Int. Res. J. of Science & Engineering, 2014; Vol 2 (2):37-43. ISSN: 2322-0015

RESEARCH ARTICLE

Comparative Study on the Protective Role of Vitamin C and Vitamin E on Mercury Induced Toxicity in *Heteropneusts fossilis*

Thakur Vinod [1*] and Kanhere RR[2]

[1] School of Life Sciences, Devi Ahilya Vishwavidyalaya, Indore-452001, MP, India.

[2] Department of Zoology, Govt. Girls Degree college, Barwani-451881, MP, India

**Address for Correspondence Email* : vinodthakursls1@gmail.com

Manuscript Details

Received : 06 January, 2014
Revised : 04 March, 2014
Received revised: 05 April 2014
Accepted: 10 April, 2014
Published: 30 April, 2014

ISSN: 2322-0015

Editor: Dr. Arvind Chavhan

Citation: Thakur Vinod and Kanhere RR. Comparative Study on the Protective Role of Vitamin C and Vitamin E on Mercury Induced Toxicity in *Heteropneusts fossilis, Int. Res. J. of Sci. & Engg*., 2014; 2 (2):37- 43.

ABSTRACT

The present investigation aimed to determine toxicological effects of mercury chloride on biochemical parameters of the widely consumed catfish *Heteropneustes fossilis*. Adult specimens were exposed to LC_{50} concentration (0.5ppm) of mercury chloride for 96 hrs. Empirical data of result obtained were subjected to statistical analysis of variance to test the effects of mercury, vitamin E and exposure period. The mean value of Liver, Intestine and gills, biochemical parameters, Protein SOD, CAT, and GSH were significantly increased from the control value. Vitamin C and Vitamin E supplementation play appositive role in detoxification of mercury toxicity specially the low dose. The result suggests that mercury chloride can negatively affect the physiology of fish. It was observation that supplementation effect of mercury.

Key words*:* Mercury Chloride, Vitamine-E, Vitamine-C, *Heteropneustes fossilis*.

INTRODUCTION

Aquatic systems are exposed to a number of pollutants that are mainly released from effluents discharged from industries, sewage treatment plants and drainage from urban and agricultural areas. These pollutants cause serious damage to aquatic life (Karbassi *et al*., 2006; Al–Masri, 2002). The contamination of fresh water with a wide range of pollutants has become a matter of great concern over the last few decades, not only because of the threat to public water supplies, but also with the damage caused to the aquatic life (Mahmoud *et al*., 2012). Fish are widely used to evaluate the health of aquatic ecosystems and physiological changes serve as biomarkers of environmental pollution. The river systems may be excessively contaminated with heavy metals released from domestic, industrial, mining and agricultural effluents (Vander Oost *et al*., 2003). Pollutant effects on fish behavior have received increasing attention over the past decade. Several recent reviews on such effects have appeared including. In Such reviews the treated fish behavioral categories included the behaviors associated with schooling, feeding, migration, aggression, fear, learning, phototropism and attraction to or avoidance of a chemical or temperature. Pitcher (1993) gave a good overview of normal fish behavior. The resulting behavioral effects may inhibit an organism's ability to capture prey, avoid predators, or successfully compete

with others. For example, a study of mosquito fish (*Gambusia affinis*) aqueous exposed to mercurial chloride demonstrated altered swimming activity and decreased swimming speed. Peter (1998) mentioned that there are three types of mercury,

1. Elemental mercury (Hg) is found in glass thermometer, button batteries, paints and dental amalgams.
2. Inorganic mercury, mercuric chloride the most toxic inorganic form has been used as a disinfectant. Mercurous chloride was used as teething powder and laxative. Mercurous fulminate is an explosive compound.
3. Organic mercury as methyl and ethyl mercury are well known as environmental contaminants and concentrated in the aquatic food chain.

