



Assessment of the Ameliorative Roles of Vitamins A, C and E on Alanine Aminotransferase (ALT) Production in *Clarias gariepinus* (Burchell, 1822) Fingerlings Exposed To Cadmium Chloride

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Abstract: The anthropogenic activities culminating in environmental pollution all over the world that usually leads to release of plethora of pollutants such as cadmium calls for concern. In the present study the effects of cadmium chloride on the production of antioxidants such as Alanine AminoTransferase (ALT) 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 Cd (00, 12mg/L, 16mg/L, 20mg/L and 24mg/L) with replicate in each case. Minimum concentration of the toxicant was taken as the concentration for each of the vitamins and administered across all treatments. 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 (Cd only with T1-T4 and replicates), CdVA (Cd+vitamin A with T1-T4 and replicates), CdVC (Cd+vitamin C with T1-T4 and replicates) and CdVE (Cd+vitamin E with T1-T4 and replicates). 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 samples exposed to Cd only, the ALT production levels in the liver of the fish showed that T1 and T4 in the 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments including the control. The control mean values in the 6th and 8th weeks of exposure, respectively are significantly higher than other treatments. T1 in the 10th week of exposure are significantly higher than other treatments. The highest ALT produced in the liver was 65.43±0.10nM/mg obtained in T4 at the end of the 6th week of exposure. T1 and T4 in the kidneys of the samples at the end of the 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments. The control mean values in the 6th and 10th weeks of exposure are significantly higher than other treatments. T4 in the 8th week of exposure are significantly higher than other treatments including the control. T4 in the 4th week of exposure recorded the highest ALT value of 71.87±0.20nM/mg in the kidney of the samples. The T3 and T4 in the gills of the samples in the 2nd and 4th weeks of exposure are significantly higher than other treatments. T2 in the 6th week of exposure are significantly higher than other treatments. The control in the 8th and 10th weeks of exposure, respectively are significantly higher than other treatments. The highest ALT produced in the gill was 62.97±0.05nM/mg obtained in T2 at the end of the 6th week of exposure. In samples exposed to CdVA, the ALT production levels in the liver indicated that T4 in both 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments. The T3 and T2 mean in the 6th and 8th weeks of exposure, respectively are significantly higher than other treatments. The highest mean ALT produced in the liver was 73.48±0.15nM/mg obtained in T3 at the end of the 6th week of exposure. T2 and T1 in the kidneys of the samples at the end of the 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments. T1 in both 6th and 8th weeks of exposure, respectively are significantly higher than other treatments. T1 recorded the highest ALT value of 81.61±0.15nM/mg in the kidney of the samples at the 4th week of exposure. The T2 and T4 in the gills of the samples in the 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments. In T3 and T4 in the 6th and 8th weeks of exposure, respectively are significantly higher than other treatments. The highest ALT produced in the gill of the fish was 66.61±0.10nM/mg obtained in T4 at the 4th week of exposure. In samples exposed to CdVC, the ALT levels in the liver indicated that T3 and T4 in the 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments. The T3 in both 6th and 8th weeks of exposure, respectively are significantly higher than other treatments. T1 in both 10th and 12th weeks of exposure are significantly higher than other treatments. The highest ALT produced in the liver was 60.43±0.15nM/mg obtained in T3 at the 6th week of exposure. In the kidney's T1 in both 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments. T3 and T2 in the kidneys of the samples at the 6th and 8th weeks of exposure, respectively are significantly higher than other treatments. The T3 and T1 in the 10th and 12th weeks of exposure, respectively are significantly higher than other treatments. The highest ALT in the kidney was 56.19±0.15nM/mg obtained in T3 at the 6th week of exposure. The T1 and T3 in the gills of the samples in the 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments. T1 in both 6th and 8th weeks of exposure, respectively are significantly higher than other treatments. T3 and T2 are significantly higher than other treatments in the 10th and 12th weeks of exposure. The highest ALT produced in the gill was 65.17±0.15nM/mg obtained in T3 at the 10th week of exposure. In samples exposed to CdVE, the ALT in the liver indicated that T2 in both 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments. The T2 and T1 in the 6th and 8th weeks of exposure, respectively are significantly higher than other treatments. T1 in both 10th and 12th weeks of exposure are significantly higher than other treatments.

Keywords: *Clarias gariepinus*, Fish organs, ALT production levels, Ameliorative roles, vitamin supplements and Cd treatments

1. 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, *Clariasgariepinus* 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, *Clariasgariepinus* is a tropical hardy species belonging to the Phylum Chordata, class Actinopterygii and family Clariidae. *Clariasspecies* 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, *Clariasspecies* is an indigenous fish occurring in freshwater throughout the country. It is suspected that apart from tilapia, *Clariasis* the most abundant cultivated fish species in Nigeria (FAO, 2003). The common species found are *Clariasgariepinus*, *Clariasanguillaris*, *Clariasbuthupogon* and *Clariaslazera*.

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). 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 and Al-Ghamdi, 2014).

