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Hepatoprotective potentials of Garlic and Vitamin A Supplements on Cadmium-Induced liver damage in Albino Wistar Rats

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Abstract

Cadmium is a non-biodegradable substance with widespread presence in the environment. Cadmium is an important occupational and environmental pollutant, and among the top hazardous substances, toxic to various animal organs. The present study investigated the hepatotoxicity of cadmium and the hepatoprotective potentials of garlic and vitamin A supplements in Wistar Rats. The animals were grouped and treated as follows: Control group received normal saline; Cadmium (Cd) group received 3mg/kg body weight (bw) of cadmium chloride; Cd+VA+GL group received 3 mg/kg bw of cadmium chloride, 400 iu/kg and 40 mg/kg bw of vitamin A and garlic supplements respectively; Cd+VA received 3 mg/kg bw of cadmium chloride and 400 iu/kg bw of vitamin A supplement and Cd+GL group received 3 mg/kg bw of cadmium chloride and 40mg/kg bw of garlic supplement. Toxic effect of cadmium and the protective effect of nutritional supplements were determined on biochemical, oxidative and histological parameters. Results showed adverse liver-specific biochemical, oxidative and histological changes in Cd exposed and untreated rats. However, treatment with garlic and vitamin A supplements of cadmium exposed rats significantly reduced concentrations of some hepatic and oxidative parameter such as liver enzymes, MDA, bilirubin and attenuated histopathological lesions, indicating ameliorative potential in cadmium-induced toxicity.

Keywords: Cadmium, antioxidants, vitamin A, garlic, toxicants

Full length article **Corresponding Author*, e-mail:*cujowundu@futo.edu.ng*

1. Introduction

Among the top 20 hazardous substances cadmium is an important occupational and environmental pollutant that is toxic to various organs and human activities have markedly increased its distribution in the global environment [1,2.3]. The extreme toxicity of cadmium has biological effects at concentration smaller than almost any commonly found mineral. The mechanism for cadmium-induced toxicity maybe multifactorial because it exerts damaging effects on the cells of various systems and tissues, such as the respiratory tract, the urinary, cardiovascular, gastrointestinal, nervous systems and the bones by having direct or indirect effects on their functions. Such effects induce degeneration or even transmutation of the cells [4,5].

Several organs have been discovered to be affected by cadmium and recent studies have identified the testis to have been exceedingly sensitive to cadmium toxicity. As a matter of fact, cadmium and other toxicants like lead, mercury and estrogenic – based compounds such as bisphenols may account for the recent fertility problem resulting to reduce sperm count and testis functions in men living in developed countries [6]. Certain antioxidants and natural antioxidants have been implicated to alleviate cadmium – induced toxicity. Studies have demonstrated the antidote capacity of garlic extracts on heavy metal [7,8,9]and pesticide (paraquate) toxicity [10].Garlic has found use in many dishes for aroma, flavour and taste enhancement [11] and a good source of dietary phytochemicals with proven antioxidant properties and ability to modulate the detoxificant system [12,13.14]. Many researchers have engaged in the study of therapeutic and medicinal values of garlic. Such studies identify garlic to have antidiabetic, anticarcinogenic, hepatoprotective, anti – platelet aggregation, antibiotic effects and also antidote to heavy metal toxicity [15]. Importantly still, Vitamin A is an antioxidant effective in the prevention of oxidative damage by reacting with free radicals. It is found to chelate catalytic metals and also act on oxygen scavengers thereby removing the excessive free radicals generated from the human body [16].Antioxidant capacity of vitamin A and carotenoids is elicited by the hydrophobic chain of polyene units that can quench singlet oxygen, neutralize thiyl radicals and combine with and stabilize peroxyl radicals.

It is important to understand the dietary control of cadmium toxicity especially in regions such as Nigeria, a developing tropical country, with poorly developed environmental management strategy. The aim of the study was to investigate and evaluate the effect of Vitamin A and garlic supplements on cadmium – induced biochemical changes in albino rats. Experimental induction of cadmium toxicity in animal

models is essential for the advancement of our knowledge and understanding of various aspects of its pathogenesis and ultimately finding new therapies and cure. The information obtained in this study could be useful to patients suffering from cadmium toxicity and serve as a guide to dieticians and nutritionists.

2. Materials and methods

2.1. Supplements and Cadmium Chloride

Garlic and vitamin A supplements were purchased from a registered pharmaceutical store, Iykesurella Pharmacy Ltd. at No. 11 Mbaise Road, Owerri, Imo State Nigeria. Cadmium chloride (BDH) was obtained from the Laboratoty of Department of Biochemistry, Federal University of Technology, Owerri.

