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Research Article

EVALUATION OF THE AMELIORATIVE ROLES OF VITAMINS A, C AND E ON ASPARTATE AMINO TRANSFERASE (AST) PRODUCTION IN *CLARIAS GARIEPINUS* (BURCHELL, 1822) FINGERLINGS EXPOSED TO LEAD NITRATE

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ABSTRACT

The presence of pollutants in the environment of an aquatic organism such as fish can lead to the production of reactive oxygen species and consequently, oxidative stress. The effects of lead nitrate on aspartate amino transferase (AST) in *C. gariepinus* and how such effects can be ameliorated through administration of vitamins were investigated. *C. gariepinus* fingerlings (whose initial weight ranged from 3-11g) were exposed to sub-lethal concentrations of Pb **(**00, 26mg/L, 44mg/L, 61mg/L and 79mg/L) with replicate in each case. 26mg/L of the vitamins in each case was administered across all bud. Fresh concentrations of both toxicant and vitamins were administered every 72 hours for a period of 12 weeks every time the water medium was changed. 3 samples of the fish were randomly selected and sacrificed from each aquarium tank every 2 weeks of the exposure period. The gills, kidneys and liver were excised from these specimens and homogenized in sodium phosphate buffer and then assayed for AST production levels in each case. From the results: In Pb only group samples, the highest mean values of AST in the liver and kidneys were obtained in T1. The highest AST in the gill was obtained in T4. In PbVA samples, the highest AST in the liver was obtained in T3. The highest AST produced in the kidneys was obtained in T2.The highest AST produced in the gill was obtained in T4. In PbVC, the highest AST values in liver and kidneys were obtained in T1. The gills' AST had its highest in T4. In PbVE, the highest AST in the liver was obtained in T1. The highest AST produced in the kidney was obtained in T4. The highest AST in the gill was obtained in T2. The kidneys of the samples of the Pb only and PbVA groups recorded the highest. The liver and the gills in the PbVC and PbVE groups, respectively produced the highest values of AST. The high levels of production of the antioxidant suggests that AST is a good biomarker of the oxidative stress elicited by the presence of the toxicant.

Keywords: *Clarias gariepinus*, AST production level, Ameliorative roles, Vitamin supplements, Pb treatment groups**.**

INTRODUCTION

Fish is a rich source of animal protein throughout the world. Due to its nutritional value (Tingman *et al*., 2010) the demand for fish food has been on the increase with increasing human population (FAO, 2010, 2012). African catfish, *Clarias gariepinus* is an important commercial fish due to its high growth rate, high consumer acceptability, and ability to withstand poor water quality, and oxygen depletion (Adewolu *et al*., 2008; Karami *et al*., 2010). The African cat fish, *C. gariepinus* is a tropical hardy species belonging to the Phylum Chordates, class Actinopterygii and family Clariidae. Colour vary widely from yellow through gray to olive or sometimes dark with dark greenish-brown markings and white belly (Stat, 2019). *Clarias* species is a widely distributed fish in Asia and Africa. In these areas, the fish is extremely popular on account of its tasty flesh, its unparalleled hardness, its rapid growth and its somewhat acceptable market price (FAO, 2003). In Nigeria, *Clarias* species is an indigenous fish occurring in freshwater throughout the country. It is suspected that apart from tilapia, *Clarias* is the most

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abundant cultivated fish species in Nigeria (FAO, 2003). The common species found are *C. gariepinus*, *C. anguillaris*, *C. buthupogon* and *C. lazera*.

The presence of pollutants in the environment of an aquatic organism such as fish can lead to the production of reactive oxygen species and consequently, oxidative stress. Heavy metals are known to elicit oxidative stress in organisms when the threshold is exceeded. Heavy metals are also known to promote oxidative damage by increasing the cellular concentration of reactive oxygen species (ROS) in fish, consequently, a response of antioxidative defences (Monteiro *et al*., 2010). Heavy metals could be essential or non-essential. Heavy metals such as Fe, Cu, Zn, Ni, Co, Cr, and Mn are vital to human only at lower concentrations, but they become more toxic when they are taken up more than the bio-recommended limits (Shilpi *et al*., 2015). It is also known that even essential metals may be toxic on the biological activities of organisms above certain concentrations (Merciai *et al*., 2014). Fish are particularly vulnerable and heavily exposed to pollutants due to feeding and living in aquatic ecosystems, because they cannot avoid pollutant harmful effects (Ahmed *et al*., 2020). Heavy metals enter fish by direct absorption from water through their gills and skin, or by ingestion of contaminated food (Ayyat *et al*., 2020). It is also known that heavy metals induce significant damage to the physiologic and biochemical processes of the fish and subsequently to fish consumers (Mehana *et al*., 2020). Among all the heavy metals, Cd, arsenic, mercury and lead pose highest degree of toxicity and that is of great concern to plants and human health (Athar *et al*., 2018). Antioxidant enzymes are crucial in their effort to decrease oxidative stress produced by exposure to toxicants (Saglam *et al*., 2014). It has also been reported that antioxidant may ameliorate, protect and remove the oxidative damage to a target organ or molecule (El Shenawy & Al Ghamdi, 2014).

