



(RESEARCH ARTICLE)



## Ameliorative effect of *Combretum dolichopentalum* in CCl<sub>4</sub>- induced oxidative stress

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### Abstract

The study evaluated the ability of the ethanol extract of *Combretum dolichopentalum* (EECD) to offer protection on rats perturbed by relatively low concentrations of carbon tetrachloride (CCl<sub>4</sub>). Five groups of 10 rats each were treated thus: Group 1 and group 2 constituting the normal and positive control group were maintained on food and water throughout the study. Group 3, 4, and 5 received 250, 500 mg/kg body weight of EECD and 50 mg/kg of silymarin for 28 days respectively. All groups except group 1 were exposed to 0.2 ml/kg body weight of CCl<sub>4</sub> intraperitoneally on day 29. Serums from the rats were assayed for antioxidant and liver specific enzyme. Lipid profile and peroxidation product as well as antioxidant molecules were determined to check the oxidative as well as the liver status of the test organism. Standard biochemical methods were employed for the assay and results were subject to statistical analysis using one way ANOVA. Administration of CCl<sub>4</sub> at 0.2 ml/kg b.w slightly altered the redox status of the rats; however, pre-treatment with the ethanol extract of *C. dolichopentalum* dose dependently stabilized the altered perturbation. This potential indicates that the crude EECD could be employed to correct minor oxidative imbalances as a result of CCl<sub>4</sub> intoxication, *C. dolichopentalum* has been reported to possess a wide array of bioactive phytochemicals, and also reported not to present significant side effects when administered. Thus *C. dolichopentalum* could be a pharmacological tool needed to offer protection on liver during toxic assault from accidentally ingested toxicants such as carbon tetrachloride.

**Keywords:** *Combretum dolichopentalum*; Oxidative stress; Carbon tetrachloride; Phytochemicals

### 1. Introduction

It is obvious that most levels of physiological function decline in oxidatively stressed organisms. An essential part of oxidative damage is followed by adverse chemical processes resulting in the apparent appearance of side products of normal metabolism; the formation of mutated, less active, and potentially toxic species of DNA, RNA, proteins, lipids and small molecules to such extent that organisms can only tolerate the accumulation of these altered bio-molecules to an extent. In this regard therefore, oxidative stress may be viewed as a struggle between Biochemistry and Chemistry. Organisms have evolved biochemical systems where the correct DNA sequences encode the correct sequences of RNA and proteins, which fold in just the right way to make both catalysts and architectural structures. The catalyst employs speed and specificity to ensure that thermodynamically favourable but kinetically unfavourable reactions occur that lead to metabolic pathways for energy generation, biosynthesis, and signal transduction. Since there are various chemical reactions for each metabolic intermediate, the provision of enzymes that catalyze just one of the possible reactions can lead to a rapid and smooth metabolic conversion of reactants to products with few side products. All these displays the beauty of biochemistry in making life possible. What antagonizes the beauty of biochemistry is chemistry itself. While enzymes can speed up reactions, it is more difficult to slow down reactions. Side reactions still go on, and the more time elapses, the more unwanted side products are formed. Importantly, these side products are not just small

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molecules, but all types of bio-molecules including nucleic acids and proteins. Almost all the molecules from micro metabolites to proteins that make up living systems are not thermodynamically stable (carbon dioxide and water may be the exceptions). Thus, from the moment that bio-molecules are bio-synthesized, a slow process of non-enzymatic decomposition begins, leading to simple products. These spontaneous side reactions over time gives rise to the modification of the biochemical species required for the orderly processes of life described above into less functional species [1]. Toxicants such as carbon tetrachloride can destabilize the oxidative status of an organism if administered orally, through dermal contact or inhalation

Carbon tetrachloride is a simple man-made molecule, present in certain cleaners, dry-cleaning agents, pesticides, and refrigerants. It is no longer present in most household products due to its high toxicity. Exposure or administration of CCl<sub>4</sub>, to a variety of species, causes centrilobular hepatic necrosis. Symptoms associated with this could be sudden and severe. Immediate symptoms can include: headache, dizziness, fatigue, confusion, and blurred vision. However, exposure to extremely high or concentrated doses may cause: vomiting, stomach pain, and death. The metabolic activation of carbon tetrachloride to the trichloromethyl radical is mediated by the microsomal enzyme. The radical formed may either react with oxygen or abstract a hydrogen atom from an available donor, yielding a secondary radical, or react covalently with lipid or protein. If the available donor is a polyunsaturated lipid, a lipid radical is formed which can undergo peroxidation [2]. This adverse reaction can only be terminated by the endogenous or exogenous presence of antioxidants.

Antioxidants are capable of preventing or inhibiting the oxidation of other molecules by oxidizing species. The roles of antioxidants are to neutralize the excess of free radicals, to protect the cells against their toxic effects and to contribute to disease prevention and defense system [3]. Exogenous antioxidants includes minerals, vitamins, flavonoids, and phenolic acids [4]

It is estimated that 80 % of metabolites/plant extracts used as drugs and sold worldwide are derived from natural products and that over 100 new natural product-based lead drugs are in clinical development [5-6]. *C.dolichopentalum* includes one of such plants. The present study evaluates the ameliorative effect of the ethanol extract of *C. dolichopentalum* on carbon tetrachloride – induced oxidative stress.

