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Oxidative Stress and Endogenous Antioxidant Vitamins: Relationship in Carbon Tetrachloride (CCl_4) Induced Experimental Rats Model

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Abstract: The role of oxidative stress in the pathogenesis of various diseases has been established. Maintaining the steady balance between reactive oxygen species and natural antioxidants is a crucial factor, and could probably serve as a major mechanism in preventing damage by oxidative stress induced by toxic substances. A comparative study and correlation analysis of malondialdehyde (MDA), serum vitamins (A, E and C) and the redox active metals (Cu, Fe and Zn) in CCl_4 induced oxidative stress in albino rats with various doses of CCl_4 were determined. The serum levels of oxidative stress markers were compared between the normal and test groups. The result showed a significant increase in the serum vitamins A and E in the CCl_4 induced oxidative stress rats compared to control rats ($P<0.05$). However, the serum level of vitamin C was not statistically different compared to control rats ($P>0.05$). MDA correlated strongly and positively with vitamin C ($r=0.94$), Vitamin A($r=0.87$), Vitamin E and Fe($r= 0.90$ and 0.87 respectively) while the serum MDA showed a weak positive relationships with Zn($r=0.02$) and Cu($r=0.47$).Thus, the increased serum levels of some antioxidant vitamins and the redox metals in the experimental rats as observed in this study could be a compensatory regulation in response to induced oxidative stress by CCl_4.The evidence from this study lends credence to the ability of endogenous antioxidants to counter the effects of free radicals generated by hepatotoxic substances and maintain the steady-state of prooxidant-antioxidant balance in the system.

Keywords: Oxidative stress, Lipid Peroxidation, Antioxidant vitamins, redox metals, CCl_4.

INTRODUCTION

Oxidative stress (OS) is the condition that occurs when the steady-state balance or equilibrium of cellular prooxidants to antioxidants is shifted in the direction of the former, creating the potential for cellular destruction; damaging all components of the cell, including proteins, lipids and DNA [1].This imbalanceis between generation and elimination of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in the system. Prooxidants are free radicals, atoms, or clusters of atoms with a single unpaired electron capable of interfering with biochemical activities due to high reactivity [2]. ROS are continuously produced during normal physiologic events and are removed by antioxidant defence mechanisms.

A Cumulative systems' oxidative damage from cellular ROS results in provocation of various diseases and cellular disorders [3]. Kim *et al*. [4] also suggested that increase production of these toxic free radicals and OS can also be induced by a variety of factors such as radiation, drugs or exposure to heavy metals and other xenobiotics (e.g. carbon tetrachloride). The carbon tetrachloride (CCl_4) intoxication in animals is an example of the experimental model that mimics oxidative stress in many human pathophysiological conditions [5]. A study demonstrated CCl_4 cellular intoxication causes free radical generation in many body tissues such as liver, kidney, heart, lung, brain and blood [6]. The toxic effect of CCl_4 probably depends on the formation of the trichloromethyl radical ($CCl_3^{\bullet}$) by hepatic microsomal cytochrome P450which react with oxygen, to form the more toxic trichloromethyl peroxyl radical ($CCl_3O_2^{\bullet}$) [7]. This free radical and other related reactive species may cause oxidative stress, which accounts forthe major network changes of cellular metabolism, increases the serum marker enzymes, DNA fragmentation, and destruction of the cells by lipid peroxidation [8].

Membrane lipids are reported as one of the primary targets of ROS. Hydroperoxides have toxic effects on cells both directly and through degradation of highly toxic hydroxyl radicals. They may also react with transition metals like iron or copper to form stable aldehydes, such as malondialdehyde (MDA), that damage cell membranes [9]. It has been reported that peroxyl radicals can remove hydrogen from lipids, producing hydroperoxides that further propagate the free-radical pathway.The accumulation of lipid peroxides introduces hydrophobic moieties and alters membrane permeability and cell function which causes the loss of hepatic integrity and depressed hepatic function resulting in hepatotoxicity and congestive hepatic failure [10]. MDA has been documented as a primary biomarker of free radical-mediated lipid damage and oxidative stress [11]. The products of lipid peroxidation (mainly in the presence of transition metal ions) initiate further free radical chain reactions and can cause oxidative damage to proteins and DNA [12].

The essential mineral nutrients zinc (Zn), copper(Cu), Iron(Fe) and selenium (Se) provide an antioxidant function to animal cells by very different mechanisms.These elements are mobilised to inhibit ROS production by being co-factors of antioxidative enzymes (copper, manganese, and zinc are co-enzymes of Superoxide dismutase(SOD), selenium is co-enzyme of Glutathione peroxidase (GPx), and iron is co-enzyme of catalase). On the other hand, relatively high concentrations of investigated elements may initiate free radical reactions [13]. Vitamins E, C and A, non-enzymatic antioxidant structures are essential for neurological antioxidant and neuroprotective function [14].