2.2. Experimental Design

Twenty-five (25) Wistar albino rats of average weight 139g were procured from the Zoological Garden Department of Agriculture, University of Nigeria Nsukka. The rats were divided into five groups of five rats and housed in five laboratory cages. The rats were allowed free access to Vital growers mesh and clean water. Acclimation lasted for seven days at the animal house of Department of Biochemistry, Federal University of Technology, Owerri (FUTO), Nigeria. Department of Biochemistry, Ethics Committee gave approval on reference number FUTO/BCH/EC/2018/14. The rats were handled according to the guidelines of the National Institute of Health [17]. After acclimatization, the rats were treated for twenty eight days as follows:

Group 1 (Control): received rat pellets and normal saline (No cadmium chloride was administered). Group 2 (Cd): received only 3mg/kg body weight (bw) of cadmium chloride. Group 3 (Cd+VA+GL) received 3 mg/kg bw of cadmium chloride, 400 iu/kg bw of vitamin A supplement and 40 mg/kg bw of garlic supplement. Group 4 (Cd+VA) received 3 mg/kg bw of cadmium chloride and 400 iu/kg bw of vitamin A supplement. Group 5 (Cd+GL) received 3 mg/kg bw of cadmium chloride and 40mg/kg bw of garlic supplement. No death was recorded throughout the period of the experiment.

2.3. Sample Collection

Blood samples were collected via ocular puncture into test tubes and this was allowed to stand for 30 min to enable clotting and spurn with a centrifuge at 3000 × g for 15 min. Serum samples obtained were stored in a refrigerator at 4°C until needed for biochemical analyses. Afterwards the rats were sacrificed and liver organ excised, rinsed in ice cold normal saline. The excised liver samples were homogenized in KCl buffer (1.15 %) in EDTA at pH 7.4 and centrifuged for 20 min at 250 × g. Aliquots of supernatant from the homogenate was used to assay oxidative stress parameters and portions of whole liver sample were stored in 0.3 % formaldehyde for histopathology.

2.3. Determination of liver function assay

Commercial kits provided by Bio Merieux France were used to assay for the activities of Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) as described by Reitman and Frankel [18]. The method described by Rec [19] was used to determine alkaline phosphatase (ALP) activity using commercial kits provided by Bio Merieux France. The method of Jendrassik and Grof [20] was used to determine total bilirubin. Total protein and albumin were determined as described by Tietz [21] and Doumas et al. [22] respectively.

2.4. Determination of oxidative parameters

To describe lipid peroxidation, the concentration, the end product of lipid peroxidation malondialdehyde (MDA) was determined by the method of Wallin et al. [23]. Serum vitamin C (ascorbic acid) concentration was determined as described by Omaye et al. [24]. Briefly: To 0.5ml of plasma, 0.5ml of water and 1ml of TCA were added, mixed thoroughly and centrifuged. To 1ml of the supernatant, 0.2ml of DTC reagent was added and incubated at 37 ^{0}C for 3 h. Finally, 1.5 ml of H_2SO_4 was added, properly mixed and allowed to stand at 25 ^{0}C for 30 min. The colour developed was read at 520 nm in spectrophotometer.

2.5. Liver histological studies

Rat liver samples which were fixed in formal saline were subjected to dehydration, clearing (dealcoholization), and infiltration processes and further embedded in paraffin as described by Okoro[25]. The samples were further treated to serial sectioning at defined thickness and stained using hematoxylin and eosin as described by Conn et al. [26]. Finally, using a light microscope adjusted to appropriate magnification (100× and 400×), the tissue sections were examined and photographed.

2.6. Statistical analysis

Results were expressed as mean and standard deviation (mean ± standard deviation) and determined for all the parameters. The data were analyzed by one-way analysis of variance (ANOVA) using SPSS program, version 18, followed by Duncan (Multiple Range-test). Values were considered significant at $p<0.05$.

3. Results and Discussions

Figures 1 and 2 shows significant increase in AST and ALP activities of Cadmium Chloride exposed and untreated Cadmium group (Cadmium) compared to control, Vitamin A and Garlic groups. However, single treatments with Vitamin A and Garlic supplements showed equal potentials to reduce AST activities, whereas Vitamin A treated group showed significant ability to reduce ALP activities compared to others. Similarly, Figure 3 showed that ALT activities were reduced non-significantly in Vitamin A (VA) and Garlic (GL) singly treated groups.

The result of serum albumin concentration (Figure 4) showed significant decrease serum albumin of Cadmium group when compared to control and supplement treated groups. Figure 5 shows significant decrease in serum total protein of Cadmium group compared to the normal control group Cd+VA+GL and Cd+GL and decreased non-significantly compared to Cd+VA. In Figure 6, total bilirubin increased significantly in Cadmium group when compared with the control group and others. However, the single and combined Vitamin A and Garlic supplement treated groups recorded a significant decrease in bilirubin concentration. Figure 7 showed a significant increase in concentration of malondialdehyde of Cadmium group when compared to control and supplement treated groups. Also, the result showed that the combined supplement and garlic groups recorded the most significant reduction in MDA concentration. Figure 8 showed a

significant decrease in serum concentration of vitamin C of Cadmium group when compared to control group. The groups treated with garlic and vitamin A showed a significant increase in concentration of vitamin C with VA treated group showing the highest concentration of Vitamin C.

