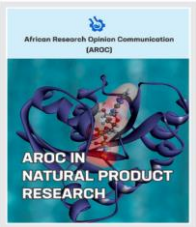
Research Article

Combretum dolichopentalum extract normalized biochemical and haematological parameters in carbon tetrachloride (CCL_4) intoxicated rats.

Favour N. Ujowundu[1], Nathan N. Oparaeche[2], Chinyere H. Onuoha[1], Haruna M. Abiola[1], Chukwudoruo S. Chieme[1], Cosmas O. Ujowundu[1]



[1*]Department of Biochemistry, Federal University of Technology Owerri, (FUTO) Nigeria
[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Madonna University Nigeria, Elele Campus, Nigeria.

Corresponding author* Favour Ntite Ujowundu**;** fnujowundu@yahoo.com
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Abstract

Background: The ethanol extract of *Combretum dolichopentalum* (EECD) is employed in Nigeria to stabilize the uterus after parturition**.** The ability of EECD to confer protection on rats destabilized by moderate concentrations of carbon tetrachloride (CCl_4) was evaluated. **Methods:** Fifty rats were assigned to 5 groups of 10 rats each. The experimental animals after acclimatization were handled accordingly: Groups 1 and 2 respectively were maintained on food and water only throughout the study. Group 3, 4, and group 5 were pre-treated with 250 mg/kg and 500 mg/kg body weight of EECD and 50 mg/kg of silymarin for 28 respectively. All groups except group 1 were intoxicated to 0.2 ml/kg body weight of CCl_4, administered via an intraperitoneal route on day 29. Serum pipetted from the blood of the rats after cardiac puncture was assayed for antioxidant enzymes, lipid peroxidation product and serum iron, zinc and biocarbonate. Haematological analysis was also conducted. **Results:** Administration of CCl_4 at 0.2 ml/kg b.w slightly increased the oxidizing species as indicated in the concentration of malondialdehyde in the rats while reducing the antioxidant enzymes; it increased the Iron and zinc concentrations and also the haematological parameters except for the white blood cells. However, this was corrected by pre-treatment with the EECD dose-dependently. **Conclusion**: These characteristics portends that the crude ethanol extract of *C. dolichopentalum* could be employed to correct minor oxidative perturbation induced by CCl_4 intoxication

Keyword: *C. dolichopentalum;* Biochemical; Haematological; Carbon tetrachloride, Antioxidant*;*

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1.0 Introduction

Ageing and various degenerative diseases associated with it are attributable to the injurious attack of reactive oxygen species on cellular constituents, including chromosomes, connective tissues and mitochondrial DNA [1]. Univalent reduction of oxygen gives rise to damaging oxidative species (reactive oxygen species, (ROS). ROS can damage many different kinds of cellular macromolecules including lipids, proteins and DNA. Damage to DNA can lead to irreversible loss or alteration of genetic information in post-mitotic cells. This produces many different oxidatively modified purines and pyrimidines, including the most commonly measured 8-oxo-7,8-dihydro-2'deoxyguanosine (8-oxodG), as well as single and double-strand breaks in both rats and human DNA and chromosomes [2-4]. Protein modifications elicited by the direct oxidative attack on lysine, arginine, proline or threonine and by secondary reaction of cysteine, histidine or lysine residues with carboxyl compounds can lead to the formation of protein carboxyl derivatives.

Biomolecules most susceptible to oxidative damage in cells are unsaturated fatty acids of cell membranes and this susceptibility increases as a function of their double bonds. Abstraction of hydrogen from unsaturated fatty acids by oxygen centred radicals mainly initiates lipid peroxidation, this is followed by the formation of hydroperoxides. A variety of derivatives including various carbonyl products results from the degradation of hydroperoxides [1,5]. Such unsaturated carbonyls include deoxyosones, enals, 2-ketoaldehydes, trienals and various reductions that are really toxic to most cellular

and extracellular biomolecules [1,5]. Many carbonyls react readily even at neutral pH and room temperature, with important biochemical groups such as amino, thiol or hydroxyl. A secondary functional group of the carbonyls increases their reactivity potential and may induce irreversible reaction products, or result in cross-linking reactions [5]. Antonio *et al.* [5] reported that 4-hydroxyl alkenals could react with nearly all amino acids under appropriate conditions. Due to its reactivity, the carbonylic products, particularly α, β-unsaturated carbonyls of lipid peroxidation, are implicated in various types of cell damage, including depletion of glutathione, protein modification, disturbance of calcium homeostasis, cell membrane destruction, tissue injuries, enzyme inhibition and disruption in DNA, RNA, and protein synthesis [1,6-7]. Besides, endogenously produced reactive oxygen species, ROS can also be induced exogenously. Contact through ingestion, inhaling, or skin with toxic substances released either from industrial plant or homemade chemicals present in certain cleaners, dry-cleaning agents, pesticides, and refrigerants etc carbon tetrachloride. The dose, duration, and administration are factors that determine the degree of harm caused when exposed to carbon tetrachloride [8].