Historically, an antioxidant has been described as any substance that interferes with the reaction of any substance with dioxygen [15]. The more mechanistic definition states that an antioxidant is any substance that hinders a free radical reaction [16]. The aim of this research was to study the relationship and compare between oxidative stress marker (malondialdehyde), antioxidant vitamins (A, C and E) and redox metals (zinc, copper and iron) in CCl_4 induced oxidative stress rats, as the importance of free radicals and ROS has attracted increasing attention over the past decade.

MATERIALS AND METHODS

Experimental design

Thirty (30) albino rats of both sexes (130-190g) were divided into two phases. They were allowed to acclimatize for one week and given access to food and water *ad-libitum* following the method of *Klein and Bayne [17].The protocols of the study were according to international Test guidelines(TG407) [18] and also* the National Institute of Health Guide for the care and use of laboratory animals [19].

Phase I

In this phase, each of the ten (10) rats was intraperitoneally induced witha single dose of 120mg/kg CCl_4 according to Alhassan *et al.*[20]. After forty-eight (48) hours, two animals were randomly selected and used as an affirmative test to ascertain the presence of hepatotoxic effects of CCl_4.The animals were sacrificed (anaesthetized with light ether anaesthesia) after one week of induction and the serum was used for determination of MDA, endogenous antioxidant levels and some redox metals.

Phase II

In the second phase, 20 rats weredivided into four groups of five rats each. Group I serves as control while groups II, III and IV were induced with 100,120 and 150mg/kg doses of CCl_4respectivelyaccording to Alhassan *et al.* [20]. The animals were sacrificed humanly and the serum antioxidant vitamins, MDA and redox metals were determined and used for comparative study.

Serum and liver sample collection

The animals were sacrificed humanelyby decapitation and the blood collected immediately into plain sample bottles. The blood was allowed to clot and then centrifugedat 3000 rpm for 10 minutes. The serum was then collected using Pasteur pipette and transferred to a smaller plain sample bottle. The animals were then dissected vertically in the abdomen using the sharp blade and the liver removed and carefully placed in a bottle containing 10% formol-saline (formalin) as a fixative. The liver samples were then taken to the department of histopathology, Aminu Kano Teaching Hospital, Kano for histopathological analysis.

Methods

Serum Malondialdehyde (MDA) was determined by the Method of Okhawa *et al*. [21]; Vitamin A by Method of Bessey *et al* [22]; Vitamin E by Fabianek *et al.* [23] and forVitamin C, the phenyl-hydrazine spectrophotometer method was used.Determination of serum Copper, Zinc and Iron metals was done by Atomic Absorption Spectrophotometer (AAS), (AA670G, Shimadzu, Japan) by AOAC [24] method.

Histopathology

Histopathological examinations of the liver samples were carried out at the department of histopathology, Aminu Kano Teaching Hospital, Kano. Haematoxylin and Eosin (H and E) staining method [25] was used.

STATISTICAL ANALYSES

GraphPad Instat 3 statistical software (2000) version was used for statistical analysis. Data were expressed as the mean ± standard deviation. Pearson's

Correlation coefficient (r) was used for correlation; comparison of mean MDA, vitamin A, C and vitamin E levels among groups by analysis of variance (ANOVA). P<0.05values were considered statistically significant.

RESULTS AND DISCUSSIONS

The study investigated the association and comparison of the oxidative stress marker, malondialdehyde (MDA), the serum vitamins (A, E, C) and the redox active metals (Cu, Fe and Zn) in CCl_4 induced oxidative stress in albino rats, and the results are presented in the figures below.

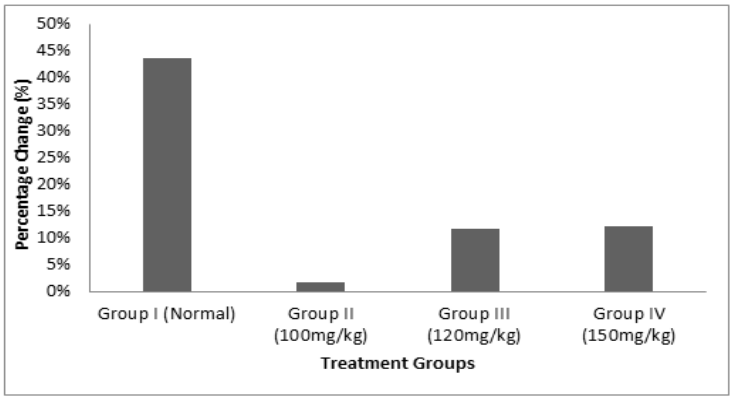

Fig-1: Percentage Change in Weight of Experimental Rats Before and After CCl_4 induction

