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Atrazine Toxicity in Nile Tilapia *[Oreochromis niloticus*].

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ABSTRACT

The herbicide atrazine is currently the most popular in the world's agriculture. The aim of the current study was to assess the toxicity of the commercial herbicide atrazine [Gesaprim] and its diverse effects on the haematological and biochemical indices in Nile Tilapia [*Oreochromis niloticus*]. By using probit analysis, the atrazine's 96-hour LC50 was determined [29.889 mg/L]. Fish were subjected to atrazine at varying concentrations [1/2 LC50=14.945 mg/l, 1/3 LC50=9.96 mg/l, 1/5 LC50 = 5.978, and 1/10 =2.989 mg/l] for a month in the study on chronic toxicity. WBC, MCHC, MCH, cholesterol, triglycerides, creatinine, uric acid, glucose, AST, and ALT demonstrated a significant increase with increasing concentrations and days [$P<0.05$], whereas RBCs, Hb, Ht, MCV, protein, albumin, and globulin demonstrated a significant decrease [$P<0.05$]. Hence, the study has demonstrated that atrazine exposure is hazardous to *O. niloticus*. Therefore, the use of the herbicide atrazine must be at a minimum level and these parameters can be used as effective guidelines for the toxic level indices for farmed tilapia fish.

Key Words:

Atrazine, chronic toxicity, hazardous, biochemical indices, probit analysis.

1. INTRODUCTION

Nowadays, more than 500 substances have been recognized as herbicides globally, and they are frequently employed in contemporary agriculture to manage weeds [1]. In Egypt , herbicides use in Egypt has increased considerably in the past few decades, During 2018, approximately 4510 tons of herbicides were utilized according to Egypt's Agricultural Pesticides Committee [2]. Due to drift, runoff, or evaporation, several herbicides that provide season-long weed control exhibit significant levels of persistence in the crop, soil, and natural water. Atrazine is an herbicide of the triazine class, It is most likely the pesticide that is applied most frequently globally. Moreover, due to the high mobility of atrazine through soil [3], it is one of the pollutants that are most frequently discovered in surface and ground rivers as a result of surface runoff from treated agricultural land [4]. Since atrazine is soluble in water, it can travel great distances [5]. Through photolysis, atrazine is progressively broken down. ATR has a varied half-life, ranging from 14 days to 4 years in soil and 6 months to several years in water

because it is resistant to microbial breakdown and is consequently stable in soil and water [6, 7]. As a result, because of its bioaccumulative and non-biodegradable characteristics, it may be hazardous to the environment because it can get into water bodies and kill fish and other aquatic organisms [8].

Fish Since exposed directly to chemicals from agricultural production through surface run-off or indirectly through the food chain of ecosystems, so they play a crucial role in determining possible danger associated with contamination in aquatic environments [9- 11].

Toxicology tests are crucial for determining an animal's susceptibility to a substance and are also helpful for determining the extent of organ damage and the ensuing physiological, biochemical, and behavioral abnormalities [12, 13]. Studies on the toxicity of atrazine in several fish species show that the reactions vary greatly depending on the dose and the species [14]. Acute, subchronic, and chronic exposure studies on the harmful effects of atrazine have shown that it can cause oxidative stress [15, 16], behavioural abnormalities [17], or biochemical changes. Atrazine has been linked to adverse effects on immunological function, the reproductive system, and the detoxification system, according to certain research [18- 20]. Atrazine exposure may also result in histopathological modifications in the liver, kidney, gills, or other fish organs [17,21]. It is crucial to gather data on the toxicity of this herbicide and its effects on a few regional species of fish in order to augment risk assessment studies of this herbicide.

O. niloticus is a species with economic and ecological relevance for fisheries and aquaculture, and because of its rusticity, rapid development, and adaptation to different production systems, it is one of the most significant species for aquaculture in Egypt [22]. *O. niloticus* are also a great test system for toxicity assessments of water contaminants because of their extensive distribution in freshwater environments, year-round availability, ease of acclimation to laboratory settings, and commercial relevance [7, 9, 23].

The aim of the present study is to determine the value of atrazine LC50 /96h in *O. niloticus* and assess the atrazine toxicity and its impacts on some haematological and biochemical indices in *O. niloticus*.

2. EXPERIMENTAL

2.1 Test chemical and fish:

For the present study commercial herbicide atrazine [6-Chloro-N2-ethyl-N4-isopropyl-1,3,5-triazine-2,4-diamine] 1,3,5-triazine] with the trade name [Gesaprim 80 WP] and purity of 80% [wt/wt] was purchased from the market. Healthy and active specimens of Tilapia Fish [initial body weight=40–50 g] were exposed to 0.05% potassium permanganate [KMnO4] for two minutes to prevent any cutaneous infections. They were subsequently acclimated for two weeks in a lab setting using semi-static systems. The experiment was held in a private fish farm established for experimental purposes and located at awlad hamam village –Damietta. The analysis of blood parameters were held in in El-serw fish farm, water pollution lab.

2.2 Determination of LC50:

Atrazine's 96-hour LC50 value was calculated using acute toxicity bioassays that were run in a laboratory setup. The aquarium was filled with atrazine that had been dissolved in filtered, distilled water. Every 24 hours, the water containing the herbicide was changed, and fresh atrazine solution was added to make up for the declining herbicide concentrations caused by the water's hydrolysis. The experiment was carried out in glass aquariums with 40 L of dechlorinated, gently aerated tap water that received continuous additional aeration. The nominal atrazine concentrations [1, 10.5, 21.0, 31.5, 42.0 and 52.5 mg/l] were randomly applied to a group of 10 fish specimens. Fish mortality was monitored for 96 hours as part of an experiment set up in triplicate to determine the test herbicide's LC50 values. Using the [24] probit analysis, the LC50 value of atrazine for *O. niloticus* was calculated to be 29.889 mg/l.

2.3 Effects of atrazine on *O. niloticus*:

Based on the 96 h LC50 values, one hundred and fifty apparently healthy *O. niloticus* were randomly divided into 5 treatments; [I- V] and were acclimated to aquaria conditions for a week before the experiment was initiated. Each treatment contained thirty fish which were maintained in three glass aquaria [40L]. Fish were exposed to different concentrations of atrazine [1/10 LC50=2.989 mg/l, 1/5 LC50 = 5.978 mg/l, 1/3 LC50=9.96 mg/l, and1/2 LC50=14.945 mg/l; groups Π− V respectively] for 30 days with three triplicates for each treatment. As a control [0.0 mg/l], a group of ten fish were kept in dechlorinated tap water [40-L], and aerated with a compressor and air stones. The water was changed totally every day. The meals were given to the fish twice daily for four weeks at a feeding rate of 3% of their real body weight each day. The exposed fish were carefully monitored for any clinical anomalies, postmortem lesions, or fatalities.

