GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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Evaluation of the ameliorative roles of Vitamins A, C and E on reduced glutathione in *Clarias gariepinus* (Burchell, 1822) fingerlings exposed to cadmium chloride

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GSC Biological and Pharmaceutical Sciences, 2021, 15(03), 052–062

Publication history: Received on 23 April 2021; revised on 29 May 2021; accepted on 31 May 2021

Article DOI: https://doi.org/10.30574/gscbps.2021.15.3.0144

Abstract

Effects of cadmium chloride on the production of antioxidants such as reduced glutathione (GSH) in *Clarias gariepinus* and how such effects can be ameliorated through administration of vitamins were investigated. *C. gariepinus* fingerlings were exposed to sub-lethal concentrations of Cd **(**00, 12mg/L, 16mg/L, 20mg/L and 24mg/L) with replicate in each case. 12mg/L each of the vitamins were administered across all bud. Fresh concentrations of both toxicant and vitamins were administered every 72 hours for a period of 12 weeks every time the water medium was changed. 3 samples of the fish were randomly selected and sacrificed from each aquarium tank every 2 weeks. The gills, kidneys and liver were excised from these specimens, homogenized in sodium phosphate buffer and then assayed for GSH production levels in each case. From the results: In Cd only group, the highest GSH level produced in the liver was 38.85±0.07µg/ml. In the liver of samples of CdVA group, the value (93.97±0.07µg/ml) increased then followed by the gill (67.72±0.13µg/ml). In CdVC, the GSH production level in the gill (39.76±0.07µg/ml) was relatively higher than livers and kidneys of the samples. In CdVE, the kidney produced the highest GSH value of 32.89±0.10µg/ml. The elicitation and utilization of the antioxidant at one point or the other were adopted by the fish in dealing with the effects of the toxicant especially in the presence of the vitamins. Higher concentrations of the vitamins could facilitate the understanding of the effects of the vitamins in mitigating the effects of the toxicant.

Keywords: *Clarias gariepinus*; GSH production level; Cd treatment groups; Ameliorative roles; Fish organs; Vitamins

1. Introduction

Fish is a rich source of animal protein throughout the world. Due to its nutritional value [29]; the demand for fish food has been on the increase with increasing human population [13, 14]. 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 [1, 19]. 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 [12]. The common species found are *Clarias gariepinus*, *Clarias anguillaris*, *Clarias buthupogon* and *Clarias lazera*.

Heavy metals induce significant damage to the physiological and biochemical processes of fish and subsequently to fish consumers [22]. Among all the heavy metals, Cd, arsenic, mercury and lead pose highest degree of toxicity and that is of great concern to both plants and human health [5]. Fish are particularly vulnerable and heavily exposed to pollutants due to feeding and living in aquatic ecosystems, because they cannot avoid pollutant harmful effects [3]. It is also known that, heavy metals enter fish by direct absorption from water through their gills and skin, or by ingestion of contaminated food [6].

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Antioxidants that facilitate or confer protective capacity on organisms could be either enzymatic or non-enzymatic. Antioxidant enzymes are crucial in their effort to decrease oxidative stress produced by exposure to toxicants [26]. It has also been reported that antioxidants may ameliorate, protect and remove the oxidative damage to a target organ or molecule [10]. 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. For instance, increase in reduced glutathione (GSH) level in fish tissues was attributed to presence of defense system to protect the fish from the oxidative stress or could appear as an antioxidant adaptation to metal exposure [21]. Also, Hermenean *et al*. [17] observed that the liver has a higher capacity and adaptability to counteract ROS compared to kidney. Glutathione has been found in all mammalian cells, and has been demonstrated that GSH is responsible for protection against ROS and RNS, detoxification of endogenous and exogenous toxins of electrophilic nature [20]. Sharma and Ansari [27] also demonstrated that GSH level in both the tissues (brain and muscles) were decreased for all exposure periods. Sisein *et al*. [28] attributed the significantly lower GSH value in the liver of *Clarias gariepinus* from Gbarantoru swamp in comparison with Niger Delta University Agricultural farm (control) partly to the increased accumulation of heavy metals which led to more utilization of GSH to detoxify metals and ROS. In the same vein, Ayoola *et al*. [7] recorded significant differences in GSH, MDA, SOD and total protein in the gills of *Hemichromis fasciatus* and *Chrysichthys nigrodigitatus* collected from polluted Lagos lagoon.