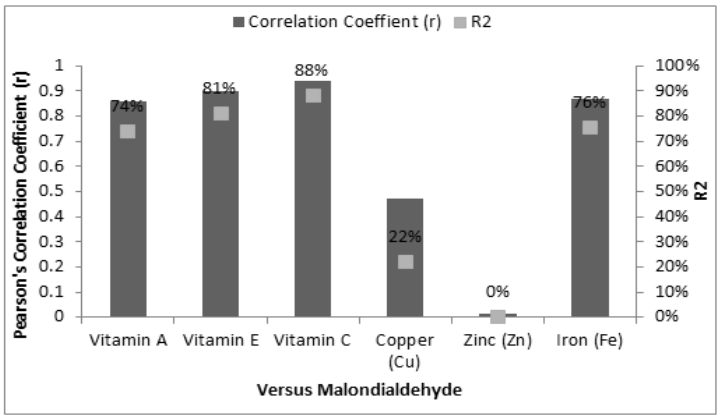

Fig-2: Correlation Coefficient (r) and Regression (R^2) values of Malondialdehyde with various Antioxidant Markers of CCl_4 Hepatotoxicity

The correlational analysis revealed the strong positive correlation between MDA with vitamin C, E and A in C>E>A order. It was notedthat the oxidative stress marker, MDA showed a positive relationship with redox ions;iron (Fe) being strongest, followed byserum copper.A weak relationship was observed in the case ofserum Zn level.

The results of the level of malondialdehyde (MDA), vitamin A, vitamin C and vitamin E and redox active metals (copper, zinc and iron) of CCl_4, induced oxidative stress in albino rats were presented in figure 3.

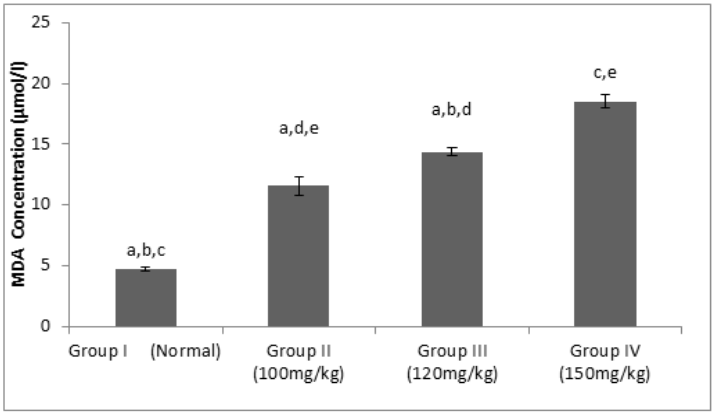

Fig-3: **Mean Values of Malondialdehyde of Experimental Animals Induced With Different Doses Of CCl_4. Values having the same superscript are significantly different at ($p<0.05$), n=5**

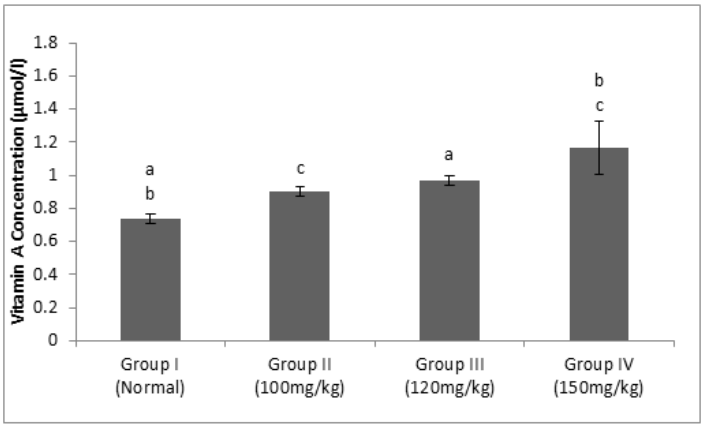

Fig-4: Mean Values of Vitamin A of Experimental Animals Induced with Different Doses of CCl_4. Values having the same superscript are significantly different at ($p<0.05$), n=5

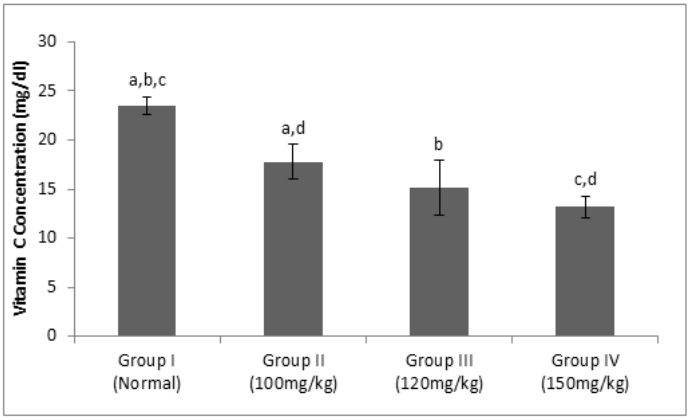

Fig-5: Mean Values of Vitamin COf Experimental Animals Induced with Different Doses of CCl_4. Values having the same superscript are significantly different at ($p<0.05$), n=5