Biochemical parameters are of fundamental importance in the physiopathological evaluation of animals (Juneja and Mahajan, 1983; Ranzani-Paiva *et al*., 1999) and often were often used when clinical diagnosis of fish physiology was applied to determine the effects of external stressors and toxic substances. Many studies indicated that biochemical changes in fish under pesticide exposure were extensively reported. Damage to blood and heamapoietic organs in fish may be associated due to either change in environmental conditions or water born pollutants. The disruption of biochemical and physiological integrity is assessable by the changes in the enzyme activities in functional organs (de la Torre *et al*., 2000, van der Oost *et al*., 2000). Selenate is found to be generally less toxic than selenite.

The role of aqueous sulphate concentrations in the differential toxicity of these selenium selenite species has not been adequately researched. Acute values for rainbow trout range from 4.2 to 32.3 mg/L, however acute values as low as 1 mg/L are reported for fathead minnows, and other values as high as 88 mg/L have been reported. Vitamin E played an essential role in elimination of mercury stress through antioxidant free radical mechanism (Ganther, 1980; Ohaida, 2005; Agarwal *et al*., 2010). The metabolic pathways of fish can be severely altered by a variety of biological, chemical and physiological factors, which could be assessed throughout several biochemical procedures. The influence of toxicant on total protein content in liver and muscle of fish has also been taken into account in evaluating response of the fish against stressors and was studied by many workers (Prabhahar *et al*., 2012). In different studies on the effect of heavy metals on fishes and water pollution were carried out for both freshwaters and marine environments (Mahmoud *et al*., 2012; Ohaida, 2005; Shakweer, 1996; Tayel and Shriadah, 1996; Mekkawy *et al*., 2008a; Mekkawy *et al*., 2008b; Mekkawy *et al*., 2011). These studies emphasized the severe effect of such heavy metals on the aquatic ecosystems. The African catfish, *Clarias gariepinus* (Burchell, 1822), was selected as the test organism in this study for its great aquaculture and commercial value in the developing country and the world. *C. gariepinus* is a benthopelagic (bottom feeder); omnivorous feeder that occasionally feeds at the surface (Teugels, 1986). *C. gariepinus*, also referred to as mudfish, is very hardy and tasty. They are able to tolerate adverse aquatic conditions, where other cultivable fish species cannot survive (Olatunde, 1983). It is widely cultivated and used as experimental fish.(Musa, S. & Omoregie) According to the aforementioned findings, the present work was suggested and aimed to study the effect of mercury and its interaction with supplementation of vitamin E on the biochemical parameters of the catfish, *Heteropneustes fossilis*.

MATERIALS AND METHODS

Classification of H. fossilis

Kingdom – Animalia
Phylum – chordate
Class – Actinopterygii
Order – Siluriformes
Family – Heteropneustidae
Genus – *Heteropneustes*
Species – *H. fossilis*

Heteropneustes fossilis is a species of air sac catfish. Commonly known as Asian stinging catfish or fossil cat it found in India, Pakistan, Nepal, Sri Lanka, Thailand and Myanmar. In Sri Lanka, this fish is called Hunga by the Sinhala speaking community, in India it's called Singhi. Especially it is found in south India in the state of kerala, it is locally called as kaari. *H. Fossilis* is found mainly in ponds, ditches, swams and marshes, but sometimes occurs in muddy rivers. It can tolerate slightly brackish water. It is omnivorous this species breeds in confined water during the mounsoon months, but can breed in ponds, derelict pond and ditches when suffient rain water accumulates. It is in great demand due to its medicinal value. Stinging catfish is able to deliver a painful sting to human. Poison that can emanate from a gland an the pectoral fin spine and has been known to be extremely painful. This species grows to a length of 30 centimetres (12 in) and is an important component of local fisheries. *H.fossilis* is also farmed and is found in the aquarium trade.

For all the experiments *H. fossilis* were obtained from local fish market. They were acclimatized in the aquarium for 7 days before experiments. The collection of experimental fishes almost same sizes (weight 30-35g) on arrival at the laboratory. The fishes (30-35g) were rendered in aquarium (n=7) containing tap water. Fish were fed on commercial feed (Taiyo pet production pvt.ltd.), nutritional content protein 32% fat 4% fibred 5% moisture 10%) feed daily. The acclimated fishes were exposed to mercuric chloride ($HgCl_2$) in glass aquarium containing 100 liter water each.