The main biological function of vitamin E is its direct influence on cellular responses to oxidative stress through modulation of signal transduction pathway [24]. Vitamins E and C supplementation can induce protective effects on certain conditions after free radical-mediated cellular damage or disruption [30]. Vitamin E (α-tocopherol) is a fat soluble antioxidant that inhibits the production of reactive oxygen species formed when fat undergoes oxidation. A study has also shown how 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, attempted to determine the effects of Cd toxicant on the production of GSH in the exposed samples and how such effects can be ameliorated by the presence of vitamin supplements.

2. Material 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). 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 00 (control), 12mg/L, 16mg/L, 20mg/L and 24mg/L, 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 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 [23] standards. The minimum concentration of the toxicant served the same concentration for the uniform vitamin administration in each treatment. 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 GSH.

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. Reduced Glutathione Bioassay

The GSH (reduced glutathione) produced in each organ of the fish from each treatment and replicate were determined from their homogenates in in the Laboratory of Step B, Federal University of Technology, Bosso Campus, Minna, Niger State. The organs (gill, liver and Kidney) were homogenized using ceramic mortar and pestle with sodium phosphate buffer. The following reagents were used for the analysis: 0.2M phosphate buffer (8.40g of NaH_2PO_4 and 9.94g of Na_2HPO_4 was dissolved in distilled water and made up to 1000 ml mark in a volumetric flask. The buffer was adjusted to pH8.0); 10% Trichloroacetic acid (10g of TCA was dissolved in distilled water and made up to 100ml in the volumetric flask); and Ellman's reagent (19.8mg of 5,5'-Dithiobis Nitro Benzoic acid (DTNB) in 100ml of 0.1 % sodium nitrate).

To 150µL of the tissue homogenate (in phosphate-saline pH 7.4), 1.5ml of 10 % TCA was added, and centrifuge at 1500g for 5 min. 1.0ml of the supernatant was treated with 0.5ml of Ellman's reagent and 3.0ml of phosphate buffer (2.0m pH 8.0). The absorbance was read at 412 nm. Estimation of Reduced Glutathione was determined by the method of Ellman [9] as described by Rajagopalan *et al*. [25]. The amount of glutathione was calculated using a GSH standard curve and expressed as micron grams of GSH formed/mg protein in each case.

2.5. Data Analysis

The antioxidant 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≤0.05 level of significance using SPSS Statistical Package (version 20.0 for Windows).

3. Results

GSH production levels in liver, kidneys and gills of *C. gariepinus* exposed to sub-lethal concentrations of $CdCl_2$ toxicant and the respective supplemented treatments with Vitamins A, C and E for a period of twelve weeks and sampled fortnightly.

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

Treatment group	1st	2nd	3rd	4th	5th	6th
CR	19.76±0.13^{m}	7.26±0.13^{f}	13.51±0.07^{i}	19.03±0.10^{l}	9.42±0.07^{a}	57.83±0.13^{f}
T1	11.64±0.10^{h}	6.47±0.13^{d}	4.54±0.13^{a}	30.21±0.07^{m}	18.00±0.03^{f}	5.79±0.07^{a}
T2	9.37±0.10^{f}	15.45±0.13^{j}	10.56±0.72^{g}	7.37±0.13^{f}	0.00±0.00	0.00±0.00
T3	12.09±0.16^{i}	6.81±0.07^{de}	12.04±0.20^{i}	4.59±0.03^{c}	11.53±0.10^{b}	0.00±0.00
T4	9.71±0.23^{fg}	6.92±0.07^{ef}	38.85±0.07^{l}	6.75±0.03^{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.
Unit of GSH mean value is µg/ml.

In the liver of the samples of *C. gariepinus* exposed to sub-lethal concentrations of $CdCl_2$, there were general low levels of production. The control mean values of GSH are significantly different from other treatments in the 2nd week of exposure. The T2 mean values in the 4th week of exposure are significantly higher than other treatments including the control. The T4 mean values of the 6th week are significantly higher than other treatments. The T1 and T3 mean values of the 8th and 10th weeks of exposure, respectively are significantly higher than other treatments. The highest GSH level in the liver exposed to this toxicant was 38.85±0.07µg/ml obtained in T4 at the end of the 6th week of exposure. (Table 1). In another development, the T2 mean values in the kidney of the samples in both 2nd and 4th weeks of exposure are significantly higher than other treatments including the control. Likewise, the T3 mean values in both 6th and 10th weeks of exposure, respectively are significantly higher than other treatments including the control. The control mean values in the 8th week of control are significantly higher than other treatments. The highest GSH mean value produced in the kidney in this case, was 23.68±0.10 µg/ml obtained in T3 at the end of the 10th week of exposure. (Table 2). On the other

