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Sublethal Effects of Chromium on Enzymatic Activities of the African Catfish: *Clarias gariepinus* (Burchell, 1822)

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Abstract

The effect of sublethal concentration (0.00, 2.00, 4.00 and 8.00 mg/l) of chromium was determined on the activities of some enzymes (creatine-kinase, lactate dehydrogenase, cholinesterase, gamma glutamyltransferase) in the plasma, liver and kidney of the exposed juvenile African Catfish *Clarias gariepinus* (length 23.00±0.86 cm and weight 96.97±5.31 g respectively) after the 7 days exposure period. The activity of lactate dehydrogenase was significantly ($p<0.05$) decrease in plasma, liver and kidney while cholinesterase showed an insignificant decrease and increase ($p>0.05$) in plasma and liver and a significant decrease ($p<0.05$) in kidney after the 7 days exposure period. The activities of gamma glutamyltransferase and creatine-kinase were insignificantly decreased ($p>0.05$) in plasma, liver and kidney except liver creatine kinase that was significantly decreased. Generally, the activities of the determined enzymes were most significant in the liver than in plasma and kidney. Therefore, sublethal concentrations of chromium have some deleterious effect on the basic activities of enzymes of the plasma, liver and kidney of *Clarias gariepinus* as revealed in this investigation.

Keywords: chromium, enzymes, freshwater fish, kidney, liver, Nigeria, plasma

Introduction

Chromium (Cr) is a naturally occurring element found in rocks, animals, plants, soil and in volcanic dust and gases. In natural water, the concentration of chromium is low and is within the range of between 1 and 2 µg/l (Moore and Ramamoorthy, 1984). Chromium is used in industry, for electroplating, steel making alloys, in chrome plating, rubber manufacturing, and leather tanning and for fertilizers (Babich *et al*., 1982). The toxicity of chromium is dependent on its chemical speciation and thus its associated health effects are influenced by the chemical exposures (Holdway, 1988). There are four states in which the chromium ion is found: Cr^{2+}, Cr^{3+}, Cr^{5+} and Cr^{6+}. It is in the hexavalent form where chromium is allowed to cross biological membranes of aquatic organisms.

Hexavalent chromium (Cr^{6+}) is mainly toxic to organisms. It can alter genetic materials and cause cancer. Doudoroff and Katz (1953) indicated that hexavalent chromium behaves toxicologically in a manner quite different from most heavy metals. Hexavalent chromium can readily penetrate gills membranes by passive diffusion and concentrate at higher levels in various organs and tissues; it can manifest its toxic action, internally as well as on the gill surface (Buhler *et al*., 1977; Knoll and Fromm, 1960). Chromium is particularly dangerous as it can accumulate in many organisms, sometimes as much as 4000 times above the level of the surrounding environment as was noted in aquatic algae (Duffus, 1980). Metal ions of high toxicity are known to cause deleterious impact on organs and blood level in fish. They form metal complexes with structural proteins, enzymes and nucleic acids and interrupt their functions. For example hexavalent chromium is relatively mobile in the environment and is actively toxic, mutagenic, tetratogenic and carcinogenic to aquatic organisms. Among the aquatic fauna, fish is the most susceptible to heavy metal contamination than any other aquatic fauna (Nwaedozie, 1998). Since, heavy metals are non-biodegradable; they can be bio-accumulated by fish, either from the surrounding water or by ingestion of food (Kumar and Mathur, 1991; Patrick and Loutit, 1978). In addition, Heath (1987) indicates that when metals reach sufficiently high concentrations in body cells they alter the physiological functioning of the fish.

Changes in many physiological and biochemical blood indices induced by environmental conditions and presence of contaminants have investigated by several authors (Kori-Siakpere *et al*., 2006; Maheswaran *et al*., 2008; Ololade and Oginni, 2010). The biochemical parameters in fish are valid for physiopathological evaluation and sensitive for detecting potential adverse effects and relatively early events of pollutant damage (Almeida *et al*., 2002; Matos *et al*., 2007; Osman *et al*., 2010).

