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RESEARCH ARTICLE

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ABSTRACT:

Rosetta branch of Nile River at Kafer El-Zayat area, Al-Gharbiah Governorate, Egypt seasonally receive a lot of pollutants such as industrial, agricultural and sewage and organisms including Nile tilapia *(Oreochromis niloticus*) may exposed to oxidative stress. For this reasons, the activities of aldehyde oxidase (AO), xanthine oxidase (XO) and protein carbonyl (PCO) in liver and white muscle of Nile tilapia (62.43 ± 6.9 g) captured from this area were assayed. The activities of AO, XO, and PCO were significantly increased as compared to control. The increase was more pronounced in autumn and winter seasons compared to other seasons. As these parameters increased in liver and white muscle as a response to these wastes in the environment seasonally it provides a useful biomarker for exposure to water pollutants. Finally, treatment of the sewage and other industrial and agricultural effluents before their entrance into Nile River recommended protecting the fish and human from deterioration effects of pollution.

KEY WORDS:

Aldehyde Oxidase, Xanthine Oxidase, Protein Carbonyl.

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INTRODUCTION:

The Nile River is the principal artery of life in Egypt, with the increased water demands and an expanding population, the demand for drainage water has increased dramatically. At the same time, pollution loads to drains also increased. It was estimated that about a quarter of the diseases facing humankind today occur due to prolonged exposure to environmental pollution (UNEP, 2010; Azimi and Sadeghi-Moghaddam, 2013). The River Nile from Aswan to El-Kanater Barrage receives wastewater discharge from 124-point sources, of which 67 are agricultural drains and the remainders are industrial sources (NWRC, 2000). Several industrial companies at Kafr El-Zayat city affect the Rosetta Branch. These industrial wastewater outfalls of the companies, El-Mobidat, El-Malyia, Salt and Soda companies, and Mashroa Eldalgamon for sewage are discharging directly at the east bank of the branch (Tayel *et al.,* 2008).

Biochemical markers of contamination are important indices used in fish toxicity and for field monitoring of aquatic pollution. They confirm contact of the specimen with specific groups of chemical compounds and clarify their further metabolic fate. Molecular biomarkers attract ever-increasing interest as early warning tools for measurement of adverse effects of environment on organisms (de la Torre *et al.,* 2005). Xenobiotic compounds in organisms undergo a series of biotransformation reactions catalyzed by detoxification enzymes, the activation of which may point to pollution exposure. Aldehyde oxidase and xanthine oxidase play a very important role in the biotransformation of drugs and xenobiotics (Beedham, 1985). When antioxidant defenses are impaired or overcome, oxidative stress may produce lipid peroxidation (LPO), protein carbonyl formation (PCO), and enzymatic inactivation (Halliwell and Chirico, 1993; Shacter, 2000). According to this detoxification, enzymes extensively used as molecular biomarkers.

The activities of aldehyde oxidase (AO), xanthine oxidase (XO) and protein carbonyl (PCO) in liver and white muscle of Nile tilapia, *Oreochromis niloticus* captured from Rosetta Branch in Kafr El-Zayat City were assayed. The findings could be beneficial in ecotoxicological researches in freshwaters as it provides data about antioxidant system response of fish exposed to different water pollutants and could open a new avenue of research in identifying and designing novel strategies, which could translate into better protection for fish and human.

MATERIAL AND METHODS:

Area of study:

Nile Tilapia (*Oreochromis niloticus*) species collected from Rosetta branch of Nile River at Kafr El-Zayat Al-Gharbia Governorate, Egypt. Five sampling localities in this area were selected according to their position relative to municipal sewage effluents and other sources of pollution. The first locality Faraon fish farm, Qasta (30° 53′ 26.55" N, 30° 47′ 50.49" E) was the cleanest, and thus can be considered a reference locality. The second locality (30° 49′ 43" N, 30° 48′ 7" E) was located downstream from the effluent discharge from Rosetta branch, Binufar, Kafr El-Zayat. This locality is receiving extensive industrial wastewater discharge because it located near the fertilizer factory in Kafr El-Zayat. The third locality was located in the front of Soda and Salt factory (30° 49′ 35" N, 30° 48′ 25" E), and receive extensive industrial wastewater discharge. The fourth locality Railway bridge (30° 49′ 7" N, 30° 48′ 46" E) was the site from which we start our tour, receive extensive urban discharge. The fifth locality (30° 48′ 51" N, 30° 48′ 37" E) was located between the bridges and receive the contamination from El Dalgamon municipal effluents. Rosetta branch of River Nile at Kafr El-Zayat area receives annually, sewage, agricultural, industrial drainage water without treatment from several drains. Some fisherman catch fish from this region and sell it in the market and it may constitute health hazard for consumers.