hand, the GSH production level in the gill indicated 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 T2 mean values in both 6th and 8th weeks of exposure, respectively are significantly higher than other treatments including the control. The T3 mean values in the 10th week of exposure are significantly higher than other treatments including the control. The highest GSH value was 17.49±0.13 µg/ml obtained in T1 of the 2nd week of exposure. (Table 3).

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

Treatment group	1st	2nd	3rd	4th	5th	6th
CR	9.88±0.07^{g}	11.70±0.13^{h}	13.80±0.03^{j}	16.13±0.13^{j}	18.80±0.10^{g}	12.55±0.10^{d}
T1	8.00±0.16^{e}	7.46±0.25^{e}	9.71±0.10^{f}	11.35±0.13^{g}	9.14±0.03^{a}	7.15±0.07^{b}
T2	13.51±0.07^{k}	20.50±0.10^{l}	9.03±0.10^{e}	2.72±0.13^{a}	0.00±0.00	0.00±0.00
T3	7.54±0.10^{d}	5.50±0.10^{c}	17.09±0.03^{k}	11.58±0.07^{g}	23.68±0.10	0.00±0.00
T4	5.28±0.10^{b}	16.07±0.10^{k}	8.40±0.13^{d}	5.73±0.10^{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.
Unit of GSH mean value is µg/ml.

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

Treatment group	1st	2nd	3rd	4th	5th	6th
CR	12.55±0.16^{j}	2.72±0.07^{a}	4.88±0.07^{a}	13.29±0.13^{i}	16.35±0.07^{d}	13.68±0.10^{e}
T1	17.49±0.13^{l}	3.63±0.13^{b}	6.58±0.07^{c}	5.62±0.16^{d}	13.34±0.56^{c}	8.23±0.03^{c}
T2	12.38±0.13^{ij}	10.67±0.07^{g}	11.58±0.07^{h}	29.71±0.03^{l}	0.00±0.00	0.00±0.00
T3	6.81±0.20^{c}	2.95±0.07^{a}	6.58±0.13^{c}	12.43±0.10^{h}	16.98±0.03^{e}	0.00±0.00
T4	4.93±0.16^{a}	12.66±0.16^{i}	5.50±0.10^{b}	3.85±0.07^{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≤0.05.
Unit of GSH mean value is µg/ml.

In the liver of the samples of *C. gariepinus* exposed to sub-lethal concentrations of $CdCl_2$, and supplemented with vitamin A, the T3 mean values of GSH are significantly different from other treatments in the 2nd week of exposure. The T1 mean values in the 4th week of exposure are significantly higher than other treatments. This mean value (93.97±0.07µg/ml) was also the highest GSH produced in the liver of the fish in this case. The T4 and T2 mean values of the 6th and 8th weeks are significantly higher than other treatments. (Table 4).

Table 4 GSH 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.

Treatment group	1st	2nd	3rd	4th	5th	6th
CR	19.76±0.13^{j}	7.26±0.13^{g}	13.51±0.07^{h}	19.03±0.10^{k}	9.42±0.07^{a}	57.83±0.13^{c}
T1	7.60±0.13^{d}	93.97±0.07^{l}	10.10±0.07^{d}	6.92±0.07^{d}	0.00±0.00	0.00±0.00
T2	5.33±0.13^{b}	5.16±0.10^{c}	0.00±0.00	16.01±0.07^{i}	0.00±0.00	0.00±0.00
T3	20.79±0.07^{k}	3.23±0.10^{b}	8.40±0.13^{c}	8.85±0.07^{ef}	0.00±0.00	0.00±0.00
T4	9.54±0.07^{f}	6.64±0.16^{e}	11.98±0.16^{f}	3.00±0.03^{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.
Unit of GSH mean value is µg/ml.