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## 2. Material and methods

### 2.1. Plant sample

Fresh leaves of *C. dolichopentalum* were harvested from a farm in Obinze in Owerri West Local Government Area of Imo state. Authentication of the plant was done by Mr. A. Ozioko, of the Bioresource Development and Conservation Program (BDGP), Research Centre at the University of Nigeria, Nsukka, Enugu State, Nigeria. The fresh leaves were plucked from their stems, washed with water and allowed to dry at room temperature. The dried samples were pulverized (using electric blender) and stored in an airtight container kept in a desiccator for 3 days.

### 2.2. Extraction

One hundred grams of the *C. dolichopentalum* powder was extracted with 1.75 L of 80 % ethanol by maceration for 48 hours, this was carried out in three separate cans and then pulled together. The sediment was removed by coarse filtration using a sieve followed by a Whatman No 1 filter paper. The extract was concentrated using a rotary evaporator under mild temperature and reduced pressure and labeled ethanol extract of *Combretum dolichopentalum* (EECD).

### 2.3. Animals

Wistar albino rats, purchased from the Animal House of the Department of Veterinary Medicine, University of Nigeria Nsukka, Enugu State, Nigeria were acclimatized for 7 days at room temperature in metal cages under 12/12 hour light and dark and were fed and maintained *ad libitum* on water and rat pellets (Pfizer Feeds, Aba, Nigeria). This study was conducted in accordance with laws and regulations for handling experimental animals as was approved by the Department of Biochemistry, FUTU.

### 2.4. Experimental design

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

**Table 1** Experimental design

Groups	Group Identity	Treatments
I	Normal control (NC)	feed and water
II	Positive control (PC)	feed, water and CCl <sub>4</sub>
III	Treated group (T <sub>250</sub> )	250 mg/kg body weight of EECD and CCl <sub>4</sub>
IV	Treated group (T <sub>500</sub> )	500 mg/kg body weight of EECD and CCl <sub>4</sub>
V	Sylimarin group	50 mg/kg body weight of Silymarin and CCl <sub>4</sub>

All the groups received food and water *ad libitum* for 28 days. On day 29, 0.2 ml/kg body weight of CCl<sub>4</sub> in liquid paraffin (2:1) was administered intraperitoneally to all groups (except normal control). The CCl<sub>4</sub> was allowed to act on the animals for 48 hr. After overnight fast and light anaesthesia with dimethyltetrachloride, the rats were sacrificed and blood collected by cardiac puncture.

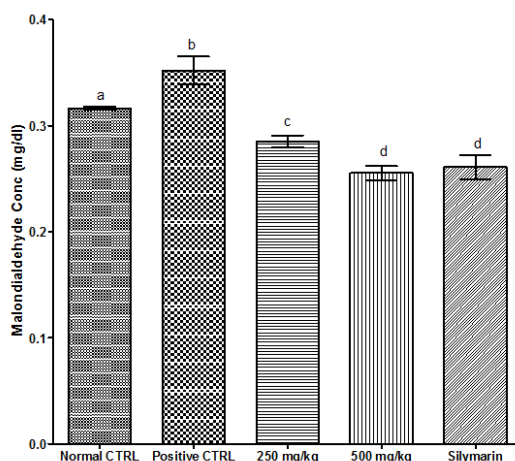
#### Blood collection

Blood samples of each animal were taken by cardiac puncture and allowed 45 minutes to clot at room temperature. After centrifugation at 600 x g for 15 minutes, the serum collected was used to assay various biochemical parameters.

#### 2.5. Biochemical assays

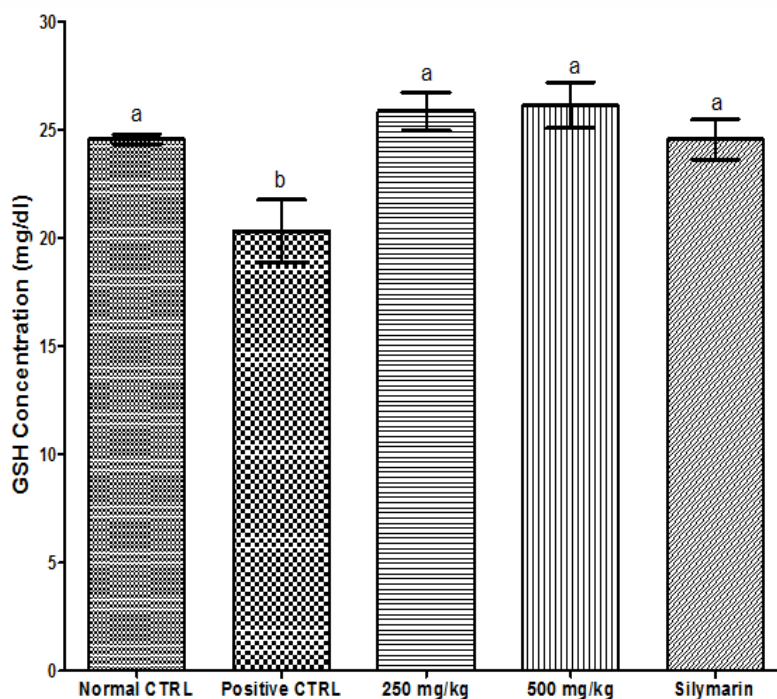
The assay method employed for serum alanine aminotransferase (ALT) activity was that of Reitman and Frankel [7]. The concentration of protein was determined according to the method of Tietz [8]. The concentration of triacylglycerides was determined according to the method of Young [9]. Determination of low-density lipoprotein (LDL)-cholesterol concentration was determined according to the method of Assman *et al.* [10]. The activity of SOD was assayed according to the method of Xin *et al* [11]. Catalase activity was determined according to the method of Aebi [12]. The concentration of GSH was determined according to the method of Raja *et al* [13]. The activity of GPx was assayed according to the method of Paglia and Valentine [14]. The concentration of MDA was determined according to the method of Wallin *et al* [15]. The concentration of serum bicarbonate was determined according to the method of Tietz [16]. The concentration of serum vitamin C was determined according to the method of Omaye *et al* [17]. The concentration of serum vitamin E was determined according to the method of Palan *et al* [18].

