

Received: 13<sup>th</sup> August-2012Revised: 16<sup>th</sup> August-2012Accepted: 18<sup>th</sup> August-2013

Research article

**EFFECT OF CONSUMPTION OF HEAVY METALS CONTAMINATED FISH (TILAPIA OREOCHROMIS) ON METABOLIC PARAMETERS IN RABBITS.**

Bolawa, O.E and Gbenle, G.O

Department of Biochemistry, College of Medicine, University of Lagos, P.M.B 12003, Idi – Araba, Lagos, Nigeria

E -mail: olatundunebolawa@yahoo.com

**ABSTRACT:** This research paper presents the biochemical effect of the consumption of heavy metals contaminated fish on metabolic parameters in rabbit. Total glucose, cholesterol, protein and levels of alkaline phosphate, alkaline aminotransferase (ALT) together with aspartate aminotransferase (AST) in the serum were measured. Compared with the control a significant decrease of total protein and total cholesterol ( $p < 0.01$ ) was ascertained in the serum of the experimental groups. Total glucose level was significantly increased in the serum of the experimental ( $p < 0.01$ ). The values of alkaline phosphate, ALT and AST significantly increased in the serum of the groups. The above results on the biochemical consumption profile indicate the toxic effect of the consumption of these contaminated fishes in rabbits.

**Key words:** markers enzymes, heavy metal toxicity, transaminases.

**INTRODUCTION**

Human activities have led to accumulation of toxic metals in the aquatic environment (Yang and Rose, 2003). Heavy metals at high concentrations can cause harmful effects on the biochemical system of fishes and this causes long term ecotoxicology effects on the organisms that eat them (Strmack and Braunbeck, 2000). The adverse input of diverse industrial wastes has aggravated the problem of contamination and sewage disposal has greatly enhanced the addition of heavy metals into the aquatic ecosystem (Karbassi et al, 2006). This problem has become complex because of the non-degradability of the inorganic pollutants. They are continuously released into the aquatic environment. Heavy metals have received particular attention among the non-degradable toxic chemicals due to their adverse effects on aquatic life forms (Dirilgen, 2001). Heavy metals are the most noxious pollutants owing to their diverse effects. Some metals are soluble in water and readily absorbed into the living organisms. Metal ions of high toxicity are known to cause deleterious impact on organs and blood level in organisms (Akahori et al, 1999). Chromium is a compound of biological interest. It is an important pollutant from industrial effluents and cause deleterious effects on living organisms (Arun Kumar et al, 2004). Cadmium is a non essential, non-biodegradable element reported to be a major contaminant that causes adverse effects on living organism (Rasmussen and Anderson, 2000; Filipovic and Raspor, 2003). Lead, a toxic contaminant metal is widely used in batteries, paint, vanishes, pipe covering welding etc. Because of its persistence in the environment, exposure to lead has become a major public health concern. Lead induces lipid peroxidation in tissues and causes irreversible damage to the systems of living organism. Lead toxicity on different biological systems and functions has been well reported (Goyen, 1993). Enzymes are necessary for normal cellular metabolism and the degenerative changes due to combined metal toxicity exhibited in the liver alter the level of its enzymes. For example aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) are released in acute and chronic liver disorders. These enzymes are biomarkers of acute hepatic damage. Thus their bioassay can serve as a diagnostic tool for assessing necrosis of the liver cells (Coppo et al, 2001).

Levels of the aforementioned heavy metals have been found in Tilapia fishes (*Oreochromis niloticus*) obtained from Carter Bridge river and Makoko river (Bolawa and Gbenle, 2010). These fishes were added to the diet of the rabbits used for this research work. The aim of the present study is therefore to determine the effects of the consumption of these fishes by rabbits on biochemical parameters (protein, albumin, glucose, cholesterol, AST, ALT, and ALP).

## MATERIALS AND METHODS

Experimental animals.

Twenty-one experimental rabbits were obtained from the Nigerian Institute of Medical Research, Yaba, Lagos.

### Fish Samples

Fish samples (Tilapia *Oreochromis*) were obtained from Carter Bridge river and Makoko rivers. These two study sites were selected because they are commercial fishing sites in Lagos, Nigeria. The fishes were dried in the oven and grinded.