2.4 Biochemical profile:

Blood samples were collected at the end of the experimental period from the caudal vessels in Eppendorf tubes. Haematological variables included white blood cell count [WBC], hemoglobin concentration [Hb], haematocrit [Ht], red blood cell count [RBC], mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH] and mean corpuscular hemoglobin concentration [MCHC] were analyzed by using a Mindary BC-2800 Auto Hematology Blood Analyzer.

Serum was obtained from blood samples by centrifugation at 3000 rpm for 15 min. Measurement of biochemical markers was conducted using the Roche Hitachi Cobas [C311]. The biochemical profile included cholesterol, triglyceride, albumin, globulin, creatinine, total bilirubin, protein, uric acid, glucose, glutamate oxaloacetate transaminase [AST/GOT] and glutamate pyruvate transaminase [ALT/GPT]

2.5 Statistical analysis

A statistical analysis [SPSS 15.0 for Microsoft Windows, SPSS Inc.] was carried out. Mean ±SD was used to describe the results. ANOVA and the student t-test were used to analyze differences in continuous variables. Each test had two tails, and the level of significance for statistical analysis was set at <0.05.

3. RESULTS

3.1 The Lethal Concentration 50 [LC] of atrazine in *O. niloticus*:

The LC was calculated according to the relationship between the survival rate and different doses of atrazine at the sequent periods [0-96 hr]. *O. niloticus* was exposed to different concentrations [1, 10.5, 21.0, 31.5, 42.0 and 52.5 mg/l] of atrazine 80% to determine the LCD50 value. *O. niloticus* at different exposure doses showed no mortality at 1, 10.5, mg/l, 10% mortality at 21 mg/l, 55.56% mortality at 31.5 mg/l, 88.89% mortality at 42 mg/l and 100% mortality at 52.5 mg/l.

Atrazine levels increased along with the rate of mortality [Fig. 1]. The groups with the greatest doses [21-52.5 mg/l] showed alterations in fish behavior. Both the control group and the experimental group exposed to atrazine at concentrations of 1 and 10.5 mg/l showed no behavioral effects. Atrazine's LC50 value for *O niloticus* after 96 hours was 29.889 mg/l.

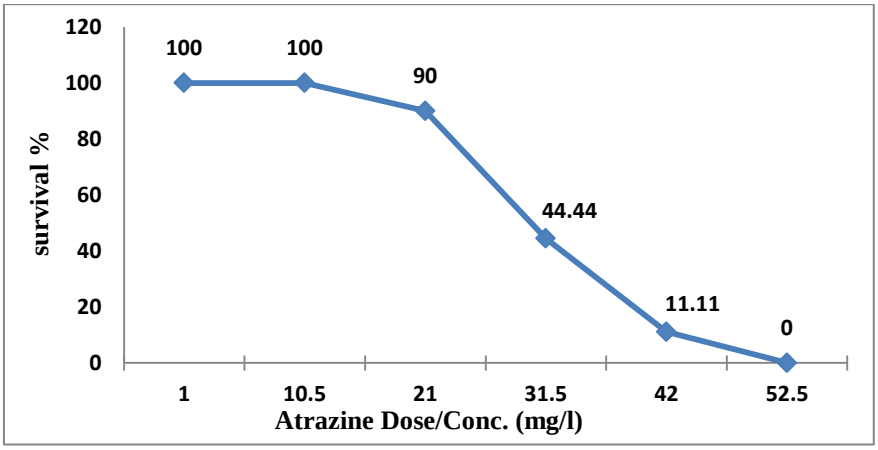

Figure [1]: Survival % of *O. niloticus* exposed to different doses of Atrazine in [LCD_{50}] experiment.

3.2 Mortality rate and behavioral changes of fish in the subacute toxicity experiment:

The toxicity of subacute doses of atrazine was conducted for 30 days by exposing *O. niloticus* to different concentrations of atrazine [1/2 LC50=14.945 mg/l, 1/3 LC50=9.96 mg/l, 1/5 LC50=5.978 mg/l, and 1/10 LC50=2.989 mg/l in groups I-V].

No changes in behavior were observed in the control group [I] and experimental group exposed to atrazine of 2.989 and 5.978 mg/l [groups II and III]

Whereas groups IV and VI showed behavioral changes as lack of responses, erratic swimming and loss of appetite compared to control and no mortality occurred in any group.

3.3Hematological Profile of fish blood exposed to subacute doses of atrazine:

The fish were exposed for 30 days to different concentrations of atrazine as: experimental group I [control= 0.0 mg/l], group II [1/10 LC50=2.989 mg/l], group III [1/5 LC50=5.978 mg/l], group IV [1/3 LC50=9.96 mg/l], and V [1/2 LC50=14.945 mg/l]. The results of hematological examination of blood samples are given in Table [1].

It is evident that, the exposure to atrazine resulted in significant changes in almost all hematological indices [Fig. 2]. A several fold significant decrease [P<0.05] in erythrocyte count [1.5-3.71 fold] subsequently led to a significant decrease in hemoglobin concentration [1.55-1.84 fold] and hematocrit value [1.27-4.42 fold] in all the experimental groups compared to the control. A non-significant increase [P<0.05] in white blood cells [1.04-1.14 fold] and MCH [1.05-114] were observed in all the experimental groups exposed to atrazine compared to the control. A significant decrease [P<0.05] in MCV was found in the experimental groups IV and V [9.96 and 14.945 mg/l, respectively], while A non-significant decrease was found in experimental groups II and III [2.989 and 5.978 mg/l, respectively] compared to the control. On the other hand, significant increase was obtained [P<0.05] in almost all groups in the case of MCHC [1.19-1.33 fold].

