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Advanced Molecular Studies on the Biomarkers of Water Pollution Effect in Fish

By

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(M.V.Sc., Kafrelsheikh University 2015)

Submitted to

Kafrelsheikh University

For The Philosophy Doctor Degree in Vet. Med .Sciences

(Biochemistry)

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2019

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List of Abbreviations

ALA.D	aminolaevulinic acid dehydratase
Ach	Acetylcholine
AChE	Acetylcholinesteras
ALA	aminolaevulinic acid
As	Arsenic
BED	Biologically effective dose
Cd	Cadmium
CAT	Catalase
CPF	Chlorpyrifos
Cr	Chromium
cDNA	Complementary deoxyribonuclic acid
Cu	Copper
CYP1	Cytochrome P450, family 1
CYP1A	Cytochrome P450, family 1, subfamily A
CYP2	Cytochrome P450, family 2
CYP3	Cytochrome P450, family 3
CYP4	Cytochrome P450, family 4

CYP	Cytocrome
CYP450	Cytocrome P450
DNA	Deoxyribonucleic acid
DMSO	Dimethylsulfoxide
EROD	Ethoxyresorufin-O-deethylase
FMO	Flavin-containing monooxygenase
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSTs	Glutathione *S*-transferases
Hb	Hemoglobin
Pb	Lead
LPO	Lipid peroxidation
LMPA	Low Melting Point Agarose
MDA	Malondialdehyde
Mn	Manganese
Hg	Mercury
MTs	Metallothioneins
MGE	Micro gel electrophoresis
MN	Micronucleus

NATs	N-acetyltransferases
Ni	Nickel
NMA	Normal Melting Agarose
OP	Organophosphate
PCV	Packed Cell Volume
PON1	Paraoxonase-1
PBS	Phosphate buffered saline
PCBs	Polychlorinated biphenyls
PAHs	Polycyclic aromatic hydrocarbons
PCR	Polymerase chain reaction
PUFA	Polyunsaturated fatty acids
K.EDTA	Potassium Ethylenediamine tetra-acetic acid
ROS	Reactive oxygen species
RT-PCR	Real time-polymerase chain reaction
RBCs	Red blood cell counts
GSH	Reduced glutathione
RTase	Reverse transcriptase
RNA	Ribonucleic acid
rpm	Rounds Per Minute

Ag	Silver
NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
SULTs	Sulphotransferases
SOD	Superoxide dismutase
Tl	Thallium
TBARS	Thiobarbituric acid reactive substances
LC50	Lethal concentration 50
TAE	Tris acetate EDTA
Tris	Trizma Base
UGTs	UDPglucronyltransferase
Zn	Zinc

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6-Summary

Pollution of the aquatic environment is a serious and growing problem caused by increasing number and amount of industrial, agricultural and commercial chemicals discharged in it causing a significant local increase in their levels in the aquatic system. The functional quality of an aquatic ecosystem is a reflection on the health of the environment.

Fish are usually sensitive to pollutants in the aquatic environment and heavily exposed to pollution because they cannot escape from the detrimental effects of pollutants .The use of fish as bio-indicators of pollution has received the attention of many searchers. Much has been documented about the accumulation of heavy metals and pesticide residues in fish collected from polluted sites.

Heavy metals and pesticides are toxic to livestock as well as human beings, which is now posing substantial threats to the local people. Both Heavy metals and pesticides are the most common form of aquatic pollutants and are of great health risk to consumers of contaminated fish. The impact of these pollutants has gained considerable interest in the field of ecotoxicology, due to their potential damaging effect on cellular components and tissues of the aquatic organisms

Physicochemical analyses provide only information about the nature of the contaminants and their concentrations in the environment, and they cannot predict bioavailability or potential effects on biota. The bio-monitoring of fish using biomarkers represents a useful tool for the assessment of aquatic pollution. Biomarkers provide the connection

between external levels of contaminant exposure, internal levels of tissue contamination, and early adverse effects in organisms.

This study aimed to evaluate the molecular, genetic, biochemical, hematological and histological markers induced by lead acetate and chlorpyrifos in *C. gariepinus* fish as a model for checking effects of pollutants in aquatic environment, and since cytochromes are the major enzymes involved in the metabolism of pollutants, Novel cytochrome genes have been suggested to be expressed in *C. gariepinus* fish. Fortunately, these genes have been identified in fish under investigation.

The study carried out on 120 fresh water *C. gariepinus* fish, of both sexes: fish were randomly divided into three groups; each group has two replicates (20 fish replicate); the first group was considered as control group ,the second group was exposed to lead acetate by a dose of 24.4 mg/L (20% of the LC_{50}) and the third group was exposed to chlorpyrifos by a dose of 1.65 mg/L (1/10 LC_{50}) , for 4 weeks , the water was changed every 2 days to avoid the accumulation of fecal matter and to maintain the toxicant concentration. The experiment was carried out in the period from mid-December to mid-January in non-breeding season.

By the end of the fourth week, whole blood and serum samples were taken for measuring hematological parameters and MDA level. Liver tissues were taken for molecular analysis, comet assay, measuring catalase activity and for histopathological examination.

This study revealed the following results:

1-Fish exposed to chlorpyrifos showed significant increase in gene expression of CYP1C1 and CYP2K, fish exposed to lead acetate showed

non-significant decrease in the expression CYP1C1 and CYP2K as compared to the control group. Real time PCR was performed before sequencing to show if the new cytochrome genes expressed in *C. gariepinus* fish or not, and this result indicate that new cytochrome genes is expressed in *C. gariepinus* fish.

2- Two of the cytochrome genes were identified for the first time in the *C.gariepinus* fish and were sequenced; showing that the nucleotide sequence of the first gene (CYP1C1) is 157 nucleotide and the second gene (CYP2K) is 163 nucleotide. The new genes were submitted to the gene bank. Alignment was carried out with other fish, which showed a very high homology between these genes and other fish genes. The CYP1C1 and CYP2K sequences are considered the first record in *C. gariepinus* fish.

3- Fish exposed to lead acetate and chlorpyrifos showed significant increase in DNA damage as revealed by comet assay, with higher damage in chlorpyrifos group than lead acetate group as compared to the control group.

4-Exposure to lead acetate and chlorpyrifos led to a significant increase in MDA level and a significant decrease in hepatic catalase activity as compared with the control fish.

5-Exposure to lead acetate and chlorpyrifos led to a significant reduction in RBCs count, PCV% and Hb concentration in comparison with the control group.

6- Liver of fish exposed to lead acetate showed hepatic vacuolation, parenchymal haemorrahge and focal leukocytic infiltration, also, liver of fish exposed to chlorpyrifos showed hepatic vacuolation and hepatic necrosis.

Conclusion

- The present results offer information about the deleterious effects of lead and chlorpyrifos and their genotoxic effect on fresh water *C.gariepinus* fish. These results could be beneficial to take preventive measure to protect the aquatic animals from these pollutants. It is suggested to limit their use in agricultural practices and of nearby water bodies to avoid the potential contamination.

- The high induction of mRNA level of CYP1C1 and CYP2K by chlorpyrifos indicated that CYP1 and 2 families can be used as potential biomarker to screen pesticides pollution. The high responsiveness of CYP1 and 2 genes suggested *C.gariepinus* fish is feasible to screen and assess pollution with pesticides.

- It can be concluded that molecular biomarkers should be considered as significant diagnostic tools for water pollution monitoring.