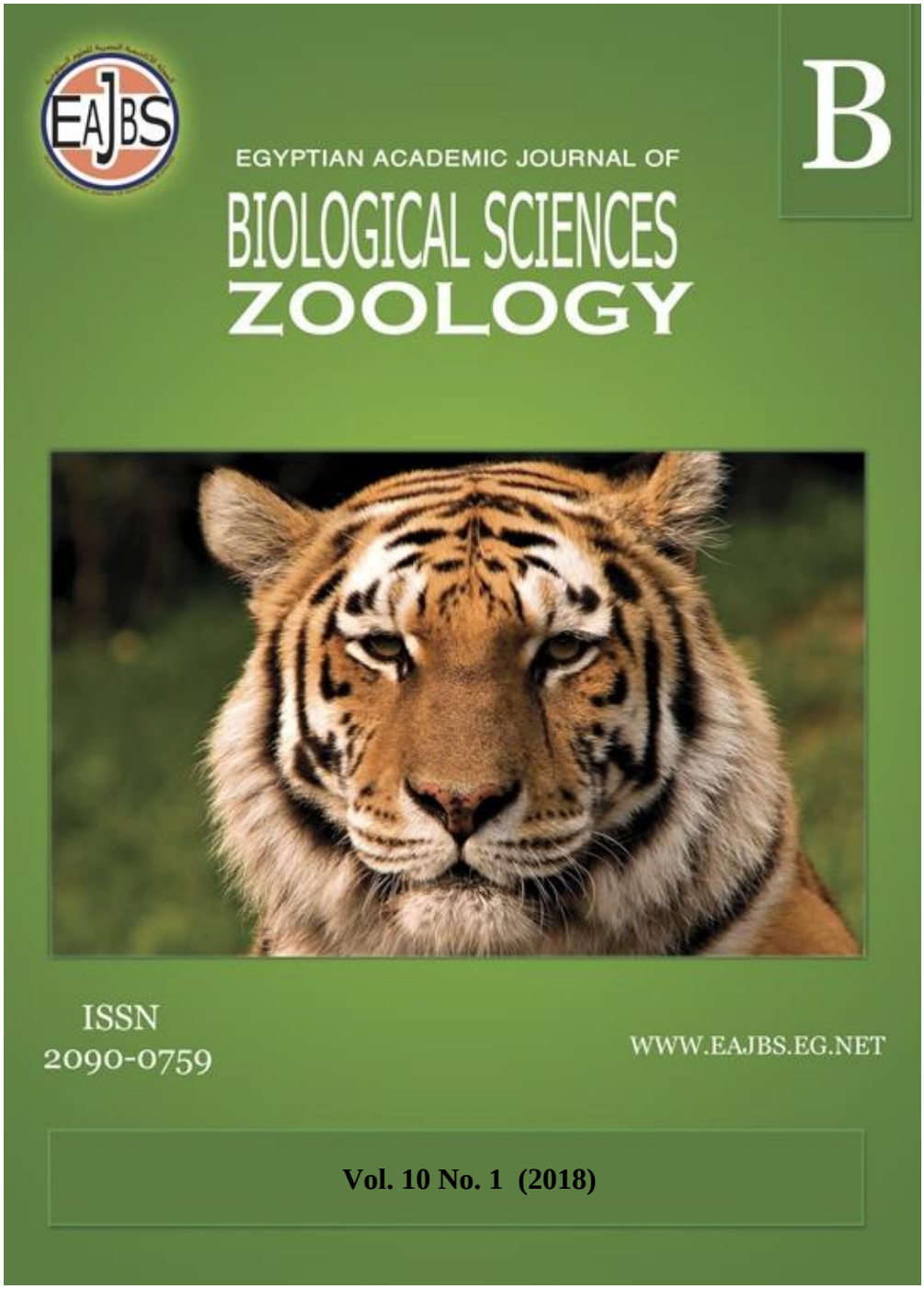

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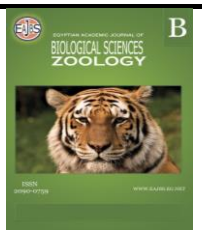

In Vivo Acute and Chronic Toxicity of Pendimethalin on Haematological and Biochemical Indices of African Catfish, *Clarias gariepinus* (Burchell 1822)

