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Assessment of DNA Damage and Apoptosis in the Brain of Freshwater Fish *Oreochromis mossambicus* Exposed to Tannery Effluent Wastewater

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Abstract The present study was designed to evaluate the effect of tannery effluent on the fingerlings of freshwater fish *Oreochromis mossambicus.* The fishes were maintained at 10% concentration of tannery effluent for a period of 30 days. Apoptosis was studied by activity of caspases. Caspase 3 and 9 activity showed significant alteration. DNA seperation by agarose gel electrophoresis shows difference in intensity of genomic DNA between 750-1000 bp in fishes exposed to tannery effluent. DNA damage was also confirmed by Comet assay which showed an increase in DNA damage and tail migration was indicated by increase in percentage of mean DNA comet tail length formation.

Keywords: *Oreochromis mossambicus, Tannery effluent, Brain, Caspases 3 and 9, Genomic DNA, Comet assay*

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1. Introduction

Tannery effluents are ranked as the highest pollutants among all industrial wastes. Recently, majority of tanning industries favour chrome tanning for processing leather. Unfortunately only fraction of chromium (Cr) is utilized in tanning process and the rest is discharged as by-product of wastewater treatment [1]. Aquatic ecosystem is the final sink for many chemicals used in industry and agriculture and has become a global problem [2]. It is well known that Cr (VI) is a potent carcinogen to humans and animals as it enters cells via surface transport systems and gets reduced to Cr (III) inducing genotoxicity [3,4]. Chromium compounds are known to have toxic, genotoxic, mutagenic and carcinogenic effects on man and animals. Genotoxicity in aquatic animals can be assessed by DNA alteration which is an important mechanism of toxicity for a variety of pollutants and therefore is often used as an indicator of pollutant effect in ecotoxicological studies [5].

Brain is the master of all living organism as it is the controlling centre for all the receptors and effector organs. Because of its structural complexity and functional diversity brain performs a number of complex biological functions that are essential for survival. The blood brain barrier (BBB) is a physical and metabolic barrier between the brain and systemic circulation which functions to protect the brain and systemic circulation from circulating drugs, toxins and xenobiotics [6].

Apoptosis is a physiological cell death, defined by internucleosomal DNA fragmentation, chromatin condensation, cellular shrinkage and membrane blebbing resulting in the formation of apoptotic bodies. Apoptotic cell death occurs in two different pathways; extrinsic or the receptor apoptotic pathway and intrinsic or the mitochondrial apoptotic pathway, both working with caspase-3 activation [7]. There are two modes of cell death, necrosis and apoptosis. Apoptosis may occur with many different pollutants such as heavy metals and insecticides.

It has been proposed in the present study, to examine the genotoxic evaluation of chromium present in tannery effluent and its effects on the brain tissue of freshwater cultivable fish *Oreochromis mossambicus.* This would provide data supporting the usefulness of freshwater fish as indicators of heavy metals. The brain tissue is analysed for caspases activity, brain genomic DNA was separated to identify the base pairs and differences in their intensities, visualization of DNA fragments by gel documentation and single cell gel electrophoresis (comet assay) was performed to identify the extent DNA damage.

2. Materials and Methods

Collection of tannery effluent: Samples were collected from Common Effluent Treatment Plant (CETP) at Pallavaram in Chennai which treats around 3000 m^3/day of wastewater. The effluent was collected at a fixed point when the discharges from all the stages of processing are released together. The samples were collected during the month of November 2019. The raw effluent was collected in different polyethylene containers of 20 litres capacity and stored in dark at room temperature till further use.

Experimental animals: Active and healthy juveniles fishes comprising fingerlings of both sexes of *Oreochromis mossambicus* weighing about 4.0 to 4.5 gms approximately and 5.5 – 7.5 cms in length was procured from Bharat seed fish farm, Budur, Poondi, Tiruvallur District and were brought to the laboratory in polythene bags containing aerated water.

Determination of lethal concentration (LC_{50}): The LC_{50} value of tannery waste water was analyzed through a static renewal bioassay technique. Preliminary screening was carried out to determine the testing chemical as described by Solbe [8]. The effluent was mixed with tap water in appropriate dilution to get wide range of concentration. A range of 7 concentrations (50, 40, 30, 25, 20, 15, 10 % / L of water) were selected for lethal dose studies. The mortality in each concentration were noted for 24, 48, 72 and 96 hours exposure. The LC_{50} value (96 hour) was found to be at 20% concentration, for fingerlings of *Oreochromis mossambicus.* From this 50% of sublethal concentration was selected for the study.