Collection of Samples:

Fish Samples:

Male Nile tilapia (*O. niloticus*) samples of 62.43 ± 6.86 g were caught and collected at each sampling site with the help of fishermen. The fish were collected from five sampling localities in Rosetta branch of Nile River in Kafr El-Zayat. After collection, fish were taken to laboratory using an icebox at 4ºC for laboratory investigations.

Tissue sampling:

The fish weight and length was registered. A small piece of white muscle from right dorsal side and right liver lobe were carefully excised on ice, avoiding squeezing the tissue, washed in ice-cold isotonic NaCl saline, blotted dry with filter paper, and weighed. The tissue was homogenized in ice-cold phosphate buffer (50 mM, pH 7.4), to nearly 10% (w/v) using Omni international homogenizer (USA) at 22,000 rpm for 20 s each with 10 s intervals. The supernatant was freeze-thawed thrice to completely disrupt mitochondria. Then the supernatant was centrifuged at 6000×g in cooling centrifuge at 4 ºC for 15 min and the yielded supernatant which contains the cytosolic and mitochondrial enzymes was saved for immediate enzyme assays. The UV/vis Spectrophotometer (JENWAY 6505, UK) used for the measurements of enzyme activities and oxidative stress biomarkers at 25ºC.

Determination of enzyme activities and oxidative stress biomarkers:

Aldehyde oxidase (AO, EC 1.2.3.1)

The AO enzyme activity was assayed according to the method of Johnson *et al*., (1984). The oxidation rate of either phethalzine was evaluated indirectly by observing the reduction of potassium ferricyanide as an artificial electron acceptor.

Xanthine oxidase (XO, EC 1.17.3.2)

The XO enzyme activity assayed according to the method of Stirpe and Corte (1969). The assay was done with or without NAD^+ to determine the capacity of the enzyme to react with O2 and NAD^+.

Protein carbonyl (PCO)

Protein carbonyl groups were quantified according to the method of Reznick and Packer (1994). Carbonyl groups react with DNPH to generate chromophoric dinitrophenylhydrazine.

Chemicals:

All chemicals used in this study were purchased from the following companies, Sigma Chemical Co. (St. Louis, MO, USA), Fisher bioreagent (USA), MP Biomedicals, ICN (USA), and Acros Belgium and were of analytical grade.

Statistical Analysis:

Data statistically analyzed and each reading (10 fishes) represents means ± standard deviation. The statistical evaluations of all data were done using one-way analysis of variance (ANOVA) followed by Dunnett's test using a computer program (GraphPad InState Software, Inc). Only one level of $P \leq 0.05$ was taken and regarded as statistically significant. Graphs and correlations between different parameters plotted using GraphPad Prism Software, Inc.

RESULTS:

Aldehyde oxidase (AO):

AO activity in liver and white muscle of Nile tilapia increased significantly ($P \leq 0.05$)

at different localities during the four seasons in comparison with their respective controls (Table 1). The increase in autumn was 1.8, 2, 2.2, and 2.4-fold in liver and 1.7, 1.9, 2.0 and 2.2-fold in white muscle at each sampling sites respectively. The increase in winter was 58.0, 68.8, 77.5, and 90.9% in liver and 44.5, 52.4, 63.0, and 74.0% in white muscle at each sampling sites respectively. The increase in spring was 26, 35.5, 45.2, and 51.8% in liver and 18.7, 24.8, 31.7, and 42.3% in white muscle at each sampling sites, respectively. The increase in summer was 18.0, 20.1, 20.7, and 21.0% in liver and 11.0, 12.2, 13.4, and 15.1% in white muscle at each sampling sites, respectively. The enzyme activity increased from lower values through summer to a maximum in the autumn in comparison with the other seasons.