Table 1. Haematological alterations in *Oreochromis niloticus* after exposure to sub-lethal concentration of atrazine

Group*	**WBC[10^3 /ml]**	**RBC [10^6/ml]**	**Hb [g/dL]**	**Ht [%]**	**MCV [Fi]**	**MCH [Pg]**	**MCHC [g/dL]**
Group I	19.7±4.6	2.6±0.7	5.9±1.5	0.84±0.2	160.5±29.2	50.4±18.9	42.2±11.6
Group II	20.5±4.9	1.7±0.4	3.8±1.1	0.66±0.1	156.7±20.7	55.6±20.3	50.31±12.6
Group III	21.0±5.1	1.2±0.3	3.8±0.9	0.47±0.1	154.8±18.7	53.0±15.4	53.0±14.7
Group IV	21.3±5.3	0.9±0.3	3.6±0.7	0.24±0.06	145.0±16.3	57.5±21.9	55.53±13.9
Group V	22.5±5.3	0.7±0.2	3.2±0.6	0.19±0.04	139.4±14.9	54.7±16.8	56.1±16.7
Significance							
Over all [ANOVA]	0.509	<0.0001	<0.0001	<0.0001	0.0004	0.6529	0.0011
Post hoc Tukey's test							
Group I *Vs.* Group II	0.517	<0.0001	<0.0001	<0.0001	0.563	0.308	0.012
Group I *Vs.* Group III	0.304	<0.0001	<0.0001	<0.0001	0.372	0.561	0.0025
Group I *Vs.* Group IV	0.315	<0.0001	<0.0001	<0.0001	0.014	0.184	0.0002
Group I *Vs.* Group V	0.078	<0.0001	<0.0001	<0.0001	0.0008	0.356	0.0004
Group II *Vs.* Group III	0.700	<0.0001	0.993	<0.0001	0.711	0.578	0.448
Group II *Vs.* Group IV	0.706	<0.0001	0.404	<0.0001	0.018	0.729	0.134
Group II *Vs.* Group V	0.259	<0.0001	0.011	<0.0001	0.0005	0.852	0.143
Group III *Vs.* Group IV	0.993	0.0003	0.341	<0.0001	0.035	0.361	0.501
Group III *Vs.* Group V	0.459	<0.0001	0.003	<0.0001	0.0008	0.684	0.448
Group IV *Vs.* Group V	0.468	0.0036	0.021	0.0004	0.170	0.581	0.880

Different atrazine concentrations: experimental group I [control= 0.0 mg/l], group II [1/10 LC50=2.989 mg/l], group III [1/5 LC50=5.978 mg/l], group IV [1/3 LC50=9.96 mg/l], and V [1/2 LC50=14.945 mg/l]. WBC: white blood cells, RBC: red blood cells, Hb: hemoglobin, Ht: haematocrit, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration. Data were expressed as mean± standard deviation [SD]*. *P*<0.05 is considered significant.

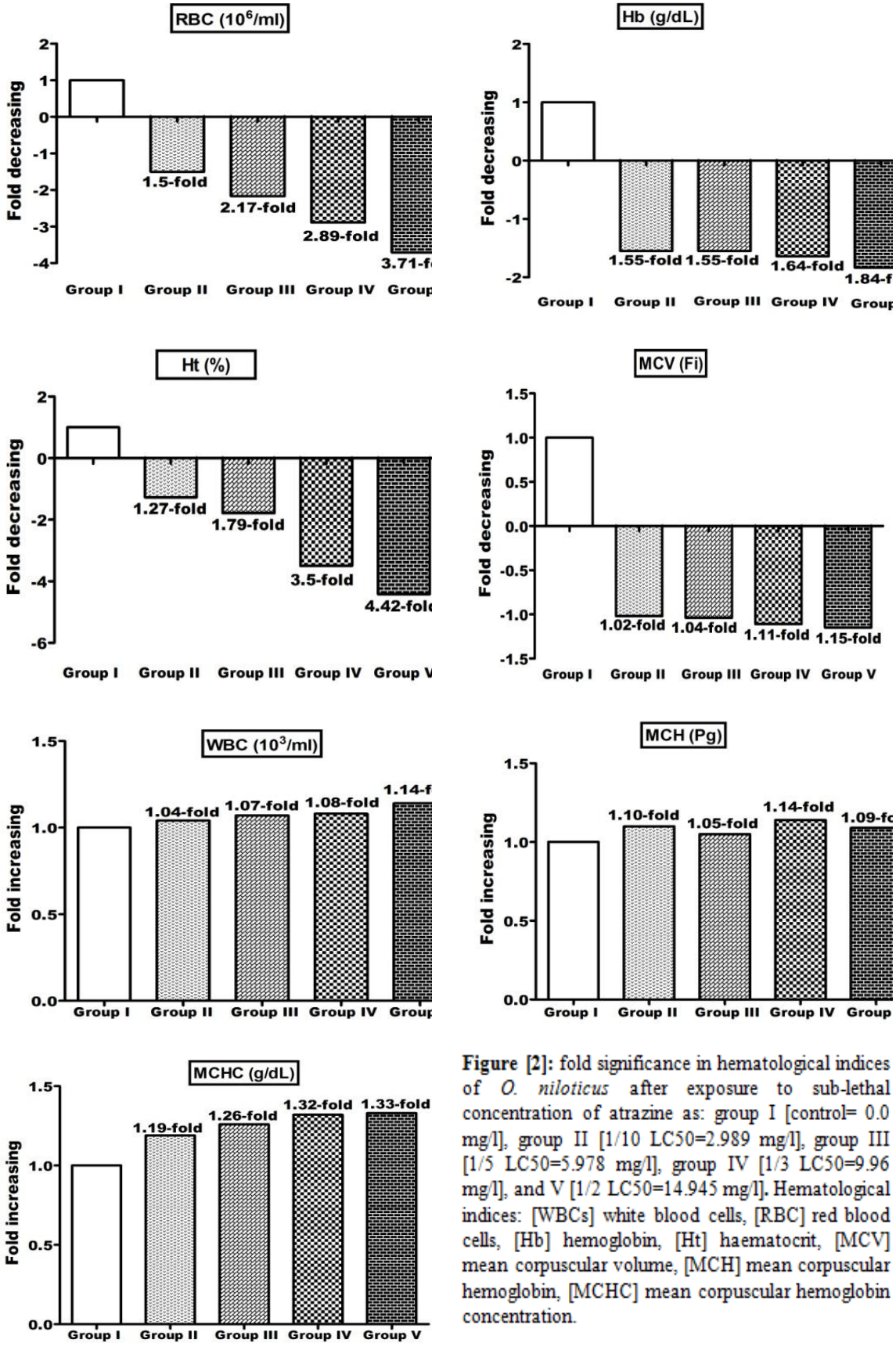

Figure [2]: fold significance in hematological indices of *O. niloticus* after exposure to sub-lethal concentration of atrazine as: group I [control= 0.0 mg/l], group II [1/10 LC50=2.989 mg/l], group III [1/5 LC50=5.978 mg/l], group IV [1/3 LC50=9.96 mg/l], and V [1/2 LC50=14.945 mg/l]. Hematological indices: [WBCs] white blood cells, [RBC] red blood cells, [Hb] hemoglobin, [Ht] haematocrit, [MCV] mean corpuscular volume, [MCH] mean corpuscular hemoglobin, [MCHC] mean corpuscular hemoglobin concentration.

