them to tolerate adverse aquatic conditions where other cultivated fish species cannot survive. Not much work has been carried out on the effect of zinc on the biochemistry of freshwater cat fish, *Clarias batrachus*. Therefore, the present study was undertaken to evaluate some serum biochemical effects resulting from the exposure of the freshwater cat fish, *Clarias batrachus* to sublethal concentrations of zinc sulphate in the water.

Materials and Methods

Healthy specimens of Indian fresh water catfish, *Clarias batrachus* were collected from local fish farm at Balrampur, U.P. and were transported in containers to the laboratory .In the laboratory, the fishes were carefully examined for any injury and then kept in 1% solution of $KMnO_4$ for few hours to get rid off dermal infection, finally they were kept in large plastic jar containing 50L of clean tap water and acclimatized for 15 days to the laboratory conditions, during which time they were fed on boiled egg yolk and commercial fish food . The fish specimen weighing 50±5 gram and measuring 15±5 cm selected for experiments. The fishes were inspected for disease conditions and general fitness. Water was changed every other day. Feeding was stopped 48 hours prior to the toxicity test, to minimize the contamination of metabolic wastes.

Srivastava and Prakash (2018) reported that the LC_{50} of zinc sulphate was 37.22mg/L at 96hours for *Clarias batrachus.* The selected sublethal concentrations zinc sulphate were 10.0mg/L, 15.0mg/L and 20.0 mg/L for 15,30 and 45 days, respectively . After 15, 30 and 45 days exposure, blood samples were collected from both the control and experimental fishes by puncturing the caudal vein to assess the effect of zinc sulphate on the serum biochemical parameters. The collected blood was allowed to centrifugal tubes, stored in a slanting position to clot for 3 minutes. It was then centrifuged at 3000 rpm for 15 minutes. The supernatant serum was separated by a fine rubber bulb pipette in separate tube. The serum samples were used for the estimation of biochemical parameters i.e. bilirubin, serum glucose, total proteins, cholesterol and enzyme activity of transaminases (SGOT and SGPT), acid and alkaline phosphatase. The serum glucose, total protein, triglycerides, cholesterol, transaminases, phosphatases and bilirubin were estimated by following standard methods. The mean values of the various biochemical parameters for the control and experimental fish were analyzed for statistical significance using the student's t-test. The calculations of statistical significance by the student's t- test at 0.01 and 0.05 levels were made using Microsoft Excel 2003.

Results and Discussion

Heavy metals are known for their strong action on biological tissues. Metal ions once absorbed into body are capable of reacting with a variety of active binding sites and thus can disturb the normal physiology of an organism. In the present study, an attempt was made to examine the sub-lethal toxic effect of zinc sulphate on the serum biochemical parameters of C*larias batrachus.* Changes in the blood biochemical values often reflect alteration of physiological state of fish. Although no mortality was observed in the present study, we found physiological effects in the fish after the exposure to zinc sulphate. Result of the quantitative estimation of serum bilirubin, glucose, cholesterol, triglycerides, acid and alkaline phosphatases, SGOT and SGPT in Clarias batrachus exposed to zinc are presented in Table 1-6.

Serum bilirubin levels were significantly decreased in *Clarias batrachus* exposed to zinc sulphate as compared to control groups (Table 1) may be hepato-dysfunction. Similar report of decreased in bilirubin level was previously recorded by Srivastava *et al.* (2007) in *Clarias batrachus* exposed to distillery effluent and also by Srivastava *et al.* (2012) in *Heteropneustes fossilis* exposed to sodium fluoride.

The serum glucose levels were significantly ($p<0.05$ & $p<0.01$) higher in zinc exposed groups of fishes as compared to control groups (Table 2). Serum glucose levels increased progressively with the increasing concentrations of metals and exposure periods under studies. Similar hyperglycemic response has reported by Hadi *et al.*, (2009)in *Tilapia zillii* exposed to aluminium and by Canli (1995) in *Cyprinus carpio* exposed to Hg, Cr and Ni. The hyperglycemic condition in metal treated fish in present study may be an effort to provide additional energy required during times of high metabolic activities such as 'fight or flight' response in order to counter the metal toxicity (Goss and Wood,1988). Another plausible reason for elevated glucose levels presently seems to be the incomplete metabolism of