Cross section of the liver of albino rats (Figure 9) stained with hematoxylin and eosin (H&E) for histological assessment of liver. The Control group showed normal structural morphology in central area, sinusoids and central vein as well as normal hepatocytes. Cadmium group showed various degenerative changes compared to the control group. The central vein showed dilatation and hydropic degeneration characterized by marked loss of uniformity and regularity of hepatic tubules. It presented a large number of inflammatory cells infiltrating the portal tract with congestion and dilatation of the portal vein radical as well as sinusoidal obliteration. Cd+VA+GL group presented decreased dilatation and hydropic degeneration. Cd+VA+GL group presented a decreased number of inflammatory cells infiltrating the portal tract. Cd+GA group showed restoration of structural morphology in central area, sinusoids and central vein as well as normal hepatocytes. Cd+GL group also showed restoration of structural morphology in central area, sinusoids and central vein as well as normal hepatocytes

Discussion: Cadmium (Cd), and metals such as arsenic, lead, mercury, and chromium, have no physiological function and is usually grouped as a toxicant [27,28,29]. A large number of different forms of environmental exposure to cadmium have been presented over the years and this is as a result of anthropogenic activities of man [30]. Human exposure to Cd comes via food, water, and cigarette smoke. On absorption Cd is very well retained in the human tissues and organ and can remain throughout life with a half-life of 25–30 years.The recorded increases in activities of serum AST and ALP of Cadmium Chloride exposed and untreated group when compared to control group is an indication of liver damage due to cadmium toxic action. Studies have shown to acute Cd exposure can induce increased levels of liver damage markers such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) in the blood and may further increase the incidence of nonalcoholic hepatitis and fatty liver [31,32]. The dietary supplements of Vitamin A and Garlic administered single and combination treatments in rat groups presenting reduced activities of liver marker enzymes indicates ameliorative potentials of these supplements with single Vitamin A treatment showed greater potential to reduce cadmium induced liver damage. These supplements may have induced increased elimination of Cadmium, thereby not allowing it to accumulate in tissues. Studies have shown that Cadmium accumulation increases liver toxicity and induces liver damage. Cadmium accumulation increases the expression of ALT, AST, GGT, MDA, and peroxidase activities [32]. Furthermore, garlic is a potential source of glutathione and has the ability to induce biosynthesis of metallothionein and these compounds protect against potential oxidative damage to tissues exposed to cadmium and lead or cadmium. Metallothionein (MT) is a cysteine (Cys)-rich protein that acts as an important antioxidant and chelator of heavy metals [33]. Metallothioneins are crucial in the metabolism of essential metals and in protection against the toxicity of metals. The elevated liver enzyme activities (AST, ALT and ALP) recorded in this cadmium chloride induced toxicity study corroborates the reports of other studies which recorded increased liver marker enzymes [34,35,36].The result of this study showed significant reduction of serum albumin and total protein concentration in Cadmium exposed group when compared to control and garlic and vitamin A supplement treated groups. As cadmium is absorbed into animal systems, it is transported in the bloodstream through erythrocytes and albumin and is then accumulated in different organs such as the kidneys and liver. In the liver cadmium can cause liver damage which leads to disturbed liver function. Liver damage can be confirmed by elevated liver function marker enzymes and histopathological findings. Liver damage is also accompanied by reduced liver function such as reduced protein synthesis [34,36,37].

Furthermore the recorded significant decrease in e serum total protein and albumin of rats exposed to cadmium and not treated with garlic and vitamin A supplements can be attributed to derangement in hepatocyte functions leading to decreased cytochrome P-450 activity and inhibition in protein metabolism in the liver [38,39]. Similarly, the present study observed increased serum concentration of total bilirubin cadmium exposed animals and not treated with garlic and vitamin A supplement indicates hepatic dysfunction which correlates with the oxidative damage in the liver due to oxidative stress [40,41]. However, animal group administered singly and combined garlic and vitamin A supplements showed significant reversal of the effect, as the serum total bilirubin of the treated rats tended to the normal control. This result agrees with the report of other researchers [42,43].

Furthermore, the result of malondialdehyde (MDA) concentration in the present showed a significant increase in concentration in animals of Cadmium group when compared to control and supplement treated groups. This indicates oxidative stress resulting in increased lipid peroxidation. Studies indicate that cadmium interference with cellular components can cause increased generation of free radicals such as reactive oxygen species (ROS) in Cd-exposed animals [43]. This was evident by the noticeable elevation witnessed in the levels of lipid peroxides in liver. However, the combined supplement and garlic groups recorded the most significant reduction in MDA concentration, indicating ameliorative potentials of the supplements. Ujowundu et al. [44] reported increased MDA concentration in uproot herbicide exposed animals and implicated to lipid peroxidation induced by free radicals generated by the herbicide.