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ABSTRACT

In vivo acute and chronic toxicity effects of pendimethalin were carried out on the haematology and biochemical parameters of juvenile *Clarias gariepinus* subjected to different acute (0.00, 0.25, 0.50, 0.75, 1.00 mg/l) and chronic (0.00, 0.05, 0.10, 0.15, 0.20 mg/l) concentrations of pendimethalin for 4 and 28 days, respectively. As concentration of the toxicant increased in both assays, there were significant ($P < 0.05$) increase in red blood cells (RBCs), haemoglobin (HB) and mean corpuscular haemoglobin concentration (MCHC), while the white blood cells (WBCs), packed cell volume (PCV), mean cell volume (MCV) and mean cell haemoglobin (MCH) significantly decreased compared to the control. Alterations in biochemical parameters in *C. gariepinus* tissues during both acute and chronic exposures exhibited similar trend of either increase or decrease in activities as the concentration of toxicant increases. In both exposures, the activities of aspartate amino transferase (AST), alanine amino transferase (ALT) and malondialdehyde (MDA) significantly increased ($P < 0.05$) in the blood, gill and liver, whereas the activities of lactate dehydrogenase (LDH), superoxide dismutase (SOD), acetylcholinesterase (AChE), glucose and protein in these tissues were significantly inhibited ($P < 0.05$) compared to the control groups, though the magnitude of induction or inhibition in each tissue differs. The study shows that exposure of *C. gariepinus* to pendimethalin toxicity could cause oxidative stress and alterations in the haemato-biochemical profile of the fish. Therefore, the use of pendimethalin should be minimized and discouraged especially around aquatic bodies so as to prevent adverse effects on the fauna inhabiting them.

INTRODUCTION

The tremendous increase in world's population necessitates the increase in food production for the teaming population. Concomitantly, this has led to the increased use of pesticides against insect infestation of food crops and weeds in order to boost crop yield. Nigeria is an agro-based country with its teeming population dependent on agricultural products. Unfortunately, due to pest and weed infestations

most of its products is lost, and this results in the application of pesticides on farmlands. Pesticides are increasingly used because they are relatively biodegradable and persistently low in the environment. However, their indiscriminate use ultimately leads to aquatic ecosystems due to runoff causing havoc to the aquatic biotic community including fish.

Pendimethalin is among the commonly used herbicides in Nigeria. Pendimethalin [N- (1-ethylpropyl)-3, 4-dimethyl-2, 6-dinitrobenzamine] is a selective pre-emergent and early post-emergent herbicide used to control annual grasses and broadleaf weeds in a variety of terrestrial fruits and vegetables. It is considered as moderately persistent herbicide that can give rise to long-lasting metabolites (Abd-algadir *et al*., 2011). Although it is undisputed that pesticides are essential in modern agriculture, there is a growing concern about possible environmental contamination from agrochemicals (Ezemonye et al., 2009). Danion et al. (2014) reported that due to repeated use of various formulations of pendimethalin, high concentrations of the chemical substance have been detected in many European aquatic ecosystems.

California Department of Pesticide Regulation (CDPR, 2008) reported that the use of pendimethalin increased by 29% in 2008, with more than 1.4 million pounds on agricultural commodities, including landscape maintenance and rights of way. Only a few authors have reported the toxicity of pendimethalin on haematological and biochemical indices of fish species such as *Oreochromis niloticus* (El-Sharkawy *et al*., 2011; El-Sayed *et al*., 2013), *Oncorhynchus mykiss* (Danion *et al*., 2014); *Channa punctata* (Tabassum *et al*., 2015; Tabassum *et al*., 2016). In tropical Africa, other than the reports of Abd-Algadir *et al*. (2011), El-Sharkawy *et al*. (2011), El-Sayed *et al*. (2013) and Moustafa *et al*. (2016) on the effects of pendimethalin in fish, no investigation has been conducted to determine the effects of pendimethalin on African tropical freshwater fishes, despite the constant use of the herbicide. Pendimethalin is highly toxic to fish and it has been classified as persistent bio-accumulative toxic (PBT) and a group C carcinogen by the United States Environmental Protection Agency (USEPA, 1997). Fish are sensitive to changes in their environment. Thus, the health of fish reflects and gives a good indication of the health status of aquatic systems. Early toxic effects of pollutants may, however, only be evident at cellular or tissue level before significant changes can be identified in fish behaviour or external appearance. *Clarias gariepinus* is one of the commercially important fish species in Africa and constitutes cheap source of proteins for the low-income earners (USAID, 2010). *C. gariepinus* is very hardy and can tolerate extreme environmental conditions, hence it is a good sentinel species for ecotoxicological assessment. Although copious amount of information exists on the effects of herbicides on fish, the impact of pendimethalin has received comparatively little attention and there is a dire need to assess and understand the effects of the herbicide on fish species. Therefore, this study is aimed to evaluate the acute and chronic effects of pendimethalin on the haematological, biochemical and oxidative stress indices of *Clarias gariepinus* following exposure.

