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Studies on atrazine induced changes in some cat fish: Aspects of female African catfish (*Clarias gariepinus*)

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Abstract
In this paper, The atrazine herbicide in adult female *Clarias gariepinus* was estimated as 13.75 mg/l Atrazine toxicity was estimated through the acute and chronic exposures of female *C. gariepinus* for 4 days and 6 weeks, respectively. Fish stayed motionless at a certain location in mid-water level for prolonged periods. The effect of Atrazine exposure on the blood picture showed a significant decrease in erythrocyte and leukocyte counts, haemoglobin content and blood indices, while the haematocrit value increased. The results indicated that the Atrazine herbicide caused alterations on behavioral, haematological and biochemical parameters of *C. gariepinus*. Therefore, strict precautions should be followed during Atrazine application to avoid polluting the aquatic environment and consequently protect fish against its toxic effect.

Keywords: Atrazine herbicide toxicity, freshwater fish, LC_{50}, clinical signs, haematological parameters

Introduction
In this study Pollution is a widespread problem in many aquatic environments. Recently, environmental pollution caused by pesticides, became a serious problem. As a consequence, residual amounts of herbicides and their metabolites have been found in drinking water and food [1], which in turn increased the concern for the possible threats to human health [2]. and toxicity risk to non-target organisms like fish [3]. Contamination of surface water has been well-documented worldwide and constitutes a major issue at local, regional, national and global levels [4].

Atrazine (ATR) herbicide belongs to S-triazine family of herbicides, which are one of the most significant water pollutants in rain, fresh, marine and ground waters. It controls or kills plants through a variety of mechanisms including the inhibition of biological processes, such as miosis, cell division, enzyme function, root growth, leaf formation, interference with the synthesis of pigments or the promotion of uncontrolled growth and inhibition of photosynthesis. So it is called photosynthesis inhibitor [5]. Atrazine has neurotoxic effects on the morphology of nerve fibres in albino rats. Also, Atrazine is concerned DNA and chromosome damage in human lymphocytes. As well as, its myelosuppressive effects in human.

Atrazine is also used in forestry and for non-selective weed control on non–crops areas. Indeed, Atrazine is well tolerated by actively growing corn or sorghum, which absorbs, metabolizes and deactivates it. The previously mentioned factors and atrazine's relatively low cost have contributed to make it a frequently used herbicide. Many haematological abnormalities were recorded in teleosts after exposure to sub-lethal levels of different chemical pesticides. Among the biochemical profiles, glucose has been extensively used as a sensitive indicator of environmental stress in fish. Protein is also one of the important biochemical parameters which are used to understand the general state of fish health and biological mechanism of metabolism under pollutant stress [6]. So, the aim of the present study is to determine the value of Atrazine LC_{50} /96h in female African catfish, *C. gariepinus* and to investigate the behavioral, haematological and biochemical changes in Atrazine exposed fish during acute and chronic exposures.

Materials and Methods
Fish and rearing conditions
A total of 200 female catfish, *C. gariepinus* with average body weight 300±50g were obtained from Abassa fish farm, El-Sharkya governorate, Egypt. Fish were transferred to the laboratory of Fish Diseases and Management Department, Faculty of Vet. Med.,

Cairo University in 150 litres well aerated fiberglass tanks. The fish were kept in identical glass aquaria measuring (80×40×40 cm) aerated with air pumps under natural photoperiod and temperature, supplied with dechlorinated tap water and left for two weeks for acclimation. Fish were examined clinically to assure the absence of any abnormalities or external lesions.

Experimental design

One hundred and ten (110) fish were divided into two groups. The 1st group was exposed to 1/2 LC_{50} (6.87mg/l) of atrazine for 4 days, where forty fish of almost the same weight and size were stocked into 4 aerated aquaria at a rate of 10 fish/aquarium, While the 2nd group of sixty fish were stocked into other 6 aerated aquaria in each of which 10 fish were stocked and exposed to 1/10 LC_{50} (1.37mg/l) of atrazine for 6 weeks. In addition to the control group. Blood samples were obtained from all groups after the 1st, 2nd, 3rd and 4th day in case of acute exposure and at the 1st, 2nd, 3rd,4th,5th and 6th week in case of chronic exposure.

Clinical investigation and postmortem examination

The exposed fish were kept under proper observation during the period of experiment for any clinical abnormalities and postmortem lesions or deaths according to the method described by Amlacher (1970).

Collection of blood samples

Under the effect of benzocaine (50mg/l), fish were anesthetized for 5 minutes according to Post (1989). Blood samples were collected from the caudal vein using plastic syringes in dry sterilized vials. The samples were divided into two parts for the haematological and biochemical investigations.