Biochemical characteristics of blood are among the important indices of the status of internal environment of the fish organism (Edsall, 1999). Changes in the biochemical profile mirror changes in metabolism and biochemical processes of the organism, resulting from the effects of various pollutants and they make it possible to study the mechanisms of the effects of the substances. Racicool *et al*. (1975) had earlier related the activities of these enzymes to the presence of pollutants in the water; hence their assay

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became a routine practice in clinical medicine to diagnose certain diseases and the extent of tissue or organ damage. Since, fish blood is sensitive to a variety of environmental changes it is possible that certain characteristics of fish blood may register effect, however many of these studies seemed to emphasize the characteristics of the blood rather than the values which are useful in pathology.

Enzymes are biochemical macromolecules which control metabolic processes of organisms, thus a slight variation in enzyme activity would affect the organisms (Roy, 2002). Enzymatic activities also provide quick screening methods for assessing the health of fish and can be used to determine the incipient lethal concentration of a toxicant. Therefore, by estimating enzyme activities in an organism, it can be easily identify a disturbance in metabolism. Studies have shown and reported the effect of heavy metal toxicant on enzymes in certain fishes. Luskova *et al*. (2002), Das *et al*. (2004) and Humtsoe *et al*. (2007), had all reported the level of transferases in different organs of different fish exposed to toxicants. Changes in plasma enzyme activity are used as indicators of tissue injury, environmental stress, or a diseased condition. The rate of increase of plasma enzyme activity depends on the concentration of an enzyme in cells, the rate of leakage caused by injury and the rate of clearance of the enzyme from plasma (Boyd, 1983)

There are limited data available on the effect/influence of chromium on the activity on enzymes like lactate dehydrogenase, gamma glutamyltransferase; creatine-kinase and cholinesterase in fish especially *Clarias gariepinus*. Works have however, been carried out on heavy metals like cadmium and zinc and its effect on transferases and dehydrogenase. Valarmathi and Azariah (1993) revealed that lactate dehydrogenase activity was significantly elevated in muscle and hepatopancrease tissues, whereas succinate dehydrogenase was suppressed in crab tissue of the muscle gills and hepatopancrease for 21 days when exposed to two sublethal concentration of 1/10 (2.8 ppm [Exp1]) and 1/3 [9.3 ppm (Exp 2)] copper chloride. Studies of energy production, adopted by *Sersama quadratum* and changes in the activities of two enzymes, lactate dehydrogenase and succinate dehydrogenase were assayed as they are sensitive to environmental pollutants (Devi, 2003). Lactate dehydrogenase activity in the tissues of the fish *Channa punctatus* was observed to be at an increase rate, when they were exposed to cadmium and copper (Sastry *et al*., 1997). Sastry and Sharma (1980) also reported decreased lactate dehydrogenase activity in the brain of *Channa punctatus* following an action of diazinon lasting 96 hr. The acute effect of diazinon in form of the pesticide Basudin 600 EW at a concentration of 32.5 mg/l on carp caused cholinesterase with substrate butryl erythiocholine the activity drop by 85% compared with that in the control group ($p<0.01$). Inactivation of cholinesterase causes a blockage of cholinergic transfer of nerve signals, paralysis, and death due to asphyxia (Voet and Voet, 1990).

Das *et al*. (2004) reported that the activity of lactate dehydrogenase activity in the gills, liver, kidney and brain increased with increased concentration of ammonia in mrigal, *Cirrhinus mrigala* (Hamilton). Kostic *et al*. (1993) showed that prolonged exposure to cadmium may enhance the activity of superoxide dismutase in some mammalian tissues, such as interscapular brown adipose tissue. Goldfish exposed to cadmium in a concentration of 20 mg Cd/l water showed that the activity of superoxide dismutase in the red blood cells significantly decreased as reported by Zikic *et al*. (2001). Investigation showed that cadmium altered the metabolism of carbohydrates, causing hyperglycemia in some marine (Thomas *et al*., 1982) and freshwater fish species (glyconeogensis) (Larsson and Haux, 1982; Zikic *et al*., 1997).