In another development, the T3, T1 and T4 mean values in the kidney of the samples in the 2nd, 4th and 6th weeks of exposure are significantly higher than other treatments. Likewise, the T2 mean values in 8th week of exposure are significantly higher than other treatments. The highest GSH mean value produced in the kidney in this case, was 33.12±0.03µg/ml obtained in T2 at the end of the 8th week of exposure. (Table 5). On the other hand, the GSH production level in the gill indicated that T1 and T3 mean values in the 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments. The T4 and T2 mean values in both 6th and 8th weeks of exposure, respectively are significantly higher than other treatments. The highest GSH value was 67.72±0.13µg/ml obtained in T4 of the 6th week of exposure. (Table 6).

Table 5 GSH 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.

Treatment group	1st	2nd	3rd	4th	5th	6th
CR	9.88±0.07^{f}	11.70±0.13^{k}	13.80±0.03^{g}	16.13±0.13^{i}	18.80±0.10^{d}	12.55±0.10^{a}
T1	9.87±0.13^{f}	9.88±0.07^{j}	12.04±0.07^{f}	8.74±0.07^{e}	0.00±0.00	0.00±0.00
T2	10.50±0.10^{g}	5.73±0.10^{d}	6.47±0.07^{b}	33.12±0.03^{l}	0.00±0.00	0.00±0.00
T3	10.56±0.13^{g}	6.40±0.10^{e}	6.01±0.52^{b}	9.08±0.13^{fg}	0.00±0.00	0.00±0.00
T4	6.30±0.10^{c}	8.57±0.10^{i}	18.29±0.07^{i}	17.38±0.07^{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.
Unit of GSH mean value is µg/ml.

Table 6 GSH 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.

Treatment group	1st	2nd	3rd	4th	5th	6th
CR	12.55±0.16^{i}	2.72±0.10^{a}	4.88±0.07^{a}	13.29±0.13^{h}	16.35±0.07^{b}	13.68±0.10^{b}
T1	20.10±0.13^{j}	5.16±0.10^{c}	6.01±0.07^{b}	3.80±0.10^{b}	16.81±0.07^{c}	0.00±0.00
T2	8.17±0.13^{e}	8.34±0.16^{hi}	29.59±0.03	34.71±0.10^{m}	0.00±0.00	0.00±0.00
T3	4.48±0.16^{a}	15.73±0.10	10.62±0.16^{e}	5.96±0.03^{c}	0.00±0.00	0.00±0.00
T4	11.07±0.16^{h}	8.23±0.10^{h}	67.72±0.13^{j}	9.14±0.10^{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.
Unit of GSH mean value is µg/ml.

Table 7 GSH 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.

Treatment group	1st	2nd	3rd	4th	5th	6th
CR	19.76±0.13^{i}	7.26±0.13^{f}	13.51±0.07^{j}	19.03±0.10^{l}	9.42±0.07^{a}	57.83±0.13^{e}
T1	14.03±0.10^{g}	12.72±0.13^{k}	15.22±0.07^{l}	5.56±0.07^{c}	9.71±0.10^{b}	10.73±0.03^{c}
T2	11.30±0.10^{e}	11.07±0.10^{i}	15.50±0.03^{m}	14.71±0.10^{j}	11.35±0.07^{c}	8.23±0.10^{b}
T3	6.98±0.10^{b}	10.33±0.07^{h}	21.07±0.03^{n}	0.00±0.00	11.41±0.10^{c}	0.00±0.00
T4	9.82±0.10^{d}	8.51±0.07^{g}	7.15±0.07^{c}	13.85±0.13^{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.
Unit of GSH mean value is µg/ml.

In the liver of the samples of *C. gariepinus* exposed to sub-lethal concentrations of $CdCl_2$, and supplemented with vitamin C, the T1 mean values of GSH are significantly different from other treatments in both 2nd and 4th weeks of exposure, respectively. The T3 and T2 mean values in the 6th and 8th weeks of exposure are significantly higher than other

treatments. Likewise, the T3 and T1 mean values in the 10th and 12th weeks of exposure are significantly higher than other treatments. The highest mean value was 21.07±0.03µg/ml obtained in T3 at the end of the 6th week of exposure. (Table 7).

In the kidneys of the samples exposed to CdVC treatments the T1 mean values in the 2nd and 10th weeks of exposure are significantly higher than other treatments, respectively. The T4 mean values in the 6th and 8th weeks of exposure are also significantly higher than other treatments. The T3 mean value (50.05±0.03µg/ml) obtained in the 4th week of exposure was the highest GSH value in the kidney of the fish. (Table 8). Likewise, the T3 mean values in 6th and 8th weeks of exposure are significantly higher than other treatments in the gills of the samples. The highest production was obtained in T1 at the 10th week of exposure with 39.76±0.07µg/ml. (Table 9).

