Original Article

Atrazine-Mediated Oxidative Stress Responses and Lipid Peroxidation in the Tissues of *Clarias gariepinus*

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Received: 24.10.2016 *Accepted: 04.12.2016*

ABSTRACT

Background: Fish have been at high risk of atrazine toxicity. Comparative atrazine toxicity on the tissues of *Clarias gariepinus* is scanty. Therefore, acute and chronic effects of atrazine on some biochemical parameters in *Clarias gariepinus* were investigated in this study.

Methods: Atrazine toxicity was determined by assessing the responses of glucose, protein, alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), superoxide dismutase (SOD), acetylcholinestarase (AChE) and malondialdehyde (MDA) in blood, gill and liver of fish exposed to both acute (0.00, 28.00, 30.00, 32.00 and 34.00 µg/l) and chronic (0.00, 7.00, 7.50, 8.00 and 8.50 µg/l) concentrations for 96 h and 28 d, respectively.

Results: In acute exposure, glucose and MDA levels showed significant (P<0.05) variations in all tissues. Protein and LDH decreased in all tissues except the latter slightly increased at 32.00µg/l in blood and liver compared to control. ALT and AChE were induced in blood but inhibited in gill and liver. SOD significantly decreased in blood but increased in gill and liver. AST was activated in blood and liver but reduced in gill. In chronic exposure, glucose, protein, SOD and AChE were inhibited in all tissues, while MDA level was induced. ALT, AST, and LDH activities were induced in blood but inhibited in gill and liver except 22.90% induction noted in liver at 8.00 µg/l atrazine.

Conclusion: Exposure to varying concentrations of atrazine induced enzymatic/metabolic alterations in *C. gariepinus*. These alterations can be used as biomarkers of atrazine toxicity in fish.

Keywords: Atrazine, Biochemical parameters, *Clarias gariepinus*, Oxidative stress, Tissues.

IJT 2017 (2): 29-38

INTRODUCTION

The use of synthetic herbicides and pesticides to bring about improved agricultural production has become an acceptable norm and it is practiced globally in contemporary times. Unfortunately, the practice has become a source of environmental concern due to indiscriminate use and undesirable impact of the chemical agent on non-target organisms. Water bodies are particularly worse hit by this practice, as it degrades their quality and affects the lives of aquatic organisms inhabiting the aquatic ecosystem. Each of these respective organisms is forced to either comply with the alterations by adopting physiological, behavioral and biochemical remedies or cease to exist.

One of the most widely used herbicides in the world is atrazine (2-chloro-4ethylamino-6-isopropylamino-s-triazine) [1, 2]. It is moderately toxic, highly water soluble, encouragingly mobile in the environment with a hydrolysis half-life of 30 d [3]. These reasons inform the general concerns about the herbicide as the most detected in streams, rivers, ponds, reservoirs and ground waters around the world [4]. In Nigerian water bodies, atrazine concentration range of between 0.00 and 0.94µg/l has been reported in water, sediment, and fish [5]. Unfortunately, aquatic organisms are exposed lifelong to these concentrations and are forced to elicit biological and biochemical responses. Worse hit by chemical pollutants are the fish residents of the aquatic ecosystem and quite a number of studies have reported and confirmed their biological and toxicological responses to atrazine. Some of these include behavioral [6], toxicological and bioavailability [3, 7], physiological [4] genetic [8] and biochemical [1, 4] responses.

These pollutants including atrazine induce oxidative stress in aquatic environments. This

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subsequently induces oxidative stress in fish by inactivating antioxidant and associated enzymes leading to decreased antioxidant potential [2]. Thus, fishes frequently come up with remarkable alterations in enzyme profile on exposure to herbicides. Mostly affected are the antioxidant defensive enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione-dependent peroxidases (GPx), glutathione reductase (GR) and acetylcholinesterase (AChE) [1]. Quantifying the intensity of atrazine in the aquatic environment and monitoring its oxidative impact on fish can be more readily achieved by using enzymatic/biochemical responses as biomarkers.

The African catfish, *Clarias gariepinus*, is a tropical fish readily found and grown in Nigerian fresh waters. Its preponderance and hardiness informed its choice for exposure to atrazine in this study. *C. gariepinus* is particularly at risk of exposure to agro-toxicants in water bodies and culture systems that are in close proximity to rice fields where large quantities of herbicides are used. Large-scale use of herbicides would be on the increase as long as the emergence of herbicides tolerant, genetically modified crops is on the increase [9]. Many reports on the oxidative stress responses of fish to herbicide intoxication are available [2, 10, 11]; however, reports regarding comparative effects of atrazine on the blood, gill and liver of *C. gariepinus* are scanty. Information on the effects of atrazine on the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and acetylcholinesterase (AChE) in *C. gariepinus* are scarce. Moreover, reports on the toxicity of atrazine on these biochemical parameters in fish focused more on acute toxicity, with few reports existing on chronic toxicity.