Vitamins A, C and E are known to play ameliorative roles in the attenuation of the effects of pollutants on organisms. Fishes survive oxidative stress by mobilizing enzymatic as well as non-enzymatic antioxidant defences (Ahmad *et al*., 2008; Van der Oost *et al*., 2003). Also, Vitamins C and E supplementations have been reported to play a positive role in detoxification of mercury toxicity especially at lower concentrations (Thakur, 2014). It can also reduce Pb and Cu levels in serum and tissues of liver and kidney as well as reduce Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST), urea and creatinine levels in Pb and Cu intoxicated male rats (Osfor *et al*., 2010). Vitamin C is known to play a crucial role in the immunological and antioxidant properties of vertebrates capable of maintaining the integrity, fluidity of membranes and capable of controlling the oxidizing reactions of fatty acids, thus keeping cellular respiration and avoiding cell death (Abdel-Warith *et al*., 2011). Non-enzymatic antioxidants such as vitamins C and E can also act to overcome oxidative stress, being a part of the total antioxidant system. They prevent the increased production of free radicals induced by oxidative damage to lipids and lipoproteins in various cellular compartments and tissues. Vitamin E (α-tocopherol) is a fat soluble antioxidant that inhibits the production of reactive oxygen species formed when fat undergoes oxidation.

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) belong to the plasma non-functional enzymes which are normally localized within the cells of liver, heart, gills, kidneys, muscle and other organs. These enzymes are liberated into the blood in pathological situations and therefore are of clinical importance. AST and ALT are highly conservative indicators in liver, and are commonly located in hepatic cytoplasm and would release into the circulation when hepatocytes necrotize (Villafranca *et al*., 2017). In the environmental studies also, blood and tissues level of AST and ALT have been measured to assess the toxic impact of aflatoxicosis and ochratoxicosis (Ellakany & Gaafar, 2002). The presence of pollutant can trigger the utilization or increased production of AST and ALT. The ameliorative role of vitamins was evident when Vitamin E and metallothionein treatments protected against Cd-induced damage of liver in grass carp by decreasing AST and ALT content, repairing organelles, and maintained the antioxidant system by elevating CAT, SOD, and GSH-Px activity and regulating related mRNA transcript expression (Feng *et al*., 2018). This research therefore, addresses the effects of Pb toxicant on AST production levels and how such effects can be attenuated to certain extent by administration of vitamin supplements.

MATERIALS AND METHODS

Samples/materials collection and Acclimatization

A total number of seven hundred and fifty (750) fingerlings of *C. gariepinus* were purchased from a commercial fish farmer and transported in 50L containers filled with water to the Old Farm Research Unit of the Department of Water, Aquaculture and Fisheries Technology, Bosso Campus, Federal University of Technology, Minna, Nigeria. The fishes were placed in fish ponds with water for acclimatization. The fishes were fed to satiation twice daily (morning and evening) with Blue Crown feed (3mm) for 14 days (2 weeks) for the acclimatization. The holding water was changed every 3 days during the period. The vitamins A, C and E granules or pellets (500g in each case) were purchased from commercial chemical stores. The toxicant, Pb (2 units of 500g) analar grades were purchased from commercial chemical stores and stored in a cool dry condition throughout the period of the experiment. These toxicants were administered according to the sub-lethal concentrations of the treatments during the chronic phase of the exposure.

Experimental Set-up

Five treatments including control with two replicates in each treatment were set-up for the Pb, Vitamin A, C and E; and the sub-lethal exposures were run for a period of twelve (12) weeks. Minimum concentration of the toxicant treatments serves the same basis for the concentration of the vitamins in each treatment group and applied across all

the buds. In order to assess long term effects of lead nitrate ($Pb(NO_3)_2$, the fishes were exposed to five sub-lethal treatments of lead nitrate concentrations corresponding to 0% (control), 15%, 25%, 35% and 45% of 96hrs LC_{50} which translated into 26mg/L as T1, 44mg/L as T2, 61mg/L as T3 and 79mg/L as T4, respectively. Each treatment was in two replicates containing 20 fish in 20L plastic aquarium for the Pb, Vitamins A, C and E supplemented exposures. The water was changed and fresh toxicant and the vitamins with the same set of concentrations were added at every 72 hours according to (OECD, 2007)standards. Three fish samples were picked at random and sacrificed from each trough on every 14th day for the twelve weeks exposure period. The liver, gills and kidney were excised, homogenized in sodium phosphate buffer solution using ceramic mortar and pestle; and stored in sample tubes, then refrigerated until needed for analyses of AST.

Preparation of sodium phosphate buffer

Sodium phosphate buffer solution (0.2 M) was prepared from the mixture of sodium dihydrogen orthophosphate with 0.1 M and disodium hydrogen orthophosphate with 0.1 M. The pH was adjusted to 8.0.

Aspartate aminotransferase (AST) Determination

Fish tissues' AST were determined as described by Reitman & Frankel, (1957) from all the treatments and replicates. Spectro-photometric method was used for the assay of AST. The homogenates were prepared in the laboratories as follow: 100µl (0.1ml) of the tissue homogenate was added into test tubes with 500µl (0.5ml) of reagent 1(buffer). The mixture was incubated for 60 minutes at 37°C. Subsequently, 500µl (0.5ml) of reagent 2 (2, 4- dinitrophenylhydrazine) was added and kept for 20 minutes at 25^0C. The reaction was terminated with the addition of 5000µl (5.0ml) of 0.4Mol/L NaOH to the mixture. The blank was prepared with 500µl (0.5ml) of reagent1 and 0.1µl (100µl) of distilled water. The absorbance was read at 546 nm.

Data Analysis

The antioxidants levels in samples exposed to sub-lethal concentrations of the toxicants as well as those treatments supplemented with vitamins were analysed using One Way Analysis of Variance followed by Duncan Multiple Range Test to separate the means where significant at $P \leq 0.05$ level of significance using SPSS Statistical Package (version 20.0 for Windows).

