Indian J. Anim. Res., 50 (3) 2016:370-373
Print ISSN:0367-6722 / Online ISSN:0976-0555

AGRICULTURAL RESEARCH COMMUNICATION CENTRE
www.arccjournals.com/www.ijaronline.in

Effect of nickel toxicity on growth parameters and hepatic enzymes in major carp

Husna Malik, Sumera Sajjad, Sobia Akhtar* and Sana Bilal

Department of Zoology, Lahore College for Women University,
Lahore. Pakistan.
Received: 30-04-2015 Accepted: 11-01-2016 DOI:10.18805/ijar.9591

ABSTRACT

Labeo rohita were used to investigate effect of nickel induced toxicity. For this two groups, control groups (N=50) and experimental groups (N=50) were further divided into group 1-4, on the basis of dosage of nickel and blood sampling. Body weight of animals was noted before and after dose treatment. It was found that body length in the experimental group did not show marked increase throughout experimental period while the effect of nickel toxicity was observed on body weight. The weight of experimental fish decreased in experimental group significantly ($p<0.05$) from first dose to the last dose. Blood plasma was analyzed for biochemical parameters (Total proteins, AST, ALT, and ALP). AST, ALT and ALP levels increased remarkably in experimental fish as compared to the control one, while the levels of total proteins decreased significantly from group 1-4. Results of the present study suggest hepatotoxicity caused by metal and role of nickel as endocrine disrupter in fish body. Nickel also causes changes in growth parameters of animal by affecting their body weight with increased dosage of metal.

Key words: ALT, AST, ALP, Fish, Nickel.

INTRODUCTION

Heavy metal accumulation has posed a great threat to aquatic systems all over the world. Large population, industrialization, urbanization and agricultural areas have boosted the conditions (Giguere, A. *et al*., 2004). Waste waters holding heavy metals released by industries are discharged into aquatic systems where they bioaccumalate in food chain, their concentration increases and becomes lethal to living organisms (Royer *et al*., 2006). Nickel and its compounds produce acute and chronic toxicity to aquatic life due to its persistence and bioaccumulation (Vieira *et al*., 2009). Nickel affects liver parameters, body weight and length. Its toxicological, physiological and histopathological alterations in rabbits, mice and minnows have been established (Pane *et al*., 2003, Bersenyi, *et al*., 2003, Doreswamy *et al*., 2004, Hoang *et al*., 2004, Gupta *et al*., 2006). However, its toxicity affects in major carps have not been studied previously. The present study was hence designed to assess the toxicological aspects of Nickel in major carp through monitoring the alterations in growth parameters, hepatic enzymes and plasma cortisol levels.

MATERIALS AND METHODS

Fish collection and maintenance: The experiment included a total of 160 fish (*Labeo rohita*) of 4.9-7.5 cm in length, 15-20g in weight. They were kept in 120L capacity aquariums of the Fish Physiology and Biochemistry laboratory, Lahore College for Women University, Lahore and acclimatized for two weeks under appropriate conditions of light, temperature, pH and aeration. Fish were fed with floating type feed having a composition of moisture 10%, crude protein 35%, crude fat 3%, fiber 3% and crude ash 10%.

Determination of median lethal concentration: Chemically pure nickel sulphate (Merck) was dissolved in distilled water for the preparation of desired stock solution for required metal dilutions. Sixty fish were used to calculate LC_{50}, for this, different concentrations of nickel, i.e. 6.0 ppm, 15.0 ppm and 30.0 ppm were administered after every 72 hours. Dose of 15.0 ppm was found to be median lethal and was used in the further experimental procedures carried out. In order to avoid the sudden stress to fish, the concentration of metal in aquarium was increased gradually. Fish were kept starved for 24 hours prior to dose treatment. Remaining hundred fish were divided into two groups; control group (N=50), which was maintained in normal water without any treatment, while the other group (N=50) as experimental which was exposed to a median-lethal concentration of nickel. The exposure mediums were continuously replenished and partly exchanged to maintain the harmful concentrations of Ni for fish throughout the experimental period.