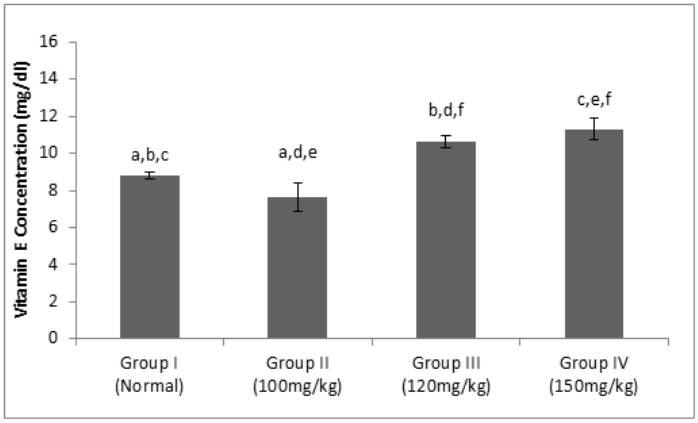

Fig-6: Mean Values of Vitamin EOf Experimental Animals Induced with Different Doses of CCl_4. Values having the same superscript are significantly different at (p<0.05), n=5

The level of MDA in CCl_4 –induced rats showed the remarkable increase with the increase in dose of the toxicant. There was a significant difference (P<0.05) between MDA of normal/control with groups II and III (Figure 3). The increased levels of serum MDA signalled an increase of cellular lipid peroxidation, an important pathogenic process that disrupts biomembranes functions and integrity.The level of serum vitamin A also increased with the increase of CCl_4 – dose and significant difference between group I and III and IV and group I and IV (figure 4) (P<0.05) of the vitamin was observed.

Serum level of vitamin C showed a decrease pattern with the increase of CCl_4 dose administered. It showed a significant difference (P<0.05) when the group I compares with II, III and IV (figure 5). The decrease in the level may be attributed to its antioxidant scavenging capacity against CCl_4 – generated free radicals.Serum vitamin E show decrease when group II compares to control (P>0.05) but the level increases with administration of larger doses of CCl_4. There was the significant increase between groups II and III, also group III with the group I and IV (P<0.05). It can, therefore, be postulated that this vitamin may likely elicit an effective response to CCl_4 induced oxidative damage.

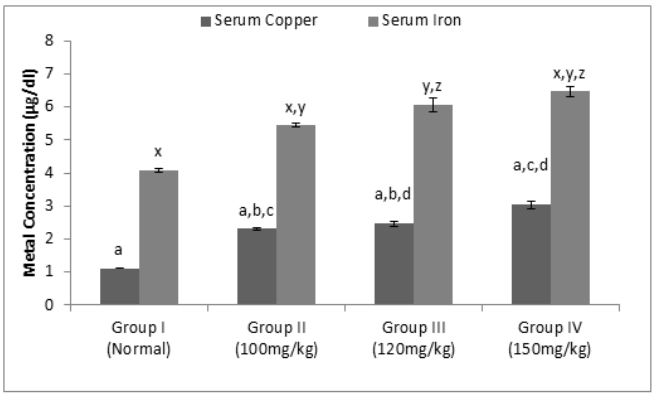

Fig-7: Serum Copper and Iron of Experimental Animals Induced with Different Doses of CCl_4. Values having the same superscript are significantly different at (p<0.05), n=5

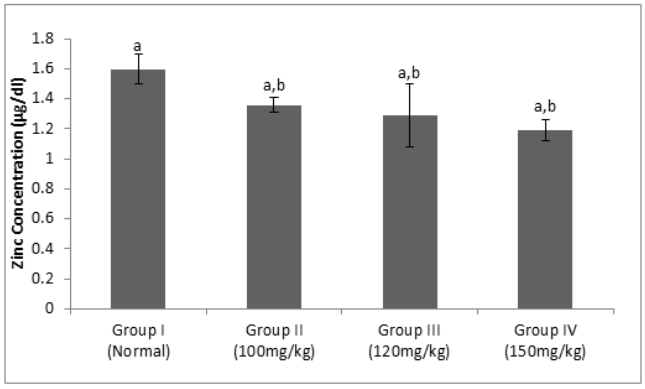

Fig-8: Serum Zinc of Experimental Animals Induced with Different Doses of CCl_4. Values having the same superscript are significantly different at ($p<0.05$), n=5

There was the remarkable elevation of serum copper level in group I compared with groups II, III and IV ($P<0.05$) (figure 7). The increase found to be dose-dependent.Likewise, Iron (Fe) shows a similar pattern with copper ($P<0.05$). However, Zinc (Zn) showed asignificant ($P>0.05$) decrease withthe increase of the dose of CCl_4 administered.

