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Evaluation of the Ameliorative Roles of Vitamins A, C and E on Aspartate Amino Transferase in *Clarias gariepinus* (Burchell, 1822) Fingerlings Exposed to Camium Chloride

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Abstract: The anthropogenic activities culminating in environmental pollution usually lead to release of plethora of pollutants such as cadmium calls for concern. The effects of $CdCl_2$ on the production of aspartate amino transferase (AST) in *C. gariepinus* and how they 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 Cd (00, 12mg/L, 16mg/L, 20mg/L and 24mg/L) with replicate in each case. In each case, 12mg/L of the vitamin was administered across all buds. 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. The various treatments group include Cd only, CdVA, CdVC and CdVE. 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. From the results: in Cd only, the highest AST produced in the liver was 135.00±0.18nM/mg in T_1. The highest AST produced in the kidneys of the fish was 145.00±0.18nM/mg in T_3. The highest AST produced in the gill was 137.97±0.09nM/mg in T_1. In CDVA samples, the highest AST produced in the liver was 132.19±0.18nM/mg in T_4. The highest AST produced in the kidneys was 113.91±0.09nM/mg in T_1. In the gills, the highest AST value was 120.94±0.36nM/mg in T_1. In the samples exposed to CdVC, the highest AST produced in the liver was 128.44±0.36nM/mg in T_1. The highest mean value of AST produced in the kidneys was 114.84±0.09nM/mg in T_3. In the gills of the samples, the highest AST value was 125.16±0.27nM/mg in T_2. In CdVE samples, the highest AST produced in the liver was 150.63±0.18nM/mg in T_2. The kidneys' highest AST value of 125.78±0.27nM/mg was recorded in T_4. In the gills of the samples, the highest AST produced in the gills of the fish was 133.28±0.09nM/mg in T_1. There were general high production levels of AST in all treatments with the highest values recorded in the liver of CdVA, CdVC and CdVE groups mostly in samples exposed to lower concentrations. The kidneys in the Cd only group however, produced the highest AST value. The high production values of AST in all treatments suggest that the enzyme is a good biomarker of oxidative stress elicited by the presence of the toxicant.

Keywords: *Clarias gariepinus*, Aspartate Amino Transferase, Ameliorative Roles, Vitamin Supplements, Cd Treatment Groups

1. Introduction

Fish is a rich source of animal protein throughout the world. It also serves both subsistent and commercial purposes in many developing countries of the world. 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 [2, 18].

The African cat fish, *Clarias gariepinus* is a tropical hardy species belonging to the Phylum Chordata, class

Actinopterygii and family Clariidae. *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 [15]. 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 abundant cultivated fish species in Nigeria [15]. The common species found are *Clarias gariepinus*, *Clarias anguillaris*, *Clarias buthupogon* and *Clarias 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 [21]. 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 [27]. It is also known that even essential metals may be toxic on the biological activities of organisms above certain concentrations [20]. Fish are particularly vulnerable and heavily exposed to pollutants due to feeding and living in aquatic ecosystems, because they cannot avoid pollutant harmful effects [4]. Heavy metals enter fish by direct absorption from water through their gills and skin, or by ingestion of contaminated food [8]. Heavy metals induce significant damage to the physiologic and biochemical processes of the fish and subsequently to fish consumers [19].

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 [7]. Antioxidant enzymes are crucial in their effort to decrease oxidative stress produced by exposure to toxicants [25]. It has also been reported that antioxidant may ameliorate, protect and remove the oxidative damage to a target organ or molecule [13].

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 [3, 29]. Also, Vitamins C and E supplementations have been reported to play a positive role in detoxification of mercury toxicity especially at lower concentrations [28]. 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 [1]. 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. The main biological function of vitamin E is its direct influence on cellular responses to oxidative stress through modulation of signal transduction pathway [23]. Vitamins E and C supplementation can induce protective effects on certain conditions after free radical-mediated cellular damage or disruption [31]. 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 [6]. The presence of pollutant can trigger the utilization or increased production of AST. For instance, cadmium in plasma of goldfish significantly increased the activities of plasma glutamic acid oxaloacetic acid-transaminase (GOT) and glutamic acid-pyruvic acid transaminase (GPT) [32].

Ellakany and Gaafar [11] reported that in *Oreochromis niloticus*, there was a marked reduction in AST in liver and muscle in response to the lower or higher level of ochratoxin. They attributed the reduced levels of aminotransfersase in various organs to tissue damage and consequently the reduction of enzyme biosynthesis for reasons related to the presence of ochratoxin. On the other hand, the ALT activities in liver and muscle were found to increase during the time course of endogenous cortisol elevation induced by ochratoxin intoxication. The results obtained also indicated that the tissue injury in toxicated fish recovered when they were fed dietary ascorbic acid because the AST and ALT activities in fish exposed to the lower or higher dose of ochratoxin + vitamin C became similar to those of control fish. 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 [16]. This research therefore, addresses the effects of Cd toxicant on AST production levels and how such effects can be attenuated to certain extent by administration of vitamin supplements.