3.4 Biochemical Indices.

Results of biochemical indices are shown in Table [2]. After 30-day exposure, there were significant alterations [P<0.05] in the concentrations of protein, albumin, globulin, glucose, cholesterol, triglyceride, uric acid, and creatinine as well as in the activities of glutamate oxaloacetate transaminase [AST] and glutamate pyruvate transaminase [ALT] compared to the control group.

A strong significant increase [P<0.05] in the total protein was found in all groups [1.26-1.42 fold] except group II with non-significant increase [1.11 fold] compared to the control [group I]. Albumin concentrations increased significantly [P<0.05] with [1.16 and 1.36 fold] in the experimental groups III and IV and increased non-significantly [P<0.05] in the other groups compared to the control [Figure 3]. On the other hand, a strong significant increase in globulin concentrations [1.15-163 fold] was found in all groups compared to the control. Glucose showed a several-fold significant increase [P<0.05] in most of the experimental groups [1.26-3.16 fold] compared to the control except in group IV [1.11 fold]. Cholesterol and triglyceride values increased significantly [P<0.05] in the experimental groups III and IV and non-significantly [P<0.05] in the experimental groups II and V compared to the control. A significant increase [P<0.05] in uric acid levels was found in most of the experimental groups [1.18-1.47 fold] while in group V the levels was increased non-significantly [1.12 fold]. On the other hand, a several-fold significant increase was observed in all experimental groups in the case of creatinine [5.5-14 fold]. AST and ALT activities in all the experimental groups were significantly higher by up to 1.17-2.09 fold for AST and 1.41-3.5 for ALT compared to the control.

Table 2. Biochemical alterations in *Oreochromis niloticus* after exposure to sub-lethal concentration of atrazine

roup*	rotein [g/dl]	lbumin [g/dl]	lobulin [g/dl]	lucose [mg/dl]	holesterol [mg/dl]	riglycerides [mg/dl]	ric acid [mg/dl]	reatinine [mg/dl]	ST [U/dL]	LT [U/dL]
Group I	1.9±0.5	0.8±0.20	1.1±0.28	38.0±9.5	82.0±21.0	85.0±24.3	1.7±0.43	0.02±0.01	96.0±25.0	12.0±4.1
Group II	2.1±0.5	0.84±0.23	1.26±0.32	79.0±19.9	87.0±22.3	92±27.0	2.5±0.63	0.12±0.02	129.0±33.3	18.0±4.3
Group III	2.4±0.6	0.93±0.24	1.47±0.37	120.0±30.1	101.0±26.3	107±30.8	2.2±0.55	0.11±0.03	112.0±30.4	17.0±3.9
Group IV	2.6±0.7	1.09±0.27	1.51±0.38	42.0±10.8	116.0±30.4	130±38.4	2.0±0.5	0.28±0.07	113.0±29.5	35.0±9.8
Group V	2.7±0.8	0.91±0.23	1.79±0.45	48.0±12.2	92.0±23.0	98.0±26.5	1.9±0.48	0.27±0.07	201.0±60.4	42.0±11.0
Significance										
***P* value [ANOVA]**	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Post hoc Tukey's test										
Group I *Vs.* Group II	0.127	0.475	0.044	<0.0001	0.375	0.294	<0.0001	<0.0001	<0.0001	<0.0001
Group I *Vs.* Group III	0.0009	0.026	<0.0001	<0.0001	0.003	0.003	0.0002	<0.0001	0.029	<0.0001
Group I *Vs.* Group IV	<0.0001	<0.0001	<0.0001	0.133	<0.0001	<0.0001	0.016	<0.0001	0.019	<0.0001
Group I *Vs.* Group V	<0.0001	0.053	<0.0001	0.0008	0.083	0.052	0.095	<0.0001	<0.0001	<0.0001
Group II *Vs.* Group III	0.039	0.143	0.022	<0.0001	0.030	0.049	0.054	0.134	0.041	0.448
Group II *Vs.* Group IV	0.0023	0.0003	0.007	<0.0001	<0.0001	<0.0001	0.001	<0.0001	0.054	0.134
Group II *Vs.* Group V	0.0009	0.243	<0.0001	<0.0001	0.396	0.389	0.0001	<0.0001	<0.0001	0.132
Group III *Vs.* Group IV	0.239	0.018	0.681	<0.0001	0.046	0.013	0.146	<0.0001	0.898	0.501
Group III *Vs.* Group V	0.106	0.743	0.004	<0.0001	0.164	0.230	0.028	<0.0001	<0.0001	0.448
Group IV *Vs.* Group V	0.608	0.007	0.012	0.048	0.001	0.0004	0.433	0.582	<0.0001	0.880

*Different atrazine concentrations [mg/l]: [Group I [control]= 0.00, Group II=2.989, Group III=5.978, Group IV=9.96, and Group V=14.945]. AST: glutamic oxaloacetic transaminase, ALT: glutamic pyruvic transaminase. Data were expressed as mean± standard deviation [SD]. *P*<0.05 is considered significant.