Growth parameters, blood collection and analyses: The body weight and length of fishes were recorded after and prior to dosage administration. It was carried out to evaluate the weekly changes in the growth of *Labeo rohita* due to

*Corresponding author's e-mail: sobiaakhtar62@gmail.com and address Lahore College for Women University Lahore.

nickel stress. Blood samples were taken both from experimental and control group, Blood sampling was done in four durations i.e. first sampling was done after fifth dose, second after sixth dose, third after seventh dose and fourth after eighth dose. About 1-1.5 ml of the blood sample was collected from fish by tail ablation with scalpel blade. Blood drawn was collected by heparinized syringe and transferred to eppendorfs, Plasma was separated by centrifugation the samples at 13,000 rpm for 15 minutes. Collected plasma was transferred into properly labeled eppendorfs and stored at -20°C till further analysis. Biochemical parameters (Total proteins, AST, ALT, and ALP) were analyzed on Semiautomatic computer based chemistry analyzer by using the end points and kinetic modes.

Statistical analyses: Mean values of different parameters were calculated and expressed as mean ± SEM. Student's t-test was used to compare the means of the same parameters among different groups while one way ANOVA was used to compare means within a same group. Probability levels of 0.05 and 0.001 were considered significant.

RESULTS AND DISCUSSION

The levels of metabolic marker enzymes in the liver of the *Labeo rohita* were determined which displayed dose exposure related effects as compared to the control group animals. The body weight of control animals showed a significant increase ($p>0.05$) during study period, while in experimental animals body weights were decreased (Table 1). Control animals showed a significant increase in the mean body weight and length while experimental animals (group 1-4) showed a decrease in the body weight in the subsequent weeks with no apparent change in body length (Table 1). Decrease in body weight of a rainbow trout was observed when exposed to heavy metal due to the stress generated by heavy metal intoxication (Fernandes *et al.*, 2007). Weight loss was also reported in brown trout and mirror carp when exposed to high levels of copper (Van Heerden *et al*. 2004). Total plasma proteins represent a decreasing trend in the experimental animals as compared to control group due to the result of hepatotoxicity (Figure1). In the present study the lower levels of total protein represent a number of pathological processes i.e. protein elimination in the urine, lowered production of liver proteins and liver damage due to heavy metal intoxication (Aboud, 2010). Verified reduction in total plasma protein concentrations can be detected as an indicator of impaired liver function. This could be attributed to either a state of hydration or change in water

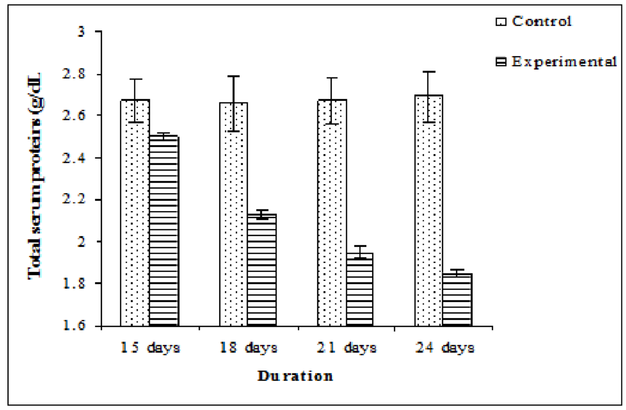

Fig 1: Mean±SEM of total serum proteins (g/dL) in control and experimental groups.

equilibrium of the fish and disturbance in the liver protein synthesis. Previous studies reported reduction in liver soluble proteins and RNA due to contamination of heavy metals (Ali *et al*., 2003).

Level of AST enzyme in the blood increased suggesting damaging effects on liver, variation in AST concentration in control groups was negligible as compared to that in experimental animals. (Figure 2). Monitoring of liver enzyme discharge into the blood has proved to be a very useful tool for toxicity studies in the liver. Previous studies revealed decrease in ALT levels in the liver of common carp, (*Cyprinus carpio*) when exposed to heavy metals like cadmium, lead, nickel and chromium (Mishra and Jain., 2009). Variations in ALT enzymes affect the energy production during processes like TCA cycle, gluconeogenic process, protein and carbohydrate metabolism, hence can help to examine metabolic changes (Raja and Muthuswami 2008).