2. Materials and Methods

2.1. 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 each) were purchased from commercial chemical stores. The toxicant, Cd (2 units of 100g) analar grades were purchased from commercial chemical stores and stored in a cool dry condition throughout the period of the experiment. This toxicant was administered according to the sub-lethal concentrations of the treatments during the chronic phase of the exposure.

2.2. Experimental Set-up

Five treatments including control with two replicates in each treatment were set-up for the Cd, Vitamin A, C and E; and the sub-lethal exposures were run for a period of twelve (12) weeks. The treatments are 0% (control), 15%, 20%, 25% and 30% which translated into 12mg/L, 16mg/L, 20mg/L and 24mg/L of the LC_{50}, respectively. The groups of treatments were tagged Cd (Cd only with T_1-T_4 and replicates), second CdVA (Cd+vitamin A with T_1-T_4 and replicates), third CdVC (Cd+vitamin C with T_1-T_4 and replicates) and fourth CdVE (Cd+vitamin E with T_1-T_4 and replicates). Each treatment was in two replicates containing 20 fish in 20L plastic aquarium for the Cd, 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 Organization for Economic Co-operation and Development [22] 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.

2.3. 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.

2.4. Aspartate Aminotransferase (AST) Determination

Fish tissues' AST were determined as described by Reitman and Frankel [24] 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°C. The reaction was terminated with the addition of 5000µl (5.0ml) of 0.4 Mol/L NaOH to the mixture. The blank was prepared with 500µl (0.5ml) of reagent$_1$ and 0.1µl (100µl) of distilled water. The absorbance was read at 546 nm. These analyses were carried out in the Drug and Vaccine Laboratory Unit of STEP B, of the Federal University of Technology, Bosso Campus, Minna, Niger State.

2.5. Data Analyses

The antioxidants levels in samples exposed to sub-lethal concentration of the toxicant 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).

3. Results and Discussions

3.1. AST Production Levels in Organs of C. gariepinus Exposed to $CdCl_2$ and the Respective Supplemented Treatments with Vitamins A, C and E

From the results of the samples exposed to sub-lethal concentrations of $CdCl_2$, the AST production levels in the liver of the fish showed that T_1 and T_4 mean values in the 2nd and 4th weeks of exposure are significantly higher than other treatments including the control. Also, the levels of AST production in this regard are relatively high in all treatments. T_1 mean values in both 6th and 8th weeks of exposure are significantly higher than other treatments including the control. Similarly, the T_3 mean values in the 10th week of exposure are significantly higher than other treatments including the control. The highest mean value of AST produced in the liver was 135.00±0.18nM/mg obtained in T_1 at the end of the 8th week of exposure. (Table 1). In another development, the T_3 mean values in both 2nd and 4th weeks of exposure are significantly higher than other treatments including the control. In like manner, T_4, T_1 and T_3 mean values in the 6th, 8th and 10th weeks of exposure, respectively are significantly higher than other treatments including the control. The highest mean value of AST produced in the kidneys of the fish was 145.00±0.18nM/mg obtained in T_3 at the end of the 4th week of exposure. (Table 2). Furthermore, the control mean values in the gills of the samples in T_1-T_3 at the end of the 2nd, 4th and 6th week of exposure, respectively are significantly higher than other treatments. T_2 and T_1 mean values in the 8th and 10th weeks of exposure are significantly higher than other treatments including the control. T_1 mean value in the 10th week of exposure recorded the highest AST production value of 137.97±0.09nM/mg. (Table 3).

From the results of the samples exposed to sub-lethal concentrations of $CdCl_2$ and supplemented with vitamin A, the AST production levels in the liver of the fish showed that T_3 mean values in the 2nd and 4th weeks of exposure are significantly higher than other treatments. Likewise, T_4 mean values in both 6th and 8th weeks of exposure are significantly higher than other treatments. The highest mean value of AST produced in the liver was 132.19±0.18nM/mg obtained in T_4 at the end of the 8th week of exposure. (Table 4). In another development, the T_2 and T_3 mean values in the kidney of the samples in the 2nd and 4th weeks of exposure are significantly

higher than other treatments. In like manner, T_1 and T_3 mean values in the 6th and 8th weeks of exposure, respectively are significantly higher than other treatments. The highest mean value of AST produced in the kidneys of the fish was 113.91±0.09nM/mg obtained in T_1 at the end of the 6th week of exposure. (Table 5). Furthermore, the control mean values in the gills of the samples in T_4 and T_2 at the end of the 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments. T_1 mean values in both 6th and 8th weeks of exposure are significantly higher than other treatments. T_1 mean value in the 6th week of exposure recorded the highest AST production value of 120.94±0.36nM/mg. (Table 6).