Table 1. Aldehyde oxidase activity (µM/min/g/wet weight tissue) in liver and white muscle of Nile tilapia in different areas during the four seasons

Seasons	Organ	Sampling sites				
		Site 1	Site 2	Site 3	Site 4	Site 5
Autumn	Liver	1.58 ± 0.14	2.91 ± 0.11*	3.27 ± 0.15*	3.53 ± 0.18*	3.78 ± 0.30*
	White muscle	0.842 ± 0.11	1.40 ± 0.07*	1.56 ± 0.06*	1.70 ± 0.09*	1.85 ± 0.16*
Winter	Liver	1.57 ± 0.08	2.49 ± 0.10*	2.66 ± 0.08*	2.79 ± 0.11*	3.0 ± 0.11*
	White muscle	0.806 ± 0.05	1.17 ± 0.10*	1.23 ± 0.08*	1.31 ± 0.06*	1.40 ± 0.06*
Spring	Liver	1.63 ± 0.17	2.05 ± 0.17*	2.21 ± 0.06*	2.37 ± 0.07*	2.48 ± 0.19*
	White muscle	0.756 ± 0.05	0.897 ± 0.04*	0.943 ± 0.04*	0.995 ± 0.05*	1.076 ± 0.06*
Summer	Liver	1.45 ± 0.09	1.70 ± 0.06*	1.73 ± 0.06*	1.74 ± 0.05*	1.75 ± 0.12*
	White muscle	0.747 ± 0.04	0.829 ± 0.06*	0.838 ± 0.03*	0.847 ± 0.03*	0.860 ± 0.05*

The sampling sites, 1 Firon fish farm (the reference site), 2 Binufar, 3 Industrial zone, 4 Railway bridge and 5 between the bridges. Each reading represents Mean ±SD of 10 fish. The difference checked by one-way ANOVA was significant at P≤0.001 and Dunnet test was significant at *P ≤ 0.05.

Xanthine oxidase (XO):

XO activity in liver and white muscle of Nile tilapia increased significantly (P ≤ 0.05) at different localities during the four seasons in comparison with their respective controls (Table 2). The increase in autumn was 2.6, 2.8, 3.0, and 3.2-fold in liver and 2.1, 2.3, 2.4, 2.5-fold in white muscle at each sampling sites, respectively. The increase in winter was 1.8, 2.0, 2.2 and 2.4-fold in liver and 1.5, 1.8, 2.0, and 2.1-fold in white muscle at each sampling sites, respectively. The increase in spring was 29.4, 33.9, 40.8, and 63.9% in liver and 18.8, 28.3, 33.8, and 44.2% in white muscle at each sampling sites, respectively. The increase in summer was 12.5, 20.7, 32.2, and 41.7% in liver and 15.5, 21.8, 26.6, and 31.8% in white muscle at each sampling sites, respectively. The enzyme activity increased from lower values through summer to a maximum in the autumn in comparison with the other seasons.

Table 2. Xanthine oxidase activity (µM/min/g/wet weight tissue) in liver and white muscle of Nile tilapia in different areas during the four seasons

Seasons	Organ	Sampling sites				
		Site 1	Site 2	Site 3	Site 4	Site 5
Autumn	Liver	0.249±0.02	0.650±0.03*	0.690±0.03*	0.751±0.05*	0.787±0.04*
	White muscle	0.182±0.01	0.377±0.02*	0.411±0.02*	0.434±0.01*	0.455±0.02*
Winter	Liver	0.298±0.03	0.527±0.05*	0.583±0.02*	0.644±0.03*	0.700±0.04*
	White muscle	0.193±0.01	0.297±0.02*	0.349±0.03*	0.377±0.015*	0.403±0.02*
Spring	Liver	0.269±0.012	0.348±0.02*	0.360±0.03*	0.379±0.02*	0.441±0.05*
	White muscle	0.194±0.010	0.231±0.015*	0.249±0.011*	0.260±0.012*	0.280±0.019*
Summer	Liver	0.282±0.01	0.317±0.02*	0.340±0.01*	0.373±0.02*	0.399±0.01*
	White muscle	0.191±0.01	0.221±0.01*	0.233±0.01*	0.242±0.03*	0.252±0.02*

The sampling sites, 1 Firon fish farm (the reference site), 2 Binufar, 3 Industrial zone, 4 Railway bridge and 5 between the bridges. Each reading represents Mean ± SD of 10 fish. The difference checked by one-way ANOVA was significant at P ≤ 0.001 and Dunnet test was significant at *P ≤ 0.05.