This study is therefore aimed to understand the acute and chronic effects of atrazine on the enzymatic/biochemical responses of *C. gariepinus*, assess which of the tissues would be more sensitive to atrazine, and the possibility of identifying a prospective biomarker of effect.

MATERIALS AND METHODS

Source of Atrazine

The herbicide, atrazine, used for the experiment was sourced from Sigma-Aldrich (St Louis, USA) and used without further purification.

Fish Maintenance and Acclimatization

Juvenile African catfish, *C. gariepinus* (average weight 11.43±2.54 gr and average length 10.92±1.42 cm, n=400) of the same brood stock were procured from the hatchery of National Institute for Freshwater Fisheries Research (NIFFR), Kainji, Nigeria. They were transported in oxygenated plastic bags containing water from hatchery to the rearing compartments in the Department of Zoology, University of Ilorin, Ilorin Nigeria; after a six-hour feeding holiday during transportation and introduction to the laboratory. The fishes were acclimatized in constantly aerated borehole water stored in 140L plastic tanks under laboratory condition for 2 wk, during which they were fed twice daily with commercial feed pellets at 3% body weight. Water parameters were monitored in the test media everyday using standard methods [12] and were found as follows: temperature 23.06±1.23 ^{o}C, pH 6.86±0.89, dissolved oxygen 7.28±2.03 mg/l, conductivity 0.89±0.35µ/sec and biological oxygen demand 21.18 ± 2.15 mg/l. The water was renewed every 48 h and a 12 h light: 12h dark photoperiod was maintained.

Experimental Design

This experimental study was divided into two phases, acute and chronic. Under acute exposure, three replicates of ten acclimatized juvenile fish each of equal weight and size were exposed for four d (96 h), to five varying definitive concentrations (0.00 µg/l, control), 28.00 µg/l, 30.00 µg/l, 32.00 µg/l and 34.00µg/l) of atrazine; prepared based on the result of the range-finding test. Fishes were not fed during this exposure period. Similarly, in chronic exposure three replicates of ten acclimatized fishes of equal weight and size were exposed for 28 d; to ¼ each of the acute concentrations i.e. 0.0 µg/l (Control), 7.00 µg/l, 7.50 µg/l, 8.00 µg/l and 8.50 µg/l atrazine solutions. The respective atrazine media were renewed every 48 h. All experimental fishes were fed twice daily with commercial feed pellets at a rate of 3% of their body weight. The experiment was carried out using a semi-static renewal method to keep the toxicant concentration constant [13].

Blood Sampling, Preparation of Tissue Supernatants and Determination of Biochemical Parameters

At the end of acute and chronic exposure sessions, respectively, fish were randomly collected from each concentration for blood sampling. Fish were lacerated at ventral part near the caudal artery using dissecting set. Blood was collected using appropriately labeled heparinized capillary tubes swiftly covered with plasticin to prevent air that can aid clotting of the blood from entering the tubes. Approximately, 0.5 ml aliquots of blood were centrifuged at 350 gr for 5 min using refrigerated centrifuge in order to separate serum for biochemical analyses. The fish were subsequently sacrificed by cervical sectioning and dissected to remove the gills and the liver. One gramme each of the gill and liver was weighed and homogenized with 5ml of 0.25 M sucrose solution in ice-cold condition.

The homogenate of each tissue was divided into two portions; first one, approximately, 150-200 µg/l were centrifuged at 14,700 g and 4 ^{o}C for 20 min to obtain the supernatant. This was used as resource base for analyzing the activities of ALT, AST, SOD, LDH and AChE. Serum and tissue activities of ALT and AST were determined by the procedure of [12]. SOD was assayed by the method of [14], while LDH was assayed following the procedure of [15]. AChE activity was measured as described by [16] and modified [17]. Lipid peroxidation was estimated by a thiobarbituric acid reactive substance (TBARS) assay [18]. The second portion of the homogenates was added to 8.1% sodium dodecyl sulfate (SDS) and 2.5M acetic acid (pH 3.34). Subsequently, 0.8% thiobarbituric acid was added to make a final volume of 2.0 ml. The mixture was placed in a microcentrifuge and incubated at 95 ^{o}C for 90 min. After cooling, it was centrifuged at 5000 gr for 10 min and optimal density established at 532 nm. TBARS levels were expressed as nmol MDA/mg [19]. Estimation of protein content was done using the method [20], while glucose level was examined [21].