### Animal Studies

Twenty-one rabbits of mean weight 6.3kg were allowed to acclimatize to laboratory condition in well-ventilated cages for two weeks. The animals were grouped into three with each group containing seven rabbits. The first group was fed with 100g of fish (obtained from Carter Bridge river) added to their feed (rabbit pellets) manufactured by Pfizer Livestock Feeds Nigeria Plc, Ikeja, Lagos State) on a daily basis for 3 months. The second group was fed with 100g of fish (obtained from Makoko river) added to their feed on a daily basis for 3 months. The third group served as the control and they were fed with just rabbit pellets for 3 months. After 3 months, the animals were anaesthetized and blood samples collected through the jugular vein. The blood sample collected was spun using a centrifuge at 4000 rpm for 35min and the serum was collected using a pipette and stored at 4°C prior to immediate determination of biochemical parameters-total glucose, total protein, total cholesterol, Glutamic oxaloacetic acid transaminase (GPT), Glutamic pyruvic acid transaminase (GPT), albumin and alkaline phosphatase. Blood glucose was estimated by using anthrone method as described by Cooper and McDaniel, 1970. Serum cholesterol was estimated as described by Plummer, 1988. Serum total protein was measured according to the procedure of Lowry et al, 1951. The activities of serum GPT, GOT and Alkaline phosphatase were estimated according to the methods of Retiman and Frankel, 1957. The data obtained from the experiments were analyzed and the results were expressed as mean± S.D. The results were evaluated using student's t-test. The values of P<0.001 were considered statistically significant.

## RESULTS

Body weights of animals during the experimental period.

Body weights of the rabbits are presented in figures 1 and 2. Body weights of both group 1 and 2 rabbits were lower than that of the control group. The reduction in body weights were not significant (P>0.005). The weight of organs in rabbits fed with fish from Carter, Bridge site and Makoko river are presented in table 1. There were significant decreases in the weights of livers and lung P<0.01 when compared with the control group that was not fed the fishes.

### Total glucose

The serum glucose level was significantly higher (P<0.01) in the rabbits fed the fish diet when compared to the control (table 2) while glucose concentration in the lung and kidney were significantly lower in the rabbits fed the fish diets when compared with the control (table 3).

### Total cholesterol

Total cholesterol in the organs significantly decreased in the experimental rabbits than the control (table 2).

### Total protein

Serum protein and albumin levels were significantly decreased in the rabbits fed fishes obtained from the study locations as compared with the control group. The protein concentrations in the lung, liver, heart and muscle were significantly higher in the rabbits fed the fish diet when compared with the control.

### Serum GOT, GPT and ALP.

Serum GOT, GPT and ALP activity of the rabbits fed the fish diet observed overall increases in their activity than control (Table 5).

**Table 1. Weight of organs of experimental and control rabbits**

Groups	Brain (g)	Heart (g)	Liver (g)	Kidney	Lung (g)
Carter Bridge group	6.74±0.30	8.23±0.50	23.16±0.20*	9.71±0.30*	4.87±0.70*
Control	6.79±0.60	5.30±0.70	41.25±1.20	11.61±0.60	9.07±0.50
Makoko group	6.70±0.80	6.82±1.20	34.04±1.00*	11.48±0.60*	7.84±0.80*

Values represent meant ± S.E.M (n=5) \* P<0.01.

**Table 2: Serum protein, albumin, glucose and cholesterol concentration in experimental and control rabbits.**

Groups	Protein mg/ml	Albumin mg/100ml	Cholesterol mg/100ml	Glucose mg/ml
Carter bridge group	25.35±0.44*	3.30±0.42*	97.00±0.20*	0.65±0.03*
Control	34.33±1.02	6.10±0.13	220.00±1.60	0.49±0.06
Makoko group	25.71±0.14*	4.60±0.12*	111.00±0.8*	0.59±0.03*

Values are expressed as mean ± S.E.M. of 5 rabbits. \* P <0.01

**Table 3. Glucose concentration in organs of control and experimental rabbits.**

Groups	Lung g/100ml	Liver g/100ml	Kidney g/100ml	Heart g/100ml	Brain g/100ml
Carter Bridge group	0.28±0.06*	1.23±0.01**	0.60±0.03*	0.74±0.08	0.22±0.06
Control	0.88±0.04	1.28±0.02	1.12±0.06	0.48±0.08	0.78±0.03
Makoko group	0.46±0.03*	0.63±0.02**	0.44±0.08*	0.41±0.01	0.68±0.05