Table 1 *AST production levels in the Liver of C. gariepinus exposed to sub-lethal concentrations of $CdCl_2$ for a period of 12 weeks.*

	1st	2nd	3rd	4th	5th	6th
CR	90.63±0.18^{n}	105.00±0.18^{l}	35.63±0.18^{b}	6.56±0.36^{a}	91.25±0.00^{c}	19.22±0.27^{a}
T_1	101.72±0.27^{o}	63.59±0.09^{h}	110.16±0.09^{n}	135.00±0.18^{o}	75.63±0.18^{a}	101.88±0.18^{f}
T_2	44.69±0.18^{k}	90.94±0.18^{k}	12.66±0.27^{a}	113.59±0.27^{m}	0.00±0.00	0.00±0.00
T_3	12.03±0.09^{c}	14.06±0.18^{e}	53.91±0.09^{d}	36.25±0.18^{h}	94.06±0.18^{d}	0.00±0.00
T_4	9.38±0.36^{b}	115.63±0.18^{m}	103.44±0.18^{k}	34.53±0.27^{g}	0.00±0.00	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 AST mean value unit is nM/mg.

Table 2. *AST production levels in the Kidney of C. gariepinus exposed to sub-lethal concentrations of $CdCl_2$ for a period of 12 weeks.*

	1st	2nd	3rd	4th	5th	6th
CR	47.03±0.09^{l}	15.31±0.18^{f}	70.00±0.18^{e}	13.59±0.81^{d}	120.31±0.18^{g}	45.47±0.45^{c}
T_1	23.91±0.09^{g}	82.19±0.18^{i}	103.91±0.27^{l}	123.75±0.18^{n}	109.69±0.18^{f}	78.75±0.36^{e}
T_2	35.94±0.18^{i}	0.47±0.09^{a}	79.84±0.27^{g}	91.88±0.18^{l}	0.00±0.00	0.00±0.00
T_3	62.34±0.27^{m}	145.00±0.18^{n}	50.00±0.18^{c}	17.81±0.18^{e}	129.84±0.27^{h}	0.00±0.00
T_4	20.16±0.09^{f}	9.53±0.09^{c}	106.56±0.18^{m}	12.66±0.27^{c}	0.00±0.00	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 AST mean value unit is nM/mg.

Table 3. *AST production levels in the Gill of C. gariepinus exposed to sub-lethal concentrations of $CdCl_2$ for a period of 12 weeks.*

	1st	2nd	3rd	4th	5th	6th
CR	41.09±0.09^{j}	152.19±0.18^{o}	120.16±0.09^{o}	9.69±0.18^{b}	100.16±0.09^{e}	42.19±0.18^{b}
T_1	14.53±0.27^{d}	20.63±0.18^{g}	97.03±0.09^{j}	23.91±0.09^{f}	137.97±0.09^{i}	48.59±0.09^{d}
T_2	4.06±0.18^{a}	88.44±0.18^{j}	95.47±0.09^{i}	84.38±0.18^{k}	0.00±0.00	0.00±0.00
T_3	19.06±0.36^{e}	2.66±0.27^{b}	79.22±0.09^{f}	47.19±0.36^{j}	83.75±0.18^{b}	0.00±0.00
T_4	25.63±0.18^{h}	11.25±0.18^{d}	88.91±0.09^{h}	38.59±0.09^{i}	0.00±0.00	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 AST mean value unit is nM/mg.

Table 4. *AST production levels in the Liver of C. gariepinus exposed to sub-lethal concentrations of $CdCl_2$ and supplemented with vitamin A for a period of 12 weeks.*

	1st	2nd	3rd	4th	5th	6th
CR	90.63±0.18^{n}	105.00±0.18^{n}	35.63±0.18^{c}	6.56±0.36^{c}	91.25±0.00^{a}	19.22±0.27^{a}
T_1	66.09±0.27^{k}	25.78±0.45^{f}	97.34±0.27^{g}	30.94±0.18^{i}	0.00±0.00	0.00±0.00
T_2	46.41±0.89^{d}	26.41±0.27^{g}	92.03±0.27^{f}	19.06±0.18^{g}	0.00±0.00	0.00±0.00
T_3	72.97±0.27^{l}	78.59±0.27^{k}	20.78±0.09^{a}	93.44±0.18^{n}	0.00±0.00	0.00±0.00
T_4	52.81±0.18^{g}	45.31±0.18^{h}	127.66±0.09^{o}	132.19±0.18^{o}	0.00±0.00	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 AST mean value unit is nM/mg.