MATERIALS AND METHODS

Fish Maintenance and Experimental Design:

Commercially available emulsifiable pendimethalin was used for the experiment. Juvenile *C. gariepinus* (average weight = 12.50 g and average length =10.50 cm) were procured from the hatcheries of the Ministry of Agriculture and

Natural Resources, Ilorin, Kwara State, Nigeria. The fish were transported to the Fisheries and Hydrobiology Laboratory, Department of Zoology, University of Ilorin, where they were acclimatized in 400-litre capacity tank for 14 days. The fish were fed twice daily at 8.00 and 18.00 hours at 3 % body weight with commercial feed pellets. Physico-chemical parameters of the test media were monitored on daily basis (APHA, AWWA, 1998) and were as follows: temperature 26.28 ± 1.53^{0}C, dissolved oxygen 6.86 ± 0.21, conductivity 231µScm^{-1}, pH 6.94 ± 0.42 and BOD 8.26 mg/l. Water in the test media was renewed every 48-hour to reduce deterioration of water quality and the prevalent photoperiod (14-hour light: 10-hour dark cycle) during the experiment was maintained.

The experiment was divided into acute and chronic phases. In acute exposure, three replicates of ten acclimatized juvenile fish were exposed for four days, to five different concentrations (0.00, 0.25, 0.50, 0.75 and 1.00 mg/l) of the toxicant; prepared based on the result of the presumptive test earlier carried out. Feeding was terminated 24-hour before the commencement of the acute test. Behavioural changes were closely monitored and mortality recorded. In chronic exposure, three replicates of ten acclimatized fish were similarly exposed using a semi-static renewal method for 28 days; to varying concentrations of pendimethalin (0.00, 0.05, 0.10, 0.15 and 0.20 mg/l) equivalent to $^{1/5}$ each of the acute concentrations. Fish were fed twice daily with a commercial feed pellet at a rate of 3% body weight.

Blood Sampling, Preparation of Tissue Homogenate and Biochemical Assay:

Blood sampling was done at the end of acute and chronic exposures. The body surface of each specimen was cleaned and blotted dry with adsorbent paper. Blood samples were collected from the caudal vessel using disposable 3-cc sterile syringes and 21-gauge needles. The samples were collected in Eppendorf tubes containing 1 % anticoagulant, ethylene diamine tetra acetic (EDTA) solution, centrifuged at 3,000 rpm for 30 minutes and used for the determination of haematological parameters i.e. red blood cell (RBC), white blood cell (WBC), haemoglobin (HB) and Packed cell volume (PCV). RBC and WBC were determined using a Neubauer haemocytometer by diluting blood with Giemsa stain at 1:30 dilution. Haemoglobin was estimated using the cyanomethemoglobin method, while the PCV was estimated according to the method described by Blaxhall and Daisley (1973). The mean cell volume (MCV), mean cell haemoglobin, and mean cell haemoglobin concentration (MCHC) were determined using the formulae proposed by Dacie and Lewis (2001).

Another portion of blood samples was collected without anticoagulant for the assessment of glucose, protein and enzymatic parameters. The fish were sacrificed and dissected to remove the gills and the liver. These tissues (approximately 1g each) were homogenized with 0.25 M sucrose solution in chilled condition using the Teflon homogenizer. The homogenates were centrifuged at 5, 000 rpm for 10 minutes at 4^{0}C. The supernatants were stored at 4^{0}C prior use. The activity of superoxide dismutase (SOD) was determined following the methods of Misra and Fridovish (1972), while the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assayed using the methods of Reitman and Frankel (1957). Total protein in the tissues was estimated spectrophotometrically following the methods of Lowry *et al*. (1951) using the bovine serum as standard, while glucose was determined by the method of Trinder (1969). Acetylcholinesterase (AChE) activity was estimated as described by Elman *et al*. (1961) and modified by Dos Santos *et al*. (2005).

