

Research Article

Research on Evaluation of the Ameliorative Roles of Vitamins A, C and E on Malondialdehyde 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 Malondialdehyde (MDA) in *C. gariepinus* and how such effects can be ameliorated through administration of vitamins were investigated. *C. gariepinus* fingerlings (whose initial weight ranged from 3-11 g) were exposed to sub-lethal concentrations of Cd (00, 12 mg/L, 16 mg/L, 20 mg/L and 24 mg/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 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. 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: samples exposed to Cd only group, the MDA production levels in the liver were generally low. The T2 and T3 mean values in the 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 exposure are significantly higher than other treatments. The highest mean value of MDA in the liver was 40.17 ± 0.05 nM/mg obtained in T3 at the end of the 2nd week of exposure. In the kidney, the control mean values in the 2nd, 4th, 8th and 10th weeks of exposure, respectively are significantly higher than other treatments. The T4 mean values in the 6th week of exposure are significantly higher than other treatments. The highest MDA mean value in this regard, was 40.05 ± 0.03 nM/mg obtained in T4 at the end of the 6th week of exposure. The control mean values in the gill of the samples are significantly higher than other treatments in the 2nd week of exposure. The T3 and T1 mean values in the 4th and 6th weeks of exposure, respectively are significantly higher than other treatments. The highest mean value of MDA in the gill was 34.98 ± 0.02 nM/mg obtained in T1 at the end of the 6th week of exposure. In the samples exposed to CdVA, MDA production levels in the liver were generally low. The T3 mean values in the 6th and 8th weeks of exposure, respectively are significantly higher than other treatments. The highest mean value of MDA in the liver was 39.52 ± 0.05 nM/mg obtained in T3 at the end of the 6th week of exposure. The T2 and T3 mean values in the 2nd and 4th weeks of exposure, respectively are significantly higher than

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other treatments. The T3 mean values in the 8th week of exposure are significantly higher than other treatments. The highest MDA mean value in the kidney in this regard, was 41.23 ± 0.34 nM/mg obtained in T3 at the end of the 4th week of exposure. The T1 and T3 mean values in the gill of the samples are significantly higher than other treatments in the 2nd and 4th weeks of exposure, respectively. The highest mean value of MDA in the gill was 40.07 ± 0.05 nM/mg obtained in T3 at the end of the 4th week of exposure. From the samples exposed to CdVC, MDA production levels in the liver indicated that T1 and T2 mean values, respectively are significantly higher than other treatments in the 2nd and 4th weeks of exposure. The T1 and T2 in the 10th and 12th weeks of exposure, respectively are significantly higher than other treatments. The highest mean value of MDA in the liver was 40.03 ± 0.05 nM/mg obtained in T4 at the end of the 6th week of exposure. The kidney's T1, T2 and T3 mean values in the 2nd, 4th and 6th weeks of exposure, respectively are significantly higher than other treatments. The T2 mean values in the 8th, 10th and 12th weeks of exposure, respectively are significantly higher than other treatments. The highest MDA mean value in this regard, was 36.67 ± 0.03 nM/mg obtained in T2 at the end of the 4th week of exposure. T1 and T2 mean values in the 10th and 12th weeks of exposure, respectively are significantly higher than other treatments. The highest mean value of MDA in the gill was 39.54 ± 0.06 nM/mg obtained in T1 at the end of the 10th week of exposure. From the results of the samples exposed CdVE, MDA production levels in the liver indicated that T1 mean values are significantly higher than other treatments in both 2nd and 4th weeks of exposure, respectively. The T2 and T3 mean values in the 6th and 8th weeks of exposure, respectively are significantly higher than other treatments. T2 mean values in the 10th and 12th weeks of exposure, respectively are significantly higher than other treatments. The highest mean value of MDA in the liver was 58.62 ± 0.03 nM/mg obtained in T2 at the end of the 6th week of exposure. The 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 the 8th and 10th weeks of exposure, respectively are significantly higher than other treatments. The highest MDA mean value in this regard, was 39.17 ± 0.03 nM/mg obtained in T3 at the end of the 4th week of exposure. The T2 and T1 mean values in the gill of the samples are significantly higher than other treatments in the 2nd and 4th weeks of exposure, respectively. T2 mean values in both 10th and 12th weeks of exposure, respectively are significantly higher than other treatments. The highest mean value of MDA in the gill was 36.81 ± 0.03 nM/mg obtained in T2 at the end of the 2nd week of exposure. The fish samples exposed to the toxicant displayed slight variations in the treatment groups and in all the organs of interest except in the CdVE group where the highest production of the antioxidant was obtained in the liver in response to the effects of the toxicant. Higher concentrations of the vitamins could facilitate better understanding of the ameliorative roles of the vitamins.

Keywords: *Clarias gariepinus*; MDA production level; Vitamin supplements; Ameliorative roles and Cd treatment groups

Introduction

Fish is a rich source of animal protein throughout the world. Cat fishes are usually reared for both subsistent and commercial consumption in many parts of developing countries of the world. 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 [1].