Table 5. *AST production levels in the Kidney of C. gariepinus exposed to sub-lethal concentrations of $CdCl_2$ and supplemented with vitamin A for a period of 12 weeks.*

	1st	2nd	3rd	4th	5th	6th
CR	47.03±0.09^{e}	15.31±0.18^{d}	70.00±0.18^{e}	13.59±0.81^{e}	120.31±0.18^{d}	45.47±0.45^{c}
T_1	55.16±0.09^{h}	45.63±0.18^{i}	113.91±0.09^{l}	16.72±0.09^{f}	0.00±0.00	0.00±0.00
T_2	77.97±0.09^{m}	14.06±0.18^{c}	33.59±0.09^{b}	31.72±0.27^{j}	0.00±0.00	0.00±0.00
T_3	51.09±0.27^{f}	90.94±0.36^{l}	101.56±0.36^{h}	34.69±0.18^{k}	0.00±0.00	0.00±0.00
T_4	57.50±0.18^{j}	71.88±0.18^{j}	106.88±0.36^{j}	4.69±0.54^{a}	0.00±0.00	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 AST mean value unit is nM/mg.

Table 6. *AST production levels in the Gill of C. gariepinus exposed to sub-lethal concentrations of $CdCl_2$ and supplemented with vitamin A for a period of 12 weeks.*

	1st	2nd	3rd	4th	5th	6th
CR	41.09±0.09^{b}	152.19±0.18^{o}	120.16±0.09^{m}	9.69±0.18^{d}	100.16±0.09^{c}	42.19±0.18^{b}
T_1	56.88±0.18^{i}	17.19±0.18^{e}	120.94±0.36^{n}	89.53±0.09^{m}	93.59±0.27^{b}	0.00±0.00
T_2	41.41±0.27^{c}	103.28±0.09^{m}	103.59±0.27^{i}	44.38±0.18^{l}	0.00±0.00	0.00±0.00
T_3	4.53±0.09^{a}	10.16±0.27^{b}	42.03±0.27^{d}	5.00±0.00^{b}	0.00±0.00	0.00±0.00
T_4	101.72±0.27^{o}	2.03±0.27^{a}	112.50±0.18^{k}	23.75±0.18^{h}	0.00±0.00	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 AST mean value unit is nM/mg.

From the results of the samples exposed to sub-lethal concentrations of $CdCl_2$ and supplemented with vitamin C, the AST production levels in the liver of the fish showed that T_3 and T_4 mean values in the 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments. Likewise, T_1 and T_4 mean values in the 6th and 8th weeks of exposure are significantly higher than other treatments. Also, T_1 in both 10th and 12th weeks of exposure are significantly higher than other treatments. The highest mean value of AST produced in the liver was 128.44±0.36nM/mg obtained in T_1 at the end of the 10th week of exposure. (Table 7). In another development, the T_1 and T_4 mean values in the kidney of the samples in the 2nd and 4th weeks of exposure are significantly higher than other treatments. In like manner, T_3 and T_2 mean values in the 6th and 8th weeks of exposure, respectively are significantly higher than other treatments. Similarly, T_3 and T_1 mean values in the 10th and 12th weeks of exposure, respectively are significantly higher than other treatments. The highest mean value of AST produced in the kidneys of the fish was 114.84±0.09nM/mg obtained in T_3 at the end of the 10th week of exposure. (Table 8). Furthermore, T_1 and T_3 mean values in the gills of the samples at the end of the 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments. T_2 and T_3 mean values in the 6th and 8th weeks of exposure are significantly higher than other treatments. T_2 mean values in 10th and 12th weeks of exposure, respectively are significantly higher than other treatments. T_2 mean value in the 12th week of exposure recorded the highest AST production value of 125.16±0.27nM/mg. (Table 9).

Table 7. *AST production levels in the Liver of C. gariepinus exposed to sub-lethal concentrations of $CdCl_2$ and supplemented with vitamin C for a period of 12 weeks.*

	1st	2nd	3rd	4th	5th	6th
CR	90.63±0.18^{o}	105.00±0.18^{m}	35.63±0.18^{b}	6.56±0.36^{b}	91.25±0.00^{e}	19.22±0.27^{b}
T_1	54.53±0.27^{l}	0.00±0.00	123.91±0.09^{l}	24.84±0.27^{h}	128.44±0.36^{k}	105.31±0.18^{h}
T_2	23.91±0.27^{c}	57.81±0.18^{i}	0.00±0.00	76.56±0.18^{l}	113.75±0.18^{i}	46.56±0.18^{e}
T_3	73.44±0.36^{n}	81.88±0.18^{k}	101.72±0.27^{g}	12.19±0.18^{d}	108.13±0.18^{h}	0.00±0.00
T_4	62.66±0.09^{m}	93.91±0.09^{l}	100.31±0.18^{f}	85.16±0.27^{m}	0.00±0.00	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 AST mean value unit is nM/mg.

