SYNERGISTIC ROLES OF VITAMINS A, C AND E ON CADMIUM INDUCED TOXICITY IN *CLARIAS GARIEPINUS* (Burchell, 1822)

BY

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MTech/SLS/2018/8320

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A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, FEDERAL UNIVERSIY OF TECHNOLOGY, MINNA, NIGERIA IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF TECHNOLOGY IN ZOOLOGY (APPLIED HYDROBIOLOGY) OF THE FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA NIGER STATE.

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ABSTRACT

Cadmium (Cd) contamination has become one of the environmental concerns that has piqued the world's attention in recent decades, resulting in both economic and health losses as a result of the negative effects triggered by Cd in living systems. Contamination of aquatic ecosystems with heavy metals such as cadmium (Cd) from various sources causes health risks in aquatic creatures, which may have an adverse effect on humans. In this study, synergistic effects of combined vitamins (A and C; A and E; A, C and E, and C and E) supplements on growth parameters, haematological parameters and tissue histopathology of *Clarias gariepinus* exposed to Cd were investigated. A total of 1000 samples of *C. gariepinus* fingerlings (with initial average weight of 12.39 g, standard length (SL), 9.98cm and total length (TL), 11.75 cm) were exposed to sub-lethal concentrations of Cd (00 (control), 4 mg/L (T1), 8 mg/L (T2), 12 mg/L (T3), 16 mg/L (T4), respectively) and supplemented with combined vitamins for a period of 8 weeks. The T2 (8 mg/L) of each of the combined vitamins were administered in each of the treatments and replicates. Fresh concentrations of both toxicant and combined vitamins were applied every 72 hours when water was changed. The weight, SL and TL were taken biweekly from 2 randomly selected samples from each treatment and replicate. Blood were collected from 2 randomly selected samples from each treatment and replicated every 4^{th} week of the exposure. These were analysed for complete blood count. The gills, liver and kidneys were also excised from 2 randomly selected sampled and preserved in 10 % formalin for the histopathological analyses. From the results: In Cadmium plus Vitamins C and E (CdVCE) treatment group had the highest TL and SL obtained in T1 were 11.55 cm and 10.13cm, respectively. A general weight loss was observed in all treatment when compared to the control. No significance differences were observed in almost all of the haematological parameters between the control and treatment groups supplemented with combined vitamins over 4 weeks of Cd exposure. However, significant fall in the levels of these parameters was observed over 8 weeks of Cd exposure in all treatment groups. Histopathological analysis revealed preserved architecture of both livers and kidneys at the lowest concentration of Cd exploited (T1) in all treatment groups, however, histological alterations were observed in higher concentrations. No histological alterations were observed in the gills of treatment groups except for T4 of groups supplemented with combined vitamins A and C, and C and E, respectively. Therefore, it is rational to infer that the combined vitamins supplements ameliorate toxic effects of Cd but only at lower concentration. Again, combined vitamins C and E, A and E, and A, C and E showed higher protective activities.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Fish are major sources of protein which are cheaply available to humans as food. *Clarias gariepinus* (African catfish) are native to Asia and African continents (Aladesanmi *et al.*, 2017). *Clarias gariepinus* are fresh water organisms, ubiquitous and can easily be cultured in any part of the world as long as the right environmental conditions are favourable (Ayanwale *et al*., 2018). *Clarias gariepinus* is a species of catfish of the family Clariidae, the air-breathing catfish. Fish are an important bioindicator species and play an increasingly important role in the monitoring of water pollution, because they respond with great sensitivity to changes in the aquatic environment (Naigaga *et al.*, 2011; Rosso *et al.*, 2013; Authman *et al.*, 2015; Aladesanmi *et al.*, 2017). The sudden death of fish can indicate heavy pollution, and the effects of exposure to sub-lethal levels of pollutants can be measured in terms of their biochemical, physiological or histological responses (Mondon *et al.*, 2001; Aladesanmi *et al.*, 2017). The binding of a toxic compound like a heavy metal with its receptor may induce cellular processes that have toxic or other adverse effects on the cell of fish (Okoye *et al.*, 2018).

Human beings are continuously exploiting the precious metals in the earth crust for technological, urbanization and social wellbeing reasons, thereby leading to the contamination or pollution of aquatic environments (Sanneh *et al*., 2011). The aquatic organisms such as fish are at the receiving end of these toxic substances which may be

lethal or sub-lethal to them. The contamination of fresh water systems with a wide range of pollutants has become a matter of global concern. Many natural aquatic bodies have been extensively contaminated with heavy metals released from domestic, industrial and other anthropogenic activities (Lakra and Nagpure, 2009). Organisms may accumulate toxic metals, which ultimately affect not only the productivity and reproductive capabilities of the organisms, but also the health of the human beings that depend on the organisms as a major source of protein. As with other aquatic animal species, fish cannot escape the detrimental effects of these pollutants (Aladesanmi *et al.,* 2017). Studies carried out on various fish species have revealed that heavy metals may alter biochemical parameters both in tissues and in blood (Shaikh *et al.*, 1999; Rani, 2000; Aladesanmi *et al.*, 2017).

Cadmium is a heavy metal and toxic at very low exposure levels and has acute and chronic effects on health and environment. Cadmium is not degradable in nature and thus, once released to the environment, stays in circulation. New releases add to the already existing deposits of cadmium in the environment. Cadmium and cadmium compounds are, compared to other heavy metals, relatively water soluble. They are therefore also more mobile in soil, generally more bioavailable and tend to bioaccumulate which eventually gets to the nearest aquatic body through seepages or run-offs. Cadmium exposure produces a wide variety of acute and chronic effects in humans. Cadmium accumulates in the human body and especially in the kidneys and can alter calcium metabolism, cause hypercalciuria and formation of stones in the kidney. High exposure can lead to lung cancer and prostate (Mallesh *et al*., 2015)

The accumulation of Cd has also been well described in different tissues of fishes (Moiseenko, 2015). Haematological indices have been recognized as valuable tools for evaluation of fish physiological status, the changes of which depend on fish species,

age, cycle of sexual maturity and diseases (Mallesh *et al*., 2015). The toxicity of Cadmium to freshwater fishes has been well reported. For example, anaemic condition was reported in *Channa punctatus* and *Oreochromis mossambicus* exposed to different doses of Cadmium (Moiseenko, 2015). Changes in serum biochemical parameters due to liver, gill and kidney dysfunction was reported by a researcher in *Oreochromisniloticus* exposed to Cadmium (Oner *et al*., 2008). It also acts as an immune-suppressant in common carp (*C. carpio*), *Oreochromis aureus, O. niloticus* and *Ictalurus melas*, respectively (Oner *et al*., 2008). Fishes were proved to be significant bioindicators of the aquatic environment so- called ecological integrity (Messaoudi *et al*., 2009). It can provide quantitative information on the ecological integrity and its health. As ornamental fishes contribute significantly to the freshwater aquaculture of India and due to their species richness, they have been selected as suitable bioindicators for heavy metal pollution (Moiseenko, 2015).

On the other hand, vitamins are organic compounds necessary in the diet to support normal fish growth and health. They are often not synthesized by fish and must be provided in the diet. The two groups of vitamins are water-soluble and fat-soluble vitamins. Water-soluble vitamins include B vitamins (thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, biotin, folic acid, and cobalamins), inositol, choline, and vitamin C (ascorbic acid). Of these, vitamin C is the most important because it is a powerful antioxidant and it enhances the immune system of fish and shrimp (Rahman *et al.*, 2018; Sahiti *et al.*, 2019). Fat-soluble vitamins include vitamins A (retinol, beta-carotene), D (cholecalciferol), E (tocopherols), and K (phylloquinone). As parts of feed ingredients, vitamins E and C have been shown to inhibit dietary lipid oxidation, thus helping to improve shelf life (Mehrad *et al.*, 2012; Khara *et al.*, 2016).

Vitamin A plays a role in healthy vision, bone and tissue growth, and reproduction. It also helps regulate immune system, which prevents and fights infections. Vitamin C is an antioxidant that maintains healthy tissue and helps the body absorb iron. It also plays a role in wound healing. Vitamin E is an antioxidant that protects red blood cells and may play a role in immune function, deoxyribonucleic acid (DNA) repair and other metabolic functions (Khara *et al.*, 2016).

Previous studies have shown that vitamin C stimulate immune responses such as macrophage activities, cell proliferation, natural killer of cell activity, complement activity, lysozyme level, leucocyte phagocytic activity, cytokine production and antibody levels (Li and Lovell, 1985) while vitamin E enhanced the natural cytotoxic activity of leucocytes (Cuesta *et al.*, 2001; Khara *et al.*, 2016) and innate immune responses in gilthead sea bream, *Sparus aurata* (Ortuno *et al.*, 2000; Khara *et al.*, 2016). Vitamin E in immune cell membranes (Beharka *et al.*, 1997; Khara *et al.*, 2016) protects macrophage membranes from peroxidative damage by free radicals and thus has a key role in fish immunity (Waagbo, 1994; Khara *et al.*, 2016).

1.2 Statement of the Research Problem

Pollution of freshwaters by heavy metals is a global trending issuein the last few decades (Ohe *et al.*, 2004; Okoye *et al.*, 2018). These pollutants disrupt the ecosystem of the recipient environment leading to a decline diversity of the aquatic organisms (Farombi *et al.*, 2007; Okoye *et al.*, 2018). The presence of heavy metals in the food web is a potential threat to the life and safety of many aquatic organisms by changing their genetic, physiological, biochemical and behavioural parameters (Scott and Sloman, 2004; Okoye *et al.*, 2018). Cadmium is not degradable in nature and very toxic at low

exposure. Since cadmium bioaccumulate in the fish even when exposed at low level, it eventually gets to human beings who occupy the highest part of the trophic level.

Deficiency of vitamins such as C and E has specific symptoms, but reduced growth is the most common symptom of any vitamin deficiency. Scoliosis (bent backbone symptom) and dark colouration have been reported from deficiencies of ascorbic acid and folic acid, respectively (Khara *et al.*, 2016; Adel and Khara, 2016; Rahman *et al.*, 2018; Sahiti *et al.*, 2019). Studies addressing how the effects of cadmium on the morphometric, histopathological and haematological features of *Clarias gariepinus* can be ameliorated or at best, attenuated by the presence of various combinations of vitamins A, C and E are rare.

1.3 Aim and Objectives of the Study

The aim of this research was to evaluate the synergistic roles of vitamins A, C and E on the effects of cadmium induced toxicity in the African catfish, *Clarias gariepinus*.

The objectives of this study were to determine:

i. growth parameters of *C. gariepinus* exposed to sub-lethal concentrations of cadmium supplemented with combinations of vitamins A, C and E.
ii. haematological parameters of *C. gariepinus* exposed to sub-lethal concentrations of cadmium supplemented with combinations of vitamins A, C and E.
iii. histopathological alterations of the tissues of *C. gariepinus* exposed to sub-lethal concentrations of cadmium supplemented with combinations of vitamins A, C and E.

1.4 Justification for the Study

Fish is an important food for humans. Thus, the contamination of food web as a result of bioaccumulation of cadmium by fish from their environments poses a great health danger to humans, the ultimate consumer. Heavy metals are of particular concern due to their persistence and un-degradable nature. The metal contamination in aquatic ecosystem is considered to be unsafe not only for fishes but, also for human beings because they consume fishes which are regarded as good sources of proteins and essential amino acids. Consumption of such staple food in contaminated forms calls for concern.

Studies have shown that antioxidative vitamins such as C and E have mitigation effects in heavy metals toxicity (Mehrpak *et al.*, 2015; Asaikkuttia *et al.*, 2016; Sahiti *et al.*, 2018). These two vitamins have also been shown to have chelating abilities, affecting the reduction in the amount of accumulated metals in different tissues to various organisms, including fish (Abdalla 2009; Donpunha *et al.*, 2011; Sahiti *et al.*, 2019). This is why the effects of toxicants on the immunity and physiology of the fish need to be put in check as soon as possible; since fish like any other vertebrate, have numerous enzymes to fight the effects of toxication (Okoye *et al.* 2018).

With all the health risks associated with the ingestion of cadmium, and deficiency of important vitamins in diets (especially vitamins with antioxidative effects), it is pertinent to assess the synergistic roles of the vitamins in ameliorating or at best, attenuating the deleterious effects of the toxicant elicited by its presence in terms of the growth parameters, histopathological and haematological alterations in the tissues of the exposed fish. This will go a long way in addressing the paucity of knowledge on how combinations of vitamins can boost the immunity of fish in helping them combat the effects of deleterious toxicants such as cadmium and enhance survival of fish and save humans, the ultimate consumer.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Biology of the African Catfish, *Clarias gariepinus*

The body of an adult African catfish is usually elongate, head large, depressed and bony with small eyes. The head is flattened, highly ossified, the skull bones (above and on the sides) forming a casque. The eyes are narrow and angular occipital process, gill openings wide, air-breathing labyrinth organ arising from gill arches; first gill arch with 24 to 110 gill rakers (Food and Agriculture Organization, FAO, 2015).The outer pectoral ray is in the form of a spine and the pelvic fin normally has six soft trays and the body is covered with a smooth scale-less skin. The skin is generally darkly pigmented on the dorsal and lateral parts of the body. The colour is uniform marbled and changes from grayish olive to blackish according to the substrate. On exposure to light the skin colour generally becomes lighter (De Graaf and Janssen, 2006).

The African catfish has four pairs of un-branched barbels, one nasal, one maxilla (longest and most mobile) on the vomer and two mandibulars (inner and outer) on the jaw. Tooth plates are present on the jaws as well as on the vomer. The major function of the barbels is prey detection. A supra-branchial or accessory respiratory organ, composed of a paired pear-shaped air-chamber containing two arborescent structures is generally present (Awaiss and Kestemont, 2008). These arborescent or cauliflower-like structures located on the secondhand fourth branchial arches are supported by cartilage and covered by highly vascularised tissue which can absorb oxygen from atmospheric air. The air chamber communicates with the pharynx and with the gill chamber. The accessory air breathing organ allows the fish to survive for many hours out of the water or for many weeks in muddy marshes (Moussa, 2000; De Graaf and Janssen, 2006; Awaiss and Kestemont, 2008). Adult *C. gariepinus* inhabit calm waters from lakes,

streams, rivers, swamps to floodplains, some of which are subject to seasonal drying. The most common habitats frequented are floodplain swamps and pools in which the catfish can survive during the dry seasons due to the presence of the accessory air breathing organs (Kamthorn and Jin, 2006).

2.2 Growth Rate (Standard Length and Weight) of African Catfish

Akinwole *et al.* (2014) carried out a study on the growth and survival of African catfish (*Clarias gariepinus*) fingerlings cultured at three stocking densities was evaluated. Fish of mean weight 5.62g were stocked in plastic tanks with three replicates for each treatment of 425 fish/m^3, 850 fish/m^3 and 1275 fish/m^3. The fish were fed with 42 % crude protein floating pellet at 5% body weight twice daily. The best growth performance and highest survival rate was obtained in 425 fish/m 3 with mean weight gain of 11.38±0.25 g while 850 fish/m 3 and 1275 fish/m^3 had 4.38±0.32 g and 3.68±0.84 g respectively. The feed conversion ratio was least in 425 fish/m^3 (1.67±0.87) while 850 fish/m^3 and 1275 fish/m^3 had 2.73±1.52 and 1.98±0.83 respectively. The mean daily weight gain of 0.27±0.01g, 0.10±0.01 g and 0.09±0.02 g were recorded in 425 fish/m^3, 850 fish/m^3 and 1275 fish/m^3. The specific growth rate was also decreasing as the stocking density increases, 425 fish/ m^3, 850 fish/m^3 and 1275 fish/m^3 had 2.26±0.05%, 1.48±0.21 % and 1.33±0.81% respectively. The survival rate was highest in 425 fish/m^3 (83.33±2.33%) while 850 fish/m^3 and 1275 fish/m^3 had 50.00±4.58 % and 44.33±5.12 % survival rate, respectively.

Chukwuemeka *et al.* (2014) carried out a study to elucidate the morphometrics of three fish species namely, *Tilapia galilaea, Tilapia aurea and Auchenoglanis occidentalis* from Tagwai Lake, Minna, using standard procedures. A total of 360 specimens of the fishes were analyzed. The results indicated the following ranges for parameters

investigated: Body Girth (BG) (3.78±0.76 to 5.48±0.84cm), and Total Gut weight (TGW) (2.10±0.84 to 3.12±1.73g); values that did not vary significantly (P>0.05) among the fish species. On the other hand, standard length (SL) (range = 10 .94±1.34 to 20.46±2. 98cm), Gut Length (GL) (range = 25.92± 6.67 to 139.77±30.56cm) and Total Body Weight (TBW) (range = 49.99±18.34 to 175.31± 66.96) varied significantly (P<0.05). Cross correlation amongst certain morphometric variables, i.e., Standard Length and Total Body Weight, Body Girth and Total Body weight, Total Gut Weight and Standard Length were strong, while Standard Length and Gut Content Weight, Gut Length and Standard Length, Gut Content Weight and Standard Length were weakly correlated. These findings no doubt support close evolutionary ties among the species, and should provide baseline information for sustainable exploitation of the fish species.

Davies *et al*. (2013) work on the growth pattern and condition of this fish in concrete tanks as there has been information on those from the wild and indoor recirculation system tanks. Length-Weight Relationship of *C. gariepinus* juveniles reared in concrete tanks in Aleluya Farm, Woji, Port Harcourt, Nigeria was studied. The fish samples were sexed, the lengths and weights measured according to standard methods. Temperature, pH, dissolved oxygen (DO) and ammonia (NH4) were determined following standard methods. The "b" value for the males was 7.74 while that of the females was 6.96 and combined sexes 7.87. The regression equation was Log W=-65.78+7.87 Log L (r = 0.90). Condition factor ranged between 1.06 (males) and 1.15 (females).

Puvaneswai and Karuppasamy (2007) conducted a study on cadmium (Cd) which pollution continues to be a global problem. Early life stages of fish appear to be especially susceptible to this form of pollution. The fish *Heteropneustes fossilis* is a useful indicator species for freshwater pollution. Investigations were carried out on acute (96 hour) and Chronic (21 day) mortality rate, accumulation of toxicant and

growth of fish larvae under Cd exposure. Ten-day old *H. fossilis* larvae were exposed to graded series of concentrations of Cd under static-renewal test conditions. Present results indicated that *H. fossilis* larvae were very sensitive even to low concentration of Cd. The No Observed Effect Concentration (NOEC), Lowest Observed Effect Concentration (LOEC) and LC_{50} values for survival were 500, 750 and 1921 μg L^{-1} for acute (96 h) and 90, 125 and 382 μg L^{-1} for chronic (21 day) exposure, respectively, while the mean NOEC, LOEC and LC_{25} values for growth were 60, 90 and 125 μg L-1 after 21 days of exposure, respectively. *H. fossilis* larvae rapidly bioaccumulated with Cd during prolonged period (21 days) of exposure. Cd accumulation in the larvae reached the level of 62.53 μg L^{-1} after 21 day of exposure, representing a two-fold increase over the use of lowest test concentration of 30 μg L-1 in their exposure media. Also, the Cd accumulation in larvae was time and concentration dependent.