Vitamins A, C and E are known to play ameliorative roles in the attenuation of the effects of pollutants on organisms. Also, Vitamins C and E supplementations have been reported to play a positive role in detoxification of mercury toxicity especially at lower concentrations (Thakur and Kanshere, 2014). 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. The main biological function of vitamin E is its direct influence on cellular responses to oxidative stress through modulation of signal transduction pathway (Pratt *et al.*, 2010). Vitamins E and C supplementation can induce protective effects on certain conditions after free radical-mediated cellular damage or disruption (Yolanda and Maria, 2012). 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 (Arenas *et al.*, 2017). The presence of pollutant can trigger the utilization or increased

production of AST and ALT. For instance, cadmium in plasma ofgoldfish significantly increased the activities of plasma glutamic acid oxaloacetic acid-transaminase (GOT) and glutamic acid-pyruvic acid transaminase (GPT) (Zikicet *al.*, 2001).

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 (Fenget *al.*, 2018). Furthermore,increased activities of AST, ALT and ALP in Indian major carps exposed to nitrite toxicity have been recorded (Das *et al.*, 2004).This research therefore, addresses the effects of Cd toxicant on ALT 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.gariiepinus*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 LC₅₀, respectively. The groups of treatments were tagged Cd (Cd only with T1-T4 and replicates), second CdVA (Cd+vitamin A with T1-T4 and replicates), third CdVC (Cd+vitamin C with T1-T4 and replicates) and fourth CdVE (Cd+vitamin E with T1-T4 and replicates).Each treatmentwas 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 every 72 hours according to Organization for Economic Co-operation and Development (OECD, 2007) standards.Three fish samples were picked at random and sacrificed from each trough on every 14thday 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 ALT.

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 pHwas adjusted to 8.0.

2.4. Alanine Aminotransferase (ALT)Determination

Fish tissues ALT were determined as described by Reitman and Frankel (1957)from all the treatments and replicates. Spectrophotometric method was used for the assay of alanine aminotransferase. The homogenates were prepared in the laboratories as follow: 100µl (0.1 ml) of the tissue homogenate was added into test tubes with 500 µl (0.5 ml) of reagent 1(buffer). The mixture was incubated for 30 minutes at 37°C in samples of *C. griiepinus* analyzed for ALT. Subsequently, 500µl (0.5ml) of reagent2 (2, 4- dinitrophenylhydrazine) was added and kept for 20minutes at 25⁰C. The reaction was

terminated with the addition of 5000 µl(5.0ml) of 0.4Mol/L NaOH to the mixture. The blank was prepared with 500 µl(0.5ml) of reagent1 and 0.1µl (100µl) of distilled water. The absorbance in each case was read at 546nm.

2.5. Data Analyses

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).

3. RESULTS AND DISCUSSIONS

ALT production levels in Liver, Kidneys and gills of *C. gariepinus* exposed to sub-lethal concentrations of CdCl₂ toxicant and the respective supplemented treatments with Vitamins A, C and E for a period of twelve weeks and sampled fortnightly

From the results of the samples exposed to sub-lethal concentrations of CdCl₂, the ALT production levels in the liver of the fish showed that T1 and T4 mean values in the 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments including the control. The control mean values in the 6th and 8th weeks of exposure, respectively are significantly higher than other treatments. T1 mean values in the 10th week of exposure are significantly higher than other treatments. The highest mean value of ALT produced in the liver was 65.43±0.10nM/mg obtained in T4 at the end of the 6th week of exposure. (Table 3.1). Furthermore, T1 and T4 mean values in the kidneys of the samples at the end of the 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments. The control mean values in the 6th and 10th weeks of exposure are significantly higher than other treatments. T4 mean values in the 8th week of exposure are significantly higher than other treatments including the control. T4 mean value in the 4th week of exposure recorded the highest ALT production value of 71.87±0.20nM/mg in the kidney of the samples. (Table 3.2). In addition to the forgoing, the T3 and T4 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, T2 mean values in the 6th week of exposure are significantly higher than other treatments. The control mean values in the 8th and 10th weeks of exposure, respectively are significantly higher than other treatments. The highest mean value of ALT produced in the gill of the fish was 62.97±0.05nM/mg obtained in T2 at the end of the 6th week of exposure. (Table 3.3).

Table3.1. ALT production levels in the Liver of *C. gariepinus* exposed to sub-lethal concentrations of CdCl₂ for a period of 12 weeks

	1 st	2 nd	3 rd	4 th	5 th	6 th
CR	48.48±0.20 ⁿ	8.73±0.15 ^d	66.70±0.05 ⁿ	65.17±0.05 ^o	48.90±0.15 ^e	23.13±0.15 ^c
T1	38.39±0.05 ^l	6.19±0.15 ^b	61.36±0.10 ^j	7.50±0.10 ^e	58.56±0.15 ^h	65.34±0.05 ^f
T2	12.71±0.10 ^f	50.68±0.10 ^k	41.78±0.05 ^e	5.93±0.20 ^d	0.00±0.00	0.00±0.00
T3	4.41±0.20 ^b	25.85±0.15 ^g	53.31±0.15 ^h	2.37±0.10 ^c	53.39±0.20 ^f	0.00±0.00
T4	11.36±0.10 ^e	51.36±0.10 ^l	65.43±0.10 ^m	2.20±0.20 ^b	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 $P \leq 0.05$. The unit for ALT mean value in each case isnM/mg.