Table 8. *AST production levels in the Kidney of C. gariepinus exposed to sub-lethal concentrations of $CdCl_2$ and supplemented with vitamin C for a period of 12 weeks.*

	1st	2nd	3rd	4th	5th	6th
CR	47.03±0.09^{j}	15.31±0.18^{b}	70.00±0.18^{d}	13.59±0.81^{e}	120.31±0.18^{k}	45.47±0.45^{d}
T_1	43.44±0.18^{i}	16.72±0.27^{c}	89.06±0.18^{e}	6.41±0.27^{a}	26.56±0.36^{a}	57.97±0.27^{f}
T_2	17.03±0.09^{b}	29.83±0.98^{g}	0.00±0.00	65.78±0.09^{k}	106.72±0.09^{g}	1.25±0.18^{a}
T_3	12.97±0.27^{a}	23.75±0.18^{e}	108.91±0.09^{j}	0.00±0.00	114.84±0.09^{j}	0.00±0.00
T_4	29.53±0.09^{e}	78.75±0.36^{j}	0.00±0.00	35.94±0.18^{i}	0.00±0.00	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 AST mean value unit is nM/mg.

Table 9. *AST production levels in the Gill of C. gariepinus exposed to sub-lethal concentrations of $CdCl_2$ and supplemented with vitamin C for a period of 12 weeks.*

	1st	2nd	3rd	4th	5th	6th
CR	41.09±0.09^{g}	152.19±0.18^{n}	120.16±0.09^{j}	9.69±0.1sa8^{c}	100.16±0.09^{f}	42.19±0.18^{c}
T_1	48.44±0.36^{k}	28.59±0.27^{f}	45.63±0.18^{c}	63.13±0.18^{j}	38.75±0.18^{b}	63.75±0.18^{g}
T_2	32.66±0.27^{f}	2.97±0.45^{a}	121.25±0.18^{k}	17.81±0.18^{g}	88.91±0.09^{d}	125.16±0.27^{i}
T_3	42.19±0.18^{h}	30.31±0.18^{h}	107.66±0.09^{h}	85.31±0.18^{n}	63.44±0.18^{e}	0.00±0.00
T_4	25.00±0.18^{d}	17.81±0.18^{d}	7.81±0.18^{a}	16.41±0.09^{f}	0.00±0.00	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 AST mean value unit is nM/mg.

From the results of the samples exposed to sub-lethal concentrations of $CdCl_2$ and supplemented with vitamin E, the AST production levels in the liver of the fish showed that T_4 and T_2 mean values in the 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments. Likewise, T_1 and T_2 mean values in the 6th and 8th weeks of exposure are significantly higher than other treatments. Also, T_2 in both 10th and 12th weeks of exposure are significantly higher than other treatments. The highest mean value of AST produced in the liver was 150.63±0.18nM/mg obtained in T_2 at the end of the 4th week of exposure. (Table 10). Furthermore, T_3 and T_4 mean values in the kidneys of the samples at the end of the 2nd and 6th weeks of exposure, respectively are significantly higher than other treatments. The 4th week of exposure has joint significance difference in T_1 and T_4. T_1 mean values in the 8th week of exposure are significantly higher than other treatments. T_4 mean value in the 6th week of exposure recorded the highest AST production value of 125.78±0.27nM/mg. (Table 11). In addition to the forgoing, the T_3 and T_1 mean values in the gills of the samples in the 2nd and 4th weeks of exposure are significantly higher than other treatments. In like manner, T_2 and T_3 mean values in the 6th and 8th weeks of exposure, respectively are significantly higher than other treatments. Similarly, T_1 mean values in both 10th and 12th weeks of exposure, respectively are significantly higher than other treatments. The highest mean value of AST produced in the gills of the fish was 133.28±0.09nM/mg obtained in T_1 at the end of the 4th week of exposure. (Table 12).

Table 10. *AST production levels in the Liver of C. gariepinus exposed to sub-lethal concentrations of $CdCl_2$ and supplemented with vitamin E for a period of 12 weeks.*

	1st	2nd	3rd	4th	5th	6th
CR	90.63±0.18^{m}	105.00±0.18^{j}	35.63±0.18^{c}	6.56±0.36^{a}	91.25±0.00^{c}	19.22±0.27^{b}
T_1	32.03±0.27^{c}	27.66±0.09^{h}	122.50±0.18^{n}	32.19±0.18^{f}	30.00±0.18^{a}	18.59±0.27^{a}
T_2	73.28±0.89^{j}	150.63±0.18^{m}	106.25±0.18^{l}	101.88±0.36^{k}	75.78±0.09^{b}	117.97±0.09^{h}
T_3	32.03±0.27^{c}	13.28±0.45^{b}	84.22±0.09^{j}	78.59±0.27^{h}	0.00±0.00	0.00±0.00
T_4	75.78±0.27^{k}	35.00±0.18^{i}	40.78±0.27^{e}	42.50±0.18^{g}	0.00±0.00	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 AST mean value unit is nM/mg.