2.3 Acute Toxicity Studies on *Clarias gariepinus* Juvenile

Guedenon *et al*. (2012) studied that assess the acute toxicity of mercury on *Clarias gariepinus*, 108 fish of mean weight 51.27 g ± 2.01 and mean length 20. 2 cm ± 0.72 were divided into six groups of six fish each. The different groups were exposed to the different concentrations of 0 mg/L, 0.3 mg/L, 0.5 mg/L, 0.8 mg/L, 1 mg/L et 1.50 mg/L for a period of 96 hours. The experiment was triplicated. The results revealed that all the fish of groups exposed to 0 mg/L of HgCl2 (control) survived whereas all the fish of groups exposed to 1 mg/L and 1.5 mg/L died. The determination of 96 hours LC50. The median lethal concentration was 0.60 mg/L with lower and upper confidence limits of 0.135 mg/L and 3.519 mg/L respectively at 95 %.

Olaifa *et al.* (2003) carried out a study to determine the Lethal and sub-lethal effects of copper on *Clarias gariepinus* juveniles were studied using a 96-hour static bioassay.

Copper (as copper-chloride, $CuCl_2$. H_2O) was used to prepare the stock solution from which five standard concentrations 0.0, 1.8, 3.2, 5.6, and 10.0 mg/L were prepared (coded A – E). Fifteen (15) juvenile *C. gariepinus* fish having a mean weight and length 5.8g and 18 cm respectively were used. The 96-hour LC_{50} estimated using the logarithm methods were 0.6, 0.71 and 0.7 mg/l for replicates 1, 2 and 3 respectively with mean as 0.67 mg/L. Haematological changes were generally not significant ($P>0.05$). Copper concentrations in bone and muscle-tissues were also determined. The mean copper concentration in bone ranged from 1.86 (treatment A) to 17.04 ppm (Treatment E) and muscle 1.29 (treatment A) to 55.5 ppm (treatment E). There were significant differences ($p<0.05$) in mortality among treatments.

Akinrotimi and Amachree, (2017) carried out a study on Seventy-Two (72) male and female African catfish *(Clarias gariepinus)* juveniles of mean length (10.74 ± 1.81 g) were exposed to different concentrations of atrazine and metolachlor (0.00 mg/L-control, 0.01, 0.02, 0.03, 0.04 and 0.05 mg/L) for 14 days to determine its effect on haematological parameters of the fish. The results obtained indicated significant ($P<0.05$) reductions with increased concentrations of the chemical in haemoglobin (Hb), Red blood Cell (RBC), packed cell volume (PCV), lymphocytes, platelets and mean corpuscular volume (MCV). The white blood cell (WBC), neutrophils, monocytes, mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentrations (MCHC) in fish exposed to the pesticides were significantly ($P<0.05$) higher than that of the control. The data obtained from this work will contribute to the base line haematological parameters for use in monitoring health status of *Clarias gariepinus* in the wild and culture medium.

Omoregie (2017) undertook an investigation on the Haematology of *Clarias gariepinus* exposed to Microcystin-LR, the bioassay experiment was conducted by exposing

Clarias gariepinus to 200 µg/L and 400 µ/L of MC-LR solutions for 14 days and 28 days to assess the haematological impacts such as estimation of red blood cells, white blood cell and Thrombocytes, haemoglobin percentage, haematocrit, and percentage changes. A dramatic reduction was observed in the RBCs in both experimental cases after 28 days; which can be attributed to haemolysis. Result showed significant impact of MC-LR on RBCs counts on duration (28 days) basis; regardless of the concentrations. The PCV after 28 days was significantly lower than that observed after 14 days, which was also significantly lower than that observed in the control set-up. The concentrations; particularly 400 µg/L MC-LR exerted significant stress on the fish. A general temporal increase was recorded in the total count of WBCs in both experimental cases. Results showed that MC-LR; particularly 400 µg/L caused significant haematological disruptions in the C. gariepinus, especially after 28 days of exposure i.e. 400 µg/L (28 days) > 400 µg/L (14 days) > 200 µg/L (28 days) > 200 µg/L (14 days). Due to the fact that natural aquatic ecosystems affected with algal bloom contain concentrations of MC-LR higher than the experimented levels, aquatic biota in affected water bodies are liable to suffer worse consequences than those observed in this experiment.

2.4 Histopatological Studies on *Clarias gariepinus*

Olojo *et al.* (2005) carried out a study on the effect of the heavy metal; lead (Pb) on the gill and liver of the African catfish (*Clarias gariepinus*) was carried out in the laboratory. One hundred and sixty (160) fingerlings of the fish were exposed to continuous exposure to sub-lethal concentrations (0.006 mg/L and 0.008 mg/L) of lead for a period of three weeks. The liver and gill of fish were removed every 9 days for histological examination. The results showed that the degree of distortion of the gills

and liver was proportional to the exposure periods and concentration of the metals was found to be dose and time dependent

Bagheri and Nezani (2012) undertook an investigation to evaluate the biochemical and histopathological effects of glyphosate on the liver of freshwater fish, *Cyprinus carpio* (Linn.) after calculating the 96 h LC_{50} of glyphosate (Roundup®41 % SL) which was 3.260 ppm. The fish fingerlings having mean wt. 3 g ±0.5 and mean length 5.5cm ±0.35 were exposed to two sub-lethal concentrations of glyphosate i.e. 25 % of LC_{50} (T_1) and 50 % of LC_{50} (T_2) for a period of 28 days. Total soluble proteins, lipids and enzymatic activities of aspartate amino transferase (AST) and alanine amino transferase (ALT) were recorded at weekly intervals and significant ($p>0.05$) decrease in protein and lipid content of the liver was continually observed till the termination of the experiment. However, the enzymatic activities of AST and ALT in liver showed a significant ($p<0.05$) increase with increasing concentrations of glyphosate and duration of exposure. The histomorphology of liver in fish exposed to glyphosate exhibited vacuolation of hepatocytes, pyknotic nuclei, degeneration of cytoplasm, and infiltration of leukocytes, necrosis and severe vasodilation in the treatments. The severity of biochemical and histological alterations was more pronounced in T2 after 28 days of exposure. The increase in activities of AST and ALT and the decrease in protein and lipid content of the liver following exposure of fish to the herbicide suggest enhanced protein catabolism, hepatocellular damage and increased utilization of energy stores to compensate for higher energy demands during stress. This indicates that the above said herbicide causes potential harm to the aquatic life.

Olurin *et al.* (2006) examined African catfish, *Clarias gariepinus* fingerlings exposed to sub lethal concentrations of herbicide, glyphosate (0, 0.05, 0.1%, v/v) over 42 days period. The gills showed marked alterations in the epithelia in response to glyphosate

treatment. There was fusion in adjacent secondary lamellae resulting in hyperplasia, with profound oedematous changes, characterized by epithelial detachment. In the liver, the enlargement of the hepatocytes was related to the concentration and duration of exposure to glyphosate. There were also large vacuoles in the hepatocytes, with pyknotic nuclei, and cytolysis that increased with concentration. Focal necrosis was also observed in the hepatocytes. It was concluded that glyphosate has a deleterious effect on the organs of *C. gariepinus.*

Ezemmuye and Ogbonioda (2010) carried out a study on *Clarias gariepinus* fingerlings exposed to lethal and sublethal concentrations of Gammalin 20 were investigated in a renewal static bioassay with particular reference to behaviour, survival, and histopathological changes. Early symptoms of gammalin 20 lethal poisoning were, respiratory distress, increased physical activity, convulsions, erratic swimming, loss of equilibrium, and increased breathing activity. Behavioural response was dose dependent and decreased with decreased concentration. The 96-hour lethal concentration (LC_{50}) value was 30 ppb. Histopathological changes of the gill, liver, and intestinal tissues of fish treated with sub-lethal concentration of gammalin 20 for twelve weeks showed gill distortion and fusion of adjacent secondary lamella as a result of hyperplasia and excessive mucus accumulation. The liver showed swelling of hepatocytes with mild necrosis, pyknosis, and vacuolation, while the intestine showed yellow bodies of the laminapropria at the tip of the mucosal fold.

2.5 Cadmium and its Effects on Organisms

Cadmium is a silver-white, blue-tinged and lustrous element with melting point of 321 ^{0}C and boiling point of 765 ^{0}C. It has an atomic weight of 112.40 g and atomic number of 48. Cadmium is one of the major heavy metals that are regarded as most toxic to

living systems. It is considered non-essential trace element to all living organisms. In most big cities, rivers and lake shores are perceived as areas mostly polluted with cadmium as a result of poor discharge of industrial wastes (Osisiogu and Aladesanmi, 2019). Cadmium itself is insoluble in water, however, its salts in forms of chlorides and sulphates are soluble in water (Omotoso *et al.*, 2019). The bioavailability of cadmium to living systems from immediate physical and chemical environments is dependent on a number of factors, such factors as adsorption and desorption from terrigenous items, pH, redox potential, chemical speciation, among others. Last decades have experienced increase in the rate of cadmium contamination in aquatic environments leading to bioaccumulation of cadmium in numerous tissues of the aquatic lives in food chain systems (Ravindran and Radhakrishnan, 2020). It is noteworthy to know that cadmium is a very toxic heavy metal for mammals and fish. As a result of increase in cadmium contamination and its toxicity, researches on cadmium toxicity have gained global attention. Rapid accumulation coupled with slow excretion of cadmium in living tissues has rendered cadmium accumulation as one of the major ecological problems. In fish, cadmium can elicit a number of structural and morphological deformities in numerous organs. The highest amount of cadmium in fish was reported in kidneys and liver (Mustafa, 2020). Cadmium has been proven to be implicated in hypertension, emphysema, damage of kidney tubules, impairment of liver functions, and cancers (Samanyika, 2017). Kidney tubules and gills' lamellae are reported to be the major sites for cadmium accumulation in acute exposure to cadmium whereas kidneys and liver are the primary sites for cadmium accumulation in the case of sub-chronic exposure to cadmium (Mustafa, 2020).

Toxic pollutants may exert their effects in several ways, depending upon the characteristics of the poison, of the receiving water, and of the biological community

the water sustains. In extreme cases, animals may be killed by the toxic heavy metal (cadmium poison). Lower concentrations of cadmium poison may exert sub-lethal toxic effects (Duffus, 2002). Some poisons appear to accumulate in the tissues of organisms during their lifetime and exert toxic effects after prolonged exposure to concentrations which are barely measurable by chemical means. It is widely suspected that some of these may pass from prey to predator organisms and achieve high concentrations in species at the top of a food web. Many poisons are known to be mutagenic, teratogenic or carcinogenic. From a biological point of view, any toxic effect is significant if it influences, or is likely to influence, the physiology or behaviour of the organism in such a way as to alter its capacity for growth, reproduction or mortality, or its pattern of dispersal, since these are the major determinants of the distribution and abundance of species (Duffus, 2002).

2.6 Effects of Cadmium on Organs of Fish

Cadmium in high doses induce structural and function alterations in various vital organs including liver, kidney, gill and intestine of fishes. Cadmium accumulates in liver of fishes in high concentrations (DeSmet and Blust, 2001). It also induces various pathological changes in liver tissues including engorgement of blood vessels, congestion, vacuolar degeneration of hepatocytes, necrosis of pancreatic cells and fatty changes in the peripancreatic hepatocytes (Rani and Ramamurthi, 1989; Dangre *et al*., 2010). Cadmium has been reported to possess nephrotoxic action in man and various animals. In fact, kidney is the principal target organ of cadmium toxicity and chronic cadmium exposure in almost all animal species is characterized by varying degree of renal damage (Romeo *et al*., 2000; Shukla and Gautam, 2004; Kumar *et al.*, 2021). Gills are also reported to act as storehouse of cadmium in experimental studies (Allen, 1995; Tao *et al*., 2000; Fafioye *et al*., 2004). Wong and Wong (2000) studied morphological

and biochemical changes in the gills of Tilapia (*Oreochromis mossambicus*) after experimental cadmium exposure. In scanning electron microscopic studies, they found an augmentation of microbridges in pavement cells and an increase in the apical membrane of chloride cells. They further reported chloride cells as a prime target of cadmium toxicity, resulting into fish hypocalcemia. Other organs like intestine and gonads of fishes also appear susceptible for ill effects of cadmium toxicity (Taylor, 1983; Kumari, and Ramkumar, 1997; Singh *et al*., 2007; Kumar *et al.*, 2021). Long exposure of cadmium produces a wide variety of acute and chronic effects in aquatic animals. Its prime site is kidney. According to the current knowledge kidney damage (renal tubular damage) is probably the critical health effect (Arain *et al*., 2015). It also creates disturbances of calcium metabolism, hypercalciuria and takes part in the formation of stones in the kidney. Several environmental variables as factors in controlling the response of fish to toxic materials as well as on the oxygen consumption of the fish, for instance, sub-lethal effects in fish, notably malformation of the spine, have been reported (Arain *et al*., 2015).

2.7 Sources of Cadmium Exposure and Bioaccumulation in Tissues

Cadmium is believed to be released to biosphere from natural and anthropogenic sources. It is an element that occurs naturally on the Earth's crust and ranked 7 of Agency for Toxic Substances and Disease Registry (ATSDR)'s "Top 20 list" (Molina-Villalba *et al*., 2015). Due to the presence of cadmium in insecticides, herbicides, sludge, fungicides, and commercially available fertilizers which are regularly used in agriculture, the percentage of cadmium in the upper soil layer has been increasing. Other sources of cadmium pollution include dental alloys, motor oil, electroplating, and car exhaust. As a result, anthropogenic activities have increased cadmium levels far higher than natural causes; with anthropogenic and natural activities contributing to

environmental cadmium pollution by 90 % and 10 %, respectively (Al-Anazi *et al*., 2015).

Furthermore, some insects can also accumulate high levels of cadmium without showing any adverse effects (Hossain *et al*., 2017). Among the tissues that accumulate cadmium, kidney is considered prime target tissue for cadmium, although liver also accumulate considerable amount of this toxic metal. After cadmium uptake through gills and intestine and some other organs, it is redistributed to these organs (Priya *et al*., 2018).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Sample Collection and Acclimatization

A total of one thousand (1000) samples of fingerlings of *C. gariepinus* was purchased from a fish farm and transported in 50 L container filled with water to the Old Research Farm Unit of Department of Water, Aquaculture and Fisheries Technology of the Federal University of Technology, Minna. The fish were distributed in 4 different plastic tanks (Aquaria) and acclimatized for a period of two weeks (14 days) in which they were fed with commercially sold feed (2 mm) morning and evening every day of the acclimatization period (Okoye *et al.*, 2018), and water was changed at least every 48 hours. Feeding was terminated 24 hour before the commencement of the exposure.

3.2 Experimental Set-Up

The vitamins A, C and E pellets (500 g each) and Cadmium-chloride (Cadmium chloride, 100 g analar grade) were purchased from commercial chemical store and stored in a cool dry place. The sub-lethal concentrations used were 4 mg/L (T1), 8 mg/L (T2), 12 mg/L (T3), 16 mg/L (T4) and 00 (control) with replicate in each case. The various treatment combination groups were CE (Cd+ Vitamin C + Vitamin E), AC (Cd+ Vitamin C + Vitamin A), AE (Cd+ Vitamin A + Vitamin E) and AEC (Cd+ Vitamin A + Vitamin E + Vitamin C). The combinations of the vitamins in each treatment group were in equal proportions. The experimental set-up ran for a period of 8 weeks (56 days). Two Samples were taken randomly from each trough on a bi-weekly basis for the morphometric parameters. The vitamin supplements concentrations were taken as twice the lowest concentration of the toxicant (8 mg/L) and administered uniformly in every treatment and its replicate. Fresh toxicant and the various vitamin combinations were

applied each time the water medium was changed at interval of every 72 hour according to Organization for Economic cooperation and Development (OECD, 2007).

3.3 Determination of Growth Parameters of *Clarias gariepinus*

The growth parameters considered during the study includes standard length (cm), total length (cm), weight (g), specific growth rate (SGR), body weight increase (BWI). They were all measured as follow:

3.3.1 Standard length

The standard length was taken from 2 randomly selected samples from each treatment and replicate and measured from the tip of the snout to the caudal lobe with a metre rule graduated in centimeters (Okoye *et al.,* 2018.

3.3.2 Total length

The total length was taken from 2 randomly selected samples from each treatment and replicate and measured from the tip of the snout to the tail end of the fish with a metre rule graduated in centimeters (Okoye *et al.,* 2018.

3.3.3 Standard weight

The weight of the fish was also determined from 2 randomly selected samples from each treatment and replicate and weighed with Camry Weighing Scale (NT241) in gram (Okoye *et al.,* 2018).

3.3.4 Weight gain

This is the difference between weight at start and at the end of the fish in a week during exposure (Okoye *et al.,* 2018).

3.3.5 Percentage weight gain

The Percentage weight gain was calculated following the methods of Okoye *et al.* (2018) as difference between final and initial weights, divided by initial weight and multiplied by 100.

3.3.6 Specific Growth Rate (SGR)

Specific growth rate (SGR), describes the daily rate of growth in the fish. It is an indices of growth in fishes (Okoye *et al.*, 2018).

3.4 Determination of Haematological Parameters of *Clarias gariepinus*

Blood sample were collected from 3 randomly selected samples every 4th week of the 8 weeks exposure periods from each treatment and replicate from each treatment group with 1ml heparinized syringe. The blood collected was stored in small ethylene diamine tetra-acetic acid (EDTA) treated vials with little quantity of the EDTA in the test tube (Ayoola, 2011). The whole blood samples were used for the estimation of haematological parameters Pack Cell Volume (PCV), Haemoglobin (Hb), Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH), Red Blood Cell (Red Blood Cell), White Blood Cell (WBC), Blood Platelets (PLT), Monocytes, Basophils, Eosinophils and Lymphocytes. The blood samples were anaylsed in the Laboratory Services Unit of the General Hospital, Minna, Niger State using Mindray Haematology Analyser (BC-5300) for full blood count.

3.5 Determination of Tissues Histopathology of *Clarias gariepinus*

The histopathology of the gills, kidneys and liver of *C. gariepinus* from each treatment and replicate were carried-out in comparison with the control samples. These organs (after excision) were preserved in 10 % neutral buffered formalin until required for

analysis. The histopathological analyses were carried-out in the Histopathology Unit of the University of Ilorin Teaching Hospital, Kwara State, Nigeria. Gills, kidneys and liver of the fish were fixed in Bouin's fluid for 24 hours; it was then dehydrated in graded ethanol concentrations and embedded in paraffin wax. Sagittal sections of 3-5µm thickness were cut and mounted on glass slides. The sections were de-paraffinized in xylene, hydrated in ethanol and stained with hematoxylin-eosin (HE). The possible changes that took place as observed in the gills, liver and kidneys were indicated from the photomicrograph prepared and observed under light microscope at × 400 magnification.

3.6 Data Analyses

The haematological parameters obtained from the combined vitamins in the various treatment groups were analysed using One Way Analysis of Variance followed by Duncan Multiple Range Test (DMRT) to separate the means were significant at $p<0.05$ level of significance using SPSS Statistical Package (version 20.0 for Windows). Length-Weight Relationship was determined using regression analysis and correlation coefficient of the length and weight measurements of the samples in all the treatments.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSIONS

4.1 Results

4.1.1 Total length (cm) of *C. gariepinus* exposed to sub-lethal concentrations of cadmium chloride and combined supplements of vitamins A, C and E.

From the results of the analysis, the total length of *C. gariepinus* exposed to sub-lethal concentrations of cadmium and supplemented with combined vitamins C and E (CE) showed no significance differences in all treatments in the first two weeks of exposure. At the 4^{th} and 6^{th} weeks of exposure, T1 mean values were significantly higher than other treatments including the control. However, there were no significance differences in all the treatments at week 8 of the exposure (Figure 4.1).