Table3.2. ALT production levels in the Kidney of *C. gariepinus* exposed to sub-lethal concentrations of CdCl₂ for a period of 12 weeks

	1 st	2 nd	3 rd	4 th	5 th	6 th
CR	65.68±0.15 ^o	21.87±0.10 ^f	68.39±0.15 ^o	12.54±0.10 ^h	54.32±0.05 ^g	16.10±0.10 ^a
T1	47.63±0.10 ^m	31.02±0.10 ^h	48.82±0.20 ^f	11.27±0.05 ^g	46.19±0.15 ^d	35.59±0.10 ^e
T2	32.71±0.20 ^j	58.14±0.10 ^m	61.70±0.10 ^k	14.92±0.20 ⁱ	0.00±0.00	0.00±0.00
T3	6.87±0.15 ^c	63.48±0.05 ⁿ	22.63±0.15 ^d	9.32±0.10 ^f	15.43±0.20 ^a	0.00±0.00
T4	12.97±0.15 ^g	71.87±0.20 ^o	13.75±0.01 ^b	46.44±0.10 ⁿ	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 $P \leq 0.05$. The unit for ALT mean value in each case isnM/mg.

Table3.3. ALT production levels in the Gill of *C. gariepinus* exposed to sub-lethal concentrations of CdCl₂ for a period of 12 weeks

	1 st	2 nd	3 rd	4 th	5 th	6 th
CR	26.61±0.20 ⁱ	10.68±0.20 ^e	7.80±0.10 ^a	19.32±0.10 ^m	77.37±0.05	24.49±0.05 ^d
T₁	7.97±0.20 ^d	43.82±0.05 ⁱ	58.05±0.05 ⁱ	19.07±0.15 ^l	37.46±0.10 ^c	21.87±1.37 ^b
T₂	2.04±0.10 ^a	7.12±0.10 ^c	62.97±0.05 ^l	0.51±0.10 ^a	0.00±0.00	0.00±0.00
T₃	33.82±0.15 ^k	0.42±0.05 ^a	13.82±0.15 ^c	17.97±0.10 ^k	19.49±0.10 ^b	0.00±0.00
T₄	21.61±0.05 ^h	45.26±0.10 ^j	50.26±0.15 ^g	16.02±0.05 ^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 unit for ALT mean value in each case isnM/mg.

From the results of the samples exposed to sub-lethal concentrations of CdCl₂, and supplemented with vitamin A, the ALT production levels in the liver of the fish indicated that T4 mean values in both 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments. The T3 and T2 mean values in the 6th and 8th weeks of exposure, respectively are significantly higher than other treatments. The highest mean value of ALT produced in the liver was 73.48±0.15nM/mg obtained in T3 at the end of the 6th week of exposure. (Table 3.4). Furthermore, T2 and T1 mean values in the kidneys of the samples at the end of the 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments. T1 mean value in both 6th and 8th weeks of exposure, respectively are significantly higher than other treatments. T1 recorded the highest ALT production value of 81.61±0.15nM/mg in the kidney of the samples at the end of the 4th week of exposure. (Table 3.5). In addition to the foregoing, the T2 and T4 mean values in the gills of the samples in the 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments. In like manner, T3 and T4 mean values in the 6th and 8th weeks of exposure, respectively are significantly higher than other treatments. The highest mean value of ALT produced in the gill of the fish was 66.61±0.10nM/mg obtained in T4 at the end of the 4th week of exposure. (Table 3.6).

Table3.4. ALT production levels in the Liver of *C. gariepinus* exposed to sub-lethal concentrations of CdCl₂ and supplemented with vitamin A for a period of 12 weeks

	1 st	2 nd	3 rd	4 th	5 th	6 th
CR	48.48±0.20 ^m	8.73±0.15 ^c	66.70±0.05 ^l	65.17±0.05 ^o	48.90±0.15 ^a	23.14±0.15 ^b
T₁	22.63±0.15 ^a	8.48±0.10 ^b	64.15±0.05 ^k	2.04±0.20 ^b	0.00±0.00	0.00±0.00
T₂	29.41±0.15 ^g	40.17±0.20 ^j	0.00±0.00	15.43±0.20 ^j	0.00±0.00	0.00±0.00
T₃	23.31±0.15 ^b	22.88±0.10 ^g	73.48±0.15 ⁿ	10.76±0.15 ^g	0.00±0.00	0.00±0.00
T₄	40.76±0.05 ^k	49.41±0.15 ^j	27.88±0.15 ^c	5.43±0.10 ^e	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 unit for ALT mean value in each case isnM/mg.