### 3. Results



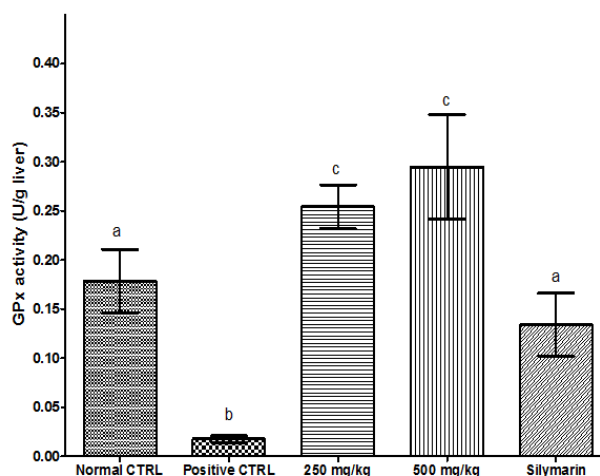
**Figure 1** Effect of EECD on malondialdehyde (MDA) concentrations in carbon tetrachloride- induced oxidative stress. Bars bearing different letters are statistically significant ( $p < 0.05$ )

Figure 1 shows a significant ( $p < 0.05$ ) increase in MDA concentrations in the positive control group compared to the normal and the groups treated with 250 mg/kg b.w and 500 mg/kg b.w EECD. However there were no significant difference ( $p > 0.05$ ) between the 500 mg/kg b.w and the silymarin treated groups.



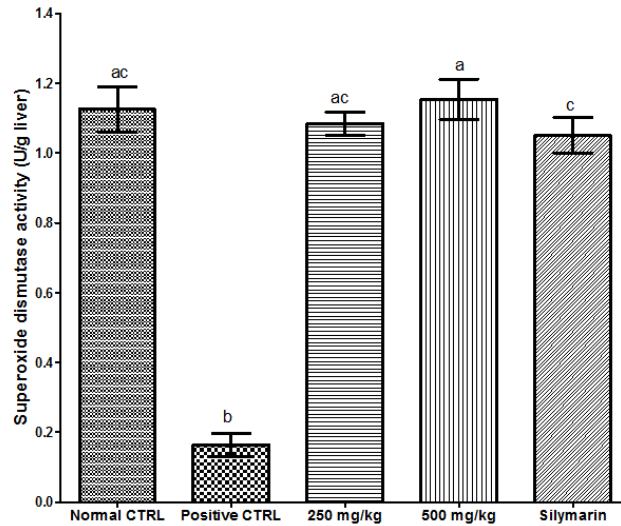
**Figure 2** Effect of EECD on glutathione (GSH) in carbon tetrachloride- induced oxidative stress. Bars bearing different letters are statistically significant ( $p < 0.05$ )

This Figure 2 shows that there was a significant ( $p < 0.05$ ) reduction in GSH concentration of the positive control compared to the normal, similarly it reveals that the treated groups showed a greater GSH concentration compared to the positive control. Nevertheless, there were no significant difference among the normal, 250 mg/kg, 500 mg/kg b.w EECD, and the silymarin treated groups.



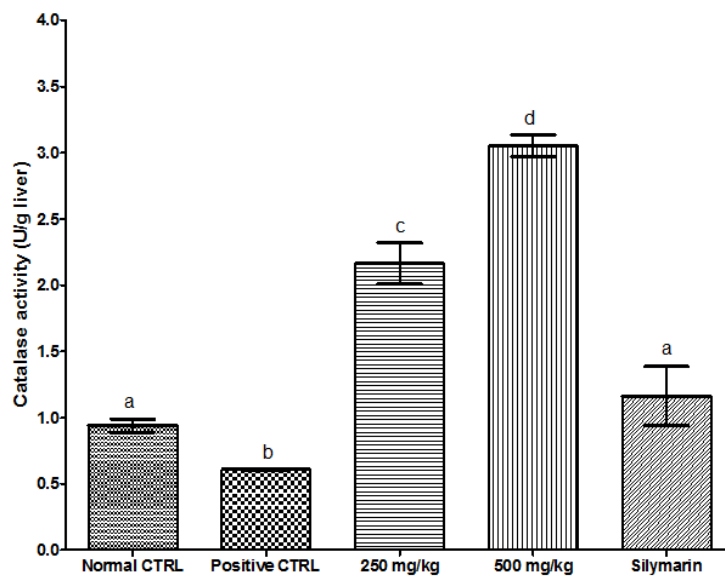
**Figure 3** Effect of EECD on glutathione peroxidase (GPx) activity in carbon tetrachloride - induced oxidative stress. Bars bearing different letters are statistically significant ( $p < 0.05$ )

The figure 3 shows that GPx activity of the positive control was reduced significantly ( $p > 0.05$ ) when compared to the normal, EECD treated groups. However there were no significant difference ( $p > 0.05$ ) between the 250 mg/kg b.w and 500 mg/kg b.w of the extract.



