Pakistan J. Zool., vol. 47(6), pp. 1777-1782, 2015.

Effect of Sodium Cyanide on Antioxidant Enzyme Activities and Lipid Peroxidation in Some Tissues of Mirror Carp (*Cyprinus carpio)*

Mustafa Kavasoğlu,[1]* Yusuf Sarıoğlu,[2] Kazım Uysal,[1] Muhammet Dönmez,[2] Sayit Altıkat,[3] İrfan Yetek[1] and Halil İsa Kuru[4]

[1]*Department of Biology, Faculty of Arts and Sciences, Dumlupınar University, Kütahya, Turkey*
[2]*Faculty of Engineering, Dumlupınar University, Kütahya, Turkey*
[3]*Department of Biochemistry, Faculty of Medicine, Dumlupınar University, Kütahya, Turkey*
[4]*Simav Vocational School of Health Services, Dumlupınar University, Kütahya, Turkey*

Abstract.- In this study, the changes of superoxide dismutase (SOD) and catalase (CAT) activities and malondialdehyde (MDA) levels in brain, muscle, liver and gill tissues of *Cyprinus carpio* was determined by addition of 0.5 mg/l cyanide into the environment of fish. The fish were exposed to cyanide for 24 and 72 h. As a result of the 24-h exposure, a significant decrease observed in MDA level in muscle and in SOD and CAT activities in brain compared to the control groups. As a result of 72-hour exposure, SOD enzyme activity and MDA levels in brain tissue were increased significantly. The CAT activity in liver was remarkably higher than other tissues of *C. carpio*. After the exposure, some tissues increased their enzyme activities as a reaction to sodium cyanide while some other tissues were adversely affected. Consequently, we can say that cyanide presence changes the activities of SOD, CAT antioxidant enzymes and MDA levels working as indicators of oxidative stress.

Key words: Cyanide, *Cyprinus carpio*, catalase, superoxide dismutase, malondialdehyde.

INTRODUCTION

Cyanide is a chemical compound possessing highly toxic potential. It is used in the production of plastic, rubber and several chemicals, also in jewelry, metal plating, leather processing, fertilizer production, pesticide and insecticide production, and in gold and silver mining premises (Way, 1984; Sousa *et al*., 2002; Kocak *et al*., 2010). About 2-3 million tons of cyanide is produced annually in industry and some areas of the cyanide leaks into water and its concentration in natural water might be in such a wide range as 0.01-10.0 mg/L (Huub *et al*., 2000). Leakage of cyanide into waste water affects first the aquatic species and subsequently affects human health considerably through food chain. Fish and other aquatic organisms are especially susceptible to cyanide exposure. Generally, the potential impact of all pollutants is greater for aquatic organisms (Okolie and Osagie, 1999; Yousafzai and Shakoori, 2011).

It is well known that cyanide and its derivatives are metabolic inhibitors (Solomonson and Spehar, 1981). After entering the body, cyanide seeps into the circulatory system and forms a complex with methemoglobin, resulting in cyanomethemoglobin. Cyanide forms a complex with cytochrome c oxidase to inhibit this enzyme (Pettersen and Cohen, 1993; David *et al*., 2008). Therefore, the cells can not use oxygen in blood, and then causing cytotoxic hypoxia and asphyxiation (Hosetti *et al*., 2011). Beside of these effects, it is thought that cyanide exposure causes rising of oxidative stress by glutathione (GSH) depletion and inhibition of a series of antioxidant enzymes (Ardelt *et al*., 1989; Hariharakrishnan *et al*., 2009). Cyanide is also a neurotoxin that causes mitochondrial dysfunction and stimulates production of intracellular reactive oxygen species (ROS) (Douglas *et al*., 2003). It is reported that cyanide exposure increases levels of ROS and reactive nitrogen species (RNS) and the amount of lipid peroxidation (Hariharakrishnan *et al*., 2009). Therefore, free radicals stimulated by xenobiotics

* Corresponding author: kavasoglu87@hotmail.com
0030-9923/2015/0006-1777 $ 8.00/0

Authors' Contributions: YS conceived and executed the project. SA, IY and HIK performed biochemical assays. MK, KU and MD supervised the work.

are deactivated by antioxidant defense system (Gumustekin *et al*., 2005). The most important enzymes of the antioxidant defense system are catalase (CAT) and superoxide dismutase (SOD) (Altikat *et al*., 2006; Ezemonye and Enuneku, 2011; Guney *et al*., 2009). Therefore, antioxidant enzymes both preserve cellular stability and play an important role in keeping free radicals away (Halliwel and Gutteridge, 1989).