Protein carbonyl:

AO activity in liver and white muscle of Nile tilapia increased significantly (P ≤ 0.05) at different localities during the four seasons in comparison with their respective controls (Table 3). The increase in autumn was 2.4, 2.7, 3.0, and 3.3-fold in liver and 2.0, 2.2, 2.5, and 2.9-fold in white muscle at each sampling sites, respectively. The increase in winter was 1.9, 2.1, 2.2, and 2.5-fold in liver and 1.7, 1.9, 2.0, and 2.3-fold in white muscle at each sampling sites, respectively. The increase in spring was 38.8, 54.0, 61.2, and 68.3% in

liver and 47.7, 51.2, 59.2, and 62.9% in white muscle at each sampling sites, respectively. The increase in summer was 30.0, 33.3, 39.0, and 46.3% in liver and 35.7, 36.7, 40.2, and 44.0% in white muscle at each sampling sites, respectively. The enzyme activity increased from lower values through summer to a maximum in the autumn in comparison with the other seasons.

Table 3. Protein carbonyl (μM/min/g/wet weight tissue) in liver and white muscle of Nile tilapia in different areas during the four seasons

Seasons	Organ	Sampling sites				
		Site 1	Site 2	Site 3	Site 4	Site 5
Autumn	Liver	0.248±0.02	0.589±0.03*	0.679±0.04*	0.750±0.07*	0.809±0.07*
	White muscle	0.127±0.01	0.246±0.02*	0.276±0.01*	0.314±0.02*	0.365±0.02*
Winter	Liver	0.236±0.02	0.446±0.04*	0.501±0.02*	0.527±0.02*	0.578±0.03*
	White muscle	0.133±0.02	0.223±0.02*	0.247±0.02*	0.267±0.02*	0.303±0.01*
Spring	Liver	0.201±0.02	0.280±0.02*	0.310±0.03*	0.325±0.03*	0.339±0.03*
	White muscle	0.100±0.01	0.148±0.03*	0.151±0.03*	0.159±0.04*	0.163±0.05*
Summer	Liver	0.206±0.01	0.268±0.03*	0.275±0.02*	0.286±0.03*	0.302±0.02*
	White muscle	0.110±0.01	0.149±0.01*	0.150±0.03*	0.154±0.03*	0.158±0.02*

The sampling sites, 1 Firon fish farm (the reference site), 2 Binufar, 3 Industrial zone, 4 Railway bridge and 5 between the bridges. Each reading represents Mean ±SD of 10 fish. The difference checked by one-way ANOVA was significant at P≤0.001 and Dunnet test was significant at *P ≤ 0.05.

DISCUSSION:

The global increase in freshwater contamination with numerous natural and industrial chemical compounds is, to date, one of the main environmental problems in the world. The aquatic environment frequently exposed to polluting processes caused by a massive input of various substances. In recent decades, ecotoxicological research has revealed links between reactive oxygen species production and environmental contamination, suggesting that biomarkers of oxidative stress may use in environmental monitoring programs. Physiological biomarkers have used to understand the effects on aquatic organisms of exposure to numerous pollutants. However, such studies have typically focused on single factors or chemical substances. Ferreira *et al.* (2008) and Sampaio *et al.* (2008 & 2010) recently analyzed the effects on some biomarkers in fish exposed to combinations of factors such as water quality and chronic ammonia exposure as an experimental environmental contaminants (Hegazi *et al.*, 2010). Many pollutants are strong oxidants (Avci *et al.*, 2005). The present work has intended for the assessment of the biochemical markers and antioxidant defenses in liver and white muscle of Nile tilapia, Nile tilapia, exposed to different water pollutants such as industrial, agricultural and sewage in the water at Rosetta Branch in Kafr El-Zayat City.