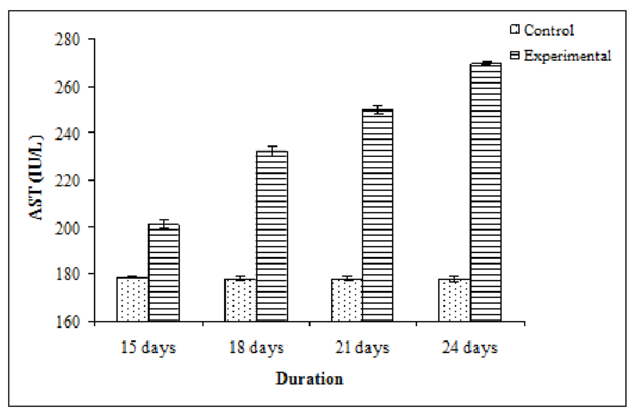

Fig 2: Mean±SEM of AST (IU/L) in control and experimental groups.

Table 1: Mean±SEM of body weight (g) and length (cm) of control and experimental groups

DAYS		15 days	18 days	21 days	24 days
Body weight (g)	Control Group	17.06± 0.56	19.82±0.76	21.64±1.27	23.58±1.73
	Experimental group	21.41±1.34	19.14±2.28	18.17±0.63	17.10±0.72
Body length(cm)	Control Group	5.68±0.14	6.74±0.23	7.94±0.17	9.26±0.20
	Experimental group	6.37±0.22	6.27±0.22	6.24±0.25	6.31±0.25

The ALT values rise in experimental group while in control group there was no significant change (Figure 3). High levels of ALT in the blood indicate impairment of liver as observed in present study. Previous studies observed that high levels of liver enzymes show hepatocellular injury (Winter *et al*., 2005). Considerable rise in AST and ALT activities in liver of animals by heavy metal pollution has been reported by many researchers (Krier and Ahmed., 2009, Ritu and Madhu., 2009). Previous investigation has reported that when stress due to toxicity takes place in the liver of freshwater fish, ALT transforms protein to main energy reserve, glycogen (Khandelwal *et al*., 2002).

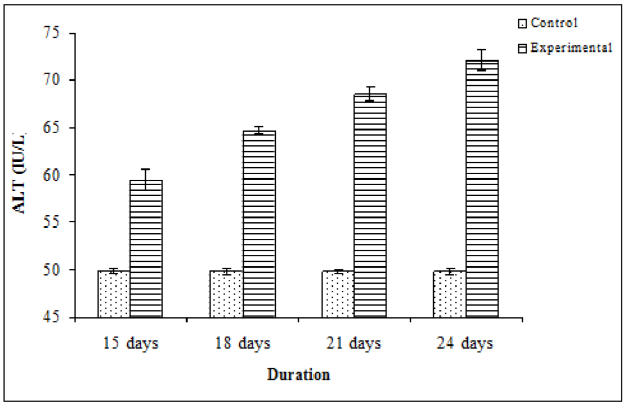

Fig 3: Mean±SEM of ALT (IU/L) in control and experimental groups.

The concentration of ALP on analysis revealed the increasing trend in experimental groups. The comparison of mean values of ALP between control and experimental animals showed significant difference. (Figure 4). The comparison of ALP between control and experimental groups indicate the probable hepatotoxicity in experimental animals which is induced by dose administration. Alkaline phosphatase (ALP) is a hydrolytic enzyme found in blood that works effectively under alkaline pH conditions and is formed from bone, liver, kidney, placenta, intestine, testes, thymus, lung and tumors or inflammation (Neiva *et al*., 2008). It was previously reported that alkaline phosphatase levels increased by liver damage, kidney dysfunction and bone disease (Kopp and Hetesa 2000, Yang *et al*., 2005).