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 [1]. The common species found are *Clarias gariepinus*, *Clarias anguillaris*, *Clarias buthupogon* and *Clarias lazera*.

Heavy metals induce significant damage to the physiologic and biochemical processes of the fish and subsequently to fish consumers [2]. 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 [3]. 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 [5].

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 [6]. It has also been reported that antioxidants may ameliorate, protect and remove the oxidative damage to a target organ or molecule [7]. 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.

MDA is an end product of lipo-peroxydation, and is considered a biomarker of oxidative stress and cellular damage [8,9]. MDA production is a well-known oxidation product of polyunsaturated fatty acids, influencing cell membrane fluidity as well as the integrity of biomembranes [10,11], and can be used as an indicator of lipid peroxidation. It is usually produced in large quantity when elicited by the presence of toxicants. For instance, [12] showed that the polluted Gbarantoru swamp in Niger Delta, Nigeria contain higher levels of heavy metals, high levels of MDA in liver cells of *C. gariepinus* and low levels of vitamins and glutathione as compared to the levels in the *C. gariepinus* harvested from Niger Delta University Agricultural Farm (Control). Lipid peroxidation is one of the major mechanisms involved in oxidative cell injury and an increase in Malondialdehyde (MDA) level is frequently observed during oxidative stress and has generally been used as a marker of oxidative damage [13]. This is usually because high levels of MDA and low activity of SOD suggest a marked effect of possible fish species exposure to environmental stress [14]. Also, MDA levels were significantly elevated in the kidney of exposed fish, while the gills and liver showed no significant increase across all exposure concentrations [15]. The significant increase in lipid oxidation (MDA) may indicate the susceptibility of lipid molecules to reactive oxygen species and the extent of oxidative damage imposed on these molecules, [15] also observed significant increase in MDA activity in the kidney and attributed it to high antioxidant (CAT, SOD and GSH) activities recorded in the study.

The main biological function of vitamin E is its direct influence on cellular responses to oxidative stress through modulation of signal transduction pathway [16]. Vitamins E and C supplementation can induce protective effects on certain conditions after free radical-mediated cellular damage or disruption [17]. 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 [18]. This research therefore, attempted to determine the effects of Cd toxicant on the production of MDA in the exposed samples and how such effects can be ameliorated by the presence of vitamin supplements.

Materials and Methods

Samples/materials collection and acclimatization

A total number of seven hundred and fifty (750) fingerlings of *C. gariepinus* were purchased from a commercial fish farmer and transported in 50 L 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 (3 mm) 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 (500 g each) were purchased from commercial chemical stores. The toxicant, Cd (2 units of 100 g) 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.

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 12 mg/L, 16 mg/L, 20 mg/L and 24 mg/L of the LC_{50} , 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 20 L 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 [19] 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 MDA.

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.

Malondialdehyde (MDA) determination

Malondialdehyde (MDA), as an *in vitro* marker of lipid peroxidation, was determined according to the fluorimetric method of Del Rio et al. [20]. A volume of 700 μ L of 0.1 M HCl and 200 μ L of sample was incubated for 20 minutes at room temperature. Subsequently, 900 μ L of 0.025 M thiobarbituric acid was added, and the mixture was incubated for 65 minutes at 37°C. Finally, 400 μ L of Tris-EDTA protein extraction buffer was added. MDA fluorescence was recorded using A Jasco FP750 spectrofluorometer (Tokyo, Japan) with A 520/549 (excitation/emission) filter. A calibration curve with MDA in the range of 0.05-5 μ M was used to calculate the MDA concentration. The results were expressed as n moles of MDA/mg protein. 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.

Data analyses

The antioxidant levels in samples exposed to sub-lethal

concentrations 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).

Results and Discussion

MDA 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 Malondialdehyde (MDA) production levels in the liver were generally low and indicated that T3 and T1 mean values, respectively are significantly higher than other treatments including the control in the 2nd week of exposure. Also, the T2 and T3 mean values in the 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 exposure are significantly higher than other treatments. The highest mean value of MDA in the liver was 40.17 ± 0.05 nM/mg obtained in T3 at the end of the 2nd week of exposure (**Table 1**). In another development, the control mean values in the 2nd, 4th, 8th and 10th weeks of exposure respectively are significantly higher than other treatments. The T4 mean values in the 6th week of exposure are significantly higher than other treatments. The highest MDA mean value in this regard, was 40.05 ± 0.03 nM/mg obtained in T4 at the end of the 6th week of exposure (**Table 2**). Furthermore, the control mean values in the gill of the samples are significantly higher than other treatments in the 2nd week of exposure. The T3 and T1 mean values in the 4th and 6th weeks of exposure, respectively 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 MDA in the gill was 34.98 ± 0.02 nM/mg obtained in T1 at the end of the 6th week of exposure (**Table 3**).