Statistical Analysis:

Data obtained were statistically analysed using parametric (one-way ANOVA) or non-parametric (Kruskall-Wallis) analysis of variance depending on the distribution and homogeneity of the data. Multiple-range test (Tukey) was used to identify differences between pendimethalin-treated fish and respective control groups. Data were expressed as means ± SD and values of $P < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

The lethal and sub-lethal effects of pendimenthalin on blood parameters of *C. gariepinus* are shown in Tables 1 and 2. During both exposures, RBC, HB and MCHC were significantly induced ($P < 0.05$) in pendimenthalin exposed groups, while WBC, PCV, MCV and MCH were significantly inhibited as compared to the control groups. Haematological parameters are widely being used to ascertain the toxic effects of toxicants on fish. Copious amount of information exists on the effects of pesticides on fish haematological parameters. Prusty et al. (2011) observations of increased RBC count, haemoglobin content and decreased PCV value of *Labeo rohita* upon exposure to fenvalerate were consistent with present results. The decreased values of MCV and MCH and increased MCHC in this study were also similar to the report of Prusty et al. (2011). The increased RBC count with concomitant increase in haemoglobin concentration might have been due to the toxic action of pendimethalin on erythropoietic organs which induced the process of erythropoiesis, and enhanced haemoglobin synthesis and MCHC level in attempt to survive the toxic stress (Shakoori, et al., 1996, Prusty, et al., 2011). The depletion of PCV value is a clear indication of comparatively toxic nature of pendimethalin despite the attempt to synthesize more but immature RBC for survival. Saravanan et al. (2011) and Ramesh et al. (2015) suggested the reduction of MCV and MCH values in fish exposed to pesticides as due to the high percentage of immature RBCs in circulation. However, the induction of MCHC level in *C. gariepinus* is indicative of sphaerocytosis (Ramesh, et al., 2015), a condition in which the RBC membrane become disrupted, causing the blood cells to be spherically shaped rather than bi-concave disc-shaped, thus affecting the osmoregulation of the fish. WBCs play an important role in the defense mechanisms of organisms. The observed inhibition of WBCs as concentration of toxicant increases is an indication of compromised immunity due to the disruption of antibody production pathway. Similar results were observed by different authors (Sopinska and Guz, 1998; Prusty et al., 2011).

Changes in biochemical parameters in the tissues of *C. gariepinus* during both acute (Tables 3-5) and chronic (Tables 6-8) exposures showed similar trend of either increase or decrease in activities as the concentration of toxicant increases. *C. gariepinus* exhibited a significant increase ($P < 0.05$) in ALT, AST and MDA activities in the blood, gill and liver in both exposures, whereas the activities of LDH, SOD, AChE, glucose and protein in these tissues (Tables 3-8) were significantly inhibited ($P < 0.05$) compared to the control groups, though the magnitude of induction or inhibition in each tissue differs; indicating the different sensitivities of each tissue to the toxicant. In this study, the acute and chronic exposures of *C. gariepinus* to different concentrations of pendimethalin enhanced ALT and AST activities throughout the exposure durations. The induction of these parameters in the tissues of *C. gariepinus* could be due to pendimethalin-induced oxidative stress and is suggestive of enhanced process of transamination, indicating tissue dysfunction.

Enhanced transamination during pesticide exposure has been attributed to the need to mitigate toxicant induced stress occasioned by increased metabolic demand for energy (Ramesh et al., 2015). Enhanced activities of ALT and AST have also been reported in *C. gariepinus* exposed to primextra herbicide (Nwani et al., 2014) and in other fishes exposed to different pesticides (Prusty et al., 2011; Saravanan et al., 2012; Owolabi and Adesida, 2015).

SOD plays an important antioxidative defense role in organisms by converting superoxide radical to hydrogen peroxide. In both exposures, SOD activity in all the tissue homogenates declined with increase in toxicant concentration demonstrating the overwhelming influence of reactive oxygen species (ROS) production on the antioxidant defense system in each tissue. Similar decline has been observed in fish following exposure to pendimethalin (El-Sayed et al. (2013) and other herbicides (Crestani et al., 2007; Saravanan et al., 2008; Nwani et al., 2010). Furthermore, the inhibition of SOD in each tissue may explain the increased MDA levels in the tissues as a result of peroxidative damage. The elevated MDA levels observed in this study are consistent with previous observations made on fish exposed to pendimethalin (El-Sayed et al., 2013; Tabassum et al., 2015) and other herbicides (Guilherme et al., 2012; Nwani et al., 2014; Owolabi and Omotosho, 2017). The significant reduction in AChE activity is consistent with the observation of Tabassum et al. (2007) and further corroborates the suggestion of Matos et al. (2007) that reduced AChE activity is related to high MDA levels. Inhibition of AChE may jeopardize some essential metabolic activities that are directly under AChE control such as orientation, prey location and escape from predators (Golombieski et al., 2008). This may have a serious implications for the survival of the species in a pendimethalin-polluted water.