Table 11. *AST production levels in the Kidney of C. gariepinus exposed to sub-lethal concentrations of $CdCl_2$ and supplemented with vitamin E for a period of 12 weeks.*

	1st	2nd	3rd	4th	5th	6th
CR	47.03±0.09^{f}	15.31±0.18^{e}	70.00±0.18^{i}	13.59±0.81^{c}	120.31±0.18^{f}	45.47±0.45^{f}
T_1	57.19±0.18^{g}	110.63±0.36^{k}	15.63±0.18^{b}	117.03±0.09^{n}	124.84±0.27^{g}	105.16±0.27^{g}
T_2	41.09±0.27^{e}	17.66±0.09^{g}	45.31±0.18^{f}	102.03±0.09^{l}	0.00±0.00	0.00±0.00
T_3	88.28±0.27^{l}	13.75±0.18^{c}	56.72±0.27^{h}	109.84±0.09^{m}	0.00±0.00	0.00±0.00
T_4	40.31±0.18^{d}	110.63±0.18^{k}	125.78±0.27^{o}	91.09±0.27^{j}	0.00±0.00	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 AST mean value unit is nM/mg.

Table 12. *AST production levels in the Gill of C. gariepinus exposed to sub-lethal concentrations of $CdCl_2$ and supplemented with vitamin E for a period of 12 weeks.*

	1st	2nd	3rd	4th	5th	6th
CR	41.09±0.09^{e}	152.19±0.18^{n}	120.16±0.09^{m}	9.69±0.18^{b}	100.16±0.09^{e}	42.19±0.18^{d}
T_1	26.88±0.54^{b}	133.28±0.09^{l}	39.38±0.18^{d}	21.72±0.27^{d}	129.22±0.09^{h}	44.06±0.18^{e}
T_2	61.56±0.18^{h}	7.19±0.18^{a}	91.09±0.09^{k}	27.50±0.18^{e}	91.88±0.18^{d}	23.59.0.27^{c}
T_3	65.00±0.18^{i}	14.53±0.09^{d}	56.41±0.27^{g}	85.47±0.09^{i}	0.00±0.00	0.00±0.00
T_4	19.53±0.09^{a}	15.47±0.27^{f}	8.75±0.18^{a}	21.72±0.27^{d}	0.00±0.00	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 AST mean value unit is nM/mg.

3.2. Discussions

3.2.1. AST Production Levels in C. gariepinus Exposed to Sub-lethal Concentrations of Cd Only

From the results of the samples exposed to sub-lethal concentrations of $CdCl_2$, the AST production levels in the liver of the fish showed that T_1 and T_4 mean values in the 2nd and 4th weeks of exposure are significantly higher than other treatments including the control. The elicitation started early in the lowest concentration and then in the highest concentration subsequently. Also, the levels of AST production in this regard are relatively high in all treatments probably due to the prevailing environmental conditions that necessitate up-regulation of the immune system. In a related development, El-Said El-Boshy *et al*. [12] reported that blood level activities of ALT and AST was significantly increased when the fish were exposed to 2, 5 and 10mg/L treatments for a period of 3 weeks. T_1 mean values in both 6th and 8th weeks of exposure are significantly higher than other treatments including the control probably due to high production and less utilization of the enzyme. This is also

probably why the highest mean value of AST produced in the liver was 135.00±0.18nM/mg obtained in T_1 as well as at the end of the 8th week of exposure. Similarly, the T_3 mean values in the 10th week of exposure are significantly higher than other treatments including the control probably due to up-regulation of the defence system as the duration and concentration of the exposure increased. The presence of pollutant in the environment of aquatic organism can trigger the utilization or increased production of AST. For instance, cadmium in plasma of goldfish significantly increased the activities of plasma glutamic acid oxaloacetic acid-transaminase (GOT) and glutamic acid-pyruvic acid transaminase (GPT) [32]. In another development, the T_3 mean values in the kidney of the sample in both 2nd and 4th weeks of exposure are significantly higher than other treatments including the control probably due to the need to counter the onslaught of the toxicant especially at the early stage of the exposure after initial elicitation and subsequently, sustenance of the tempo as the duration of exposure increased since, the highest mean value of AST produced in the kidneys of the fish was 145.00±0.18nM/mg obtained in T_3 as well at the end of the 4th week of exposure, and that the same T_3 mean values are significantly higher than other treatments in the 10th week of exposure. This is also probably why T_4 and T_1mean values in the 6th and 8th weeks of exposure, respectively are significantly higher than other treatments including the control due to the need for up-regulation and sustenance. In like manner, increased activities of AST, ALT and ALP in Indian major carps exposed to nitrite toxicity have been reported [10]. Furthermore, the control mean values in the gills of the samples in T_1-T_3 at the end of the 2nd, 4th and 6th week of exposure, respectively are significantly higher than other treatments. This is probably because the gills may not be very sensitive in detecting the production of the enzyme and perhaps the available concentrations have been engaged in dealing with the effects of the toxicant. Probably as the duration of exposure increased there were improved production levels and less utilization of the available enzyme. This is probably why T_2 and T_1 mean values in the 8th and 10th weeks of exposure are significantly higher than other treatments including the control; and the highest AST production mean value (137.97±0.09nM/mg) was also obtained in T_1 in the 10th week of exposure.