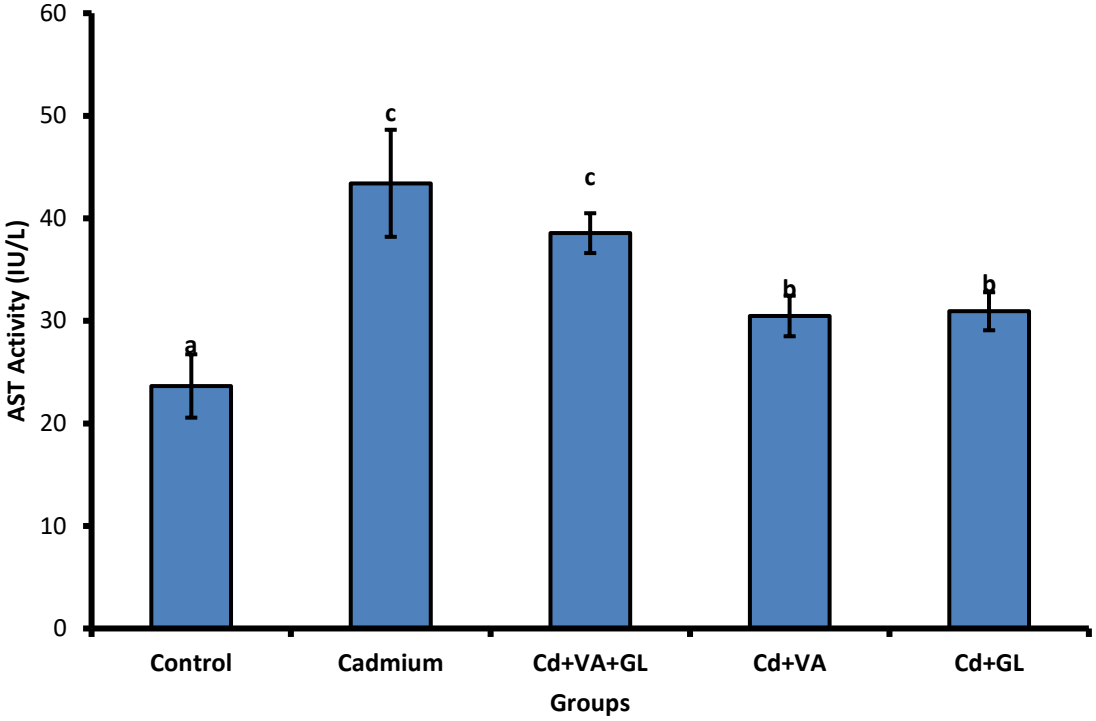

Figure 1: Serum AST activities of Wistar rats exposed to Cadmium chloride and treated with dietary supplements-Vitamin A (VA), Garlic (GL) singly and Combined (VA+GL). Barrepresent mean ± standard deviation of quadruple determinations. Bars with different letters are significant at $p < 0.05$. Cd represents Cadmium.

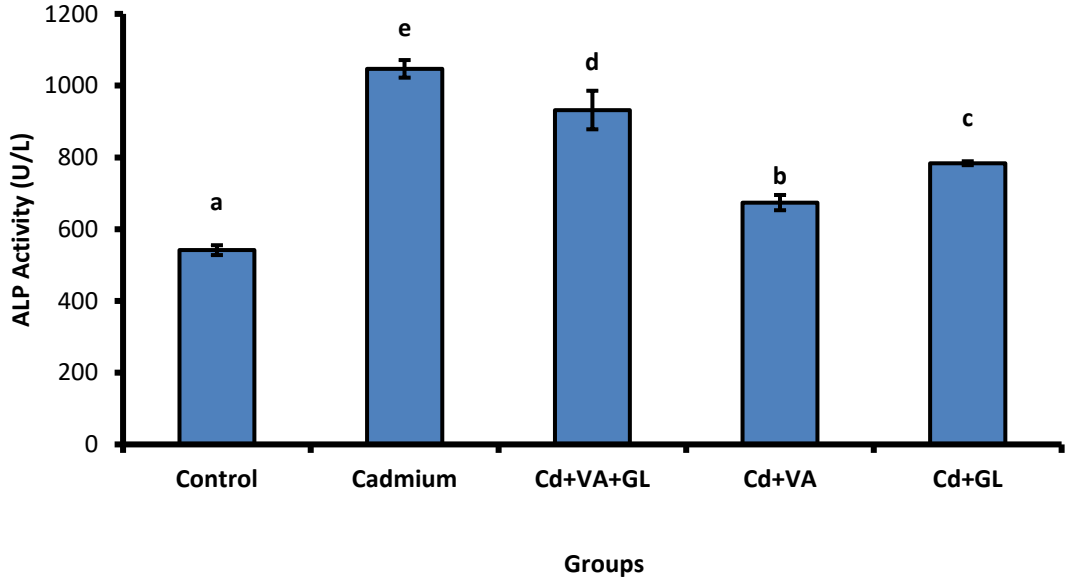

Figure 2: Serum ALP activities of Wistar rats exposed to Cadmium chloride and treated with dietary supplements-Vitamin A (VA), Garlic (GL) singly and Combined (VA+GL). Barrepresent mean ± standard deviation of quadruple determinations. Bars with different letters are significant at $p < 0.05$. Cd represents Cadmium