Rajamanickam and Muthuswamy (2008) reported that lactate dehydrogenase exhibited a significant decrease in the first day compared to the control and progressively increased in the successive exposure period in the liver when the common carp *(Cyprinus carpio* L.) was exposed to a combined heavy metal solution for a period of 1, 8, 16 and 32 days, indicating that an exposure of the common carp to a sublethal concentration of combined heavy metals causes liver damage and continuous accumulation of toxic heavy metals by common carp may affect hepatic function and cause cellular degeneration.

The aim of the study is to determine the sublethal effect of chromium on enzymatic parameters such as creatine-kinase, cholinesterase, lactate dehydrogenase and gamma glutamyltransferase in selected organs (liver and kidney) and serum of the African Catfish (*Clarias gariepinus*) and to ascertain selected organs/tissue is most affected by chromium after the 7 days of exposure.

Materials and methods

Apparently healthy live specimens of *Clarias gariepinus* (mean weight, 96.97±5.31 g; mean length 23.00±0.86 cm) obtained from a commercial fish farm in Igbide community, Isoko South Local Government Area of Delta State, Nigeria; and transported to the Animal and Environmental Biology Research Laboratory, Delta State University, Abraka where they were kept in large plastic drums supplied with clean borehole water. Fish were acclimatized to the experimental conditions for two weeks. Mortality during the period of acclimatization was less than 2%.

Stock solution of chromium as potassium chromate (K_2CrO_4) was prepared from 1 g standard AnalaR grade granules in 1 litre of deionised water to form 100% concentration. From this stock solution, various concentrations used in the investigations were prepared by dilution.

Triplicates of the same experimental concentration design were conducted. For each triplicate, a set of four tanks were stocked with 20 randomly selected fish. At the end of the acclimatization period, each tank was randomly assigned to one of three treatments, plus control. Three tanks were dosed for each testing concentration of chromium as potassium chromate (K_2CrO_4): 2 mg/l, 4 mg/l, 8 mg/l of K_2CrO_4 and one received no K_2CrO_4 (0 mg/l; control).

The experimental tanks consisted of large plastic containers of 60 l capacity, filled to half their capacities and covered with a lid made of fine polyethylene gauze screen of 1 mm mesh size, to prevent the fish from jumping out of the containers. Experimental fish were fed daily with Catfish feed (Dizengoff; 4.5 mm; Protein 42%, Fat 13%, Fibre 1.9% and Ash 1.2%) at 3% of their body weights. The fish were not fed 24 hours prior to the experimental period, as well as during the experimental period, which lasted 192 hours. Natural photoperiod was maintained during the acclimation and experimental period.

The water quality parameters of the experimental set up with potassium dichromate toxicant bioassay and control were monitored. The procedures for determination of the water quality parameters were determined according to APHA (1998) and presented in Tab. 1.

The test was performed using a semi-static renewal method in which the exposure medium was exchanged everyday to maintain toxicant strength and level of dissolved oxygen as well as minimizing the level of ammonia excretion during this experiment.

Two fish were randomly caught individually using a small hand net from each experimental tank at the end of the exposure period of seven days. The experiments were conducted three times, yielding a total of six fish for each treatment at each sampling time.

Blood from the selected fish was drawn from the caudal vessels with a heparinised disposable plastic syringes and a hypodermic needle. The use of plastic syringe is a necessary precaution with fish blood, because contact with glass results in decreased coagulation time (Smith *et al*., 1952). Plasma was obtained by centrifugation and diluted 1:20 with deionised water. The diluted plasma was then stored in a refrigerator at-4°C and later analysis were conducted for the creatine kinase, gamma glutamyltransferase, lactate dehydrogenase and cholinesterase. After blood collection, the fishes were sacrificed. The liver and kidney were removed from the fish and pulverized in a laboratory mortar and pestle. Two ml of 10% sucrose solution was added to the homogenate and then centrifuged for 15 min at 4800 rpm. The supernatant was strained through glass wool to remove the lipids; then the clear supernatant kept refrigerated at 4°C until analysis. All determinations were carried out in duplicates for each sample.