Assay of lipid per oxidation:

LPO was determined using the protocol of Ohkawa *et al*. (1979) as modified by Jamall and Smith (1985). In brief, it was determined by the reaction of 2-thiobarbuturic acid with malondialdehyde (MDA),one of the major products formed by peroxidation of lipids. Amount of MDA was measured by taking the absorb ate at 532nm (extinction coefficient=1.56×10^2), using a Shimadzu UV-1700 spectrophotometer. The method is based on the principle that the peroxidation products of membrane lipid majority being malondialdehyde (MDA),when heated with thiobarbituric acid (TBA) in acidic medium, form pink coloured complexes.

Estimation of superoxide dismutase activity:

The endogenous SOD activity was determined using the pyrogallol (1, 2, and 3- benzenetriol) autoxidation inhibition assay following the protocol of Marklund and Marklund (1974). The rate of autoxidation was determined by recording the increase in the absorption at 420 nm. Diethylenetriaminepeces of Fe^{++}, Ca^{++} and Mn^{++}.

Estimation of catalase activity:

CAT activity was estimated by considering the method of Aebi (1983), based on the estimation of amount of hydrogen peroxide (H_2O_2) decomposed by catalase to H_2O and O_2. The reaction proceeds in two steps, first the enzyme combines with one molecule of H_2O_2 to from an enzyme-substracte complex, which then reacts with another molecule of H_2O_2 to form oxygen and water. This decomposition of H_2O_2 can be following directaly by recording the decrease in its absorbance at 240 nm. The difference in extinction (Δe 240) per unit time is the measure of the catalase activity in the tissue.

Estimation of reduced glutathione:

Glutathione primarily protects the thiol groups of the molecules and the membranes content was measured by taking the absorbance of the product formed by the reaction of Ellman's reagent with GSH at 412 nm. (Extinction coefficient, E= 1.36×10^4) following the method of Ellman (1959) with little modification Beutler *et al* (1963) using 5, 5' –dithio-bis(-2-nitribenzoic acid) DTNB reagent (Ellman' reagent). The sulfhydryl group of GSH reacts with DTNB to produce a yellow- colored 5- thio-2- nitrobenzoic acid (TNB). The amount of TNB produced is directly proportional to the concentration of GSH in the sample. Measurement of the absorbance of TNB at 412 nm provides an accurate estimation of GSH in the sample.

Determination of tissue protein:

Tissue protein estimation was done by the routine method of Lowry *et al*. (1951) using bovine serum albumin as standard. The aromatic amino acid (tyrosine, tryptophan and phenylalanine) residues present in a protein react with the Folin-Ciocateau reagent to give a colored complex. The color formed is due to the reaction of the alkaline copper with the protein and the reduction of the phosphomolybdate by tyrosine, and tryptophan present in the protein. The intensity of the color depends on the amount of these aromatic amino acids present in the protein.

RESULTS AND DISCUSSIONS

Experiment 1: In this experiment twenty one healthy fish were divided into three groups of seven each. While animals in group one served as control and those in group two and three were exposed to LC_{50} of mercury chloride (0.5 ppm). However, fish in group three were also exposed simultaneously to Vitamin C (0.20 mg/l) and vitamin E (0.15 mg/l diluted in 1 ml of ethanol) in water for 96 hours. After 4 days the experiment was terminated by exposing the fishes to anaesthesia. After sacrificing liver was removed and washed thoroughly with phosphate buffered saline (PBS, 0.1 M, pH 7.4) and 10% tissue homogenate was prepared for the estimation of LPO, SOD, CAT activities and GSH and Protein content to observe the impact of vitamin E on mercury induced toxicity.

The LC_{50} of $HgCl_2$ increased hepatic LPO ($P<0.01$) and reduced the activities of SOD and CAT ($P<0.01$ and $P<0.001$ respectively) and GSH and protein content ($P<0.05$ and $P<0.01$, respectively). While simultaneous exposure of Vitamin C and Vitamin E to *Heteropneustes*

fossilis reduced hepatic LPO (P<0.001) with a concomitant increase in SOD and CAT (P<0.01 for both) and hepatic GSH and protein content (P<0.001 for both) significantly, as compared to respective control values .

