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PROTEIN METABOLISM AND ACETYLCHOLIN - ESTERASE IN TILAPIA FISH AS AN INDICATOR TO EVALUATE THE POLLUTION IN LARGE DRAINS IN EGYPT

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ABSTRACT: Environmental pollution is one of the most deleterious agents to the biological life. The aquatic environment with its water quality is considered the main factor controlling the state of health and disease in both cultured and wild fish. Therefore, it is necessary to study the impact of deleterious effects of pollutants in the aquatic ecosystem. Some biochemical parameters such as total soluble protein, transaminases, alkaline phosphatase and acetylcholinesterase were determined among two seasons in fish samples (*Oreochronnis niloticus*) collected from two drains El-Bats and El-Wadi draining Qarun Lake, El Fayoum Governorate, Egypt. The data showed a significant increase in total soluble protein, ALP, ALT in the liver and muscle tissues in the two seasons while the AST and ACh-E were inhibited in the same fish samples.

Key words: Pollution, biochemical parameters, ACh-E, AST, ALP, total soluble protein.

INTRODUCTION

The aquatic environment is the ultimate recipient of pollutants produced by natural and anthropogenic sources. Accumulation and persistence of some toxicants as metals and pesticides in the environment represent a threat to biological life as witnessed by chronic and acute poisoning of aquatic organisms.

The study of the biological response of organisms to different environmental conditions and the quantitative evaluation of their physiological status are being considered as a successful approach for the assessment of environmental quality (Mohamed *et al.*, 2003). Aquatic animals, inhabiting polluted water, their bodies tend to accumulate many chemicals in high concentrations even when the ambient environmental contamination levels are low (Colombo *et al.*, 1995). A potentially hazardous

situation for the entire food chain. Once a toxicants enter an organism, several biochemical and physiological responses occur which may be adaptive or may lead to toxicity. The biochemical processes represent the most sensitive and relatively early events of pollutant damage. Thus, it is important that pollutant effects be determined and interpreted in biochemical terms, to delineate mechanisms of pollutant action, and possibly ways to mitigate adverse effects (Ghousia, 2004).

Qarun Lake, one of five large lakes in Egypt, is an inland closed basin of about 40 km length, 5-7 km mean width and an average depth of 4.2 m. It lies in an arid region, occupying the deepest part of Fayoum province in the western desert of Egypt (Meshal and Marcos, 1981). The lake receives agricultural water and sewage through two main drains, El-Batts and El-Wadi in addition to other small drains.

El-Bats drain extending about 50.9 Km receives drainage water as well as crude sewage from the eastern and north eastern part of El-Fayoum depression. The annual average (207.6 millions m^3) of drainage water is discharged into the eastern part of the lake. On the other hand, El-Wadi Drain (48.5 km long), receives drainage water from the middle region of El-Fayoum depression. The annual average of its drainage water is 103.03 million m^3 discharged into the middle part of the lake (Sabae, 1996). The investigation of the response of several enzyme systems to pesticide or heavy metals or both will contribute to full understanding the possible sublethal effects of such pollutants as a partial of the natural matrix in environment. Transamination is catalyzed by enzymes termed transominases or aminotransaminase. Transaminases are important and critical enzymes in biological processes, they play a role in amino-acids catabolism and biosynthesis (Martin *et al.*, 1985). Alkaline phosphatase (ALP) is a membrane-bound enzyme related to the transport of various metabolites (Lin *et al.*, 1976). It has been also proposed as a good biomarker in ecotoxicology because of its sensitivity to metabolic salts (Boge *et al.*, 1992). Acetylcholinesterase hasa potential biomarker (Laport *et al.*, 1996). It plays a vital role in maintaining the normal neural functioning of the sensory (Peakoll, 1992).So; this study aimed to evaluate the status of freshwater fish (*Oreochromis niloticus)* inhabited in polluted water using biochemical parameters such as ALT, AST, total protein and neurotoxicity using acetylcholinesterase activity.