From the results of the samples exposed to sub-lethal concentrations of CdCl₂ and supplemented with vitamin A, the Malondialdehyde (MDA) production levels in the liver were generally low and indicated that T2 and T4 mean values, respectively are significantly higher than other treatments in the 2nd and 4th weeks of exposure. Also, the T3 mean values in the 6th and 8th weeks of exposure respectively

Table 1: Production of MDA 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	34.22 ± 0.03 ^m	14.82 ± 0.03 ^f	7.60 ± 0.03 ^d	15.86 ± 0.05 ^h	7.76 ± 0.02 ^e	10.88 ± 0.03 ^g
T1	36.18 ± 0.07 ⁿ	11.95 ± 0.03 ^d	21.51 ± 0.02 ^j	4.90 ± 0.03 ^d	28.80 ± 0.02 ⁱ	0.54 ± 0.07 ^b
T2	23.92 ± 0.07 ^h	32.60 ± 0.03 ^l	10.38 ± 0.30 ^e	4.17 ± 0.27 ^c	6.62 ± 0.03 ^d	12.18 ± 0.03 ^h
T3	18.64 ± 0.05 ^e	28.59 ± 0.02 ^j	11.39 ± 0.03 ^g	3.43 ± 0.03 ^b	13.01 ± 0.03 ^f	0.00 ± 0.00
T4	32.78 ± 0.03 ^l	8.96 ± 0.05 ^c	40.03 ± 0.05 ⁿ	3.45 ± 0.05 ^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. MDA unit of each mean value is nM/mg.

Table 2: Production of MDA 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	27.83 ± 0.03 ^j	39.77 ± 0.03 ^k	24.19 ± 0.02 ^h	51.49 ± 0.06 ^j	56.35 ± 0.06 ⁱ	0.14 ± 0.03 ^a
T1	26.00 ± 0.05 ⁱ	37.76 ± 0.02 ^j	5.21 ± 0.05 ^a	9.43 ± 0.67 ^g	13.48 ± 0.03 ^e	4.89 ± 0.05 ^c
T2	4.31 ± .03 ^b	0.35 ± 0.02 ^a	8.43 ± 0.03 ^d	2.85 ± 0.05 ^c	0.00 ± 0.00	0.00 ± 0.00
T3	2.37 ± 0.06 ^a	11.28 ± 0.02 ^d	10.91 ± 0.05 ^e	6.69 ± 0.02 ^f	4.66 ± 0.02 ^a	0.00 ± 0.00
T4	11.9 5 ± 0.06 ^c	11.60 ± 0.77 ^{cd}	40.05 ± 0.03 ^o	6.79 ± 0.05 ^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. MDA unit of each mean value is nM/mg.

Table 3: Production of MDA 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	34.22 ± 0.03 ^k	14.82 ± 0.03 ^e	7.60 ± 0.03 ^e	15.86 ± 0.05 ^g	7.75 ± 0 .02 ^a	10.88 ± 0.03 ^c
T1	18.80 ± 0.03 ^d	20.47 ± 0.03 ^g	0.98 ± 0.03 ^a	20.93 ± 0.03 ^h	0.00 ± 0.00	0.00 ± 0.00
T2	38.27 ± 0.05 ^o	14.08 ± 0.03 ^d	13.68 ± 0.05 ⁱ	12.69 ± 0.03 ^e	0.00 ± 0.00	0.00 ± 0.00
T3	14.91 ± 0.06 ^c	7.34 ± 0.05 ^b	39.52 ± 0.05 ^o	29.24 ± 1.14 ⁱ	0.00 ± 0.00	0.00 ± 0.00
T4	3.29 ± 0.06 ^a	25.54 ± 0.02 ^j	24.70 ± 0.05 ^m	0.91 ± 0.05 ^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. MDA unit of each mean value is nM/mg.

are significantly higher than other treatments. The highest mean value of MDA in the liver was 39.52 ± 0.05 nM/mg obtained in T3 at the end of the 6th week of exposure (**Table 4**). In another development, the T2 and T3 mean values in the 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments. The MDA level of production in the 6th week was very low, and the T2 mean values are significantly higher than in other treatments. The T3 mean values in the 8th week of exposure are significantly higher than other treatments. The highest MDA mean value in this regard, was 41.23 ± 0.34nM/mg obtained in T3 at the end of the 4th week of exposure (**Table 5**) Furthermore, the T1 and T3 mean values in the gill of the samples are significantly higher than other treatments in the 2nd and 4th weeks of exposure, respectively. The T2 and T1 mean values in the 6th and 8th weeks of exposure, respectively are significantly higher than other treatments. The highest mean value of MDA in the gill was 40.07 ± 0.05 nM/mg obtained in T3 at the end of the 4th week of exposure (**Table 6**).