There are only a few studies concerning the determination of the levels of cyanide in natural water sources in Turkey. Özdemir and Sırıken (2006) measured cyanide levels as 0.02 ppm being the highest in Afyonkarahisar (Turkey) well water sources. Also, oxidative stress on aquatic organisms caused by cyanide toxicity is not a much studied area. For this reason, we aimed to research on the antioxidant enzymes and lipid peroxidation levels in brain, muscle, liver and gill tissues of mirror carp against cyanide toxicity.

MATERIALS AND METHODS

In this study, 36 mirror carps of average weights of 50 g were used. The fish were acclimatized to aquarium (100x40x30 cm) conditions for 15 days. Fish were fed daily with commercial food by 2% of their total weights. The experiments were conducted in 12 aquariums: 6 aquariums as control and 6 aquariums containing 0.5 mg/L cyanide water. Three fishes were placed to each aquarium. Sodium cyanide (NaCN) was used as a cyanide source. The experiments were carried out at 2 different times (24 h and 72 h). Eighteen fishes in 6 aquaria (3 control and 3 experimental) were dissected after 24 h, while another set of 18 fishes in 6 aquaria (3 control and 3 experimental) were dissected after 72 h. At the end of the experiment, the fishes were anaesthetized with ethanol and dissected to obtain their brain, muscle, liver and gill tissues. They were stored at -80°C until analysis.

Prior to biochemical analysis, the tissues were homogenized in 50 µM, refrigerated sodium-phosphate buffer pH7.4 containing 0.25 M sucrose at 8000 rpm for 5 min using homogenizer. Homogenates were centrifuged at +4°C at 10,000 rpm for 30 min. The resultant supernatant was used to determine SOD and CAT enzyme activities and MDA levels.

Malondialdehyde (MDA) assay

MDA was determined by the double heating method of Draper and Hadley (1990). The principle of the method was spectrophotometric measurement of the pink color produced during the reaction of thiobarbituric acid (TBA) with MDA at 532 nm. The concentration of MDA was calculated from the standard chart of MDA–TBA complex by using 1,1,3,3-71 tetraethoxypropane on behalf of MDA. Results were compared to the amount of protein sample and were expressed as nmol/mg protein.

Determination of CAT activity

CAT activity was measured according to the method of Aebi (1984). The principle of the assay is based on the determination of the rate constant of hydrogen peroxide decomposition by CAT enzyme. One katal (1 kat) of CAT equals the enzyme activity that recognized 1 mol of hydrogen peroxide in a second at 37°C. CAT activity was measured by observing the change on absorbance of sample and blank for a minute by spectrophotometricaly at 240 nm. Results were compared with the amount of protein sample and were expressed as U/mg protein.

Determination of SOD activity

The determination method of SOD Activity depends on the spectrophotometric measurement of SOD's inhibition effect on autoxidation of 6-hydroxyidopamine (6-OHDA) (Crosti *et al*., 1987). Since the curve of autoxidation speed is stabile in the first minute, this reaction was measured spectrophotometricaly at 490 nm until the 60th seconds of oxidation. Results were compared to the amount of protein sample and were expressed as U/mg protein.

Protein determination

Total tissues protein concentration was determined by the Lowry protein assay (Lowry *et al*., 1951), using bovine serum albumin as a Standard.

Table I.- Effect of cyanide on catalase (CAT) and superoxide dismutase (SOD) enzyme activities and malondialdehyde (MDA) levels in some tissues of mirror carp. (The data with * is different compared to the control group, p<0.05).