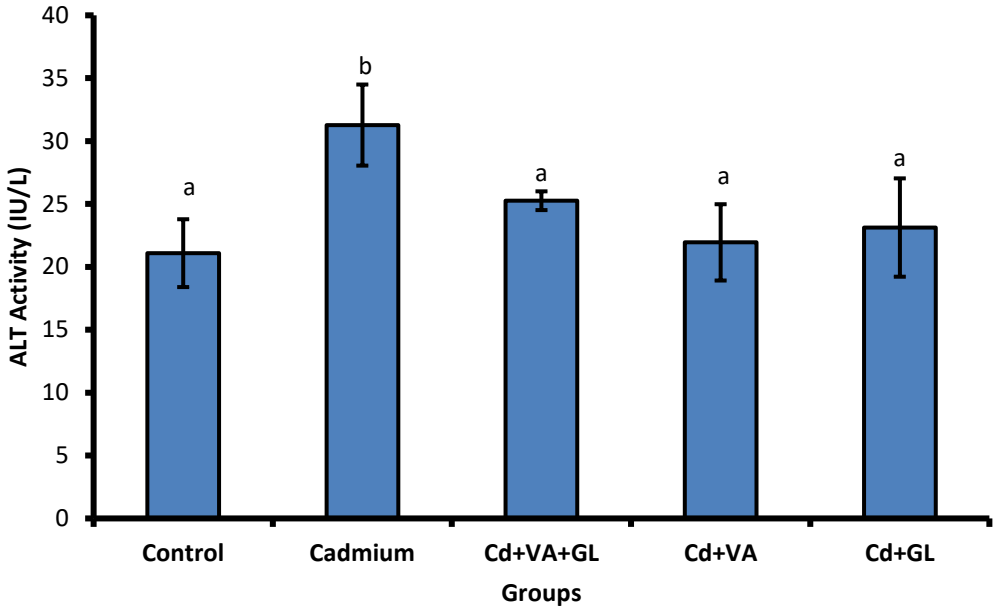

Figure 3: Serum ALT activities of Wistar rats exposed to Cadmium chloride and treated with dietary supplements-Vitamin A (VA), Garlic (GL) singly and Combined (VA+GL). Barrepresent mean ± standard deviation of quadruple determinations. Bars with different letters are significant at $p < 0.05$. Cd represents Cadmium

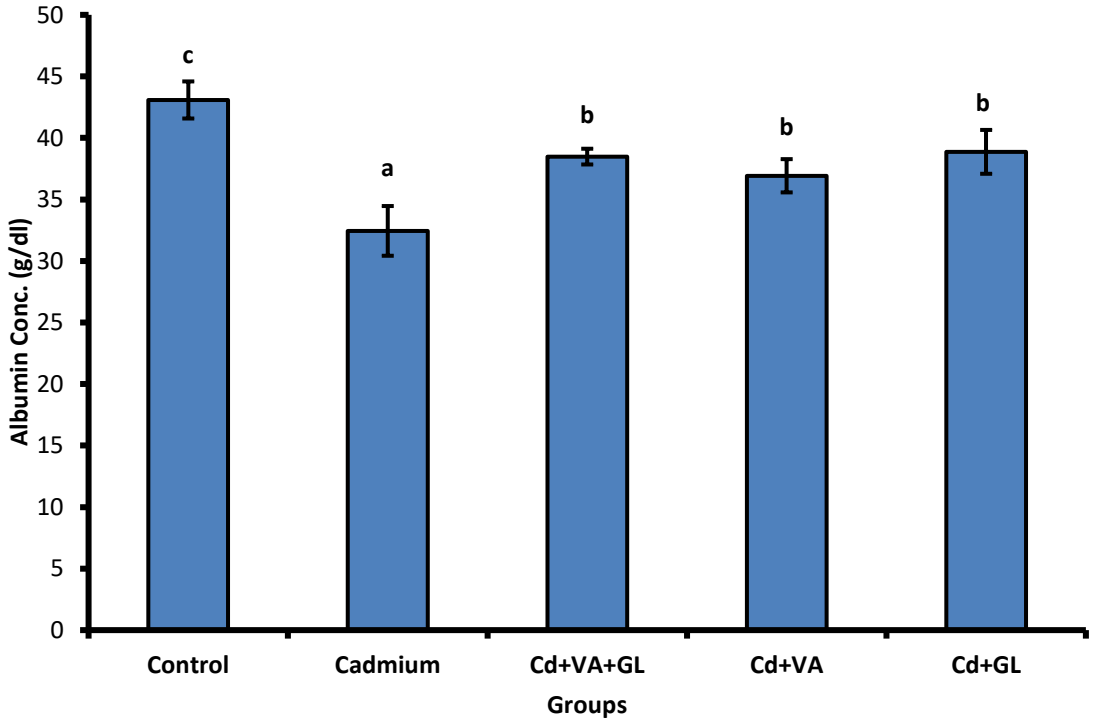

Figure 4: Concentration of albumin of Wistar rats exposed to Cadmium chloride and treated with dietary supplements-Vitamin A (VA), Garlic (GL) singly and Combined (VA+GL). Barrepresent mean ± standard deviation of quadruple determinations. Bars with different letters are significant at $p < 0.05$. Cd represents Cadmium.