In many biomonitoring studies, the liver is the main target organ for investigation because of its fast answer to environmental influences, high metabolic activity, and essential function in the organism. Although white muscle has a lower metabolic rate, its importance for investigation is of great significance to humans because of its nutritional importance, especially in the case of commercially important fish species such as Nile tilapia. The current experiment demonstrated that the activities of AO and XO in liver and white muscle of Nile tilapia collected from polluted sites significantly increased as compared to control fish collected from Faraon fish farm, the reference site. The increase was more predominant in autumn and winter season than in spring and summer. To understand the cause of this increase in AO and XO activities, we tried to demonstrate the biochemical nature of these enzymes. Aldehyde oxidase and xanthine oxidase related to group of enzymes known as the molybdenum containing hydroxylases (Rajagopalan and Johnson, 1992). In addition to the microsomal monoxygenases, aldehyde oxidase and xanthine oxidase play a very important role in the biotransformation of drugs and xenobiotics (Beedham, 1985). These enzymes are widely distributed throughout the animal kingdom. Aldehyde oxidase and xanthine oxidase are very closely related enzymes (Kitamura *et al.* 2006) in terms of their general chemical structure, biochemical characteristics, and amino acid sequences (Kundu *et al.,* 2007). These two enzymes are complex metalloflavoenzymes that contain one flavin adenine dinucleotide (FAD), two non-identical iron sulfur centers [2Fe-2S], and a molybdenum cofactor as prosthetic groups (Borges *et al.,* 2002). AO generates superoxide anion and hydrogen peroxide but in contrast to XO, AO seems to be a permanent oxidase, with no activity towards NAD^+.

The enzyme XO plays a crucial role in the production of uric acid, catalyzing the oxidation of hypoxanthine and xanthine. Uric

acid acts as a scavenger of hydroxyl radicals, singlet oxygen, hypochlorous acid, oxoheme oxidants, and hydroperoxyl radicals. During the reoxidation of XO, molecular oxygen acts as an electron acceptor, producing superoxide radical and hydrogen peroxide. Consequently, XO considered an important biological source of superoxide radicals. When acting as an NADH oxidase, XO is a generator of superoxide, a powerful reactive oxygen species (ROS). Due to their highly reactive nature, these ROS affect various molecular components of the cell, with excess amounts leading to cell degeneration and death. XO is present in nearly all species. In mammalian tissues, XO was found predominantly in the liver and intestine (Harrison, 2002). However, it also contributes to oxidative metabolism of certain xenobiotics. Studies on AO and XO have shown that modulation of enzyme activities, cofactor availability, substrate concentration and oxygen tension all affect rates of intracellular reactive oxygen species production (Clarke *et al*., 1995). Excessive production of ROS, which react with various cell components such as lipids, proteins or nucleic acids results in cell damage. Consequently, AO has implicated in pathophysiology of alcohol liver injury, visual processes, synthesis of retinoic acid and reperfusion tissue injury (Beedham, 2002; Al-Omar *et al.,* 2004). Retinoic acid is an important hormone in the differentiation and development of neurons and glia as well as cell-cell signaling in the central nervous system (Chandrasekaran *et al.,* 2000). Recently, it has shown that altered retinoic acid synthesis could implicate in the etiology of Parkinson's disease and schizophrenia. Alternatively, AO is a source of oxygen radicals, which may contribute to these diseases (Mira *et al.,* 1995; Hegazi and Hasanein, 2010). Although xanthine oxidase generates reactive oxygen species, it should note that in vivo, the enzyme exists predominantly as dehydrogenase, reacting with NAD^+, whereas AO reacts exclusively with oxygen (Turner *et al.,* 1995). The present increase in the activity of AO and XO may greatly contribute to an increased rate of ROS generation as result to the exposure for a great input of pollutants sewage, agricultural and industrial wastes, through a number of drains into the River Nile. Consequently, results indicate that elevated levels of XO and AO considered as a more specific marker in monitoring environmental stress due to pollution.