	CAT (nkat/mg protein) Means±S.E.M.	SOD (nkat/mg protein) Means±S.E.M.	MDA (nmol/mg protein) Means±S.E.M.
Liver			
Control	1414.02±102.36	255.95±23.47	0.71±0.04
Cyanide 24h	1339.42±98.41	255.98±20.18	0.70±0.05
72h	1320.57±71.64	216.92±26.56*	0.85±0.04
Brain			
Control	42.00±3.72	121.07±10.12	1.2±0.09
Cyanide 24h	37.95±3.16*	98.15±11.28*	1.14±0.11
72h	37.50±4.08	153.35±11.87*	1.63±0.10*
Muscle			
Control	35.17±2.93	51.40±4.45	1.91±0.14
Cyanide 24h	42.35±3.68*	57.20±4.69*	1.26±0.09*
72h	42.28±3.27*	55.85±4.02	0.93±0.08*
Gill			
Control	27.35±2.09	33.68±3.93	1.19±0.07
Cyanide 24h	33.90±2.64*	40.57±3.42*	1.24±0.11
72h	32.47±3.13*	45.57±4.04*	1.29±0.09

Statistical analysis

All data were expressed as the mean values ± SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA). The statistical analyses were performed using SPSS statistical version 15.0 software package.

RESULTS

MDA levels in the brain tissues of mirror carp exposed to cyanide for 72 h were increased significantly compared to the control group ($p<0.05$). However, no significant change occurred in the MDA levels in the brain tissues of the fish exposed to 0.5 mg/L concentration of cyanide for 24 h compared to the control group ($p>0.05$). Although MDA levels in the liver tissues of the fish exposed to cyanide for 24 and 72 h were increased compared to the control group, it was not statistically significant ($p>0.05$). MDA levels in the muscle tissues of the fish exposed to cyanide for both 24 and 72 h were decreased significantly compared to the control group ($p<0.05$). However, no significant change occurred in the MDA levels in the gill tissues of the fish exposed to cyanide for 24 and 72 h compared to the control group ($p>0.05$) (Table I).

CAT enzyme activity in the brain tissues of the fish exposed to 0.5 mg/L concentration of cyanide for 24 h was decreased significantly compared to the control group ($p<0.05$). While CAT enzyme activity in the brain tissues of the fish exposed to cyanide for 72 h was decreased considerably, it was not statistically significant ($p>0.05$). Even though, CAT enzyme activity in the liver tissues of the fish exposed to cyanide for both 24 and 72 h was low compared to the control group, it was not statistically significant ($p>0.05$). Contrary to the brain and muscle tissues, an increase was observed in CAT enzyme activity in the muscle and gill tissues ($p<0.05$) (Table I).

When the SOD enzyme activity in mirror carp exposed to 0.5 mg/L concentration of cyanide for 24 h was compared to the control group, a significant decrease ($p<0.05$) was observed in the brain tissues, whereas a significant increase ($p<0.05$) occurred in the fish exposed for 72 h. Although an increase was observed in the SOD enzyme activity in the liver of the fish exposed to cyanide for 24 h compared to the control group and

this increase was not statistically significant ($p>0.05$). A decrease ($p<0.05$) was determined in the SOD enzyme activity in the liver of the fish exposed to cyanide for 72 h. SOD activity was increased in the muscle tissues in both groups; however, a significant increase was determined only in the group exposed for 24 h ($p<0.05$). In the gill tissues, a significant increase ($p<0.05$) was determined in the SOD activity in the fish exposed to cyanide for both 24 and 72 h compared to the control group (Table I).

