



STUDIES ON SOME BIOCHEMICAL PARAMETERS OF THE EARLY DEVELOPMENTAL STAGES OF THE AFRICAN CATFISH

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ABSTRACT

In this paper, Atrazine is considered the most common aquatic herbicide used. So, the present study aimed at studying atrazine-induced changes in some biochemical characteristics during early embryonic stages of *Clarias gariepinus*. These characteristics include two metabolic enzymes, lactate hydrogenase (LDH) and glucose-6-phosphate dehydrogenase (G6PDH), two oxidative enzymes, glutathione reductase (GSH) and superoxide dismutase (SOD), two thyroid hormones, triiodothyronine (T3) and thyroxine (T4), lipid peroxidation (LPO), DNA damage and patterns of protein fractions of different early developmental stages.

Key words: lactate hydrogenase., triiodothyronine, lipid peroxidation, pollution,

INTRODUCTION & REVIEW :

In this study Fish are widely used to evaluate the health of aquatic ecosystems and physiological changes serve as biomarkers of environmental pollution¹. Aquatic systems are exposed to a number of pollutants that are mainly released from effluents discharged from industries, sewage treatment plants and drainage from urban and agricultural areas. These pollutants cause serious damage to aquatic life^{2,3}.

The contamination of fresh water with a wide range of pollutants has become a matter of great concern over the last few decades, not only because of the threat to public water supplies, but also with the damage caused to the aquatic life. The river systems may be excessively contaminated with heavy metals released from domestic, industrial, mining and agricultural effluents⁴. Pollutant effects on fish behavior have received increasing attention over the past decade. Several recent reviews on such effects have appeared including⁵⁻¹⁰. In Such reviews the treated fish behavioral categories included the behaviors associated with schooling, feeding, migration, aggression, fear, learning, phototropism and attraction to or avoidance of a chemical or temperature. Pitcher¹¹ gave a good overview of normal fish behavior. The resulting behavioral effects may inhibit an organism's ability to capture prey, avoid predators, or successfully compete with others^{12,13}. For example, a study of mosquito fish (*Gambusia affinis*) aqueous exposed to mercurial chloride demonstrated altered swimming activity and decreased swimming speed¹⁴. Similarly, Alvarez¹⁵ noted concentration dependent effects of maternally-transferred MeHg in larval Atlantic croaker (*Micropogonias undulatus*), including decreased response speed and increased response time to a vibratory stimulus.

Mercury is generally found at very low concentrations and is very reactive in the environment. Total mercury levels are generally lesser than 10 ng/g in crustal materials such as granites, feldspars and clays¹⁶, and in the range of 40 to 200 ng/g in soils and sediments that are not directly impacted by

anthropogenic discharges. Generally, the majority of mercury in aquatic systems is inorganic forms (about 95 to 99%) and is found in sediments rather than the dissolved phase.

In Egypt, different studies on the effect of heavy metals on fishes and water pollution were carried out for both freshwaters and marine environments. These studies emphasized the severe effect of such heavy metals on the aquatic ecosystems. The African catfish, *Clarias gariepinus* (Burchell, 1822), was selected as the test organism in this study for its great aquaculture and commercial value in Egypt and elsewhere in the developing world. *C. gariepinus* is a benthopelagic (bottom feeder); omnivorous feeder that occasionally feeds at the surface. *C. gariepinus*, also referred to as mudfish, is very hardy and tasty. They are able to tolerate adverse aquatic conditions, where other cultivable fish species cannot survive. It is widely cultivated and used as experimental fish.

According to the aforementioned findings, the present work was suggested and aimed to study the effect of mercury and its interaction with supplementation of selenium and vitamin E on the biochemical parameters of the Nile catfish, *Clarias gariepinus*.

MATERIALS AND METHODS

Gametes were collected from mature specimens of the African catfish, *C. gariepinus* from the river Nile. The fertilized eggs were incubated in dechlorinated tap water (pH = 7.7, dissolved oxygen 88-94% saturation, temperature 27-29°C and photoperiod 12:12 (light:dark). Under atrazine influences, different measurements of enzyme activities, electrophoretic protein analysis, lipid peroxidation, DNA fragmentation, Total Protein (TP) and hormones were carried out.

The basic statistics, means, standard errors and ranges were estimated. The patterns of variation due to developmental stages and atrazine doses and their interaction were studied by two way analysis of variance using the SPSS package at the 0.05 significance level. Levene's test of equality of error variance of the dependent variables was applied, with rejection of the null hypothesis for raw, log-transformed and SQRT-transformed data. So, the homogeneity of variance was assumed for raw data. The Tukey-HSD test was considered for multiple comparisons. Moreover, the Dunnett, t-test was applied, measuring the control against other treatments in each developmental stage.