From the results of the samples exposed to sub-lethal

concentrations of CdCl₂ and supplemented with vitamin C, the Malondialdehyde (MDA) production levels in the liver indicated that T1 and T2 mean values, respectively are significantly higher than other treatments in the 2nd and 4th weeks of exposure. Also, the T4 and T1 mean values in the 6th and 8th weeks of exposure, respectively are significantly higher than other treatments. The production levels in the 8th week of exposure are very low in all treatments. The T1 and T2 in the 10th and 12th weeks of exposure, respectively are significantly higher than other treatments. The highest mean value of MDA in the liver was 40.03 ± 0.05 nM/mg obtained in T4 at the end of the 6th week of exposure (**Table 7**). In another development, the T1, T2 and T3 mean values in the 2nd, 4th and 6th weeks of exposure, respectively are significantly higher than other treatments. The T2 mean values in the 8th, 10th and 12th weeks of exposure, respectively are significantly higher than other treatments. The highest MDA mean value in this regard, was 36.67 ± 0.03 nM/mg obtained in T2 at the end of the 4th week of exposure (**Table 8**). Furthermore, the T1 and T3 mean

Table 4: Production of MDA 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	34.22 ± 0.03 ^k	14.82 ± 0.03 ^e	7.60 ± 0.03 ^e	15.86 ± 0.05 ^g	7.75 ± 0.02 ^a	10.88 ± 0.03 ^c
T1	18.80 ± 0.03 ^d	20.47 ± 0.03 ^g	0.98 ± 0.03 ^a	20.93 ± 0.03 ^h	0.00 ± 0.00	0.00 ± 0.00
T2	38.27 ± 0.05 ^o	14.08 ± 0.03 ^d	13.68 ± 0.05 ⁱ	12.69 ± 0.03 ^e	0.00 ± 0.00	0.00 ± 0.00
T3	14.91 ± 0.06 ^c	7.34 ± 0.05 ^b	39.52 ± 0.05 ^o	29.24 ± 1.14 ⁱ	0.00 ± 0.00	0.00 ± 0.00
T4	3.29 ± 0.06 ^a	25.54 ± 0.02 ^j	24.70 ± 0.05 ^m	0.91 ± 0.05 ^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. MDA unit of each mean value is nM/mg.

Table 5: Production of MDA 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	27.83 ± 0.03 ^h	39.77 ± 0.03 ^m	24.19 ± 0.02 ^l	51.49 ± 0.06	56.35 ± 0.06 ^d	0.14 ± 0.03 ^a
T1	19.10 ± 0.05 ^e	16.99 ± 0.03 ^f	1.58 ± 0.03 ^b	13.82 ± 0.05 ^f	0.00 ± 0.00	0.00 ± 0.00
T2	30.77 ± 0.02 ⁱ	1.67 ± 0.03 ^a	8.82 ± 0.05 ^f	2.90 ± 0.05 ^b	0.00 ± 0.00	0.00 ± 0.00
T3	19.77 ± 0.03 ^f	41.23 ± 0.34 ^o	4.49 ± 0.03 ^d	12.67 ± 0.02 ^e	0.00 ± 0.00	0.00 ± 0.00
T4	11.76 ± 0.03 ^b	38.66 ± 0.03 ^l	4.17 ± 0.03 ^c	0.24 ± 0.06 ^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. MDA unit of each mean value is nM/mg.

Table 6: Production of MDA 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	31.18 ± 0.05 ^j	23.38 ± 0.03 ^j	12.11 ± 0.02 ^h	10.86 ± 0.47 ^d	20.68 ± 0.05 ^c	2.11 ± 0.02 ^b
T1	38.20 ± 0.06 ^m	7.85 ± 0.05 ^c	16.88 ± 0.05 ^j	3.75 ± 0.03 ^c	13.52 ± 0.03 ^b	0.00 ± 0.00
T2	18.75 ± 0.03 ^d	22.85 ± 0.05 ^h	34.91 ± 0.03 ⁿ	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
T3	26.09 ± 0.05 ^g	40.07 ± 0.05 ⁿ	10.72 ± 0.02 ^g	2.71 ± 0.02 ^b	0.00 ± 0.00	0.00 ± 0.00
T4	36.74 ± 0.07 ^l	35.28 ± 0.03 ^k	22.80 ± 0.05 ^k	2.76 ± 0.05 ^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. MDA unit of each mean value is nM/mg.

Table 7: Production of MDA 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	34.22 ± 0.03 ^m	14.82 ± 0.03 ^f	7.60 ± 0.03 ^d	15.86 ± 0.05 ^h	7.76 ± 0.02 ^e	10.88 ± 0.03 ^g
T1	36.18 ± 0.07 ⁿ	11.95 ± 0.03 ^d	21.51 ± 0.02 ^j	4.90 ± 0.03 ^d	28.80 ± 0.02 ⁱ	0.54 ± 0.07 ^b
T2	23.92 ± 0.07 ^h	32.60 ± 0.03 ^l	10.38 ± 0.30 ^e	4.17 ± 0.27 ^c	6.62 ± 0.03 ^d	12.18 ± 0.03 ^h
T3	18.64 ± 0.05 ^e	28.59 ± 0.02 ^j	11.39 ± 0.03 ^g	3.43 ± 0.03 ^b	13.01 ± 0.03 ^f	0.00 ± 0.00
T4	32.78 ± 0.03 ^l	8.96 ± 0.05 ^c	40.03 ± 0.05 ⁿ	3.45 ± 0.05 ^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. MDA unit of each mean value is nM/mg.