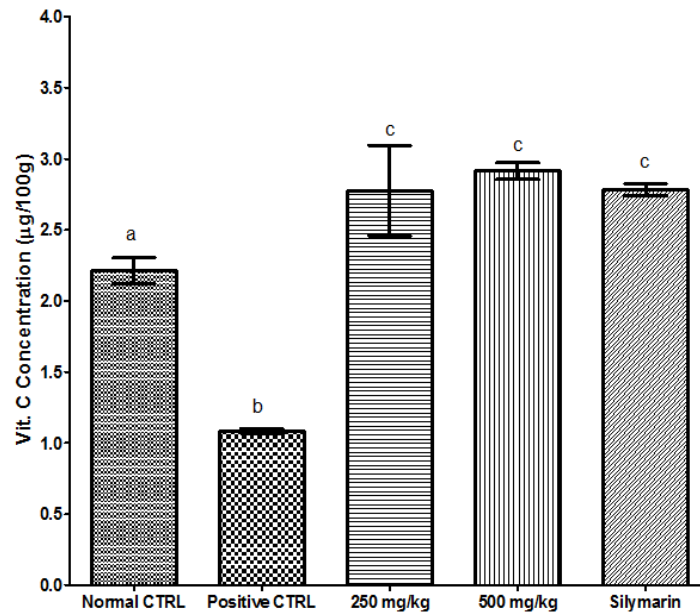
**Figure 4** Effect of EECD in superoxide dismutase (SOD) activity in carbon tetrachloride -induced oxidative stress. Bars bearing different letters are statistically significant ( $p < 0.05$ )

Figure 4 shows that in the positive control group, the activity of SOD reduced significantly ( $P < 0.05$ ) when compared to those of the normal control, 250 mg/kg and 500 mg/kg b.w EECD treated group. Also revealed was the non-significant difference ( $p > 0.05$ ) between the normal and 250 mg/kg b.w EECD treated group.



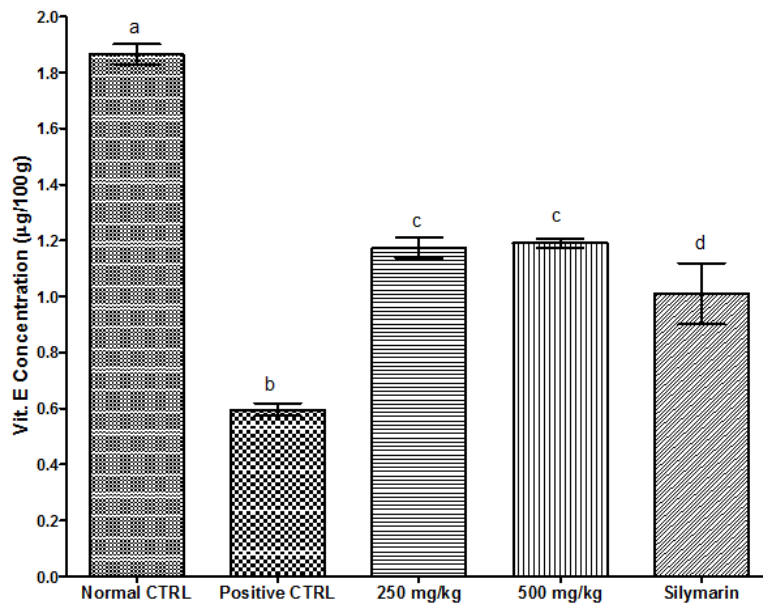
**Figure 5** Effect of EECD on catalase activity in carbon tetrachloride intoxicated rats. Bars bearing different letters are statistically significant ( $p < 0.05$ )

This Figure (5) shows that catalase activity in the positive control animals was reduced significantly ( $p < 0.05$ ) when compared to normal control, silymarin, 250 mg/kg and 500 mg/kg b. w EECD treated groups. There were no significant difference ( $p > 0.05$ ) between the normal and the silymarin treated groups.



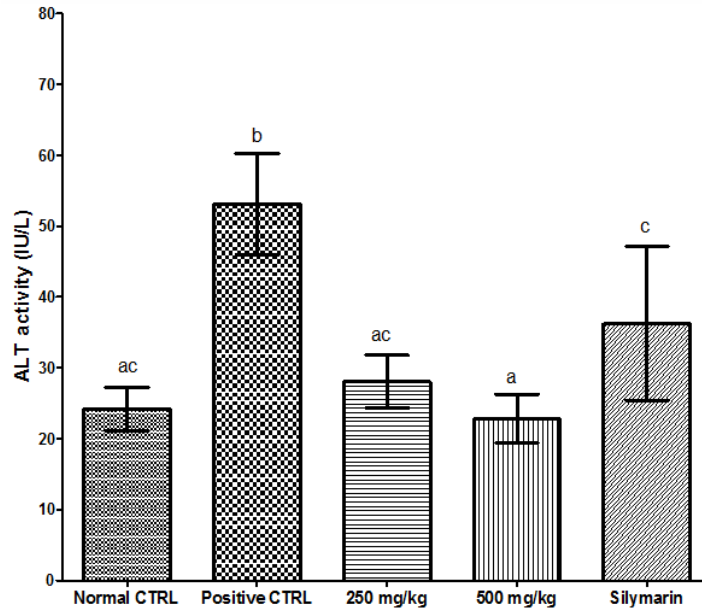
**Figure 6** Effect of EECD on vitamin C concentration in carbon tetrachloride- induced hepatotoxicity. Bars bearing different letters are statistically significant ( $p < 0.05$ )

Figure 6 indicates that vitamin C concentration of the positive control was reduced significantly ( $p < 0.05$ ) when compared to the normal, silymarin, 250 mg/kg and 500 mg/kg b.w EECD treated group. Also observed were the non-significant difference ( $p > 0.05$ ) among the silymarin, the 250 and 500 mg/kg b.w EECD treated groups.