RESULTS AND DISCUSSION

Metabolic enzymes: G6PDH and LDH: The atrazine doses have different adverse impacts on 2 metabolic enzymes (G6PDH and LDH) during early embryonic development of *Clarias gariepinus*. The atrazine-induced activity of G6PDH (Table 1, Fig. 1) was fluctuated significantly with developmental progress ($p < 0.001$) with a peak in 12 h-PFS and a sharp decrease in 24 h-PFS directly after hatching. Such sharp decrease may refer to the fact that zygotic gene of G6PDH is activated during this later stage. Similarly, significant fluctuation ($p < 0.001$) in G6PDH activity was evident under atrazine doses with no special trends towards decrease or increase in each developmental stage studied.

The atrazine-induced activity of LDH (Table 2, Fig. 2) was fluctuated significantly in some cases and increased in others with developmental progress ($p < 0.001$) with a sharp decrease in 24 h-PFS directly after hatching. Such sharp decrease may refer to the fact that zygotic gene of LDH is activated during this later stage.

Table 1: Effect of different doses of atrazine on the activity of G6PDH (Mean±SE) during early developmental stages of the African catfish *Clarias gariepinus*

Embryonic stages*	Control	0.1 mg L ⁻¹ exposure	1 mg L ⁻¹ exposure	5 mg L ⁻¹ exposure	10 mg L ⁻¹ exposure	20 mg L ⁻¹ exposure	100 mg L ⁻¹ exposure	200 mg L ⁻¹ exposure
4 h-PFS	833.4±20.9 ^{ab} (807.6-874.9)	325.3±11.2 ^{ab} (302.8-336.5)	628.1±11.2 ^a (605.7-639.4)	1256.3±22.4 ^{ab} (1211.4-1278.7)	213.1±11.2 ^a (201.9-235.6)	535±3.4 ^a (528.3-538.4)	628.1±11.2 ^a (605.7-639.4)	863.7±11.2 ^a (841.3-874.9)
8 h-PFS	493.5±11.2 ^{ab} (471.1-504.8)	616.9±11.2 ^{ab} (605.7-639.4)	527.2±11.2 ^a (504.8-538.4)	459.9±11.2 ^{ab} (437.5-471.1)	527.2±11.2 ^a (504.8-538.4)	426.2±11.2 ^{ab} (403.8-437.5)	179.5±11.2 ^{ab} (168.3-201.9)	751.5±22.4 ^{ab} (706.7-773.9)
12 h-PFS	1121.7±29.7 ^{ab} (1076.8-1177.7)	1895.62±11.2 ^{ab} (1884.4-1918.1)	2882.7±95.8 ^{ab} (2692-2994.9)	2781.8±44.7 ^a (2692-2826.6)	3477.2±224.3 ^{ab} (3028.5-3701.5)	2075.1±78.5 ^{ab} (1951.7-2220.9)	1783.5±51.4 ^{ab} (1682.5-1850.8)	1951.7±51.4 ^{ab} (1850.8-2019)
16 h-PFS	392.6±29.7 ^{ab} (336.5-437.5)	661.8±11.2 ^{ab} (639.4-673)	493.5±44.9 ^{ab} (403.8-538.4)	1311.2±1.1 ^a (1308.9-1312.4)	1985.4±67.3 ^{ab} (1850.8-2052.7)	515.9±11.2 ^a (504.8-538.4)	1940.5±11.2 ^a (1918.1-1951.7)	1334.8±11 ^{ab} (1312.4-1346)
24 h-PFS	56.1±11.2 ^{ab} (33.7-67.3)	89.7±11.2 ^{ab} (67.3-100.9)	123.4±11 ^{ab} (100.9-134.6)	123.4±11.2 ^{ab} (100.9-134.6)	190.7±11.2 ^a (168.3-201.9)	157±11.2 ^{ab} (134.6-168.3)	123.4±11.2 ^{ab} (100.9-134.6)	264.7±4.5 ^a (255.7-269.2)

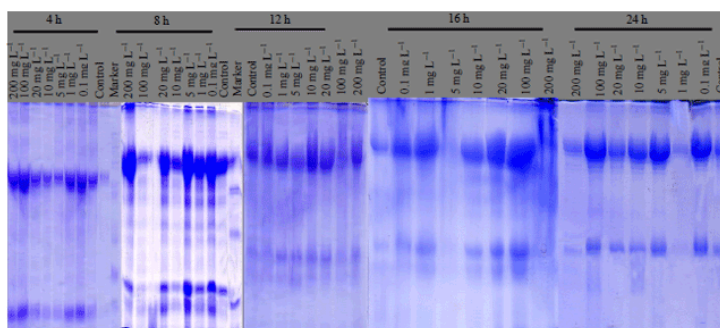


Fig. 1: Protein fractions identified in different larval stages of *C. gariepinus* under different doses of atrazine in comparison with control *h-PFS: Hours post fertilization stages