RESULTS AND DISCUSSIONS

AST production levels in Liver, Kidneys and gills of *C. gariepinus* exposed to sub-lethal concentrations of $PbNO_3)_2$ toxicant and the respective supplemented treatments with Vitamins A, C and E for a period of twelve weeks and sampled fortnightly. In the samples exposed to sub-lethal concentrations of $Pb(NO_3)_2$, the AST production levels in the liver of the fish indicated that the control mean values in the 2nd and 4th weeks of exposure are significantly higher than other treatments. The T1 mean values in the 6th and 8th weeks of exposure are significantly higher than other treatments including the control. The T1 and T2 mean values in the 10th week of exposure are significantly higher than other treatments including the control. The mean values of T3 in the 12th week of exposure are significantly higher than other treatments including the control. The highest mean value of AST in the liver was 124.68 ± 0.19nM/mg obtained in T1 at the 6th week of exposure. (Table 1). On the other hand, the T3, T4 and T1 mean values in the kidney of the fish are significantly higher than other treatments including the control in the 2nd, 4th and 6th weeks of exposure, respectively. However, the mean values of T4 are significantly higher than other treatments including the control in the 8th week of exposure. Meanwhile, the control mean values are significantly higher than other treatments at 10th week of exposure. The highest mean value in this regard was 141.40 ± 0.10nM/mg obtained in T1 at the 12th week of exposure. This value is also significantly higher than other treatments including the control (Table 2). Furthermore, in the gills of the sample, the T1 mean values in the 2nd week of exposure are significantly higher than other treatments including the control. However, the control mean values in the 4th, 6th and 10th weeks of exposure are significantly higher than other treatments. The T2 mean values in the 8th week of exposure are significantly higher than other treatments including the control. The highest AST mean value produced in the gill in this case was 124.21 ± 0.28nM/mg obtained in T4 at the end of the 12th week of exposure (Table 3).

In another development, the samples of fish exposed to sub-lethal concentrations of $Pb(NO_3)_2$ and supplemented with vitamin A the AST production in the liver indicated that T1 mean values are significantly higher than other treatments in the 2nd week of exposure. At the 4th and 8th weeks of exposure, respectively the T4 and T1 mean values are significantly higher than other treatments. T3 and T4 mean values in the 10th and 12th weeks of exposure are significantly higher than other treatments. The highest mean value of 113.75±0.19nM/mg at the 10th week was obtained in T3 (Table 4). On the other hand, the ^{AST} mean values produced in the kidneys indicated that T1 and T2 mean values in the 2nd and 4th weeks of exposure are significantly higher than other treatments. However, at the T2 mean values in both 8th and 12th week of exposure are significantly higher than other treatments. The highest AST production value in this case was 139.86±0.19nM/mg obtained in T2 at the 12th week (Table 5). Furthermore, in the gills of the fish samples, the T4 mean values in the 2nd week of exposure are significantly higher than other treatments. The T1, T3 and T4 mean values in the 8th, 10th and 12th weeks of exposure, respectively are significantly higher than other treatments. The highest AST production value in the gill in this case was 115.78±0.10nM/mg obtained in T4 at the 12th week of exposure. (Table 6).

Table 1. AST production levels in the Liver of *C. gariepinus* exposed to sub-lethal concentrations of $Pb(NO_3)_2$ for a period of 12 weeks.

	1st	2nd	3rd	4th	5th	6th
CR	90.62 ± 0.18^{k}	105.00 ± 0.19^{k}	86.90 ± 0.06^{f}	6.56 ± 0.37^{b}	81.25 ± 5.78^{g}	19.21 ± 0.28^{b}
T1	87.03 ± 0.10^{j}	11.40 ± 0.28^{b}	124.68 ± 0.19^{k}	21.25 ± 0.19^{g}	119.06 ± 0.19^{l}	104.53 ± 0.10^{i}
T2	50.93 ± 0.37^{g}	20.00 ± 0.19^{e}	78.50 ± 0.12^{e}	53.59 ± 0.28^{i}	119.68 ± 0.19^{l}	94.53 ± 0.28^{g}
T3	98.90 ± 0.28^{l}	39.06 ± 0.19^{g}	23.89 ± 0.16^{b}	3.28 ± 0.28^{a}	5.31 ± 0.19^{c}	115.78 ± 0.28^{j}
T4	9.53 ± 0.46^{a}	69.84 ± 0.01^{i}	119.68 ± 0.19^{i}	100.31 ± 0.19^{m}	5.78 ± 0.28^{c}	84.84 ± 0.28^{f}

Mean values and standard errors with different alphabets along the column are significantly different from each other at P≤0.05. The unit for AST means value in each case is nM/mg.

Table 2. AST production levels in the Kidney of *C. gariepinus* exposed to sub-lethal concentrations of $Pb(NO_3)_2$ for a period of 12 weeks.

	1st	2nd	3rd	4th	5th	6th
CR	47.03 ± 0.01^{f}	15.31 ± 0.19^{c}	70.00 ± 0.19^{c}	13.59 ± 0.82^{e}	120.31 ± 0.19^{m}	45.46 ±0.46^{c}
T1	17.50 ± 0.55^{c}	96.00 ± 0.13^{j}	103.28 ± 0.28^{h}	74.84 ± 0.28^{j}	94.53 ± 0.10^{i}	141.40±0.10^{o}
T2	62.65 ± 0.28^{h}	16.71 ± 0.10^{d}	34.98 ± 0.08^{c}	14.53 ± 0.10^{f}	86.40 ± 0.28^{h}	62.18±0.11^{d}
T3	68.28 ± 0.28^{i}	0.93 ± 0.19^{a}	97.65 ± 0.10^{g}	14.53 ± 0.10^{f}	35.78 ± 0.28^{f}	74.84±0.28^{e}
T4	39.68 ± 0.19^{d}	106.09 ± 0.28^{k}	20.00 ± 0.19^{a}	129.21 ± 0.28^{n}	2.49 ± 0.19^{a}	119.21±0.19^{l}

Mean values and standard errors with different alphabets along the column are significantly different from each other at P≤0.05. The unit for AST means value in each case is nM/mg.