Exposure of CCl_4, to a variety of species, causes haematological disorders, centrilobular hepatic necrosis besides other symptoms [9]. This study is aimed at demonstrating the effects of the ethanol extract of *C. dolichopentalum*, against oxidative stress induced by CCl_4 as *C. dolichopentalum* has shown the presence of a wide range of pharmacologically active constituents capable of scavenging free radicals [9-12].

2.0 Materials and Methods

2.1 Collection and authentication of plant material

C. dolichopentalum leaves were harvested from Obinze in Owerri West Local Government Area of Imo state. The fresh plant was authenticated by Mr A. Ozioko, of the Bioresource Development and Conservation Program (BDCP), Research Centre at the University of Nigeria, Nsukka, Enugu State, Nigeria.

2.2 Preparation of the plant extract

C. dolichopentalum leaves were plucked from their stems washed with water and allowed to air dry at room temperature. The dried samples were pulverized (using an electric blender) and stored in an airtight container. Three hundred grams of the pulverized *C. dolichopentalum* powder was extracted with 1.75 L of 80 % ethanol by maceration for 2 days, this was done in three separate cans of 100 g each and then pulled together. The residue was removed by filtration using a sieve followed by a Whatman No 1 filter paper. The concentration of the extract was carried out using a rotary evaporator under mild temperature and reduced pressure and labelled ethanol extract of *C. dolichopentalum* (EECD).

2.3 Experimental animals

The experimental animals, Wistar albino rats of male species weighing 150-200 g, were purchased from the Animal House of the Department of Veterinary Medicine, University of Nigeria Nsukka, Enugu State, Nigeria. The animals were acclimatized for 7 days at room temperature in metal cages under 12/12 hours' light and dark and were maintained *ad libitum* on water and rat pellets (Pfizer Feeds, Aba, Nigeria). This study was carried out in accordance with laws and regulations for handling experimental animals as was approved by the Department of Biochemistry, Federal University of Technology Owerri.

2.4 Experimental Design

Fifty (50) Wistar albino rats weighing between 150 and 200 g were used for this prophylactic study. The rats were assigned to 5 groups of ten rats each after 7 days of acclimatization. This study was designed as shown in Table 1.

Table 1: Experimental treatments

Groups	Group Identity	Treatments
I	Normal control (NC)	Water only
2	Negative control (NGC)	water + CCl_4
3	Treated group (T_{250})	250 mg/kg EECD + CCl_4
4	Treated group (T_{500})	500 mg/kg EECD + CCl_4
5	Sylimarin group	50 mg/kg Silymarin + CCl_4

All groups received food and water *ad libitum* for 4 weeks. On day 29, 0.2 ml/kg body weight of CCl_4 in liquid paraffin (2:1) was administered intraperitoneally to all groups (except normal control). The CCl_4 was allowed to act on the animals for 2 days. The rats were sacrificed and blood was collected by cardiac puncture after overnight fast and light anaesthesia with dimethyl tetrachloride. Liver tissue of the animals was obtained, washed in 1.15 % KCl buffered solution and dabbed with paper, weighed and prepared for homogenization.

2.5 Blood collection

Blood samples of each animal were collected by cardiac puncture into EDTA bottles for haematological parameters and plain bottles for biochemical parameters. Blood samples collected into plain bottles were allowed 45 minutes to clot at room temperature, thereafter centrifuged at 600 x g for 15 minutes; the serum collected was used to assay various biochemical parameters.

2.6 Biochemical Assays

SOD activity was assayed according to the method of Xin et al. [13]. This method studies the activity of SOD as it converts superoxide to hydrogen peroxide in the presence of a detector iodonitrozolium violet. The activity of GPx was determined according to the method of Paglia et al. [14] based on the principle that GPx catalyzes the oxidation of GSH by hydroperoxide. In the presence of glutathione reductase (GR) and NADPH, the oxidized (GSSG) glutathione is immediately converted to the reduced form with concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance of NADPH at 340 nm is measured in a spectrophotometer. The concentration of MDA was determined according to the method of Wallin et al. [15].

Malondialdehyde (MDA) reacts with thiobarbituric acid (TBA) to form a red or pink coloured complex which in acid solution, absorbs maximally at 532 nm. Bicarbonate was determined according to the methods of Tietz et al. [16] using CO_2 gas electrodes. Atomic absorption spectrophotometer based on Beer-Lambert's principle was employed for the determination of iron and zinc concentrations.

2.7 Haematological analysis

An automated haematology analyzer machine (Mindray BC 2300, USA) was used for the haematological analysis of the following haematological component: red blood cell count (RBC), haemoglobin concentration (Hb), packed cell volume (PCV), mean cell haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean cell volume (MCV). White blood cell count (WBC) and platelet count.

2.8 Statistical analysis

Data collected from results were subject to statistical analysis using Statistical Package for Social Science (SPSS) version 16 and presented as means ± standard error of the mean. One-way analysis of variance (ANOVA) at $p < 0.05$ were used for comparing the significant differences between treatment groups.