DISCUSSION

Cyanide is an intracellular neurotoxin stimulating ROS production (Douglas *et al*., 2003). An increase was determined in hydrogen peroxide and superoxide anion levels after cyanide treatment (Ardelt *et al*., 1994; Daya *et al*., 2000). Cyanide shows functional toxic effects in different tissues as a result of hydrogen peroxide accumulation (David *et al*., 2008). Intracellular calcium, which increases after cyanide treatment, causes lipid peroxidation and generates ROS causing neuronal damages (Johnson *et al*., 1987). An increase was reported in lipid peroxidation in the brain where cyanide causes neurotoxicity (Ardelt *et al*., 1994; Daya *et al*., 2000). In this study, compared to the other tissues, an increase was found in the MDA levels, the final product of lipid peroxidation, in the brain tissues of the fish exposed to cyanide. In a rat study, no significant change was observed in the liver lipid peroxidation levels as a result of chronic cyanide induction (Mathangi *et al*., 2011). In this study, no significant change was seen in the liver tissue, but a decrease was observed in the muscle and gill tissues. In another study, different results were obtained among fish tissues in terms of MDA levels (Huang *et al*., 2007). It was accepted that protection against lipid peroxidation differs from tissue to tissue (Maranesi *et al*., 2004). It is well-known that cyanide leads to neurotoxicity in neuronal membranes due to oxidative damage because of lipid peroxidation (Daya *et al*., 2000; Deepa *et al*., 2003). Various experimental studies revealed that acute and chronic cyanide poisoning leads to oxidative stress (Mills *et al*., 1996; Douglas *et al*., 2003; Abdel-Zaher *et al*., 2011).

A relationship was found between temporary changes in most antioxidant enzyme activities and membrane lipid peroxidation occurring in time-dependent manner (Mathangi *et al*., 2011). Because of inhibition of various antioxidant enzymes by cyanide, it is thought that oxidative stress plays an important role in cyanide neurotoxicity (Ardelt *et al*., 1989). It was found that cyanide given to rats in toxic dosage inhibits antioxidant enzymes like GP-x and depletes non-enzymatic antioxidants like intracellular GSH (Abdel-Zaher *et al*., 2011). It was also reported that cyanide causes an increase in lipid peroxidation and a decrease in GSH levels (blood, brain and liver) and GP-x (liver and brain), SOD (brain and liver) and CAT (blood and brain) enzyme activities (Mathangi *et al*., 2011). Okolie and Iroanya (2003) reported inhibition of SOD and CAT in liver, kidney and lung tissues of the cyanide-treated rabbits. Such enzymes containing metal porphyrins as peroxidase, catalase and xanthine oxidase are inhibited strongly by cyanide (Yamamoto, 1989). Fish also have been tending to be affected by cyanide toxicity. It was reported that cyanide caused the protein deformation in *Tilapia zillii*'s liver tissues (Cheng *et al*., 2001). Hosetti *et al*. (2011) reported that copper cyanide complex decreased the total protein, glycogen and pyruvate amounts; increased amino acids, urea, protease and lactase levels as statistically significant in a freshwater fish *Catla catla*. CAT activity of *Cyprinus carpio* exposed to lethal (1 mg/L) and sub lethal (0.066 mg/L) concentrations of sodium cyanide showed inhibition (David *et al*., 2008). CAT activity can be a good diagnostic tool of biological tracing program for sodium cyanide poisoning (David *et al*., 2008). Brain CAT, GP-x and GR activities after 30 min. after cyanide exposure and SOD activities after 60 min. decreased significantly (Ardelt *et al*., 1989). Aquatic organisms are vulnerable to cyanide toxicity and synergic effects of cyanide's toxic effects may also inhibit CAT system (David *et al*., 2008). It was found that as a result of oxidative damage, cyanide inhibits brain antioxidant defense mechanism (Gunesekar *et al*., 1996). It was observed that SOD and CAT enzyme activities in the brain tissue are inhibited in this study. Significant inhibition was observed in the SOD enzyme activity of the liver

tissue after only 72 h of cyanide exposure but no significant change was seen in the CAT and, after 24 h of exposure, in the SOD activities. Contrary to the literature, however, SOD and CAT enzyme activities in the muscle and gill tissues were stimulated. Since the liver of fish has metabolic tissues, CAT activities in liver of *C. carpio* were extremely higher than the other tissues. It was stated that SOD and CAT activities increased in the fish living in contaminated water and this condition could be used as a marker of environmental pollution (Rodriguez-Ariza *et al*., 1993). It was determined that CAT enzyme has an important role in detoxification of hydroxyl groups in the muscle tissue (Jiang *et al*., 2010). The changes in the enzyme activity levels might have an effect on up-regulation of these enzymes as a result of damaged oxidative metabolism and cellular processes (Al-Ghanim and Mahboob, 2012).