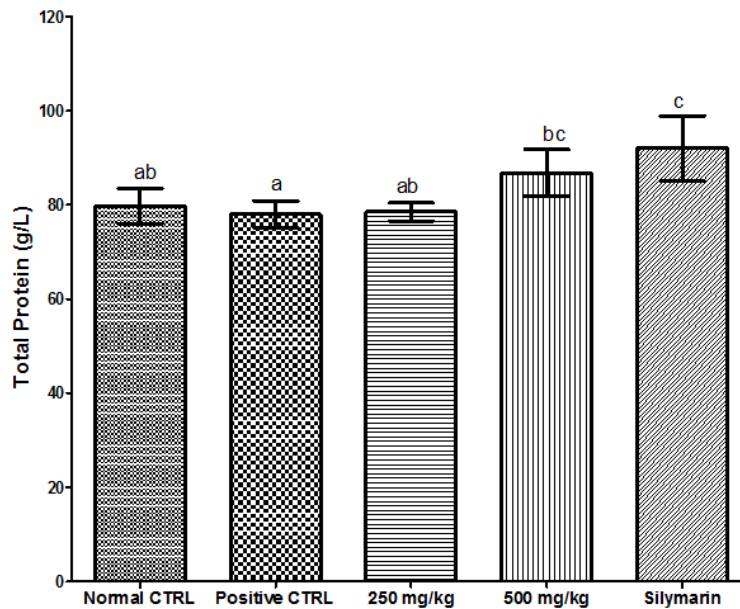
**Figure 7** Effect of EECD in Vitamin E concentration in carbon tetrachloride- induced hepatotoxicity. Bars bearing different letters are statistically significant ( $p < 0.05$ )

Figure 7 show that vitamin E concentration of the positive control was significantly ( $p < 0.05$ ) reduced when compared to the normal control, 250 mg/kg and 500 mg/kg b.w EECD treated rats. Observation of the non-significant difference ( $p > 0.05$ ) between the 250 and 500 mg/kg b.w EECD treated groups was also made.



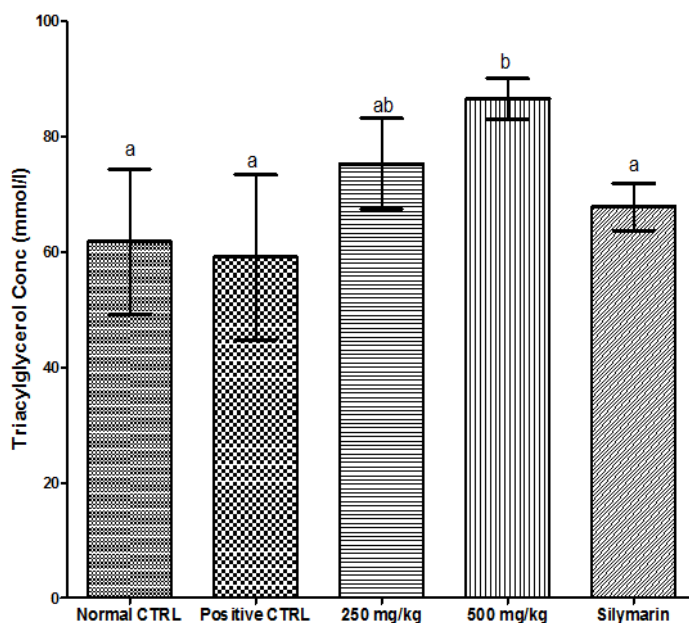
**Figure 8** Effects of ethanol extracts of *C. dolichopentalum* on serum alanine amino transferase (ALT) activity in carbon tetrachloride- induced hepatotoxicity. Bars bearing different letters are statistically significant ( $p < 0.05$ )

Figure 8 shows that ALT activity in the positive control were elevated significantly ( $p < 0.05$ ) when compared to normal control, EECD treated group at 250 mg/kg b.w. and 500 mg/kg b. w as well as the silymarin groups. A non-significant difference ( $p > 0.05$ ) between the normal and 250 mg/kg b.w EECD treated groups was also observed.



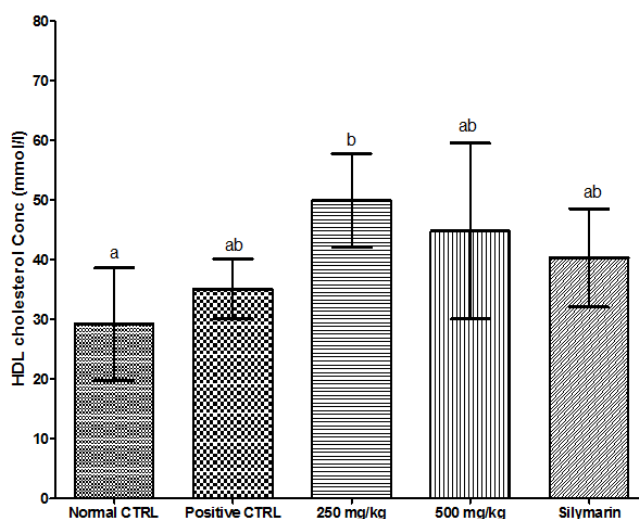
**Figure 9** Effect of EECD on total protein concentration in carbon tetrachloride- induced hepatotoxicity. Bars bearing different letters are statistically significant ( $p < 0.05$ )

This result as shown in Figure 9 shows that total protein concentration of the positive control reduced non-significantly ( $p > 0.05$ ) when compared to the normal control and 250 mg/kg b. w treated group. There were also no significant difference ( $p > 0.05$ ) between the normal and the 250 mg/kg b.w EECD treated groups. Treatment with EECD at 500 mg/kg b.w and the silymarin showed significantly ( $p < 0.05$ ) elevated total protein concentrations.