Table3.5. ALT production levels in the Kidney of *C. gariepinus* exposed to sub-lethal concentrations of CdCl₂ and supplemented with vitamin A for a period of 12 weeks

	1 st	2 nd	3 rd	4 th	5 th	6 th
CR	65.68±0.15 ^o	21.87±0.10 ^f	68.39±0.15 ^m	12.54±0.10 ⁱ	54.32±0.05 ^b	16.10±0.10 ^a
T₁	32.04±0.10 ^j	81.61±0.15 ⁿ	61.70±0.10 ^j	38.56±0.05 ⁿ	0.00±0.00	0.00±0.00
T₂	43.14±0.24 ^l	8.73±0.15 ^c	43.48±0.15 ^f	0.51±0.00 ^a	0.00±0.00	0.00±0.00
T₃	36.61±0.10 ^j	21.19±0.10 ^e	56.36±0.05 ^h	38.05±0.05 ^m	0.00±0.00	0.00±0.00
T₄	24.66±0.05 ^d	5.85±0.15 ^a	58.39±0.15 ⁱ	4.24±0.10 ^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 unit for ALT mean value in each case isnM/mg.

Table3.6. ALT production levels in the Gill of *C. gariepinus* exposed to sub-lethal concentrations of CdCl₂ and supplemented with vitamin A for a period of 12 weeks

	1 st	2 nd	3 rd	4 th	5 th	6 th
CR	26.61±0.20 ^e	10.68±0.20 ^d	7.80±0.10 ^a	19.32±0.10 ^k	77.37±0.05 ^c	24.49±0.05 ^c
T₁	31.87±0.20 ^h	53.14±0.15 ^l	20.00±0.10 ^b	9.07±0.15 ^f	0.00±0.00	0.00±0.00
T₂	57.12±0.10 ⁿ	23.31±0.15 ^h	28.14±0.10 ^d	5.17±0.15 ^d	0.00±0.00	0.00±0.00
T₃	29.32±0.20 ^f	49.58±0.15 ^k	52.46±0.15 ^g	10.93±0.15 ^h	0.00±0.00	0.00±0.00
T₄	23.39±0.10 ^c	66.61±0.10 ^m	38.82±0.10 ^e	33.56±0.20 ^l	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 unit for ALT mean value in each case isnM/mg.

From the results of the samples exposed to sub-lethal concentrations of CdCl₂, and supplemented with vitamin C, the ALT production levels in the liver of the fish indicated that T3 and T4 mean values in the 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments. The T3 mean values in both 6th and 8th weeks of exposure, respectively are significantly higher than other treatments. Likewise, T1 mean values in both 10th and 12th weeks of exposure are significantly higher than other treatments. The highest mean value of ALT produced in the liver was 60.43±0.15nM/mg obtained in T3 at the end of the 6th week of exposure. (Table 3.7). In another development, kidney's T1 mean values in both 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments. Also, T3 and T2 mean values in the kidneys of the samples at the end of both 6th and 8th weeks of exposure, respectively are significantly higher than other treatments. The T3 and T1 mean values in the 10th and 12th weeks of exposure, respectively are significantly higher than other treatments. The highest ALT production value of 56.19±0.15nM/mgin the kidney of the samples was obtained in T3 at the end of the 6th week of exposure. (Table 3.8).In addition to the forgoing, the T1 and T3 mean values in the gills of the samples in the 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments. T1 mean values in both 6th and 8th weeks of exposure, respectively are significantly higher than other treatments. Likewise, T3 and T2 mean values are significantly high than other treatments in the 10th and 12th weeks of exposure. The highest mean value of ALT produced in the gill of the fish was 65.17±0.15nM/mgobtained in T3 at the end of the 10th week of exposure. (Table 3.9).

Table3.7. ALT production levels in the Liver of *C. gariepinus* exposed to sub-lethal concentrations of CdCl₂ and supplemented with vitamin C for a period of 12 weeks

	1 st	2 nd	3 rd	4 th	5 th	6 th
CR	48.48±0.20 ⁿ	8.73±0.15 ^d	66.70±0.05 ^l	65.17±0.05 ⁿ	48.90±0.15 ^g	23.14±0.15 ^e
T1	29.75±0.05 ^k	7.29±0.10 ^c	60.00±0.10 ^j	9.15±0.10 ^b	57.29±0.10 ^j	22.97±0.24 ^d
T2	13.22±0.20 ^c	9.32±0.10 ^e	0.00±0.00	15.51±0.15 ^g	9.75±0.05 ^a	19.32±0.10 ^c
T3	40.17±0.10 ^m	13.82±0.15 ^j	60.43±0.15 ^k	32.04±0.10 ^j	45.00±0.05 ^f	0.00±0.00
T4	34.49±0.05 ^l	26.53±0.15 ^m	53.39±0.20 ^h	15.00±0.15 ^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 unit for ALT mean value in each case isnM/mg.