Values represent mean ± S.E.M (n=5) \*P<0.05, \*\*P<0.01

**Table 4: Protein concentrations in the organs of control and experimental rabbits**

Groups	Lung mg/ml	Liver mg/ml	Kidney mg/ml	Heart mg/ml	Muscle mg/ml
Carter Bridge group	1.25±0.01*	2.20±0.12*	1.43±0.06*	1.68±0.06	1.73±0.04*
Control	0.30±0.50	0.80±0.01	0.75±0.01	0.24±0.05	0.65±0.02
Makoko group	1.30±0.03*	2.50±0.10*	0.13±0.04	0.42±0.60	1.80±0.05*

Values represent meant± S.E.M (n=5) \*P<0.05

**Table 5. Enzyme levels in the serum of rabbits.**

Groups	Alkaline phosphate u/L	AST u/L	ALP u/L
Carter bridge group	287.50±15.01 <sup>a</sup>	100.00±8.40 <sup>a</sup>	31.00±2.64 <sup>b</sup>
Control	131.25±12.30	66.50±7.20	27.30±2.14
Makoko group	216.50±14.25 <sup>a</sup>	105.00±9.50 <sup>a</sup>	33.00±2.58 <sup>b</sup>

Values are expressed as mean± S.E.M of 5 rabbits

Values carrying different superscript horizontally are significantly different (P<0.0.1

## DISCUSSION

Environmental stressors such as heavy metal exposure may change the biochemical parameters (Yang and chen, 2003). The measurement of serum biochemical parameter can be useful as diagnostic tool in toxicology to find the general health status of the rabbits and target organs affected by toxicants (McDonald and Grosell, 2006). The present study demonstrated that the rabbits exposed to a fish diet gotten from polluted riverine areas displayed a significant elevation in the level of blood glucose after the exposure periods. Similar observations have been reported by Partap and Bonga (1990). High levels of blood glucose are caused by disorders in carbohydrate metabolism. A variety of stressors stimulate the adrenal tissues resulting in increased level of circulating glucocorticoids and catecholamine. The elevation in blood glucose level is a response to the increase rate of glycogenolysis or gluconeogenesis. Hyperglycemic response in this study is an indication of a disruption due to enhanced breakdown of liver glycogen and mediated by reduced insulin secretory activity (Hontela et al, 1996). Plasma proteins were decreased significantly with exposure of the rabbit to the fish diet. This could be due to renal excretion, impaired protein synthesis or due to liver disorder (Vutukuru, 2005). The decrease of plasma protein could also result from the breakdown of protein into amino-acids, first and possibly into nitrogen and other elementary molecules. Similar reduction in protein has been reported in *Heterobranchus bidorsalis* and *Clarias gariepinus* following exposure to sublethal effect of cadmium toxicity (Korisiakpere et al 2006). Alkaline phosphatase is composed of several isoenzymes that are present in practically all tissues of the body, especially in cell membranes. It catalyzes the hydrolysis of monophosphate esters and has wide substrate specificity. The functional activity of the enzyme was found to increase during the exposure with heavy metals as an adaptive response in mitigating the metal toxicity. Increase stimulation of alkaline phosphatase has previously been found in such pathological processes as liver impairment, kidney dysfunction and bone disease (Yang and Chen, 2003). This supports our present study with increased activity of alkaline phosphatase in the experimental rabbits. Transaminases play an important role in carbohydrate and amino-acid metabolism in the tissue of organisms (Atroshi et al, 2000). The data of the present study showed that exposure of the rabbits to the fish diet caused significant elevations in the activities of blood serum GOT and GPT levels. Several reporters showed that these blood enzymes were highly increased in the fish treated with cadmium and copper (Singh and Roddy, 1990). Increase in blood enzymatic activity is either due to (i) leakage of these enzymes from hepatic cells into the blood stream, (ii) increased synthesis and (iii) enzyme induction of these enzymes (Shakoori et al, 1990). These enzymes liberated into the blood stream when the hepatic parenchyma cells are damaged. The present study showed that the fish diet altered the entire biochemical metabolism in the rabbit by changing the levels of total glucose, total protein, alkaline phosphatase, GOT and GPT in the blood samples. Such changes in biochemical levels under the effect of heavy metal toxicity (from the fish gotten from polluted riverine areas) might result in impairment of energy requiring vital processes. This gives us an idea about the health status of the fish population in these riverine area and the rabbits feeding on them.