In the combined vitamins A and C (AC) treatment group, the total lengths mean values in T1-T3 are significantly higher than the T4 mean values at the 6^{th} week f exposure. There are however, no significance differences in the mean values of the 2^{nd}, 4^{th} and 8^{th} weeks of exposure in all treatments and control (Figure 4.2). The total length mean values of the samples of the combined vitamins A and E (A, E) treatment group showed no significance difference ($p>0.05$) in all treatments throughout the exposure periods excepts at the 6^{th} week (Figure 4.3). In the combined vitamins A, C and E treatment group, there were no significance differences in all the treatments throughout the exposure period. However, the T4 mean values were significantly lower than other treatments (Figure 4.4).

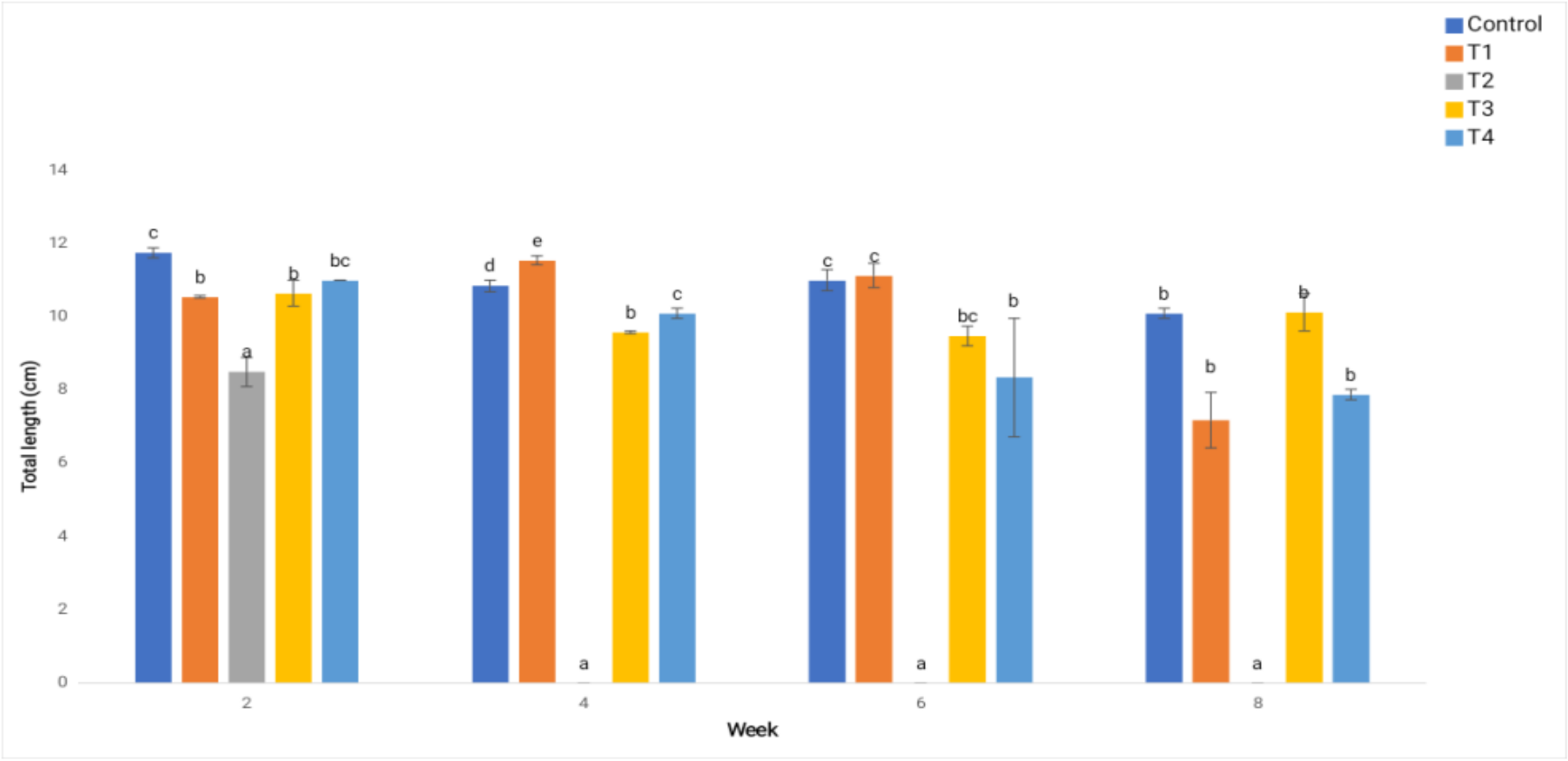

Figure 4.1: Total length of *C. gariepinus* exposed to Sub-lethal Concentrations of Cadmium chloride and Combined Vitamins C and E for a period of 8 weeks Each bar represents mean ± standard error of mean (SEM) of three replicates for each treatment. Bars with different superscripts in the same week are significantly different at $p < 0.05$. [a] denotes least value while [b] denotes higher value. T1= 4 mg/L, T2 = 8 mg/L, T3 = 12 mg/L, T4 = 16 mg/L

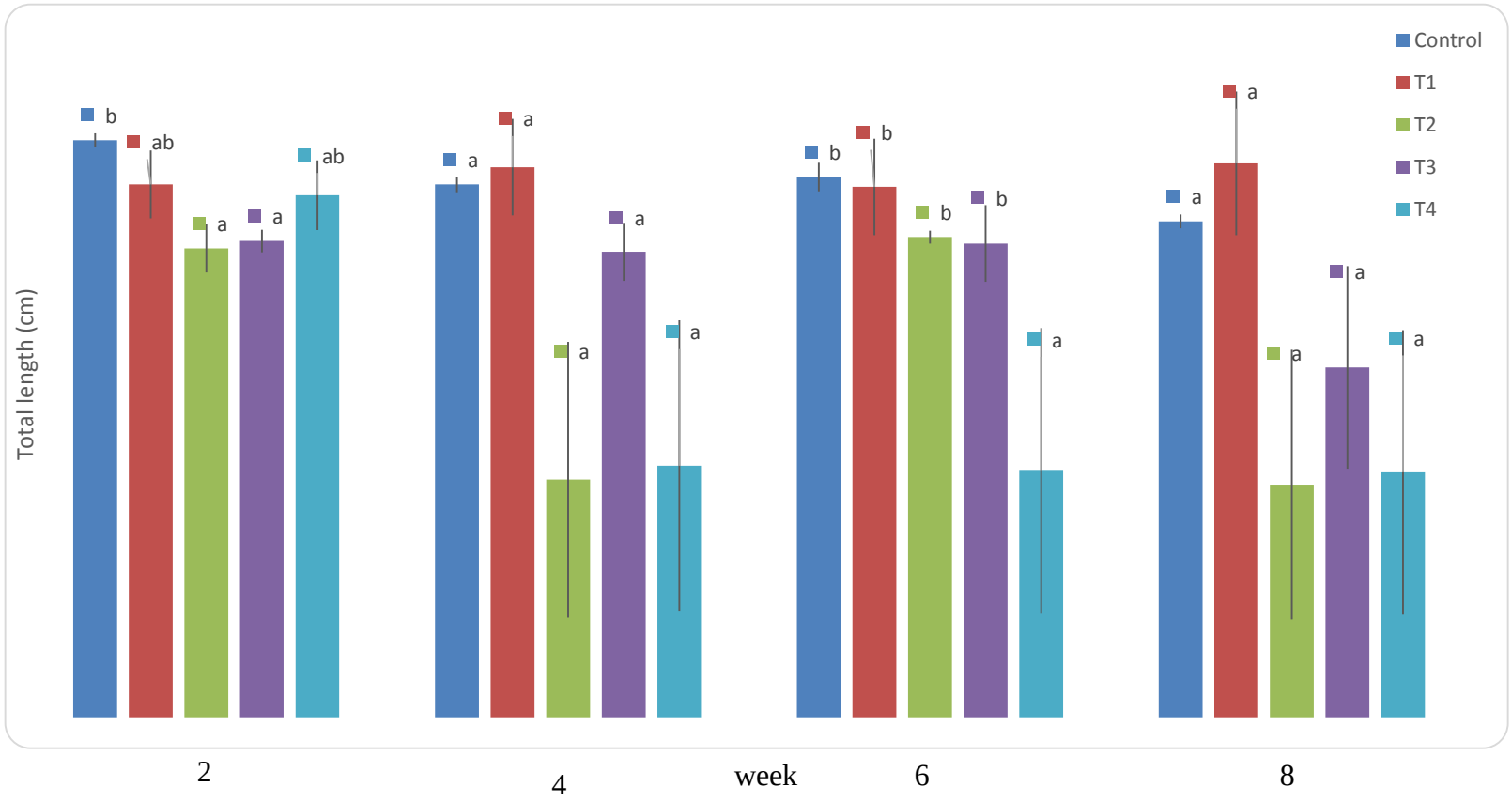

Figure 4.2: Total length of *C. gariepinus* exposed to Sub-lethal Concentrations of Cadmium chloride and Combined Vitamins A and C for a Period of 8 weeks. Each bar represents mean ± standard error of mean (SEM) of three replicates for each treatment. Bars with different superscripts in the same week are significantly different at $p < 0.05$. [a] denotes least value while [b] denotes higher value. T1= 4 mg/L, T2 = 8 mg/L, T3 = 12 mg/L, T4 = 16 mg/L

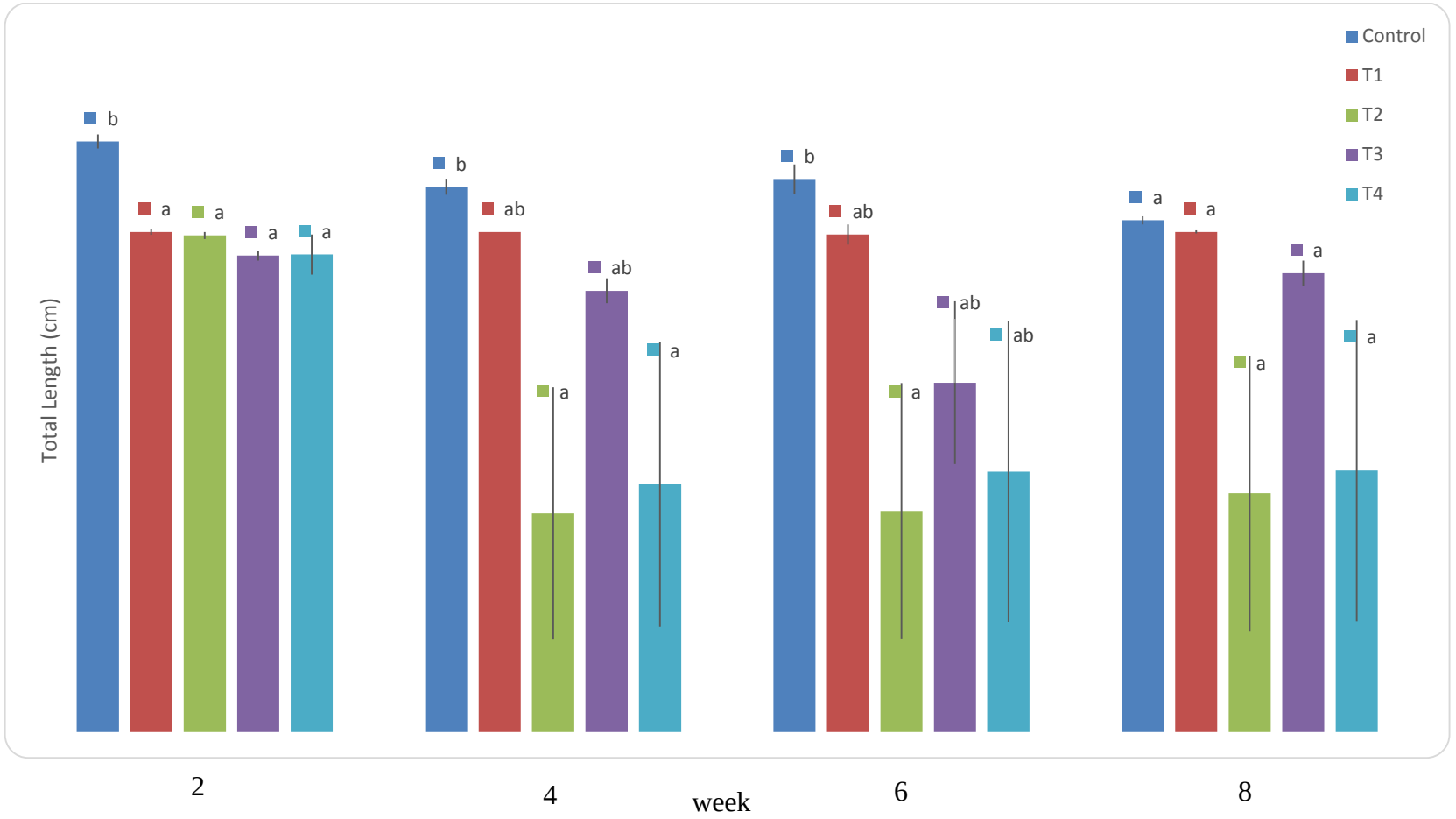

Figure 4.3: Total length of *C. gariepinus* Exposed to Sub-lethal Concentrations of Cadmium chloride and Combined Vitamins A and E for a Period of 8 weeks. Each bar represents mean ± standard error of mean (SEM) of three replicates for each treatment. Bars with different superscripts in the same week are significantly different at $p < 0.05$. [a] denotes least value while [b] denotes higher value. T1= 4 mg/L, T2 = 8 mg/L, T3 = 12 mg/L, T4 = 16 mg/L

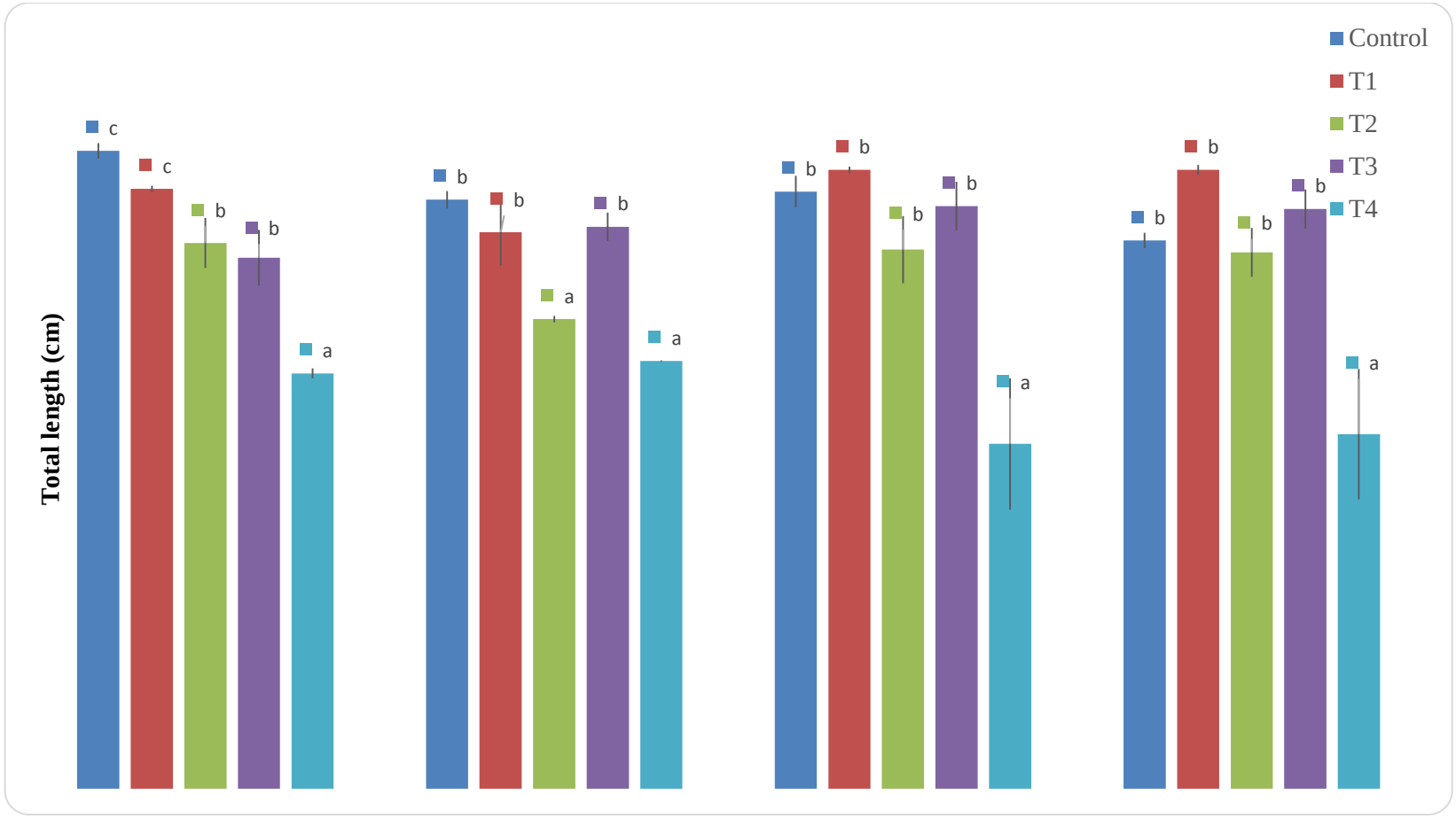

Figure 4.4: Total length of *C. gariepinus* Exposed to Sub-lethal Concentrations of Cadmium chloride and Combined Vitamins A E and C for a Period of 8 weeks. Each bar represents mean ± standard error of mean (SEM) of three replicates for each treatment. Bars with different superscripts in the same week are significantly different at $p < 0.05$. [a] denotes least value while [b] denotes higher value. T1= 4 mg/L, T2 = 8 mg/L, T3 = 12 mg/L, T4 = 16 mg/L.

4.1.2 Standard length (cm) of *C. gariepinus* exposed to sub-lethal concentrations of cadmium chloride and combined supplements of vitamins A, C and E

In the samples exposed to sub-lethal concentrations of Cadmium chloride and supplemented with combined vitamins C and E, there were no significance differences in all treatments for the 8 weeks exposure period except in T1 mean values at the 4^{th} week of exposure which were significantly different from the other treatments (Figure 4.5).

For the combined vitamins A and C supplements there were no significance differences in the mean values at the 2^{nd} week of exposure. However, from the 4^{th} -8^{th} weeks of exposure only the T1 mean values are significantly higher than other treatments (Figure 4.6). The result of the analysis for vitamins A and E combination implied that the T2 mean values at 4^{th} and 6^{th} weeks of exposure were significantly lower than other treatments (Figure 4.7).

The sub-lethal exposure of the samples of *C. garienpinus* to Cadmium chloride with combination of vitamins A, E and C supplements for the period of 8^{th} weeks indicated that no significance differences in all the treatment except the T4 mean values from the 2^{nd} -8^{th} weeks of exposure that were only significantly lower than other treatments (Figure 4.8).