Data Analysis

Data analyses were carried out using parametric (one-way ANOVA) or non-parametric (Kruskall-Wallis) analysis of variance depending on the distribution of the data and the homogeneity. Multiple-range test (Tukey test) was used to identify differences and values of $P<0.05$ were considered significant.

RESULTS

Tables 1-6 show the varying levels of biochemical parameters in the blood, gill and liver of *C. gariepinus* from control and atrazine-exposed groups. During acute exposure, glucose levels in the blood (Table 1) showed the highest and significant ($P<0.05$) variations, constituting +51% increase over the control value at atrazine concentration of 30.00 µg/l (Figure 1), whereas in the gill (Table 2) a significant increase ($P<0.05$) constituting +28.66% at 32.00 µg/l of atrazine (Figure1) was observed. However, in the liver (Table 3) glucose level increased in a concentration-dependent manner. Protein level significantly decreased ($P<0.05$) in all the tissues, with the highest decrease (-27.45%, Figure 1) at 34.00 µg/l in the blood and liver of *C. gariepinus*, whereas in the gill, highest decrease (-42.86%, Figure 1) was recorded at 32.00 µg/l of atrazine. The activities of the transaminase enzymes (ALT and AST) were significantly induced ($P<0.05$) with increase in concentrations in the blood (Table 1), but the opposite was the case in the gill (Table 2). In the liver (Table 3), only the activity of ALT was inhibited in a concentration-dependent manner while that of AST was significantly induced ($P<0.05$) as concentration increased. LDH activities were significantly decreased ($P<0.05$) in all the tissues of the exposed fish except at 32.00 µg/l where a slight but significant ($P<0.05$) induction by 2.00% in the blood and 1.56% in the liver were recorded (Figure 1). SOD activity was inhibited in the blood except at 32.00 µg/l atrazine concentration, while in the gill and liver the activities were induced, the highest induction being recorded at 32.00 µg/l in gill (20%, Figure 1) and at 34.00 µg/l in the liver (87.50%, Figure 1). In the gill and liver, the activities of AChE were inhibited, while it was induced in the blood as compared to the control. TBARS content showed significant elevation ($P<0.05$) in all the tissues (Tables 1-3) with a uniform trend in the gill (Table 2) and liver (Table 3) but no trend in the blood (Table 1).

In chronic exposure, glucose, and protein levels compared to their corresponding control groups significantly ($P<0.05$) decreased in all the tissues (Tables 4-6) with highest percentage mean decrease (Figure 1) recorded at the highest

concentration of atrazine. At the highest concentration in each case, the decrease were in the order -35.71%, -22.73%, -10.53% in gill, liver and blood, respectively for glucose and -52.94%, -24.82%, -22.45% in liver, gill and blood respectively for protein (Figure 1).

Similarly, the transaminase enzymes' (ALT and AST) and LDH activities were significantly (P<0.05) inhibited by increasing concentrations of atrazine in the gill (Table 5) and liver (Table 6) of *C. gariepinus* compared to their respective control groups, except at 8.00 µg/l where LDH activity in the liver showed 22.90% increase over the control value (Figure 1). In contrast, transaminases' activities in blood (Table 4) were significantly (P<0.05) increased in a concentration-dependent manner, showing the highest percentage increase in both enzymes at the highest concentration of atrazine (Figure 1); whereas LDH showed no trend. In the blood (Table 4), SOD and AChE activities decreased significantly (P<0.05) at lower concentrations of atrazine but at the highest concentration, a slight increase of +4.03% and +41.67%, respectively were observed (Figure 1). The activities of SOD and AChE in the gill and liver were significantly (P<0.05) inhibited in a concentration-dependent manner. TBARS contents measured as MDA were significantly induced in all the tissues (Tables 4-6) compared to their control groups.

All the parameters investigated showed different sensitivities to atrazine in each tissue. In acute assay (Tables 1-3, Figure 1), three of the parameters (AST, LDH and AChE) were highly susceptible to atrazine (given their high percentage alteration i.e. decrease or increase) in blood, two (protein and ALT) in gill and three (SOD, MDA, and glucose) in liver. In chronic assay, AST and AChE were highly modified in blood, glucose and ALT in gill and protein, LDH, SOD and MDA in liver (Tables 4-6, Figure 1).

Table 1. Biochemical changes in the blood of *C. gariepinus* exposed to different concentrations of atrazine for 96 h (4 d).