Experimental Design: Acclimated freshwater fish fingerlings of *Oreochromis mossambicus* were divided into two groups consisting of 5 fishes in each group.

Group I: Control group - Fishes maintained in dechlorinated toxicant free tap water

Group II: Experimental group - Fishes maintained in tannery effluent

All the groups of fishes were maintained in 30 L of water. Fishes of control group was maintained in 30 L tap water. Group-II fishes were exposed to sublethal concentration (10%) of the tannery waste water for a period of 30 days. No mortality was recorded during the period of study. The experimental set up was maintained promptly with the renewal of effluent water daily in group- II. The tubs were aerated with air stones attached to an air compressor to saturate oxygen. The fishes were fed with commercial fish feed twice a day daily (2% of their body weight). Commercial food pellets with ingredients consisting of fish meal, wheat flour, soybean meal, yeast, vitamins and minerals were fed. Left over feed if any was removed by siphoning, two hours post feeding to reduce contamination with food remains. Faecal residues were removed daily through suction.

At the end of the experiment, fishes were euthanized by decapitation of cervical region and brain tissues were carefully removed, washed twice in ice cold physiological saline (0.9 N) solution and weighed. Tissue samples were homogenized and processed as required for different molecular analysis.

Analysis of chromium in raw tannery effluent : The raw tannery effluent total chromium content , hexavalent and chromium content was analysed by following the standard methods as given by APHA [9].

Activity of Caspases and Apoptosis: The commercially available lysis buffer for tissues were used. The cells were resuspended in 50 µL of chilled cell lysis buffer and homogenated. The cells are incubated on ice for 10 minutes and then centrifuged at 10,000 x g for 1 minute. The supernatant (cytosolic extract) are transferred to a fresh tube and put on ice for immediate assay. Caspases activity was determined by the method of Baharara Javad *et al.,* [10] by using Caspase-3 assay kit (colorimetric) (ab39401) and Caspase- 9 assay kit (colorimetric) (ab65608).

Isolation of genomic DNA by Agarose Gel Electrophoresis and fragmentation studies: Isolation of DNA was performed by the method of Basnakian and Jill James [11].

Collection and Cellular dissociation of brain tissue for comet assay: The control and experimental fishes were euthanized, the brain were excised immediately and washed with phosphate buffer solution (PBS), the brain tissues were transferred to microtubes for the cellular dissociation to be used in the comet assay. The method for cellular dissociation was based on Monteiro *et al*., [12].

3. Results and Discussion

Content of Chromium in raw tannery effluent: The content of chromium in the raw tannery effluent was 25.33 ppm and hexavalent chromium was 19.45 ppm. The values obtained were beyond the tolerance limit.

Caspases activity: The percentage activity of caspase 3 and caspase 9 was increased in brain of the fishes exposed to tannery effluent by 7.23 % and 6.70 % respectively, when compared with the control percentage activity baseline value (Figure 1a & Figure 1b).

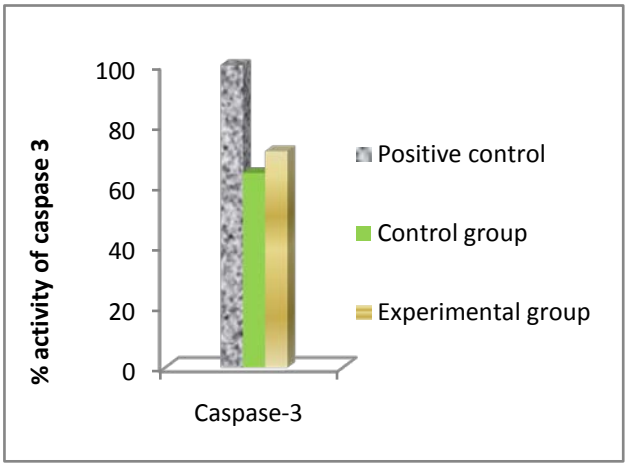

Figure 1a. Effect of tannery effluent on the caspase-3 activity in brain of freshwater fish *Oreochromis mossambicus* (Values are (n=2) and expressed as % activity of caspase-3)