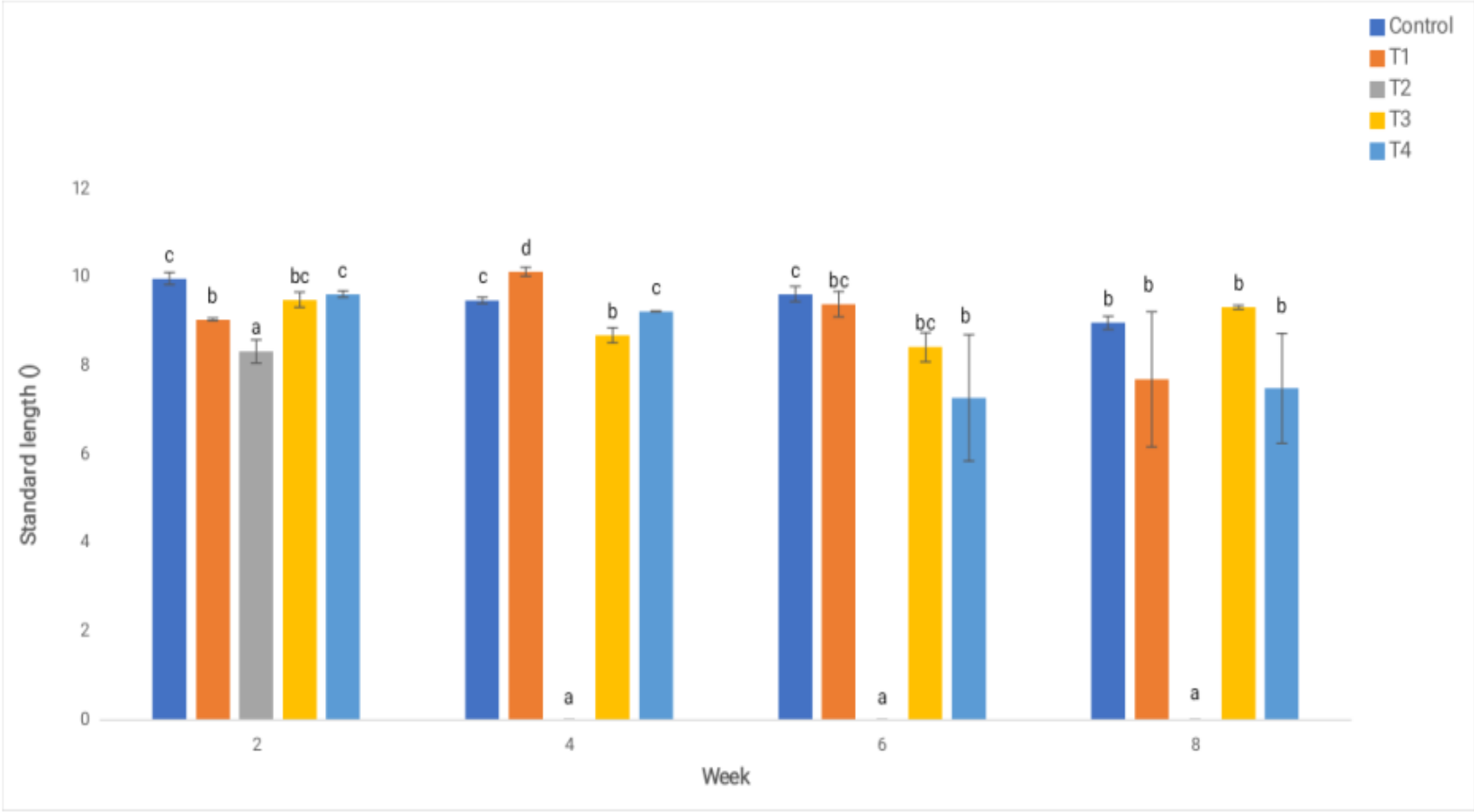

Figure 4.5: Standard length of *C. gariepinus* exposed to Sub-lethal Concentrations of Cadmium chloride and Combined Vitamins C and E for a Period of 8 weeks. Each bar represents mean ± standard error of mean (SEM) of three replicates for each treatment. Bars with different superscripts in the same week are significantly different at $p < 0.05$. [a] denotes least value while [d] denotes highest value. T1= 4 mg/L, T2 = 8 mg/L, T3 = 12 mg/L, T4 = 16 mg/L

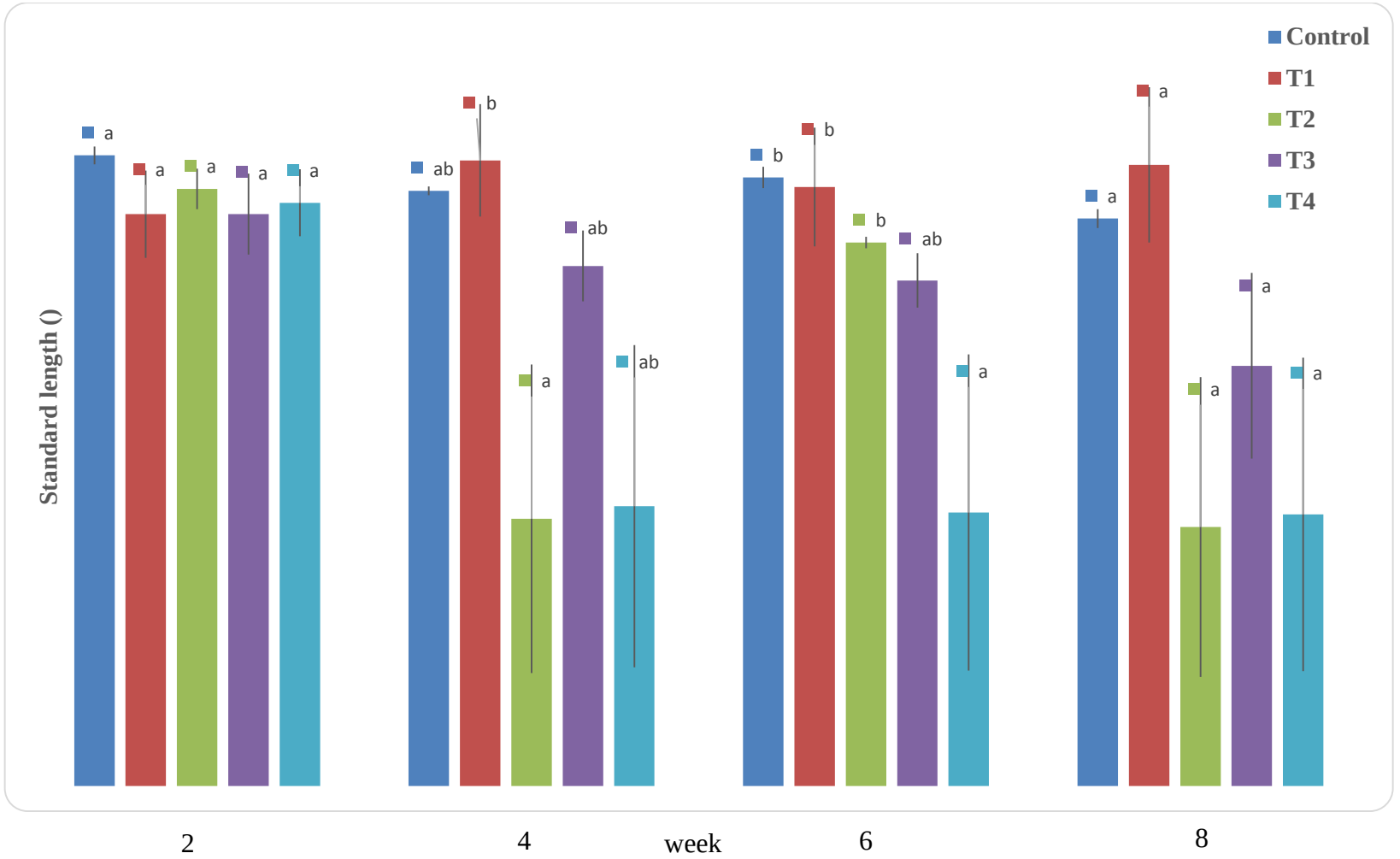

Figure 4.6: Standard length of *C. gariepinus* exposed to Sub-lethal Concentrations of Cadmium chloride and Combined Vitamins A and C for a Period of 8 weeks. Each bar represents mean ± standard error of mean (SEM) of three replicates for each treatment. Bars with different superscripts in the same week are significantly different at $p < 0.05$. [a] denotes least value while [b] denotes highest value. T1= 4 mg/L, T2 = 8 mg/L, T3 = 12 mg/L, T4 = 16 mg/L

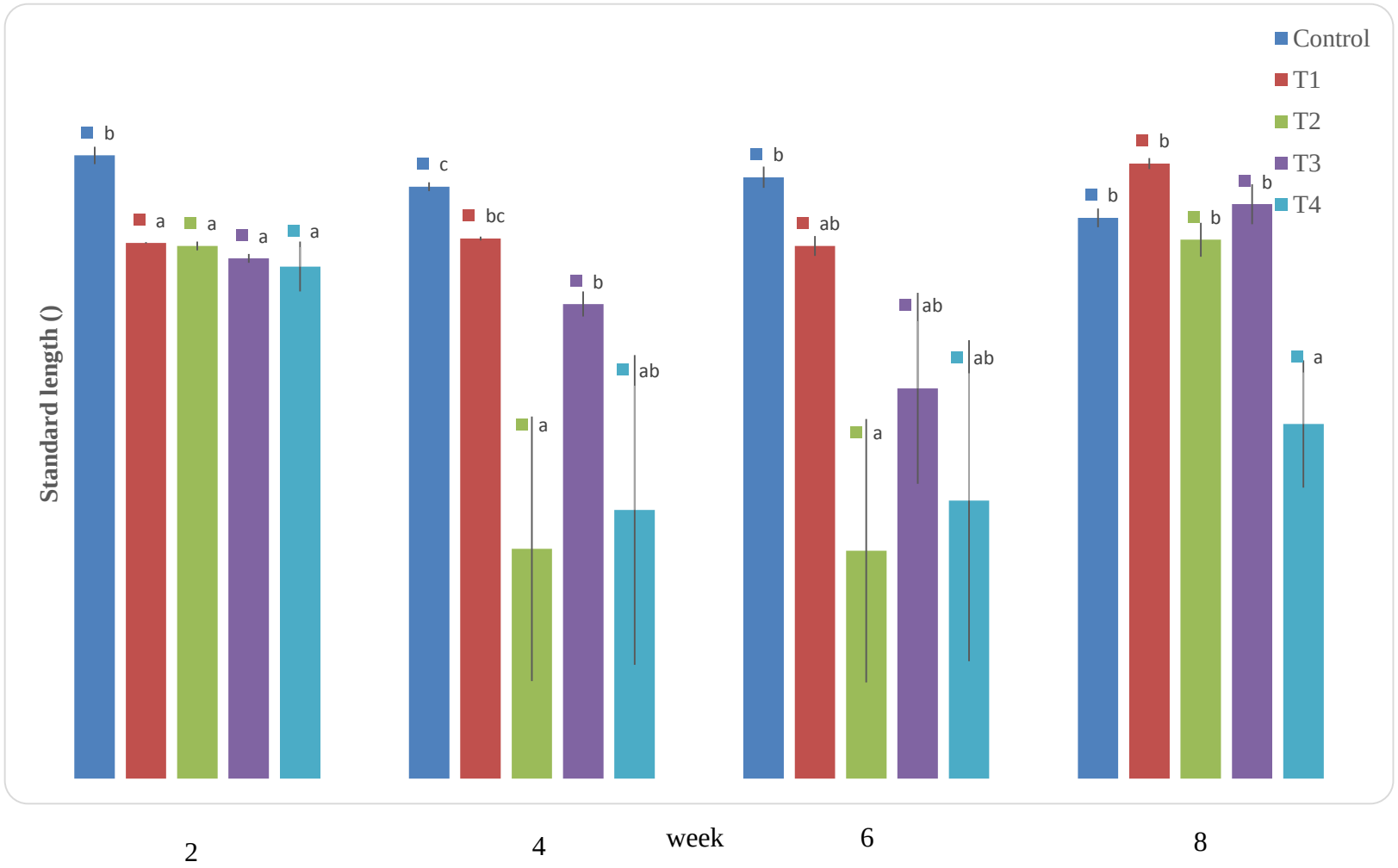

Figure 4.7: Standard length of *C. gariepinus* exposed to Sub-lethal Concentrations of Cadmium chloride and Combined Vitamins A and E for a Period of 8 weeks. Each bar represents mean ± standard error of mean (SEM) of three replicates for each treatment. Bars with different superscripts in the same week are significantly different at $p < 0.05$. [a] denotes least value while [b] denotes highest value. T1= 4 mg/L, T2 = 8 mg/L, T3 = 12 mg/L, T4 = 16 mg/L

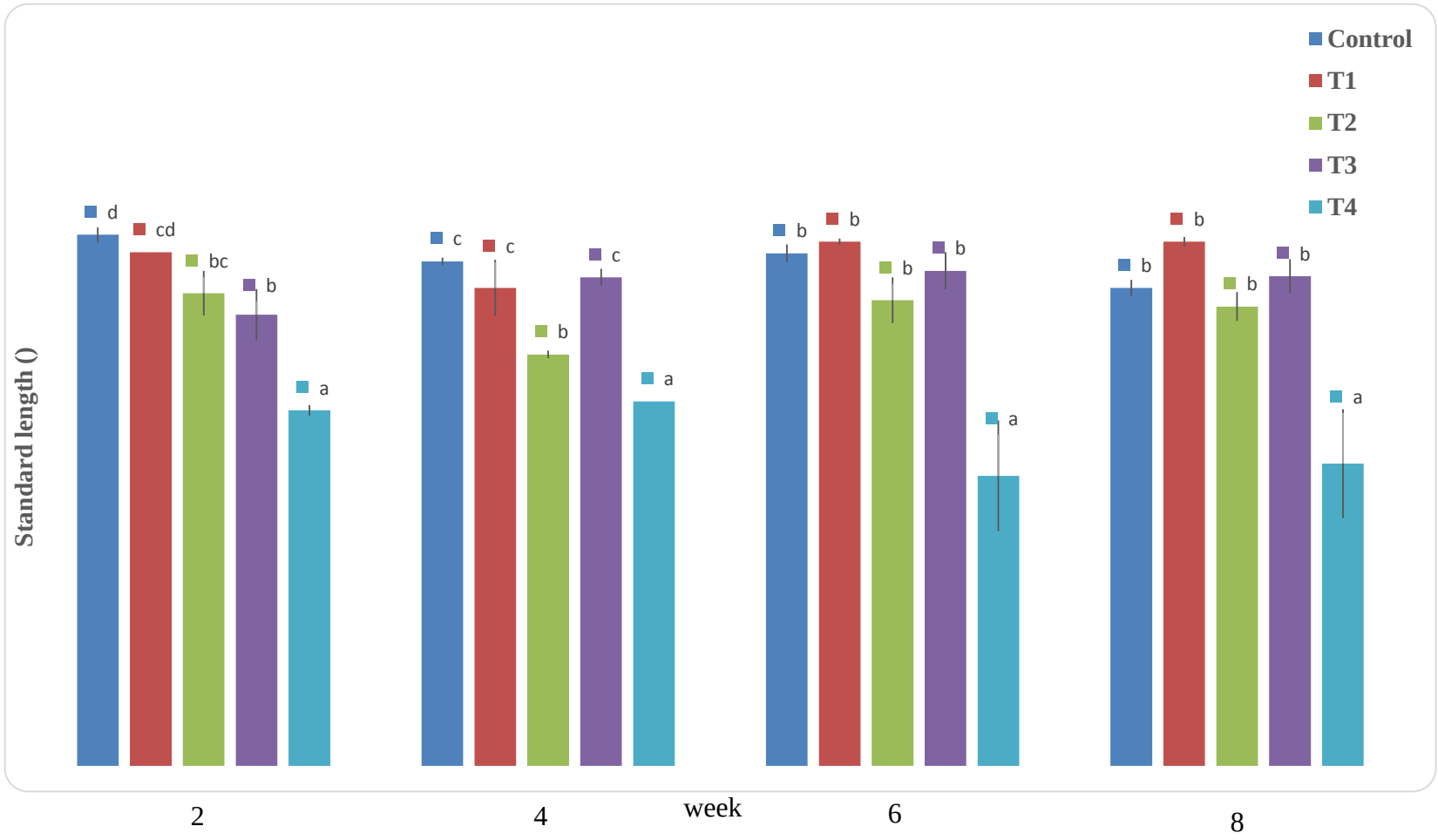

Figure 4.8: Standard length of *C. gariepinus* Exposed to Sub-lethal Concentrations of Cadmium chloride and Combined Vitamins A, E and C for a Period of 8 weeks. Each bar represents mean ± standard error of mean (SEM) of three replicates for each treatment. Bars with different superscripts in the same week are significantly different at $p < 0.05$. [a] denotes least value while [c] denotes highest value. T1= 4 mg/L, T2 = 8 mg/L, T3 = 12 mg/L, T4 = 16 mg/L

4.1.3 Weight (g) of *C. gariepinus* exposed to sub-lethal concentrations of cadmium chloride and combined supplements of vitamins A, C and E.

The control mean values of the weight of the samples of CE treatment group were significantly higher than all the other treatments during the 8 weeks exposure except in week 4. T2 mean values in week 2 were significantly lower than other treatments. On the other hand, the T1 mean values in week 4 were significantly different from other treatments (Figure 4.9).

The results of the samples exposed to combined vitamins A and C group showed that the T1 in the 2^{nd} week is significantly higher than other treatments at the 4^{th} and 8^{th} week. On the other hand the T2 mean values in the 2^{nd} week are significantly higher than that of the 4^{th}-8^{th} weeks (Figure 4.10).

The weight of the samples exposed to vitamins A and E combinations implies that no significance difference in all the treatments except at the 6^{th} week where the control mean values were significantly higher than other (Figure 4.11). In the combined vitamins A, C and E treatment group, there is no significance difference in all the treatments except T2 mean value at the 4^{th} week which was significantly lower than other treatments (Figure 4.12).