	Concentration (µg/l)				
Parameter	**0.00**	**28.00**	**30.00**	**32.00**	**34.00**
Glucose	2.72±0.02^{a}	2.60±0.02^{a}	4.11±0.02^{c}	3.10±0.02^{b}	3.00±0.21^{b}
Protein	51.0±0.20^{e}	47.0±0.02^{d}	43.0±0.02^{c}	40.0±0.20^{b}	37.0±0.20^{a}
ALT	18.7±0.20^{a}	19.7±0.20^{b}	22.1±0.20^{c}	23.5±0.20^{d}	40.0±0.20^{e}
AST	157.0±0.20^{a}	233.0±0.20^{b}	319.0±0.02^{a}	264.0±020^{c}	664.0±0.02^{e}
LDH	100.0±0.02^{d}	79.0±0.20^{a}	85.0±0.20^{b}	102.0±0.20^{e}	92.0±0.20^{c}
SOD	122.0±0.02^{a}	110.0±0.20^{a}	115.0±0.02^{ab}	123.0±0.02^{bc}	120.0±0.02^{bc}
AChE	10.0±0.20^{a}	10.0±0.20^{a}	12.0±0.20^{b}	16.0±0.20^{d}	14.0±0.20^{c}
MDA	2.60 ± 0.02^{a}	2.60±0.02^{a}	3.40±0.20^{b}	3.20±0.20^{b}	2.80±0.10^{a}

Values are means of 3 replicates and Means ± SD with the same superscript in the same row are not significantly different (P>0.05) ALT=Alanine aminotransaminase (IU/l), AST=Aspartate aminotransaminase (IU/l), LDH=Lactate dehydrogenase (IU/l), SOD=Superoxide dismutase (IU/l), AChE=Acetylcholinestarase (IU/l), MDA=Malondialdehyde (nmol MDA/mg).

Table 2. Biochemical changes in the gill of *C. gariepinus* exposed to different concentrations of atrazine for 96 h (4 d).

	Concentration (µg/l)				
Parameter	**0.00**	**28.00**	**30.00**	**32.00**	**34.00**
Glucose	31.4±0.20^{b}	37.0±0.30^{d}	29.2±0.20^{a}	40.40±0.20^{e}	32.8±0.10^{c}
Protein	14.0±0.20^{d}	10.0±0.30^{b}	12.0±0.10^{c}	8.0±0.20^{a}	12.0±0.10^{c}
ALT	248±0.20^{c}	176.4±0.20^{d}	81.8±0.20^{c}	72.0±0.10^{b}	63.4±0.10^{a}
AST	422.0±0.10^{e}	286.0±0.20^{d}	180.0±0.20^{c}	90.0±0.30^{b}	80.0±0.20^{a}
LDH	136.0±2.00^{d}	124.0±2.00^{a}	130.0±1.00^{bc}	132.0±1.00^{c}	128.0±2.00^{b}
SOD	85.0±0.30^{a}	90.0±0.10^{b}	98.0±0.20^{c}	102.0±1.00^{e}	100.0±0.10^{d}
AChE	20.0±1.00^{e}	18.0±1.00^{c}	14.0±0.20^{a}	16.0±0.30^{b}	15.0±0.10^{ab}
MDA	14.0±0.10^{a}	16.0±0.20^{b}	16.0±0.30^{b}	17.0±0.20^{c}	18.0±0.10^{d}

Values are means of 3 replicates and Means±SD with the same superscript in the same row are not significantly different (P>0.05) ALT=Alanine aminotransaminase (IU/l), AST =Aspartate aminotransaminase (IU/l), LDH=Lactate dehydrogenase (IU/l), SOD=Superoxide dismutase (IU/l, AChE =Acetylcholinestarase (IU/l), MDA=Malondialdehyde (nmol MDA/mg).

Table 3. Biochemical changes in the liver of *C. gariepinus* exposed to different concentrations of atrazine for 96 h (4 d).

	Concentration (µg/l)				
Parameter	**0.00**	**28.00**	**30.00**	**32.00**	**34.00**
Glucose	9.30±0.02^{a}	10.0±0.20^{b}	11.0±0.02^{c}	15.0±0.20^{d}	20.1±0.20^{e}
Protein	24.0±0.20^{e}	22.0±0.02^{d}	20.0±0.20^{c}	17.0±0.02^{b}	16.0±0.20^{a}
ALT	15.2±0.02^{e}	14.9±0.00^{d}	19.3±0.20^{c}	12.3±0.02^{b}	10.7±0.02^{a}
AST	142.0±0.20^{a}	180.0±0.20^{b}	204.0±0.30^{c}	236.0±020^{d}	268.0±0.20^{d}
LDH	256.0±0.30^{d}	212.0±0.20^{a}	216.0±0.30^{c}	260.0±0.20^{e}	228.0±0.20^{b}
SOD	128.0±0.20^{a}	196.0±0.20^{a}	208.0±0.20^{c}	231.0±0.30^{c}	240.0±0.30^{e}
AChE	36.0±0.30^{e}	27.0±0.20^{a}	32.0±0.20^{c}	34.0±0.20^{d}	30.0±0.02^{b}
MDA	22.0±0.10^{a}	24.0±0.02^{b}	26.0±0.02^{c}	28.0±0.20^{d}	30.0±1.00^{e}