MATERIALS AND METHODS

Sample Preparation

The present study was carried out on fresh-water fish, the Nile tilapia (*Tilapia nilotica*) which collected from the two drains: El-Bats drain and El-Wadi drain. Fish samples (2 kg) from each site were collected and transported to the laboratory in ice box. Then the fish were sacrificed and the muscle tissue and organs (liver and brain) were isolated to determine the biochemical parameters (total soluble protein, aspartate amino-transferase, alanine aminotransfe-rase, alkaline phosphatase and acetylcholinesterase) as follows:-

Determination of ALP, AST and ALT

The isolated muscle and liver (1 g) was homogenized in 10 ml of 0.25 M ice-cold sucrose solution using an ice-Jacketed homogenizer with a motor-driven Teflon coated pestle. The homogenate was centrifuged in cold centrifuge machine at 3000 xg for 20 min at 4 °C to remove nuclei and cell debris. A clear cell-free extract was used for assay of ALP, AST, ALT enzymes and total soluble protein using spectrophotometer apparatus. The conditions of the enzymes and protein are:-

1-The activity of plasma aminotransferases, alanine amino transferase (ALT) and aspartate aminotransferase (AST) were colorimetrically determined according to the method adopted by Reitman and Frankel (1957).

2-Plasma alkaline phosphatase (ALP) activity was kinetically estimated according to the method of Hausdman *et al.* (1967).

3-Total soluble protein was determined according to the method of Bradford (1976).

Determination of Acetylcholine-sterase (ACh–E)

The liver and brain was homogenised (10% w/v) in 0.1 M phosphate buffer (pH 7.5). The homogenates were centrifuged at 5000 x g for 10 minutes at 4°C. The resultant supernatant was used as the enzyme source for the estimation of ACh-E activity. The ACh-E activity was estimated in brain and liver tissues by the method described by Ellman *et al.*, (1961).

Statistical Analysis

All data were subjected to statistical analysis by one – way analysis of variance (ANOVA) test by Gad, (2001) using SPSS software for Windows version 10.Aprobability of $p \leq 0.05$; $p \leq 0.01$ and $p \leq 0.001$ as the level of

significance unless stated otherwise

RESULTS AND DISCUSSION

Biomarkers were chosen to detect sublethal biochemical and cellular changes in organisms exposed to toxicants. The utility of these methods lies to their ability to provide an early warning of toxicants stress in organisms and of the whole environment quality of aquatic ecosystem (Mohamed *et al.*, 2003).

Proteins are the most important and abundant macromolecules in living organisms, which play a vital role in architecture and physiology of cell and cellular metabolism. Concerning, the data recorded in Tables 1 & 2 pointed out the total soluble protein of muscle and liver was increased in the samples collected from El-Bats and El-Wadi drains through the two seasons (Summer& Winter) where, the significant increased protein reached 32.85, 29.71 and 32.91 (mg / g)as compared with 28.33 (mg / g) in control of fish muscles except, the total soluble protein (27.95 mg / g) after the summer in El-Bats drain was reduced. However, the significant increase of protein in liver was 41.43, 47.79, 33.97 and 43.43 (mg / g) as compared with (33.82 mg / g) in control. These results were agreed with Talha *et al.*, 1998. The generally increase or decrease in total soluble protein indicated great amount of proteins are synthesized or damaged by the liver, where increased protein are needed for repair of damaged cell organelle and tissue regeneration. Furthermore, a compensatory production of enzymes lost as a result of tissue necrosis or to meet increased demand to detoxify the insecticide might necessitate enhanced synthesis of enzyme proteins (Gill *et al.*, 1990). While decreased protein content may be attributed to the destruction/necrosis of cells and consequently impairment in protein synthesis machinery (Bradbury *et al.*; 1987).