3.2.2. AST Production Levels in C. gariepinus Exposed to Sub-lethal Concentrations of Cd Supplemented with Vitamin A

From the results of the samples exposed to sub-lethal concentrations of $CdCl_2$ and supplemented with vitamin A, the AST production levels in the liver of the fish showed that T_3 mean values in the 2nd and 4th weeks of exposure are significantly higher than other treatments. The increased production levels of AST in the higher concentrations in both early and later stages of the exposure were probably necessitated by the need for drastic action against the onslaughts of the toxicant as the duration increases. Ensibi *et al*. [14] have indicated that the activities of antioxidant enzymes may be elevated or inhibited by biochemical stress depending on the intensity and the duration of the stress applied as well as on the susceptibility of the exposed species. The production levels were not significant in lower levels in the liver probably because of the shield provided by the vitamin. In the same vein, T_4 mean values in both 6th and 8th weeks of exposure are significantly higher than other treatments; and the highest mean value of AST produced in the liver (132.19±0.18nM/mg) was also obtained in T_4 at the end of the 8th week of exposure probably due to the need for up-regulation of the defence system. In another development, the T_2 and T_3 mean values in the kidney of the samples in the 2nd and 4th weeks of exposure are significantly higher than other treatments. The elicitation of the production of the AST in the kidney commenced in T_2 and subsequently in T_3 as the duration of the exposure increased probably due to the initial needs for up-regulation of the immune system to ensure survival. In like manner, T_1 mean values in the 6th week of exposure are significantly higher than other treatments with 113.91±0.09nM/mg as the highest AST produced in the kidneys of the fish also in T_1 at the same period of exposure probably due to less utilization in the presence of the vitamin. Furthermore, the control mean values in the gills of the samples in T_4 and T_2 at the end of the 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments probably due to the initial need for the up-regulation of the body's defence system and the early elicitation subsequently. Also in the gills as the duration of exposure increased the production levels probably become more with less utilization in lower concentrations, and also probably due to the succoring presence of the vitamin. This may be the reason why T_1 mean values in both 6th and 8th weeks of exposure are significantly higher than other treatments with the highest value of 120.94±0.36nM/mg at the 6th week of exposure.

3.2.3. AST Production Levels in C. gariepinus Exposed to Sub-lethal Concentrations of Cd Supplemented with Vitamin C

From the results of the samples exposed to sub-lethal concentrations of $CdCl_2$ and supplemented with vitamin C, the AST production levels in the liver of the fish showed that T_3 and T_4 mean values in the 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments. The need for defence of the body's system against the effects of the toxicant probably prompted the increased production of the enzyme in the higher concentrations. This is also probably why the T_4 mean values are significant in the 8th week of exposure. T_1 mean values in the 6th, 10th and 12th weeks of exposure, respectively are significantly higher than other treatments; and is also the highest mean value of AST produced in the liver (128.44±0.36nM/mg) at the end of the 10th week of exposure. This is probably due to the need for high production of the enzyme at later stages of the exposure but in the presence of the vitamin there may have been less utilization in combating the effects of the toxicant. Similar