Values are means of 3 replicates and Means ± SD with the same superscript in the same row are not significantly different (P>0.05) ALT = Alanine aminotransaminase (IU/l), AST =Aspartate aminotransaminase (IU/l), LDH=Lactate dehydrogenase (IU/l), SOD=Superoxide dismutase (IU/l, AChE =Acetylcholinestarase (IU/l), MDA=Malondialdehyde (nmol MDA/mg)

Table 4. Biochemical changes in the blood of *C. gariepinus* exposed to different concentrations of atrazine for 28 d.

Parameter	Concentration (µg/l)				
	0.00	**7.00**	**7.50**	**8.00**	**8.50**
Glucose	3.80±0.10^{a}	3.50±0.02^{c}	3.20±0.10^{ab}	3.40±0.01^{c}	3.40±0.02^{bc}
Protein	49.0±1.00^{d}	49.0±0.20^{d}	43.0±1.00^{c}	41.0±0.10^{b}	38.0±0.10^{a}
ALT	23.9±0.30^{a}	24.3±0.10^{b}	27.4±0.20^{c}	28.3±0.02^{d}	44.6±0.20^{e}
AST	205.0±0.30^{a}	279.0±0.10^{b}	315.0±0.20^{c}	363.0±0.20^{d}	690.0±0.02^{e}
LDH	112.0±1.00^{e}	88.0±0.20^{a}	97.0±0.30^{b}	109.0±1.00^{d}	105.0±0.20^{c}
SOD	124.0±1.00^{c}	112.0±1.00^{a}	113±1.00^{a}	119.0±1.00^{b}	129.0±1.00^{d}
AChE	12.0±0.10^{c}	10.0±1.00^{a}	11.0±0.10^{b}	13.0±0.30^{d}	17.0±0.20^{e}
MDA	3.30±0.20^{b}	3.60±0.02^{b}	4.20±0.02^{c}	4.00±0.10^{c}	3.40±0.10$_{ab}$

Values are means of 3 replicates and Means±SD with the same superscript in the same row are not significantly different (P>0.05) ALT=Alanine aminotransaminase (IU/l), AST =Aspartate aminotransaminase (IU/l), LDH=Lactate dehydrogenase (IU/l), SOD=Superoxide dismutase (IU/l), AChE = Acetylcholinestarase (IU/l), MDA=Malondialdehyde (nmol MDA/mg)

Table 5. Biochemical changes in the gill of *C. gariepinus* exposed to different concentrations of atrazine for 28 d.

	Concentration (µg/l)				
Parameter	**0.00**	**7.00**	**7.50**	**8.00**	**8.50**
Glucose	14.0±0.10^{e}	13.0±0.20^{d}	12.0±0.30^{c}	11.0±0.20^{b}	9.0±0.10^{a}
Protein	41.9±0.10^{d}	38.7±0.10^{c}	33.8±0.10^{b}	34.0±0.10^{b}	31.5±0.20^{a}
ALT	253.0±1.00^{e}	180.5±0.10^{d}	78.5±0.10^{c}	75.0±1.00^{b}	67.9±0.10^{a}
AST	425.0±0.10^{e}	290.0±1.00^{d}	186.0±0.10^{c}	97.0±2.00^{b}	84.0±2.00^{e}
LDH	147.0±0.10^{e}	133.0±0.10^{a}	141.0±0.20^{c}	143.0±1.00^{d}	138.0±0.30^{b}
SOD	106.0±1.00^{d}	105.0±0.20^{d}	97.0±0.10^{c}	91.0±2.00^{b}	84.0±0.10^{a}
AChE	21.0±0.10^{d}	19.0±0.10^{c}	16.0±0.10^{a}	17.0±1.00^{b}	17.0±1.00^{b}
MDA	15.0±0.20^{a}	16.0±0.20^{b}	16.0±0.10^{b}	18.0±0.30^{c}	19.0±0.20^{d}

Values are means of 3 replicates and Means±SD with the same superscript in the same row are not significantly different (P>0.05) ALT =Alanine aminotransaminase (IU/l), AST =Aspartate aminotransaminase (IU/l), LDH=Lactate dehydrogenase (IU/l), SOD=Superoxide dismutase (IU/l, AChE = Acetylcholinestarase (IU/l), MDA= Malondialdehyde (nmol MDA/mg)

Table 6. Biochemical changes in the liver of *C. gariepinus* exposed to different concentrations of atrazine for 28 d.