The enzymes aminotransferases are the strategic link between carbohydrate and protein metabolism, as they interconvert the metabolites such as α-ketoglutarate, pyruvate and oxaloacetate on one hand to glutamate, alanine and aspartate on other hand, respectively. Aminotransferases play a vital role in the utilization of amino acids for oxidation and/or for gluconeogenesis (Rod Well, 1988). In the present study, the aspartate aminotransferase (AST) activity was inhibited in the muscles tissues of fish samples collected from the two drains since, data

showed 184.93, 255.20, 131.22 and 167.33 U / g in comparison with 333.82 U/g in control. The same trend was in the activity of AST in the liver except, the increase in AST activity after winter in El-Bats drain since data showed 146.58, 350.44, 143.91 and 245.05 U / g in comparison with 320.99 U / g in control. The depression in the activity of AST may be due to the formation of complex compounds with AST protein in the liver (Hashem, 1980) or accumulation of toxic materials such as heavy metals in this organs which its concentration is built up over time (Heath, 1995).

The ALT activity of muscles tissues exhibited a marked decrease in the two drains after summer and winter where, data recorded 45.51, 85.53, 48.41 and 65.12 U/g as compared with control (101.38 U/g) in muscles. In contract, the activity of ALT showed a significant increase in the liver tissues at the two drains after winter and non significant increase at El–Wadi drain after summer. While, the activity of enzyme was reduced after summer in El–Bats drain. and winter at the two drains except, the activity of enzyme was reduced after summer in El-Bats drain. Since, data showed 94.89, 152.02, 102.47 and 210.23 U/g as compared with control (101.38 U/g). The enhanced activity of ALT in the liver may attributed to the increased tissues utilization of amino acids through these reactions to compensate the energy demand warranted by toxic materials (Begum, 2004), or due to cell damage.

Alkaline phosphatase (ALP) is a group of enzymes which hydrolysed phosphate esters at optimal pH_S of about 10. It occurs in many organs including intestine, bone and liver (Walmsley and White, 1994).

Data presented in Tables 1 & 2, showed a significant increase in ALP activity in the muscles tissues at two drains under the investigation except an insignificant increase at El–Bats drain after summer since the data recorded in this study were 9.57, 18.47, 37.61 and 30.64 U/g as compared with 8.83 U/g in control. Also, the increase in ALP activity was noticed in the liver tissues at the two drains after winter and summer except, ALP activity was reduced after winter at El-Batts drain where, the data recorded 9.01, 4.71, 6.13 and 9.81 U/g as compared with control (5.52 U/g). The increase in ALP

Table 1. Total soluble protein, transaminases and alkaline phosphatase in muscle of fish collected from polluted drains

Variable	Control	El-Bats		El-Wadi	
		After summer	After winter	After summer	After winter
AST (U/g)	333.82 ± 12.68	184.93 ± 2.47***	255.20 ± 9.08***	131.22 ± 3.94***	167.33 ± 5.24***
ALT (U/g)	101.38 ± 9.09	45.51 ± 2.89***	85.53 ± 6.94	48.41 ± 5.33**	65.14 ± 7.86***
ALP (U/g)	8.83 ± 1.49	9.57 ± 1.29	18.47 ± 2.15***	37.61 ± 6.73***	30.64 ± 0.44***
T.S.P. (mg/g)	28.33 ± 1.18	27.95 ± 2.03	32.85 ± 1.45	29.71 ± 0.95	32.91 ± 1.14

AST: Aspartic Aminotransferase. ALT: Alanine Aminotransferase.
ALP: Alkaline Phosphatase. T.S.P: Total Soluble Protein.
Significant differences is indicated by * $p \leq 0.05$; ** $p \leq 0.01$;
*** $p \leq 0.001$ when compared with control values.

Table 2. Protein metabolic enzymes and alkaline phosphatase in liver of fish collected from polluted drains

Variable	Control	El-Bats		El-Wadi	
		After summer	After winter	After summer	After winter
AST (U/g)	320.99 ± 7.78	146.58 ± 11.89***	350.44 ± 12.19**	143.91 ± 8.04***	245.05 ± 3.9***
ALT (U/g)	101.38 ± 9.09	94.89 ± 7.27	152.02 ± 10.740***	102.47 ± 4.96	210.23 ± 9.05***
ALP (U/g)	5.52 ± 0.71	9.01 ± 1.34*	4.71 ± 0.74	6.13 ± 0.20	9.81 ± 0.59**
T.S.P. (mg/g)	33.82 ± 0.77	41.43 ± 0.13***	47.79 ± 2.53***	33.97 ± 0.62	43.43 ± 1.54***

AST: Aspartic Aminotransferase. ALT: Alanine Aminotransferase.
ALP: Alkaline Phosphatase. T.S.P: Total Soluble Protein.
Significant differences is indicated by * $p \leq 0.05$; ** $p \leq 0.01$;
*** $p \leq 0.001$ when compared with control values.

activity may be due to alterations in their active sites induced by pesticides and/or disruption of membranes due to toxic action of toxicant (Nicholls *et al.*, 1989).

