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Determination of Glutathione Related Enzymes and Cholinesterase Activities in *Oreochromis niloticus* and *Clarias gariepinus* as Bioindicator for Pollution in Lake Manzala

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Abstract: The northern lakes of Egypt have always acted as a buffer zone between the drainage system in the Nile Delta and the Mediterranean Sea. Most of these lakes are exposed to high inputs of agricultural drainage water, sewage and industrial wastewater, like lake Manzala,which influence the living organisms like fish. In the present study the effects of pollution in southern region of lake Manzala on some biochemical parameters in liver, brain and muscles of *Oreochromis niloticus* and *Clarias gariepinus* were studied. Samples of the two studied fish were collected seasonally (January to December 2007) from the southern region of lake Manzala. Liver glutathione reductase, glutathione S-transferase and brain acetylcholinesterase enzymes as well as total protein and lipid contents in muscle were determined. The present results showed that liver GSH and GST of *O. niloticus* and *C. gariepinus* were increased significantly in spring and summer seasons, brain AchE activity was inhibited significantly and total protein and lipid contents in muscles were decreased significantly in spring and summer seasons compared to the control fish. It was concluded that pollution of the lake Manzala adversly affect enzymatic activities, physiological functions and meat quality of its fish.

Key words: Glutathione · Acetylcholine esterase · *O. niloticus* · *Clarias gariepinus* · Lake Manzala

INTRODUCTION

Today, most environmental problems are attributed to the production and release of toxic chemicals capable of interacting with the environment and disrupting the ecosystem. The toxic chemicals produce stress on the aquatic organisms like fish. Fish in ponds, lakes and rivers can not avoid exposure to these chemicals, that suspended or dissolved in water, being less than land animals to move to favorable regions to a void unfavorable condition [1]. Egyptian costal lakes act as temporary reservoirs for drainage water and often are highly contaminated with anthropogenic materials. This is true particularly for Manzala Lake. Moreover, fish production in the lakes have virtually ceased due to discharge of industrial and agricultural waste water [2].

Lake Manzala is considered an important source of fish in Egypt for many reasons. First it has the largest overall area of the natural lakes in Egypt. Second, it is connected to the Mediterranean Sea from the north-norteastern coast and also to the Nile Damietta Branch at Damietta.Third, it is surrounded by five governorates which share the food resources among their populations [3]. The lake was a brackish environment, but recently it changed to nearly eutrophic lake [4].

Previous studies have reported that exposure of fish to pollutants(agricultural, industrial and sewage) evolved antioxidative defense system which include enzymes such as glutathione peroxidase, glutathione S-transferase, superoxide dismutase and catalase that show general high activity in the liver, a major organ for xenobiotic [5, 6]. These enzymes present in all organisms metabolize many pollutants in order to protect the organisms against the deleterious effects of xenobiotics. It was suggested that some of these enzymes can constitute good molecular bioindicators for oxidative stress and can also indicate the magnitude of response in vertebrate population chronically exposed to contaminants, such as metals and other xenobiotic [7].Glutathione (L-Y glutamyl-L cysteinyl glycine GSH) is probably the most abundant natural low molecular weight thiol that is detected in virtually all living cells of the vertebrates often in millimeter concentration it is a central player in a series of critical cell function including, transport of amino acid across cell membrane,

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catalyzing disulfide exchange ereactions, serving as co-enzyme for certain enzymes, maintaining thiol group in proteins and detoxifying peroxides and free radicals as well as reactive toxic intermediates free radicals [8]. It was reported [9] that, GSH concentration depends on the metabolic rate and the levels of oxidative stress. Glutathione S-transferase (GST) is a super-family of mainly cytosolic conjugated enzymes which use the tripeptide glutathione (GSH), instead of glucoronic acid. It was also reported that GST is super-family of enzyme that can express detoxification activity through some of there isozymes; glutathione peroxidase, towards lipid hyhroperoxides generated by pollutants or organic contaminants [10]. Acetylcholinesterase (AchE) is the enzyme that cleaves the transmitter acetylcholine in cholinergic synapse and neuromuscular junction [11]. The normal function of acetylecholine enzyme (AchE) is to terminate neurotransmission due to acetylcholine (Ach) that has been liberated at cholinergic nerve ending response to nervous stimuli [12] Inhibition of AchE activity in fish is considered a specific biomarkers of exposure to pesticides pollutants (Organochlorine and pyrethroids)[13].