**Figure 10** Effect of EECD on serum triacylglyceride concentration in carbon tetrachloride- induced hepatotoxicity. Bars bearing different letters are statistically significant ( $p < 0.05$ )

Figure 10 shows a non-significant ( $p > 0.05$ ) reduction in triacylglyceride concentration of the positive control compared to the normal control and the silymarin treated group. Nevertheless, there was a significant ( $p < 0.05$ ) difference between the 250 and 500 mg/kg b.w EECD treated groups.

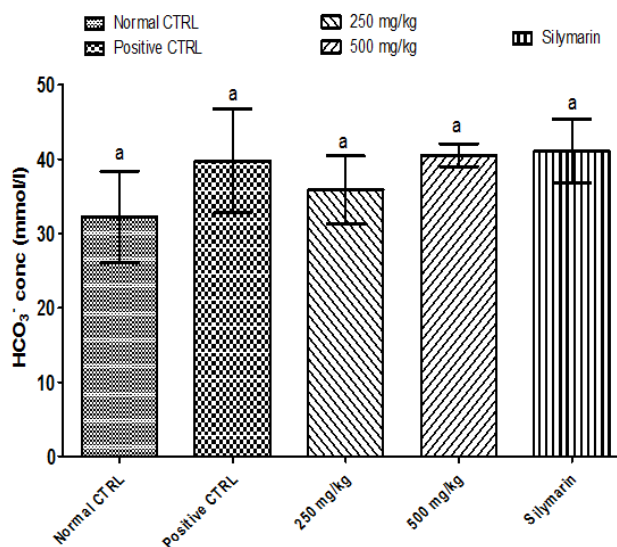


**Figure 11** Effect of EECD on HDL- cholesterol in  $CCl_4$  -intoxicated rats. Bars bearing different letters are statistically significant ( $p < 0.05$ )

Figure 11 shows a non-significant ( $p > 0.05$ ) difference in high-density lipoprotein cholesterol concentration in the positive control group compared to the EECD treated at 500 mg/kg b.w and the silymarin groups. However there was a significant difference between the normal and the EECD treated group at 250 mg/kg b. w EECD.

Figure 12 shows a non-significant ( $p > 0.05$ ) increase in the serum bicarbonate concentration of the positive control group compared to the normal. Also observed was a non-significant ( $p > 0.05$ ) difference between the positive control and both the EECD and silymarin treated groups.





**Figure 12** Effect of EECD in serum bicarbonate ( $\text{HCO}_3^-$ ) in  $\text{CCl}_4$ -induced oxidative stress. Bars bearing different letters are statistically significant ( $p < 0.05$ )

#### 4. Discussion

The hepatotoxicity of carbon tetrachloride has probably been more extensively studied than that of any other hepatotoxin and there is now a wealth of data available. Its toxicity has been studied both from the biochemical and pathological view points and therefore the data available provides particular insight into mechanisms of toxicity especially on liver. The liver is the most important organ which plays pivotal role in regulating various physiological processes in the body. It is involved in metabolism, secretion and storage, detoxication and synthesis of metabolites. Therefore, damage to the liver inflicted by hepatotoxic agents is of grave consequences [19]. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages [20]. The liver is the main target organ of  $\text{CCl}_4$  toxicity due to its high content of cytochrome P-450 [21].  $\text{CCl}_4$  is converted through hepatic microsomal cytochrome P-450 into trichloromethyl free radicals ( $\cdot\text{CCl}_3$  or  $\cdot\text{CCl}_3\text{OO}$ ) [22], which in turn initiate lipid peroxidation process [23, 24]

Figure 1 shows a significant ( $p < 0.05$ ) increase in MDA concentrations in the positive control group compared to the normal and the groups treated with 250 mg/kg b.w and 500 mg/kg b.w EECD. However there were no significant difference ( $p > 0.05$ ) between the 500 mg/kg b.w and the Silymarin treated groups. Due to the reactivity of carboxylic products, particularly  $\alpha$ ,  $\beta$ -unsaturated carbonyls of lipid peroxidation, are implicated in various types of cell damage, including tissue injuries, cell membrane destruction, depletion of glutathione, protein modification, disturbance of calcium homeostasis, retardation of respiration, enzyme inhibition and decreased DNA, RNA and protein synthesis [25-28].

The group of rats intoxicated with  $\text{CCl}_4$  and pretreated with EECD showed decreased MDA concentrations. The reduction of  $\text{CCl}_4$  induced oxidative stress by the anti-lipid peroxidative activity of the EECD, might be due to the extract ability to prevent the penetration of  $\text{CCl}_4$  into the liver cells, thus preventing the activation of the toxicants, since activation of  $\text{CCl}_4$  is required for lipid peroxidation to occur [29].