Table 8 GSH 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.

Treatment group	1st	2nd	3rd	4th	5th	6th
CR	9.88±0.10^{d}	11.70±0.13^{j}	13.80±0.03^{k}	16.13±0.13^{k}	18.80±0.10^{h}	12.55±0.10^{d}
T1	12.09±0.16^{f}	8.74±0.07^{i}	8.57±0.03^{e}	11.01±0.07^{d}	19.76±0.13^{i}	8.23±0.03^{b}
T2	7.49±0.20^{c}	5.90±0.07^{c}	0.00±0.00	4.99±0.07^{b}	0.00±0.00	8.34±0.03^{b}
T3	5.84±0.16^{a}	50.05±0.03^{m}	10.28±0.10^{g}	12.15±0.07^{e}	13.46±0.10^{e}	0.00±0.00
T4	7.38±0.07^{c}	1.47±0.13^{a}	10.56±0.07^{h}	14.08±0.07^{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.
Unit of GSH mean value is µg/ml.

Table 9 GSH 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.

Treatment group	1st	2nd	3rd	4th	5th	6th
CR	12.55±0.16^{f}	2.72±0.07^{ab}	4.88±0.07^{a}	13.29±0.13^{g}	16.35±0.07^{f}	13.68±0.10^{e}
T1	17.32±0.10^{h}	3.97±0.07^{b}	7.38±0.07^{d}	0.00±0.00	39.76±0.07^{j}	6.75±0.03^{a}
T2	17.55±0.10^{h}	16.24±0.07^{l}	6.70±0.07^{b}	12.49±0.07^{f}	18.06±0.07^{g}	12.60±0.07^{d}
T3	9.88±0.07^{d}	6.07±0.16^{d}	11.53±0.10^{i}	22.83±0.13^{m}	12.04±0.20^{d}	0.00±0.00
T4	19.88±0.07^{i}	7.04±2.10^{e}	8.91±0.10^{f}	4.25±0.03^{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.
Unit of GSH mean value is µg/ml.

Table 10 GSH 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

Treatment group	1st	2nd	3rd	4th	5th	6th
CR	19.76±0.13^{k}	7.26±0.11^{d}	13.51±0.07^{cd}	19.03±0.10^{i}	9.42±0.07^{a}	57.83±0.13^{h}
T1	8.80±0.10^{e}	6.07±0.49^{c}	13.63±0.07^{cd}	10.67±0.07^{f}	15.05±0.10^{c}	10.39±0.10^{c}
T2	11.24±0.07^{g}	7.26±0.20^{d}	14.99±0.20^{d}	8.00±0.10^{c}	11.47±0.10^{b}	14.71±0.10^{f}
T3	7.60±0.13^{c}	3.80±0.23^{b}	9.48±0.10^{b}	15.22±0.13^{h}	0.00±0.00	0.00±0.00
T4	5.84±0.16^{b}	15.22±0.13^{i}	8.12±0.36^{b}	10.28±0.03^{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.
Unit of GSH mean value is µg/ml.

In the liver of the samples of *C. gariepinus* exposed to sub-lethal concentrations of $CdCl_2$, and supplemented with vitamin E, the T2, T4 and T2 mean values of GSH are significantly different from other treatments in the 2nd, 4th and 6th weeks of exposure, respectively. Similarly, the T3, T1 and T2 mean values in the 8th, 10th and 12th weeks of exposure are significantly higher than other treatments. The highest GSH mean value in this regard was 15.22±0.13µg/ml obtained in both T4 and T3 at the end of the 4th and 8th weeks of exposure, respectively. (Table 10).

Table 11 GSH 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

Treatment group	1st	2nd	3rd	4th	5th	6th
CR	9.88±0.07^{f}	11.70±0.13^{g}	13.80±0.03^{d}	16.13±0.13	18.80±0.10^{e}	12.55±0.10^{d}
T1	11.24±0.13^{g}	8.91±0.03^{f}	9.31±0.07^{b}	7.15±0.07^{b}	26.35±0.07^{f}	4.65±0.07^{b}
T2	7.83±0.13^{c}	8.23±0.10^{e}	0.00±0.00	10.28±0.10^{e}	0.00±0.00	0.00±0.00
T3	6.24±0.07^{b}	32.89±0.10	9.25±0.03^{b}	13.34±0.10^{g}	0.00±0.00	0.00±0.00
T4	4.99±0.07^{a}	3.29±0.07^{b}	14.03±0.03^{d}	5.67±0.07^{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.
Unit of GSH mean value is µg/ml.