Haematological parameters

The first part of blood samples were examined immediately using Sodium citrate as an anticoagulant for the following parameters: Red and white blood cell count using improved Neubauer haemocytometer. Haemoglobin content was estimated calorimetrically using Randox kits as described by Van Kampen and Zijlstra (1961). Haematocrit value (Ht) was performed using haematocrit centrifuge at 12.000 r.p.m. Blood Indices: mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated according to Gupta (1977).

Biochemical examination

The second part of blood samples were allowed to clot at room temperature and centrifuged at 3000 r.p.m. for 10 minutes. Total protein was determined by Biuret test according to Bradford (1976). Serum albumin (ALB) was measured by using Stanbio kits as described by Dumas and Biggs (1972). Globulin (GLOB) was calculated according to the method described by Coles (1986). A/G ratio was calculated as the ratio of serum albumin to serum globulin.

Statistical analysis

Data were statistically analyzed using analysis of variance, one way "ANOVA", and Duncan's multiple range test to evaluate comparison between means at $P< 0.05$ (SPSS, 2004).

Results and Discussion

To study the toxic effects of Atrazine on female *C. gariepinus*, it was necessary to determine its half lethal concentration (LC_{50}) /96h. Results revealed that the (LC_{50})/96h of Atrazine is 13.75 mg/l (Table 1).

Table 1: Half lethal concentration (LC_{50})/96h of Atrazine in female *C. gariepinus.*

Atrazine Conc. (mg/l)	No. of alive fish	No. of dead fish	a	b	a x b
0.00	8	0	0	0	0
2. 50	7	1	2.5	0.5	1.25
5.00	6	2	2.5	1.5	3.75
10.00	4	4	5	3	15
20.00	2	6	10	5	50
40.00	0	8	20	7	140
	∑a x b =210				

The result is nearly similar to those of [7, 8] who recorded that LC_{50} values of Atrazine for bluegill sunfish *Lepomis macrochirus* and toads *Rhinella arenarum* at stage 25 were 16 mg/l and 14.41 mg/l, respectively. But the results disagreed with those of [9, 10] who reported that the LC_{50}/96h of Atrazine were 9.37, 76, 4.3 and 10.2 mg/l for *Oreochromis niloticus*, *Cyprinus carpio*, *Poecilia reticulate* and *Rhamdiá quelen*, respectively. The differences in results may be attributed to the toxic effects with respect to age, size, health and fish species [11].

The clinical signs in female *C. gariepinus* associated with Atrazine exposure were manifested by fish excitation, trials to jump out of the aquarium, moving towards the air source, loss of appetite, sluggish or restlessness swimming and staying motionless at a certain location generally at mid-water level for prolonged periods. Detached fins and tail, rapid opercular movement were also observed. Finally the opercular movement stopped gradually before death with oozing of blood from the gill cover as a consequence of the rupture in gill blood vessels (Fig.1). Swelling and inflammation of the genital opening (Fig.2). Abnormal skin pigmentation in the form of fading (Fig.3) and excessive mucous secretion in gills and skin were common. While the postmortem examination revealed different degrees of congestion, haemorrhage and enlargement of the spleen (Fig.4) and haemorrhage in all internal organs especially the ovaries (Fig.5). Enlargement and discoloration of liver with distension of the gall bladder were also recorded as prominent lesions (Fig. 6 & 7). Concerning the results of total protein, albumin, globulin and A/G ratio (Tables 2 & 3).

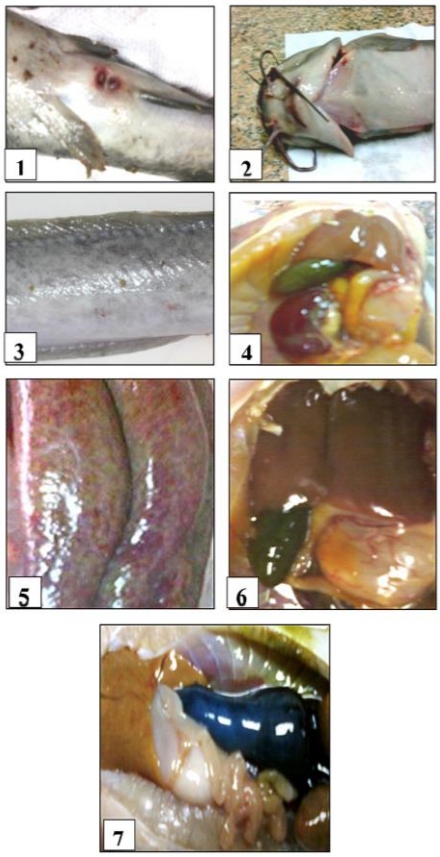

Table 2: Effect of acute exposure to Atrazine on serum glucose and protein pattern of female *C. gariepinus.*