Cholinesterases are widely distributed among animals, both vertebrates and invertebrates (De la Torre *et al*, 2002). The inhibition of acetylcholinesterase in particular is well documented as a specific biomarker target for assessing the exposure of non-target aquatic organisms to orgnophosphorus and carbamate insecticides (Weiss, 1958).

Concerning, the environmental pollution in the two drains (El-Bats and El-Wadi), the acetylcholinesterase activity in both brain and liver was affected, where the data recorded in Table 3, were 97.26, 79.67, 85.69 and 60.94 nmol / min / mg protein as compared with 193.13 nmol / min / mg protein in brain of the control fish. While the liver ACh-E was 79.63, 62.50, 78.18 and 95.83-nmol / min/mg protein as compared with control (118.84-nmol / min / mg protein). The acetylcholinesterase activity was significantly reduced reduced in both organs at both drains in winter and summer except, in significantly reduced was noticed in liver at El-Wadi drain after winter.

Table 3. Brain and liver acetylcholinesterase activity (nmol/min/mg protein) of fish collected from polluted drains.

Variable	Control	El-Bats		El-Wadi	
		After summer	After winter	After summer	After winter
Brain	193.13 ± 14.98	97.26 ± 2.85***	79.67 ± 4.02***	85.69 ± 0.8**	60.94 ± 1.29***
Liver	118.85 ± 17.02	79.63 ± 1.46***	62.50 ± 1.46**	78.18 ± 9.21**	95.83 ± 3.52

Significant differences is indicated by * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$ when compared with control values.

The inhibition in ACh-E activity may be due to a secondary outcome of conformational changes in the enzyme due to binding metals with a large number of functional sulphydryl groups and as a direct effect derived from their ability to bind covalently to serine at the active site of ACh-E-site (Szabo and Nemcsok, 1992).

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تمثيل البروتين و الأسيتيل كولين استيريز فى سمك البلطى كمؤشر لتقييم تلوث المصارف الكبيرة بجمهورية مصر العربية

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٢.المعمل المركزي للمبيدات- مركز البحوث الزراعية – الدقي – جيزة

التلوث البيئي من أخطر العوامل في الحياة البيولوجية. كما تعتبر البيئة المائية و جودة مياهها العامل الرئيسي المتحكم في الحالة الصحية و المرضية للأسماك المنزرعة و البرية. و لذلك فانه من الضروري دراسة التأثير السمي للملوثات في النظام البيئي وقد تم تقدير بعض المعايير البيوكيميائية مثل البروتينات الكلية الذائبة و إنزيمات النقل الأمينى و إنزيم الفوسفاتيز القاعدي و كذلك إنزيم الأسيتيل كولين استيريز فى عينات أسماك البلطى النيلى التي تم جمعها من مصرفي البطس والوادي و اللذين يصبان فى بحيرة قارون- محافظة الفيوم- جمهورية مصر العربية: و لقد أوضحت البيانات زيادة معنوية فى البروتينات الذائبة فى عينات الكبد والعضلات لأسماك البلطى النيلى تحت الاختبار وكذلك أظهرت البيانات زيادة فى نشاط إنزيم الفوسفاتيز القاعدي فى الكبد والعضلات بينما زاد نشاط إنزيم الألانين أمينو ترانسفيريز في أنسجة الكبد فقط وعلى العكس من ذلك فقد بينت النتائج تثبيط إنزيم الاسبارتات أمينو ترانسفيريز فى أنسجة الكبد والعضلات وكذلك تثبيط إنزيم الأسيتيل كولين استيريز فى كبد ومخ الأسماك خلال موسمي الدراسة.