Table 3. AST production levels in the Gill of *C. gariepinus* exposed to sub-lethal concentrations of $Pb(NO_3)_2$ for a period of 12 weeks.

	1st	2nd	3rd	4th	5th	6th
CR	41.09 ± 0.01^{e}	152.18 ± 0.19^{m}	120.15 ± 0.10^{j}	9.68 ± 0.19^{c}	100.15 ± 0.10^{k}	12.18 ± 0.19^{a}
T1	118.75 ± 0.18^{n}	116.71 ± 0.10^{l}	79.37 ± 0.19^{e}	90.46 ± 0.28^{k}	94.68 ± 0.37^{j}	117.34 ± 0.46^{k}
T2	13.43 ± 0.19^{b}	31.71 ± 0.28^{f}	112.50 ± 0.19^{i}	93.75 ± 0.19^{l}	13.12 ± 0.19^{e}	101.56 ± 0.19^{h}
T3	117.03 ± 0.10^{m}	44.53 ± 0.10^{h}	71.56 ± 0.19^{d}	38.90 ± 0.28^{h}	3.75 ± 0.37^{b}	46.71 ± 0.18^{c}
T4	104.00 ± 0.46^{o}	31.25 ± 0.37^{f}	47.03 ± 0.28^{d}	10.31 ± 0.19^{d}	9.68 ± 0.19^{d}	124.21 ± 0.28^{m}

Mean values and standard errors with different alphabets along the column are significantly different from each other at P≤0.05. The units for AST mean value in each case is nM/mg.

Table 4. AST production levels in the Liver of *C. gariepinus* exposed to sub-lethal concentrations of $Pb(NO_3)_2$ and supplemented with vitamin A for a period of 12 weeks.

	1st	2nd	3rd	4th	5th	6th
CR	90.62 ± 0.19^{k}	105.00 ± 0.19^{m}	0.00 ± 0.00	6.50 ± 0.37^{a}	81.25 ± 5.78^{g}	19.21 ± 0.28^{a}
T1	101.56 ± 0.55n	6.56 ± 0.19a	0.00 ± 0.00	110.93 ± 0.19m	87.96 ± 0.10h	75.15 ± 0.10d
T2	43.59 ± 0.90e	23.28 ± 0.00e	0.00 ± 0.00	39.21 ± 0.10f	54.37 ± 0.19d	94.06 ± 0.19h
T3	94.37 ± 0.19m	15.46 ± 0.28d	0.00 ± 0.00	23.59 ± 0.46e	113.75 ± 0.19m	103.59 ± 0.10k
T4	38.59 ± 0.00c	66.56 ± 0.19l	0.00 ± 0.00	22.81 ± 0.19d	10.93 ± 0.19a	110.62 ± 0.37l

Mean values and standard errors with different alphabets along the column are significantly different from each other at P≤0.05. The units for AST mean value in each case is nM/mg.

Table 5. AST production levels in the Kidney of *C. gariepinus* exposed to sub-lethal concentrations of $Pb(NO_3)_2$ and supplemented with vitamin A for a period of 12 weeks.

	1st	2nd	3rd	4th	5th	6th
CR	47.03 ± 0.10f	15.31 ± 0.19c	70.00 ± 0.19a	13.59 ± 0.82c	120.31 ± 0.19o	45.46 ± 0.46c
T1	63.90 ± 0.28j	31.71 ± 0.28h	0.00 ± 0.00	103.59 ± 0.28l	30.93 ± 0.19b	82.34 ± 0.10e
T2	29.06 ± 0.19b	37.34 ± 0.10k	0.00 ± 0.00	127.96 ± 0.10o	65.00 ± 0.19e	139.86 ± 0.19o
T3	55.31 ± 0.19g	0.00 ± 0.00	0.00 ± 0.00	53.59 ± 0.28h	79.06 ± 0.19f	126.09 ± 0.28n
T4	13.43 ± 0.19a	33.90 ± 0.28j	0.00 ± 0.00	111.09 ± 0.28n	96.25 ± 0.19i	102.65 ± 0.10j

Mean values and standard errors with different alphabets along the column are significantly different from each other at P≤0.05. The units for AST mean value in each case is nM/mg.

Table 6. AST production levels in the Gill of *C. gariepinus* exposed to sub-lethal concentrations of $Pb(NO_3)_2$ and supplemented with vitamin A for a period of 12 weeks.

	1^{st}	2^{nd}	3^{rd}	4^{th}	5^{th}	6^{th}
CR	41.09 ± 0.10^{d}	152.18 ± 0.19^{n}	120.15 ± 0.10^{b}	9.68 ± 0.19^{b}	100.15 ± 0.10^{j}	42.18 ± 0.19^{b}
T_1	93.12 ± 0.19^{l}	28.75 ± 0.37^{g}	0.00 ± 0.00	97.96 ± 0.10^{k}	107.30 ± 0.10^{l}	99.37 ± 1.09^{i}
T_2	59.84 ± 0.28^{h}	10.46 ± 0.10^{b}	0.00 ± 0.00	40.62 ± 0.19^{g}	32.96 ± 0.28^{c}	86.25 ± 0.37^{f}
T_3	60.46 ± 0.10^{i}	32.96 ± 0.28^{i}	0.00 ± 0.00	69.37 ± 0.37^{i}	115.46 ± 0.28^{n}	93.43 ± 0.19^{g}
T_4	114.53 ± 0.10^{o}	26.87 ± 0.19^{f}	0.00 ± 0.00	73.90 ± 0.10^{j}	106.56 ± 0.19^{k}	115.78 ± 0.10^{m}

Mean values and standard errors with different alphabets along the column are significantly different from each other at P≤0.05. The units for AST mean value in each case is nM/mg.