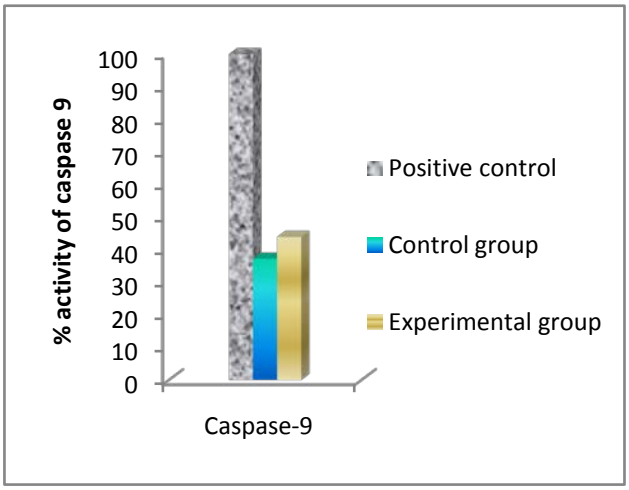

Figure 1b. Effect of tannery effluent on the caspase-9 activity in brain of freshwater fish *Oreochromis mossambicus* (Values are (n=2) and expressed as % activity of caspase -9)

Isolation of genomic DNA and its separation: Brain DNA of *Oreochromis mossambicus* from tannery exposed effluent and control was analysed on agarose gel, the presence of DNA fragments could be detected between 750 bp and 1000bp when compared with molecular marker and difference in intensities of base pair subunits were noticed (Figure 2).

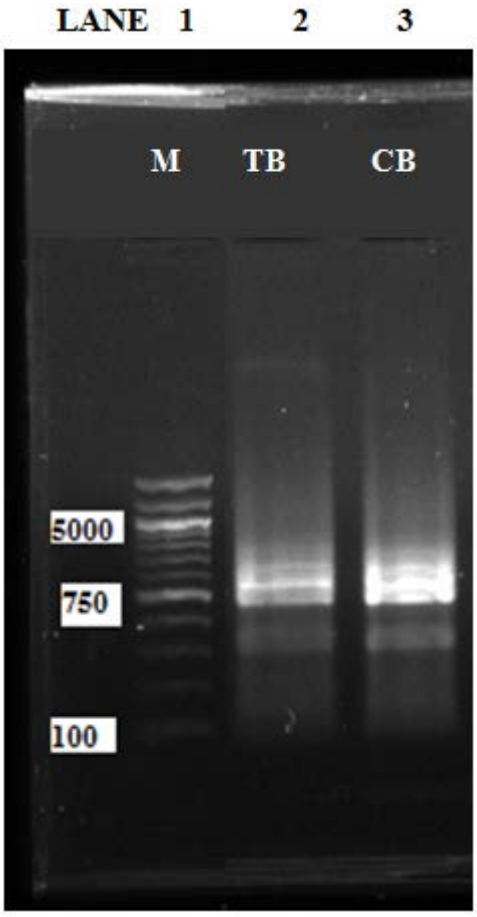

Figure 2. Separation of DNA by agarose gel electrophoresis and identification of genomic DNA in brain of freshwater fish *Oreochromis mossambicus* fingerlings exposed to tannery effluent (Lane -1: Molecular marker, Lane -2 Test brain (TB) and Lane- 3 Control brain (CB))

Comet studies: Fishes exposed to tannery effluent show variable score of DNA damage, the formation of comet tails in brain cells was significantly higher by 3.28% ($p < 0.001$) in fishes exposed to tannery effluent when compared to the control group of fishes (Figure 3 & Figure 4).

The brain has a large membrane surface due to axon extensions and neuronal dendrites that are rich in polyunsaturated fatty acids and high intake of oxygen, making them particularly vulnerable to reactive oxygen species (ROS) attack [13]. The generation of ROS triggered by oxidative stress is related to apoptotic cell death via the mitochondrial apoptosis pathway. Bcl-2, which plays an important role in promoting cell survival, pro-apoptotic protein actions, regulates the antioxidant pathway and ROS generation sites to prevent apoptosis and cellular damage [14].