Experiment 2: Twenty one healthy fish were divided into three groups of seven each. While animals in group one served as control and those in group two and three were exposed to LC_{50} of mercury chloride (0.5 ppm). However, fish in group three were also exposed simultaneously to Vitamin C (0.20 mg/l) and vitamin E (0.15 mg/l diluted in 1 ml of ethanol) in water for 96 hours. After 4 days the experiment was terminated by exposing the fishes to anaesthesia. After sacrificing intestine was removed and washed thoroughly with phosphate buffered saline (PBS, 0.1 M, pH 7.4) and 10% tissue homogenate was prepared for the estimation of LPO, SOD, CAT activities and GSH and Protein content to observe the impact of vitamin E on mercury induced toxicity.

Exposure with $HgCl_2$ to fish increased intestinal LPO (P<0.01) and reduced the activities of SOD and CAT (P<0.001 for both) and GSH and protein content (P<0.05 and P<0.01, respectively). However, simultaneous exposure of Vitamin C and vitamin E to *H. fossilis* reduced intestinal LPO (P<0.001). Simultaneous exposure with vitamin C and vitamin E increased SOD and CAT (P<0.001 and P<0.01 respectively) and GSH and protein content (P<0.01 and P<0.001 respectively) significantly, as compared to respective control values**.**

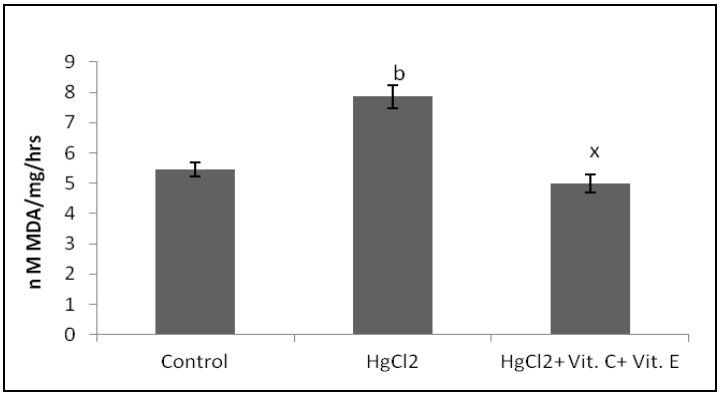
Experiment 3: Twenty one healthy fish were divided into three groups of seven each. While animals in group one served as control and those in group two and three were exposed to LC_{50} of mercury chloride (0.5 ppm). However, fish in group three were also exposed simultaneously to Vitamin C (0.20 mg/l) and vitamin E (0.15 mg/l diluted in 1 ml of ethanol) in water for 96 hours. After 4 days the experiment was terminated by exposing the fishes to anaesthesia. After sacrificing gills were removed and washed thoroughly with phosphate buffered saline (PBS, 0.1 M, pH 7.4) and 10% tissue homogenate was prepared for the estimation of LPO, SOD, CAT activities and GSH and Protein content to observe the impact of vitamin E on mercury induced

Table 1: Effect of simultaneous exposure with Vitamin C (0.20 mg/l) and Vitamin E (0.15mg/l) for 96 hrs on mercury chloride induced (0.5pmm) toxicity on hepatic LPO (nM MDA/mg/hour), tissue protein (mg/ml) and on the activities of SOD (U/mg protein), CAT (µM H_2O_2 decomposed/min/mg protein) and GSH (µM/mg protein) in *Heteropneustes fossilis*.

Parameter	Control	$HgCl_2$	$HgCl_2$+ Vit. C+ Vit. E
Protein	3.63±0.22	2.78±0.16[b]	4.57±0.19[x]
SOD	5.62±0.28	3.74±0.13[b]	4.21±0.14[y]
CAT	30.96±1.07	15.75±0.78[a]	44.30±1.28[y]
GSH	28.92±1.03	23.17±0.85[c]	35.38±1.67[x]

Data are mean ± SEM. (n = 7); [a], $P < 0.001$; [b], $P < 0.01$ and [c], $P < 0.05$ as compared to the respective values of control group; [x], $P < 0.001$ and [y], $P < 0.01$ as compared to the respective values of $HgCl_2$ treated group.