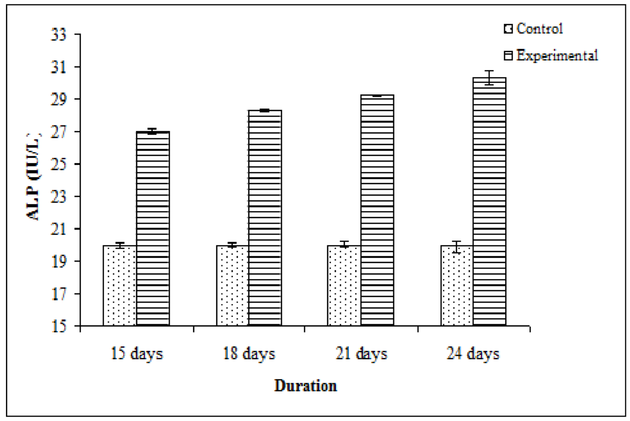

Fig 4: Mean±SEM of ALP (IU/L) in control and experimental groups.

Mean plasma cortisol concentration was compared for each group between animals of control and experimental groups. For all the sampling days of experiment, mean plasma cortisol level of experimental group was observed to be significantly higher than that of control group (Zhang *et al*. 2010) It was demonstrated that rapid rise in glucocorticoids and catecholamines from internal cells of fish are accompained by cortisol elevation due to nickel toxicity (Yang *et al*., 2005). It was reported that exposure of fish to nickel sulphate has deleterious effects on feeding behaviour, growth and reproduction due to chronic cortisol elevation and had no effect on swimming performance (Harper and wolf 2009, Zhang *et al*., 2010).

CONCLUSION

From the present study, it is concluded that nickel when given at a sub lethal dose shows toxic effects in major carp species of fish. An apparent change in biochemical parameters i.e, total plasma proteins and augmented levels of liver enzymes (ALT, AST, ALP) were observed in the experimental animals as compared to control ones. Continuous administration of the metal salt resulted in decline in the body weight, total plasma protein, suggesting probability of liver damage. Furthermore, the results provide evidence that enzymes are diagnostic tools of aquatic pollution.

REFERENCES

Aboud, O. A. S. A. (2010). Impact of lead, mercury and cadmium on the immune response of *Oreochromis niloticus*. *New York Science Journal*. **3:** 12-16.

Ali, A., Al-Ogaily, S. M., Al-Asgah, N.A. and Gropp, J. (2003). Effect of sublethal concentrations of copper on the growth performance of *Oreochromis niloticus*. *Journal of Applied Ichthyology*, **19:**183-188.

Bersenyi, A., Fekete, S. G., Szocs, Z. and Berta, E. (2003). Effect of ingested heavy metals (Cd, Pb and Hg) on haematology and serum biochemistry in rabbits. *Actaveterinaria Hungarica*, **51:**297-304.

Doreswamy, K., Shrilath, B., Raheshkumar, T. and Muralidhara. (2004). Nickel-induces oxidative stress in testis of mice: evidence of DNA damage and genotoxic effects. *Journal of Andrology*, **25:** 996-1003.

Fernandes, A. F., Ferreira-Cardoso, J. V., Garcia-Santos, S., Monteiro, S. M., Carrola, J., Matos, P. and Fontaínhas-Fernandes, A. (2007). Histopathological changes in liver and gill epithelium of Nile tilapia, *Oreochromis niloticus*, exposed to waterborne copper. *Pesq. Vet. Bras.* **27:**103-109.

Giguere, A., Campbell, P. G. C., Hare, L., McDonald, D. G. and Rasmussen, J. B. (2004). Influence of lake chemistry and fish age on cadmium, copper and zinc concentrations in various organs on indigenous yellow perch (percaflavescens). *Canadian Journal of Marine Biology and Assessment, UK,* **53:**721-739.

Gupta, N., Gupta, D. K., Verma, V. K. and Krishna, G. (2006). Zinc-induced changes on chromosomes of a fresh water teleost, *Heteropneustes fossilis* (Bloch). *Asian Journal of Experimental Sciences,* **20:**281-288

Harper, C. and Wolf, J. C. (2009). Morphologic Effects of the Stress Response in Fish. *Institute of Laboratory Animal Research Journal,* **50:** 387-396.