According to the literature, it was shown that brain is the target organ in cyanide toxicity (Borowitz *et al*., 1992; Gunesekar *et al*., 1996). The data acquired from this study also revealed that the brain is one of the most affected organs by the cyanide toxicity. Increase in the enzyme activities of muscle and gill tissues is thought to decrease MDA levels. It is also thought that the response of antioxidant system to cyanide toxicity protects the cell against the oxidative stress caused by cyanide. Because the liver is a rich organ in terms of enzyme activity, it might have been affected by cyanide toxicity less than the other tissues. Consequently, it was observed that the antioxidant response of mirror carp exposed to cyanide was different among fish tissues.

REFERENCES

ABDEL-ZAHER, A.O., ABDEL-HADY, R.H., ABDEL MONEIM, W.M. AND SALIM, S.Y., 2011. Alpha-lipoic acid protects against potassium cyanide-induced seizures and mortality. *Exp. Toxicol. Pathol.*, **63**: 161–165.

AEBI, H., 1984. Catalase *in vitro*. *Methods Enzymol.*, **105**: 121-126.

AL-GHANIM, K.A. AND MAHBOOB, S., 2012. Effect of sodium cyanide on the activities of some oxidative enzymes and metabolites in *Clarias gariepinus*. *Afr. J. Biotechnol.*, **11**: 9849-9854.

ALTIKAT, S., COBAN, A., CIFTCI, M. AND OZDEMIR, H., 2006. *In vitro* effects of some drugs on catalase purified from human skin. *J. Enzym. Inhibit. Med. Chem.*, **21**: 231-234.

ARDELT, B.K., BOROWITZ, J.L. AND ISOM, G.E., 1989. Brain lipid peroxidation and antioxidant protectant mechanisms following acute cyanide intoxication. *Toxicology*, **56**: 147-154.

ARDELT, B.K., BOROWITZ, J.L., MADUH, E.U., SWAIN, S.L. AND ISOM, G.E., 1994. Cyanide-induced lipid peroxidation in different organs: subcellular distribution and hydroperoxide generation in neuronal cells. *Toxicology*, **89**: 127-137. doi:10.1016/0300-483X(94)90221-6

BOROWITZ, J.L., KANTHASAMY, A.G. AND ISOM, G.E., 1992. Toxicodynamics of cyanide. In: *Chemical warfare agents* (ed. S.M. Somani), Academic Press, San Diego, pp. 209-236.

CHENG, Y., LIU, T. AND LIN, S., 2001. Cyanide-Induces alterations to the biophysical conformations of the isolated fish liver. *Ecotoxicology*, **10**: 71 - 77.

CROSTI, N., SERVIDEI, T., BAJER, J. AND SERRA, A., 1987. Modification of 6-hydroxydopamine technique for the correct determination of superoxide dismutase. *J. Clin. Chem. Clin. Biochem.*, **25**: 265-266.

DAVID, M.M., MUNASWAMY, V.V., HALAPPA, R.R. AND MARIGOUDAR, S.R., 2008. Impact of sodium cyanide on catalase activity in the freshwater exotic carp, *Cyprinus carpio* (Linnaeus). *Pestic. Biochem. Physiol.*, **92**: 15-18. doi:10.1016/j.pestbp.2008.03.013

DAYA, S.S., WALKER, R.B. AND ANOOPKUMAR-DUKIE, S.S., 2000. Cyanide-induced free radical production and lipid peroxidation in rat brain homogenate is reduced by aspirin. *Metab. Brain Dis.*, **15**: 203-210. doi:10.1023/A:1011163725740

DEEPA, S.M., RODERICK, B.W., BEVERLEY, D.G. AND SANTY, D., 2003. 6-Hydroxymelatonin protects against cyanide induced oxidative stress in rat brain homogenates. *J. Chem. Neuroanat.*, **26**: 103-107. doi:10.1016/S0891-0618(03)00034-6