Parameter	Concentration (µg/l)				
Glucose	**0.00**	**7.00**	**7.50**	**8.00**	**8.50**
Glucose	22.0±0.20^{e}	21.0±0.20^{d}	19.0±0.30^{c}	18.0±0.10^{b}	17.0±0.10^{a}
Protein	20.4±0.10^{e}	14.9±0.20^{d}	11.1±0.10^{c}	10.2±0.20^{b}	9.60±0.10^{a}
ALT	21.4±0.20^{e}	20.1±0.20^{d}	19.3±0.20^{c}	16.9±0.10^{b}	16.2±0.10^{a}
AST	269.0±1.00^{e}	236.0±2.00^{d}	204.0±1.00^{c}	143.0±2.00^{a}	181.0±1.00^{b}
LDH	262.0±0.20^{d}	221.0±1.00^{b}	153.0±1.00^{a}	268.0±2.00^{e}	234.0±0.02^{b}
SOD	243.0±0.20^{e}	232.0±0.10^{d}	208.0±0.10^{c}	199.0±0.30^{b}	183.0±1.00^{a}
AChE	35.0±0.20^{d}	28.0±1.00^{a}	36.0±1.00^{e}	33.0±0.20^{c}	30.0±0.30^{b}
MDA	21.0±0.10^{a}	24.0±0.02^{b}	27.0±0.10^{c}	29.0±0.02^{d}	29.0±0.20^{d}

Values are means of 3 replicates and Means ± SD with the same superscript in the same row are not significantly different (P>0.05) ALT =Alanine aminotransaminase (IU/l), AST =Aspartate aminotransaminase (IU/l), LDH Lactate dehydrogenase (IU/l), SOD= Superoxide dismutase (IU/l, AChE = Acetylcholinestarase (IU/l), MDA = Malondialdehyde (nmol MDA/mg)