Table 2: Effect of different doses of atrazine on the activity of LDH (Mean±SE) during early developmental stages of the African catfish *Clarias gariepinus*

Embryonic stages*	Control	0.1 mg L ⁻¹ exposure	1 mg L ⁻¹ exposure	5 mg L ⁻¹ exposure	10 mg L ⁻¹ exposure	20 mg L ⁻¹ exposure	100 mg L ⁻¹ exposure	200 mg L ⁻¹ exposure
4 h-PFS	171.6±0.39 ^{ab} (171-172.3)	131.7±3.6 ^{ab} (126.3-138.5)	105.1±1 ^a (103.5-106.9)	130.6±0.6 ^{ab} (129.9-131.9)	111.8±0.4 ^a (111.1-112.3)	167±1.4 ^{ab} (164.8-169.7)	137.5±0.8 ^a (136-138.4)	278±0.7 ^a (276.9-279.2)
8 h-PFS	95.3±3.3 ^{ab} (88.7-98.9)	83.4±1.2 ^{ab} (82.1-85.7)	85.1±0.9 ^{ab} (83.4-86.1)	64.1±0.9 ^{ab} (63.2-65.9)	63.1±1.5 ^{ab} (61-65.9)	72.4±0.1 ^{ab} (72.2-72.5)	150.2±0.9 ^{ab} (148.6-151.6)	166.2±0.7 ^{ab} (164.8-167.1)
12 h-PFS	647.2±0.8 ^{ab} (646-648.9)	481.2±0.11 ^{ab} (480.9-481.3)	816.2±0.7 ^{ab} (815.1-817.4)	915.7±0.8 ^{ab} (914-916.6)	171.3±0.24 ^{ab} (170.9-171.7)	464.3±12 ^{ab} (450-488.2)	790.4±0.96 ^{ab} (788.5-791.6)	1054.3±0.23 ^{ab} (1053.9-1054.8)
16 h-PFS	487.4±0.81 ^{ab} (485.9-488.6)	631.8±0.83 ^{ab} (630.2-632.8)	691±0.6 ^a (690.1-692.2)	758±0.25 ^{ab} (757.6-758.4)	552.6±1.1 ^{ab} (550.5-553.7)	618.5±1.4 ^{ab} (615.8-620.2)	2199.9±5.1 ^{ab} (2190.8-2208.3)	1342.7±1.3 ^{ab} (1340.3-1344.8)
24 h-PFS	507.3±0.17 ^{ab} (507-507.6)	99±0.06 ^{ab} (98.9-99.1)	197.5±0.2 ^a (197.1-197.8)	136.4±2.4 ^{ab} (131.8-139.8)	98.2±7.4 ^{ab} (85.7-111.2)	57.3±3 ^a (52.7-63)	111.5±0.7 ^a (110.1-112.3)	120.2±0.8 ^a (118.7-121.5)

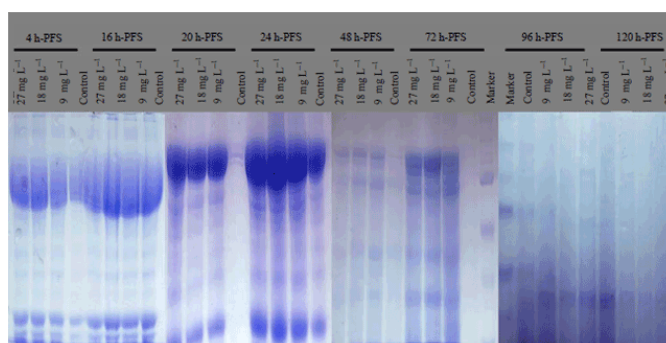


Fig. 2: Protein fractions identified in different larval stages of *C. gariepinus* under different doses of atrazine in comparison with control, h-PFS: Hours post fertilization stages

CONCLUSION :

It is concluded that atrazine induces oxidative stress, DNA damage, lipid peroxidation and endocrine disruption on the developmental stages of African catfish *Clarias gariepinus*. Most of these parameters could be used as indicators of environmental aquatic pollution. However, fluctuations in T3 and T4 activities in the developmental stages of *C. gariepinus* were insignificant in most cases and referred to the fact that it may not be related to atrazine doses but to the stage of development.

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