Parameters / Days	Glucose (mg/dl)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio (g/dl)
Control	58.33±16.10^{d}	7.31±0.39^{a}	2.34±0.09^{a}	4.97±0.29^{a}	0.47±0.01^{c}
1st day	105.77±5.87^{c}	4.77±0.06^{b}	1.82±0.01^{b}	2.96±0.07^{b}	0.61±0.02^{b}
2nd day	115.39±6.39^{bc}	3.19±0.01^{c}	1.48±0.03^{cd}	1.70±0.02^{c}	0.87±0.03^{a}
3rd day	151.92±5.09^{a}	3.34±0.02^{c}	1.55±0.01^{c}	1.79±0.01^{c}	0.86±0.01^{a}
4th day	143.59±8.97^{ab}	2.92±0.02^{c}	1.38±0.01^{d}	1.55±0.01^{c}	0.89±0.01^{a}

Table 3: Effect of chronic exposure to Atrazine on serum glucose and protein pattern of female *C. gariepinus.*

Parameters / Weeks	Glucose (mg/dl)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio (g/dl)
Control	58.33±16.10^{c}	7.31±0.39^{a}	2.34±0.09^{a}	4.97±0.29^{a}	0.47±0.01^{c}
1st week	82.05±8.41^{bc}	5.84±0.15^{b}	2.14±0.05^{a}	3.53±0.07^{b}	0.61±0.03^{b}
2nd week	109.94±7.03^{ab}	4.79 ±0.28^{c}	1.72±0.25^{b}	3.08±0.09^{c}	0.56±0.08^{bc}
3rd week	106.41±25.25^{abc}	4.84±0.12^{c}	2.12±0.04^{a}	2.72±0.14^{cd}	0.78±0.05^{a}
4th week	126.28±17.70^{ab}	3.77±0.05^{d}	1.45±0.02^{bc}	2.32±0.03^{d}	0.62±0.03^{b}
5th week	138.47±16.65^{a}	2.97±0.01^{e}	1.32±0.01^{c}	1.64±0.01^{e}	0.80±0.01^{a}
6th week	153.84±1.11^{a}	2.93±0.01^{e}	1.37±0.01^{c}	1.56±0.03^{e}	0.88±0.01^{a}

Proteins are the most important and abundant macromolecules in living organisms, which play a vital role in architecture and physiology of the cell and in cellular metabolism. Also, serum proteins play an important role in the maintenance of osmotic balance between the circulating blood and the tissue membrane. Results revealed hypoproteinaemia, hypoglobulinaemia, hypoalbuminaemia and increased A/G ratio in Atrazine exposed *C. gariepinus* during short- and long-term exposure in comparison to the control groups. These results are supported by [12, 13].

The reduction in protein could be attributed to adjustment of the fish to its new environmental condition as a result of stress response [14]. So, under stress condition, fish secrete high amounts of catecholamine which deplete glycogen reserves. Unlike mammals, fish consume protein and do not store it in the body tissue for muscle energy when carbohydrate source is absent and hence, the exposed fish meet their extra energy requirements from body proteins, which are mobilized to produce glucose for fish by the process of gluconeogenesis. The depletion of the protein fractions in body tissues may be due to their degradation and possible utilization of degraded products for metabolic purposes. The decrease in serum globulin may be due to the increase in antibodies as a result of atrazine effect. Also, α-globulin decreased in response to the inflammatory reaction of the pesticide. On the other hand, these results disagree with Glusczak *et al.* (2007) who recorded a marked increase in serum total protein level of silver catfish, *R. quelen* exposed to glyphosate herbicide. In addition, Moraes *et*

al. (2009) and Velisek *et al*. (2011) recorded no significant changes in serum total protein and albumin concentrations of *Leporinus obtusidens* and *Cyprinus carpio* exposed to (376 µg/l) clomazone herbicide and (0.2 and 2 µg/l) terbutryn for 90 days and 28, 90 days, respectively. This may be attributed to different tested chemicals and the differences in exposure periods or fish species.

Conclusion

In conclusion, of this study showed that acute and chronic exposures to Atrazine were associated with changes in the behavioral, haematological and biochemical parameters of African catfish, *C. gariepinus*. The alterations of these parameters may provide a better understanding of the toxicological endpoint of aquatic pollutants and to ascertain a safer level of these chemicals in the aquatic environment for protection of aquatic organisms.

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