From the results of the analysis of the samples of fish exposed to sub-lethal concentrations of $Pb(NO_3)_2$ and supplemented with vitamin C, the T3 and T1 mean values in the 4^{th} and 8^{th} weeks of exposure, respectively are significantly higher than other treatments. Similarly, theT1 and T2 mean values in the 10^{th} and 12^{th} week of exposure are significantly higher than other treatments. The highest mean value was 129.06±0.37 nM/mg nM/mg obtained in T1 at the 10^{th} week of exposure (Table 7). In another development, the AST mean values produced in the kidneys indicated that the T3 mean values in both 2^{nd} and 4^{th} weeks of exposure respectively are significantly higher than other treatments. The T2 mean values in the 8^{th} week are significantly higher than other treatment. The mean values of T1 and T3 in the 10^{th} and 12^{th} weeks of exposure are significantly higher than other treatments. The highest AST mean values in the kidneys of the fish were 112.81±0.19nM/mg obtained in T1 at the 10^{th} week of exposure (Table 8). Furthermore, the gills' AST production levels indicated that T2 mean values in the 2^{nd} and 4^{th} weeks of exposure, respectively are significantly higher than other treatments. Likewise, T4 mean values in the 8th and 10^{th} weeks of exposure respectively are significantly higher than other treatments. The T2 mean values in the 12^{th} week of exposure are significantly higher than other treatments. The highest AST mean value in the gill was 125.78±0.28 obtained in T4 at the 8^{th} week of exposure (Table 9).

Table 7. AST production levels in the Liver of *C. gariepinus* exposed to sub-lethal concentrations of $Pb(NO_3)_2$ and supplemented with vitamin C for a period of 12 weeks.

	1^{st}	2^{nd}	3^{rd}	4^{th}	5^{th}	6^{th}
CR	90.62 ± 0.19^{o}	105.00 ± 0.19^{m}	0.00 ± 0.00	6.56 ± 0.37^{a}	81.25 ± 5.78^{e}	19.21 ± 0.28^{b}
T_1	46.71 ± 0.28^{k}	72.96 ± 0.28^{k}	0.00 ± 0.00	58.43 ± 0.37^{i}	129.06 ± 0.37^{o}	82.50 ± 0.19^{i}
T_2	37.81 ± 0.37^{i}	23.59 ± 0.10^{f}	0.00 ± 0.00	8.28 ± 0.10^{b}	97.34 ± 0.28^{g}	122.65 ± 0.28^{n}
T_3	22.65 ± 0.10^{d}	116.71 ± 0.10^{n}	0.00 ± 0.00	28.59 ± 0.28^{h}	82.34 ± 0.28^{f}	30.31 ± 0.19^{d}
T_4	71.25 ± 0.19^{n}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	112.18 ± 0.19^{l}	84.37 ± 0.19^{j}

Mean values and standard errors with different alphabets along the column are significantly different from each other at P≤0.05. The units for AST mean value in each case is nM/mg.

Table 8. AST production levels in the Kidney of *C. gariepinus* exposed to sub-lethal concentrations of $Pb(NO_3)_2$ and supplemented with vitamin C for a period of 12 weeks.

	1^{ST}	2^{ND}	3^{RD}	4^{TH}	5^{TH}	6^{TH}
CR	47.03 ± 0.10^{l}	15.31 ± 0.19^{e}	70.00 ± 0.19^{a}	13.59 ± 0.62^{f}	120.31 ± 0.19^{n}	45.46 ± 0.46^{f}
T1	30.46 ± 0.10^{g}	7.34 ± 0.28^{b}	0.00 ± 0.00	13.12 ± 0.19^{e}	112.81 ± 0.19^{m}	79.53 ± 0.28^{h}
T2	32.65 ± 0.28^{h}	7.65 ± 0.28^{c}	0.00 ± 0.00	25.31 ± 0.19^{g}	102.03 ± 0.10^{k}	97.81 ± 0.19^{l}
T3	47.96 ± 0.10^{m}	30.93 ± 0.19^{g}	0.00 ± 0.00	10.78 ± 0.10^{d}	97.81 ± 0.19^{h}	100.00 ± 0.19^{m}
T4	29.53 ± 0.10^{f}	3.43 ± 0.19^{a}	0.00 ± 0.00	0.00 ± 0.00	44.21 ± 0.28^{c}	0.00 ± 0.00

Mean values and standard errors with different alphabets along the column are significantly different from each other at P≤0.05. The units for AST mean value in each case is nM/mg.

Table 9. AST production levels in the Gill of *C. gariepinus* exposed to sub-lethal concentrations of $Pb(NO_3)_2$ and supplemented with vitamin C for a period of 12 weeks.

	1st	2nd	3rd	4th	5th	6th
CR	41.09±0.10j	152.18±0.19	120.15±0.10b	9.68±0.19c	100.15±0.10j	42.18±0.19e
T1	9.68±0.19a	39.21±0.10i	0.00±0.00	69.21±0.10k	38.75±0.19a	56.87±0.19g
T2	27.18±0.19e	89.53±0.28l	0.00±0.00	62.65±0.10j	69.84±0.28d	94.06±0.19k
T3	16.56±0.19b	58.28±0.28j	0.00±0.00	109.68±0.19l	42.65±0.10b	18.90±0.28a
T4	19.68±0.19c	30.46±0.28h	0.00±0.00	125.78±0.28m	100.00±0.19i	27.18±0.37c

Mean values and standard errors with different alphabets along the column are significantly different from each other at P≤0.05. The units for AST mean value in each case is nM/mg.