Tab. 1. Mean (± standard error) value of monitored water quality parameters of chromium concentration and the control of fish *Clarias gariepinus* exposed to sublethal concentrations of chromium during the 7 days exposure period

Water quality parameters	Concentrations of chromium (mg/l)			
	0.00	2.00	4.00	8.00
Temperature (^{0}C)	29.67 (1.86)	29.33 (1.67)	29.33 (1.67)	29.33 (1.67)
Dissolved oxygen (mg/l)	9.45 (2.29)	9.17 (1.21)	8.89 (1.11)	8.43 (0.46)
Total alkalinity (mg/l)	1.20 (0.10)	0.60 (0.12)	0.57 (0.15)	0.83 (0.20)
Free carbon (iv) oxide (mg/l)	1.67 (0.29)	0.70 (0.25)	0.90 (0.25)	0.77 (0.34)

Plasma biochemical parameters of the creatine-kinase, gamma glutamyltransferase, lactate dehydrogenase and cholinesterase were all determined colorimetrically using commercial diagnostic kits following the manufacturer's instruction; using a spectrophotometer (Spectrumlab 21A, Lenjguang Tech, China). The various procedures used in the determination of enzymatic parameters in the experimental fish are presented below.

Immunoinhibition kinetic UV method of Spinreact kit chemicals SAU, Spain was used to determine the activity of creatine kinase in the sample tissue and organ which was based on the principle of an antibody to the anti-CK-M inhibiting completely CK-MM and subunit (M) of the CK-MB. The rate of NADPH formation, measured photometrically, is proportional to the catalytic concentration of CK-B present in sample (Gerhardt, 1979).

The IFCC Enzymatic colorimetric method of Cromatest kit Linear chemicals, Barcelona, Spain was used was used to determine the activity of gamma glutamyltransferase in the sample tissue and organ which was based on the principle of γ glutamyltransferase catalyzing the transfer of a γ-glutamyl group from γ-glutamyl-3-carboxy-4-nitroanilide to glycylglycine with the formation of L-γ-glutamyl-glycylglycine and 5-amino-2-nitrobenzoate. The amount of 5-amino-2-nitro-benzoate formed, monitored kinetically at 405 nm, is proportional to the enzyme activity present in the sample (IFCC, 1983).

The SFBC UV Enzymatic method of the Cromatest kit, linear chemical Barcelona, Spain was used was used to determine the activity of lactate dehydrogenase in the sample tissue and organ which was based on the fact that in the presence of reduced nicotinamide adenine dinucleotide (NADH) at pH 7.5, lactate dehydrogenase catalyses the reduction of pyruvate to lactate (P-L). The reaction was monitored kinetically at 340 nm by the rate of decrease in absorbance resulting from oxidation of NADH to NAD^+ proportional to the activity of LDH present in the sample.

Total enzymatic colorimetric method of Cromatest kit linear chemical, Barcelona Spain was used to determine the activity of cholinesterase in the sample tissue and organs which was based on the principle of cholinesterase catalyzing the hydrolysis of butrylthiocholine substrates forming butyrate and thiocholine. The latter reduces 5, 5 mercapto bis-2-nitrobenzoic acid (DMNB) to 5-mercapto-2-nitrobenzoate (5-MNBA), a coloured compound.

The results obtained were subjected to analysis for mean and standard error. The mean values of treatment were subjected to statistical analysis using one-way analysis of variance (ANOVA) to test for the level of significance between the various sublethal concentration of chromium. Comparison of the means were analyzed the Bonferroni posttest. All statistical analyses were performed using the

software programme (GraphPad Prism® Software version 5.0, San Diego, CA). Results were considered significant at the 95% confidence level (P<0.05).