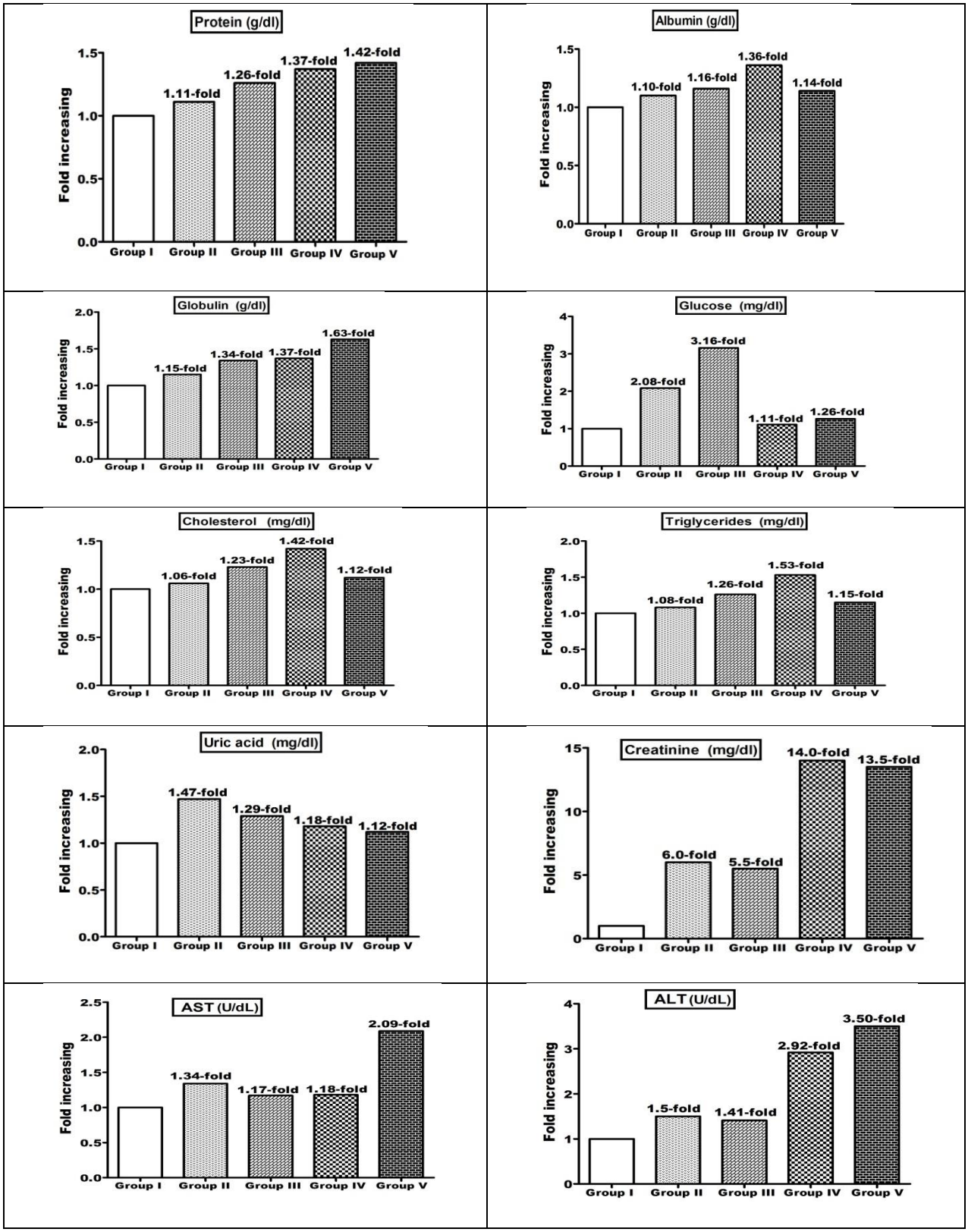

Figure [3]: Fold significance in hematological indices of *O. niloticus* after exposure to sub-lethal concentration of atrazine as: group I [control= 0.0 mg/l], group II [1/10 LC50=2.989 mg/l], group III [1/5 LC50=5.978 mg/l], group IV [1/3 LC50=9.96 mg/l], and V [1/2 LC50=14.945 mg/l]. AST: glutamic oxaloacetic transaminase, ALT: glutamic pyruvic transaminase..

4. DISCUSSION

Previous research that evaluated the effects of atrazine on fish survival at concentrations of 100 µg/l or greater showed variations in fish susceptibility to the chemical. *Salmo salar* exposed to 100 µg atrazine/l for 21 days was reported to have died [9%] [25]. *O. mykiss* treated to a high concentration of 55 µg atrazine/l followed by a bacterial challenge was also reported to have died [26].

On the other hand, studies reported no effects on survival were observed in several fish *[Salmo salar*, *Prochilodus lineatus, Oncorhynchus mykiss*, *Cyprinus carpio* and *Rhamdia quelen species* at concentrations up to 100 µg atrazine/L [27-31, 15, 19]. This may be due to the difference in the concentration of the used atrazine and/or fish species difference. The impact of atrazine on aquatic life, particularly fish physiology, has been described [33-39]. When compared to the control group, *O. nitoticus* subjected to atrazine showed a decrease in its haematological profile, including RBC, Hb, and Ht. As a result of atrazine's potential interference with the haem or globin synthesis pathway, a decrease in Hb content may be a sign of a loss in Hb synthesis as well as a decrease in oxygen carrying ability.

Also, Ht value was significantly decreased with the reduction in RBC count and the increase the concentration of atrazine for exposure periods [30 days]. Retraction of RBC can cause a substantial drop in hematocrit, which causes fish anaemia. The reason for the low numbers could be an increase in RBC breakdown or a decrease in RBC synthesis. Hematological changes were determined to be significant [$p<0.05$]. WBC counts increased during atrazine's hazardous exposure period. It suggests that fish can evolve a coping strategy to deal with the hazardous stress.

similar studies agreed with our findings and reported a significant decrease in values of RBCs count, Ht, and Hb content compared to the control groups after exposure to atrazine [40]. According to [33], *O. nitoticus* and *Chrysichthyes auratus'* red blood cell, HBG, and HCT levels decreased after exposure to 3 and 6 mg/l atrazine. Moreover, Puigdoller et al. [25] described the significant increase in HCT level in Atlantic salmon following atrazine exposure. [41] found that the quantity of erythrocytes in *C. carpio* decreased when exposed to 0.1mg/l ATR.

Changes in erythrocyte amount suggest a remedy for the body's lack of oxygen caused by the death of its gills, and fluctuations in erythrocyte level show a release of erythrocytes from the blood [42]. In addition, Prasad et al. [32] observed that Tilapia mosambica that had been exposed to 1.1 mg/l of atrazine had suffered damage to its gill lamellae, which causes a reduction in respiratory volume. The decrease in red blood cell quantity is caused by erythropoiesis' reluctance and an increase in the degree of erythrocyte destruction in hematopoietic structures, both of which are trending downward [43].

In fish exposed to atrazine, [44] demonstrated a notable decrease in red blood cells, Hb, and PCV and identified the toxic effects of atrazine on the liver, kidney, and spleen. Schizothorax plagiostomus exposed to atrazine underwent considerable changes in all haematological parameters, according to Akhtar et al in 2021 [39]. Higher concentrations had a significant impact on those exposed to them.

Ramesh et al. [45] reported catastrophic effects of atrazine on the haematology of Cyprinus carpio and hypothesised that atrazine significantly changed the evaluated haematology factor intensities. Fish's homeostatic system is susceptible to reversible changes brought on by environmental pollutants because blood cell indices like MCV, MCH, and MCHC are so sensitive.

Throughout the course of the experiment in the current investigation, the levels of MCH and MCHC considerably rose in comparison to the control. These increases might signify macrocytic anaemia [46, 47]. A variety of reactions to atrazine were observed in *Schizothorax plagiostomus*, which resulted in a notable decrease in MCH, MCHC, monocytes, and lymphocytes in the group exposed to 4 ppm [38].

In order to identify problems in the liver and other tissues, it is crucial to measure blood biochemical markers [48,49]. The fish's protein concentration could be used as a stress indication as well as a general

health indicator [44]. We observed increases in albumin and total protein during the experiment. Similar findings in common carp[Cyprinus carpio L.] were reported by Blahova et al., [16].

According to [50], an increase in energy demand may increase protein consumption, where the protein is converted into energy, and cause a decrease in protein serum. Blood chemistry imbalance is a significant indicator of kidney disease. Increased kidney protein production into the circulation results from kidney damage, and it may also cause finger-sized fish's protein serum levels to drop.