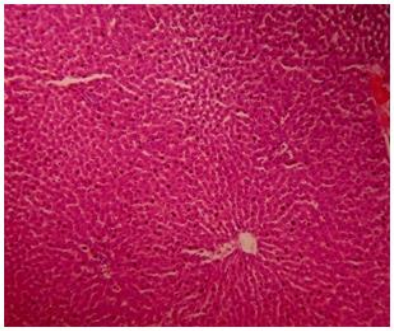

Fig-9: Normal (Group I) Showing normal hepatocytes

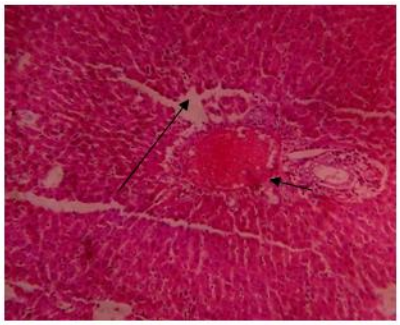

Fig-10: Group II-induced with 100mg/kg CCl_4showing mild necrosis

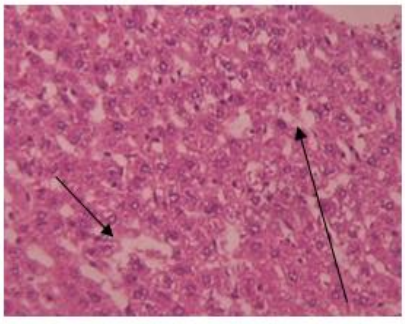

Fig-11: Group II-induced with 120mg/kg CCl_4 showing liver necrosis

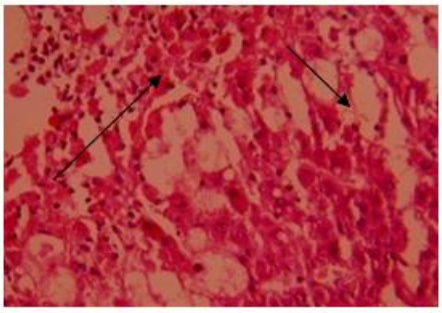

Fig-12: Group II-induced with 150mg/kg CCl_4 showing mild necrosis

Free radical scavenging activities of biomolecules is generally the reported mechanism for antioxidants to inhibit lipid oxidation.Antioxidants (inhibitors of lipid peroxidation) is important not only for the preservation of food but also for the defenceof living cells against oxidative damage [26]. Antioxidants are classified into two mechanistic groups: those that inhibit or retard the formation of free radicals from their unstable precursors (initiation) and those that interrupt the radical chain reaction (propagation and branching). The former ones are called preventive antioxidants and the latter one's chain-breaking antioxidants [27].

Liver diseases are mostly mediated by reactive oxygen species (ROS) which play a significant role in the development of tissue injury and pathological conditions in the living system[28]. Single or repeated administrationof graded dosesof CCl_4 is one of the common methods employedto investigate the possible molecular mechanisms of hepatic damage in rats. This model has been implemented in various studies for the deposition of extracellular matrix in the cases of liver cirrhosis and fibrosis [29].

A toxic substance such as CCl_4 when administrated is distributed and deposited to organs such as the liver, brain, kidney, lung and heart [30]. The reactive metabolite trichloromethyl radical ($^{\bullet}CCl_3$) and trichloromethyl peroxide radical ($CCl_3O_2\bullet$) have been formed from the metabolic conversion of CCl_4 by cytochrome P450 [31]. As O_2 tension rises, a greater fraction of $^{\bullet}$ the CCl_3 present in the system reacts very rapidly with O_2 and more reactive free radicals, like $CCl_3OO^{\bullet}$ is generated from $^{\bullet}CCl_3$. These free radicals initiate the peroxidation of membrane polyunsaturated fatty acids (PUFA), cell necrosis, GSH depletion, membrane damage and loss of antioxidant enzyme activity [32].

A measure of the organism's redox status is the balance between oxidative factors – namely products of lipids peroxidation (peroxide radicals, malondialdehyde), endogenous and exogenous antioxidant substances (antioxidative enzymes, low-molecular antioxidants or cations of divalent metals) [13].

Excessive production of ROS induces the system to crave for more antioxidants such as vitamins C, A and E in order toscavenge these free radicals. The increased levels of lipid peroxidation products (MDA) observed, generally induces compensatory changes expressed by enhanced production and activity of serum antioxidative vitamins and serum redox metals.These findings agree with previous studies of Balahoroglu *et al*. [33], Khan and Ahmad [34] and Kiziler *et al*. [35]. The increased MDA observed may also support the hypothesis of Sengupta *et al*. [36] who suggested that decrease in the levels of antioxidant accelerate the lipid peroxidation thereby generating more MDA. It also causes inactivation of enzymes and receptors in membranes and thus changes membrane molecular properties.