LDH activity appears to be related to the levels of glucose in the tissues of fish in this study. As LDH activity decreased, glucose levels also decreased. The reduction in LDH activity shows that glycolytic activities decreased in the tissues, hence the reduction in glucose levels in all the tissues. This could be due to the prevailing toxicant-induced stress which triggered the release of energy substrates to the tissues to meet the energy required for survival. The reduced protein levels are suggestive of its rapid utilisation to generate energy, more so, that the stored energy substrate might have been used up for tissue repair. Proteins are used as alternative energy source in stressed conditions (Singh et al., 2010). Many authors have reported hypoglycaemic and hypoproteinaemic conditions in fish exposed to various toxicants (Kasthuri and Chandran, 1997; Satyendra and Srivastava, 2015; Nwani et al., 2014; Owolabi and Omotosho, 2017).

In summary, pendimethalin causes lipid membrane damage in *C. gariepinus* by affecting the antioxidant defense system; and hence altered the haematological and biochemical parameters of the fish. These parameters could therefore be useful in monitoring pendimethalin pollution in water bodies.

Table 1: Haematological parameters of *C. gariepinus* exposed to varying concentrations of Pendimethalin for 96 hours

Parameter	Concentration (mg/l)				
	0.00 (Control)	0.25	0.50	0.75	1.00
RBC (10^{12}/l)	2.21 ± 0.26^{a}	2.28 ± 0.34^{b}	3.29 ± 0.29^{c}	3. 71 ± 0.37^{d}	4.29 ± 0.34^{e}
HB (g/dl)	4.20 ± 0.16^{a}	4.70 ± 0.09^{b}	5.15 ± 0.15^{c}	5.69 ± 0.12^{d}	6.29 ± 0.17^{e}
PCV (%)	23.29 ± 1.29^{d}	23.09 ± 1.13^{d}	21.89 ± 1.67^{c}	19.22 ± 0.89^{b}	17.20 ± 0.72^{a}
WBC (10^{9}/l)	2. 72 ± 0.15^{e}	2. 58 ± 0.10^{d}	2. 26 ± 0.06^{c}	1.98 ± 0.04^{b}	1.89 ± 0.03^{a}
MCV (fl)	49.88 ± 0.08^{e}	43.67 ± 0.05^{d}	40.87 ± 0.06^{c}	38.02 ± 0.08^{b}	30.97 ± 0.05^{a}
MCH (pg)	62.78 ± 1.03^{d}	61. 54 ± 1.05^{d}	59. 77 ± 1.06^{c}	52.67 ± 0.03^{b}	45.78 ± 0.02^{a}
MCHC (g/dl)	57.34 ± 1.27^{a}	64.18 ± 1.39^{b}	67.01 ± 1.22^{c}	73. 09 ± 1.52^{d}	78. 11 ± 1.64^{e}

Data are represented as mean ± SD of three replicates. Means along the same row with different superscripts are significantly different (P < 0.05). RBC = Red blood cell, HB = haemoglobin, PCV = Packed cell volume, WBC = White blood cell, MCV = Mean cell volume, MCH = Mean cell haemoglobin, MCHC = Mean cell haemoglobin concentration

Table 2: Haematological parameters of *C. gariepinus* exposed to varying concentrations of Pendimethalin for 28 days

Parameter	Concentration (mg/l)				
	0.00 (Control)	0.05	0.10	0.15	0.20
RBC (10^{12}/l)	2.26 ± 0.31^{a}	2.31 ± 0.28^{a}	3.18 ± 0.36^{b}	3.53 ± 0.32^{c}	4. 23 ± 0.37^{d}
HB (g/dl)	4.51 ± 0.18^{a}	4.80 ± 0.21^{b}	5.14 ± 0.23^{b}	5.82 ± 0.19^{b}	6.18 ± 0.20^{b}
PCV (%)	24.10 ± 1.16^{d}	23.86 ± 1.19^{c}	22.81 ±1.10^{c}	19.83 ± 1.08^{b}	16.81 ± 1.10^{a}
WBC (10^{9}/l)	3.01 ± 0.15^{e}	2.82 ± 0.07^{d}	2.49 ± 0.10^{c}	2.24 ± 1.14^{b}	1.92 ± 0.09^{a}
MCV (fl)	46.22 ± 0.07^{d}	42.58 ± 0.09^{c}	38.73 ± 0.06^{b}	39.46 ± 0.08^{b}	34.24 ± 0.06^{a}
MCH (pg)	62.22 ± 1.08^{e}	59.10 ± 1.10^{d}	56.32 ± 1.04^{c}	52.73 ± 1.11^{b}	48.75 ± 1.07^{a}
MCHC (g/dl)	49.62 ± 1.14^{a}	53.76 ± 1.08^{b}	56.13 ± 1.06^{c}	60. 09 ± 1.12^{d}	63.11 ± 1.20^{e}