Table3.8. ALT production levels in the Kidney of *C. gariepinus* exposed to sub-lethal concentrations of CdCl₂ and supplemented with vitamin C for a period of 12 weeks

	1 st	2 nd	3 rd	4 th	5 th	6 th
CR	65.68±0.15 ^o	21.87±0.10 ^l	68.39±0.15 ^m	12.54±0.10 ^d	54.32±0.05 ⁱ	16.10±0.10 ^a
T1	23.73±0.10 ^g	49.58±0.05 ^o	51.19±0.10 ^g	6.95±0.20 ^a	34.07±0.10 ^b	35.34±0.15 ^h
T2	9.24±0.15 ^b	15.68±0.15 ^k	0.00±0.00	51.36±0.10 ^l	41.78±0.05 ^d	16.61±0.20 ^b
T3	7.37±0.15 ^a	10.59±0.15 ^f	56.19±0.15 ⁱ	10.85±0.10 ^c	49.15±0.10 ⁿ	0.00±0.00
T4	16.36±0.15 ^e	1.67±0.10 ^a	5.09±0.10 ^a	22.80±0.15 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 unit for ALT mean value in each case isnM/mg.

Table3.9. ALT production levels in the Gill of *C. gariepinus* exposed to sub-lethal concentrations of CdCl₂ and supplemented with vitamin C for a period of 12 weeks

	1 st	2 nd	3 rd	4 th	5 th	6 th
CR	26.61±0.20 ^j	10.68±0.20 ^g	7.80±0.10 ^b	19.32±0.10 ^h	77.37±0.05 ^l	24.49±0.05 ^f
T ₁	26.36±0.05 ⁱ	2.29±0.15 ^b	49.15±0.10 ^f	51.87±0.20 ^m	37.71±0.05 ^c	19.32±0.10 ^c
T ₂	18.56±0.15 ^f	12.04±0.10 ⁱ	9.32±0.10 ^c	19.32±0.10 ^h	42.12±0.15 ^e	33.56±0.20 ^g
T ₃	24.32±0.05 ^h	36.44±0.10 ⁿ	16.78±0.10 ^d	12.97±0.15 ^e	65.17±0.15 ^k	0.00±0.00
T ₄	14.24±0.10 ^d	11.61±0.05 ^h	26.61±0.10 ^e	50.09±0.24 ^k	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 unit for ALT mean value in each case isnM/mg.

From the results of the samples exposed to sub-lethal concentrations of CdCl₂, and supplemented with vitamin E, the ALT production levels in the liver of the fish indicated that T2 mean values in both 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments. The T2 and T1

mean values in the 6th and 8th weeks of exposure, respectively are significantly higher than other treatments. T1 mean values in both 10th and 12th weeks of exposure are significantly higher than other treatments. The highest mean value of ALT produced in the liver was 76.70±0.15nM/mg obtained in T1 at the end of the 8th week of exposure. (Table 3.10). In another development, kidney's T1 and T2 mean values in 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments. Also, T2 and T1 mean values in the kidneys of the samples at the end of the 6th and 8th weeks of exposure, respectively are significantly higher than other treatments. The highest ALT production value of 71.78±0.05nM/mgin the kidney of the samples was obtained in T2 at the end of the 6th week of exposure. (Table 3.11). Furthermore, the T3 and T2 mean values in the gills of the samples in the 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments. T2 and T4 mean values in the 6th and 8th weeks of exposure, respectively are significantly higher than other treatments. T1 mean values in both 10th and 12th weeks of exposure, respectively are significantly higher than other treatments. The highest mean value of ALT produced in the gill of the fish was 73.48±0.05nM/mgobtained in T1 at the end of the 12th week of exposure. (Table 3.12).

Table3.10. ALT production levels in the Liver of *C. gariepinus* exposed to sub-lethal concentrations of CdCl₂ and supplemented with vitamin E for a period of 12 weeks

	1 st	2 nd	3 rd	4 th	5 th	6 th
CR	48.48±0.20 ⁿ	8.73±0.15 ^a	66.70±0.05 ^m	65.17±0.05 ⁿ	48.90±0.15 ^d	23.14±0.15 ^c
T1	17.37±0.05 ^e	16.44±0.10 ^g	56.10±0.20 ^j	76.70±0.15 ^o	49.58±0.15 ^e	75.59±0.10 ^h
T2	39.07±0.15 ^m	21.10±0.05 ^j	57.88±0.05 ^k	12.63±0.05 ^d	31.10±0.15 ^c	16.61±0.20 ^b
T3	18.22±0.05 ^f	19.32±0.10 ⁱ	50.85±0.20 ^g	7.88±0.05 ^a	0.00±0.00	0.00±0.00
T4	21.10±0.05 ^g	17.20±0.15 ^h	53.82±0.05 ^h	34.32±0.05 ^k	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 unit for ALT mean value in each case isnM/mg.