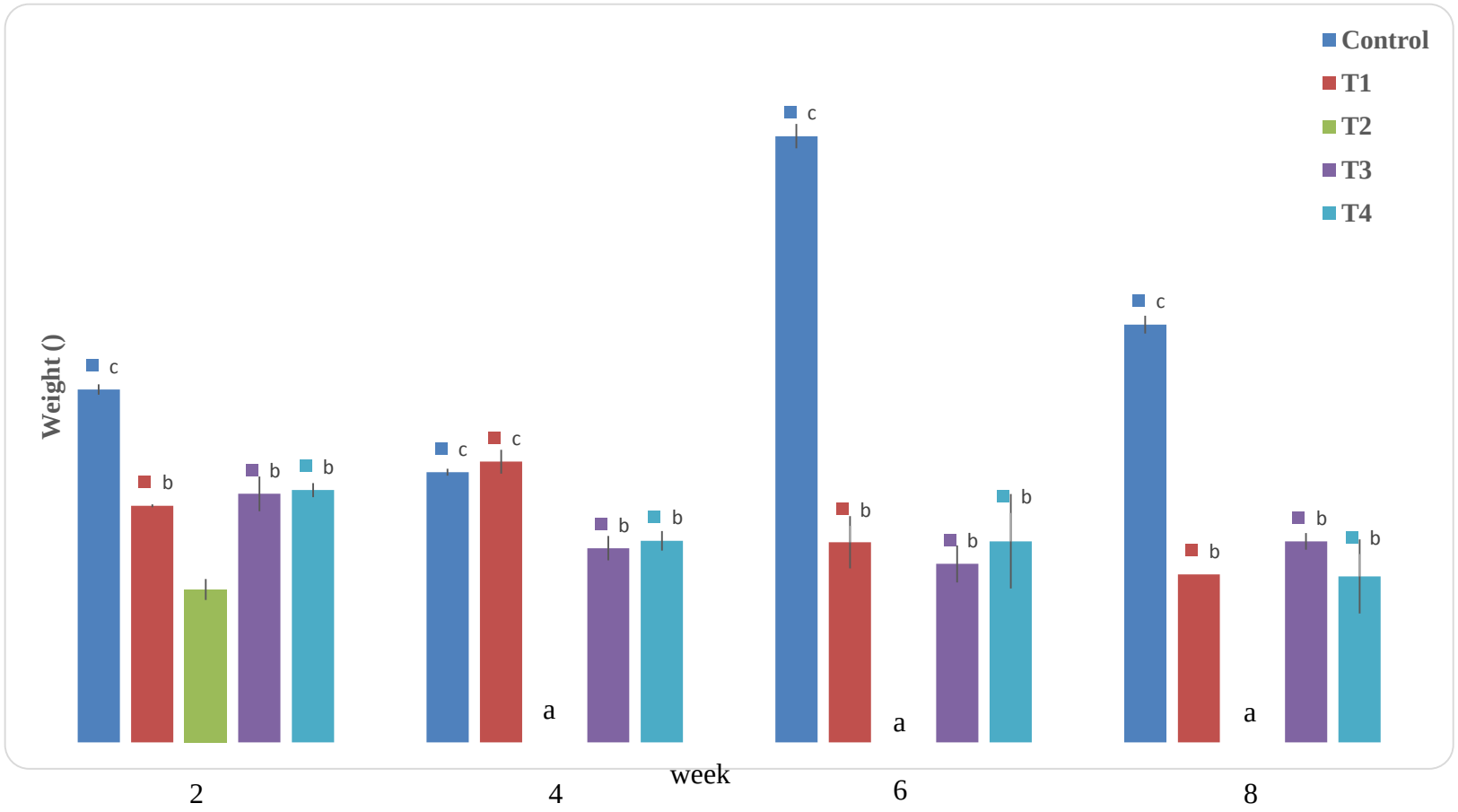

Figure 4.9: Weight of *C. gariepinus* Exposed to Sub-lethal Concentrations of Cadmium chloride and Combined Vitamins C and E for a Period of 8 weeks. Each bar represents mean ± standard error of mean (SEM) of three replicates for each treatment. Bars with different superscripts in the same week are significantly different at $p < 0.05$. [a] denotes least value while [c] denotes highest value. T1= 4 mg/L, T2 = 8 mg/L, T3 = 12 mg/L, T4 = 16 mg/L

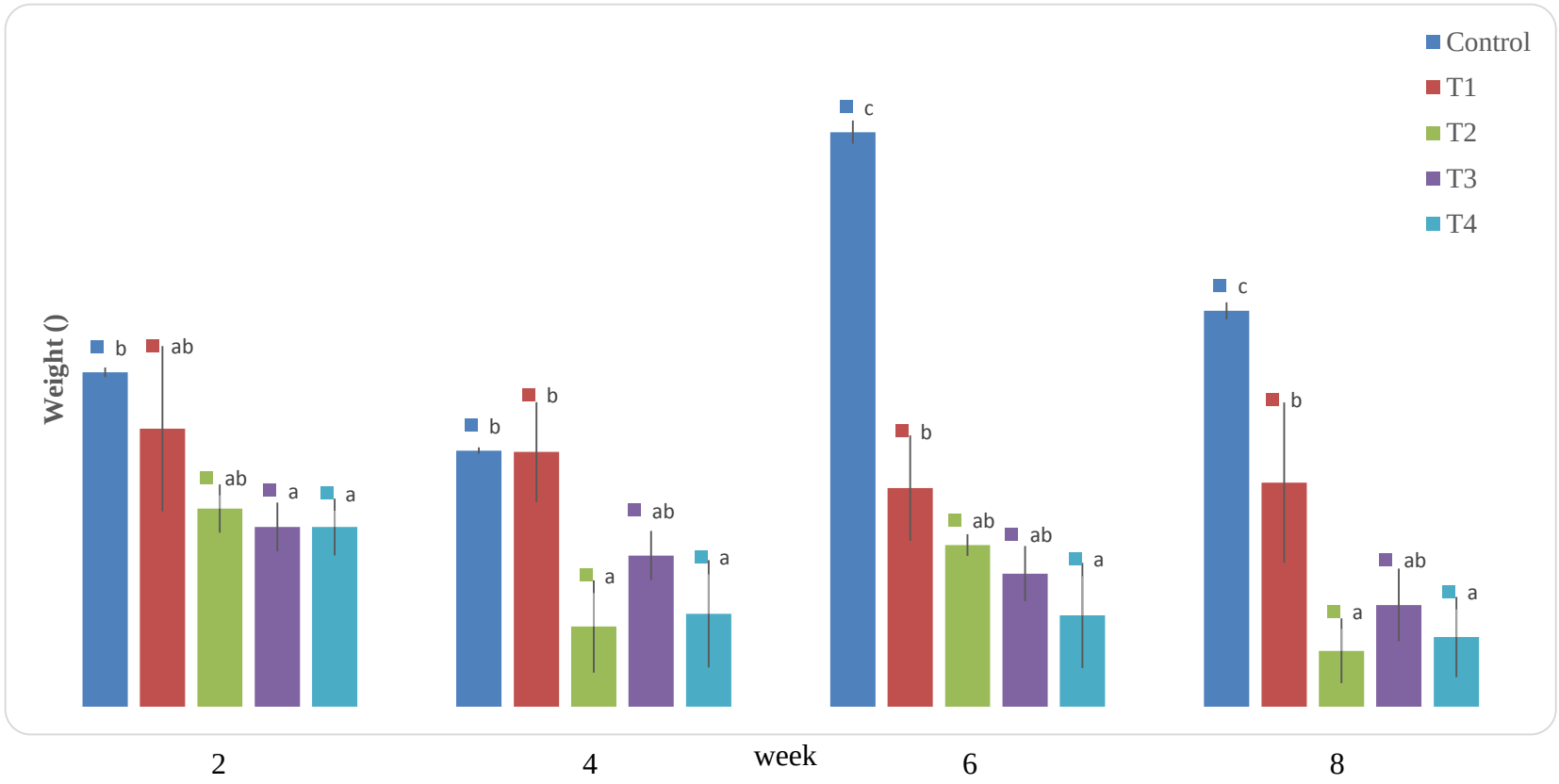

Figure 4.10: Weight of *C. gariepinus* Exposed to Sub-lethal Concentrations of Cadmium chloride and Combined Vitamins A and C for a Period of 8 weeks. Each bar represents mean ± standard error of mean (SEM) of three replicates for each treatment. Bars with different superscripts in the same week are significantly different at $p < 0.05$. [a] denotes least value while [c] denotes highest value. T1= 4 mg/L, T2 = 8 mg/L, T3 = 12 mg/L, T4 = 16 mg/L

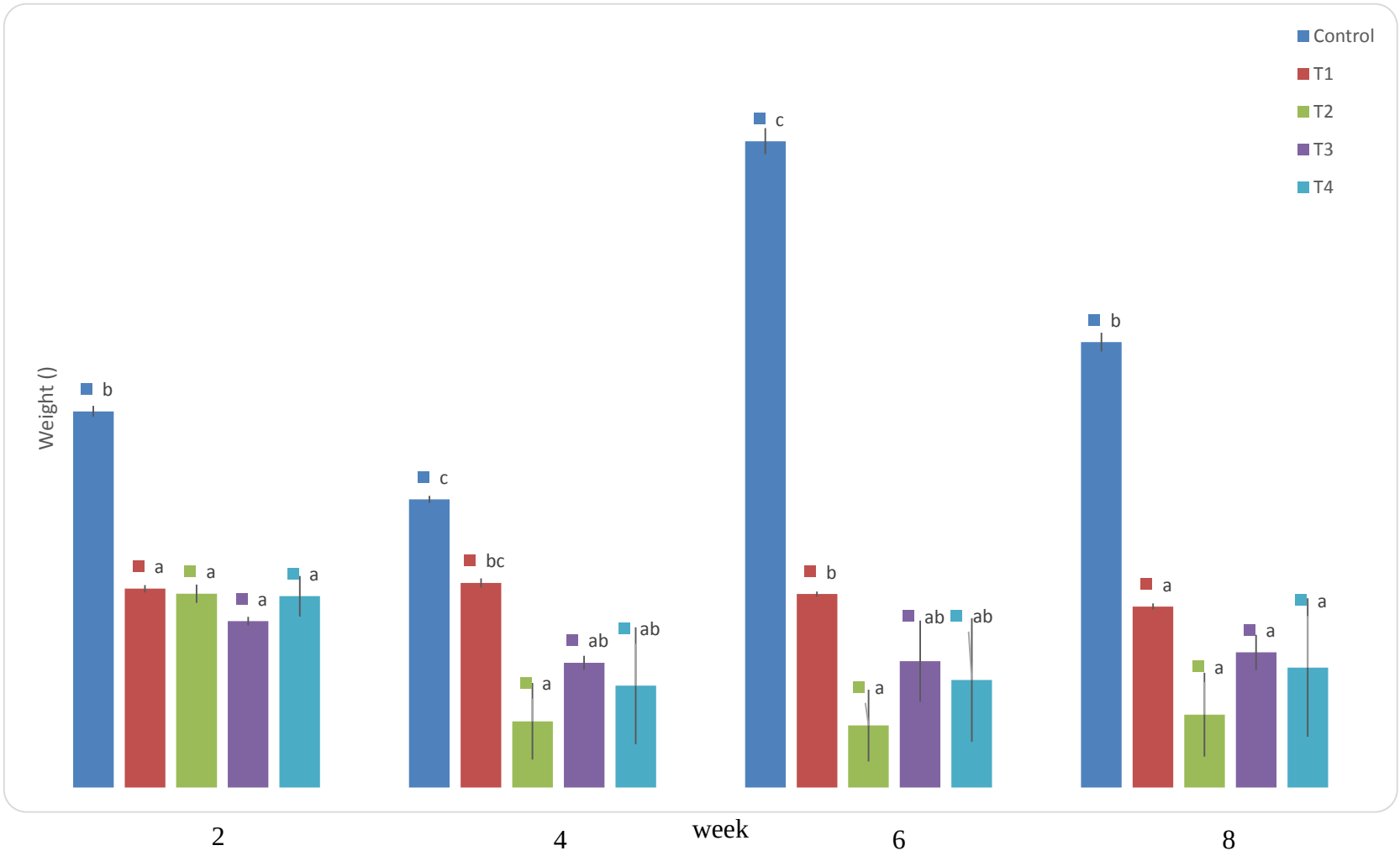

Figure 4.11: Weight of *C. gariepinus* Exposed to Sub-lethal Concentrations of Cadmium chloride and Combined Vitamins A and E for a Period of 8 weeks. Each bar represents mean ± standard error of mean (SEM) of three replicates for each treatment. Bars with different superscripts in the same week are significantly different at $p < 0.05$. ^{a} denotes least value while ^{c} denotes highest value. T1= 4 mg/L, T2 = 8 mg/L, T3 = 12 mg/L, T4 = 16 mg/L

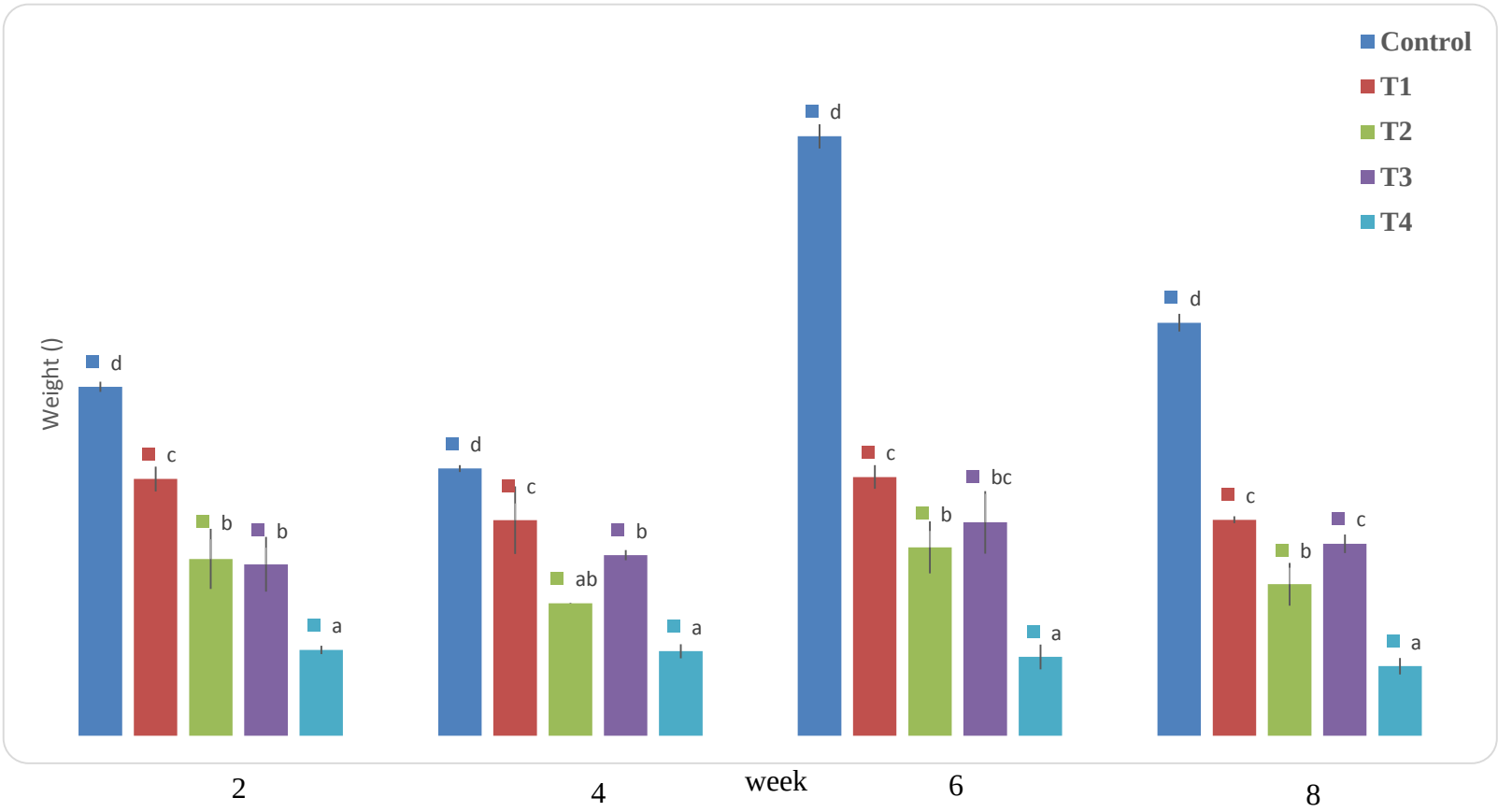

Figure 4.12: Weight of *C. gariepinus* Exposed to Sub-lethal Concentrations of Cadmium chloride and Combined Vitamins A, E and C for a Period of 8 weeks. Each bar represents mean ± standard error of mean (SEM) of three replicates for each treatment. Bars with different superscripts in the same week are significantly different at $p < 0.05$. [a] denotes least value while [c] denotes highest value. T1= 4 mg/L, T2 = 8 mg/L, T3 = 12 mg/L, T4 = 16 mg/L

4.1.4 Weight derivatives of *C. gariepinus* exposed to sub-lethal concentrations of cadmium chloride and combined supplements of vitamins A, C and E.

The growth derivatives of *C. gariepinus* exposed to sub-lethal concentrations of Cadmium chloride and combined supplements of vitamins A, C and E for a period of 8 weeks recorded the least weight gain of -0.44 g with % weight gain and specific growth rate of -15 and -5.50 respectively while the combined vitamins A and C had the highest weight gain of 6.30 with %weight gain and specific growth rate of 61 and -78.75, respectively

Table 4.1: Weight Derivatives of *C. gariepinus* Exposed to Sub-lethal Concentrations of Cadmium chloride Supplemented with Combined Vitamins C and E

Treatment (mg/L)	Final weight (g)	Initial weight (g)	Weight gain (g)	Weight gain (%)	Specific Growth Rate (%)
0	14.67	12.39	2.28	18	-28.50
4	5.90	8.31	-2.41	-29	30.13
8	0.00	5.37	-5.37	-100	67.13
12	7.06	8.73	-1.67	-19	20.88
16	5.83	8.86	-3.03	-34	37.88

Table 4.2: Weight Derivatives of *C. gariepinus* exposed to Sub-lethal Concentration of Cadmium Chloride Supplemented with Combined Vitamin A and C for a period of 8 weeks.

Treatment (mg/L)	Final weight (g)	Initial weight (g)	Weight gain (g)	Weight gain (%)	Specific Growth Rate (%)
0	14.67	12.39	2.28	18	-28.50
4	16.6	10.3	6.30	61	-78.75
8	2.07	7.34	-5.27	-71	65.88
12	3.77	6.66	-2.89	-43	36.13
16	2.58	6.66	-4.08	-61	51.00

Table 4.3: Weight Derivatives of Samples of *C. gariepinus* exposed to Sub-lethal Concentration of Cadmium Chloride Supplemented with Combined Vitamin A and E for a period of 8 weeks

Treatment (mg/L)	Final weight (g)	Initial weight (g)	Weight gain (g)	Weight gain (%)	Specific Growth Rate (%)
0	14.67	12.39	2.28	18	-28.5
4	5.96	6.55	-0.59	-9	7.38
8	2.40	6.38	-3.98	-62	49.75
12	4.45	5.48	-1.03	-19	12.88
16	3.95	6.30	-2.35	-37	29.38

Table 4.4: Weight Derivatives of *C. gariepinus* exposed to Sub-lethal Concentration of Cadmium Chloride Supplemented with Combined Vitamin A and E for a period of 8 weeks.

Treatment (mg/L)	Final weight (g)	Initial weight (g)	Weight gain (g)	Weight gain (%)	Specific Growth Rate (%)
0	14.67	12.39	2.28	18	-28.50
4	7.67	9.912	-1.45	-16	28.03
8	5.38	6.28	-0.9	-14	11.25
12	6.82	6.09	0.73	12	-9.13
16	2.47	2.91	-0.44	-15	5.50

4.1.5 Haematological Parameters of *C. gariepinus* exposed to sub-lethal concentrations of cadmium chloride and combined supplements of vitamins A, C and E.

The haemoglobin, Pack Cell voume (PCV), eosinophils and monocytes levels of control were significantly higher ($p<0.05$) than all the treatments of *C. gariepinus* exposed to sub-lethal concentrations of Cd and treated with combined vitamins C and E for a period of 4 weeks. However, monocytes level of T4 was significantly higher ($p<0.05$) than other treatments. There were no significant differences in the levels of MCV, MCH, MCHC, lymphocytes, neutrophils and basophils levels in all treatments. The RBC concentration of the control was significantly higher than those of 4 – 12 mg. Also, PLT level of control was significantly higher than those of T1 and T3 but not significantly different from those of T2 and T4. The WBC concentration of all combined vitamins CE treatments were significantly higher than that of control, with T2 having the highest value (Table 4.5).

The haemoglobin, PCV, MCV, MCH levels of the control were observed to be significantly higher ($p<0.05$) than those of other treatments of the CE treatment group at 8^{th} week of exposure. No significant differences were observed in the levels of MCHC, RBC, PLE, WBC, lymphocytes, neutrophils levels in the control and other treatments except for T2. The level of basophils in T4 were significantly higher ($p<0.05$) than the control and other treatments. No significant differences were observed in the levels of eosinophils and monocytes (Table 4.6).