The problems of pollution in Lake Manzala have been extremely increased in the last years these problems includes, presence of high concentrations of heavy metals and pesticides in both water and varies fish organs that affected fish fertility, reproduction and production. Most of these toxic substances exceed the international agreed threshold levels in food stuffs which of course adversely affected the health of human fish consumes. Based on the results of a previous study [14], the concentrations of heavy metals (Zn, Mn, Pb and Cu) and the pesticides (lindine, aldren. o.p DDT, and atrazin)in water samples and fish organs (liver, muscle and gills) collected from southern region of lake Manzala more than permissible levels recommended by [15]. Pollution of lake Manzala had received the attention of many workers. The effects of pollutants on antioxidant defense agents and AchE activity as well as total protein and total lipids contents in muscles of fish collected from the lake not been examined so fare, since most of the field studies concerned with physical and chemical environment of the lake [16], microbial pollution of the lake [17], water quality in the southern region [18], histological change in fish organs [19], accumulation of heavy metals, pesticides residues in water and fish organs and the changes in biochemical parameters in fish blood [14], So the aim of the present study is to evaluate the impacts of pollutants (agricultural, sewage and industrial drainage) of the southern region of lake Manzala on antioxidant defense agents(like GSH and GST) in the liver of *O. niloticus* and *C. gariepinus* the most abundant fish species in the lake as well as to determine the brain AchE activity, total protein and total lipids in muscles of fish.

MATERIALS AND METHODS

Study Area: Lake Manzal is economically the most important Delta lake in Egypt.The surface area of the lake has been decreasing steadily over the past few decades. Now, the area of the lake Manzala had been reduced to only 120 km^2 [20]. A total amount of 7500 million cubic meters of untreated industrial, sewage and agricultural drainage, is discharges annually into the lake via numbers of drains [21,22]. The southern region of the lake receives agricultural and industrial drainage water without treatment from several drains. The main drain Bahr-El-Baker E,l-Genka and El-Bashtir region. Some fisherman catch fish from this region and sell it in the market and it may constitute health hazard for consumers.

Sampling and Analysis: Samples of *Oreochromis niloticus* and *Clarias gariepinus*, weighing 58-80 g and 125-278 g respectively, were collected seasonally during a period extending from January to December 2007 along the southern region of lake Manzala (polluted area). During the same periods samples of both studied fish with comparable weight and mangemental conditions were collected from Al-Kanater Al-Khyria fish farm station (unpolluted area), [23, 24]. Fish were sacrificed to remove the epiaxial muscle on the dorsal surface, the entire liver and brain from each one. Samples from such organs were immediately homogenized in ice cold distilled water. Thereafter, centrifugation was carried out at 3000 r.p.m for 15 min. The supernatant was collected and stored at 20°C for biochemical analysis.

Determination of Reduced Glutathione (GSH) Content: GSH content in liver was determined by using the methods described by [25]. The method depends on precipitation of protein, using tungestate sulphuric acid solution and formation of a yellow color after reaction with 5, 5 dithiobis-nitrobenzoic acid (DTNB). The intensity of the color was measured at 412 nm. Glutathione content was expressed as mg/100 g wet weight.

Determination of Glutathiontransferase (GST) Activity: The enzyme was determined in the liver according to the method of [26] using 1-chloro-2,4-dinitrobenzene

(CDNB)as a substrate.The enzymes activity were expressed as Umol /min / mg protein wet weight.

Determination of Acetylcholinesterase (AchE) Activity: The enzymes was determined in brain tissues according to [27] by using cholinesterase kit (Diamond company). The enzyme activity was expressed as U/g wet wt.

Determination of Total Protein Content in Muscle: Total protein content was determined according to the method described by [28].

Determination of Total Lipids Content in Muscle: Total lipids content in muscle was determined according to the method of [29].

Statistical Analysis: Data were analyzed using student's *t-test* [30].

RESULTS

Table 1 represents the change in liver GSH for *O. niloticus* and *C. gariepinus* collected from Manzala lake during the four season of the study year. Results indicated that GSH in the studied fish was not changed significantly during winter season, but it tended to increased ($P \leq 0.01$)) during spring and summer ($P \leq 0.001$) seasons as compared to the control fish.

Liver GST activity of *O. niloticus* and *C. gariepinus* was recorded in Table 2.The activities showed a non significant change for both investigated fish during the winter season, however,during the spring and summer seasons the activities were increased ($P \leq 0.001$).

The change in AchE activity in brain of *O. niloticus* and *C. gariepinus* coll ected from Manzala lake during four seasons were shown in Table 3. The brain AchE activities of the studied fish were not changed significantly at winter season, but it showed inhibition at spring and summer season compared to the control fish.

Table 1: Liver glutathione reductase (GSH) mg/100 g wet wt. of *O. niloticus* and *C. gariepinus* from Manzala lake (Mean±SE)

	Control *O.niloticus*	Manzala lake *O.niloticus*	Control *C. gariepinus*	Manzala lake *C. gariepinus*
Season	M±S.E	M±S.E	M±S.*E*	M±S.E
Winter	0.90±0.20	1.1±0.08	1.23±0.07	1.32±0.12
Spring	1.2±0.30	2.0±0.07**	1.45±0.06	2.61±0.10**
Summer	1.4±0.50	2.4±0.01***	1.56±0.04	2.9±0.08***
Autumn	1.12±0.22	1.8±0.1*	1.91±0.06	2.1±0.08*

Number of Fish L =12, ** P <0.01, *** P <0.001

Table 2: Liver Glutathion S-Trasferase (GST) activity U/mg wet wt.of *O. niloticus* and *C. gariepinus* from Manzala lake.