## REFERENCES

- Akahori A, Gabryelak T, Jozwiak and Gondko R, (1999): Zinc induced damage to carp (*Cyprinus carpio* .L.) erythrocyte in vitro. *Biochem Mol. Biol. Int.* 47:89-98.
- Altroshi F, Rizzo A, Samkari S, Biege I, Westemark P and Veijalainen P, (2000).
- Arun Kumar, Rana KS et al (2004): Bioconcentration of chromium, copper and iron in Indian major carp *Labeo rohita* (Han). *Indian .J. Environ and Ecoplan.* 8(1):217-219.
- Coppo JA, Mussart NB and Fioranelli SA, (2001): Physiological variations of enzymatic activities in blood of bull frog, *Ranacatesbeiana* (Shaw, 1820). *Rev. Vet* 12/13: 22-27.
- Dirilgen N, (2001): Accumulation of heavy metals in fresh water organisms, assessment of toxic interactions. *Tur. Chem.* 25:173-179.
- Filipovic V and Raspor B, (2003): Metallothionein and metal levels in cytosol of liver, kidney and brain in relation to growth parameters of *Mullus surmuletus* and *Liza aurata* from the Eastern Adriatic Sea. *Water research*, 37:3253-3262.

- Goyer RA, (1993): Lead toxicity: current concerns. *Environ Health Perspect* 100: 177-187.
- Hontela A, Daniel C et al (1996): Effect of acute and subacute exposure to cadmium on the inter renal and thyroid function in rainbow trout, *Oncorhynchus mykiss*. *Aquat. Toxicol.* 35: 171-182.
- Karbassi AR, Bayati T and Moathar F, (2006): Origin and chemical partitioning of heavy metals in river bed sediments. *Int. J. Environ Sci Tech*, 3:35-42.
- Kori-Siakpere O, Ake JEG et al (2006): Sub lethal effects of cadmium on some selected hematological parameters of *Heteroclaris*. *Intel. J. Zool. Reser.* 2(1): 77-83
- Liver enzyme activates of rats exposed to Ochratoxin A and T-2 Toxin with antioxidants. *Bull Environ. Contam. Toxicol.* 64:586-592.
- Lowry OH, Rosenberg NJ et al (1951): Protein measurement with Folin Phenol reagent. *J. Biol Chem.* 193, 265-275.
- McDonald MD and Grosell M,(2006): Maintaining osmotic balance with an aglomerular kidney. *Com. Biochem. Physiol.* 143: 447-458.
- Partap HP and Bonga SEW, (1990): Effect of water-borne cadmium on plasma cortisol and glucose in the cichlid fish, *Oreochromis mossambicus*. *Comp. Biochem. Physiol.* 95; 313-317.
- Rasmussen AD and Anderson O, (2000): Effects of cadmium exposure on volume regulation in the lungworm, *Arenicola marina*. *Aquat. Toxicol* 48:151-164.
- Reitman S, Frankel S, (1957): A colorimetric method for the determination of serum of glutamic oxaloacetic and glutamic pyruvic transaminase. *Am J. Clin Pathol*, 28:56-63
- Shakoori AR, Alam J, et al (1990): Biochemical effect of bifenthrin (talsar) administered orally for one month on the blood and liver of rabbit. *Proc. Pak. Congr. Zool.* 10:61-81.
- Singh HS and Reddy TV, (1990): Effect of copper sulphate on hematology, blood chemistry and hepatosomatic index of Indian catfish, *Heteropneustes fossilis* (Bloch) and its recovery. *Ecotoxic. Environ. Saf.* 20:30-35
- Strmack M and Braunbeck T, (2000): Isolated hepatocytes of Rainbow trout, *Oncorhynchus mykiss* as a tool to discriminate between differently contaminated small river system. *Toxicol In vitro.*14: 361-377.
- Vutukur SS, (2005): Acute effect of hexavalent chromium on survival, oxygen consumption, hematological parameters and some biochemical profiles of the Indian major carps, *Labeo rohita*. *Int. J. Environ Res Public Health.* 2 (3) 456-462.
- Yan H and Rose NL, (2003): Distribution of Hg in the lake sediments across the UK, *Sci Total Environ* 304:391-404.
- Yan JL and Chen HC, (2003): Serum metabolic enzyme activities and hepatocyte ultrastructure of common carp after gallium exposure. *Zoological Studies*, 42:455-461.
- Yang J and Chen HC, (2003): Effects of gallium on common carp, *Cyprinus carpio*; Acute test, serum biochemistry and erythrocyte morphology. *Chemosphere*, 53:877-882.