In another development, the T1 and T3 mean values in the kidney of the samples in the 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments. Likewise, the T4 and T2 mean values in the 6th and 8th weeks of exposure are significantly higher than other treatments. The highest GSH mean value produced in the kidney in this case, was 32.89±0.10µg/ml obtained in T3 at the end of the 4th week of exposure. (Table 11).

Table 12 GSH 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

Treatment group	1st	2nd	3rd	4th	5th	6th
CR	12.55±0.16^{h}	2.72±0.07^{a}	4.88±0.07^{a}	13.29±0.13^{g}	16.35±0.07^{d}	13.68±0.10^{e}
T1	8.34±0.16^{d}	12.66±0.23^{h}	11.70±0.07^{c}	5.39±0.10^{a}	16.24±0.07^{d}	18.40±0.13^{g}
T2	8.68±0.23^{de}	7.38±0.07^{d}	4.71±0.10^{a}	15.22±0.13^{h}	27.60±0.13^{g}	3.91±0.10^{a}
T3	17.66±0.10^{j}	2.43±0.10^{a}	11.70±0.07^{c}	9.08±0.13^{d}	0.00±0.00	0.00±0.00
T4	13.57±0.30^{i}	7.26±0.07^{d}	19.65±2.36^{e}	19.48±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.
Unit of GSH mean value is µg/ml.

On the other hand, the GSH production level in the gill indicated that T1 and T3 mean values in the 2nd and 4th weeks of exposure, respectively are significantly lower than other treatments. The T4 mean values in both 6th and 8th weeks of exposure, respectively are significantly higher than other treatments. T2 and T1 in the 10th and 12th weeks of exposure, respectively are significantly higher than other treatments. The highest GSH value was 27.60±0.13µg/ml obtained in T2 of the 10th week of exposure. (Table 12).

4. Discussion

GSH production levels in C. *gariepinus* exposed to sub-lethal concentrations of $CdCl_2$ toxicant and the respective supplemented treatments with Vitamins A, C and E.

In the liver of the samples of *C. gariepinus* exposed to sub-lethal concentrations of $CdCl_2$, there were general low levels of production. This is probably because cadmium is deleterious, and as such led to utilization of the antioxidant in combating the effects posed. This is also probably why the control mean values of GSH are significantly higher than other treatments in the 2nd week of exposure. As the duration increased more GSH productions were probably elicited to up-regulate the body's defense systems in order to counteract the effects of the toxicant especially in higher

concentration treatments. Hence, the T2 mean values in the 4th week of exposure are significantly higher than other treatments including the control, the T4 mean values of the 6th week are significantly higher than other treatments. Likewise, the T1 and T3 mean values of the 8th and 10th weeks of exposure, respectively are significantly higher than other treatments and the highest GSH level in the liver exposed to this toxicant was 38.85±0.07µg/ml obtained in T4 at the end of the 6th week of exposure; since the defensive homeostatic mechanism of cells and tissues combat metal intoxication either by sequestering the metal in a harmless way or by enhanced excretion of the toxic metal [8].

In another development, the T2 mean values in the kidney of the samples in both 2nd and 4th weeks of exposure are significantly higher than other treatments including the control. The production levels of the antioxidant were triggered early in T2 most likely to upstage the effects of the toxicant before the fish can actually adapt to the prevailing conditions in its environment. Subsequently, it was the turn of a higher concentration as the duration increases. This is probably why the T3 mean values in both 6th and 10th weeks of exposure, respectively is significantly higher than other treatments including the control; and the highest GSH mean value produced in the kidney in this case, was 23.68±0.10µg/ml obtained also in T3 at the end of the 10th week of exposure. Similar report was given by Saglam *et al.* [26] that there were increased activity of glutathione in the liver and kidney of fresh water fish, *Oreochromis niloticus* exposed to cadmium and copper toxicants. Also, Fatima *et al*. [15] reported that there were reduced activities of superoxide dismutase (SOD), catalase (CAT) and GSH in both species, and significant distortions in histology of liver, kidney and brain of affected fishes. On the other hand, the GSH production level in the gill indicated 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 gill being the portal of entry, the elicitation took place in the lowest concentration treatment. Subsequently, the T2 mean values in both 6th and 8th weeks of exposure, respectively are significantly higher than other treatments including the control; and the T3 mean values in the 10th week of exposure are significantly higher than other treatments including the control. The highest GSH value was 17.49±0.13µg/ml obtained in T1 of the 2nd week of exposure. This is probably because the antioxidant produced early in the exposure were not put to too much utilization as it the case in higher concentrations.