report was given by Shalaby [26] who stated that total protein content and AST became normalized_similar to those of the control upon administration of Vitamin C. In another development, the T_1 and T_4 mean values in the kidney of the samples in the 2nd and 4th weeks of exposure are significantly higher than other treatments. This is likely an up-regulation of the defence system from the lowest concentration and subsequently, in the highest concentration as the duration increases. This is also probably why T_3, $T_2$and T_1 mean values in the 6th, 8th and 12th weeks of exposure, respectively are significantly higher than other treatments. Similarly, T_3 mean values in the 10th weeks of exposure, respectively are significantly higher than other treatments; and the highest mean value of AST produced in the kidneys of the fish (114.84±0.09nM/mg) was also obtained in T_3 at the end of the 10th week of exposure probably due to the need for up-regulation of the body's immune system to counteract the effects of the toxicant. Findings by Ali *et al*. [5] on amelioration of vitamin C indicated that the levels of creatinie (28.3±1.1µmol/L), Cystatin C (1932.5±38.5ng/ml), Uric acid (4.8±0.1mg/day) and ALP (51.6±1.1IU/L) were significantly (P 0.05) increased due to administration of mercuric chloride but in the presence of vitamin C, the effects of mercuric choride on creatinine 921.9±1.4µmol/L), Cystatin C (1676.2±42.2ng/ml), Uric acid (3.9±0.1mg/day) and ALP (43.3±0.8IU/L) were less compared to metal-exposed specimens. They also reported that similar results were also obtained in rabbits treated with cadmium chloride and vitamin C, and also with co-administration of both metals and vitamin C. Also, Bakare *et al*. [9] posited that concomitant increase in the activities of ALT and AST in the serum of treated mice indicates acute hepato-cellular injury. Furthermore, T_1 and T_3 mean values in the gills of the samples at the end of the 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments probably for the purpose of defence against early and subsequent onslaughts of the effects of the toxicant especially as the duration increases. This is also probably why the T_2 and T_3 mean values in the 6th and 8th weeks of exposure are significantly higher than other treatments. T_2 mean values in 10th and 12th weeks of exposure, respectively are significantly higher than other treatments; and T_2 mean value in the 12th week of exposure also recorded the highest AST production value of 125.16±0.27nM/mg probably because after elicitation for the up-regulation of the body's immune systems there may be less utilization in combating the effects of the toxicant. In like manner, activities of the hepatic enzymes lactate dehydrogenase (LDH), alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) were found to be significantly elevated, particularly in summer [30].

3.2.4. AST Production Levels in C. gariepinus Exposed to Sub-lethal Concentrations of Cd Toxicant and Supplemented with Vitamin E

From the results of the samples exposed to sub-lethal concentrations of $CdCl_2$ and supplemented with vitamin E, the AST production levels in the liver of the fish showed that T_4 and T_2 mean values in the 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments. Again, there were elicitations and increased production of the enzyme in the highest concentration at early stage of the exposure probably to combat the onslaught of the toxicant; and subsequently in the lower concentrations as the duration of exposure increases probably due to the succoring presence of the vitamin. This is also probably why T_1 and T_2 mean values in the 6th and 8th weeks of exposure are significantly higher than other treatments. Perhaps this also applies to T_2 which in both 10th and 12th weeks of exposure are significantly higher than other treatments; and the highest mean value of AST produced in the liver (150.63±0.18nM/mg) was also obtained in T_2 at the end of the 4th week of exposure probably because the enzymes produced in the higher concentrations have been put to greater use than in lower concentrations. This is in conformity with the findings of Feng *et al*. [16] who reported that, 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. Furthermore, T_3 and T_4 mean values in the kidneys of the samples at the end of the 2nd and 6th weeks of exposure, respectively are significantly higher than other treatments due to the probable need for up-regulations of the body's defence system in tackling the effects of the toxicant especially at the inception. Similar finding was reported by Kaoud *et al*. [17] when they stated that exposure of Nile Tilapia to Cd resulted in significant increase in plasma AST and ALT, and also caused structural damages. The 4th week of exposure has joint significance difference in T_1 and T_4 probably because there were needs for up- regulation in all the treatments especially at the early stage of the exposure. This may also be why T_4 mean value in the 6th week of exposure recorded the highest AST production value of 125.78±0.27nM/mg. T_1 mean values in the 8th week of exposure are significantly higher than other treatments probably due to the presence of the vitamin and attendant less utilization of the enzyme in this lowest concentration. In addition to the forgoing, the T_3 and T_1 mean values in the gills of the samples in the 2nd and 4th weeks of exposure are significantly higher than other treatments probably due to the need for early protection against the toxicant in T_3 and subsequently in T_1 as the duration increased. Similarly, T_1 mean values in both 10th and 12th weeks of exposure, respectively are significantly higher than other treatments; and the highest mean value of AST produced in the gills of the fish (133.28±0.09nM/mg) was also obtained in T_1 at the end of the 4th week of exposure probably the vitamin was sufficient in providing the immune boost and hence, less utilization of the enzyme. This is also likely why T_2 and T_3 mean values in the 6th and 8th weeks of exposure, respectively are significantly higher than other treatments.

4. Conclusions and Recommendations

There were general high production levels of AST in all treatments. The highest mean production values were recorded in the liver of CdVA, CdVC and CdVE groups mostly in samples exposed to lower concentrations. The kidneys in the Cd only group however, produced the highest AST value. The high production values of AST in all treatments suggest that the enzyme is a good biomarker of oxidative stress elicited by the presence of the toxicant. This also buttresses the importance of these organs in the detoxifications of the effects of xenobiotics in their immediate environment.

The highest AST mean value produced in the Cd only group is 145.00±0.18nM/mg, CdVA is 132.19±0.18nM/mg, CdVC is 128±0.36nM/mg and CdVE is 150.63±0.18nM/mg to attenuate the effects of the toxicant at one point and organ or the other.

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