3.0 Results

3.1 Effects of EECD on antioxidant enzymes and lipid peroxidation in CCl_4 intoxicated rats

Results of the present study revealed a significant ($p<0.05$) increase in MDA concentrations in the negative control group compared to the normal and the EECD treated groups. However, there was no significant difference ($p>0.05$) between the 500 mg/kg b.w and the Silymarin treated groups. Pre-treatment with EECD before intoxication with CCl_4 showed decreased MDA concentrations (Figure 1A). The GPx activity of the negative control was reduced significantly ($p>0.05$) when compared to the normal, EECD treated groups. But there was no significant difference ($p>0.05$) between the 250 mg/kg b.w and 500 mg/kg b.w of the extract (Figure 1B). Furthermore, there was a significant ($P<0.05$) reduction in SOD activity of the negative control group, when compared to those of the normal control, 250 and 500 mg/kg b.w EECD treated groups (Figure 1C). However, no significant difference ($p>0.05$) in the SOD activities between the normal and 250 mg/kg b.w EECD treated group.

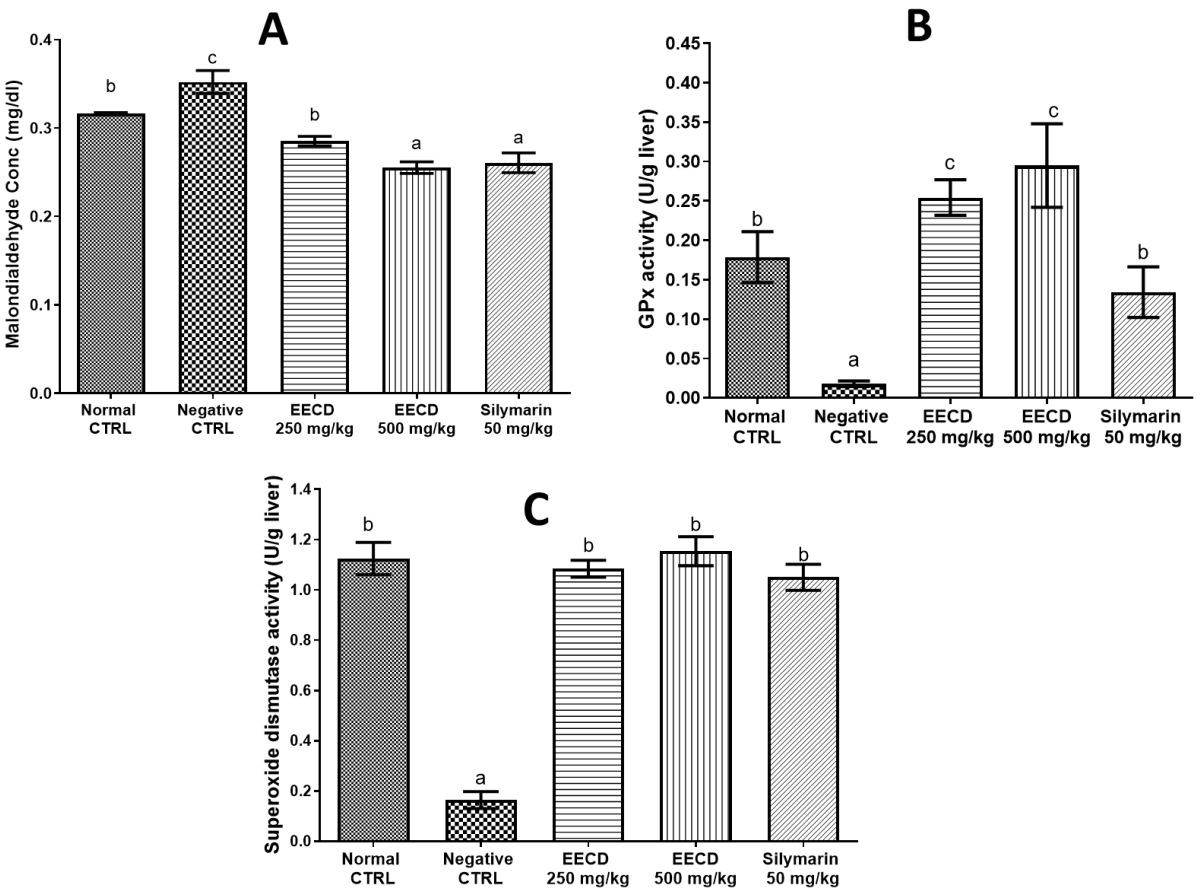

Figure 1: Effects of ethanol extract of *Combretum dolichopentalum* (EECD) on antioxidant enzymes and lipid peroxidation in CCl_4 intoxicated rats. Bar graph showing the effects of EECD on (A) malondialdehyde (MDA) concentrations, (B) glutathione peroxidase (GPx) and (C) superoxide dismutase (SOD) activities in CCl_4 -induced oxidative stress. Bars bearing different letters are statistically significant ($p<0.05$).