values in the gill of the samples are significantly higher than other treatments in the 2nd and 4th weeks of exposure, respectively. The T4 and T2 mean values in the 6th and 8th weeks of exposure, respectively are significantly higher than other treatments. T1 and T2 mean values in the 10th and 12th weeks of exposure, respectively are significantly higher than other treatments. The highest mean value of MDA in

the gill was 39.54 ± 0.06 nM/mg obtained in T1 at the end of the 10th week of exposure (**Table 9**).

From the results of the samples exposed to sub-lethal concentrations of CdCl₂ and supplemented with vitamin E, the Malondialdehyde (MDA) production levels in the liver indicated that T1 mean values are significantly higher

than other treatments in both 2nd and 4th weeks of exposure, respectively. Also, the T2 and T3 mean values in the 6th and 8th weeks of exposure, respectively are significantly higher than other treatments. T2 mean values in the 10th and 12th weeks of exposure, respectively are significantly higher than other treatments. The highest mean value of MDA in the liver was 58.62 ± 0.03 nM/mg obtained in T2 at the end of the 6th week of exposure (Table 10). In another development, the 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 the 8th and 10th weeks of exposure, respectively are significantly

higher than other treatments. The highest MDA mean value in this regard, was 39.17 ± 0.03 nM/mg obtained in T3 at the end of the 4th week of exposure (Table 11). Furthermore, the T2 and T1 mean values in the gill of the samples are significantly higher than other treatments in the 2nd and 4th weeks of exposure, respectively. The T1 and T2 mean values in the 6th and 8th weeks of exposure, respectively are significantly higher than other treatments. T2 mean values in both 10th and 12th weeks of exposure, respectively are significantly higher than other treatments. The highest mean value of MDA in the gill was 36.81 ± 0.03 nM/mg obtained in T2 at the end of the 2nd week of exposure (Table 12).

Table 8: Production of MDA 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	27.83 ± 0.03^i	39.77 ± 0.03^n	24.19 ± 0.02^l	51.49 ± 0.06^i	56.35 ± 0.06^l	0.14 ± 0.03^a
T1	21.55 ± 0.05^f	22.34 ± 0.05^h	5.19 ± 0.06^a	9.01 ± 0.67^e	3.40 ± 0.02^a	8.57 ± 0.06^e
T2	14.26 ± 0.03^d	36.67 ± 0.03^m	0.00 ± 0.00	9.49 ± 0.03^e	35.30 ± 0.05^i	9.29 ± 0.05^f
T3	5.05 ± 0.03^a	30.88 ± 0.03^k	6.62 ± 0.03^c	2.34 ± 0.05^a	22.37 ± 0.09^h	0.00 ± 0.00
T4	6.09 ± 0.05^c	5.21 ± 0.05^a	5.70 ± 0.06^b	4.26 ± 0.03^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 $P \leq 0.05$. MDA unit of each mean value is nM/mg.

Table 9: Production of MDA 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	31.18 ± 0.05^j	23.38 ± 0.03^l	12.11 ± 0.02^i	10.86 ± 0.47^f	20.68 ± 0.05^g	2.11 ± 0.02^c
T1	36.51 ± 0.05^o	21.46 ± 0.05^g	23.78 ± 0.02^k	4.73 ± 0.03^{cd}	39.54 ± 0.06^k	2.23 ± 0.03^c
T2	23.89 ± 0.06^g	5.84 ± 0.06^b	11.83 ± 0.02^h	12.37 ± 0.06^g	5.86 ± 0.07^c	3.50 ± 0.02^d
T3	5.56 ± 0.03^b	45.07 ± 0.05^o	10.77 ± 0.02^f	10.72 ± 0.02^f	5.10 ± 0.03^b	0.00 ± 0.00
T4	31.95 ± 0.03^k	14.54 ± 0.03^e	28.87 ± 0.05^m	4.77 ± 0.09^{cd}	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 $P \leq 0.05$. MDA unit of each mean value is nM/mg.

Table 10: Production of MDA 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	34.22 ± 0.03^m	14.82 ± 0.03^d	7.60 ± 0.03^c	15.86 ± 0.05^h	7.76 ± 0.02^d	10.88 ± 0.05^f
T1	37.87 ± 0.03^o	16.72 ± 0.03^e	0.48 ± 0.05^a	2.43 ± 0.05^b	4.87 ± 0.07^b	2.62 ± 0.05^c
T2	31.67 ± 0.03^k	9.47 ± 0.05^b	58.62 ± 0.03^k	17.60 ± 0.06^i	14.70 ± 0.02^e	4.82 ± 0.06^e
T3	33.41 ± 0.05^l	27.64 ± 0.06^j	11.79 ± 0.05^f	23.22 ± 0.05^k	0.00 ± 0.00	0.00 ± 0.00
T4	23.78 ± 0.03^f	13.73 ± 0.07^c	12.09 ± 0.03^g	1.97 ± 0.02^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 $P \leq 0.05$. MDA unit of each mean value is nM/mg.