Exposure to CCl_4 elevates tissue levels of free copper,iron and zinc due to release from stores and sequestering mechanisms, an event that precedes cytotoxicity and tissue damage in the organisms, when these elements are not functionally or tightly bound to sequestering proteins, they can; as part of low molecular mass complex, catalyze unwanted electron transfer reactions with consequent formation of reactive and damaging species such as hydroxyl radical. They can induce oxidative stress by catalysing the conversion of superoxide and hydrogen peroxide to more potent oxidants such as hydroxyl radical, which can cause tissue injury by initiating lipid peroxidation and oxidation of proteins and nucleic acids [12]. Increase levels of MDA were observed in this study, which represents an important finding to support the documented hypothesis, that depression of the antioxidant defence potential in the liver of experimental rats as a result of different doses of CCl_4 induction.

The increased concentration of Cu^{+2}, Zn^{+2} and Fe^{+2}, determined in our study could have had an additional influence on the level of oxidative stress through Fenton reaction. These elements are mobilised to inhibit ROS synthesis by being co-factors of antioxidative enzymes (copper and zinc are co-enzymes of SOD and iron is co-enzyme of catalase). On the other hand, relatively high concentrations of investigated

elements may initiate free radical reactions [13]. This will probably increase the MDA and more antioxidants mobilized in response to increased oxidative stress in the test group by CCl_4.

However, excess copper can cause problems because it can oxidize proteins and lipids, bind to nucleic acids and enhance the production of free radicals [37]. It is thus important to have mechanisms that will maintain the amount of copper in the body within normal limits [37]. Copper after ingestion in the diet is transported to the liver bound to albumin, then is taken up by liver cells, and part of it is excreted in the bile. Copper also leaves the liver attached to ceruloplasmin, thereby protecting the cells from free-radical injury [37]. Thus, the high level of serum copper could be due to increase absorption of copper from the gastrointestinal tract by the animals.

Zinc functions as an antioxidant only at specific sites or tissues, and is not a required cofactor for an antioxidant enzyme. Although Zn plays a structural role in the enzyme, superoxide dismutase, the activity of this enzyme is not decreased in Zn deficiency and its activity is usually depressed at high Zn intakes [38]. Zn may function as a site-specific antioxidant by two mechanisms. Firstly, it competes with Fe and Cu for binding to cell membranes and some proteins, displacing these redox-active metals and making them more available for binding to ferritin and metallothionein, respectively. Secondly, Zn binds the sulfhydryl groups in proteins, protecting them from oxidation. Zn status does not directly control tissue peroxide levels but can protect specific molecules against oxidative and peroxidative damage. The correlation analysis shows a weak positive correlation of the zinc levels with the MDA in CCl_4 induced oxidative stress [38].

Iron is another essential trace element present in almost all cells of the body. The human body requires iron for the synthesis of the oxygen-carrying protein called haemoglobin found in red blood cells and myoglobin which is also a protein found in muscles.This iron deposits in oxidative stress can exceed the storage and detoxification capacity of ferritin and also fully saturates transferrin and leads to the formation of free iron which accumulates in blood and tissues. This free iron will then, cause the formation of very harmful compounds, such as hydroxyl radical (OH). The hydroxyl radicals are highly reactive and attack lipids to form lipid peroxides which contribute to oxidative stress [38].

This situation may imply that the CCl_4 can disturb RBC antioxidant mechanisms and so erythrocytic damage could relate to the lipid peroxidation [39], and hence increase in serum iron. In addition, MDA, a highly reactive bifunctional molecule, has been shown to be able to cross-link RBC phospholipids and proteins, affecting membrane fluidity and impairing various membrane functions, which causes a decrease in RBC survival [40].

From the histopathological analysis, CCl_4 poisoning caused fatty and hydropic vacuolation and necrosis of cells in parenchymal cells near the hepatic veins which are in accordance with the study of Cameron and Karunaratne [41]. Fatty liver or liver steatosis was observed in the liver of experimental animals after 48hours of CCl_4 induction. The animals left untreated for the period of the study showed the formation of fibrous tissue in the liver from one portal triad to another (bridging fibrosis).Kumar *et al*. [42] also reporteda generally irreversible hepatic damage as deposition of collagen has consequences on patterns of hepatic blood flow and perfusion of hepatocytes.Fibrosis progresses to cirrhosis which is the end stage of the chronic liver disease. All three characteristics of cirrhosis as stated by Kumar *et al*. [42] have been significantly observed in these experimental animals at à dose of 150mg/kg.

CONCLUSION

The relationship of some biochemical and histomorphological alterations induced by various doses of CCl_4 has been studied. The presence of apositive correlation between oxidative stress marker, MDA, antioxidant vitamins (especially vitamin E) and redox elements was observed in the experimental rats. The increased level of antioxidant vitamins and redox metals in this study may probably be attributed to the increased utilisation as endogenous antioxidants to neutralise the effect of increased ROS thereby maintaining prooxidant-antioxidant balance in the animal model.