In the liver of the samples of *C. gariepinus* exposed to sub-lethal concentrations of $CdCl_2$, and supplemented with vitamin A, the T3 mean values of GSH are significantly different from other treatments in the 2nd week of exposure. At this stage there was probably an urgent need to up-regulate the defense system to counter the xenobiotic in the environment of the fish. The impact was subsequently felt in T1 mean values in the 4th week of exposure where they are significantly higher than other treatments; and at this low concentration there were probably less utilization in combating the effects posed by the toxicant due to the presence of the vitamin in the water matrix. This is also probably why this mean value (93.97±0.07µg/ml) was the highest GSH produced in the liver of the fish in this case. The T4 and T2 mean values of the 6th and 8th weeks are significantly higher than other treatments probably because the responses are both duration and concentration dependent. Jamakala and Rani [18] stated that when *Oreochromis mossambicus* was exposed to Cd toxicity there was elevation of bioaccumulation of Cd in muscle tissues but upon supplementation with Zn or Ca accumulation of the toxicant was progressively reduced in all the test tissues.

In another development, the T3, T1 and T4 mean values in the kidneys of the samples in the 2nd, 4th and 6th weeks of exposure are significantly higher than other treatments. Likewise, the T2 mean values in 8th week of exposure are significantly higher than other treatments. The production level in the kidney was elicited early in the T3 and subsequently in other treatments as the duration increases. The elicitation of the antioxidant in T2 was probably less utilized in this later stage of the exposure, hence, this GSH mean value (33.12±0.03 µg/ml) produced in the kidney in this case, and obtained in T2 at the end of the 8th week of exposure was also the highest. Furthermore, the GSH production level in the gill indicated that T1 and T3 mean values in the 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments. The detection started early with the lowest concentration probably due to less utilization and the presence of the vitamin, hence its availability. However, as the concentration and duration increased the T4 and T2 mean values in both 6th and 8th weeks of exposure, respectively are significantly higher than other treatments; and the highest GSH value (67.72±0.13 µg/ml) was also obtained in T4 of the 6th week of exposure.

In the liver of the samples of *C. gariepinus* exposed to sub-lethal concentrations of $CdCl_2$, and supplemented with vitamin C, the T1 mean values of GSH are significantly different from other treatments in both 2nd and 4th weeks of exposure, respectively. The availability of the antioxidant for detection in this lowest concentration may be due to less utilization and the succor provided by the vitamin. Subsequently, the need for higher production of the antioxidant probably becomes more sacrosanct in dealing with the toxicant as the duration of exposure increases. This is probably why the T3 and T2 mean values in the 6th and 8th weeks of exposure are significantly higher than other treatments; and the highest mean value (21.07±0.03 µg/ml) was also obtained in T2 at the end of the 6th week of exposure. Likewise, the T3 and T1 mean values in the 10th and 12th weeks of exposure are significantly higher than other treatments probably due to the need for up-regulation of the body's defense at later stages of the exposure especially in the lowest concentration

of the toxicant. In another development, the kidney displayed varying production levels of the antioxidant and the T2 mean values in 8th week of exposure are significantly higher than other treatments. The highest GSH mean value produced in the kidney in this case, was (50.05±0.03 µg/ml) obtained also in T3 at the end of the 4th week of exposure. At this stage of exposure, the need for up-regulation of the body's immune system probably becomes more pertinent in dealing with the deleterious effects of the toxicant in the kidney. On the other hand, the GSH production levels in the gill indicated that T1 and T3 mean values in the 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments. The gill which is the first point of entry to the toxicant probably elicited and detected the onslaught of the lowest concentration. This is probably due to the underutilization of the antioxidant and the presence of the vitamin in the environment of the fish. The T1 mean value (39.76±0.07µg/ml) obtained in the 10th week of exposure was the highest GSH value in the gill of the fish probably due to the up-regulation of the body's defence system.