Table 10. AST production levels in the Liver of *C. gariepinus* exposed to sub-lethal concentrations of $Pb(NO_3)_2$ and supplemented with vitamin E for a period of 12 weeks.

	1^{st}	2^{nd}	3^{rd}	4^{th}	5^{th}	6^{th}
CR	90.62 ± 0.19^{h}	105.00 ± 0.19^{k}	0.00 ± 0.00	6.56 ± 0.37^{c}	81.25 ± 5.78^{e}	19.21 ± 0.28^{b}
T1	84.84 ± 0.28^{g}	135.78 ± 0.28^{m}	23.75 ± 0.37^{a}	37.96 ± 0.28^{f}	91.56 ± 0.19^{g}	122.34 ± 0.28^{l}
T2	99.53 ± 0.10^{m}	42.81 ± 0.19^{g}	72.34 ± 0.28^{d}	0.00 ± 0.00	93.75 ± 0.19^{h}	0.00 ± 0.00
T3	94.68 ± 0.37^{i}	45.78 ± 0.28^{i}	110.00 ± 0.19^{j}	0.00 ± 0.00	128.28 ± 0.10^{n}	82.96 ± 0.28^{i}
T4	99.06 ± 0.19^{l}	22.81 ± 0.19^{e}	119.53 ± 0.19^{l}	95.00 ± 0.19^{h}	120.31 ± 0.19^{m}	90.93 ± 0.19^{j}

Mean values and standard errors with different alphabets along the column are significantly different from each other at P≤0.05. The units for AST mean value in each case is nM/mg.

Table 11. AST production levels in the Kidney of *C. gariepinus* exposed to sub-lethal concentrations of $Pb(NO_3)_2$ and supplemented with vitamin E for a period of 12 weeks.

	1^{st}	2^{nd}	3^{rd}	4^{th}	5^{th}	6^{th}
CR	47.03 ± 0.10^{d}	15.31 ± 0.19^{b}	70.00 ± 0.19^{c}	13.59 ± 0.82^{e}	23.75 ± 0.19^{b}	45.46 ± 0.46^{e}
T1	96.71 ± 0.28^{k}	45.62 ± 0.19^{h}	106.71 ± 0.10^{h}	44.53 ± 0.10^{g}	53.75 ± 0.37^{c}	73.59 ± 0.28^{h}
T2	75.15 ± 0.10^{f}	72.50 ± 0.37^{j}	120.15 ± 0.10^{m}	0.00 ± 0.00	53.75 ± 0.37^{c}	32.81 ± 0.19^{c}
T3	94.84 ± 0.10^{j}	29.53 ± 0.28^{f}	109.53 ± 0.10^{i}	0.00 ± 0.00	82.96 ± 0.28^{f}	68.59 ± 0.10^{g}
T4	25.31 ± 0.19^{b}	20.46 ± 0.10^{c}	89.06 ± 0.19^{f}	5.62 ± 0.19^{b}	56.87 ± 0.37^{d}	123.90 ± 0.10^{m}

Mean values and standard errors with different alphabets along the column are significantly different from each other at P≤0.05. The units for AST mean value in each case is nM/mg.

Table 12. AST production levels in the Gill of *C. gariepinus* exposed to sub-lethal concentrations of $Pb(NO_3)_2$ and supplemented with vitamin E for a period of 12 weeks.

	1st	2nd	3rd	4th	5th	6th
CR	41.09 ± 0.10^{c}	152.18 ± 0.19^{n}	120.15 ± 0.10^{m}	9.68 ± 0.19^{d}	100.15 ± 0.10^{k}	42.18 ± 0.19^{d}
T_1	101.40 ± 0.10^{o}	134.53 ± 0.10^{l}	113.59 ± 0.28^{k}	0.93 ± 0.19^{a}	4.84 ± 0.28^{a}	123.90 ± 0.10^{m}
T_2	100.00 ± 0.19^{n}	153.12 ± 0.19^{o}	91.25 ± 0.19^{g}	0.00 ± 0.00	96.09 ± 0.10^{i}	11.09 ± 0.28^{a}
T_3	66.09 ± 0.10^{e}	3.43 ± 0.18^{a}	48.43 ± 0.19^{b}	0.00 ± 0.00	99.84 ± 0.28^{j}	62.96 ± 0.28^{f}
T_4	20.31 ± 0.37^{a}	22.65 ± 0.10^{d}	82.50 ± 0.19^{e}	0.00 ± 0.00	110.62 ± 0.37^{l}	91.87 ± 0.19^{k}

Mean values and standard errors with different alphabets along the column are significantly different from each other at $P \leq 0.05$. The units for AST mean value in each case is nM/mg.

In the samples exposed to sub-lethal concentrations of $Pb(NO_3)_2$ and supplemented with vitamin E, the AST production in the liver of the fish indicated that, T2,T1,T4, T4, T3 and T1 mean values across their respective columns in the 2nd to the 12th weeks of exposure are significantly higher than other treatments. The highest mean value in this perspective was 135.78±0.28 nM/mg obtained in T1 at the 4th week of exposure (Table10). In addition to the forgoing, the T1 mean values in the kidneys of the samples in 2nd week of exposure are significantly higher than other treatments. The T2 mean values in both 4th and 6th weeks of exposure are significantly higher than other treatments. T3 and T4 mean values in the 10th and 12th week of exposure are significantly higher than other treatments. The highest mean value of AST produced in the kidney was123.90 ± 0.10 nM/mg obtained in T4 at the end of the 12th week of exposure (Table 11). On the other hand, the T1, T2 and T1mean values of AST produced in the gill at the end of the 2nd, 4th and 6th weeks of exposure, respectively are significantly higher than other treatments. Also, T4 and T1 mean values in the 10th and 12th weeks of exposure are significantly higher than other treatments. The highest mean value in this case was 153.12 ± 0.19nM/mg obtained in T2 at the end of the 4th week (Table 12).