Table 1: Effect of Zinc sulphate on the serum Bilirubin (mg/ml) level in *C. batrachus*

Experiment Set.	15 Days Mean±SD (↓ %)	30 Days Mean±SD (↓ %)	45 Days Mean±SD (↓ %)
Control	0.58±0.32	0.60±0.28	0.61±0.23
10 mg/L	0.48±0.16 (20.83)	0.46±0.33 (23.33)	0.48±0.31 (21.31)
20mg/L	0.45±0.38 (22.41)	0.43±0.21* (28.33)	0.39±0.18** (36.06)
30mg/l	0.41±0.33* (29.31)	0.39±0.28** (35.00)	0.31±0.41** (49.18)

*Significant at p< 0.05 ; ** significant at p< 0.01.

Table 2: Effect of Zinc sulphate on the serum glucose (mg/dl) level in *C.batrachus*

Experiment Set.	15 Days Mean±SD (↑ %)	30 Days Mean±SD (↑ %)	45 Days Mean±SD (↑ %)
Control	11.10±0.20	11.20±0.30	11.30±0.33
10 mg/L	13.30±0.10 (19.81)	13.45±0.18 (20.18)	13.85±0.19 (22.56)
20mg/L	15.45±0.15* (39.19)	15.90±0.19* (41.96)	16.15±0.20* (42.92)
30mg/l	17.18±0.18** (57.77)	17.78±0.22** (58.75)	17.98±0.20** (59.11)

*Significant at p< 0.05; ** significant at p< 0.01.

Table 3: Effect of Zinc sulphate on the serum protein (g/dl) level in *C. batrachus*

Experiment Set.	15 Days Mean±SD (↓ %)	30 Days Mean±SD (↓ %)	45 Days Mean±SD (↓ %)
Control	3.98±0.51	3.78±0.48	3.65±0.50
10 mg/L	2.90±0.52 (27.13)	2.79±0.18 (26.19)	2.75±0.38* (24.65)
20mg/L	2.60±0.53* (34.67)	2.38±0.23** (37.03)	2.75±0.38* (32.05)
30mg/l	2.45±0.18** (38.44)	2.30±0.20** (39.15)	2.10±0.38** (42.46)

*Significant at p< 0.05; ** significant at p< 0.01.

blood sugars (Zikic *et al.*, 2001). Thus it can be calculated that zinc affects glucose dynamics in *Clarias batrachus* in order to obtain more energy to withstand and overcome the existing stress condition.

Proteins are highly sensitive to heavy metals and happen to be one of the earliest indicators of heavy metal poisoning. In the present study zinc induced gradual and significant decline in the serum proteins contents in all zinc exposure fishes groups (Table 3). This hypoproteinemia in the present study can be attributed to the enhanced proteolysis. Proteolysis seems to offer a physiological mechanism in a bid to provide energy to cope up with the stressful situation caused by metal toxicity. Depletion in protein level in metal exposed fish thus might be due to its enhanced use to build up new cells or enzymes to reduce the stress. Blood serum protein is a fairly labile biochemical system, precisely reflecting the condition of the organism and the changes happening to it under influence of internal and external factors (Shalaby *et al.*, 2006). Thus, it can be calculated that decline in serum protein content can be attributed to the increased cost of homeostasis, tissue repair and detoxification during stress. Therefore, the influence of metal on total protein concentration which act as potential metabolic biomarker of fish has been taken into consideration in evaluating the response to stressors and consequently the increasing demand for energy.

Triglycerides represent the major energy reserve in the fish. Serum triglycerides levels are usually used to evaluate the metabolic status of an organism. In the present study it is clear from table that triglycerides undergo significant ($p<0.05$ & $P<0.01$) increase in all the groups of zinc exposed fishes when compared to control (Table 4) and hence lead to hypertriglyceridemic conditions may be due to impairment in liver function as also pointed by Hadi *et al.*, (2009). As such, various lipolytic enzymes which function locally and convert triglycerides into fatty acids and glycerol probably get released in blood due to the degeneration of liver cells leaving triglycerides unprocessed. Thus, it seems that reduced rate of lypolysis ultimately results in the elevated serum triglycerides levels.