Caspase-3 activation is important for the execution phase of apoptosis involving pesticide exposure [15,16]. In our study, caspase-3 activity was increased in the brain tissue of the fishes exposed to tannery effluent. This reveals that toxic compounds present in tannery effluent may activate caspase-3 and may induce brain cell apoptosis in freshwater fish *Oreochromis mossambicus.* Two possible mechanisms may be mentioned in this event are ROS generation may cause caspase 3 activation and increased Ca may induce apoptotic cell death [17]. The observed DNA damage is possibly due to direct interactions of toxic compounds present in tannery effluent with cellular DNA due to ROS generation causing strand breaks in DNA [18].

Cytochrome-C promotes activation of caspase-9 followed by downstream events leading to apoptosis as evident from increase in the activity of casapse-9 in the brain tissue of fishes exposed to tannery effluent. Caspase-9 triggers mitochondrial morphological changes and ROS production [19]. It is clear from the present study that tannery effluent like other environmental toxicants activates the expression of apoptotic genes and down regulates the expression of anti-apoptotic genes after activation of caspase-9 and caspase-3 which is required for efficient execution of apoptosis [20]. Expression of apoptotic genes was identified by upregulation of caspase 3 and caspase 9 after the exposure of zebrafish to monocrotophos [21]. Oxidative stress has been reported to inhibit expression of Bcl-2 mRNA in zebrafish. Similar mechanisms could be suggested in the present study for the increase in caspase activity.

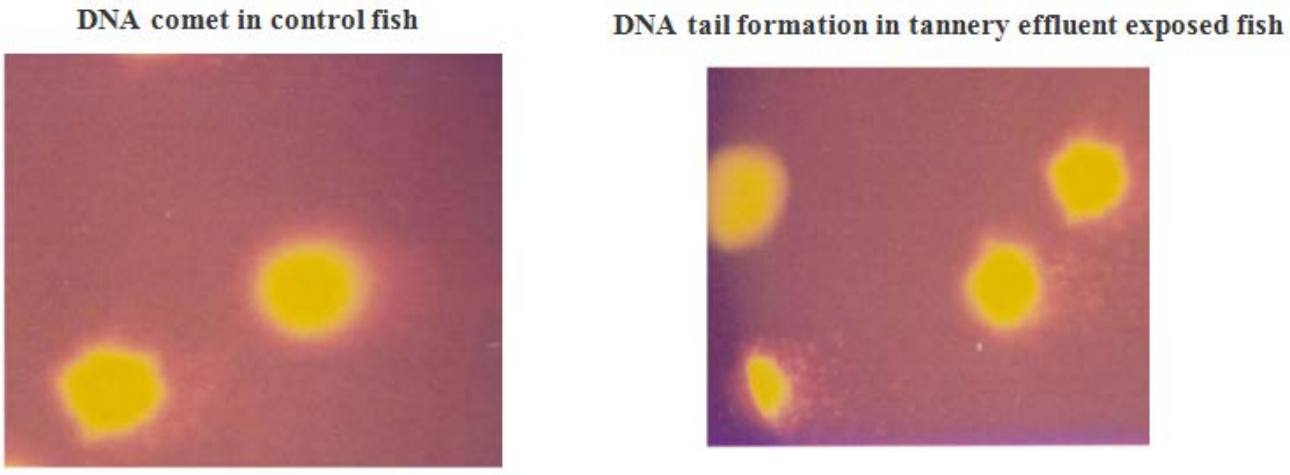

Figure 3. Comet Assay and identification of DNA damage in brain of freshwater fish *Oreochromis mossambicus* fingerlings exposed to tannery effluent

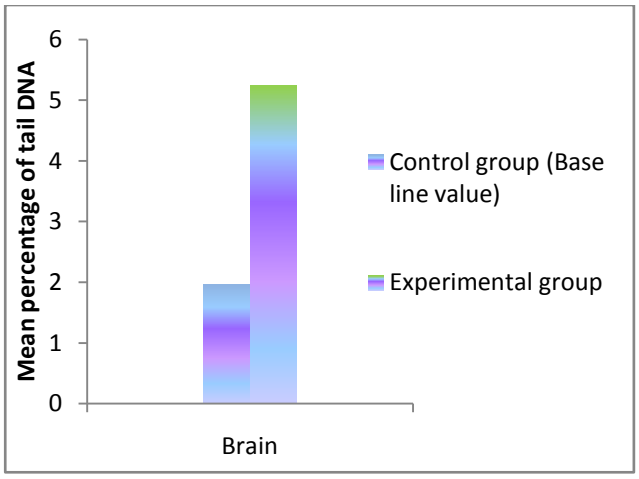

Figure 4. Effect of tannery effluent on the mean percentage of tail DNA by Comet assay in brain of freshwater fish *Oreochromis mossambicus* (Values are (n=2) and expressed as Mean percentage of tail DNA formed)