Table 11: Production of MDA 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	27.83 ± 0.03 ^h	39.77 ± 0.03 ^m	24.19 ± 0.02 ⁱ	51.49 ± 0.06 ^o	56.35 ± 0.06 ^h	0.14 ± 0.03 ^a
T1	29.33 ± 0.02 ⁱ	19.05 ± 0.02 ^g	10.35 ± 0.02 ^e	14.59 ± 0.09 ^g	3.45 ± 0.05 ^a	0.00 ± 0.00
T2	19.24 ± 0.05 ^d	38.99 ± 0.03 ^k	7.48 ± 0.05 ^b	22.87 ± 0.03 ^j	0.00 ± 0.00	0.00 ± 0.00
T3	8.94 ± 0.03 ^a	39.17 ± 0.03 ^l	11.69 ± 0.02 ^f	5.65 ± 0.06 ^d	0.00 ± 0.00	0.00 ± 0.00
T4	17.37 ± 0.03 ^c	23.31 ± 0.02 ⁿ	41.76 ± 0.62 ^j	30.58 ± 0.07 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 ≤ 0.05. MDA unit of each mean value is nM/mg.

Table 12: Production of MDA 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	31.18 ± 0.05 ^j	23.38 ± 0.03 ^{hi}	12.11 ± 0.02 ^g	10.86 ± 0.47 ^f	20.68 ± 0.05 ^f	2.11 ± 0.02 ^b
T1	20.17 ± 0.05 ^e	23.75 ± 0.06 ⁱ	29.84 ± 0.07 ^m	9.80 ± 0.05 ^e	5.17 ± 0.05 ^c	3.43 ± 0.03 ^d
T2	36.81 ± 0.03 ⁿ	5.37 ± 0.03 ^a	10.33 ± 0.06 ^d	28.50 ± 0.05 ^m	30.21 ± 0.07 ^g	18.55 ± 0.05 ^g
T3	23.50 ± 0.03 ^g	17.50 ± 0.03 ^f	14.73 ± 0.03 ^h	24.31 ± 0.03 ^l	0.00 ± 0.00	0.00 ± 0.00
T4	10.91 ± 0.05 ^b	0.00 ± 0.00	14.75 ± 0.02 ^h	3.41 ± 0.02 ^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. MDA unit of each mean value is nM/mg.

Discussion

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

From the results of the samples exposed to sub-lethal concentrations of CdCl₂ the Malondialdehyde (MDA) production levels in the liver were generally low and indicated that T3 and T1 mean values, respectively are significantly higher than other treatments including the control in the 2nd week of exposure. The general low production levels may be due to the utilization of the available MDA to deal with the deleterious situations. This could also be the reason why there were mortalities as the concentration and duration of exposure increased, and perhaps the body's defence system overwhelmed [21] reported that decreased level of GSH indicates high LPO and MDA. Increased GSH level and decreased MDA concentration in the liver of *C. gariepinus* exposed to Cu: Cd mixture indicated the ability of the fish to overcome the stress created by the mixture of toxicant. Also, the T2 and T3 mean values in the 6th and 10th weeks of exposure, respectively are significantly higher than other treatments including the control. This is probably because as the duration of the exposure increased there were corresponding need to up-regulate the production

level, hence, the body's defence in countering the effects of the toxicants. The control mean values in the 8th week of exposure are significantly higher than other treatments. This is probably because the MDA produced in the treatments have been utilized in dealing with the effects of the toxicant. The highest mean value of MDA in the liver was 40.17 ± 0.05 nM/mg obtained in T3 at the end of the 2nd week of exposure. In line with the foregoing, similar finding by Li et al. [22] has demonstrated that the MDA contents increase with exposure time and dose-dependency in crab, *Sinopotamon henanense* exposed to Cd. Also, when *Gammarus pulex* was exposed to 32 µg/L of Cd there was an increase in LPO [23]. In another development, the control mean values in the kidneys of the samples in the 2nd, 4th, 8th and 10th weeks of exposure, respectively are significantly higher than other treatments. The control mean values are higher than other treatment probably due to the utilization of the available MDAs in the exposed treatments to respond and counter the deleterious effect of the toxicant. In the highest concentration however, there was probably an up-regulation of the immune system. Perhaps, this is why T4 mean values in the 6th week of exposure are significantly higher than other treatments, and the highest MDA mean value (40.05 ± 0.03 nM/mg) in this regard, was also obtained in T4 at the end of the 6th week of exposure. Similar finding by Ensibi et al. [24] reported that cadmium chloride directly influenced significant increase in MDA levels after 48 and