In the samples exposed to sub-lethal concentrations of $Pb(NO_3)_2$, the AST production levels in the liver of the fish indicated that the control mean values in the 2nd and 4th weeks of exposure are significantly higher than other treatments. The T1 mean values in the 6th and 8th weeks of exposure are significantly higher than other treatments including the control. The production rate of AST is high in lower concentrations than in higher concentration in the liver of the samples probably because at lower concentrations lesser concentration of AST is needed in combating the effects of the toxicant; hence greater availability in the already elicited production. This is also probably why the T1 and T2 mean values in the 10th week of exposure are significantly higher than other treatments including the control, as well as the production of the highest mean value of AST (124.68±0.19 nM/mg) in the liver which was obtained in T1 at the 6th week of exposure. In line with this, (Adeyemi, Adewale, & Oguma, 2014) reported that the liver aspartae aminotransferase activity (AST) showed a significant reduction after exposure to either Pb or cypermethrin alone. However, the mean values of T3 in the 12th week of exposure are significantly higher than other treatments including the control probably because, at this stage of the exposure there was the need for the up-regulation of the body's defence system to counter the effects of the toxicant. Similar report was given by Abdel-Warith *et al*., (2020) when they posited that hepatic enzyme activities of AST and ALT displayed a significant increase with increasing concentrations and exposure time. Also, sub-lethal concentrations of lead acetate (28.2 and 14.1 ppm) caused an increase of glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) activities in fish *Cirrihinus mrigala* which indicate liver damage (Chavan & Muley, 2014).

On the other hand, the T3, T4 and T1 mean values in the kidney of the fish are significantly higher than other treatments including the control in the 2nd, 4th and 6th weeks of exposure, respectively. However, the mean values of T4 are significantly higher than other treatments including the control in the 8th week of exposure. There is probably constant build up to upstage the deleterious effects of the toxicants. The highest mean value in this regard was 141.40±0.10 nM/mg obtained in T1 at the 12th week of exposure. This value is probably indicative of the fact that there was less utilization of AST in the lowest concentration especially towards the end of the research in the kidney of the fish. In line with this, (Michael *et al*., 2018) reported that AST, ALT and LDH in fish exposed to atrazine were significantly increased with increasing atrazine across the treatments relative to control. Furthermore, in the gills of the sample, the T1 mean values in the 2nd week of exposure are significantly higher than other treatments including the control. This is probably due to differences in the concentration produced and the concentration utilized in combating the effects of the toxicant. However, the control mean values in the 4th, 6th and 10th weeks of exposure are significantly higher than other treatments. This may be due to the utilization of the AST produced at these stages of the exposure such that the concentrations in the un-exposed samples are higher. The highest AST mean value produced in the gill in this case was 124.21 ± 0.28nM/mg obtained in T4 at the end of the 12th week of exposure. At this stage of exposure, there is probably the need for sustained production of AST to

counteract the effects of the toxicant (Markiewicz-Górka *et al*., 2015) reported that aspartate aminotransferase (AST) activity and bilirubin concentration also increased significantly in the animal group exposed to all three metals and correlated positively with blood Cd, Pb, and Mn. Also, GOT and GPT levels increased significantly in starry flounder, *Platichthys stellatus* exposed to hexavalent chromium (Ko *et al*., 2019).

In another development, the samples of fish exposed to sub-lethal concentrations of $Pb(NO_3)_2$ and supplemented with vitamin A, the AST production in the liver indicated that T1 mean values are significantly higher than other treatments in the 2nd week of exposure. This is probably because at lower concentration in the presence of the vitamin and at early stage of the exposure the utilization is minimal after being triggered by the presence of the toxicant. At the 4th and 8th weeks of exposure, respectively the T4 and T1 mean values are significantly higher than other treatments. At higher concentrations and at later stages of the exposure, T3 and T4 mean values in the 10th and 12th weeks of exposure are significantly higher than other treatments probably depicting the constant need for improvement in the immune system of the body to counter the effects of the toxicant. This is also probably why the highest mean value of 113.75±0.19 nM/mg at the 10th week was obtained in T3. On the other hand, the AST mean values produced in the kidneys indicated that T1 and T2 mean values in the 2nd and 4th weeks of exposure are significantly higher than other treatments. This is probably because the rate of utilization in higher concentration is higher than in lower concentration. Also, the T2 mean values in both 8th and 12th week of exposure are significantly higher than other treatments and the highest AST production value in this case (139.86±0.19 nM/mg) was also obtained in T2 at the 12th week.

This concentration (T2) probably proved to be the threshold of the elicitation of the effects of the toxicant in the kidney of the fish. Furthermore, in the gills of the fish samples, the T4 mean values in the 2nd week of exposure are significantly higher than other treatments. This probably suggests, early up-regulation of the body' immune system especially at the portal of entry to the toxicant. At later stages of the exposure the need for sustenance of the defence system was probably why the T1, T3 and T4 mean values in the 8th, 10th and 12th weeks of exposure, respectively are significantly higher than other treatments; and the highest AST production value in the gill in this case (115.78 ± 0.10nM/mg) was also obtained in T4 at the 12th week of exposure. The presence of vitamin A probably must have mitigated the effects of the toxicant in lower concentrations in the gills of the fish. (Shokrzadeh *et al*., 2012) reported that, the rats exposed to diazinon in combination with vitamin A, E and C separate groups displayed significant reduction in ALT and AST activities compared to diazinon group. Also, toxic effects of As exposure on *P. stellatus* indicate how GOT and GPT valuesincreased with increasing arsenic concentration as well as the duration increases (Han *et al*., 2019).