From the results of the samples of *C. gariepinus* treated with combined vitamins A and C, the haemoglobin, PCV, RBC and monocytes levels of the control group were significantly higher than the other treatments. RBC mean values of the control are significantly higher than the other treatments. The T4 mean value on the other hand is

significantly higher than T1-T3. MCV level rof T1 was observed to be significantly higher than the control and other treatments except T2. No significant differences were observed in the levels of MCH, MCHC, lymphocytes, neutrophils, and basophils in all the treatments including the control. PLT level of control is significantly higher than those of T1 and T2. The WBC levels of all treatments were significantly higher than the control, although T1 and T2 were significantly higher than T3 and T4 (Table 4.7).

Furthermore, at the end of 8 weeks of exposure, there were significant rise in the levels of haemoglobin, PCV, MCV, MCH, basophils of the control than all treatments. However, no significant differences were observed in the levels of MCHC, PLT, WBC, lymphocytes, eosinophils and monocytes in all treatments including the control. The basophils level of control was found to be significantly higher than those of the treated groups. (Table 4.8).From the results of the samples of *C. gariepinus* exposed to sub-lethal concentrations of Cadmium chloride and supplemented with combined vitamins A and E, the control mean values of PCV and HB are significantly higher than other treatment with T4 significantly higher than T1-T3. Also, no significant differences were observed in the levels of MCV, MCH, MCHC, RBC, PLT, neutrophils, basophils in all treatments including the control. WBC levels of all treatments except for T4 are higher than that of the control, with T2 having the highest value. Lymphocytes levels of all the treatments were significantly higher than the control. No significant difference was observed in the mean values of eosinophils in all treatments including the control (Table 4.9).

The haemoglobin, PCV, MCV and MCH levels of the control are significantly higher than the treatments after 8 weeks of Cadmium chloride exposure and supplemented with combined vitamins A and E. However, no significant differences were observed in the levels of MCHC, RBC, WBC, lymphocytes, neutrophils, eosinophils and monocytes in

all treatments including the control. The PLT level of the control and T1 were significantly higher than other treatments (Table 4.10).

In the samples exposed to ACE treatment group after 4 weeks of exposure, the haemoglobin and PCV mean values of the control and T4 are significantly higher than other treatments. No significant differences were observed in the levels of MCV, MCH, MCHC, RBC, neutrophils, basophils and monocytes in the control and other treatments. T1-T3 mean values of WBC are significantly higher than the control and T4 (Table 4.11).

However, after 8 weeks of exposure to Cadmium chloride and supplementation with combined vitamins A, C and E, the haemoglobin, PCV, MCV and MCH levels of the control are significantly higher other treatments. No significant differences were observed in the levels of MCHC, RBC, WBC, lymphocytes, neutrophils, eosinophils and monocytes in all treatment including the control. However, the PLT level of the control was not significantly different from those of other treatments except T1 that is significantly lower. The basophils level recorded in T1 was significantly higher than other treatments including the control (Table 4.12).

Table 4.5: Haematological Parameters of *C. gariepinus* exposed to Sub-lethal Concentrations of Cadmium Chloride and Combined Vitamins C and E Supplements for a Period of 4 weeks.

Parameter	Treatments (mg/L)				
	Control (0)	4	8	12	16
Hb (g/dL)	6.10 ± 0.51^{b}	3.10 ± 0.50^{a}	3.80 ± 0.50^{a}	4.00 ± 0.49^{a}	4.20 ± 0.56^{a}
PCV (%)	18.00 ± 1.15^{c}	9.00 ± 0.89^{a}	11.40 ± 0.91^{ab}	12.00 ± 1.07^{ab}	12.60 ± 1.24^{b}
MCV (fi)	40.00 ± 2.31^{ab}	45.00 ± 3.12^{b}	40.00 ± 2.02^{ab}	37.00 ± 1.81^{ab}	35.00 ± 2.38^{a}
MCH (pg)	13.00 ± 1.44^{a}	15.00 ± 0.50^{a}	13.00 ± 1.24^{a}	12.00 ± 1.43^{a}	11.97 ± 1.46^{a}
MCHC (g/dL)	33.00 ± 1.73^{a}	34.00 ± 2.45^{a}	33.00 ± 3.78^{a}	33.00 ± 1.58^{a}	33.00 ± 1.49^{a}
RBC (10^{12}/L)	4.50 ± 0.50^{c}	2.00 ± 0.31^{a}	2.80 ± 0.14^{ab}	3.20 ± 0.42^{b}	3.60 ± 0.33^{bc}
PLT (10^{6}/L)	28.00 ± 1.73^{b}	22.00 ± 1.08^{a}	25.00 ± 1.25^{ab}	21.00 ± 2.45^{a}	24.00 ± 2.00^{ab}
WBC (10^{12}/L)	227.00 ± 4.04^{a}	324.00 ± 5.48^{d}	386.00 ± 4.38^{e}	295.00 ± 4.38^{c}	251.00 ± 3.79^{b}
LYMPHOCYTES (%)	32.00 ± 0.87^{a}	35.00 ± 1.66^{a}	42.00 ± 2.81^{b}	37.00 ± 2.44^{ab}	31.00 ± 1.43^{a}
NEUTROPHILS (%)	64.00 ± 0.58^{ab}	64.00 ± 3.03^{ab}	57.00 ± 1.43^{a}	62.00 ± 2.81^{ab}	67.00 ± 3.16^{b}
BASOPHILS (%)	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
EOSINOPHILS (%)	1.00 ± 0.10^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
MONOCYTES (%)	3.00 ± 0.29^{c}	1.00 ± 0.12^{a}	1.00 ± 0.07^{a}	1.00 ± 0.05^{a}	2.00 ± 0.33^{b}

values are presented as mean ± standard error of mean (SEM) of three replicates. Values with different superscripts in a column are significantly different at $p<0.05$.[a] denotes least value while [b] denotes higher value. Hb= Haemoglobin, PCV= Packed Cell Volume, MCV= Mean Corpuscular Volume, MCHC = Mean Corpuscular Haemoglobin Count, RBC= Red Blood Cell Count, PLT = Platelet Count, WBC = White Blood Cell Count

Table 4.6: Haematological Parameters of *C. gariepinus* exposed to Sub-lethal Concentrations of Cadmium chloride and Combined Vitamins C and E Supplements for a period of 8 weeks.

Parameter	Treatments (mg/L)				
	Control (0)	4	8	12	16
Hb (g/dL)	9.70±0.51^{c}	3.80±0.31^{b}	0.00±0.00^{a}	3.70±0.31^{b}	3.50±0.31^{b}
PCV (%)	29.00±1.73^{c}	11.40±0.81^{b}	0.00±0.00^{a}	11.10±0.51^{b}	10.50±1.09^{b}
MCV (fi)	120.00±2.89^{d}	43.00±1.47^{c}	0.00±0.00^{a}	44.00±3.75^{c}	18.00±1.43^{b}
MCH (pg)	40.00±4.62^{c}	14.00±0.50^{b}	0.00±0.00^{a}	15.00±1.21^{b}	15.00±1.24^{b}
MCHC (g/dL)	33.00±1.15^{b}	33.00±2.01^{b}	0.00±0.00^{a}	33.00±1.44^{b}	33.00±1.33^{b}
RBC (10^{12}/L)	2.40±0.14^{b}	2.60±0.434^{b}	0.00±0.00^{a}	2.50±0.50^{b}	2.40±0.07^{b}
PLT (10^{6}/L)	246.00±4.62^{b}	242.00±3.18^{b}	0.00±0.00^{a}	250.00±5.43^{b}	242.00±2.64^{b}
WBC (10^{12}/L)	10.20±0.72^{b}	10.90±^{b}	0.00±0.00^{a}	10.00±1.66^{b}	9.70±0.65^{b}
LYMPHOCYTES (%)	64.00±2.31^{b}	64.00±1.49^{b}	0.00±0.00^{a}	57.00±5.16^{b}	58.00±3.18^{b}
NEUTROPHILS (%)	34.00±3.46^{b}	35.00±2.00^{b}	0.00±0.00^{a}	42.00±2.06^{b}	37.00±2.81^{bb}
BASOPHILS (%)	2.00±0.08^{c}	1.00±0.14^{b}	0.00±0.00^{a}	1.00±0.05^{b}	3.00±0.26^{d}
EOSINOPHILS (%)	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
MONOCYTES (%)	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}

values are presented as mean ± standard error of mean (SEM) of three replicates. Values with different superscripts in a column are significantly different at $p<0.05$.^{a} denotes least value while ^{b} denotes higher value. Hb= Haemoglobin, PCV= Packed Cell Volume, MCV= Mean Corpuscular Volume, MCHC = Mean Corpuscular Haemoglobin Count, RBC= Red Blood Cell Count, PLT = Platelet Count, WBC = White Blood Cell Count

Table 4.7: Haematological Parameters of *C. gariepinus* exposed to Sub-lethal Concentrations of Cadmium chloride and Combined Vitamins A and C Supplements for a period of 4 weeks.

Parameter	Treatments (mg/L)				
	Control (0)	4	8	12	16
Hb (g/dL)	6.10 ± 0.51^{b}	3.10 ± 0.33^{a}	3.40 ± 0.59^{a}	3.80 ± 0.12^{a}	3.90 ± 0.31^{a}
PCV (%)	18.00 ± 1.15^{b}	9.30 ± 1.08^{a}	10.20 ± 1.42^{a}	11.40 ± 0.91^{a}	11.70 ± 1.80^{a}
MCV (fi)	40.00 ± 2.31^{ab}	51.00 ± 3.16^{c}	48.00 ± 1.47^{bc}	43.00 ± 4.95^{ab}	36.00 ± 1.23^{a}
MCH (pg)	13.00 ± 1.44^{ab}	17.00 ± 1.07^{b}	16.00 ± 2.04^{ab}	14.00 ± 1.24^{ab}	12.00 ± 0.55^{a}
MCHC (g/dL)	33.00 ± 1.73^{a}	33.00 ± 2.81^{a}	33.00 ± 2.04^{a}	33.00 ± 2.60^{a}	33.00 ± 1.99^{a}
RBC (10^{12}/L)	4.50 ± 0.50^{c}	1.80 ± 0.24^{a}	2.10 ± 0.31^{ab}	2.60 ± 0.26^{ab}	3.20 ± 0.31^{b}
PLT (10^{6}/L)	28.00 ± 1.73^{c}	21.00 ± 1.54^{ab}	19.00 ± 1.72^{a}	28.00 ± 1.66^{c}	26.00 ± 2.00^{bc}
WBC (10^{12}/L)	227.00 ± 4.04^{a}	401.00 ± 5.92^{d}	385.00 ± 1.99^{d}	327.00 ± 7.24^{c}	273.00 ± 5.07^{b}
LYMPHOCYTES (%)	32.00 ± 0.87^{a}	35.00 ± 1.66^{a}	33.00 ± 1.47^{a}	34.00 ± 2.04^{a}	33.00 ± 2.64^{a}
NEUTROPHILS (%)	64.00 ± 0.58^{a}	62.00 ± 4.35^{a}	64.00 ± 3.34^{a}	64.00 ± 3.38^{a}	65.00 ± 5.05^{a}
BASOPHILS (%)	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
EOSINOPHILS (%)	1.00 ± 0.10^{b}	1.00 ± 0.13^{b}	1.00 ± 0.05^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
MONOCYTES (%)	3.00±02.9b	2.00±0.08a	2.00±0.18a	2.00±0.18a	2.00±0.21a

values are presented as mean ± standard error of mean (SEM) of three replicates. Values with different superscripts in a column are significantly different at $p<0.05$.[a] denotes least value while [b] denotes higher value. Hb= Haemoglobin, PCV= Packed Cell Volume, MCV= Mean Corpuscular Volume, MCHC = Mean Corpuscular Haemoglobin Count, RBC= Red Blood Cell Count, PLT = Platelet Count, WBC = White Blood Cell Count

Table 4.8: Haematological Parameters of *C. gariepinus* Exposed to Sub-lethal Concentrations of Cadmium chloride and Combined Vitamins A and C Supplements for a Period of 8 weeks.

Parameter	Treatments (mg/L)				
	Control (0)	4	8	12	16
Hb (g/dL)	9.70±0.51^{b}	5.00±0.44^{a}	4.90±0.47^{a}	4.80±0.28^{a}	4.70±0.42^{a}
PCV (%)	29.00±1.73^{b}	15.00±1.43^{a}	14.80±1.47^{a}	14.40±1.24^{a}	14.00±1.24^{a}
MCV (fi)	120.00±2.89^{b}	63.00±3.16^{a}	59.00±2.23^{a}	55.00±2.68^{a}	56.00±3.16^{a}
MCH (pg)	40.00±4.62^{b}	20.00±1.47^{a}	19.00±1.24^{a}	18.00±1.22^{a}	19.00±0.90^{a}
MCHC (g/dL)	33.00±1.15^{a}	33.00±2.00^{a}	30.00±2.04^{a}	33.00±2.81^{a}	33.00±1.24^{a}
RBC (10^{12}/L)	2.40±0.14^{a}	2.40±0.12^{a}	2.50±0.20^{a}	2.60±0.12^{a}	2.50±0.21^{a}
PLT (10^{6}/L)	246.00±4.62^{bc}	251.00±4.56^{c}	251.00±3.39^{c}	236.00±3.95^{ab}	224.00±4.93^{a}
WBC (10^{12}/L)	10.20±0.72^{a}	12.20±0.89^{a}	12.60±0.72^{a}	11.20±0.88^{a}	10.10±0.89^{a}
LYMPHOCYTES (%)	64.00±2.31^{a}	68.00±2.81^{a}	57.00±2.00^{a}	64.00±3.20^{a}	67.00±4.93^{a}
NEUTROPHILS (%)	34.00±3.46^{a}	31.00±1.08^{a}	43.00±2.38^{b}	35.00±1.82^{a}	33.00±2.43^{a}
BASOPHILS (%)	2.00±0.08^{c}	1.00±0.03^{b}	0.00±0.00^{a}	1.00±0.02^{b}	0.00±0.00^{a}
EOSINOPHILS (%)	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
MONOCYTES (%)	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}

Values are presented as mean ± standard error of mean (SEM) of three replicates.Values with different superscripts in a column are significantly different at $p<0.05$. ^{a} denotes least value while ^{b} denotes higher value. Hb= Haemoglobin, PCV= Packed Cell Volume, MCV= Mean Corpuscular Volume, MCHC = Mean Corpuscular Haemoglobin Count, RBC= Red Blood Cell Count, PLT = Platelet Count, WBC = White Blood Cell Count

Table 4.9: Haematological Parameters of *C. gariepinus* exposed to Sub-lethal Concentrations of Cadmium chloride and Combined Vitamins A and E Supplements for a Period of 4 weeks.

Parameter	Treatments (mg/L)				
	Control (0)	4	8	12	16
Hb (g/dL)	6.10 ± 0.51^{c}	3.80 ± 0.41^{a}	4.30 ± 0.43^{ab}	4.90 ± 0.56^{abc}	5.70 ± 0.31^{bc}
PCV (%)	18.00 ± 1.15^{c}	11.40 ± 0.33^{a}	12.90 ± 0.41^{ab}	14.70 ± 0.72^{b}	17.10 ± 0.74^{c}
MCV (fi)	40.00 ± 2.31^{ab}	42.00 ± 1.33^{b}	38.00 ± 0.43^{ab}	36.00 ± 1.43^{a}	37.00 ± 2.00^{ab}
MCH (pg)	13.00 ± 0.29^{a}	14.00 ± 1.56^{a}	12.00 ± 0.71^{a}	12.00 ± 0.72^{a}	12.00 ± 0.74^{a}
MCHC (g/dL)	33.00 ± 1.73^{a}	33.00 ± 1.67^{a}	33.00 ± 2.60^{a}	33.00 ± 1.39^{a}	33.00 ± 1.47^{a}
RBC (10^{12}/L)	4.50 ± 0.50^{b}	2.70 ± 0.44^{a}	3.40 ± 0.55^{ab}	4.00 ± 0.20^{ab}	4.50 ± 0.43^{b}
PLT (10^{6}/L)	28.00 ± 1.73^{a}	27.00 ± 1.96^{a}	22.00 ± 1.62^{a}	25.00 ± 1.67^{a}	22.00 ± 1.81^{a}
WBC (10^{12}/L)	227.00 ± 4.04^{a}	308.00 ± 4.62^{c}	362.00 ± 4.04^{d}	276.00 ± 5.48^{b}	234.00 ± 2.31^{a}
LYMPHOCYTES (%)	32.00 ± 0.87^{a}	38.00 ± 0.98^{b}	37.00 ± 1.67^{b}	39.00 ± 1.43^{b}	37.00 ± 1.09^{b}
NEUTROPHILS (%)	64.00 ± 0.58^{a}	58.00 ± 2.02^{a}	58.00 ± 3.23^{a}	57.00 ± 0.74^{a}	61.00 ± 3.97^{a}
BASOPHILS (%)	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
EOSINOPHILS	1.00 ± 0.10^{b}	1.00 ± 0.04^{b}	1.00 ± 0.12^{b}	1.00 ± 0.06^{b}	0.00 ± 0.00^{a}
MONOCYTES	3.00 ± 0.29^{b}	3.00 ± 0.26^{b}	4.00 ± 0.24^{c}	3.00 ± 0.08^{b}	2.00 ± 0.08^{a}

Values are presented as mean ± standard error of mean (SEM) of three replicates. Values with different superscripts in a column are significantly different at $p<0.05$. ^{a} denotes least value while ^{b} denotes higher value. Hb= Haemoglobin, PCV= Packed Cell Volume, MCV= Mean Corpuscular Volume, MCHC = Mean Corpuscular Haemoglobin Count, RBC= Red Blood Cell Count, PLT = Platelet Count, WBC = White Blood Cell Count

Table 4.10: Haematological Parameters of *C. gariepinus* exposed to Sub-lethal Concentrations of Cadmium chloride and Combined Vitamins A and E Supplements for a Period of 8 weeks.

Parameter	Treatments (mg/L)				
	Control (0)	4	8	12	16
Hb (g/dL)	9.70±0.51^{b}	5.80±0.23^{a}	5.40±0.54^{a}	5.50±0.29^{a}	5.80±0.51^{a}
PCV (%)	29.00±1.73^{b}	17.40±0.70^{a}	16.40±0.71^{a}	16.50±1.40^{a}	17.40±0.74^{a}
MCV (fi)	120.00±2.87^{b}	70.00±1.73^{a}	68.00±2.31^{a}	69.00±4.10^{a}	73.00±2.77^{a}
MCH (pg)	40.00±4.62^{b}	23.02±1.46^{a}	22.00±1.15^{a}	23.00±1.42^{a}	24.00±1.44^{a}
MCHC (g/dL)	29.67±4.37^{a}	33.00±3.18^{a}	32.00±2.02^{a}	33.00±2.60^{a}	33.00±1.09^{a}
RBC (10^{12}/L)	2.40±0.14^{a}	2.50±0.05^{a}	2.40±0.57^{a}	2.40±0.08^{a}	2.40±0.42^{a}
PLT (10^{6}/L)	246.00±4.62^{c}	238.00±3.46^{bc}	226.00±3.46^{ab}	220.00±3.12^{a}	215.00±4.91^{a}
WBC (10^{12}/L)	10.20±0.72^{a}	11.40±1.10^{a}	11.40±0.78^{a}	9.80±0.73^{a}	11.00±1.39^{a}
LYMPHOCYTES (%)	64.00±2.31^{ab}	57.00±1.15^{a}	59.00±2.89^{a}	63.00±1.73^{ab}	67.00±2.01^{b}
NEUTROPHILS (%)	34.00±3.46^{a}	42.00±2.31^{a}	38.00±3.75^{a}	35.00±2.38^{a}	33.00±4.33^{a}
BASOPHILS (%)	2.09±0.16^{c}	1.00±0.12^{b}	2.00±0.06^{c}	2.00±0.41^{c}	0.00±0.00^{a}
EOSINOPHILS (%)	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
MONOCYTES (%)	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}

Values are presented as mean ± standard error of mean (SEM) of three replicates. Values with different superscripts in a column are significantly different at $p<0.05$. ^{a} denotes least value while ^{b} denotes higher value. Hb= Haemoglobin, PCV= Packed Cell Volume, MCV= Mean Corpuscular Volume, MCHC = Mean Corpuscular Haemoglobin Count, RBC= Red Blood Cell Count, PLT = Platelet Count, WBC = White Blood Cell Count

Table 4.11: Haematological Parameters of *C. gariepinus* exposed to Sub-lethal Concentrations of Cadmium chloride and Combined Vitamins A, C and E Supplements for a Period of 4 weeks.