DOUGLAS, C.J., KRISHNAN, P., LI, L., PALUR, G.G., YAN, S., JOSEPH, L.B. AND GARY, E.I., 2003. cyanide enhancement of dopamine-induced apoptosis in mesencephalic cells involves mitochondrial dysfunction and oxidative stress. *Neurotoxicology*, **24**: 333-342. doi:10.1016/S0161-813X(03)00042-1

DRAPER, H.H. AND HADLEY, M., 1990. Malondialdehyde determination as index of lipid peroxidation. *Methods Enzymol.*, **186**: 421-431.

EZEMONYE, L.I.N. AND ENUNEKU, A.A., 2011. Biochemical alterations in Hoplobatrachus occipitalis exposed to sub lethal concentrations of cadmium. *Turk. J. Fish. aquat. Sci.*, **11**: 485–489.

GUMUSTEKIN, K., CIFTCI, M., COBAN, A., ALTIKAT, S., AKTAS, O., GUL, M., TIMUR, H. AND DANE, S.,

2005. Effects of nicotine and vitamin E on glucose 6-phosphate dehydrogenase activity in some rat tissues in vivo and in vitro. *J. Enzyme Inhib. med. Chem.*, **20**: 497-502. doi:10.1080/14756360500277384

GUNESEKAR, P.G., SUN, P.W., KANTHASAMY, A.G., BOROWITZ, J.L. AND ISOM, G.E., 1996. Cyanide induced nephrotoxicity involves nitric oxide and reactive oxygen species generation after n -methyl-D aspartate receptor activation. *J. Pharmacol. exp. Therapeut.*, **277**: 150-155.

GUNEY, T., YILDIZ, B., ALTIKAT, S., KURAL, N. AND ALATAS, O., 2009. Decreased antioxidant capacity and increased oxidative stress in patients with juvenile idiopathic arthritis. *J. Pediatr. Sci.*, **1**: 1-6.

HALLIWELL, B. AND GUTTERIDGE, J.M.C., 1989. *Free radicals in biology and medicine*, Ed 2. Clarendon Press, Oxford.

HARIHARAKRISHNAN, J., SATPUTE, R.M., PRASAD, G.B.K.S. AND BHATTACHARYA, R., 2009. Oxidative stress mediated cytotoxicity of cyanide in LLC-MK2 cells and its attenuation by alpha-ketoglutarate and N-acetyl cysteine. *Toxicol. Lett.*, **185**: 132-141. doi:10.1016/j.toxlet.2008.12.011

HOSETTI, B.B., PRAVEEN, D. AND SHWETHA, A., 2011. Metabolic changes in the freshwater fish *Catla catla*, under copper cyanide intoxication. *Int. J. Pharmaceut. biol. Arch.*, **2**: 874-879.

HUANG, D.J., ZHANG, Y.M., SONG, G., LONG, J., LIU, J.H. AND JI, W.H., 2007. Contaminants-induced oxidative damage on the carp *Cyprinus carpio* collected from the upper Yellow River, China. *Environ. Monitor. Assess.*, **128**:483–488.

HUUB, J.G., ELISABETH, B. AND HENRY, F., 2000. Cyanide toxicity and cyanide degradation in anaerobic wastewater treatment. *Water Res.*, **34**: 2447-2454. doi:10.1016/S0043-1354(99)00418-2

JIANG, W.D., FENG, L., LIU, Y., JIANG, J., HU, K., LI, S.H. AND ZHOU, X.Q., 2010. Lipid peroxidation, protein oxidant and antioxidant status of muscle , intestine and hepatopancreas for juvenil Jian carp (*Cyprinus carpio* var. Jian) fed graded levels of myo-inositol. *Fd. Chem.*, **120**: 692-697.

JOHNSON, J.D., CONROY, W.G., BURRIS, K.D. AND ISOM, G.E., 1987. Peroxidation of brain lipids following cyanide intoxication in mice. *Toxicology*, **46**: 21-28.