Alteration in the cholesterol level of blood is the indication of liver dysfunction. In the present study significant increase in cholesterol values have been observed in all the zinc exposed groups of fishes as compared to controls (Table 4). This hypercholesterolemia in metal treated fishes m ight be due to the degenerative changes in the liver tissue. Since homeostasis of lipids is one of the principal functions of liver, any change in serum cholesterol concentration may thus be a clear indicative of liver dysfunction. Moreover, it seems that the rise in cholesterol levels (which are potential energy reserves) in response to

Table 4: Effect of Zinc sulphate on the serum Total Lipid level in *C. batrachus*

Experiment Set.	15 Days Mean±SD (↑ %)	30 Days Mean±SD (↑ %)	45 Days Mean±SD (↑ %)
Triglyceride(mg/dl)			
Control	49.80±0.31	53.62±0.33	52.85±0.28
10 mg/L	52.32±0.35 (5.06)	56.28±0.32 (4.96)	56.30±0.24 (9.57)
20mg/L	54.58±0.41 (9.60)	59.32±0.42 (10.63)	60.42±0.41* (14.32)
30mg/l	56.18±0.18* (12.81)	63.28±0.31* (18.01)	64.18±0.34** (21.43)
Cholesterol (mg/dl)			
Control	72.20±0.22	78.38±0.33	78.65±0.53
10 mg/L	80.32±0.18* (11.24)	83.15±0.32 (6.08)	86.18±0.31 (9.57)
20mg/L	83.18±0.42* (15.21)	86.18±0.18 (9.95)	89.14±0.18* (13.34)
30mg/l	85.72±0.26** (18.72)	90.25±0.34* (15.14)	91.21±0.42* (15.97)

*Significant at p< 0.05 ; ** significant at p< 0.01.

unfavourable condition possibly may be one of the way these intoxicated fishes employ to strengthen the excess energy reserves which are required by them to mitigate the effects of stress. Liver releases various lipolytic enzymes in the blood which convert cholesterol into bile. Impairment in the liver tissue under the stress of zinc exposure, by inhibiting the release of such enzymes which catabolize cholesterol may be another probable causative of increment in the serum cholesterol content in *Clarias batrachus.*

Thus triglycerides and cholesterol are known to participate in the rise of total lipid. The rise of these energy reserves in response to pollution could be due to the fact that excess energy reserves (as glucose, triglycerides and cholesterol) are required by organisms to mediate the effects of stress (Lee

Table 5: Effect of Zinc sulphate on the serum phosphatases level in *C. batrachus*

Experiment Set.	15 Days Mean±SD (↑ %)	30 Days Mean±SD (↑ %)	45 Days Mean±SD (↑ %)
Acid phosphatase (U/L)			
Control	1.34±0.78	1.36±0.72	1.38±0.48
10 mg/L	1.38±0.32 (2.99)	1.40±0.18 (2.94)	1.44±0.32 (4.34)
20mg/L	1.42±0.48 (5.97)	1.48±0.12 (8.82)	1.52±0.28 (10.14)
30mg/l	1.65±0.28* (23.13)	1.68±0.18* (23.52)	1.72±0.33* (23.91)
Alkaline phosphatases(U/L)			
Control	3.28±0.56	3.30±0.45	3.34±0.26
10 mg/L	4.22±0.18 (28.65)	4.43±0.28* (34.24)	4.02±0.26 (20.36)
20mg/L	4.28±0.30* (30.48)	4.78±0.31** (44.84)	4.92±0.28** (47.31)
30mg/l	4.52±0.18* (37.80)	4.98±0.32** (50.90)	5.22±0.34** (56.28)

*Significant at $p< 0.05$; ** significant at $p< 0.01$.