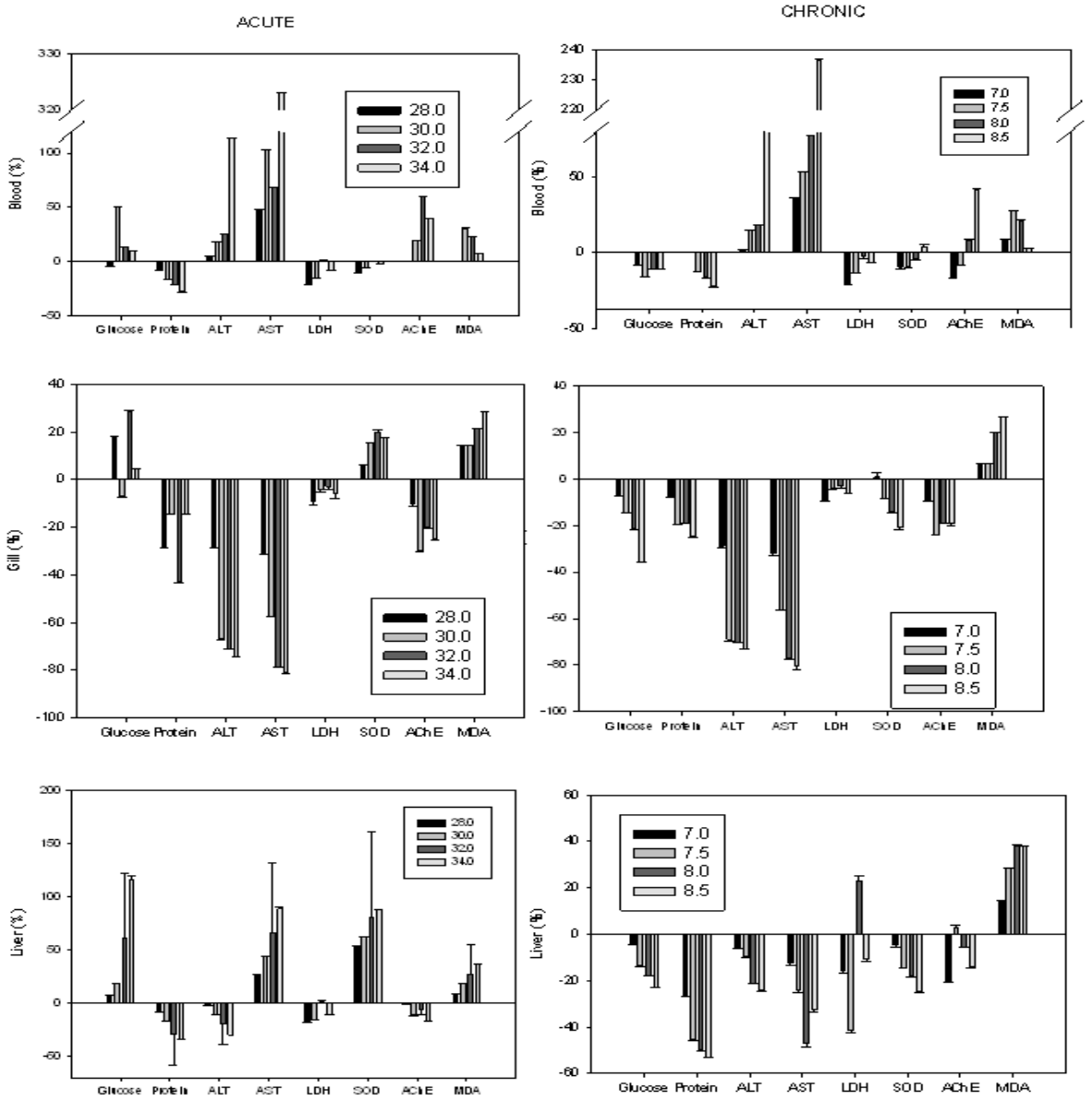

Figure 1. Percentage increase(+) or decrease(-) in biochemical parameters of different tissues of *C.gariepinus exposed* to varying concentrations of atrazine (µg/l)for 96 h (Acute) and 28 d(chronic).

DISCUSSION

The present study generally shows that *C. gariepinus* exposure to varying concentrations of atrazine-induced oxidative stress as demonstrated by significant concentration-dependent changes in almost all the biochemical parameters investigated. This is probably due to accumulation of the pollutant in various tissues of the fish. Atrazine has been rapidly accumulated in various tissues of fish [22]. The varying levels of glucose observed in all the tissues during acute and chronic exposures are indicative of disruption in carbohydrate metabolism. The attempt to cope with increased energy demand under atrazine-induced hypoxic condition might have been responsible for the elevation of glucose levels during the acute exposure. The excess utilization of energy for metabolic processes and combating toxic stress that subsequently led to exhaustion after a prolonged and continued atrazine-induced hypoxia explains the reduction in glucose level in all the tissues. Similar hypoglycaemic conditions in fish species in response to atrazine intoxication have been reported [23].

The reduction in protein content of all the tissues investigated is indicative of intense proteolytic activities that might have resulted in the production of free amino acids used in tricarboxylic cycle for energy production. The depletion of protein as glucose decreased may be due to the rapid utilization of protein in these tissues when the fish is under stress condition. The blood, gill and liver are important target organs of xenobiotic accumulation and exhibit biochemical alterations following toxicant accumulation. The percentage protein inhibition in all the tissues during acute and chronic exposures (Tables 1-6, Figure 1) was not less than 2-fold of the corresponding control values; the gill and liver seemingly having higher folds. Thus, it follows that upon exposure; large amount of atrazine is made available to these tissues and may interfere with the functional integrity of the tissues. Therefore, the depletion in protein level could also be attributed, in part, to the damaging effects of atrazine on these tissues. Similar trend in protein reduction due to atrazine toxicity and other herbicides have been reported in many fishes [23-25].

The significantly elevated aminotransferase's (ALT and AST) activities in the blood of fish during short-term exposure, with concomitant decrease in the gill and liver during prolonged exposure, could be due to cellular degradation of these tissues; resulting in interference with the normal physiological and biochemical processes such as Kreb's and tricaboxylic cycles and subsequent leakage into the bloodstream [24]. These results are similar to [25] observations that acute sub-lethal concentrations of atrazine caused a significant increase in ALT and AST of *C. carpio*. The AST is a mitochondrial enzyme found in the blood, heart, kidney and skeletal muscle [26]. The induction of ROS due to mitochondrial damage following atrazine intoxication may explain the elevation of AST in the blood of *C. gariepinus*. The present results agree with the observations in *C. carpio* [25]. Furthermore, the dynamics of ALT and AST activities during short-term and prolonged exposures appear to be related to the reduction of protein in the tissues of atrazine-exposed fishes. This seems obvious as increased levels of these enzymes in stress conditions have stimulatory effects on gluconeogenic process to generate energy for detoxification process, homeostatic maintenance and tissue repairs. Thus, increased levels of these enzymes in the serum and liver of atrazine-exposed fishes is in response to oxidative stress and could be an attempt to supply enough energy needed to survive the toxic stress.