Results

The results of enzymatic parameters obtained following sublethal exposure of the African catfish: *Clarias gariepinus* to the various concentrations of chromium over a period of 7 days are presented herein

The activity of creatine kinase in the African catfish *Clarias gariepinus* exposed to sublethal concentrations of chromium after 7 days exposure period is as presented in Fig. 1 which showed insignificant (P>0.05) and significant (P<0.05) difference in the plasma, liver and kidney activity. Plasma creatine kinase activity showed insignificant decrease (P>0.05) as the concentrations of chromium increased revealing 9.49 (1.83), 8.66 (1.09), 4.54 (1.70) and 4.05 (1.20) µkat/l at 0.00, 2.00, 4.00 and 8.00 mg/l chromium while liver activity was insignificant decrease (P>0.05) in the least concentrations (2.00 mg/l) and significant decrease (P<0.05) in other concentration (4.00 mg/l and 8.00 mg/l) respectively revealing 13.21 (2.78), 6.19 (1.52), 4.74 (1.36) and 2.06 (0.98) µkat/l at 0.00, 2.00, 4.00 and 8.00 mg/l chromium. The activity of creatine kinase in the kidney showed insignificant increase (P>0.05) in the least concentration and insignificant decrease (P>0.05) in the last two concentrations (4 mg/l and 8 mg/l) revealing 9.49 (1.83), 10.93 (2.17), 9.49 (3.88) and 9.31 (3.01) µkat/l at 0.00, 2.00, 4.00 and 8.00 mg/l chromium. Statistically, it was revealed that the significant difference of creatine kinase in fish was highest in liver, less in kidney and least in plasma.

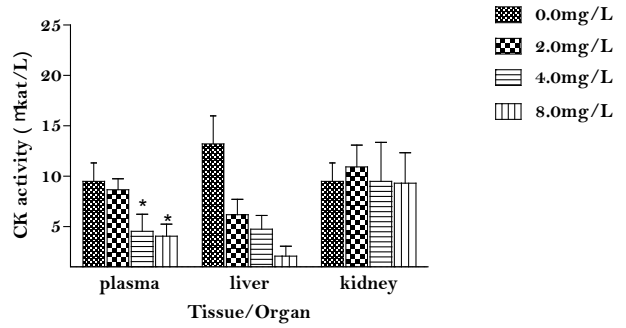

Fig. 1. Creatine kinase activity in *Clarias gariepinus* exposed to sublethal concentrations of chromium after 7days exposure period. Each column represents the mean value and vertical bars indicate the standard error of the means. Asterisk represents significant difference between the control and experimental group at 0.05 level

The activity of gamma glutamyltransferase in plasma, liver and kidney of the African catfish *Clarias gariepinus* exposed to sublethal concentrations of chromium after the 7 days exposure period is as presented in Fig. 2 which showed insignificant difference (P>0.05) in the plasma, liver and kidney. The activity of gamma glutamyltransferase in serum showed insignificant decrease (P>0.05) as the concentrations of chromium increased. Liver gamma glutamyltransferase activity was insignificant increases (P>0.05) as the concentrations of chromium increased revealing 21.61 (5.69), 40.13 (32.93), 33.21 (15.45) and 43.21 (10.33) at 0.00, 2.00, 4.00 and 8.00 mg/l concentrations of chromium while kidney gamma glutamyltransferase activity showed insignificant decrease (P>0.05). Statistically, it was revealed that the significant difference of gamma glutamyltransferase in fish was highest in plasma, less in liver and least in kidney.

The activity of lactate dehydrogenase in plasma, liver, kidney of the African catfish *Clarias gariepinus* exposed to sublethal concentrations of chromium after the 7 days period is as presented in Fig. 3 which showed significant decrease in plasma, liver and kidney. The activity of lactate dehydrogenase in plasma shows insignificant decrease (P>0.05) in 2.00 mg/l and significant decrease (P<0.05) in 4.00 mg/l and 8.00 mg/l chromium revealing 1.52 (0.10), 1.38 (0.14) 0.57 (0.17) and 0.27 (0.02) µkat/l at 0.00, 2.00, 4.00 and 8.00 mg/l chromium respectively. The activity of lactate dehydrogenase in liver showed significant decrease (P<0.05) as the concentrations of chro-