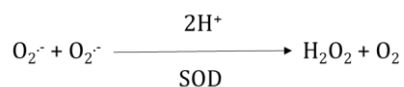
Figure 2 shows that there was a significant ( $p < 0.05$ ) reduction in GSH concentration of the positive control compared to the normal, similarly it reveals that the treated groups showed a greater GSH concentration compared to the positive control. Nevertheless, there were no significant difference among the normal, 250 mg/kg, 500 mg/kg b.w EECD, and the silymarin treated groups. Glutathione (GSH), together with its coupled enzyme is one of the most important antioxidant defense lines in the body. Upon need, the level of GSH can become up regulated; this is indicated by an increase in NADPH generation. The importance of GSH becomes particularly evident when the redox balance is disrupted due to excessive GSH consumption as shown in the positive control; this greatly facilitated the development of toxicity caused by pro-oxidant xenobiotics[30]. Lipid peroxidation response to glutathione depletion was alleviated significantly ( $P < 0.05$ ) by the ethanol extract of *C. dolichopentalum* in a dose dependent manner just as the standard drug (silymarin). This may be attributed to the ability of the EECD to keep the cellular thiol redox status in the reduced form.

The figure 3 shows that GPx activity of the positive control was reduced significantly ( $p > 0.05$ ) when compared to the normal, EECD treated groups. However there were no significant difference ( $p > 0.05$ ) between the 250 mg/kg b.w and 500 mg/kg b.w of the extract.

Glutathione peroxidase (a selenium-containing enzyme) is the most important enzyme for the extraperoxisomal inactivation of  $H_2O_2$  [31]. A reduction in GSH will in turn reduce the activity of GPx as is evident in the intoxicated but untreated group (positive control). This is because GSH is a substrate for GPx as it provides the electrons needed to reduce  $H_2O_2$  or hydroperoxides [32]. Treatment with the plant extract was able to correct this anomaly, possibly by providing scavengers for ROS. [33, 34] also reported a restoration of GPx activity after intoxication with  $CCl_4$  and subsequent treatment with plant extract.

Figure 4 shows that in the positive control group, the activity of SOD reduced significantly ( $P < 0.05$ ) when compared to those of the normal control, 250 mg/kg and 500 mg/kg b.w EECD treated group. Also revealed was the non-significant difference ( $p > 0.05$ ) between the normal and 250 mg/kg b.w EECD treated group. While figure (5) shows that catalase activity in the positive control animals was reduced significantly ( $p < 0.05$ ) when compared to normal control, silymarin, 250 mg/kg and 500 mg/kg b.w EECD treated groups. There were no significant difference ( $p > 0.05$ ) between the normal and the silymarin treated groups.

Several lines of evidence support the assumption that removal of superoxide by SOD is indeed a detoxication reaction [35]. Basically, the cytosolic form (Cu, Zn - SOD) is more susceptible to the preoxidants [36].



While the mitochondrial form of SOD can be induced under oxidative stress, the activities of SOD and catalase decrease significantly ( $P < 0.05$ ) in the group of rats intoxicated with  $CCl_4$  only. This decrease in the antioxidant enzyme activity might be attributed to the exhaustion of these antioxidant enzymes. In enzyme catalysed reaction, a short initial phase (pre-steady state or burst), where the enzyme – substrate complex is formed and free enzyme decreases, the turnover rate (enzyme activity) is low in this phase, according to the principle of mass conservation. Total enzyme is the summation of the bound and free enzyme complex [37]. As free enzyme reduces, the enzyme activity drops. This may explain the observed decrease in the activities of SOD, CAT, and GPx in oxidatively stressed system.

The pretreated groups showed increases in SOD and CAT activities probably due to the presence of flavonoids such as isorhamnetin, apigenin, luteolin, kaempferol and quercetin in EECD [38]; these are hydroxylated compounds that act as scavengers of ROS and RNS. The capacity of flavonoids to act as antioxidant depends upon their molecular structure. The position of hydroxyl group and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities.

Rats intoxicated with  $CCl_4$  showed significant ( $P < 0.05$ ) reduction in vitamin C and E concentrations. Although, a vitamin to man, guinea pigs and monkeys, vitamin C is not a vitamin to rats since rats possess the enzyme L-gluconolactone oxidase needed to convert ketogluconolactone to ascorbic acid in the uronic acid pathway. Its deficiency is manifested in scurvy, fragile capillaries, poor dentine formation in children and delayed wound healing due to deficient formation of collagen [39]. Decrease in vitamin C and E concentrations can be linked to depletion of the antioxidants as a result of oxidative stress caused by  $CCl_4$ .

Figure 6 indicates that vitamin C concentration of the positive control was reduced significantly ( $p < 0.05$ ) when compared to the normal, silymarin, 250 mg/kg and 500 mg/kg b.w EECD treated group. Also observed was the non-significant difference ( $p > 0.05$ ) among the silymarin, the 250 and 500 mg/kg b.w EECD treated groups. Figure 7 show that vitamin E concentration of the positive was significantly ( $p < 0.05$ ) reduced when compared to the normal control, 250 mg/kg and 500 mg/kg b.w EECD treated rats. Observation of the non-significant difference ( $p > 0.05$ ) between the 250 and 500 mg/kg b.w EECD treated groups was also made. Vitamin C and E concentrations were restored in the rat groups pre-treated with EECD before intoxication with  $CCl_4$ . This evidently shows the antioxidant properties of the extract against ROS [40]. Vitamin C functions synergistically with other antioxidants. Alpha-tocopherol consists of a phenolic group which can reduce lipid peroxy radicals. It has been claimed to be the most important lipid – soluble antioxidant. Its hydrophobic nature dictates its location exclusively in the cell membrane and that it protects membranes from oxidation by reacting with lipid peroxy radicals [41, 42]. This reaction produces oxidised  $\alpha$  –

tocopheroxyl radicals that can be recycled back by ascorbate, which in turn is kept in its reduced state by GSH [43, 44, 32].