72 hrs of exposure in the treated copepods hinting that the copepods had suffered from oxidative damage. Furthermore, the control mean values in the gill of the samples are significantly higher than other treatments in the 2nd week of exposure. This is probably because in the exposed samples the MDAs produced were utilized in dealing with the situation at hand. The T3 and T1 mean values in the 4th and 6th weeks of exposure, respectively are significantly higher than other treatments. As the duration of exposure increased the exposed treatments may have improved upon the production level of the MDA to counteract the effects. Similarly, [25] reported how exposure of *Labeo rohita* to zinc led to significant increase in peroxidase in all treatment in comparison with the control group after 30 days of exposure. Subsequently, the MDAs may have been overwhelmed or utilized in dealing with the situation and as such, the control mean values in the 8th and 10th weeks of exposure, respectively are significantly higher than other treatments. The highest mean value of MDA in the gill was 34.98 ± 0.02 nM/mg obtained in T1 at the end of the 6th week of exposure probably due to the elevation of the MDA production levels even in the lowest concentration; however, this was not sustained as this became lower than the value of the control samples.

From the results of the samples exposed to sub-lethal concentrations of CdCl₂ and supplemented with vitamin A, the Malondialdehyde (MDA) production levels in the liver were generally low and indicated that T2 and T4 mean values, respectively are significantly higher than other treatments in the 2nd and 4th weeks of exposure. The elicitation started early in T2 and subsequently in higher concentrations as the duration of exposure increased probably to boost the fighting capacity of the samples to deal with the effects of the toxicant. This is also probably why the T3 mean values in the 6th and 8th weeks of exposure, respectively are significantly higher than other treatments and the highest mean value of MDA in the liver was 39.52 ± 0.05 nM/mg obtained in T3 as well at the end of the 6th week of exposure. Similar finding by Gopalkrishnan and Rao, [26] indicated that a notable decrease was detected in the activities of the enzymes and in the level of other metabolites (such as SOD, Catalase, GSH, ascorbic acid and total sulphhydryl groups, ATPase, succinic dehydrogenase, phosphorylase, glycogen and protein levels) together with a significant increase in the LPO, glycogen and inorganic arsenic levels after arsenic administration; supplementation of vitamin A to the arsenic treated mice brought about no significant variation in these antioxidants and metabolic indices in comparison to the control; revealing amelioration

by vitamin A on arsenic exerted metabolic and neurotoxic effects in mice. In another development, the T2 and T3 mean values in the 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments. Likewise, the elicitation started early in T2 and subsequently T3 where the highest was obtained in order to deal with the menace at hand. The MDA levels of production in the 6th week were very low, and the T2 mean values are significantly higher than other treatments probably due to the utilization of the available MDA to counteract the effects of the toxicant especially in higher concentrations at this stage of exposure. This is also probably why the T3 mean values in the 8th week of exposure are significantly higher than other treatments. Furthermore, the T1 and T3 mean values in the gill of the samples are significantly higher than other treatments in the 2nd and 4th weeks of exposure, respectively. In the gill the elicitation started early in the lowest concentration. The T2 and T1 mean values in the 6th and 8th weeks of exposure, respectively are significantly higher than other treatments probably due to high production of the enzyme and less utilization in these lower concentrations. The highest mean value of MDA in the gill was 40.07 ± 0.05 nM/mg obtained in T3 at the end of the 4th week of exposure. At this treatment there were probably needs for up-regulation of the body's immune system to counter the effects of the toxicant especially at this early stage of the exposure.

From the results of the samples exposed to sub-lethal concentrations of CdCl₂ and supplemented with vitamin C, the Malondialdehyde (MDA) production levels in the liver indicated that T1 and T2 mean values, respectively are significantly higher than other treatments in the 2nd and 4th weeks of exposure. The elicitation started early in the lower concentrations and then probably sustained due to lesser utilization in comparison to higher concentrations, and the presence of the vitamin serving as immune booster. This may also be the reason why T1 and T2 in the 10th and 12th weeks of exposure, respectively are significantly higher than other treatments principally due to low concentration of the toxicant and lesser utilization. The production levels in the 8th week of exposure are very low in all treatments probably due to utilization of the available and, or newly produced MDAs in dealing with the effects of the toxicant in all treatments. The highest mean value of MDA in the liver was 40.03 ± 0.05 nM/mg obtained in T4 at the end of the 6th week of exposure probably due to up-regulation of MDA to counter the effects of the toxicant. Similar report by Banaee et al. [27] indicated that exposure of *Cyprinus carpio* to CdCl₂ significantly increased AST, LDH, CPK, catalase and MDA activity, administration of chitosan or

vitamin C alone or in combination with each other was effective in regulating ALT, CPK and catalase activity; although administration of vitamin C and chitosan caused a significant decrease in MDA, ALT and LDH, these enzymes were still significantly higher than those in the control group.