In this study DNA banding patterns were analysed by the isolation of genomic DNA from brain tissue and its separation on an agarose gel. Distinct bands were observed between 750-1000bp in the control brain tissue of *Oreochromis mossambicus*, while disappearance or less prominent appearance of base pairs in DNA subunits between 750-1000bp were observed in experimental fishes. Such changes may be attributed to the effect of tannery effluent. Similar changes in DNA banding pattern were observed in brain of *Channa punctatus* exposed to methyl parathion [22]. DNA smearing with less intensity which could be an indicator of necrosis was observed in experimental brain tissue, between 750bp-1000bp suggesting that long-term exposure may upregulate both apoptotic and necrotic pathways in cells [23].

The comet assay studies revealed have that tail length/ movement was maximum. The present study showed a consistently enhanced DNA fragmentation and formation of distinct tail from the region of the head. DNA in brain of fishes exposed to tannery effluent for 30 days. The DNA breaks as evidenced by tail movement are possibly due to defective apoptosis, or excessive production of reactive oxygen species. From the comet assay results we can also conclude that the mechanism of DNA damage can be described as follows: The Cr (VI) enters the system either by ingestion or by absorption as an oxyanion. The absorbed chromium is metabolically reduced to Cr (V), Cr (IV) and finally to reduced trivalent (III) form. These reduced forms have been shown to induce a wide range of genomic DNA damage, which make chromium to inhibit DNA replication [24].

4. Conclusion

Raw tannery wastewater without the required primary, secondary or tertiary treatment, when released from tanning industries directly into natural water bodies or when used for agriculture or aquaculture practices without proper recycling may have adverse negative effects on life aquatic organisms. To ensure the protection of water bodies, environmental monitoring report need to be done periodically in order to limit and prevent adverse environmental contaminations.

References

[1] Noorjahan, C.M., Physicochemical Characteristics, Identification of bacteria and biodegradation of industrial effluent, *Journal of Bioremediation and Biodegradation*, 5, 219-223, 2014.

[2] Adeogun, A.O. and A.V. Chukwuka. Toxicity of industrial wastewater acting singly or in joint-ratios on *Clarias gariepinus*, *American. Journal of Environmental. Sciences*, 8, 366-375, 2012.

[3] Matsumoto. S.T., Mantovani, M.S., Malagutti, M.J.A., Dias, A.L., Fonseca, I.C.and Marin-Morales, M.A., Genotoxicity mutagenicity of water contaminated with tannery effluent, as evaluated by the micronucleus test and comet assay using the fish (*Oreochromis niloticus)* and chromosome aberrations in onion root-tips, *Genetics and Molecular Biology*, 29, 148-158, 2006.

[4] Beyersmann, D. and Hartwig, A, Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms, *Archives of Toxicology*, 82, 493-512, 2008.

[5] Frenzilli G., Nigro, M.and Lyons, B.P.,. The comet assay for the evaluation of genotoxic impact in aquatic environments, *Mutation Research*. 681.80-92, 2009.

[6] Eliceiri, B.P., Gonzalez, A.M. and Baird, A., Zebrafish model of the blood brain barrier. Morphological and permeability studies. *Methods in Molecular Biology*, 686, 371-378, 2011.

[7] Monteiro, S.M., dos Santos, N.M., Calejo, M., Fontainhas - Fernandes, A and Sousa, M., Copper toxicity in gills of the teleost fish, *Oreochromis niloticus*, Effects in apoptosis induction and cell proliferation, *Aquatic Toxicology*, 94 (3), 219-228, 2009.

[8] Solbe JF. Freshwater. In: Handbook of Ecotoxicology. Peter Calins (ed) Blackwell Science Ltd. Osney meed OX 20EL 68, 3, 1975.