Table3.11. ALT production levels in the Kidney of *C. gariepinus* exposed to sub-lethal concentrations of CdCl₂ and supplemented with vitamin E for a period of 12 weeks

	1 st	2 nd	3 rd	4 th	5 th	6 th
CR	65.68±0.15 ^o	21.87±0.10 ^k	68.39±0.15 ⁿ	12.54±0.10 ^c	54.32±0.05 ^f	16.10±0.10 ^a
T1	31.36±0.10 ^j	43.39±0.10 ^m	61.44±0.15 ^l	47.97±0.20 ^m	19.41±0.15 ^a	54.41±0.10 ^f
T2	2.19±0.10 ^a	47.29±0.10 ^o	71.78±0.05 ^o	45.85±0.05 ^l	0.00±0.00	0.00±0.00
T3	10.43±0.15 ^b	46.70±0.05 ⁿ	11.53±0.10 ^c	21.19±0.10 ^h	0.00±0.00	0.00±0.00
T4	31.27±0.05 ⁱ	15.34±0.05 ^f	19.07±0.05 ^d	18.14±0.10 ^e	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 unit for ALT mean value in each case isnM/mg.

Table3.12. ALT production levels in the Gill of *C. gariepinus* exposed to sub-lethal concentrations of CdCl₂ and supplemented with vitamin E for a period of 12 weeks

	1 st	2 nd	3 rd	4 th	5 th	6 th
CR	26.61±0.20 ^h	10.68±0.20 ^c	7.80±0.10 ^a	19.32±0.10 ^f	77.37±0.05 ^h	24.49±0.05 ^d
T1	15.00±0.15 ^d	11.27±0.05 ^d	40.85±0.10 ^f	19.41±0.15 ^g	63.48±0.05 ^g	73.48±0.05 ^g
T2	33.98±0.05 ^k	41.02±0.20 ^l	54.07±0.10 ⁱ	11.61±0.05 ^b	28.22±0.15 ^b	40.17±0.10 ^e
T3	35.93±0.20 ^l	9.15±0.10 ^b	24±0.15 ^b	24.66±0.05 ⁱ	0.00±0.00	0.00±0.00
T4	11.02±0.10 ^c	14.92±0.20 ^e	36.19±0.15 ^e	27.63±0.10 ^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 unit for ALT mean value in each case isnM/mg.

4. DISCUSSIONS

4.1. ALT Production Levels in *C. gariepinus*exposed to Sub-Lethal Concentrations of Cd Toxicant and the Respective Supplemented Treatments with Vitamins A, C and E

Transaminases such as GOT (Glutamate oxalo transaminase) and GPT (Glutamate pyruvate transaminase) represent useful biomarkers for biomonitoring of chemical pollutants in aquatic organisms, in which altered levels of transaminases indicate compensatory mechanism against impaired metabolism (Ramesh *et al.*, 2014; Sathyaet *al.*, 2012).From the results of the samples

exposed to sub-lethal concentrations of CdCl₂, the ALT production levels in the liver of the fish showed that T1 and T4 mean values in the 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments including the control. The elicitation started early in the lowest concentration and then in the highest concentration subsequently probably to combat the prevailing condition posed by the effects of the toxicant. In line with this, Younis *et al.* (2012) reported that the levels of gluconeogenic enzymes are low under normal conditions, and Zinc increased sera AST and ALT activities in *O. niloticus* at both short and long exposure periods. The control mean values in the 6th and 8th weeks of exposure, respectively are significantly higher than other treatments. The oxidative stress generated by the xenobiotic may have culminated in the utilization of the available concentration of the enzyme in the treatments, hence, making them lower than the control mean values in these stages of the exposure; since it is known that the activity of AST and ALT enzymes in blood (any organ) can be used as a stress indicator (Awad-Elkareem *et al.*, 2014). T1 mean values in the 10th week of exposure are significantly higher than other treatments. At this stage, there may be less utilization especially in the lowest treatments in combating the effects of the toxicant. The highest mean value of ALT produced in the liver was 65.43±0.10 nM/mg obtained in T4 at the end of the 6th week of exposure probably due to the usual need for up-regulation of the body's defence system. Findings from El-Said El-Boshy *et al.* (2014) indicate that blood level activities of ALT and AST was significantly increased when the fish were exposed to 2, 5 and 10 mg/L treatments for a period of 3 weeks. Furthermore, T1 and T4 mean values in the kidneys of the samples at the end of the 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments. The triggering of the production of the enzyme started early in the lowest treatment and subsequently, the need for up-regulation of the defence system in the highest concentration at the end of the 4th week of exposure. The control mean values in the 6th and 10th weeks of exposure are significantly higher than other treatments probably when the available ALT has been put to use in other treatments. T4 mean values in the 8th week of exposure are significantly higher than other treatments including the control; and T4 mean value in the 4th week of exposure recorded the highest ALT production value of 71.87±0.20 nM/mg in the kidney of the samples probably depicting the importance of the enzyme in mitigating the effect of the toxicant in combating the effects of the toxicant at these different stages of the exposure. In addition to the foregoing, the T3 and T4 mean values in the gills of the samples in the 2nd and 4th weeks of exposure are significantly higher than other treatments probably ensuring survival at these stages given that the concentrations are high necessitating immune up-regulation. This may also apply to T2 mean values in the 6th week of exposure which are significantly higher than other treatments, and the highest mean value of ALT produced in the gill of the fish was 62.97±0.05 nM/mg obtained in T2 as well as at the end of the 6th week of exposure. The control mean values in the 8th and 10th weeks of exposure, respectively are significantly higher than other treatments. Similar finding indicated that Glucose, AST, ALT, Creatinine and cortisol increased significantly with increasing Zn concentration and exposure time, peaking at day 56 (Abdel-Tawwab *et al.*, 2013).