According to Hussein et al. [33] , the total protein of *Chrysichthyes auratus* and *Oreochromis niloticus* decreased because of globulin reduction, demonstrating the toxin atrazine's impact on the fish immune system. According to Gluth and Hanke [51], carp species exposed to atrazine for 72 hours at a concentration of close to 100 g/L showed a significant decrease in blood protein concentrations as a result of the fish group's blood's thinning impact.

A sensitive biomarker of environmental stress for any chemical pollutant, including pesticides, has been demonstrated to be blood glucose [52]. The current study found that fish produced more glucose to provide more energy to combat the toxicity of atrazine, as seen by the rise in value of glucose compared to the control. Recent research suggests that the hypoxic conditions brought on by the herbicide atrazine may be responsible for the drop in glucose levels in the blood following poisoning. Hussein et al. [33] discovered a notable drop in blood serum glucose. The harmful effects of the toxin on the fish's kidney may be the cause of this decline [53]. Inhibiting glycogenolysis and glycogenesis under toxic stress results in increased glucose synthesis [54]. The sub-lethal exposure to atrazine at [1/2 LC50=14.945 mg/l, 1/3 LC50=9.96 mg/l, 1/5 LC50=5.978 mg/l, and 1/10 LC50=2.989 mg/l] resulted in a significant increase in glucose concentration, demonstrating the response of exposed fish to metabolic stress.

In the current study, all experimental groups have significantly higher cholesterol levels than the control group. All steroid hormones are built on a foundation of cholesterol. When it does, as a result of the production of cortisol, a lot of cholesterol is required [55]. In the Akhtar, et al. [39] study, all experimental groups exposed to various amounts of atrasine experienced a significant rise in cholesterol.

Triglyceride is a primary source of oils and fats that flow into the circulation and is used as a form of storage for fats. The results showed that there were higher levels of triglyceride content than in the control group. According to Yang and Chen [56], elevated levels are a result of hepatitis and a lack of glycogen storage capacity. Glycogen not only gives the body energy, but it also serves as a key marker for lipid metabolism and the body's nutritional status.

By raising atrazine concentrations, uric acid and creatinine levels in fish serum considerably rose. Both variables are common screening markers for renal structural integrity and function. The elevated levels of uric acid and creatinine showed that atrazine toxicity had a significant impact on kidney function, possibly as a result of the water-born atrazine's effect on glomeruli filtration rate and/or pathological kidney alterations that led to dysfunction. Similar findings were made by Akhtar et al. [39], who discovered a significant rise in creatinine levels in all experimental groups exposed to various atrazine dosages.

AST and ALT, which are liver-specific enzymes, are more accurate indicators of hepatotoxicity. Several researchers have documented the alterations in the enzyme kinetics of the organs and blood of fish subjected to various contaminants or stressors [57]. For instance, Akhtar et al. [39], reported that levels of LDH, ALP, AST, and ALT had changed significantly. ALT and AST levels both significantly rose. ALP activity increased in the experimental groups [1 ppm and 2 ppm], and then decreased in the experimental groups [3ppm and 4ppm].

5. CONCLUSION

This study concluded that atrazine is lethal *to O. niloticus* and can cause changes in biochemical and hematological parameters. Therefore, the use of the herbicide atrazine must be at a minimum level and these parameters can be used as effective guidelines for the toxic level indices for farmed tilapia fish.

6. REFERENCES

[1] Zhang Y, Yang J, Shi R, Su Q, Yao L, Li P [2011] Determination of acetanilide herbicides in cereal crops using accelerated solvent extraction, solid-phase extraction and gas chromatography-electron capture detector. J Sep Sci 34:1675–1682.

[2] Agricultural Pesticides Committee [APC], Egypt. [2019] http://www.apc.gov.eg/AR/.

[3] Phyu YL, Warne MS, Lim RP [2006] Toxicity and bioavailability of atrazine and molinate to the freshwater fish [Melanotenia fluviatilis] under laboratory and simulated field conditions. Sci Total Environ 356:86–99.

[4] Zheng L, ZhangY,Yan Z, Zhang J, Li L, ZhuY, ZhangY, Zheng X,Wu J, Liu Z [2017] Derivation of predicted no-effect concentration and ecological risk for atrazine better based on reproductive fitness. Ecotox Environ Safe 142:464–470.

[5] Stara, A., Kouba, A., & Velisek, J. [2018]. Biochemical and histological effects of sub-chronic exposure to atrazine in crayfish Cherax destructor. *Chemico-biological interactions*, *291*, 95-102.

[6] Guan, Y. H., Ma, J., Ren, Y. M., Liu, Y. L., Xiao, J. Y., Lin, L. Q., & Zhang, C. [2013]. Efficient degradation of atrazine by magnetic porous copper ferrite catalyzed peroxymonosulfate oxidation via the formation of hydroxyl and sulfate radicals. Water research, 47[14], 5431-5438.

[7] Kanu, K. C., Otitoloju, A. A., & Amaeze, N. H. (2022). Survival analysis of African catfish and Nile tilapia briefly exposed to complex pesticide mixtures. *The Journal of Basic and Applied Zoology*, *83*(1), 1-15.

[8] Mhadhbi, L.; Beiras, R. Acute Toxicity of Seven Selected Pesticides [Alachlor, Atrazine, Dieldrin, Diuron, Pirimiphos-Methyl, Chlorpyrifos, Diazinon] to the Marine Fish [Turbot,Psetta M_axima]. Water. Air. Soil Pollut. 2012, 223, 5917–5930.DOI: 10.1007/s11270-012-1328-9.

[9] de Campos Ventura, B., de Angelis, D. D. F., & Marin-Morales, M. A. [2008]. Mutagenic and genotoxic effects of the Atrazine herbicide in Oreochromis niloticus [Perciformes, Cichlidae] detected by the micronuclei test and the comet assay. *Pesticide Biochemistry and Physiology*, *90*[1], 42-51.

[10] Nwani, C. D., Lakra, W. S., Nagpure, N. S., Kumar, R., Kushwaha, B., & Srivastava, S. K. [2010]. Toxicity of the herbicide atrazine: effects on lipid peroxidation and activities of antioxidant enzymes in the freshwater fish Channa punctatus [Bloch]. *International journal of environmental research and public health*, *7*[8], 3298-3312.

[11] Van Der Kraak, G. J., Hosmer, A. J., Hanson, M. L., Kloas, W., & Solomon, K. R. [2014]. Effects of atrazine in fish, amphibians, and reptiles: An analysis based on quantitative weight of evidence. *Critical reviews in toxicology*, *44*[sup5], 1-66.