3.2 Effects of EECD on zinc, iron and bicarbonate concentrations in CCl_4 intoxicated rats

Analysis of serum electrolyte concentrations revealed a non-significant ($p>0.05$) increase in the serum bicarbonate concentration of the negative control group compared to the normal. Also observed was a non-significant ($p>0.05$) difference between the negative control and both the EECD and silymarin treated groups (Figure 2A). There was a significant ($p<0.05$) increase in the serum iron concentration of the negative control group compared to the normal control and treated (EECD and silymarin) groups (Figure 2B). The serum concentration of zinc was also significantly ($p<0.05$) elevated in the negative control group compared to the normal, EECD treated groups (250 mg/kg b.w and 500 mg/kg b. w) and the silymarin treated group (Figure 2 C). However, no significant ($p>0.05$) difference was observed between the 250 mg/kg and 500 mg/kg b.w EECD treated groups.

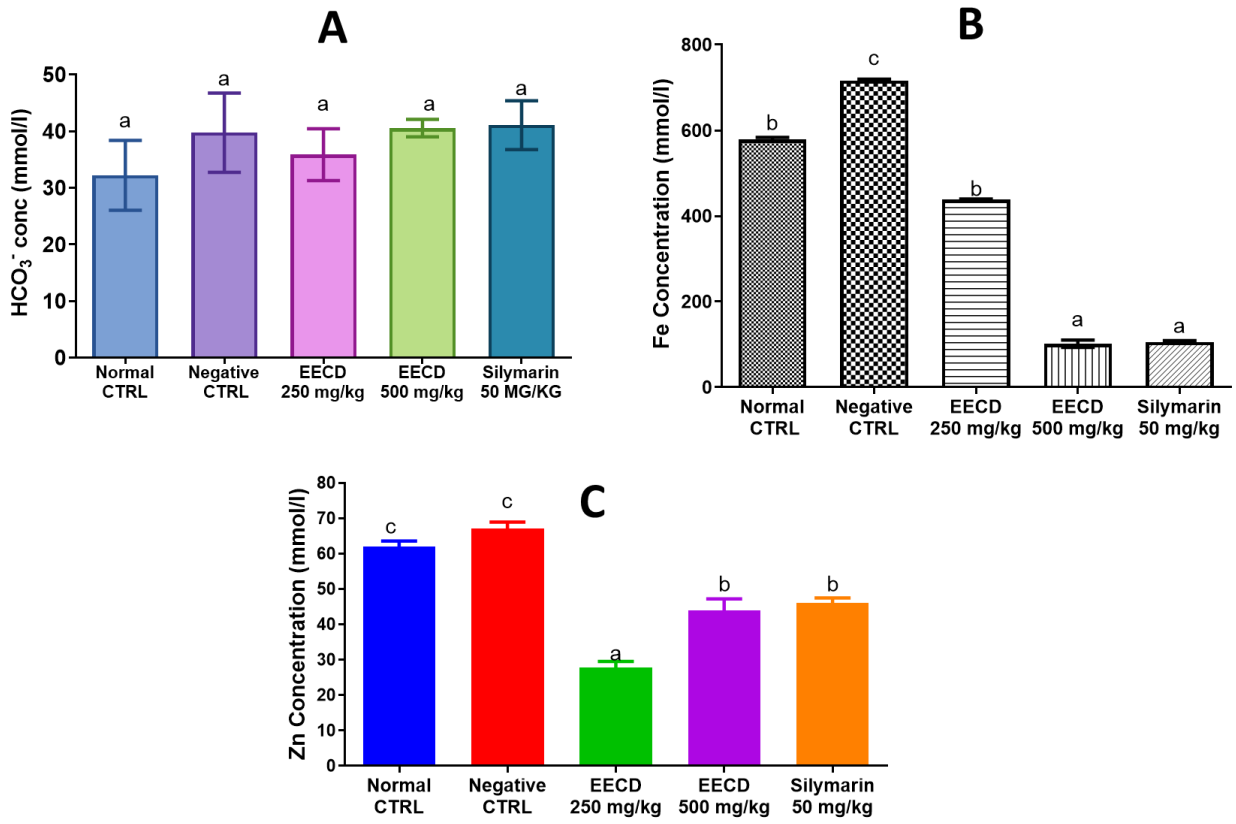

Figure 2: Effects of ethanol extract of *Combretum dolichopentalum* (EECD) on serum zinc, iron and bicarbonate concentrations in CCl_4 intoxicated rats. Bar graph showing the effects of EECD on (A) serum bicarbonate (HCO_3) concentration, (B) iron and (C) zinc (SOD) concentrations in CCl_4 -induced oxidative stress. Bars bearing different letters are statistically significant ($p<0.05$).