Data are represented as mean ± SD of three replicates. Means along the same row with different superscripts are significantly different (P < 0.05). RBC = Red blood cell, HB = haemoglobin, PCV = Packed cell volume, WBC = White blood cell, MCV = Mean cell volume, MCH = Mean cell haemoglobin, MCHC = Mean cell haemoglobin concentration

Table 3: Biochemical alterations in the blood of *C. gariepinus* exposed to varying concentrations of Pendimethalin for 96 hours

Parameter	Concentration (mg/l)				
	0.00 (Control)	0.25	0.50	0.75	1.00
ALT	16.26± 0.31^{a}	18.50± 0.28^{b}	22.64± 0.36^{c}	25.01± 0.32^{d}	26.29 ± 0.37^{d}
AST	28.92 ± 0.18^{a}	28.56± 0.21^{a}	32.01 ± 0.23^{b}	34.23± 0.19^{c}	37.62± 0.20^{d}
LDH	231.00± 48.31^{e}	210.00± 51.08^{d}	198.01±37.08^{c}	180.15± 37.08^{b}	172.28± 36.25^{a}
SOD	165.09± 1.39^{c}	153.12± 1.85^{b}	149.24±1.68^{b}	140.18± 1.46^{a}	135.66 ± 1.78^{a}
AChE	21.19± 0.04^{c}	20.23± 0.07^{c}	18.10± 0.06^{b}	15.12± 0.04^{a}	15.34± 0.06^{a}
Glucose	23.67± 0.07^{c}	23.28±0.05^{c}	19.10± 0.04^{b}	18.00± 0.05^{b}	16.34± 0.04^{a}
Protein	9.35± 0.04^{c}	9.16± 0.03^{c}	8.57± 0.04^{b}	8.21±0.04 ^{b}	6.24±0.02 ^{a}
MDA	4.06±0.02^{a}	7.02±0.06^{b}	7.28±0.04^{b}	9.22±0.06^{c}	9.38±0.05^{c}

Data are represented as mean ± SD of three replicates. Means along the same row with different superscripts are significantly different (P < 0.05). ALT-Alanine aminotransferase (IU/l), AST-Aspartate aminotransferase (IU/l), LDH-Lactate dehydrogenase (IU/l), SOD-Superoxide dismutase (IU/l), AChE-Acetylchinesterase (IU/l), Glucose-(mg/dl), Protein-(mg/dl), MDA-Malondialdehyde (nmol MDA/mg).

Table 4: Biochemical alterations in the gill of *C. gariepinus* exposed to varying concentrations of Pendimethalin for 96 hours

Parameter	Concentration (mg/l)				
	0.00 (Control)	0.25	0.50	0.75	1.00
ALT	42.03 ± 0.28^{a}	45.09± 0.31^{b}	49.12 ± 0.36^{c}	53.09 ± 0.30^{d}	52.96± 0.37^{c}
AST	54.07 ± 1.08^{a}	58.14±1.11^{b}	63.20 ± 1.23^{c}	62.84 ±1. 59^{c}	65.23± 1.42^{d}
LDH	265.10 ± 43.66^{d}	248.18 ± 37.15^{c}	247.22 ±38.20^{c}	237.56± 41.08^{b}	216.82± 40.09^{a}
SOD	234.74 ± 2.48^{e}	226.39 ± 2.87^{d}	217.98 ± 2.91^{c}	198.90± 2.84^{b}	188 ± 2.79^{a}
AChE	28.37 ± 0.02^{e}	25.69± 0.04^{d}	23.17± 0.06^{c}	20.79 ± 0.04^{b}	18.99 ± 0.03^{a}
Glucose	30.23 ± 1.04^{e}	27.92± 1.02^{d}	25.03 ± 1.04^{c}	23.21± 0.93^{b}	19.89 ± 0.97^{a}
Protein	16.29 ± 0.14^{e}	14.19± 0.08^{d}	12.53 ± 0.06^{c}	9.95± 0.04^{b}	7.98 ± 0.02^{a}
MDA	18.60 ± 0.27^{a}	23.02 ± 0.31^{b}	23.58 ± 0.36^{b}	26.09 ± 0.41^{c}	27.91 ± 0.44^{d}

Data are represented as mean ± SD of three replicates. Means along the same row with different superscripts are significantly different (P < 0.05). ALT-Alanine aminotransferase (IU/l), AST-Aspartate aminotransferase (IU/l), LDH-Lactate dehydrogenase (IU/l), SOD-Superoxide dismutase (IU/l), AChE-Acetylchinesterase (IU/l), Glucose-(mg/dl), Protein-(mg/dl), MDA- Malondialdehyde (nmol MDA/mg).