In the liver of the samples of *C. gariepinus* exposed to sub-lethal concentrations of $CdCl_2$, and supplemented with vitamin E, the T2, T4 and T3 mean values of GSH are significantly different from other treatments in the 2nd, 4th and 6th weeks of exposure, respectively. In this case there were early elicitation of the antioxidant in the presence of the vitamin and subsequently increase in its production as the concentration and duration of exposure increases. Similarly, the T3, T1 and T2 mean values in the 8th, 10th and 12th weeks of exposure are significantly higher than other treatments. This is probably because at later stages of the exposure there is the need for production of the antioxidant for sustenance and survival. The highest GSH mean value in this regard was 15.22±0.13µg/ml obtained in both T4 and T3 at the end of the 4th and 8th weeks of exposure, respectively probably due to the need for up-regulation of the antioxidant production levels despite utilization. Yulin *et al*. [31] reported how cell viability was significantly reduced following Cd exposure, but the vitamin C supplementation (though not vitamin E) attenuated the increase in cell viability, vitamin C can increase the antioxidation response and MT and immune-related gene expression. In another development, the T1 and T3 mean values in the kidney of the samples in the 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments; and the highest GSH mean value produced (32.89±0.10 µg/ml) was also obtained in T3 at the end of the 4th week of exposure. In this case, the sensitivity of the kidney is probably brought to bear in the presence of the vitamin at early stages of the exposure. In line with this, Ezedom *et al*. [11] stated that the Cd-contaminated diet was found to be more toxic to the kidney while the arsenic was found to be more toxic to the liver and that alterations in enzymatic activities and levels of MDA and GSH were also recorded. Subsequently, the T4 and T2 mean values in the 6th and 8th weeks of exposure are significantly higher than other treatments. This again is probably because there must be up-regulation of the defense system of the body at the later stages of the exposure. On the other hand, the GSH production level in the gill indicated that T1 and T3 mean values in the 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments; and the T4 mean values in both 6th and 8th weeks of exposure, respectively are significantly higher than other treatments. The same reasons given above are probably also tenable such that increased production in the early stages had to be improved upon at later stages of the exposure in the higher concentrations. T2 and T1 in the 10th and 12th weeks of exposure, respectively are significantly higher than other treatments. The highest GSH value was 27.60±0.13µg/ml obtained in T2 of the 10th week of exposure. In these lower concentrations and later stages of the exposure the elicited production levels were probably sustained with lesser utilization, and also likely due to the presence of the vitamin in the environment of the fish samples. In the work of Adi *et al*. [2] Cd exposure led to decrease in SOD, CAT, GR, GPx activities and a concomitant increase in LPx and GST activities, and that Ca+Zn and vitamin E administration with Cd significantly reversed Cd-induced perturbation in oxidative stress marker enzymes. They also found that vitamin E exhibited more inhibitory activity against Cd than did Ca+Zn, and it protected against Cd-induced nephrotoxicity. Furthermore, the vitamin E and C mixture modulated the oxidative stress induced by ZnONPs (zinc oxide nano-particles) [4].

5. Conclusion

The various treatment groups displayed different levels of production of the antioxidant. There were general low production level in the Cd only group with the control mean values significantly higher than other treatments and the highest production was recorded in the liver. The liver recorded the highest GSH value of 38.85±0.07µg/ml.

There were improved production level of the antioxidant in the CdVA group and the highest production was also recorded in the liver as 93.97±0.07µg/ml.

The CdVC group produced the highest GSH level in the kidneys of the fish with 50.05±0.03µg/ml; while the highest production of the antioxidant in the CdVE group was recorded in the kidney with 32.89±0.10µg/ml.

The elicitation and utilization of the antioxidant at one point or the other were adopted by the fish in dealing with the effects of the toxicant. Higher concentrations of the vitamins could facilitate the understanding of the effects of the vitamins in mitigating the effects of the toxicant.

Compliance with ethical standards

Acknowledgments

The authors wish to appreciate the department of Water, Aquaculture and Fisheries Technology and their technical staff for allowing access to their facilities and the assistance provided by the staff during the exposure period. We also appreciate the services of STEP B of the University for facilitating the assay of the antioxidants.

Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

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