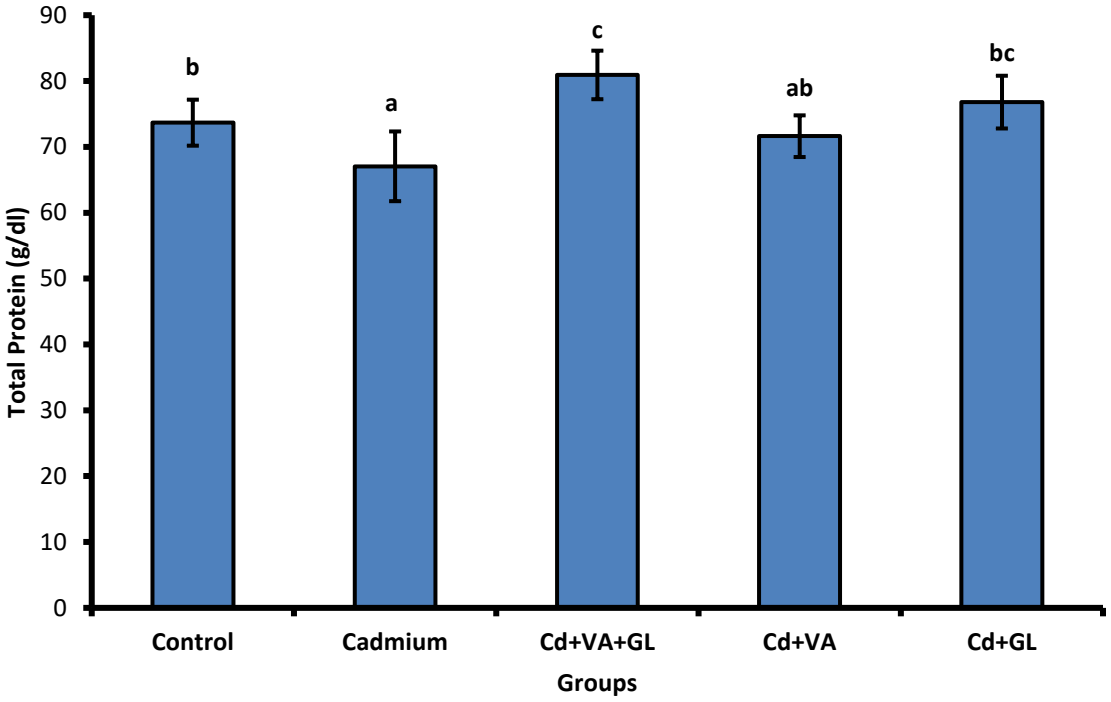

Figure 5: Concentration of total protein of Wistar rats exposed to Cadmium chloride and treated with dietary supplements-Vitamin A (VA), Garlic (GL) singly and Combined (VA+GL). Barrepresent mean ± standard deviation of quadruple determinations. Bars with different letters are significant at $p < 0.05$. Cd represents Cadmium.

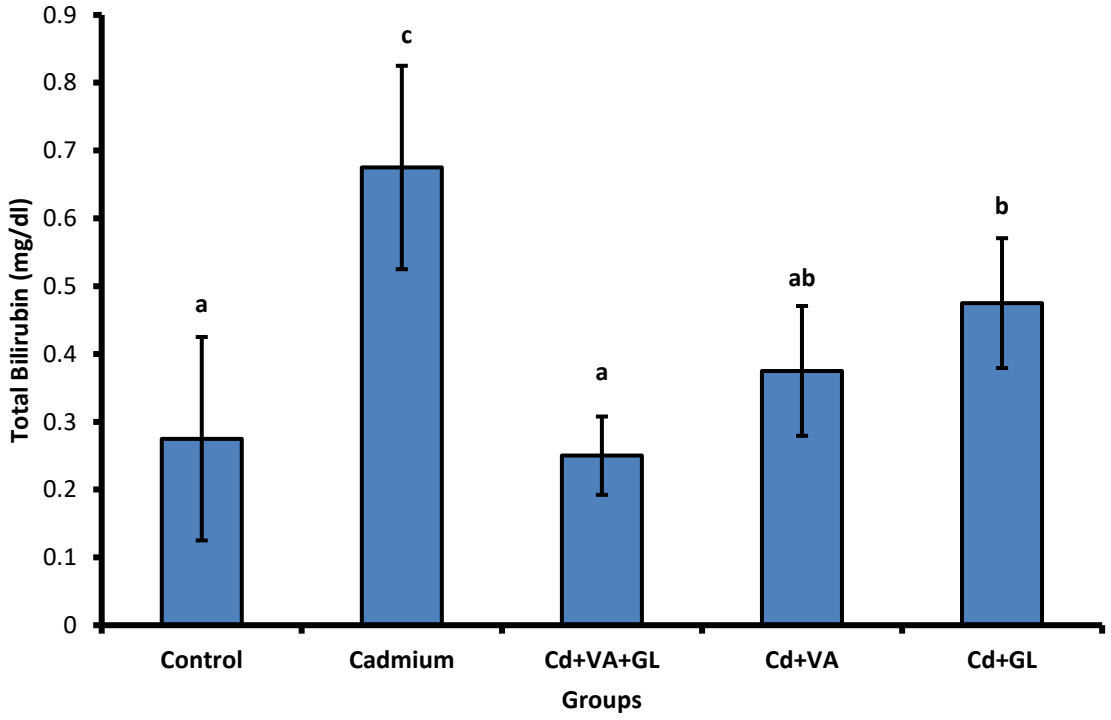

Figure 6: Concentration of bilirubin of Wistar rats exposed to Cadmium chloride and treated with dietary supplements-Vitamin A (VA), Garlic (GL) singly and Combined (VA+GL). Bar represent mean ± standard deviation of quadruple determinations. Bars with different letters are significant at $p < 0.05$. Cd represents Cadmium.