Table 5: Biochemical alterations in the liver of *C. gariepinus* exposed to varying concentrations of Pendimethalin for 96 hours

Parameter	Concentration (mg/l)				
	0.00 (Control)	0.25	0.50	0.75	1.00
ALT (IU/l)	48.10 ± 0.31^{a}	53.32 ± 0.48^{b}	54.98 ± 0.39^{b}	57.92 ± 0.42^{c}	60.13 ± 0.47^{d}
AST (IU/l)	56.23 ± 0.58^{a}	59.47± 0.51^{b}	64.30 ± 0.49^{c}	67.25 ± 0.59^{d}	68.27 ± 0.63^{d}
LDH (IU/l)	277.27 ± 40.16^{e}	251.36 ± 41.92^{d}	228.58 ± 42.20^{c}	197.66 ± 44.18^{b}	190.49 ± 43.70^{a}
SOD (IU/l)	272.61 ± 1.25^{e}	263.85 ± 1.27^{d}	257.92 ± 1.34^{c}	251.48 ± 1.24^{b}	244. 73 ± 1.49^{a}
AChE (IU/l)	32.08 ± 0.17^{d}	29.85 ± 0.19^{d}	25.71 ± 0.16^{c}	22.95 ± 0.18^{b}	19.21 ± 0.16^{a}
Glucose	28.51 ± 0.58^{d}	27.94 ± 0.47^{d}	25.48 ± 0.44^{c}	22.86 ± 0.41^{b}	19.89 ± 0.37^{a}
Protein	15.98 ± 0.02^{e}	15.25 ± 0.04^{d}	12.80 ± 0.02^{c}	10.75 ± 0.04^{b}	7.73 ± 0.03^{a}
MDA	14.92 ± 0.35^{b}	17.84 ± 0.37^{b}	22. 28 ± 0.33^{b}	25.60 ± 0.41^{b}	26.76 ± 0.42^{b}

Data are represented as mean ± SD of three replicates. Means along the same row with different superscripts are significantly different (P < 0.05). ALT-Alanine aminotransferase, AST-Aspartate aminotransferase, LDH-Lactate dehydrogenase, SOD-Superoxide dismutase, AChE-Acetylchinesterase, Glucose-(mg/dl), Protein-(mg/dl), MDA- Malondialdehyde (nmol MDA/mg).

Table 6: Biochemical alterations in the blood of *C. gariepinus* exposed to varying concentrations of Pendimethalin for 28 days

Parameter	Concentration (mg/l)				
	0.00 (Control)	0.25	0.50	0.75	1.00
ALT	26.24 ± 1.21^{a}	28.90± 1.28^{b}	31.19 ± 1.39^{b}	34.09 ± 1.42^{c}	38.02 ±1.47^{d}
AST	33.45 ± 1.58^{a}	39.21 ± 1.51^{b}	42.67 ± 1.49^{c}	46.08 ± 1.46^{d}	48.95 ± 1.53^{e}
LDH	186.16 ± 20.86^{e}	173.29 ± 31.92^{d}	160.73 ± 32.20^{c}	156.91 ± 34.18^{b}	149.90 ± 37.60^{a}
SOD	148.46 ± 5.35^{e}	141.96 ± 4.67^{d}	136.88 ± 5.01^{c}	129. 83 ± 4.81^{b}	122. 37 ± 4.69^{a}
AChE	18.98 ± 0.38^{d}	17.14 ± 0.31^{d}	15.37 ± 0.35^{c}	13.91± 0.38^{b}	10.78 ± 0.34^{a}
Glucose	20.02 ± 0.26^{d}	19.01± 0.26^{d}	15.37 ± 0.32^{c}	13.89 ± 031^{b}	10.92± 0.35^{a}
Protein	13.78 ± 0.05^{e}	10.06 ± 0.04^{d}	10.13 ± 0.03^{c}	9.21 ± 0.04^{b}	6.98 ± 0.03^{a}
MDA	7.03 ± 0.15^{b}	11.32± 0.18^{b}	12.99 ± 0.22^{b}	13.57± 0.24^{b}	15.06 ± 0.22^{b}

Data are represented as mean ± SD of three replicates. Means along the same row with different superscripts are significantly different (P < 0.05). ALT-Alanine aminotransferase (IU/l), AST-Aspartate aminotransferase (IU/l), LDH-Lactate dehydrogenase (IU/l), SOD-Superoxide dismutase (IU/l), AChE-Acetylchinesterase (IU/l), Glucose-(mg/dl), Protein-(mg/dl), MDA- Malondialdehyde (nmol MDA/mg).