From the results of the analysis of the samples of fish exposed to sub-lethal concentrations of $Pb(NO_3)_2$ and supplemented with vitamin C, the T3 and T1 mean values in the 4th and 8th weeks of exposure, respectively are significantly higher than other treatments. At the early stage of exposure there was probably the need for up-regulation of the defence system in the higher concentrations. The presence of the vitamin most likely ensured less utilization of AST produced due to the onslaught of the toxicant. This is probably why they are produced in greater concentrations at later stages of the exposure. Similarly, the same scenario played out inT1 and T2 mean values in the 10th and 12th week of exposure which are significantly higher than other treatments and the highest mean value (of 129.06±0.37nM/mg) was also obtained in T1 at the 10th week of exposure. In line with this, (Morina *et al*., 2013) showed how glucose, hepatic alanine transaminase (ALT) and aspartate transaminase (AST) levels along with erythrocyte profile are more convenient biomarkers of water pollution and can be used for early detection for pollution effects on fishes. At these stages of the exposure the fish samples have also grown bigger in lower concentrations than in higher ones. In another development, the AST mean values produced in the kidneys indicated that the T3 mean values in both 2nd and 4th weeks of exposure, respectively are significantly higher than in other treatments. The mean values increased in T3 at early stages of the exposure probably because the immune system of the body needs to be increased at this concentration in order to deal with the effects of the toxicant. In addition to this, at lower concentrations and increased duration of the exposure the other treatments come to play perhaps, when the effects have overwhelmed the initial succor provided by the vitamin. This is probably why the T2 mean values in the 8th week are significantly higher than in other treatment. Also, the mean values of T1 and T3 in the 10th and 12th weeks of exposure are significantly higher than other treatments.

The highest AST mean value in the kidneys (112.81±0.19nM/mg) of the fish was also obtained in T1 at the 10th week of exposure. Furthermore, the gills' AST production levels indicated that T2 mean values in the 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments. The need for up-regulation of the immune system in the gill was probably elicited at these stages of exposure; the T2 mean values in the 12th week of exposure are also significantly higher than other treatments when the effects of the toxicant had probably overwhelmed the effects of the vitamin. Likewise, T4 mean values in the 8th and 10th weeks of exposure respectively are significantly higher than other treatments, and the highest AST mean value in the gill was 125.78±0.28nM/mg obtained in T4 at the 8th week of exposure. At these later stages when the duration and concentration increased the need for sustained production of AST probably became more pertinent. In conformity with this, Abdel-Warith *et al*. (2020) reported that hepatic enzyme activities of aspartate amino transferase (AST) and alanine aminotransferase (ALT) displayed a significant increase with increasing concentrations and exposure time.

In the samples exposed to sub-lethal concentrations of $Pb(NO_3)_2$ and supplemented with vitamin E, the AST production in the liver of the fish indicated that, T2 and T1 in the 2nd and 4th weeks of exposure were significant and the highest mean value (135.78±0.28 nM/mg) was also obtained in T1 at the 4th week. The AST production levels were probably not utilized once triggered or elicited but retained in the lower concentrations till the end of the research. T4, T4, T3 mean values in the 6th, 8th and 10th weeks of exposure were significantly higher than other treatments in their respective treatments probably due to the need to up-regulate the defence system to counter the effects of the toxicant (Arenas *et al*., 2017) posited that AST and ALT are highly conservative indicators in liver, and are commonly located in hepatic cytoplasm and would release into the circulation when hepatocytes necrotize. In addition to the foregoing, the T1 mean values in the kidneys of the samples in 2nd week of exposure are significantly higher than other treatments. The T2 mean values in both 4th and 6th weeks of exposure are significantly higher than other treatments. At the early stages of the exposure the production levels of AST were high. The initial surge and elicitation of AST were not as high as in the higher concentration treatments probably due to the presence of the vitamin in the water matrix. This is probably why T3 and T4 mean values in the 10th and 12th week of exposure are significantly higher than other treatments and the highest mean value of AST produced in the kidney (123.90 ± 0.10nM/mg) was also obtained in T4 at the end of the 12th week of exposure. In line with these observations, (Mohamed *et al*., 2019) reported a marked significant decrease in AST, ALT, urea, creatinine ($P \leq 0.05$) was observed in DMSA groups and also how administration of DMSA improved the histopathological alterations in fish liver and kidney. On the other hand, the T1, T2 and T1 mean values of AST produced in the gill at the end of the 2nd, 4th and 6th weeks of exposure, respectively are significantly higher than other treatments and the highest mean value (153.12 ± 0.19nM/mg) in this case was also obtained in T2 at the end of the 4th week. This is probably because at these lower concentrations the AST produced were not so much engaged in dealing with the deleterious effects of the toxicant unlike in the higher concentrations; hence its availability and significance. The presence of the vitamin especially in the lower concentrations was probably also a contributing factor in the ability of the fish to deal with the effects of the toxicant.

CONCLUSIONS AND RECOMMENDATIONS

The AST production levels were varied from one treatment group to the other. The kidneys of the samples of the Pb only and PbVA groups recorded the highest value of the antioxidant. The liver and the gills in the PbVC and PbVE groups, respectively produced the highest values of AST. The high levels of production of the antioxidant suggest that AST is a good biomarker of the oxidative stress elicited by the presence of the toxicant. The highest mean values in the Pb only group was 141.40±0.10nM/mg, PbVA was 139.86±0.19nM/mg, PbVC was 129.06±0.37nM/mg and PbVE was 153.12±0.19nM/mg. AST can be adopted as quick check of the oxidative stress elicited by the presence of the toxicant. The mitigative effects of the vitamins can be explored further by administering higher concentrations of the vitamins for better understanding of the mechanisms of amelioration in the presence of the toxicant.

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