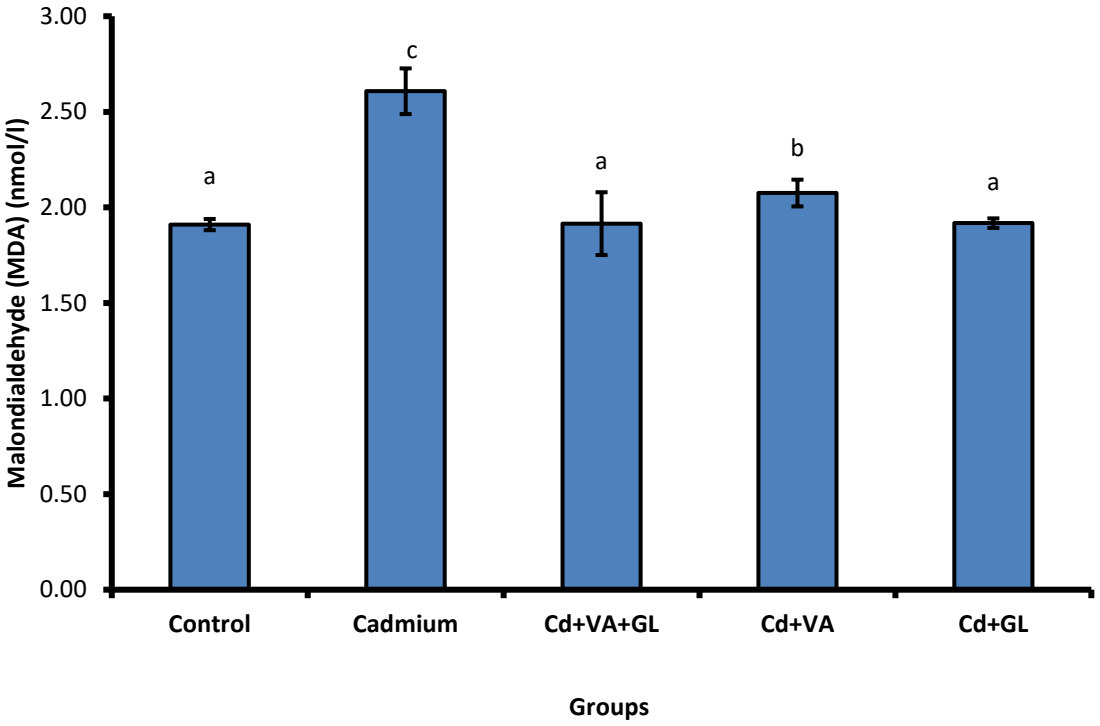

Figure 7: Concentration of malondialdehyde of Wistar rats exposed to Cadmium chloride and treated with dietary supplements-Vitamin A (VA), Garlic (GL) singly and Combined (VA+GL). Bar represent mean ± standard deviation of quadruple determinations. Bars with different letters are significant at $p < 0.05$. Cd represents Cadmium.

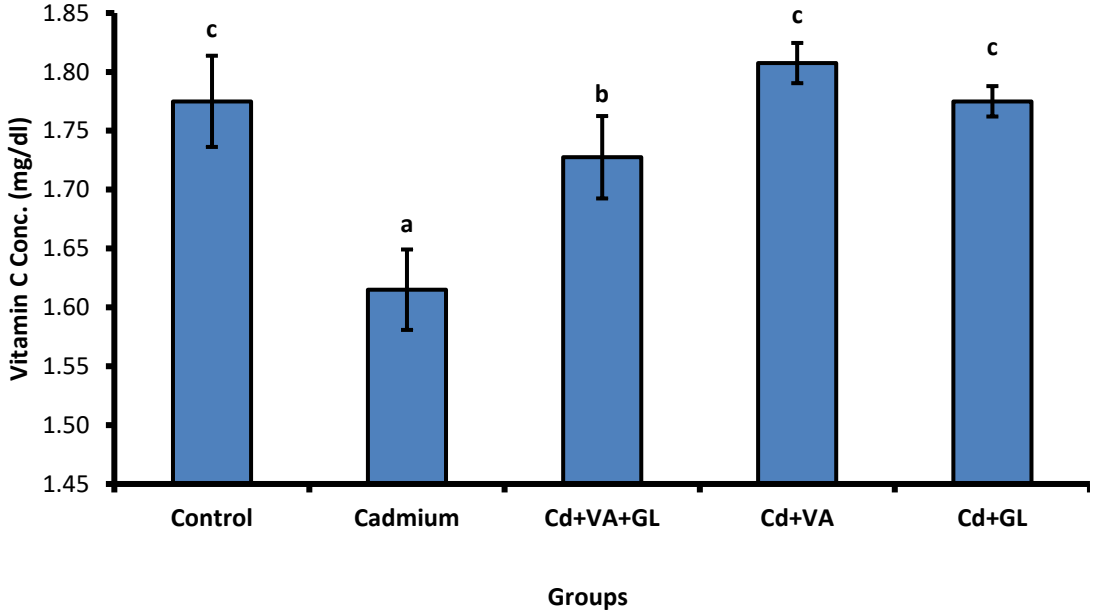

Figure 8: Concentration of vitamin C of Wistar rats exposed to Cadmium chloride and treated with dietary supplements-Vitamin A (VA), Garlic (GL) singly and Combined (VA+GL). Barrepresent mean ± standard deviation of quadruple determinations.