[12] Matsumoto, S. T., Mantovani, M. S., Malaguttii, M. I. A., Dias, A. L., Fonseca, I. C., & Marin-Morales, M. A. [2006]. Genotoxicity and mutagenicity of water contaminated with tannery effluents, as evaluated by the micronucleus test and comet assay using the fish Oreochromis niloticus and chromosome aberrations in onion root-tips. *Genetics and Molecular Biology*, *29*[1], 148-158.

[13] Zhang, T., Qu, Z., Li, B., & Yang, Z. [2019]. Simultaneous Determination of Atrazine, Pendimethalin, and Trifluralin in Fish Samples by QuEChERS Extraction Coupled with Gas Chromatography-Electron Capture Detection. *Food Analytical Methods*, 1-8.

[14] Elia, A.C., Waller, W.T., Norton, S.J., 2002. Biochemical responses of bluegill sunfish [Lepomis macrochirus, Rafinesque] to atrazine induced oxidative stress. Bull. Environ. Contam. Toxicol. 68, 809–816.

[15] Mela, M., Guiloski, I. C., Doria, H. B., Randi, M. A. F., de Oliveira Ribeiro, C. A., Pereira, L., ... & de Assis, H. S. [2013]. Effects of the herbicide atrazine in neotropical catfish [Rhamdia quelen]. Ecotoxicology and environmental safety, 93, 13-21.

[16] Blahova, J., Modra, H., Sevcikova, M., Marsalek, P., Zelnickova, L., Skoric, M., & Svobodova, Z. [2014]. Evaluation of biochemical, haematological, and histopathological responses and recovery ability of common carp [Cyprinus carpio L.] after acute exposure to atrazine herbicide. BioMed Research International, 2014.

[17] Plhalova, L., Blahova, J., Mikulikova, I., Stepanova, S., Dolezelova, P., Praskova, E., ... & Svobodova, Z. [2012]. Effects of subchronic exposure to atrazine on zebrafish [Danio rerio]. Polish journal of veterinary sciences, 15[3].

[18] Tillitt, D. E., Papoulias, D. M., Whyte, J. J., & Richter, C. A. [2014]. Atrazine Reduces Reproduction in Fathead Minnow *[Pimephales promelas]*—Raw Data Report [No. 805]. US Geological Survey.

[19] Kreutz, L. C., Barcellos, L. J. G., dos Santos, E. D., Pivato, M., & Zanatta, R. [2012]. Innate immune response of silver catfish [Rhamdia quelen] exposed to atrazine. Fish & shellfish immunology, 33[4], 1055-1059.

[20] Fu Y, Li M, Liu C, Qu JP, Zhu WJ, Xing HJ, Xu SW, Li S [2013] Effect of atrazine and chlorpyrifos exposure on cytochrome P450 contents and enzyme activities in common carp gills. Ecotoxicol Environ Saf 94:28–36.

[21] Paulino, M. G., Souza, N. E. S., & Fernandes, M. N. [2012]. Subchronic exposure to atrazine induces biochemical and histopathological changes in the gills of a Neotropical freshwater fish, Prochilodus lineatus. Ecotoxicology and environmental safety, 80, 6-13.

[22] Watanabe, W. O.; Losordo, T. M.; Fitzsimmons, K.; Hanley, F.Tilapia Production Systems in the Americas: Technological Advances, Trends, and Challenges. Rev. Fish. Sci. 2002, 10, 465–498. DOI: 10.1080/20026491051758.

[23] Finney, D.J., 1971. Probit Analysis. 1st Edn., Cambridge University Press, Cambridge, ISBN-10: 0521135907, pp: 272.

[24] Nieves-Puigdoller et al. 2007] Nieves-Puigdoller K, Björnsson BT, and McCormick SD. [2007]. Effects of hexazinone and atrazine on the physiology and endocrinology of smolt development in Atlantic salmon. Aquat Toxicol, 84, 27–37.

[25] Shelley LK, Ross PS, Miller KM, Kaukinen KH, and Kennedy CJ. [2012]. Toxicity of atrazine and nonylphenol in juvenile rainbow trout [Oncorhynchus mykiss]: Effects on general health, disease susceptibility and gene expression. Aquat. Toxicol, 124–125, 217–26 aquatox.2007.05.011.

[26] Matsumoto J, Hosmer AJ, and Van Der Kraak G. [2010]. Survival and iono-regulatory performance in Atlantic salmon smolts is not affectedby atrazine exposure. Comp Biochem Physiol C, 152, 379–84.

[27] Shenoy K. [2012]. Environmentally realistic exposure to the herbicide atrazine alters some sexually selected traits in male guppies. PLoS ONE, 7, e30611.

[28] Fu, Y., Li, M., Liu, C., Qu, J. P., Zhu, W. J., Xing, H. J., ... & Li, S. [2013]. Effect of atrazine and chlorpyrifos exposure on cytochrome P450 contents and enzyme activities in common carp gills. Ecotoxicology and environmental safety, 94, 28-36.

[29] Wang LL, Liu T, Wang C, Zhao FQ, Zhang ZW, Yao HD, et al. [2013]. Effects of atrazine and chlorpyrifos on the production of nitric oxide and expression of inducible nitric oxide synthase in the brain of common carp [*Cyprinus carpio* L.]. Ecotoxicol Environ Safety, 93, 7–12.

[30] Blahova, J., Modra, H., Sevcikova, M., Marsalek, P., Zelnickova, L., Skoric, M., & Svobodova, Z. [2014]. Evaluation of biochemical, haematological, and histopathological responses and recovery ability of common carp [Cyprinus carpio L.] after acute exposure to atrazine herbicide. BioMed Research International, 2014.

[31] Prasad, T. A. V., Srinivas, T., Rafi, G. M., & Reddy, D. C. [1991]. In Vivo Effect of Atrazine on Haematology and O2 Consumption in Fish Tilapia mossambica . Biochemistry International, 23, 157-161.

[32] Hussein, S. Y., El-Nasser, M. A., & Ahmed, S. M. [1996]. Comparative Studies on the Effects of Herbicide Atrazine on Freshwater Fish Oreochromis niloticus and Chrysichthyes auratusat Assiut, Egypt. Bulletin of Environmental Contamination and Toxicology, 57, 503-510. https://doi.org/10.1007/s001289900218

[33] Grobler, E., Van Vuren, J. J. J., & Du Preez, H. H. [1989]. Routine Oxygen Consumption of Tilapia sparrmanii [Cichlidae] Following Acute Exposure to Atrazine. Comparative Biochemistry and Physiology Part C: Comparative Pharmacology, 93, 37-42.