Biomarkers of oxidative stress directly connected with changes of ROS concentration in organism (Ahmad *et al.,* 2004). When antioxidant defenses are impaired or overcome, oxidative stress may produce lipid peroxidation (LPO), protein carbonyl formation (PCO), and enzymatic inactivation (Halliwell and Chirico, 1993; Shacter, 2000).

The results have indicated that the degree of PCO in the examined liver and white muscle of *O. niloticus* tissues was sensitive to seasonal difference and metabolic products including wastes and toxicants in the environment. In addition, show a significant increase at the four different sites compared to reference site.

Oxidative stress results when the effectiveness of antioxidant defenses is insufficient to deal with the production of reactive oxygen species (ROS). ROS can induce damage to DNA, lipids, and proteins. The oxidation of proteins results in the production of stable carbonyl groups, which can use as a measure of oxidative injury. During the past several years, protein carbonyl levels have also been measured in fish tissues (Lushchak *et al.* 2005; Parvez and Raisuddin, 2005). Reactive oxygen species generally damage all biomolecules due to their catalytic properties. In vivo, Protein carbonylation is a metal (Copper or Iron) accelerated modification occurring to protein side chain of many amino acids such as lysine, arginine, proline or histidine to produce carbonyls. The hypochlorous acid (HOCL) a major endogenously produced oxidizing species released by an enzyme myeloperoxidase; predominantly induce the protein carbonylation (Kettle and Winterbourn, 1997). The protein carbonyl content (PCC) resulted by oxidation makes the protein resistant to hydrolysis and functional inactivation of proteins in serum or plasma, cellular components, membrane proteins etc. Since, protein is major constituents of all forms of the biological system, the exact conformation and three dimensional folding are highly connected to the protein functions, the restore of nativity of protein is crucial. Thus, critical evaluation of protein carbonyl content serves as biomarkers of protein oxidative damage in various conditions (Shi *et al.,* 2005). Therefore, in our experiment the modulation of a significant and non-significant increase in protein carbonyl levels in fish from different polluted locations could be indicative of exposure of fish to xenobiotics. Of the two possible responses, an increase would likely be the more severe. Water pollutants can cause oxidative damage in biological systems and their measurement has become a useful measure of exposure of aquatic organisms to pollutants.

CONCLUSION

The present findings indicate that Rosetta branch, Kafr El-Zayat water; due to the continuous discharge of different pollutants contained toxic constituents that caused change in the biochemical markers and antioxidant defenses in liver and white muscle of Nile tilapia. The antioxidant defense system of fish responded differently to

pollutants exposures with differing sources. Consequently, the activities of AO, XO, and PCO in the examined fish tissues were sensitive to metabolic products including wastes and toxicants in the environment so it provide a useful biomarker for environmental managers in investigating the exposure of fish to water pollutants. However, to protect the human public health and fish from pollution and reduce environmental risk, it could recommended that treatment of the agricultural, industrial and sewage effluents should be carried out before their discharge to the River Nile.

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المؤشر الحيوى للإجهاد التآكسدى الديهيد اوكسيديز وزانثين اوكسيديز لسمك البلطى المعرض للتلوث فى نهر النيل

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تهتم الدراسة بتقييم المؤشر الحيوى للإجهاد التآكسدى فى الكبد والعضلات البيضاء لسمكة البلطى التى تم تجمعيها من فرع رشيد ،مدينة كفر الزيات- محافظة الغربية،مصر. حيث تم دراسة تآثير الملوثات المائية فى هذهالمنطقةوالتى تتعرض لصرف كميات كبيرة من مياه الصرف والملوثات الصناعية والزراعية فى اسماك المياه العذبه البلطى النيلى (62.43+6.86 جم) . أظهرت النتائج زيادة ذات دلالة احصائية عند القيمه $p \leq 0.05$.ووجد أن هناك زيادة ملحوظة خلال فصلي الخريف والشتاء مقارنة بفصلي الصيف والربيع . وتعتبر المؤشر الحيوي للإجهاد التأكسدي ألدهيد أوكسديز و زانثين أوكسيديز مؤشرات حيوية تستخدم للتعرف على تلوث البيئة المائية للكائنات البحرية. حيث هذه التغيرات تعتبر كدلالات بيولوجية للكشف عن تعرض الاسماك للمياه الملوثة.