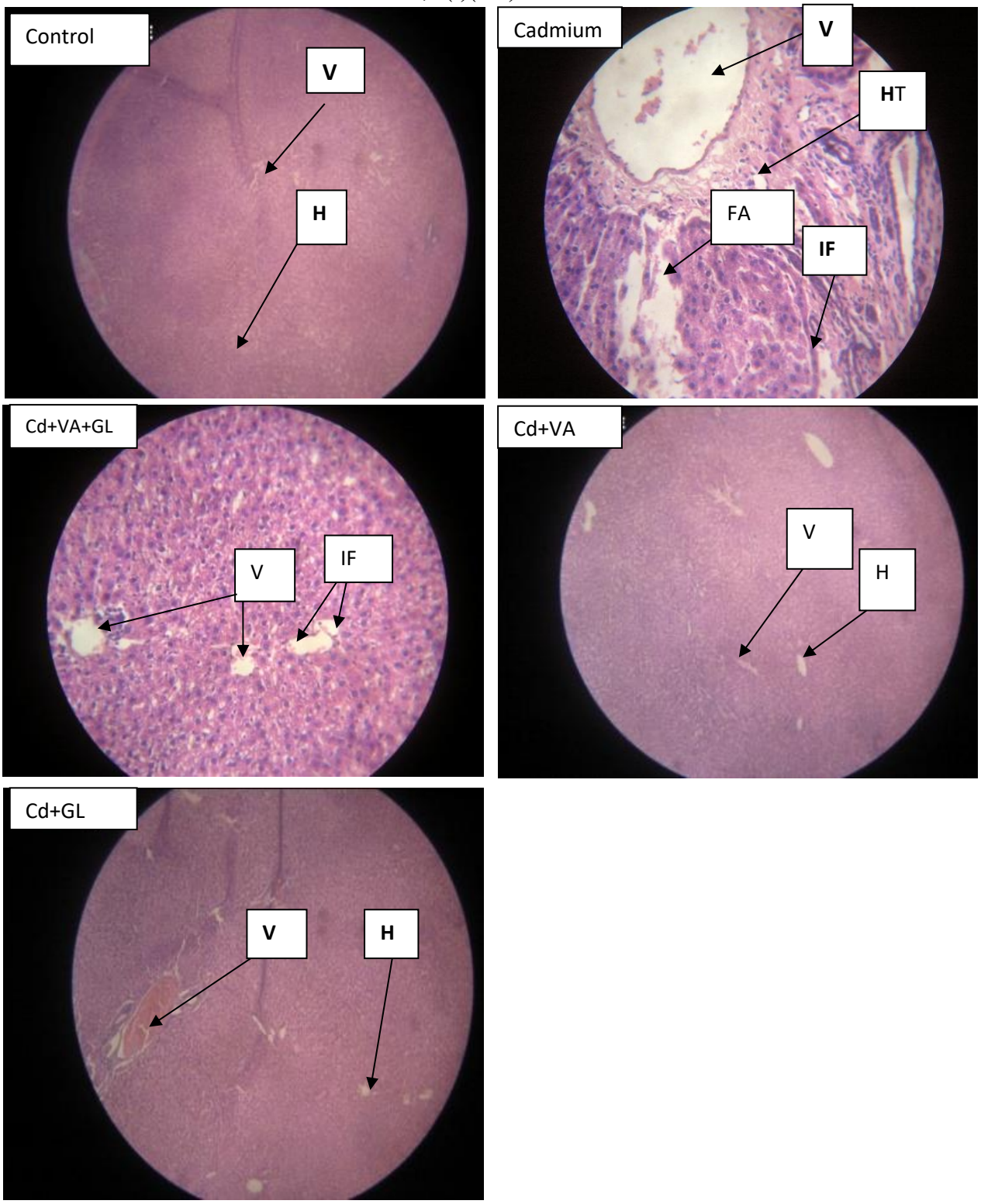

Figure 9: Histological assessment of liver sections stained with hematoxylin and eosin of Wistar rats exposed to Cadmium chloride and treated with dietary supplements-Vitamin A (VA), Garlic (GL) singly and Combined (VA+GL). Labels: V indicates central vein, H indicates hepatic parenchyma, F indicates fatty materials, IF indicates inflammatory cells.

Serum vitamins C concentration decreased significantly in the cadmium group than in the control group. The result indicated that vitamin C may have been used up to moping the free radicals generated by cadmium-induced toxicity. Previous reports showed that cadmium-induced toxicity has been alleviated by administration L-ascorbic acid [45].These biochemical results were confirmed by the observations recorded from the histopathological assessment of the liver which revealed that Cadmium chloride stimulated hepatocellular injuries in exposed rats. The histopathological sections of the various groups showed cadmium-induced degenerative changes such as severe hepatocytenecrosis, fatty changes, degenerative signs and inflammatory cell infiltrations in cadmium group compared to the normal control group. These results were consistent with the acute and chronic effects of cadmium reported earlier [46,47]. However, significant restoration in the structural pattern of the liver sections were recorded in the groups exposed to cadmium and treated with and treated with Vitamin A (VA) and Garlic (GL) dietary supplements in single and combined forms.

4. Conclusions

In conclusion, administration of vitamin A and garlic dietary supplements could provide significant protection against cadmium-induced toxicity in animals in its free radical-scavenging capacities, attenuating oxidative induced hepatic damage.

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