3.3 Effects of EECD on haematological parameters in CCl_4 intoxicated rats

Haematological analysis revealed a significant ($P<0.05$) increase in the RBC count of the negative control group compared to the normal, and the 500 mg/kg EECD treated group. However, there was no significant ($p>0.05$) difference among the negative control, 250 mg/kg EECD treated and silymarin groups (Figure 3A). There was a significant ($P<0.05$) increase in the haemoglobin concentration of the negative control, EECD (250 and 500 mg/kg) treated and silymarin treated groups when compared to the normal. A non-significant ($p>0.05$) difference in the levels of haemoglobin was also observed between the negative control, EECD (250 and 500 mg/kg) and silymarin treated groups (Figure 3B). Results also reveal a significant ($P<0.05$) reduction in WBC count of the negative control group compared to the normal, EECD (250 and 500 mg/kg) and silymarin treated groups. However, there was no significant ($p>0.05$) difference in the levels of the WBC counts among the EECD treated (250 and 500 mg/kg) and the silymarin treated groups (Figure 3C). The platelet count was significant ($P<0.05$) elevated in the negative control group when compared to the normal group, EECD (250 and 500 mg/kg) and silymarin treated groups. However, there was no significant ($p>0.05$) difference in the platelet counts among the normal group, EECD treated, and silymarin treated groups (Figure 3D).

Similarly, a significant ($P<0.05$) increase in the levels of the PCV has been observed in the negative control and the treatment (EECD and silymarin) groups when compared to the normal control. However, the PCV levels in the negative control group and the treated groups (EECD and the silymarin) were not significantly ($p>0.05$)

different from each other (Figure 4A). The MCH (Figure 4B) and MCHC (Figure 4C) concentrations in the normal control group were not significantly ($p>0.05$) different from the levels in the negative control and treatment groups. However, a significant increase in the MCV was observed in rats treated with 500 mg/kg EECD when compared with normal and other experimental groups (Figure 4D).

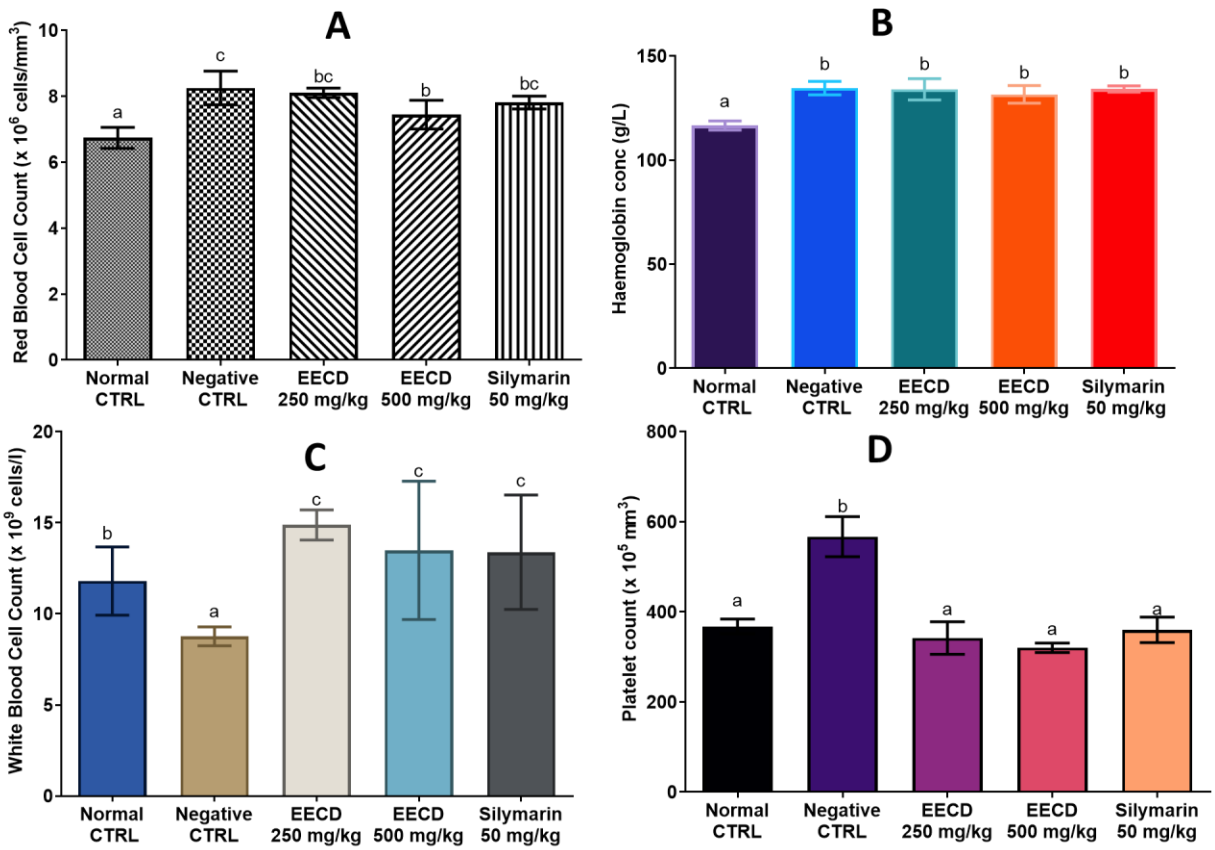

Figure 3: Effects of ethanol extract of *Combretum dolichopentalum* (EECD) on (A) red blood cell (RBC), (B) haemoglobin concentration, (C) white blood cell count (WBC) and (D) platelet levels in CCl_4 intoxicated rats. Bars bearing different letters are statistically significant ($p<0.05$).