From the results of the samples exposed to sub-lethal concentrations of CdCl₂, and supplemented with vitamin A, the ALT production levels in the liver of the fish indicated that T4 mean values in both 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments. This is most likely because of the need for up-regulation of the immune system of the body in the highest concentration especially at the early stages of the exposure when the fish are probably not yet adapted to the effects of the toxicant. Lower concentrations are probably un-perturbed at these stages due to the presence of the vitamin. This is probably why the T3 and T2 mean values in the 6th and 8th weeks of exposure, respectively are significantly higher than other treatments since the duration of exposure has increased and the production levels of the enzyme may have to be upgraded to counter the effects of the toxicant. This may also be the reason why the highest mean value of ALT produced in the liver (73.48±0.15 nM/mg) was also obtained in T3 at the end of the 6th week of exposure. Furthermore, T2 and T1 mean values in the kidneys of the samples at the end of the 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments. This is probably due to high production level of the enzyme and less utilization of same in combating the effects of the toxicant. This is also probably why T1 mean value in both 6th and 8th weeks of exposure, respectively are significantly higher than other treatments and the highest ALT production value of 81.61±0.15 nM/mg in the kidney of the samples at the end of the 4th week of exposure was also obtained in T1. This high production

value may also be as a result of the sensitivity of the kidney in the presence of the vitamin in the lowest concentration of the exposure. In addition to the foregoing, the T2 and T4 mean values in the gills of the samples in the 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments probably because of the need for up-regulation and sustenance of the enzyme production level to counter the effects of the toxicant. In like manner, T3 and T4 mean values in the 6th and 8th weeks of exposure, respectively are significantly higher than other treatments, and the highest mean value of ALT produced in the gill of the fish was 66.61 ± 0.10 nM/mg obtained in T4 as well at the end of the 4th week of exposure. This may also be due to improved production level of the enzyme to combat the effects of the toxicant especially as the duration of the exposure increased. Literatures addressing the ameliorative roles of vitamin A in ameliorating the toxic effects of heavy metals in cat fishes are rare. However, Bashandy and Alhazza (2008) reported how CdCl₂ significantly elevated blood hydroperoxide, AST, ALT, ALP, cholesterol and hepatic cadmium level and that the beneficial roles of β -carotene resulted in reducing the harmful effects of the toxicant.

From the results of the samples exposed to sub-lethal concentrations of CdCl₂, and supplemented with vitamin C, the ALT production levels in the liver of the fish indicated that T3 and T4 mean values in the 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments. The significance in the highest concentrations probably depicts the need for improvement in the body's defence mechanisms to counter the effects of the toxicant especially at the early stages of the exposure. This is probably why the increase in the enzyme production was sustained in T3 mean values in both 6th and 8th weeks of exposure, respectively which are significantly higher than other treatments as the duration of the exposure progresses. This is also probably why the highest mean value of ALT produced in the liver was 60.43 ± 0.15 nM/mg obtained in T3 at the end of the 6th week of exposure. Similar finding by Effiong and Onyeze (2017) indicated that the highest effects were observed at 12.8 mg/L (highest concentration of the exposure) having total protein of 8.51 mmol/L, AST (14.39 mmol/L), ALT (16.99 mmol/L), ALP (66.0 mmol/L), Uric acid (0.68 mg/L) and creatinine (20.7 mg/L). T1 mean values in both 10th and 12th weeks of exposure are significantly higher than other treatments probably due to the need for up-regulation of the body's defence system in the presence of the vitamin as the duration increases culminating in less utilization. In another development, kidney's T1 mean values in both 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments probably due to the need for early elicitation and less utilization in the presence of the vitamin. Also, T3 and T2 mean values in the kidneys of the samples at the end of both 6th and 8th weeks of exposure, respectively are significantly higher than other treatments probably because as the duration and concentration of exposure increases the need for up-regulation became even more important in combating the effects of the toxicant where the succoring effects of the vitamin can no longer be solely relied upon. This may also be the reason why the highest ALT production value of 56.19 ± 0.15 nM/mg in the kidney of the samples was obtained in T3 at the end of the 6th week of exposure. The T3 and T1 mean values in the 10th and 12th weeks of exposure, respectively are significantly higher than other treatments probably also in the light of increased production and less utilization as the duration of exposure increased. In addition to the foregoing, the T1 and T3 mean values in the gills of the samples in the 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments. Early elicitation of the production of the enzyme in the lowest concentration and in the higher concentration subsequently, to combat the effects in the presence of the vitamin which probably ensures less utilization, hence its availability. This is also likely why T1 mean values in both 6th and 8th weeks of exposure, respectively are significantly higher than other treatments. Likewise, T3 and T2 mean values are significantly high than other treatments in the 10th and 12th weeks of exposure; and the highest mean value of ALT produced in the gill of the fish was 65.17 ± 0.15 nM/mg obtained in T3 as well at the end of the 10th week of exposure probably necessitated by the need for improved production and sustenance of the enzyme to counter the effects of the toxicant especially at later stages of the exposure. Similar report by Mozhdeganloo *et al.* (2016) indicated that permethrin significantly increased ALT, AST and DH activities in the liver perfusion medium and MDA level in liver tissue; the values of GSH and total antioxidant capacity (FRAP) in the liver tissue were significantly decreased due to permethrin administration, but PTN-0.64+ vitamin C group increased the values of GSH and FRAP, and decreased the level of MDA and the activities of hepatic enzymes when compared to the PTN-0.64 only group.