Figure 1: Effect of simultaneous exposure with Vitamin C (0.20 mg/l) and Vitamin E (0.15mg/l) for 96 hrs on mercury chloride induced (0.5pmm) toxicity on hepatic LPO (nM MDA/mg/hour) in *Heteropneustes fossilis.*



Each vertical bar represents the mean ± SEM. (n = 7); [a], $P < 0.001$; [b], $P < 0.01$ and [c], $P < 0.05$ as compared to the respective values of control group; [x], $P < 0.001$ and [y], $P < 0.01$ as compared to the respective values of $HgCl_2$ treated group.

Table 2: Effect of simultaneous exposure with Vitamin C (0.20 mg/l) and Vitamin E (0.15mg/l) for 96 hrs on mercury chloride induced (0.5pmm) toxicity on intestinal LPO (nM MDA/mg/hour), tissue protein (mg/ml) and on the activities of SOD (U/mg protein), CAT (µM H_2O_2 decomposed/min/mg protein) and GSH (µM/mg protein) in *Heteropneustes fossilis*.

Parameter	Control	$HgCl_2$	$HgCl_2$+ Vit. C+ Vit
Protein	4.63±0.07	2.50±0.09[b]	4.95±0.91[x]
SOD	5.62±0.21	3.05±0.14[a]	6.39±0.25[x]
CAT	30.96±1.17	16.84±0.87[a]	24.67±1.76[y]
GSH	28.92±1.53	23.37±0.59[c]	28.08±1.29[y]

Data are mean ± SEM. (n = 7); [a], $P < 0.001$; [b], $P < 0.01$ and [c], $P < 0.05$ as compared to the respective values of control group; [x], $P < 0.001$ and [y], $P < 0.01$ as compared to the respective values of

toxicity.

It was observed that exposure with $HgCl_2$ to fish increased gills LPO ($P<0.01$) and reduced the activities of SOD and CAT ($P<0.001$ for both) and GSH and protein content ($P<0.05$ and $P<0.01$, respectively). However, simultaneous exposure of Vitamin C and vitamin E to *H. fossilis* reduced LPO ($P<0.001$) in gills. Interestingly, simultaneous exposure with vitamin C and vitamin E increased SOD and CAT ($P<0.01$ and $P<0.001$ respectively) activities and GSH and protein content ($P<0.01$ and $P<0.001$ respectively) significantly in gills, as compared to respective control values.

DISCUSSION

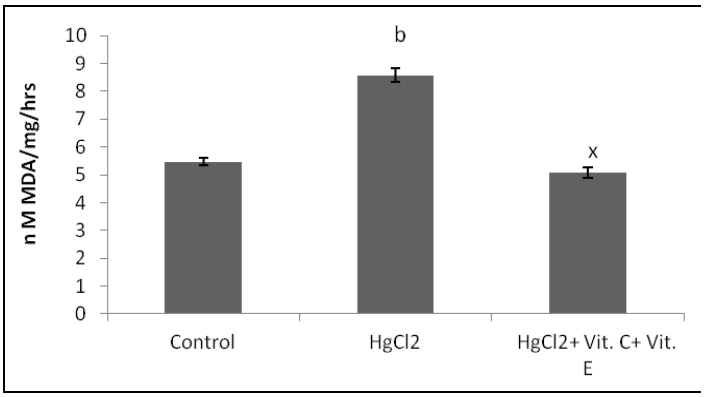
To investigate the possible ameliorative effects of Vitamin C and Vitamin E on the oxidative damage induced by mercury chloride toxicant was studied on *H. fossilis* with reference to some biological markers (tissue protein and LPO) and antioxidant enzymes (SOD, CAT and GSH). The effect of Vitamin C, Vitamin E and combination of both Vitamin C and Vitamin E were studied on *H. fossilis* organs such as liver, intestine and Gill.