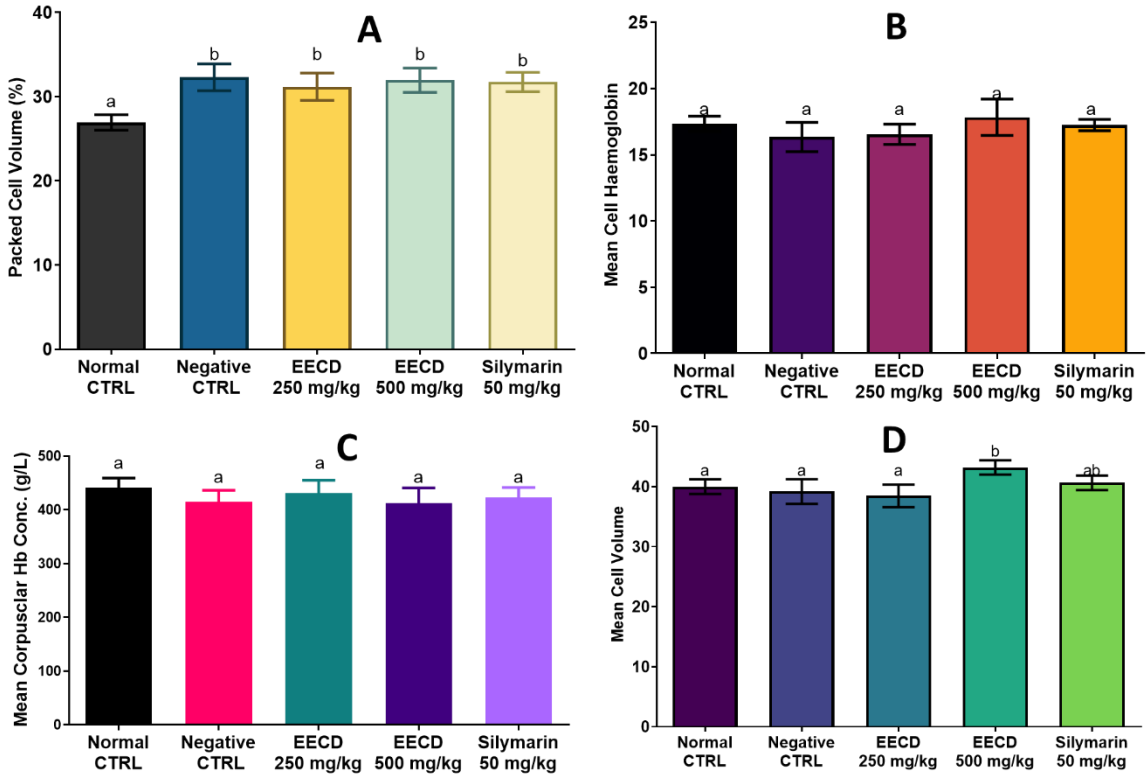

Figure 4: Effects of ethanol extract of *Combretum dolichopentalum* (EECD) on (A) packed cell volume (PCV), (B) mean cell haemoglobin (MCH), (C) mean cell haemoglobin concentration (MCHC), and (D) mean cell volume (MCV) in CCl_4 intoxicated rats. Bars bearing different letters are statistically significant ($p<0.05$).

4.0 Discussion

The toxicity of carbon tetrachloride has been elucubrated both from the biochemical and pathological viewpoints and as a result, the data available provides particular insight into mechanisms of toxicity especially on the liver [2-4]. The key organ which plays a crucial role in regulating various physiological processes in the body is the liver. It is associated with detoxication, metabolism, secretion, storage, and synthesis of metabolites. This is the reason, injury to the liver due to hepatotoxic agents may be of grave consequences [17]. Most hepatotoxic agents destroy liver cells mainly by initiating lipid peroxidation and other oxidative damages [18]. The liver is the major target organ of CCl_4 toxicity owing to its high content of cytochrome P-450 [19], however, it is also known that the red blood cell is metabolically active towards pharmacologically active endogenous and exogenous substances, it is evident that the RBC displays moderate cytochrome P-450-like activity, not excluding its ability to catalyse various other transformations of a range of drugs [20].

Trichloromethyl free radicals ($\cdot CCl_3$ or $\cdot CCl_3OO$) is produced from CCl_4 mediated by hepatic microsomal cytochrome P-450 [21], which in turn initiate the lipid peroxidation process [22-23]. Carboxylic products, such as the α, β – unsaturated carbonyls of lipid peroxidation, are very reactive, thus inflicts various types of cell damage, such as cell membrane destruction, depletion of glutathione, disturbance of calcium homeostasis, enzyme inhibition, decreased DNA, RNA and protein synthesis, protein modification, retardation of respiration, and tissue injuries [1,9, 24]. The anti-lipid peroxidative activity of the EECD as shown by the reduction of CCl_4 induced oxidative stress might be as a result of the extract's ability to prevent the penetration of CCl_4 into the liver cells, thus preventing the activation of the toxicants, since activation of CCl_4 is a prerequisite for lipid peroxidation to occur [24].