[34] Srinivas, T., Prasad, T. A. V., Raffi, G. M., & Reddy, D. C. [1991]. Effect of Atrazine on Some Aspects of Lipid Metabolism in Fresh Water Fish. Biochemistry International,23, 603-609.

[35] Prasad, T. A. V., Srinivas, T., Reddy, J. S., & Reddy, D. C. [1995]. Atrazine Toxicity on Transport Properties of Hemocyanin in the Crab Oziotelphusa senex senex. Ecotoxicology and Environmental Safety, 30, 124-126. https://doi.org/10.1006/eesa.1995.1015.

[36] Phyua YL, Warne MStJ, Lim RP [2006]. Toxicity and bioavailability of atrazine and molinate to the freshwater fish [Melanotenia fluviatilis] under laboratory and simulated field conditions. Sci. Total Environ 356: 86-99.

[37] Ahmad, W., Sultan, A., Jan, S. M. H., Ali, A., Rahman, I. U., Usman, H., Sher, A., Khan, N., Suleman, Khan, Z., & Khan, A. [2021]. Environmental Risk of Atrazine [Herbicide] to Brain,Gills and Liver Tissues of Fish Ctenopharyngodon idell [Valenciennes, 1844]. Journal of Geoscience and Environment Protection, 9, 1-16.

[38] Akhtar N., Khan M. F., Tabassum S., Zahran E. [2021] Adverse effects of atrazine on blood parameters, biochemical profile and genotoxicity of snow trout [Schizothorax plagiostomus]. Saudi Journal of Biological Sciences 28 [2021] 1999–2003.

[39] Kanu, K. C., Okoboshi, A. C., & Otitoloju, A. A. (2023). Haematological and biochemical toxicity in freshwater fish Clarias gariepinus and Oreochromis niloticus following pulse exposure to atrazine, mancozeb, chlorpyrifos, lambda-cyhalothrin, and their combination. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *270*, 109643.

[40] Horn, G., Hanke, W., 1980. Hmatologische Vernderungen bei karpfen unter dem Einflu v o n Atrazin. Forschungsbericht der Arbeitsgruppe kotoxikologie amZoologischen Institut der Universitt, Karlsruhe, In Hanke G. Untersuchungen berden Einfluvon Schadstoffen auf Fische.

[41] Drastichova, J., Svobodova, Z., Luskova, V., Machova, J., 2004. Effect of cadmium on hematological indices off common carp, Cyprinus carpio [L.]. Bull. Environ. Contam. Toxicol. 72, 725–732.

[42] Joshi, P.K., Bose, M., Harish, D., 2002. Changes in certain hematological parameters in a siluroid cat fish, Clarias baterchus [L.] exposed to cadmium chloride. Pollut. Res. 21, 129–131.

[43] Rehwoldt, R.E., 1978. Investigations into the acute toxicity and some chronic effects of selected herbicides on several freshwater fishes. App. Eco. J. 4, 316–327.

[44] Ramesh, M., Srinivasan, R., Saravana, M., 2009. Effect of atrazine [Herbicide] on blood parameters of common carp Cyprinus carpio [Actinopterygii: Cypriniformes]. Afr. J. Environ. Sci. Technol. 3, 453–458.

[45] Talas SZ, Gulhan MF. 2009. Effects of various propolis concentrations on biochemical and hematological parameters of rainbow trout [Oncorhynchus mykiss]. Ecotoxicology and Environmental Safety 72: 1994–1998.

[46] Saravanan M, Usha Devi K, Malarvizhi A, Ramesh M. 2012. Effects of Ibuprofen on hematological, biochemical and enzymological parameters of blood in an Indian major carp, Cirrhinus mrigala. Environmental Toxicology and Pharmacology 34: 14–22.

[47] Banaee, M., Sureda, A., Mirvaghefi, A. R., & Ahmadi, K. [2011]. Effects of diazinon on biochemical parameters of blood in rainbow trout [Oncorhynchus mykiss]. *Pesticide biochemistry and physiology*, *99*[1], 1-6.

[48] El-matary, A.F., Sheta, M.B., Beheary, S.M., [2021]. Integrated comparative impacts of using dietary supplementation plant wastes [Opuntia ficus-indica, Moringa Oleifera and Telfairia occidentalis] on hemato-biochemical blood status of Oreochromis niloticus exposed to mercury toxicity. Egyptian Journal of Aquatic Biology & Fisheries, Vol. 25 [1]: 25 – 45.

[49] Chatterjee, N., Pal, A.K., Manush, S.M., Das, T., Mukherjee, S.C., 2004. Thermal Tolerance and oxygen consumption of Labeo rohita and Cyprinus carpio early fingerlings acclimated to three different temperatures. J. Therm. Biol 29, 265–270. https://doi.org/10.1016/j.jtherbio.2004.05.001.

[50] Gluth, G., Hanke, W., 1985. A comparison of physiological changes in carp, Cyprinus carpio, induced by several pollutants at sublethal concentrations. I. The dependency on exposure time. Ecotoxicol. Environ. Saf. 9, 179–188. https://doi.org/10.1016/0147-6513[85]90020-X.

[51] Banaee M., [2012]. Adverse effect of insecticides on various aspects of fish's biology and physiology," in Insecticides—Basic and Other Applications, S. Soloneski and M. Larramendy, Eds., chapter 6,

[52] Braunbeck, T., Storch, V., Nagel, R., 1989. Sex-specific reaction of liver ltrastructure in zebra fish [Brachydanio rerio] after prolonged sublethal exposure to 4- nitrophenol. Aquat. Toxicol. 14, 185–202.https://doi.org/10.1016/0166-445X [89]90016-7.

[53] Mohammod Mostakim, G., Zahangir, M., Monir Mishu, M., Rahman, M., & Islam, M. S. [2015]. Alteration of blood parameters and histoarchitecture of liver and kidney of silver barb after chronic exposure to quinalphos. *Journal of toxicology*, *2015*.

[54] Kazemi, R., Pourdehghani, M., Yousefi Jourdehi, A., Yarmohammadi, M. and Nasri Tajan, M., [2010] Cardiovascular system physiology of aquatic animals and applied techniques of fish hematology. Shabak published book, 2010, 194 P.

[55] Yang J-L., Chen H-C. [2003] Effects of gallium on common carp [Cyprinus carpio]: acute test, serum biochemistry, and erythrocyte morphology, Chemosphere 53 ;877–882

[56] Bordin, E. R., Yamamoto, F. Y., Mannes, Y., Munhoz, R. C., Muelbert, J. R. E., de Freitas, A. M., ... & Ramsdorf, W. A. (2023). Sublethal effects of the herbicides atrazine and glyphosate at environmentally relevant concentrations on South American catfish (Rhamdia quelen) embryos. *Environmental Toxicology and Pharmacology*, *98*, 104057.