Table 7: Biochemical alterations in the gill of *C. gariepinus* exposed to varying concentrations of Pendimethalin for 28 days

Parameter	Concentration (mg/l)				
	0.00 (Control)	0.25	0.50	0.75	1.00
ALT	46.29 ± 1.40^{a}	51.08 ± 1.48^{b}	54.32 ± 1.29^{c}	58.22 ± 1.47^{d}	60.87 ± 1.47^{e}
AST	58.17 ± 1.39^{a}	62.07 ± 1.71^{b}	65.11 ± 1.63^{c}	72.18 ± 1.74^{d}	76.34 ± 1.63^{e}
LDH	202.43 ± 44.62^{e}	189.52 ± 46.61^{d}	174.51 ± 51.00^{c}	168.06 ± 50.11^{b}	162.63 ± 53.10^{a}
SOD	169.26 ± 16.25^{e}	165.04 ± 17.18^{d}	160.07 ± 14.61^{c}	152.76 ± 16.88^{b}	147.03 ± 13.59^{a}
AChE	29.33 ± 0.29^{d}	26.05 ± 0.29^{d}	22.85 ± 0.31^{c}	19.93 ± 0.38^{b}	15.99 ± 0.36^{a}
Glucose	31.37 ± 0.25^{d}	27.67 ± 0.32^{d}	25.48 ± 0.33^{c}	20.87 ± 0.36^{b}	17.84 ± 0.37^{a}
Protein	18.91 ± 0.07^{e}	15.74 ± 0.04^{d}	13.68 ± 0.06^{c}	9.95 ± 0.04^{b}	5.98 ± 0.03^{a}
MDA	12.68 ± 0.32^{a}	15.23 ± 0.37^{b}	19.03 ± 0.36^{c}	23.04 ± 0.31^{d}	23.96 ± 0.42^{d}

Data are represented as mean ± SD of three replicates. Means along the same row with different superscripts are significantly different ($P < 0.05$). ALT-Alanine aminotransferase (IU/l), AST-Aspartate aminotransferase (IU/l), LDH-Lactate dehydrogenase (IU/l), SOD-Superoxide dismutase (IU/l), AChE-Acetylchinesterase (IU/l), Glucose-(mg/dl), Protein-(mg/dl), MDA- Malondialdehyde (nmol MDA/mg).

Table 8: Biochemical alterations in the liver of *C. gariepinus* exposed to varying concentrations of Pendimethalin for 28 days

Parameter	Concentration (mg/l)				
	0.00 (Control)	0.25	0.50	0.75	1.00
ALT	49.61 ± 0.67^{a}	52.65 ± 0.73^{b}	55.03 ± 0.68^{c}	59.13 ± 0.72^{d}	63.06 ± 0.77^{e}
AST	61.21 ± 0.86^{a}	64.95 ± 0.78^{b}	67.01 ± 0.99^{c}	73.50 ± 0.94^{d}	77.09 ± 0.93^{e}
LDH	198.28 ± 37.61^{e}	190.66 ± 40.02^{d}	186.30 ± 42.30^{c}	178.92 ± 43.14^{b}	171.63 ± 43.90^{a}
SOD	159.88 ± 11.35^{e}	152.73 ± 14.50^{d}	149.39 ± 16.53^{c}	144.48 ± 10.90^{b}	138.91 ± 12.95^{a}
AChE	34.27 ± 4.85^{d}	29.86 ± 3.99^{d}	26.55 ± 4.81^{c}	20.84 ± 4.68^{b}	17.99 ± 3.96^{a}
Glucose	27.06 ± 0.05^{d}	24.84 ± 0.06^{d}	20.77 ± 0.05^{c}	17.93 ± 0.07^{b}	15.78 ± 0.07^{a}
Protein	25.53 ± 0.02^{a}	24.96 ± 0.04^{a}	$20.34b \pm 0.06^{c}$	17.98 ± 0.04^{d}	14.87 ± 0.04^{e}
MDA	16.72 ± 0.35^{b}	19.67 ± 0.37^{b}	19.59 ± 0.35^{b}	22.98 ± 0.39^{b}	27.12 ± 0.41^{b}

Data are represented as mean ± SD of three replicates. Means along the same row with different superscripts are significantly different ($P < 0.05$). ALT-Alanine aminotransferase (IU/l), AST-Aspartate aminotransferase (IU/l), LDH-Lactate dehydrogenase (IU/l), SOD-Superoxide dismutase (IU/l), AChE-Acetylchinesterase (IU/l), Glucose-(mg/dl), Protein-(mg/dl), MDA- Malondialdehyde (nmol MDA/mg).

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