In another development, the kidneys' T1, T2 and T3 mean values in the 2nd, 4th and 6th weeks of exposure, respectively are significantly higher than other treatments. In the kidney of the fish, one of the major organs of detoxification, the trigger of the production of MDA started early; and continued the trends in higher concentrations as the duration of exposure increased. Again, this may be attributed to less utilization of the produced MDA in lower concentrations as well as the presence of the vitamin. This is also probably why the T2 mean values in the 8th, 10th and 12th weeks of exposure, respectively are significantly higher than in other treatments, and the highest MDA mean value in this regard, was 36.67 ± 0.03 nM/mg obtained in T2 as well at the end of the 4th week of exposure. Furthermore, the T1 and T3 mean values in the kidney of the samples are significantly higher than other treatments in the 2nd and 4th weeks of exposure, respectively. In line with this, [28] demonstrated how Cd toxicant was ameliorated with Zn and Ca when they reported that there was significant reduction in Cd bioaccumulation with Zn and Ca supplements both individually and in combination depicting the vital roles of these elements in heavy metal detoxification with maximum decrease recorded in fish kidney (6.996 ± 0.284 µg/gm wet wt) of the tissue of *Oreochromis mossambicus* supplemented with only Ca which indicates that Ca is a better mitigator than either Zn or its combination with Ca.

In the gill, the first point of contact to the internal environment, and where Reactive Oxygen Species (ROS) are primarily generated culminating in oxidative stress due to the presence of xenobiotics exhibited early detection of the toxicant; and consequent production of MDA to counter the effects of the toxicant. In line with these assertions, [29] posited that increasing Cd concentration led to an increase in ROS and resulted ultimately in membrane lipid peroxidation at higher Cd concentrations. Subsequently, probably the need for up-regulation of the body's immune system was why the T4 and T2 mean values in the 6th and 8th weeks of exposure, respectively are significantly higher than other treatments. T1 and T2 mean values in the 10th and 12th weeks of exposure, respectively are significantly higher than other treatments, and the highest mean value of MDA in the gill was 39.54 ± 0.06 nM/mg obtained in T1 as well at the end of the 10th week of exposure is probably a testimony to up-regulation of the body's immune system

for survival as the duration of exposure increased and most likely less utilized in the lower concentrations and in the presence of the vitamin. This is in agreement with [30] who reported that in treatments supplemented with vitamin C there were increased malondialdehyde index compared to those of the control group when rainbow trout were exposed to diazinon.

From the results of the samples exposed to sub-lethal concentrations of CdCl₂ and supplemented with vitamin E, the Malondialdehyde (MDA) production levels in the liver indicated that T1 mean values are significantly higher than other treatments in both 2nd and 4th weeks of exposure, respectively. This early detection in lowest concentration may be due to less utilization in the presence of the vitamin. As the concentration and duration of exposure increased the T2 and T3 mean values in the 6th and 8th weeks of exposure, respectively became significantly higher than other treatments. This is also probably why T2 mean values in the 10th and 12th weeks of exposure, respectively are significantly higher than other treatments; and the highest mean value of MDA in the liver (58.62 ± 0.03 nM/mg) was also obtained in T2 at the end of the 6th week of exposure. In conformity with this, [31] reported that exogenous vitamin E and metallothionein could effectively reduce the Cd-caused Malondialdehyde (MDA) in liver of grass carp. In another development, the T1 and T3 mean values of the kidney in the 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments. At one point or the other the need for up-beat on the immune system is sacrosanct. This is probably due to up-regulation in higher concentration and as the duration of exposure increases. This is probably why the T4 and T2 mean values in the 8th and 10th weeks of exposure, respectively are significantly higher than in other treatments. The highest MDA mean value in this regard, was 39.17 ± 0.03 nM/mg obtained in T3 at the end of the 4th week of exposure probably due to the need for up-regulation especially at this early stage of the exposure. Furthermore, the T2 and T1 mean values in the gill of the samples are significantly higher than other treatments in the 2nd and 4th weeks of exposure, respectively. This early elicitation and sustenance of the production of MDA levels through-out the duration of the exposure is most likely due to the initial need for up-regulation and less utilization in the presence of the succor provided by the vitamin in the lower concentrations of the toxicant. This is also probably why the T1 and T2 mean values in the 6th and 8th weeks of exposure, respectively are significantly higher than other treatments. T2 mean values in both 10th and 12th weeks of exposure, respectively are significantly higher

than other treatments; and the highest mean value of MDA in the gill was 36.81 ± 0.03 nM/mg obtained in T2 as well at the end of the 2nd week of exposure probably due to the same reasons adduced above.

Conclusion and Recommendations

The fish samples exposed to the toxicant displayed slight variations in the treatment groups and in all the organs of interest except in the CdVE group where the highest production of the antioxidant was obtained in the liver in response to the effects of the toxicant. The highest MDA mean value obtained in the liver is 58.62 ± 0.03 nM/mg. Higher concentrations of the vitamins could facilitate better understanding of the ameliorative roles of the vitamins

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