Table 6: Effect of Zinc sulphate on the serum Transaminases level in *C. batrachus*

Experiment Set.	15 Days Mean±SD (↑ %)	30 Days Mean±SD (↑ %)	45 Days Mean±SD (↑ %)
SGOT(U/L)			
Control	65.05±0.08	66.10±0.20	66.70±0.32
10 mg/L	69.42±0.43 (6.72)	70.81±0.30 (7.12)	71.31±0.52 (6.91)
20mg/L	71.38±0.42 (9.73)	72.18±0.18 (10.11)	73.72±0.16 (10.52)
30mg/l	74.42±0.43* (14.40)	74.38±0.28* (12.52)	76.82±0.42* (15.17)
SGPT(U/L)			
Control	32.35±0.41	32.83±0.32	33.05±0.18
10 mg/L	38.18±0.33 (18.02)	46.42±0.12* (4139)	49.18±0.32* (48.35)
20mg/L	45.18±0.28* (39.65)	58.18±0.14** (77.21)	55.10±0.35* (66.21)
30mg/l	50.45±0.23* (55.95)	62.18±0.28** (89.39)	65.25±0.38** (96.83)

*Significant at $p< 0.05$; ** significant at $p< 0.01$.

et al.,1983). Since homeostasis of lipids is one of the principal liver functions, any change in serum triglyceride concentration is used as an indicator of liver dysfunction (Kaplan *et al.*,1988). In addition, the abnormal accumulation of fats (both cholesterol as well as triglycerides) in experimental animals could be due to induced imbalance between fat production and utilization (Moore *et al.*,1988).

Serum enzymes such as acid and alkaline phosphatases are membrane bound lysosomal enzymes and the sensitive biomarkers in ecotoxicology as they provide an early warning of potentially hazardous alterations in contaminated aquatic organisms. These enzymes synthesized in liver catalyze the hydrolysis of monophosphate esters and their activities usually find relation to cellular damage. Alkaline phosphatase is a polyfunctional enzyme acts as transphosphorylase at alkaline p^H and plays an important role in mineralization of the skeleton of aquatic animals and in membrane transport activities (Bernt *et al.*, 2001 and Lan *et al.*, 1995). Acid phosphatase is a hydrolyzing enzyme and act as a good indicator of stress conditions in biological systems (Verma et al., 1980). In the present study significantly increased in serum phosphatases activity was found in zinc exposed fishes compared to control (Table 5). Hadi *et al.* (2009) also advocated increase in enzymatic activities of fish under stress of xenobiotics. Jiraungkoorskul *et al.* (2003) and Thangamalathi *et al.* (2016) showed that the change in ALP activity was a result of physiological and functional alteration in metal exposed fish.

Liver is an important organ metabolism and detoxification of xenobiotic as well as biocides in the fish (Sharma *et al.*, 2007).The serum glutamate pyruvate transaminases (SGPT) and serum glutamate oxalate transaminase (SGOT) produced in liver, on the other hand, play an important role in protein and amino acid metabolism. In liver of healthy fishes, transaminases are located in mitochondria. Any variation in the concentration of these enzymes thus very clearly reflects the status of hepatic condition of the fish.

Thus SGOT and SGPT are markers of liver function. The elevated levels of SGOT and SGPT in zinc induced *Clarias batrachus* indicate the liver damage (Table 6). Similar report of increased in the level of transaminases was shown in fluoride induced fish (Srivastava *et al.*, 2012). Therefore, elevated level of these enzymes in serum of zinc induced *Clarias batrachus* indicated liver damage and disruption of normal liver function.

Thus metal intoxication appears possibly results in liberation of these enzymes (phosphatases and transaminases) into circulation which might have been due to the damage of the hepatic tissue under the state of stress created by metal toxicity. Due to the impaired cellular architecture, these enzymes it appears may get released from the hepatic cells which have become necrotic with degenerated cell membranes. Once released from the liver cells, these enzymes enter the general circulation and thus may lead to increase in their concentration in the blood.

On the basis of present results and discussion, it can be concluded that heavy metal, zinc induced marked serum biochemical alterations in *Clarias batrachus*. Examination of biochemical parameters thus can be useful as a diagnostic tool in fish toxicology to identify their general health status and target organs affected by toxicant.

Metal concentrations in the aquatic environment is seen as potential threat for aquatic organisms that are exposed to significant amount of heavy metals as a consequence of industrial, agricultural and anthropogenic activities. From the observations of present data it can be concluded that, although heavy metal, zinc is an essential trace element for various physiological processes but at high concentration it poses toxic metabolic stress hence altered the serum biochemistry of fishes thereby affecting human population. Also, it can be concluded that the fishes can effectively used as monitors or water quality.

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