Parameter	Treatments (mg/L)				
	Control (0)	4	8	12	16
Hb (g/dL)	6.10 ± 0.51^{b}	4.40 ± 0.44^{a}	4.80 ± 0.22^{a}	5.60 ± 0.31^{ab}	6.40 ± 0.29^{b}
PCV (%)	18.00 ± 1.15^{c}	13.20 ± 0.72^{a}	14.40 ± 0.51^{ab}	16.80 ± 0.50^{bc}	19.20 ± 1.08^{c}
MCV (fi)	40.00 ± 2.31^{a}	41.00 ± 1.15^{a}	37.00 ± 1.73^{a}	37.00 ± 1.73^{a}	37.00 ± 2.60^{a}
MCH (pg)	13.00 ± 1.44^{a}	13.00 ± 1.07^{a}	12.00 ± 0.29^{a}	12.00 ± 1.15^{a}	13.00 ± 0.87^{a}
MCHC (g/dL)	33.00 ± 1.73^{a}	33.00 ± 0.58^{a}	33.00 ± 1.44^{a}	33.00 ± 2.60^{a}	33.00 ± 2.77^{a}
RBC (10^{12}/L)	4.50 ± 0.50^{ab}	3.20 ± 0.24^{a}	3.80 ± 0.56^{ab}	4.50 ± 0.26^{ab}	5.10 ± 0.43^{b}
PLT (10^{6}/L)	28.00 ± 1.73^{b}	24.00 ± 2.31^{ab}	21.00 ± 1.15^{a}	24.00 ± 2.02^{ab}	21.00 ± 1.15^{a}
WBC (10^{12}/L)	227.00 ± 4.04^{b}	272.00 ± 2.87^{d}	342.00 ± 4.04^{e}	253.00 ± 1.73^{c}	217.00 ± 2.02^{a}
LYMPHOCYTES (%)	32.00 ± 0.87^{a}	38.00 ± 2.02^{b}	28.00 ± 1.44^{a}	38.00 ± 1.44^{b}	39.00 ± 2.02^{b}
NEUTROPHILS (%)	64.00 ± 0.58^{ab}	58.00 ± 1.73^{a}	67.00 ± 2.89^{b}	59.00 ± 2.60^{a}	58.00 ± 1.44^{a}
BASOPHILS (%)	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	1.00 ± 0.14^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
EOSINOPHILS (%)	1.00 ± 0.10^{b}	0.00 ± 0.00^{a}	1.00 ± 0.20^{b}	1.00 ± 0.20^{b}	1.00 ± 0.12^{b}
MONOCYTES (%)	3.00 ± 0.29^{ab}	4.00 ± 0.87^{b}	3.00 ± 0.52^{ab}	2.00 ± 0.45^{a}	2.00 ± 0.48^{a}

Values are presented as mean ± standard error of mean (SEM) of three replicates. Values with different superscripts in a column are significantly different at $p<0.05$[a] denotes least value while [b] denotes higher value. Hb= Haemoglobin, PCV= Packed Cell Volume, MCV= Mean Corpuscular Volume, MCHC = Mean Corpuscular Haemoglobin Count, RBC= Red Blood Cell Count, PLT = Platelet Count, WBC = White Blood Cell Count

Table 4.12: Haematological Parameters of *C. gariepinus* exposed to Sub-lethal Concentrations of Cadmium chloride and Combined Vitamins A, C and E Supplements for a Period of 8 weeks

Parameter	Treatments				
	Control (0)	**4**	**8**	**12**	**16**
Hb (g/dL)	9.70±0.51^{b}	3.70±0.27^{a}	4.60±0.70^{a}	3.90±0.41^{a}	3.70±0.33^{a}
PCV (%)	29.00±1.73^{b}	11.10±0.91^{a}	13.90±0.62^{a}	11.70±0.31^{a}	11.10±2.01^{a}
MCV (fi)	120.00±2.89^{b}	46.00±2.89^{a}	53.00±1.73^{a}	48.00±2.31^{a}	47.00±3.46^{a}
MCH (pg)	40.00±4.62^{b}	15.00±2.31^{a}	17.00±1.15^{a}	16.00±2.89^{a}	15.00±0.58^{a}
MCHC (g/dL)	33.00±1.15^{a}	33.00±2.31^{a}	30.00±2.89^{a}	33.00±1.73^{a}	33.00±1.73^{a}
RBC (10^{12}/L)	2.40±0.14^{a}	2.40±0.10^{a}	2.60±0.15^{a}	2.40±0.12^{a}	2.40±0.33^{a}
PLT (10^{6}/L)	246.00±4.62^{b}	232.00±4.04^{a}	249.00±5.20^{b}	247.00±2.89^{b}	236.00±2.89^{ab}
WBC (10^{12}/L)	10.20±0.72^{a}	11.00±0.51^{a}	9.70±0.57^{a}	10.70±1.24^{a}	9.80±0.55^{a}
LYMPHOCYTES (%)	64.00±2.31^{ab}	58.00±2.31^{a}	64.00±2.31^{ab}	67.00±1.15^{b}	62.00±1.15^{ab}
NEUTROPHILS (%)	34.00±3.46^{a}	37.00±1.73^{a}	35.00±1.15^{a}	32.00±1.73^{a}	38.00±2.31^{a}
BASOPHILS (%)	2.00±0.08^{c}	3.00±0.08^{d}	1.00±0.05^{b}	0.00±0.00^{a}	0.00±0.00^{a}
EOSINOPHILS (%)	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
MONOCYTES (%)	0.00±0.00^{a}	1.00±0.06^{b}	0.00±0.00^{a}	1.00±0.06^{a}	0.00±0.00^{a}

Values are presented as mean ± standard error of mean (SEM) of three replicates. Values with different superscripts in a column are significantly different at $p<0.05$. Hb= Haemoglobin, PCV= Packed Cell Volume, MCV= Mean Corpuscular Volume, MCHC = Mean Corpuscular Haemoglobin Count, RBC= Red Blood Cell Count, PLT = Platelet Count, WBC = White Blood Cell Count

4.1.6 Histopathological analysis of some organs of *C. gariepinus* exposed to sub-lethal concentrations of Cadmium chloride and treated with combined vitamins A, C and E for 8 weeks

The photomicrographs showed preserved liver architecture with normal sinusoids (S), central vein (CV) and hepatocytes (H) which is comparable to the control except for T1 of AC which showed massive necrosis (N). However, as the Cd concentration increases, the severity of liver damage increased with photomicrographs showing widen sinusoids (WS), distorted sinusoids (DS), distorted central vein (DCV), vacuolation (V) and necrosis (N). Nevertheless, combined vitamins AC and CE were able to protect liver from Cd hepatotoxicity at up to 8mg/L of Cadmium chloride (T2). (Plates Ia and Ib).

Similar to the effects observed with the livers of *C. gariepinus* exposed to sub-lethal concentrations of Cd and treated with combined vitamins A, C and E. The photomicrographs of their kidneys showed preserved kidneys architecture at the lowest concentration of Cd used (T1) for all the treated groups with the kidneys showing normal glomerular (G), Bowman's capsule (BC) and showed no difference from the control except for AC. However, T2-T4 of all the treatment groups supplemented with all combined vitamins showed kidney damage features which include tubular vacuolation (TV), widened Bowman's capsule (WBC), distorted Bowman's capsule (DBC), shrink Bowman's capsule (SBC) (Plates IIa and IIb).

The photomicrographs of gills of all experimental groups revealed preserved architecture with gills showing normal primary lamellae (P), secondary lamellae (S). Although, gills T4 of *C. gariepinus* treated with combined vitamins AC and CE showed little damages (in the forms of epithelial detachment (ED) and hemorrhage (H)) in the gills at this concentration (Plates IIIa and IIIb).

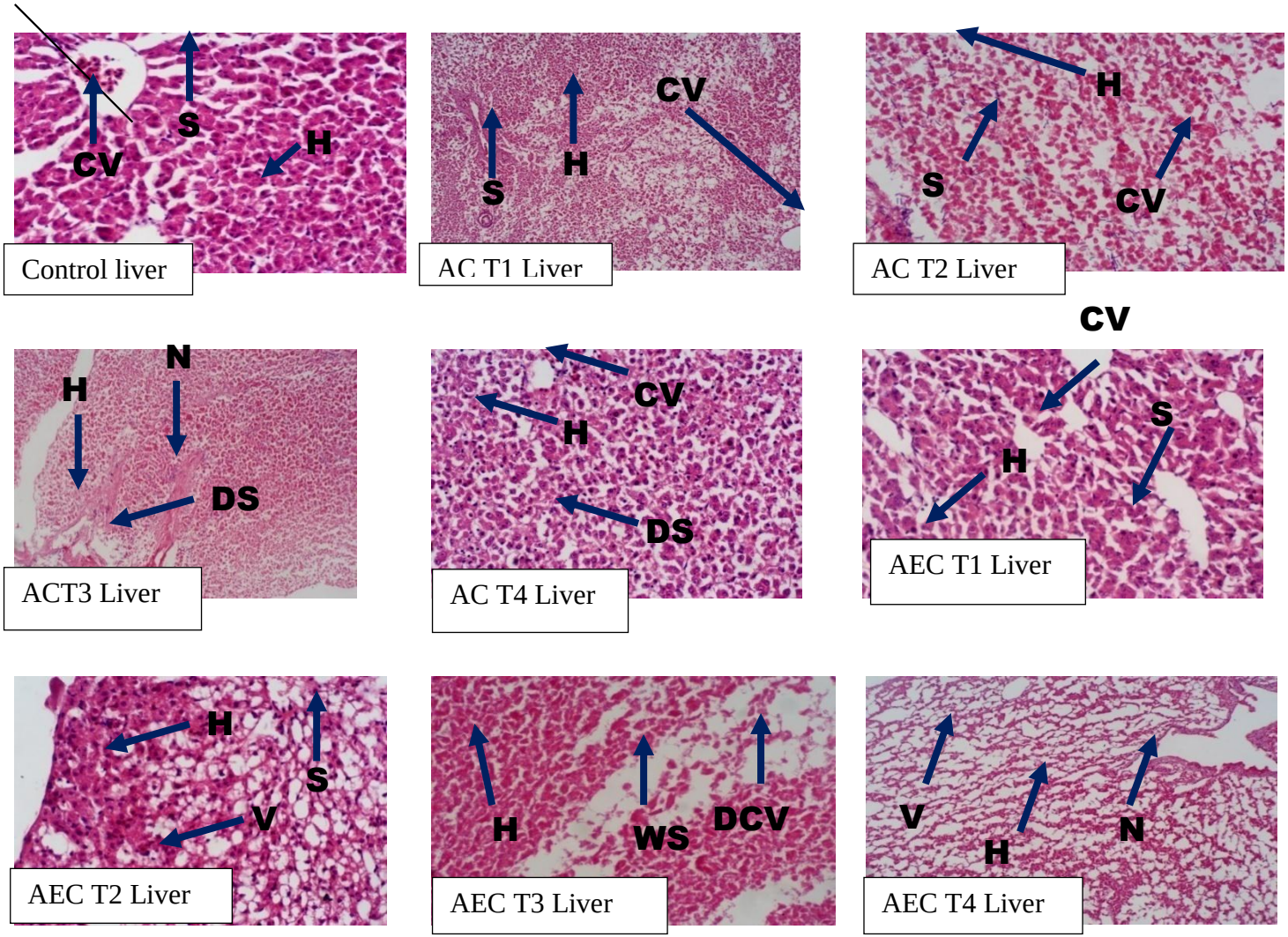

Plate Ia - Photomicrographs of livers of *C. gariepinus* Exposed to Various Cadmium Concentrations and Treated with Combined Vitamins for 8 weeks. Key: S= sinusoids, CV= central vein, H= hepatocytes, N= necrosis, WS= widen sinusoids, DS= distorted sinusoids, DCV= distorte d central vein, V= vacuolation.

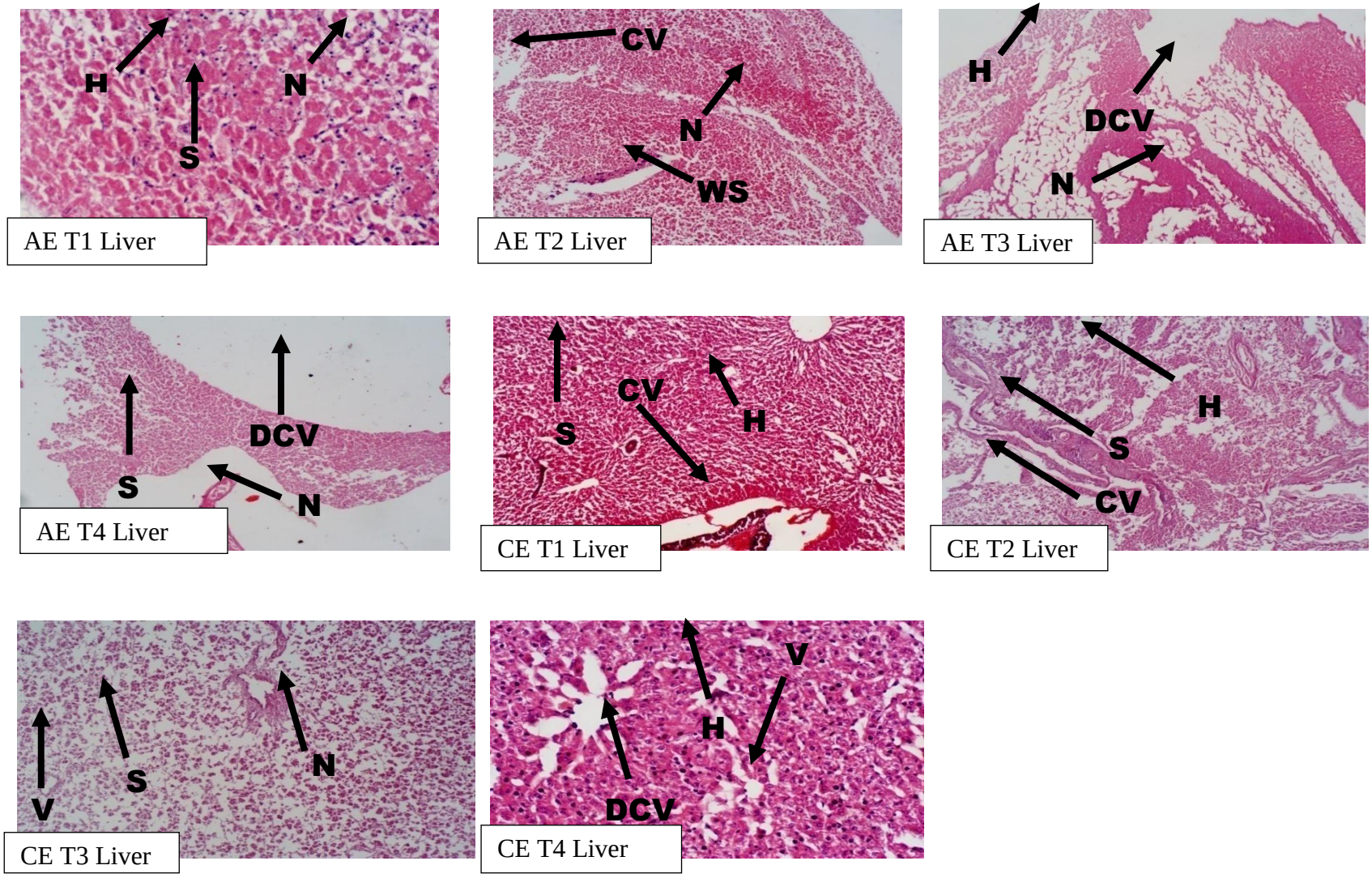

Plate Ib - Photomicrographs of Livers of *C. gariepinus* Exposed to Various Cadmium Concentrations and Treated with Combined Vitamins for 8 weeks. Key: S= sinusoids, CV= central vein, H= hepatocytes, N= necrosis, WS= widen sinusoids, DS= distorted sinusoids, DCV= distorted central vein, V= vacuolation

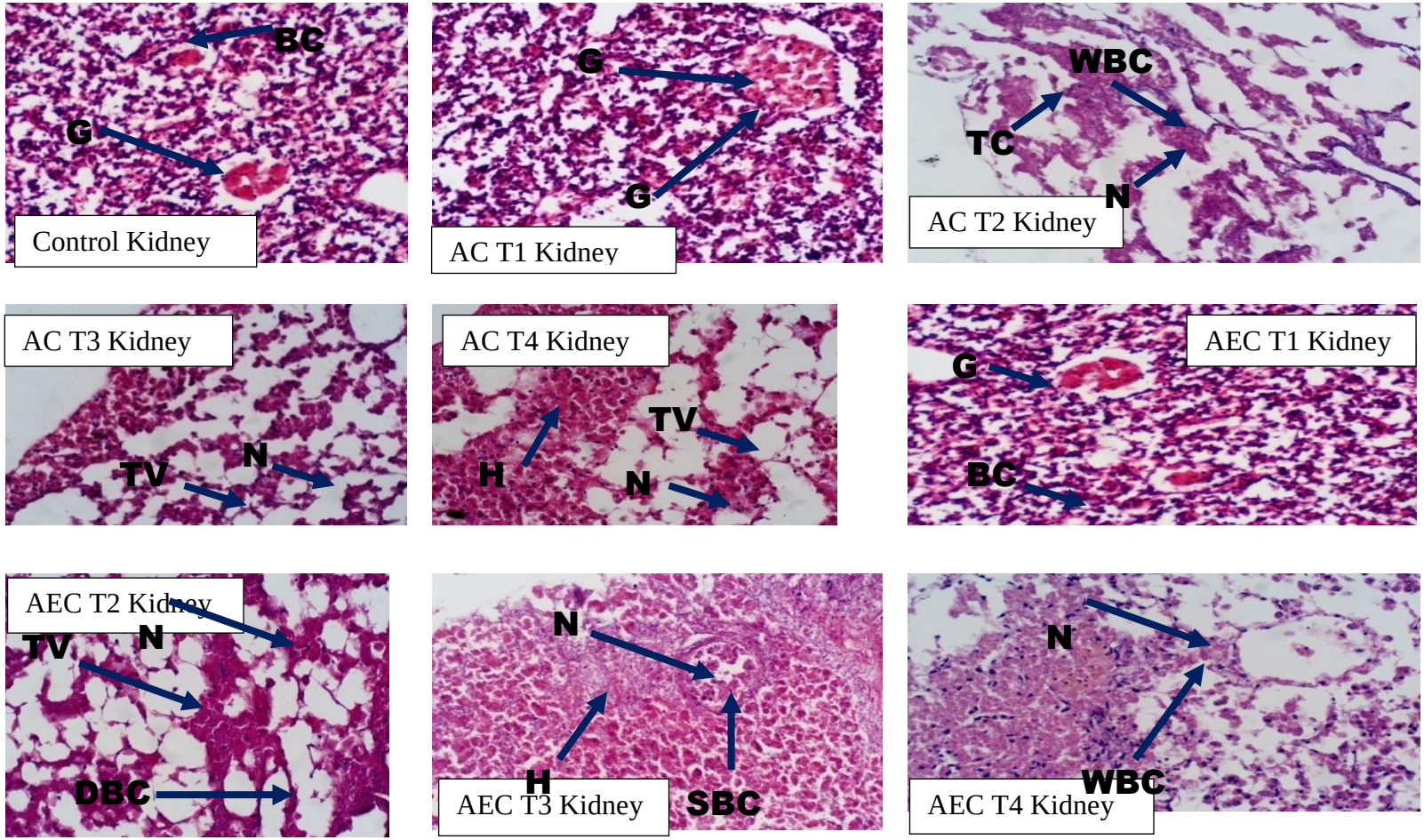

Plate IIa - Photomicrographs of Kidneys of *C. gariepinus* Exposed to Various Cadmium Concentrations and Treated with Combined Vitamins for 8 weeks. Keys: G= glomeruli, BC= Bowman's capsule, TV= tubular vacuolation, WBC= widen Bowman's capsule, DBC= distorted Bowman's capsule, SBC= shrink Bowman's capsule.