KOCAK, S., DUNDAR, Z.D., DEMIRCI, S., CANDER, B. AND DOGAN, H., 2010. Cyanide intoxication: a case report. *J. Acad. Emerg. Med.*, **1**: 11-14.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. AND RANDALL, R.J., 1951. Protein measurement with the Folin phenol reagent. *J. biol. Chem.*, **193**: 265–275.

MARANESI, M., ZAMBONIN, L., TOLOMELLI, B., CABRINI, L. AND BOCHICCHIO, D. 2004. Effects of different dietary amounts of LCPUFA n3 and vitamin B 6 on lipid composition and antioxidant defences in rat kidney. *J. Nutri. Biochem.*, **15**: 396-401. doi:10.1016/j.jnutbio.2003.09.006

MATHANGI, D., SHYAMALA, R., VIJAYASHREE, R., RAO, K., RUCKMANI, A., VIJAYARAGHAVAN, R. AND BHATTACHARYA, R., 2011. Effect of alpha-ketoglutarate on neurobehavioral, neurochemical and oxidative changes caused by sub-chronic cyanide poisoning in rats. *Neurochem. Res.*, **36**: 540-548. doi:10.1007/s11064-010-0376-z

MILLS, E., GUNASEKAR, P., PAVLAKOVIC, G. AND ISOM, G., 1996. Cyanide-induced apoptosis and oxidative stress in differentiated PC12 cells. *J. Neurochem.*, **67**: 1039-1046.

OKOLIE, N.P. AND IROANYA, C.U., 2003. Some histologic and biochemical evidence for mitigation of cyanide-induced tissue lesions by antioxidant vitamin administration in rabbits. *Fd. Chem. Toxicol.*, **41**: 463-469. doi:10.1016/S0278-6915(02)00224-7

OKOLIE, N.P. AND OSAGIE, A.U., 1999. Liver and kidney lesions and associated enzyme changes induced in rabbits by chronic cyanide exposure. *Fd. Chem. Toxicol.*, **37**: 745-750. doi:10.1016/S0278-6915(99)00059-9

OZDEMIR, M. AND SIRIKEN, B., 2006. Determination of cyanide levels in well water in the Afyonkarahisar region. *Vet. J. Ankara Unv.*, **53**: 37 – 40.

PETTERSEN, J. AND COHEN, S., 1993. The effects of cyanide on brain mitochondrial cytochrome oxidase and respiratory activities. *J. appl. Toxicol.*, **13**: 9-14.

RODRIGUEZ-ARIZA, A., PEINADO, J., PUEYO, C. AND LOPEZ-BAREA, J., 1993. Bio chemical indicators of oxidative stress in fish from polluted littoral areas. *Canadian J. Fish. aquat. Sci.*, **50**: 2568-2573.

SOLOMONSON, L.P. AND SPEHAR, A.M., 1981. Cyanide as a metabolic inhibitor. In *Cyanide in biology* (eds. B. Vennesland, E.E. Conn, C.J. Knowles, J. Westley and F. Wissing), Academic Press, London, pp. 11-28.

SOUSA, A.B., SOTO-BLANCO, B., GUERRA, J., KIMURA, E. AND GORNIAK, S., 2002. Does prolonged oral exposure to cyanide promote hepatotoxicity and nephrotoxicity? *Toxicology*, **174**: 87-95.

WAY, J.L., 1984. Cyanide Intoxication and its Mechanism of Antagonism. *Annu. Rev. Pharmacol. Toxicol.*, **24**: 451. doi:10.1146/annurev.pa.24.040184.002315

YAMAMOTO, H.A., 1989. Hyperammonemia, increased brain neutral and aromatic amino acid levels, and encephalopathy induced by cyanide in mice. *Toxicol. appl. Pharmacol.*, **99**: 415-420.

YOUSAFZAI, A. M. AND SHAKOORI, A. R, 2011. Hepatic responses of a freshwater fish against aquatic pollution. *Pakistan J. Zool.*, **43**: 209 – 221.

(Received 31 December 2014, revised 9 February 2015)