Hoang, T. C., Tomasso, J. R. and Klaine, S. J. (2004). Influence of water quality and age on nickel toxicity to fathead minnows (*Pimephales promelas). Environmental Toxicology and chemistry*, **23:**86-92.

Khandelwal, S., Shukla, L. J. and Shanker, R. (2002). Modulation of acute cadmium toxicity by *Emblica officinalis* fruit in rat. *Indian Journal of Experimental Biology,* **40:** 564-570.

Kopp, R. and Hetesa, J. (2000). Changes of hematological indices of juvenile carp carp (*Cyprinus carpio* L.) under influence of Natural population Cyanobacterial water blooms. *Acta Veterinaria Brno*, **69:**131-137

Krier, M. and Ahmed, A.(2009).The asymptomatic outpatient with abnormal liver function tests. *Clinical Liver Diseases*, **13:**167–177.

Mishra, M. and Jain, S. K. (2009). Effect of natural ion exchanger chabazite for remediation of Lead toxicity: An experimental study in teleost fish *Heteropneustes fossilis. Asian Journal of Experimental Science*, **23:**39-44.

Neiva, B., De Lima, L., Flora, R. D., Lang, F., E., Bauermann, M. D., Loro, L., Lucia, V. and Baldisserotto, B. (2008). Lipid peroxidation and superoxide dismutase activity in silver catfish *(Rhamdia quelen)* juveniles exposed to different dissolved oxygen levels. *Ciência Animal Brasileira,* **9:** 811-814.

Pane, E. F., Smith, C., McGeer, J. C. and Wood, C. M. (2003). Mechanisms of acute and chronic water borne nickel toxicity in the freshwater cladoceran, *Daphnia magna. Environmental Science and Technology*, **37:**4382-4389.

Raja, V. and Muthuswami, N. (2008). Effect of heavy metal induced toxicity on metablic biomarkers in common carp (*Cyprinus carpio* L.). *Maejo International Journal of Science and Technology*, **2:**192-200

Ritu, S. and Madhu, K. (2009). Role of *Panax ginseng* as an antioxidant after cadmium- induced hepatic injuries. *Food and Chemical Toxicology*, **47:** 769-773.

Royer, T.V., David, M.B.and Gentry, L. E. (2006). Timing of riverine export of nitrate and phosphorus from agricultural watersheds in Illinois: implications for reducing nutrient loading to the Mississippi River. *Environmental Science and Technology*, **40:** 4126-4131.

Vieira, R. L., Gravato, C., Soares, A. M. V. M., Morgado, F. and Guilhermino, L. (2009). Acute effects of copper and mercury on the estuarine ûsh *Pomatoschistus microps* : Linking biomarkers to behaviour. *Chemosphere*, **76 :** 1416–1427

Van Heerden, D., Vosloo, A. and Nikinmaa, M. (2004). Effects of short-term copper exposure on gill structure, methallothionein and hypoxia-inducible factor-1á (HIF-1á) levels in rainbow trout (*Oncorhynchus mykiss*). *Aquatic Toxicology*.**69:**271-280.

Winter, M. J., Verweij, F., Garofalo, E., Ceradini, S., McKenzie, E. J., Williams, M. A., Taylor, E. W., Butler, P. J., van der Oost, R. and Chipman, J. K. (2005). Tissue levels and biomarkers of organic contaminants in feral and caged chub (*Leuciscus cephalus*).from rivers in the West Midlands, UK. *Aquatic Toxicology,* **73:** 394-405.

Yang, R. W., Shao, Z. X., Chen, Y. Y., Zand, Y. and Wang, W. J. (2005). Lipase and pancreatic amylase Activites in diagonosis of acute pancreatic in patients with hyperamylasemia. *Hepatobillary and Pancreatic Disease International*, **4:** 600-603.

Zhang, Y., Salinas, I., Li, J., Parra, D., Bjork, S., Xu, Z., LaPatra, S. E., Bartholomew, J. and Sunyer, J. O. (2010). IgT, a primitive immunoglobulin class specialized in mucosal immunity *Nature Immunology,* **11:** 827-835.