Recently Muthu and Krishnamoorthy (2012) reported the ameliorative role of vitamin E and vitamin E on mercuric chloride induced toxicity in rats.

Another experiment was conducted to investigate the impact of mercury chloride for 96 hours, LC_{50} of mercury chloride with 0.20 mg/l of vitamin C supplementation. Promising results were obtained for vitamin C supplementation fish groups. In the liver homogenate of *H. fossilis* analysis revealed significant improvement in all five biochemical parameters for Vitamin C supplementation groups. This significant reduction in hepatic LPO clearly supports the hypothesis that the potent antioxidant Vitamin C protect against the oxidative

$HgCl_2$ treated group.

Figure 2: Effect of simultaneous exposure with Vitamin C (0.20 mg/l) and Vitamin E (0.15 mg/l) for 96 hrs on mercury chloride induced (0.5pmm) toxicity on intestinal LPO (nM MDA/mg/hour) in *Heteropneustes fossilis.*



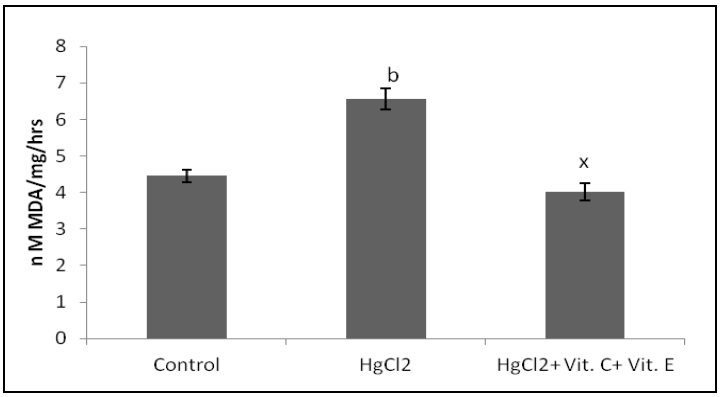
Each vertical bar represents the mean ± SEM. (n = 7); [a], $P < 0.001$; [b], $P < 0.01$ and [c], $P < 0.05$ as compared to the respective values of control group; [x], $P < 0.001$ and [y], $P < 0.01$ as compared to the respective values of $HgCl_2$ treated group.

Table 3: Effect of simultaneous exposure with Vitamin C (0.20 mg/l) and Vitamin E (0.15 mg/l) for 96 hrs on mercury chloride induced (0.5pmm) toxicity on gills LPO (nM MDA/mg/hour), tissue protein (mg/ml) and on the activities of SOD (U/mg protein), CAT (µM H_2O_2 decomposed/min/mg protein) and GSH (µM/mg protein) in *Heteropneustes fossilis*.

Parameter	Control	$HgCl_2$	$HgCl_2$+ Vit. C+Vit. E
Protein	3.63±0.08	2.36±0.06[b]	3.99±0.07[x]
SOD	5.62±0.23	3.62±0.32[a]	4.89±0.33[y]
CAT	30.96±1.70	17.91±.81[a]	35.43±1.69[x]
GSH	28.92±1.23	22.57±0.89[c]	26.62±1.45[y]

Data are mean ± SEM. (n = 7); [a], $P < 0.001$; [b], $P < 0.01$ and [c], $P < 0.05$ as compared to the respective values of control group; [x], $P < 0.001$ and [y], $P < 0.01$ as compared to the respective values of $HgCl_2$ treated group.

Figure 3: Effect of simultaneous exposure with Vitamin C (0.20 mg/l) and Vitamin E (0.15mg/l) for 96 hrs on mercury chloride induced (0.5pmm) toxicity on Gills LPO (nM MDA/mg/hour) in *Heteropneustes fossilis*



Each vertical bar represents the mean ± SEM. (n = 7); [a], $P < 0.001$; [b], $P < 0.01$ and [c], $P < 0.05$ as compared to the respective values of control group; [x], $P < 0.001$ and [y], $P < 0.01$ as compared to the respective values of $HgCl_2$ treated group.

damage induced by mercury chloride poisoning in *H. fossilis*. The level of all three antioxidant enzymes was observed high as compared to control groups.