From the results of the samples exposed to sub-lethal concentrations of CdCl₂, and supplemented with vitamin E, the ALT production levels in the liver of the fish indicated that T2 mean values in both 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments. This is probably indicative of the elicitation point or threshold in the production of the enzyme which are then sustained to combat the deleterious effects of the toxicant in the presence of the vitamin. As the duration of the exposure increases there were probably continuous productions and less utilization especially in the lower concentrations. This may be the reason why the T2 and T1 mean values in the 6th and 8th weeks of exposure, respectively are significantly higher than other treatments. This may also account for why T1 mean values in both 10th and 12th weeks of exposure are significantly higher than other treatments; and the highest mean value of ALT produced in the liver was 76.70±0.15nM/mg obtained in T1 as well at the end of the 8th week of exposure. In line with this, just as stated earlier, Feng *et al.* (2018) demonstrated how Cd administration resulted in damage of liver function and morphology in liver, which was expressed as the increased content of AST and ALT, rupture of organelles and decrease of CAT, SOD and GSH-Px activity; however, vitamin E and MT treatments protected against Cd-induced damage of liver in grass carp by decreasing AST, ALT content, repairing organelles and maintained the antioxidant system by elevating CAT, SOD and GSH-Px activity and regulating related mRNA transcript expression. In another development, kidney's T1 and T2 mean values in 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments. This may also be as a result of the high production of the enzyme and less utilization in lower concentrations in the presence of the succoring effects of the vitamin at early stages of the exposure and even as the duration progresses. This is probably why T2 and T1 mean values in the kidneys of the samples at the end of the 6th and 8th weeks of exposure, respectively are significantly higher than other treatments; and the highest ALT production value of 71.78±0.05nM/mg in the kidney of the samples was obtained in T2 as well at the end of the 6th week of exposure. Furthermore, the T3 and T2 mean values in the gills of the samples in the 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments. The defence systems probably have to be improved upon early in these treatments to counter the effects of the toxicant. This need for improved ALT production probably continued in T2 and T4 mean values in the 6th and 8th weeks of exposure, respectively which are significantly higher than other treatments. T1 mean values in both 10th and 12th weeks of exposure, respectively are significantly higher than other treatments; and the highest mean value of ALT produced in the gill of the fish was 73.48±0.05nM/mg obtained in T1 at the end of the 12th week of exposure probably due to the fact that at this stage the concentration of the toxicant is low and the produced enzyme in the presence of the vitamin are less utilized in combating the effects of the toxicant. Moreover, at this stage there were high mortalities in higher concentrations where the effects of the enzyme as well as vitamin could no longer sustain the samples. Similar finding by Tuncsoy *et al.* (2015) reported how exposure to Cu-CT mixture decreased sera ALT activity on day 1 compared with Cu alone; exposure to Pb-CT mixture decreased sera AST and ALT activities on day 15 compared with Pb alone due to the reactive amino groups of chitosan which forms complexes between metal ions and the polymer chain.

5. CONCLUSIONS AND RECOMMENDATIONS

The kidneys of the Cd only and CdVA groups had the highest ALT production values while the liver and gills in the CdVE and CdVC groups, respectively produced the highest ALT values. The high production values of ALT indicate physiological perturbations and as such, a good biomarker of oxidative stress elicited by the presence of the toxicant.

The highest ALT mean values produced in the various treatment groups include: Cd only (71.87±0.20nM/mg), CdVA (81.61±0.15nM/mg), CdVC (65.17±0.15nM/mg) and CdVE (76.70±0.15nM/mg) indicative of the various mechanisms adopted by the samples in dealing with the presence of the toxicant. These high production rates in CdVA kidneys and all the three organs in CdVE treatments in lower concentrations of the toxicant are indicative of, to some extent the ameliorative roles of the vitamins against the effects of the toxicant.

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