[9] APHA - American Public Health Association. Standard Methods for examination of water and wastewater. Washington DC, USA. 20th Edition, 1998.

[10] Baharara Javad, Ramezani, Divsalar, Mousavi and Seyedarabhi, Induction of apoptosis by green synthesized gold nanoparticles through activation of caspase-3 and 9 in human cervical cancer cells, *Avicenna Journal of Medical Biotechnology*, 8(2), 75-83. 2016.

[11] Basnakian, A.G. and James, S.J, A rapid and sensitive assay for the detection of DNA fragmentation during early phases of apoptosis. *Nucleic Acids Research*, 22 (13), 2714-2715, 1994.

[12] Monteiro V, D. Cavalcante, G.S.M.,. Vilela, M.B.F.A., Sofia, S.H. and Martinez, C.B.R. *In vivo* and *in vitro* exposures for the evaluation of the genotoxic effects of lead on the Neotropical freshwater fish *Prochilodus lineatus*, *Aquatic Toxicology*, 291-298, 7, 2011.

[13] Wang, X. and Michaelis, E.K., Selective neuronal vulnerability to oxidative stress in the brain. *Frontiers of Aging and Neuroscience*, 2, 12-20, 2010.

[14] Deng, J., Yu, L. and Liu, C., Hexabromocyclododecane - induced developmental toxicity and apoptosis in zebrafish embryos, *Aquatic Toxicology*, 93(1), 29-36, 2009.

[15] Piner. P. and Uner N., Oxidative and apoptotic effects of lambda-cyhalothrin modulated by piperonyl butoxide in the liver of *Oreochromis niloticus*, *Environmental Toxicology and Pharmacol*ogy, 33 (3), 414-420, 2012.

[16] Topal A, Atamanalp M, Oruc E, Kirici M and Kocaman EM, Apoptotic effects and glucose-6-phosphate dehydrogenase response in liver and gill tissues of rainbow trout treated with chlorpyrifos,. *Tissue and Cell Research*, 4 , 490-496 , 2014.

[17] Misra, S., Hamilton, C and Niyogi, S., Induction of oxidative stress by selenomethionine in isolated hepatocytes of rainbow trout (*Oncorhynchus mykiss*), *Toxicology invitro.*, 26; 621-629, 2012.

[18] Cao, J., Chen, J., Wang, J., Klerks, P.and Xie, L.,. Effects of sodium fluoride on MAPK's signaling pathway in the gills of a freshwater teleost, *Cyprinus carpio. Aquatic. Toxicology*. 16(152C), 164-172, 2014.

[19] Matthew Brentnall, Luis Rodriguez-Menocal, Rebeka Ladron De Guevara, Enrique Cepero and Lawrence H Boise Caspase-9, caspase-3 and caspase-7 have distinct roles during intrinsic apoptosis. *BMC Cell Biology*, 1471 (21), 14-32, 2013.

[20] Gao, D., Xu, Z., Qiao, P., Liu, S., Zhang, L., He, P., Zhang, X., Wang, Y. and Min, W., Cadmium induces liver cell apoptosis through caspase-3A activation in purse red common carp (*Cyprinus carpio*), *PLOSONE*, 8 (12), 2013.

[21] Kuppuswamy, J.M. and Seetharaman, B. Monocrotophos based pesticide alters the behavior response associated with oxidative indices and transcription of genes related to apoptosis in adult Zebrafish (*Danio rerio*) brain. *Biomedical and Pharmacology Journal*, 13 (3), 1291-1304, 2020.

[22] Veeraiah, K. , Padmavathi ,P., Tata Rao, S. and Vivek, C.H., Methyl parathion (50%EC) induced changes in protein and DNA banding pattern in the fish *Channa punctatus*, *International Journal of Bioassays*, 4(1), 3632-3637, 2015.

[23] Datta, S., Saha, D.R., Ghosh D, Majumdar T, Bhattacharya, S and Mazumder, S. Sub-lethal concentration of arsenic interferes with the proliferation of hepatocytes and induces *in vivo* apoptosis in *Clarias batrachus* (L), *Comparative Biochemistry and Physiology*, Part C, 145, 339-349, 2007.

[24] Nickens, K.P., Patierno, R. and Ceryak, S., Chromium genotoxicity: A double-edged sword, *Chemical Biological Interaction*, 188, 276-288, 2010.