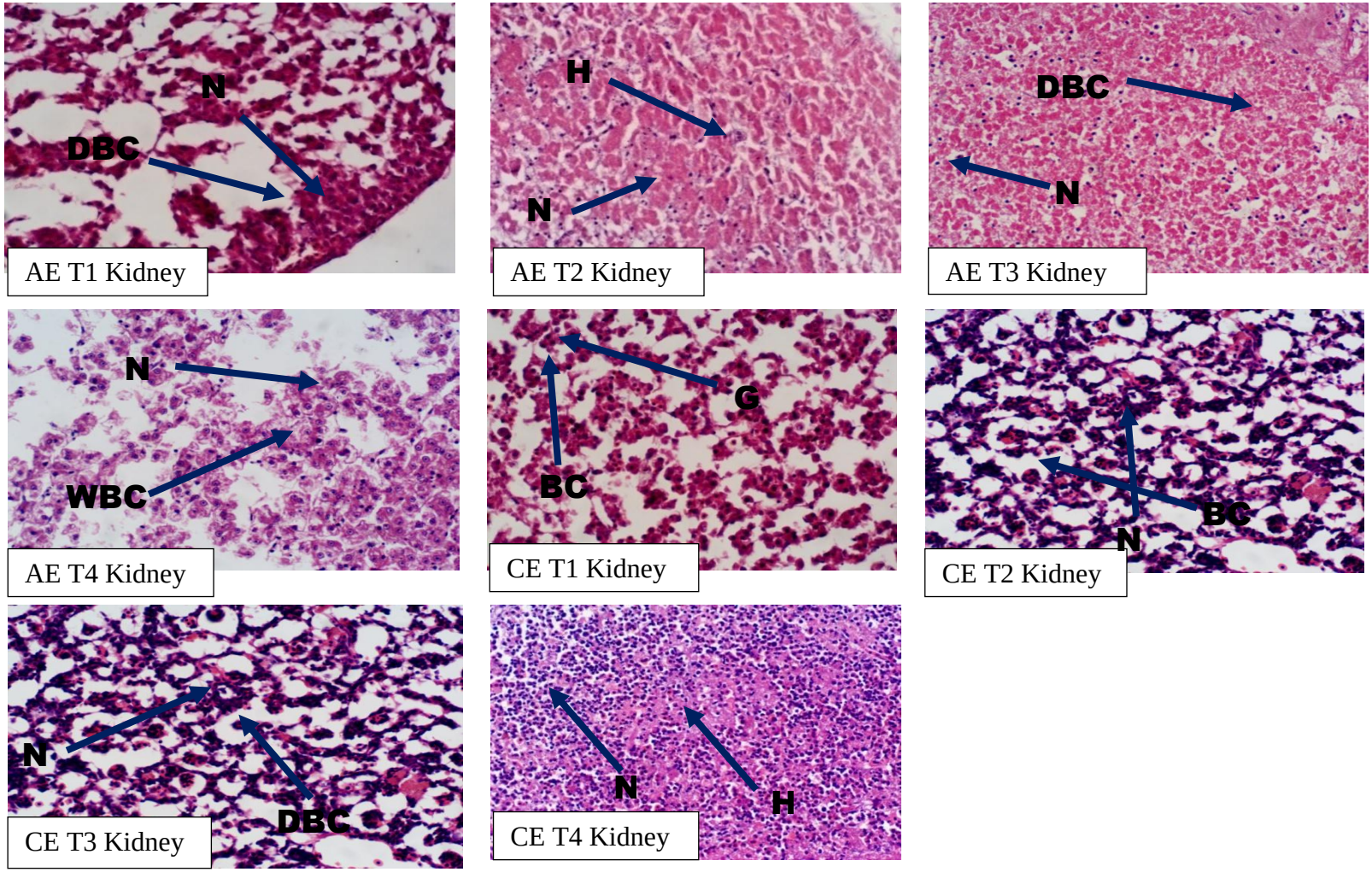

Plate IIb - Photomicrographs of Kidneys of *C. gariepinus* Exposed to Various Cadmium Concentrations and Treated with Combined Vitamins for 8 weeks. Keys: G= glomeruli, BC= Bowman's capsule, TV= tubular vacuolation, WBC= widen Bowman's capsule, DBC= distorted Bowman's capsule, SBC= shrink Bowman's capsule.

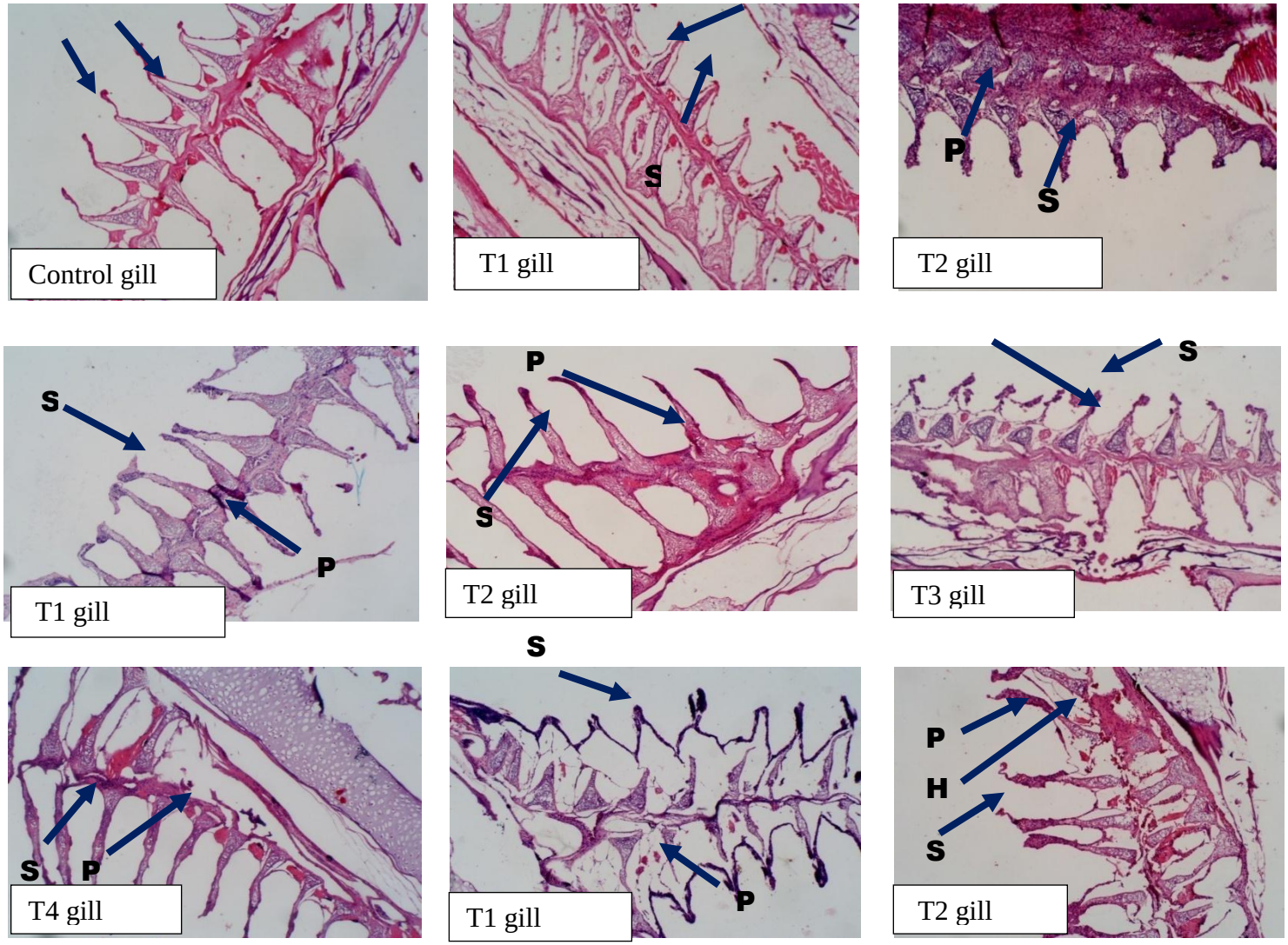

Plate IIIb - Photomicrographs of Gills of *C. gariepinus* Exposed to Various Cadmium Concentrations and Treated with Combined Vitamins for 8 weeks. Keys: P= primary lamellae, S= secondary lamellae, ED= epithelial detachment, H= hemorrhage

4.2 Discussion

4.2.1 Morphometric parameters of *C. gariepinus* exposed to sub-lethal concentrations of cadmium chloride and combined supplements of vitamins A, C and E.

Bioaccumulation of toxic metals like cadmium has one of its prominent effects to be growth inhibition following chronic exposure (Zebral *et al*., 2018). In this study, at 2nd week of exposure, the lengths of the control were significantly higher than the treatments except for T3 of group supplemented with combined vitamins C and E; T1 and T4 of groups supplemented with combined vitamins A and C; T1 of groups supplemented with combined vitamins A, C and E, respectively. However, as the period of exposure increases, no significant differences were observed between the lengths of treatment groups (T1-T4) supplemented with combined vitamins as there were no significant differences observed between the control and almost all the treatment groups supplemented with combined vitamins at later weeks (weeks 4, 6 and most especially 8) indicating the long-term ameliorative effect of combined vitamins on the lengths of *C. gariepinus* exposed to sub-lethal concentrations of Cd. The ability of all the combined vitamins to restore length reduction induced by Cd could be traceable to their antioxidant properties (Mumtaz *et al*., 2020) as Cd has been shown to elicit its toxic effect via oxidative stress (Kitamura and Hiramatsu, 2010; Zhang *et al*., 2020).

There was a general decrease in weight in all treatment groups compared to the control throughout the experimental period, most especially at later weeks (weeks 6 and 8) of the experiment. The loss in weight observed in all the treatment groups could be as a result of increased energy utilization by the *C. gariepinus* in treatment groups (T1-T4) induced by Cd leading to increased metabolism which in turn result in energetic deficiency and consequently weight loss. Similar observation was made by Rahman *et al*. (2018) who reported highest weight for control and the least for Cd-exposed groups

with weight decreasing with increase in cadmium concentration. Ko *et al*. (2019) also reported weight loss in *Platicthys stellatus* when they were exposed to different chromium concentrations for a period of 2 weeks. Also, growth performance was found to reduce with increase in the concentrations of zinc which is contrary to that of the control group, and the maximum feed intake and feed conversion of the control were observed to be significantly higher than those of groups exposed to varying concentrations of zinc (Abdel-Tawwab *et al.,* 2018). Thus, another possible cause of weight loss in this study could be loss of appetite induced by Cd exposure

4.2.2 Haematological parameters of *C. gariepinus* exposed to sub-lethal concentrations of Cadmium chloride and combined supplements of vitamins A, C and E

Haematological parameters are promising indicators of physiological changes in fish under stress and thus are extensively used in toxicological investigations and environmental monitoring (Kavitha *et al*., 2010). The haemoglobin, PCV and RBC are usually decreased in fish exposed to Cd which may be as a result of red blood cells destruction and or inhibition of RBCs production (Eriegha and Ekokotu, 2017).). However, the significant decrease in haemoglobin, PCV, and RBC levels observed in treatment groups supplemented with combined vitamins C and E, and A and C when compared to the control could be as a result of haemolytic or inhibitory effect of Cd on erythrocytes synthesis in these treatments. However, the absence of significant differences in the levels of these parameters in treatment groups (T3 and T4) supplemented with combined vitamins A and E, and A, C and E could be traceable to the ability of the combined vitamins to prevent haemolysis or inhibitory effect of Cd on erythropoiesis during the first 4 weeks of exposure. However, significant decreased levels in these parameters in all treatment groups over 8 weeks of exposure, could be

due to the toxic effects such as hemophilia, red cell shrinkage and or gills injury associated with chronic exposure to Cd (Saravanan *et al*., 2011). The decreased levels of these parameters in this study are in agreement with the findings of Odo *et al.* (2017) who reported decreased levels of PCV, Hb and RBC in *C. gariepinus* exposed to Vestaline (Pendimethalin) herbicide for a period of 8 weeks. However, the increased RBC level was attributed to the blood cell reserve coupled with cell shrinkage owing to osmotic alterations of the blood by the action of the metal as this was not compensated with increase in the levels of Hb and PCV. Also, these results agree with report of Mekkawy *et al*. (2020) who observed reduced levels of haemoglobin, PCV and RBC in Cd-exposed fish. On the other hand, no significant differences observed in the levels of MCH, MCHC and MCV between the control and all treatment groups supplemented with combined vitamins over 8 weeks of exposure. Exposure to toxic heavy metal like cadmium often produce alteration in the levels of MCV, MCH, MCHC (Khalesi *et al*., 2017). Decreased levels of MCV, MCH complemented the fall in the levels of Hb, PCV and RBC over 8 weeks of exposure observed in treatment groups supplemented with combined vitamins. This is in accordance with the finding of Khalesi *et al*. (2017) who reported increased MCV level and decreased MCH of *Oreochromis niloticus* exposed to Cd (Khalesi *et al*., 2017). The MCHC level is a good indicator of red blood cell swelling Effiong *et al*. (2019). Thus, maintained levels of MCHC in the treatment groups comparable to the control invariably implies that combined vitamins supplements maintain the integrity of the RBC which would have been otherwise destructed by Cd deleterious nature.

Leucocytes and its differentials have been shown to be involved in immunological response to physiological stress and their numbers increase significantly with increase in stress. Such increase in WBC count occurs by increased rate of lymphoperisis or

enhanced release of lymphocytes from lymphoid tissues (Michael, 2018). Thus, non-significant differences observed in the levels of WBC and its differentials imply that the combined vitamins supplements were able to suppress Cd-induced stress.

4.2.3 Histopathological analysis of some organs of *C. gariepinus* exposed to sub-lethal concentrations of Cadmium chloride and treated with combined vitamins A, C and E for 8 weeks

Pathological changes in fish are strong indicators of exposure to environmental toxicants. Histopathology has been widely used as biomarker in assessing the health of fish exposed to Cd both in laboratory and field (Annabi *et al*., 2011). Klimaczewski *et al*. (2018) confirmed the histopathological evaluation in fish as an extremely valuable tool to determine the toxicopathic impacts of material because they may better reflect the real health state of the animal than other biomarker/diagnosis methods. The highest amount of cadmium in fish was reported in kidneys, liver and gills causing histological alterations in these organs, the severity of damage often increases with increase in concentration of toxicants (Turner *et al*., 2020). There were no histological alterations observed in T1 of all treatment groups of combined vitamins supplements which could be attributed to the antioxidant properties of the combined vitamins supplements (Mumtaz *et al*., 2020). However, the histological alterations observed in the livers and kidneys which include necrosis of the hepatocytes, distorted central vein, vacuolations, tubular vacuolations, shrinked Bowman's capsule, widened Bowman's capsule among others could be as a result of toxicity of Cd at higher concentration in these organs which surpasses the activities of combined vitamins supplements. This finding agrees with that of Capriello *et al*. (2019) who found damages in the brain tissue of zebrafish with severity increasing with increase in Cd concentration. Also, Morcillo *et al*. (2017) reported conclusive histological alterations in multiple organs of *Dicentrarchus labrax*

exposed to waterborne Cd. The absence of histological alterations in gills of all the treatment groups further buttress the potentials of the combined vitamins supplements most especially combined vitamins in ameliorating deleterious effects of Cd.

CHAPTER FIVE

5.0 CONCLUSION, RECOMMENDATIONS AND CONTRIBITION OF RESEARCH TO KNOWLEDGE

5.1 Conclusion

The result obtained in the growth parameters of *C. gariepinus* exposed to sub-lethal concentrations of Cd and supplemented with combined vitamins, it is clear that the combined vitamin C and E possess promising potentials in alleviating toxic effects of Cd on growth parameters of *C. gariepinus* as it had the least reduction in weight compared to other combined vitamins supplements explored in this study. The ability of the combined vitamins C and E supplement to restore some of the haematological parameters further buttress the point that the supplement could be used in alleviating toxic effect of Cd as it showed potential restorative effect on Cd-induced alterations in haematopoietic system alongside combined vitamins A, C and E supplement. However, the combined vitamins supplements possess more beneficial properties over short period of exposure to Cd as there were no significance differences in almost all haematological parameters between the control and the treatments of the combined vitamins supplements in the first 4 weeks of exposure

From results obtained in the histopathological evaluations, it is rational to infer that the combined vitamins C and E supplement is the best among others due to its ameliorative potential on liver and kidneys as it maintained preserved architecture of these organs at lower concentration of Cadmium chloride (T1) similar to other combined vitamins coupled with its beneficial effects on growth and haematological parameters

5.2 Recommendations

The use of varied concentrations of combined vitamin C and E should be considered in further researches as the ameliorative potentials of this combined vitamins supplement could be concentration-dependent.

Standardizing the concentration of the combined vitamins for ameliorating toxic effects of toxicants should be a major concern to be able to administer appropriate concentrations capable of ameliorating the effects of the toxicant when needed.

Further research should also be carried out to elucidate the effects of the combined vitamins on the physiology of the fish in the absence of the toxicant.

5.3 Contribution of Research to Knowledge

The thesis revealed that the combined vitamin supplements ameliorate toxic effects of cadmium (Cd) but only at lower concentration. The vitamin (A and C; A and E; A, C and E, and C and E) supplements on haemoglobin concentration (6.40±0.29 g/dL) and pack cell volume (19.20±1.08%) pack cell volume showed a significant ($P<0.05$) increase at higher concentration over 4 weeks exposure on *Clarias gariepinus*. Thus, haemoglobin concentration (3.70±0.33g/dL) and pack cell volume (11.10±2.01%) parameters were between the control and treatment groups.

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