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REFERENCES

1. Valko, M., Leibfrittz, D., Monocol, J., Cronin, M., Mazur, M., and Tesler, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *Int. J. of Biochem and Cell Biology 39*(1), 44-84.
2. Aguiar, C. C. T., Analia, B. A., Paulo, V. P. A., Neuma, R. D., Chaves, E. M. C., Danielle, S. M., Woods, D. T., Marta, M. F., & Vasconcelos, S. M. N. (2012). Review Article Oxidative Stress and Epilepsy: *Oxidative Med. and Cell. Longevity*. *10*, 1155-1165.

3. Halliwell, B. (1991). The biological toxicity of free radicals and other reactive oxygen species. Free radicals and food additives. *Edited by: Aruoma OI, Halliwell B. 1991, Taylor & Francis Inc: PA, 41-45*.
4. Kim, H. J., Odendhal, S., & Bruckner, J. V. (1990). Effect of oral dosing vehicles on the acute hepatotoxicity of carbon tetrachloride in rats. *Toxicol Appl Pharmacol., 102,* 34-49.
5. McGregor, D., & Lang, M. (1996). Carbon tetrachloride: genetic effects and other modes of action. *Mutat Res., 366,* 181-195.
6. Dashti, H., Jeppsson, B., Hagerstrand, I., Hultberg, B., Srinivas, U., Abdulla, M., & Bengmark, S. (1989). Thioacetamide and carbon tetrachloride-induced liver cirrhosis. *Eur Surg Res., 21*, 83-91.
7. Behar-Cohen, F. F., Heydolph, S., Faure, V., Droy-Lefaix, M. T., Courtois, Y., & Goureau, O. (1996). Peroxynitrite cytotoxicity on bovine retinal pigmented epithelial cells in culture. *Biochem Biophys Res Commun, 226,* 842-849.
8. Bhadauria, M., Nirala, K. S., & Shukla, S. (2008). Multiple treatments of *Propolis* ameliorates carbon tetrachloride-induced liver injuries in rats. *Food Chem Toxicol, 46,* 2703–2712.
9. Halliwell, B., & Chirico, S. (1993). Lipid peroxidation, Its mechanism, measurement and significance. *Am. J. Clin. Nutr*, *57*, 715S-724S.
10. Khan, R. A., Khan, M. R., & Sahreen, S. (2012). CCl_4-induced hepatotoxicity: protective effect of rutin on p53, CYP2E1 and the antioxidative status in a rat. *BMC Compl Alternative Med, 12*,178.
11. Shodehinde, S. A., & Oboh, G. (2013). Antioxidant properties of aqueous extracts of unripe Musa paradisiaca on sodium nitroprusside induced lipid peroxidation in rat pancreas in vitro. *Asian pacific journal of tropical biomedicine*, *3*(6), 449-457.
12. Vassalle, C., Petrozzi, L., Botto, N., Andreassi, M. G., & Zucchelli, G. C. (2004). Oxidative stress and its association with coronary artery disease and different atherogenic risk factors. *Journal of internal medicine*, *256*(4), 308-315.
13. Bartosz, G. (2004). *The second face of oxygen*. Free radical in nature editor 2004, PWN, Warsaw, Poland.
14. Stephensen, C. B. (2001). Vitamin A, infection, and immune function. *Annual review of nutrition*, *21*(1), 167-192.
15. Terney, A. L. Jr., & Sorokin, V. (1997). Redox, radicals and antioxidants. Baskin, S.I. Salem, H. eds. Oxidants, antioxidants and free radicals: 1-21. Taylor and Francis Washington D.C.
16. Powell, S. W., & Saul, R. (2014). Zinc and Health: Current Status and Future Directions. The Antioxidant Properties of Zinc. *The J. of nutr,10*, 1155-1165.
17. Klein, H. J., & Bayne, K. A. (2007). Establishing a culture of care, conscience, and responsibility: Addressing the improvement of scientific discovery and animal welfare through science-based performance standards. *ILAR journal*, *48*(1), 3-11.
18. OECD (2006). Repeated Dose 28-day oral Toxicity Study in Rodents; Updated with Parameters for endocrine effects. http://www.oecd.org/dataoecd.
19. NIH. (1996). NIH Guide for the Care and use of Laboratory Animals. NIH Publication, No. 23-83.
20. Alhassan, A. J., Sule, M. J., Aliyu, S. A., & Aliyu, M. D. (2009). Ideal hepatotoxicity model in rats using Carbon Tetrachloride (CCl 4). *Bayero Journal of Pure and Applied Sciences*, *2*(2), 185-187.
21. Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical biochemistry*, *95*(2), 351-358.
22. Bessey, O. A., Lowry, O. H., BEOCK, M., & Lofez, J. A. (1946). The determination of vitamin A and carotene in small quantities of blood serum. *Journal of biological chemistry*, *166*, 177-188.
23. Fabianek, J., Defilippi., T. Richards., & Herp, A. (1967). Micromethod for Tocopherols Determination in blood serum. *American Assoc. of Clin. Chemistry*, *14*(5), 456-462.
24. AOAC. (2005). *Official Methods of Analysis of Association of Analytical Chemist*. 18th Edn. Gaithersburg, USA. Maryland. 20877-2417.
25. Spector & Goldman (eds.) (2006).“*Preparation of Cells and Tissues for Fluorescence Microscopy,”* Chapter 4, in *Basic Methods in Microscopy*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA.
26. Barbaste, M., Verge, S., Dumas, M., Soulet, S., Nay, B., Arnaudinaud, V., ... & Vercauteren, J. (2002). Dietary antioxidants, peroxidation and cardiovascular risks. *Journal of Nutrition Health and Aging*, *6*(3), 209-223.
27. Barclay, L. R. C., Vinqvist, M. R., Mukai, K., Goto, H., Hashimoto, Y., Tokunaga, A., & Uno, H. (2000). On the antioxidant mechanism of curcumin: classical methods are needed to determine antioxidant mechanism and activity. *Organic letters*, *2*(18), 2841-2843.
28. Mada, S. B., Inuwa, H. M., Abarshi, M. M., Mohammed, H. A., & Aliyu, A. (2014). Hepatoprotective Effect of Momordica charantia Extract against CCl4 Induced Liver Damage in Rats.
29. Weber, L. W., Boll, M., & Stampfl, A. (2003). Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. *Critical reviews in toxicology*, *33*(2), 105-136.
30. Ko, K. M., Ip, S. P., Poon, M. K. T., Wu, S. S., Che, C. T., Ng, K. H., & Kong, Y. C. (1995). Effect of a lignan-enriched fructus schisandrae extract on hepatic glutathione status in rats:

protection against carbon tetrachloride toxicity. *Planta Medica*, *61*(02), 134-137.

31. Kim, S. H., Jang, Y. P., Sung, S. H., Kim, C. J., Kim, J. W., & Kim, Y. C. (2003). Hepatoprotective dibenzylbutyrolactone lignans of Torreya nucifera against CCl4-induced toxicity in primary cultured rat hepatocytes. *Biological and Pharmaceutical Bulletin*, *26*(8), 1202-1205.
32. Agarwal, M., Srivastava, V. K., Saxena, K. K., & Kumar, A. (2006). Hepatoprotective activity of Beta vulgaris against CCl 4-induced hepatic injury in rats. *Fitoterapia*, *77*(2), 91-93.
33. Balahoroğlu, R., Dülger, H., Özbek, H., Bayram, I., & Şekeroğlu, M. R. (2008). Protective effects of antioxidants on the experimental liver and kidney toxicity in mice. *Eur J Gen Med*, *5*(3), 157-164.
34. Khan, M. R., & Ahmed, D. (2009). Protective effects of Digera muricata (L.) Mart. on testis against oxidative stress of carbon tetrachloride in rat. *Food and Chemical Toxicology*, *47*(6), 1393-1399.
35. Kiziler, A. R., Aydemir, B., Gulyasar, T., Unal, E., & Gunes, P. (2008). Relationships among iron, protein oxidation and lipid peroxidation levels in rats with alcohol-induced acute pancreatitis. *Biological trace element research*, *124*(2), 135-143.
36. Sengupta, S., Wehbe, C., Majors, A. K., Ketterer, M. E., DiBello, P. M., & Jacobsen, D. W. (2001). Relative roles of albumin and ceruloplasmin in the formation of homocystine, homocysteine-cysteine-mixed disulfide, and cystine in circulation. *Journal of Biological Chemistry*, *276*(50), 46896-46904.
37. Murray, R. K., & Keeley, F. W. (2006). Micronutrients and vitamins In: Murray RK, Granner DK, Mayes PA, Rodwell VW. *Harper's illustrated biochemistry*,.
38. Prabhu, R., Prabhu, V., & Prabhu, R. S. (2009). Iron overload in beta thalassemia: a review. *J Biosci Tech*, *1*(1), 20-31.
39. Friedman, M. J. (1979). Oxidant damage mediates variant red cell resistance to malaria. *Nature*, *280*(5719), 245-247.
40. Sugihara, T., Rawicz, W., Evans, E. A., & Hebbel, R. P. (1991). Lipid hydroperoxides permit deformation-dependent leak of monovalent cation from erythrocytes. *Blood*, *77*(12), 2757-2763.
41. Cameron, G. R., & Karunaratne, W. A. E. (1936). Carbon tetrachloride cirrhosis in relation to liver regeneration. *The Journal of Pathology*, *42*(1), 1-21.
42. Kumar, V., Abbas A., & Fausto N. (2004). *Robbins and Cotran Pathologic Basis of Disease.* 7TH edition, Elsevier, New Delhi, India, P 1525.