The most important enzyme for the extraperoxisomal inactivation of H_2O_2 is Glutathione peroxidase (a selenium-containing enzyme) [2]. It is observed that a reduction in GSH will in turn reduce the activity of GPx as is evident in the intoxicated but untreated group (negative control). This is because GSH is a substrate for GPx as it provides the electrons needed to reduce H_2O_2 or hydroperoxides [26-27]. Correction of this anomaly was achieved probably due to the free radical scavenging activity of the extract. Ujowundu *et al* [28-29], reported similar restoration of GPx activity after intoxication with CCl_4 and subsequent treatment with plant extract.

There is evidence that supports the assumption that the removal of superoxide by Superoxide dismutase (SOD) is a detoxication reaction [30]. Basically, the most susceptible SOD to the prooxidants is the cytosolic form (Cu, Zn – SOD [31] whereas the mitochondrial form of SOD can be induced under oxidative stress. The activity of SOD decreased significantly ($P<0.05$) in the group of rats intoxicated with CCl_4 only. This decrease in SOD activity might be ascribed to the depletion of these antioxidants enzymes. In enzyme catalysed reaction, a short initial pre-steady state or burst, where the enzyme-substrate complex is formed and unbound enzyme decreases, the rate of turnover (enzyme activity) is low in this state, following the principle of mass conservation. Total enzyme is the summation of the bound and unbound enzyme [32]. As the unbound enzyme reduces, the enzyme activity decreases. This may account for the observed decreases in the activities of SOD and GPx in oxidatively stressed systems.

The pH of blood needs to be maintained around (7.35–7.45), for this is required of life, and relatively small variability in pH value of blood can portend severe metabolic consequences. The main buffers in the blood are haemoglobin in the erythrocytes and bicarbonate ion (HCO_3^-) in the plasma. The bicarbonate buffer system is the primary buffer of blood and also makes up the alkali reserve [33]. Bicarbonate buffers neutralize strong and non-volatile acids (such as HCl, H_2SO_4, and lactic acid) entering the extracellular fluid (ECF), converting these acids into weak and volatile acids; H_2CO_3 thus formed is diffusible, and eliminated by diffusion of CO_2 through the alveoli of the lungs. A significant ($P<0.05$) increase in [HCO_3^-] was observed in the CCl_4 treated group which depicts a condition that might lead to alkalosis. The condition is associated with an absolute or relative increase in [HCO_3^-]. An increase in the alkali reserve is the most frequent cause of clinically observed alkalosis. However, maintenance of acid-base balance was not observed in the treated group, but an increase in alkali reserve in a dose-dependent fashion was shown in the treated groups. That is to say that, the EECD and the silymarin groups may have initiated a compensatory mechanism that raised pulmonary respiration. This increased ventilation would result in loss of CO_2 and reduction in [H_2CO_3] with sequel increase in [HCO_3^-], increasing NH_3 formation, H^+ excretion compared to K^+ excretion in the distal tubule and HCO_3^- reabsorption. The treated groups rather portend exposure to metabolic acidosis (a reduction in plasma level of HCO_3^-).

Intracellular redox-active transition metals notably Fe^{2+}/Fe^{3+} and Cu^+/Cu^{2+} have been implicated in catalyzing oxidative stress due to their Fenton activity [34,26]. Thus, iron chelators like desferrioxamine (DFO) preclude the binding of H_2O_2 to catalytically active iron and thus inhibit the formation of hydroxyl radicals [35]. The results as shown in Figure 2 recorded a significant ($P<0.05$) increase in the iron concentration of the negative control group compared to the normal. Within the cells, the highest concentration of free, redox-active iron is found in the lysosomes. If an oxidant damages the lysosomal membrane, then iron leaks out into the cytosol, where it can participate in Fenton reactions and greatly aggravate oxidative damage [26].

Treatment with both concentrations of EECD caused a significant ($P<0.05$) reduction of iron concentrations of the liver in a dose-dependent manner. If lysosomal degradation of metalloprotein is inhibited, then oxidant stress-induced cell injury can be greatly diminished. Because intracellular iron and other catalytically active transition metals are potentially dangerous to a cell, they are either tightly bound to proteins or low-molecular ligands or sequestered in specific pools [26]. It appears that EECD enhanced the sequestering of iron judging from the lower concentration of iron in the treated group compared to the normal.

Zinc is an important constituent of proteins, yet would be highly toxic if the free concentrations

of zinc in the cell increased beyond a certain critical level. A significant ($P<0.05$) increase in zinc concentration was recorded in the negative control group (Figure 2) compared to the normal. This indicates that toxicants could interfere with normal zinc homeostasis. Toxicants indirectly displace zinc from certain key proteins or directly by causing bioavailability of exceedingly high intracellular zinc concentration which induces a stress response. This can either activate or disrupt signalling pathways. If via multiple parallel pathways, xenobiotics can shift the overall equilibrium towards an unfavourable response [36]. Increasing cellular [$Zinc^{2+}$] levels activate the metal sensor, MTF-1 (metal-response-element-binding transcription factor) which transmigrates in the nucleus and activates genes involved in the homeostasis of metals, such as those of metallothionein (MT), metal efflux carriers and antioxidant enzymes (enzymes involved in GSH synthesis). Pretreatment with the EECD prevented the disruption of zinc homeostasis significantly ($P<0.05$).