LDH is an important glycolytic enzyme in anaerobic pathway of carbohydrate metabolism. In this study, the trend in LDH activity is in contrast to that noticed in aminotransferase enzymes. LDH activities significantly decreased in all the tissues during both exposures, except the slight increase observed in the blood and liver of fish at 32µg/l during acute exposure. Decreased LDH activities in the tissues may be due to increased tissue damage by atrazine. However, the slight (about 2%, Figure 1) induction of LDH activities at 32 µg/l atrazine during acute exposure is suggestive of a slight compensatory attempt to subsist on pyruvate, the end product of glycolysis, to produce lactate that can, in turn, be use to cope with the rapid demands of energy. During prolonged exposure, the impact of atrazine became overwhelming as evidenced in decreased activities of LDH in tissues that also corroborated the reduction of protein in these tissues. Besides, protein was probably the only obvious alternative energy substrate available to *C. gariepinus* under

atrazine toxicity. Decreased LDH activities in fish tissues treated with other pesticides have been observed [27].

SOD is an important antioxidant catalyzing the conversion reaction of superoxide radical to hydrogen peroxide and molecular oxygen. In this study, similar SOD response pattern was observed in the blood during the short-term and prolonged exposures, but the patterns in gill and liver in both exposures differed. The decrease in SOD activity in the blood of atrazine-exposed fish irrespective of the exposure duration could be due to a net increase in the level of superoxide radical. This explanation may also hold true for the activity profile of the enzyme in gill and liver during prolonged exposure. Attenuation in SOD activity in the presence of herbicides was also reported in different species [28, 29]. However, the increased activity of the enzyme in gill and liver during acute exposure appears to be an adaptive response to increased generation of these free radicals. Fish exposed to atrazine increases SOD activity in their tissues [2, 10]. At higher concentration of atrazine, i.e. at 32.00 µg/l for acute and 8.50 µg/l for chronic, the increased SOD activity recorded in the blood could also be an adaptive response since it is known that SOD is inducible, the level of which depend on the increasing need to protect against toxic oxidation [30]. Thus, at these concentrations, the only means for the tissues to reduce deterioration due to oxidative stress is to stimulate the activity of SOD to scavenge excess radicals.

One of the manifestations of free radical mediated processes is lipid peroxidation and it has been used extensively as an effective biomarker of toxic pollutants in fish. The increased LPO observed in all tissues during both exposures may be due to ROS production, which could cause peroxidative injury to membrane lipids of all the tissues and ultimate cell apoptosis [31]. In this study, peak level of LPO in gill and liver of *C. gariepinus* coincided with peak activities of SOD during the acute exposure. The stimulation of SOD was capable enough to counteract the lipid peroxidation induced by ROS in these tissues. However, the marked decrease in SOD activity in the gill and liver paralleled by enhanced LPO levels, as observed during the chronic exposure, demonstrates compromised antioxidant defenses in the face of prolonged intoxication and this could contribute to the damage of the tissues. In contrast to this observation, *Prochilodus lineatus's* gill did not exhibit concentration-dependent response to atrazine toxicity and acute exposure to concentration less than 25 µg/l did not induce SOD responses neither did it change LPO levels in the fish's gill [4].

The activity of AChE has been widely used as a specific biomarker of pesticide biomarker in aquatic organisms. In the present study, acute atrazine exposure elicited significantly (P<0.05) induced AChE activity in the blood, while in gill and liver the activities were inhibited. In chronic exposure, all the tissues demonstrated concentration-dependent inhibition in AChE activities. The decrease in AChE activity is suggestive of atrazine toxicity which might have caused accumulation of acethylthiocholine at neuromuscular junction and disruption of synaptic transmission in the cholinergic system of the fish. Induction or inhibition of AChE in fish tissues can influence cholinergic transmission process, thus eliciting undesirable effects [32]. Inhibition of AChE has been reported in fishes exposed to different pesticides [33].

The differences observed in the activities of all parameters investigated in each tissue are suggestive of tissue-specific responses and this could be a consequence of metabolic differences and antioxidant capacity of each tissue. Differences in oxidative stress among tissues of fishes exposed to herbicides have been reported and these depend on species, feeding, habitat, and duration of exposure [28]. The overlap in the sensitivities of each parameter to atrazine in each tissue confirmed their potential use in risk assessment of atrazine pollution, though the liver seems to be more sensitive.

CONCLUSION

Exposure of *C. gariepinus* to varying concentrations of atrazine causes oxidative stress and significant alterations in enzymatic and metabolic parameters in the tissues investigated. These alterations, however, depend on the defensive ability of the tissue examined. These parameters can be considered as potential complementary biomarkers used in the assessment of atrazine toxicity.

ACKNOWLEDGEMENTS

Prof. Adeolu T. Ande is gratefully acknowledged for his useful suggestions. The

authors are also grateful to Miss Adeoye, Messrs Erinle, Olu-Bello and Yekeen for their technical assistance during the experiment. The authors declare that there are no conflicts of interests.

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