Further, an experiment was especially designed and conducted to standardize the dose of Vitamin E. The toxic free environment was chosen to fix the dose of vitamin E. Here, three different concentrations of Vitamin E such 0.10 mg/l, 0.15 mg/l and 0.20 mg/l were used along with a control group that received no vitamin supplementation. In liver tissue homogenate analysis showed that vitamin E supplementation greater improvement in respective of all biochemical parameters considered under this study. The protein content was increased nearly two fold with a dose of 0.2 mg/l of vitamin E supplement. The low level of LPO (Table 10 and Figure 8) in the vitamin E supplementation group suggest that a need for a potent antioxidant to protect the liver cells of *H. fossilis* against environmental toxicant. Other antioxidant enzymes were also affluent in case of vitamin E supplementation group in comparison to control group. The effective dose vitamin E was fixed as 0.15 mg/ml for further experimentation.

Furthermore, an experiment was conducted by including the toxic environment (LC_{50} of mercury chloride for 96 hours). As done for vitamin C, here also three fish groups were considered in the present study that a mercury chloride toxicant group, mercury chloride with vitamin E supplementation and a control group that received no vitamin supplement. In the liver analysis result showed a slight improvement in the protein content for vitamin E supplement group. However, the improvement of protein was poor in comparison to vitamin C supplementation.

REFERENCES

1. Aebi H Catalase invitro. In: Colowick SP, Kaplan NO (eds) Method in enzymology, Academic, New York, 1983, 121-126.
2. Agarwal R, Goel SK, Chandra R and Behari JR. Role of vitamin E in preventing acute mercury toxicity in rat. *Environ. Toxicol. Pharmacol*,. 2010; 29, 70-78.
3. Al–Masri M, Aba S, Khalil AH and Al–Hares Z. Sedimentation rates and pollution history of a dried lake. Sci.Total.Environ, 2002; 293 (1 – 3):177 – 189.
4. Beutler, E., Duron, O., Kelly, B.M., 1963. Improved method for the determination of blood glutathione. J. Lab. Clin. Med. 61, 882–888.
5. de la Torre FR, Saliba A and Ferrari L. Biomarkers assessment in juvenile Cyprinus carpio exposed to waterborne cadmium. *Environ. Pollut.*, 2000; 109: 277-262.
6. El-Deek, MS and Ahmed NS. Trace metals concentrations in some fish families from the Mediterranean Sea. *Egypt. Ass. J. Agricul. Sci.*, 1996; 27, 233-240.
7. Ellman GL. Tissue sulfhydryl groups. *Arch. Biochem. Biophys*., 1959; 82: 70–77.
8. Ganther HE. Interactions of vitamin E and selenium with mercury and silver. *Ann. NY Acad. Sci.*, 1980; 355, 212-226.
9. Jamall IS, Smith JC. Effects of cadmium on glutathione peroxidase, superoxide. dismutase, and lipid peroxidation in the rat heart: a possible mechanism of cadmium cardiotoxicity. *Toxicol Appl Pharmacol*. 1985; 80:33-42.
10. Juneja C and Mahajan C. Hematological and haemopoietic changes in fishChanna punctatusdue to mercury pollution in water, *Indian J. Anim. Res*., 1983;17 (2):63–71.
11. Karbassi R, Bayati I, Moattar F. Origin and chemical partioning of heavy metals in riverbed sediments. *Int. J. Environ. Sci. Tech*, 2006; 3(1): 35 – 42.
12. Lowry OH, Rosenbrough NG, Farr AL, Randall RJ. Protein measurement with folin phenol reagent. *J Biol*., 1951; 193:265–275.
13. Marklund S and Marklund G. Superoxide dismutase: involvement of superoxide anion radical in auto oxidation of pyragallol and a convenient assay for superoxide dismutase. *Eur J Biochem*., 1974; 47: 469–474
14. Mekkawy IA, Mahmoud UM, Wassif ET and Naguib M. Effects of cadmium on some haematological and biochemical characteristics of Oreochromis niloticus(Linnaeus, 1758) dietary supplemented withtomato paste and vitamin E. *Fish Physiol Biochem.*, 2011, DOI10.1007/s10695-010-9418-3.
15. Mekkawy IAA, Mahmoud UM and Ibrahim ATA. Heavy metal distribution and their corresponding damage effect in some organs of Bagrus bajad (Forsskal, 1775) from three localities at Assiut. *Egypt. J. Egypt. Ger. Soc. Zool*., 2008a; 54: 119-137.
16. Mekkawy IAA, Mahmoud UM and Ibrahim ATA. Heavy metal distribution in some organs of Oreochromis niloticus(Linnaeus, 1758) collected from three localities at Assiut, Egypt and the corresponding tissue damage. *J. Egypt. Ger. Soc. Zoo*l., 2008b; 55: 97-127.
17. Musa S and Omoregie E. Haematological changes in mudfish, Clarias gariepinus(Burchell) exposed to malachite green. *J. Aqua. Sci*., 1999; 14, 32-37.
18. Ohaida AMI. The Effect of Lead and its Interaction With Supplementation of Selenium and Vitamin E on the Growth Performance, Biochemical and Physiological Characteristics, Histopathology and Cytopathology of Oreochromis niloticus. Ph.D. Thesis, Faculty of Science. Assiut Univ., Assiut, Egypt, 218pp (2005).