	Control *O.niloticus*	Manzala lake *O.niloticus*	Control *C. gariepinus*	Manzala lake *C. gariepinus*
Season	M±S.E	M±S.E	M±S.E	M±S.E
Winter	1.22±0.07	1.25±0.12	1.32±0.05	1.30±0.10
Spring	1.52±0.08	2.4±0.20**	1.63±0.07	2.66±0.08**
Summer	1.55±0.06	2.6±0.18**	1.68±0.09	2.86±0.09***
Autumn	1.32±0.07	1.35±0.15	1.45±0.06	1.50±0.07

Number of Fish L=12, ** P<0.01, *** P<0.001

Table 3: Brain acetylcholinesterase activity U ml/mg wet wt. of *O. niloticus* and *C. gariepinus* from Manzala lake(Mean±SE)

	Control *O.niloticus*	Manzala lake *O.niloticus*	Control *C. gariepinus*	Manzala lake *C. gariepinus*
Season	M±S.E	M±S.E	M±S.*E*	M±S.E
Winter	0.81±0.03	0.78±0.02	0.71±0.01	0.68±0.02
Spring	1.01±0.04	0.75±0.22**	1.12±0.02	0.77±0.03**
Summer	1.25±0.04	0.63±0.12***	1.61±0.04	0.65±0.021***
Autumn	1.09±0.2	0.96±0.05	0.96±0.05	0.98±0.023

Number of Fish L group=12, ** P<0.01, *** P<0.001

Table 4 represents the change in total protein and lipids contents in muscle of *O. niloticus* and *C. gariepinus* collected from Manzala lake during four season of the study year. The result indicated that there was a significant ($P<0.01$) decrease in total protein in

Table 4: Total protein and lipid contents(g/100 g wet wt.)in muscles of *O. niloticus* and *C.gariepinus* from Manzala lake(Mean±SE)

	Muscle protein content (g/100g wet wt.)				Muscle lipid content (g/100 g wet wt.)			
	Control O. n	Manzala O.n	Control C.g	Manzala C. g	Control O. n	Manzalal O.n	Control C.g	Manzal C. g.
Season	M±S.E	M±SE.	M±SE	M±SE	M±SE	M±SE.	M±S.E	M±SE
Winter	16.7 ±0.8	11.0±0.4**	17.2±0.9	12±1.9**	0.64±0.06	0.68±0.05	1.4±0.1	1.5±0.03
Spring	15.9±0.6	13.0±0.6**	17.1±0.6	16.4±0.21	0.72±0.02	0.43±0.01*	1.1±0.02	0.82±0.02*
Summer	16.0±0.4	12.0±0.3**	16.3±0.8	15.5±0.5	0.74±0.04	0.31±0.01**	1.04±0.04	0.64±0.03**
Autumn	15.9±0.6	15.0±0.5	16.9±0.6	16.1±0.7	0.68±0.03	0.72±0.03	1.2±0.03	1.10±0.01

Number of Fish L group =12. O. n: *Oreochromis niloticus*. C. g: *Clarais gariepinus*. *P<0.01, **P<0.001

muscle of the two studied fish during the winter season. But in spring and summer seasons total protein in muscle of *C. gariepinus* was not changed significantly compared to the control fish, while in *O. niloticus* total protein was still decreased ($P<0.01$).Total lipids content in muscle of *O. niloticus* and *C. gariepinus* did not reveal any significant different in winter season, however in spring and summer season there was a highly significant decrease in total lipid ($P<0.01$).

DISCUSSION

Lake Manzala is affected by continuous and steady flow of pollutants, including sewage, agricultural and industrial wastes, through a number of drains. This pollutants have serious effects on the aquatic ecosystem,including fish, since these wastes contain a variety of toxic organic as well as inorganic compounds. The impact of these toxic compounds on aquatic ecosystems can be assessed by the measurement of their external levels in the surrounding water or by determining some biochemical parameters in fish that respond specially to the degree and type of contamination.one of these parameters are the Xenobiotic metabolizing enzymes glutathione reductase (GSH), glutathione S-trans ferase (GST), which bio-transform different toxic agents to water soluble products and used as important biomarkers for environmental condition [31]. Fish exposed to pollutants (agricultural, sewage and industrial wastes) is thought to generate free radicals specially reactive oxygen species (ROS) with subsequent alteration in fish antioxidant defense such as Glutathione (GSH., L-and glutamyle L-cysteinyl-glycine)which is probably the most abundant cellular thiol, that is detected virtually in all tissues but, its concentration, in general is high in the liver (as a major organ for antioxidant defense in fish) [32]. Apparently, GSH is important in protecting against deleterious effects of the cell exposed to ROS by reacting with them to form glutathione disulphide(G-S-S-G). This antioxidant effect occurs spontaneously through GSH or may also catalyzed by glutathione S-transferase (GST ; an enzyme for which GSH is utilized as substrate [33].