The total mass of red blood cells (RBC) in the circulatory system is regulated within a narrow limit, thus adequate red cells are always available to provide sufficient transport of oxygen from the lungs to the tissues. The cells do not become so numerous that they can impede blood flow. Any condition that causes the quality of oxygen transported to the tissues to decrease ordinarily, increases the rate of RBC production as seen in the CCl_4–intoxicated rats (negative group) in this study. Destruction of major portions of bone marrow by various diseases of the circulation that cause decreased tissue blood flow might result in hypoxia. Hypoxia causes a marked increase in erythropoietin production and the erythropoietin, in turn, enhances RBC production until the hypoxia is relieved [37]. Compared to the negative control, the treated group was able to offer little protection against the secondary polycythaemic conditions. The silymarin group however offered better protection.

Many compounds of diverse structure including insecticides, carcinogens and others, when administered to a mammal can result in a marked increase in hepatic δ–ALA synthetase. This occurs because most of the drugs are metabolized by cytochrome P450. During the metabolism of CCl_4, consumption of haem by cytochrome P450 is greatly increased; this, in turn, diminishes the cellular concentration of haem, leading to derepression of δ–ALA synthetase with a corresponding increase in the ratio of haem synthesis [32]. Furthermore, the increase in RBC may also result in an increase in the synthesis of haemoglobin to fill the RBC [38].

The packed cell volume (PCV), mean cell haemoglobin concentration (MCHC) and mean cell volume (MCV) are used in the investigation of anaemia. The levels of PCV are increased in all forms of polycythaemia. In the present study, the PCV values of the intoxicated group are significantly higher than those of the normal control. PCV is the part of the whole blood occupied by red cells. Thus if the negative control group recorded a high RBC and Hb values, it is only rational that the PCV values of the intoxicated group increased. This may not be as a result of health conditions, but only a response to poor delivery of oxygen from the lungs to the tissues.

Low MCHC values are found in iron deficiency anaemia and other conditions in which the red cells are microcytic and hypochromic. The MCV values reflect the size of the red blood cell, while MCH and MCHC reflect the haemoglobin content of the blood cells. RBC indices are therefore used to diagnose types of anaemia. The values obtained for RBC count in the EECD treated rats indicate that the extract tends to balance the irregularities in the RBC cell count.

The liver harbours a large percentage of resident macrophages (Kupffer cells) and specific resident T cells (YδT cells) that have been implicated in certain forms of immune cell-mediated toxicity induced by xenobiotics. The results of this study showed a significant decrease in the WBC of the negative control group compared to that of the normal. The effects of xenobiotics which can modulate the function of the immune system at several levels can have two opposing consequences. If xenobiotics push the normal function of the immune system out of balance, the result can be either an immuno-suppression or an immuno-overestimation. Either direction portends danger and results in toxicity; immunosuppression can result in a diminished resistance against infections. CCl_4 is bio-activated in the liver and induces centrilobular

necrosis [39]. Thus CCl_4 could have suppressed the maturation and development of immune cells, and thus caused immunosuppression [40].

Platelet counts can be used to investigate thrombocytopenia and thrombocytosis conditions. This study showed a significant increase in platelet numbers, probably caused by myeloproliferative diseases, such as polycythaemia vera, following tissue injury. The polycythemic condition agrees with this study since the intoxicated rats (NGC) showed increases in RBC, which defines polycythaemia vera. But pretreatment with EECD alleviated this increase. Platelets play important role in blood coagulation, haemostasis and blood thrombus formation. The action of platelets, though quite beneficial in initiating the reaction to injury may actually be harmful in conditions such as coronary occlusion [41]. In that case, platelet function may delay reperfusion and help to cause re-occlusion of the vessel. This study is in line with the work done by Gilberto [42] who used Gossypitrin against the hepatotoxicity of carbon tetrachloride. Aqueous extract of *Podophyllum hexadrum* and *X. aethiopica* was also shown to exhibit antioxidant against CCl_4 induced stress [43-44].

5.0 Conclusion

The results of the present study suggested that the crude ethanol extract of *Combretum dolichopentalum* could be employed to correct minor oxidative perturbation induced by CCl_4 intoxication. This study discovered an increase in alkali reserve in a dose-dependent fashion in the groups treated with ethanol extract of *C.dolichopentalum*, this implies that EECD could be employed to neutralize strong and non-volatile acids in cases of increased plasma acidity, Pre-treatment with EECD alleviated an increase in platelet concentration as they can be harmful in conditions such as coronary occlusion.

Conflict of interest: The authors declared no conflict of interest.

Author Contributions: The work was conducted in collaboration with all authors. FNU and COU designed the study, wrote the protocol, performed the statistical analysis, managed the literature searches and wrote the draft of the manuscript. NO, CHO, HMA, and CSC participated in managing the analyses of the study, managed the literature searches, and writing the draft of the manuscript. All authors read and approved the final version of the manuscript

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