19. Ohkawa H, Ohishi N and Yagi K. A New Assay Method for Lipid Peroxides Using a Methylene Blue Derivative, *Biochem. Int.,* 1979; *10*, 205–211.
20. Olatunde AA. Strategies for increasing freshwater fish production in the Green Revolution Programme. Pages 70-75. In, Proceedings of Second Annual Conference o the Fisheries Society of Nigeria (FISON). Calabar, January (1983).
21. Peter V. Emergency toxicology. Philadelphia, Lippincott-Raven publishers (1998).
22. Pitcher T. Behaviour of Teleost fishes. London, Champan and Hall (1993)
23. Prabhahar C, Saleshrani K, Tharmaraj K and Vellaiyan M. Studies on the Effect of Cadmium Compound on the Biochemical Parameters of Fresh Water Fish in Cirrhinus mrigala, *International Journal of Pharmaceutical & Biological Archives*, 2012; 3(1):69-73.
24. Ranzani-Paiva M, Salles F, Eiras J, Ishikawa C and Alexandrino A, Análise hematológica de curimbatá (Prochilodus scrofa), pacu (Piractus mesopotamicus) e tambaqui (Colossoma macropomum) das estações de piscicultura do instituto de pesca, Boletim do Instituto de Pesca,1999; 25: 77–83
25. Shakweer LM and Abbas MM. Effect of sex on the concentration levels of some trace metals in Oreochromis niloticus of Lake Edku and Sardinella aurita of the Mediterranean waters. *Egypt. Bull. Nat. Inst. of Oceanogr. and Fish*., A.R.E., 1996; 22, 121-141.
26. Tayel FTR and Shriadah MMA. Fe, Cu, Mn, Pb, and Cd in some fish species from western Harbor of Alexandria. *Egypt. Bull. Nat. Inst. of Oceanogr. and Fish.* A.R.E., 1996; 22, 85-96.
27. Teugels G. A systemic revision of the African species of the genus Clarias(Pisces, Clariidae). *Ann. Musee Royal'Afri. Cent*., 1986; 247: 1-199.
28. van der Oost R, Satumalay AI and Vermeulen NPE. Validation of inland water pollution assessment using biomarker responses in caged carp (Cyprinus carpio). *Mar. Environ. Res*., 2000; 50: 431-432.
29. Vander Oost R, Beyer J, Verneykebm NPE. Fish bioaccumulation and biomarkers in environmental risk assessment. A review. *Environ .Toxicol. Pharmacol.*, 2003; 13: 57 –149.