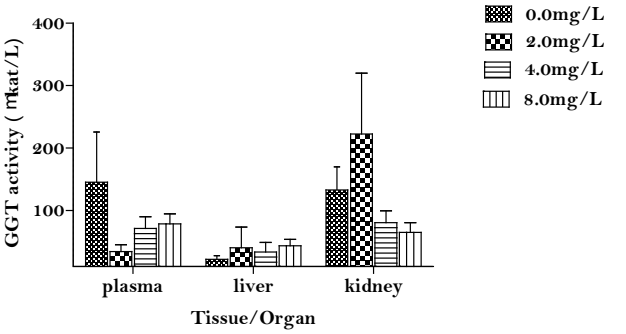

Fig. 2. Gamma glutamyltransferase activity in *Clarias gariepinus* exposed to sublethal concentrations of chromium after 7days exposure period. Symbol as presented in Fig. 1

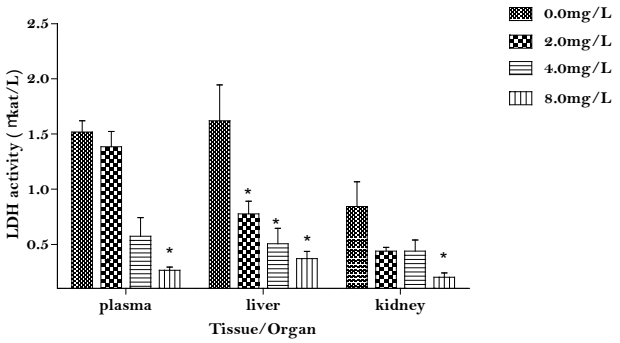

Fig. 3. Lactate dehydrogenase activity of *Clarias gariepinus* exposed to sublethal concentrations of chromium after 7 days. Symbol as presented in Fig. 1

mium increased revealing 1.62 (0.32), 0.78 (0.12), 0.51 (0.14) and 0.37 (0.06) μkat/l at 0.00, 2.00, 4.00 and 8.00 mg/l chromium. The activity of lactate dehydrogenase in kidney showed insignificant decrease (P>0.05) in the first two concentrations, 2.00 mg/l and 4.00 mg/l and significant decrease (P<0.05) in 8.00 mg/l of chromium revealing 0.84 (0.22), 0.44 (0.03), 0.44 (0.10) and 0.20 (0.04) μkat/l at 0.00, 2.00, 4.00 and 8.00 mg/l concentrations of chromium. Statistically, it was revealed that the significant difference of lactate dehydrogenase in fish was highest in liver, less in plasma and least in kidney.

The activity of cholinesterase in the plasma, liver and kidney of the African catfish *Clarias gariepinus* exposed to sublethal concentrations of chromium after 7 days exposure period is as presented in Fig. 4 which showed significant (P<0.05) and insignificant (P>0.05) difference in serum, liver and kidney. The activity of cholinesterase in plasma revealed an insignificant decrease (P>0.05) as the concentrations of chromium increased. The activity in liver cholinesterase also showed insignificant decrease (P>0.05) in two concentrations, 2.00 mg/l and 8.00 mg/l and an insignificant increase (P>0.05) in 4.0 mg/l. In kidney the activity of cholinesterase showed significant decrease (P>0.05) as the concentrations of chromium increased.

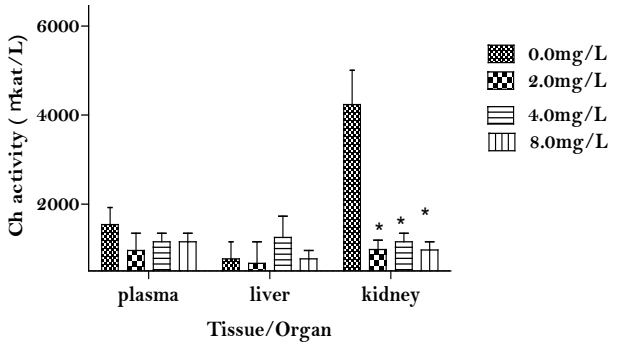

Fig. 4. Cholinesterase activity of *Clarias gariepinus* exposed to sublethal concentrations of chromium after 7days exposure period. Symbols as in Fig.1

Discussion

Enzymes are necessary for normal cellular metabolism including that of the liver and the degenerative changes due to the combined metal toxicity in the liver alter the level of a number of its enzymes.