The present study revealed that (GSH) and (GST) in liver of *O. niloticus* and *C. gariepinus* collected from southern region of lake Manzala markedly increased during spring and summer season as compared to the control fish collected from Al-Kanater Al-Khyria fish farm (unpolluted area) [23,24]. The increase in GSH and GST in the present study may be due to the high environmental temperature during spring and summer which increase oxygen consumption and aerobic metabolisms by fish and consequently increased (ROS) production followed by enhanced antioxidant capacity in fish [34]. In this respect [35] Suggested that antioxidant defense is enhanced in marine and freshwater species during spring and summer when compared to low temperature period. Moreover the increase GST activity could be related to the enzymes remarkable sensitivity to a large variety of pollutants in southern region of lake Manzala [14,36,37].It has also suggested that the increased GST activity in response to pollution could be related to fish adaptation to the continuous exposure to pollutants, even if their concentrations in the environment still meet acceptable sanitary conditions [38]. The induction of GSH and GST in the liver of *O. niloticus* and *C. gariepinus* collected from Manzala lake in the present investigation is in accordance with that recorded in liver and kidney of *O. niloticus* captured from sewage polluted sites [39]; in liver of Rainbow trout exposed to cadmium [40]; in liver of Cyprinus carpio exposed to polychlorinated biphenyle [41]; in liver of *Oncorhynchus mykiss* exposed to trinitrotoluene for 72 h [42] and in liver of *O. niloticus* exposed to malathion, phenol and lannate for 3 weeks [43].

The present results indicated marked inhibition in the brain AchE activity of *O. niloticus* and *C. gariepinus* collected from Manzala lake. Mean while this enzyme showed lower activity during spring and summer and no change during winter and autumn seasons. The inhibition of brain AchE activity may be associated with fish exposure to various pollutants as agricultural pesticides and herbicides as well as metals. In this context, it has accepted that pesticide demonstrated acute toxicity in fish through inhibition of brain AchE activity [13, 44]. The same toxic effect was observed with herbicides in the work of [45, 46] who reported that fish respond to herbicide stress by a reduction of brain Ache activity. With regard to the effect of metal pollution, several studies indicated that heavy metals such as zinc and mercury can be considered as environmental inhibitors for AchE activity in fish [13]. Inhibition of AchE causes accumulation of the neurotransmitter, acetylcholinesterase the synapse and blocking the neurotransmission in the respiratory centers of the brain or neuromuscular junction of the respiratory system which lead to death [47].

In various fish species, proteins are of importance as, structural compounds, bio catalysts and hormones for control of growth and differentiations [48]. So variation in fish proteins could be used as bio-indicator for monitoring physiological status of the tested fish.

In the present study examined *O. niloticus* and *C. gariepinus* collected from southern region of lake Manzala exhibited lowered protein value in muscle during winter season,it was suggested that protein decline during winter is probably a metabolic adaptation to the food shortage in environment [49]. During this period of inadequate food supply (starvation period) energy required for metabolic maintenance may be provided from utilization of protein reserves which mainly accumulate in the muscle tissues [50], but in spring and summer seasons total protein of *C. gariepinus* was not changed significantly compared to the control fish Table 4, In *O. niloticus* total protein steel decrease during spring and summer. During autumn season total protein of the two studied fish was not changed significantly. Beside the above mentioned this protein depletion could be attributed to change in the lake water quality as a result of the discharged effluents from different sources, including hydrocarbon found in sewage wastes [51] and heavy metals found in the industrial and agricultural effluents [52, 53]. This may be explained in light that, exposure to metals (as Cu and Zn) may led to high accumulation in the gills that cause damage in their structure and a reduction in the rate of oxygen consumption causing short

to different pollutants [58,59] It was concluded that fish (*O. niloticus* and *C. gariepinus)* inhabiting Manzala lake showed worse physiological status. This was indicated by elevations of the liver (GSH and GST), inhibition of brain AchE and decreases in total proteins and lipids content in muscles. The obtained data also indicated that the seasonal variation during the study period greatly affected the tested physiological parameters with maximal changes during summer and minimal during winter.

In order to decrease the pollution effect, the lake outlets should be activated to renewal the lake water, biological treatment of the sewage and industrial wastes and removal of the bottom polluted sediment from the lake.

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