Creatine kinase is an enzyme found in several tissue types of the body including muscles and bone. Its function is to catalyze the conversion of creatine to phosphocreatine by splitting itself in the conversion of ATP. The activity of *Clarias gariepinus* exposed to sublethal concentrations of chromium showed an insignificant and a significant decrease in serum and liver and also an insignificant increase in kidney respectively. Luskova *et al*. (2002) reported an increase in the mean valve of creatine kinase activity when investigating the effect of diazinon on blood plasma biochemistry in carp (*Cyprinus carpio* L.)

Gamma glutamyltransferase is an enzyme of the liver produced by the microsomes and is widely distributed in cells that are involved in the secretion and absorption of bile. It is a useful laboratory marker as it acts as an indicator of any liver cells damages. Its activity in the *Clarias gariepinus* exposed to sublethal concentrations of chromium revealed insignificant decrease in serum and an insignificant increase in liver and kidney. Similar changes were also observed by Celik *et al*. (1996) in the study of the effect of some pesticides on the activity of liver and erythrocytes enzymes of fish.

The lactate dehydrogenase is anaerobic enzyme involved in the conversion of pyruvate to lactate in the Embden Meyerhoff pathway. Lactate dehydrogenase is a cytoplasmic enzyme. It is generally associated with cellular metabolic activity. It acts as a pivotal enzyme between the glycolytic pathway and tricarboxylic acid cycle. The enzyme shows an increasing activity during a strenuous muscle exercise (Rajamanickam and Muthuswamy, 2008). Lactate dehydrogenase is released from the liver after its cellular damage (Ceron *et al*., 1997) this may be the reason for the significance decrease in the liver lactate dehydrogenase; thus suggesting that its activity is a sensitive index to measure influence of external factors as supported by the findings of Sastry and Sharma (1980) who reported decreased in lactate dehydrogenase activity in the brain of C*hanna punctatus* exposed to diazinon. Palikova *et al*. (2004) also reported significant decrease in lactate dehydrogenase activity of Carp (*Cyprinus carpio* L.*)* with long term exposure to cyanobacteria extract during the early life stages. Rajamanickam and Muthuswamy (2008) reported a decrease in the level of lactate dehydrogenase in the liver homogenate of common carp in a one day exposure to heavy metals with subsequent increase afterwards. The increased level of the lactate dehydrogenase may be due to an alternative aerobic glycolytic pathway in conversion of lactate to pyruvate for the production of glucose which is a major source of energy during stress induced by heavy metals. The variation of lactate dehydrogenase activity can thus be used as another sensitive index for assessing heavy metal toxicity.

Cholinesterase is an important enzyme needed for the proper functioning of the nervous system of fishes. The activity showed as insignificant decrease and increase in serum and liver and a significant decrease in kidney. These findings have been supported by Aguiar *et al*. *(*2004) who revealed that cholinesterase activity decreased in different fishes. Svoboda *et al*. (2001) reported a significant decrease of cholinesterase in the kidney. Hassanein (2002) reported a fall in the acetylcholinesterase activity in various fish species. Inhibition of acetylcholinesterase causes accumulation of neurotransmitter, acetylcholine (Ach) at the synapse and blocking the neurotransmission in the respiratory centre of the brain or neuromuscular junction

respiratory centre of the brain or neuromuscular junction respiratory system which leads to death. In addition, reduced acetylcholinesterase will be unable to maintain a normal upright position in water resulting in uncontrolled drifting, inability to protect themselves from predation, beaching and other hazards.

Conclusions

In conclusion, the results of this study show that the fishes were stressed after being exposed to sublethal concentrations of chromium. The heavy metal chromium altered the activity of the enzymes significantly thus result in the instable physiological state of the fish. Therefore, sublethal concentrations of chromium have some deleterious effect on the basic activities of enzymes of the plasma, liver and kidney of the experimental fish, *Clarias gariepinus*.

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