Figure 8 shows that ALT activity in the positive control were elevated significantly ( $p < 0.05$ ) when compared to normal control, EECD treated group at 250 mg/kg b.w. and 500 mg/kg b. w as well as the silymarin groups. A non-significant difference ( $p > 0.05$ ) between the normal and 250 mg/kg b.w EECD treated groups was also observed. Alanine aminotransferase (ALT) has been considered liver specific. In rat liver, ALT is found almost exclusively in periportal hepatocytes, and this is consistent with the enzyme role in gluconeogenesis.  $CCl_4$  intoxicated rats showed a significant ( $P < 0.05$ ) increase in alanine aminotransferases, compared to the normal group. After damage to hepatic parenchymal cells, the enzyme leaks into the serum and peak activities are found 24 to 48 h after a toxic insult [45-48]. Decrease in activities of the transaminases of EECD pre-treated rats compared to untreated group (PC) indicates stabilization of plasma membrane as well as repair of hepatic tissue damage caused by  $CCl_4$ . This effect may be as a result of the tannin content of the plant, which renders the membrane less permeable to chemical injury [[49, 38]. This effect is in agreement with the commonly accepted view that serum levels of transaminases return to normal with healing of hepatic parenchymal and the regeneration of hepatocytes [50].

This result as shown in Figure 9 shows that total protein concentration of the positive control reduced non-significantly ( $p > 0.05$ ) when compared to the normal control and 250 mg/kg b. w treated group. There was also no significant difference ( $p > 0.05$ ) between the normal and the 250 mg/kg b.w EECD treated groups. Treatment with EECD at 500 mg/kg b.w and the silymarin showed significantly ( $p < 0.05$ ) elevated total protein concentrations. The extent of liver damage was further assessed by estimating serum levels of total protein (T.P), Result showed a non-significant ( $P < 0.05$ ) decrease in T.P in  $CCl_4$  intoxicated rats (PC) when compared with the control (NC). A fall in protein and albumin concentrations has been reported in severe parenchymal liver damage, especially from poisoning with  $CCl_4$  [51,52]. This reflects synthetic incapability of the liver of rats intoxicated with  $CCl_4$ . Thus this will alter the liver's ability to synthesis plasma protein that will be actively involved in fluid exchange, binding and transport function, buffering action and enzymatic activities [39].

Figure 10 shows a non-significant ( $p > 0.05$ ) reduction in triacylglyceride concentration of the positive control compared to the normal control and the silymarin treated group. Nevertheless, there was a significant ( $p < 0.05$ ) difference between the 250 and 500 mg/kg b.w EECD treated groups. Normal levels of lipids in the liver are the result of maintenance of a proper balance between factors that tend to increase and decrease the fat content of the liver.  $CCl_4$  is involved in the production of fatty liver as shown by the result of TG in the intoxicated group compared to the normal.  $CCl_4$  produces fatty liver by the following mechanisms: interference with synthesis of apo-protein required to be incorporated in lipoprotein complex, interference with the secretory mechanism itself or interference with conjugation of the lipid moiety with lipoprotein apo-protein [39]. Pre-treatment with EECD at 500 mg/kg b.w prevented a drastic drop in TG. However, the silymarin group gave a better result.

Figure 11 shows a non-significant ( $p > 0.05$ ) difference in high-density lipoprotein cholesterol concentration in the positive control group compared to the EECD treated at 500 mg/kg b.w and the silymarin groups. However there was a significant difference between the normal and the EECD treated group at 250 mg/kg b. w EECD. The results of this study show an increase in HDL-cholesterol concentration in the intoxicated group compared to the normal. HDL-cholesterol has been demonstrated to influence the binding and uptake of LDL-cholesterol by the peripheral cells.

Maintenance of the pH of blood (7.35–7.45) is one of the prerequisites of life and relatively small changes in pH value of blood can lead to severe metabolic consequences. The primary buffers in blood are haemoglobin in the erythrocytes, and bicarbonate ion ( $HCO_3^-$ ) in the plasma. Bicarbonate buffer system is the chief buffer of blood and also constitutes the alkali reserve [39]. Neutralization of strong and non-volatile acids (such as HCl,  $H_2SO_4$ , lactic acid) entering the ECF is achieved by the bicarbonate buffers. Strong and non-volatile acids are converted 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.

Figure 12 shows a non-significant ( $p > 0.05$ ) increase in the serum bicarbonate concentration of the positive control group compared to the normal. Also observed was a non-significant ( $p > 0.05$ ) difference between the positive control and both the EECD and silymarin treated groups. The  $CCl_4$  treated group showed a significant ( $P < 0.05$ ) increase in  $[HCO_3^-]$  a condition which might lead to alkalosis. The condition is characterized by an absolute or relative increase in  $[HCO_3^-]$ . Primary alkali excess or increase in the alkali reserve is the most frequent cause of clinically observed alkalosis. However, the treated group did not show maintenance of acid- base balance, but an increase in alkali reserve in a dose dependent fashion. In other words, the EECD and the silymarin groups might have stimulated a compensatory mechanism that increased pulmonary respiration. This increased ventilation would result in  $CO_2$  loss and reduction in  $[H_2CO_3]$  with subsequent increase in  $[HCO_3^-]$ , increasing  $NH_3$  formation,  $H^+$  excretion compared to  $K^+$  excretion in distal

tubule and  $\text{HCO}_3^-$  reabsorption. The treated groups rather acted like they were exposed to metabolic acidosis (a reduction in plasma level of  $\text{HCO}_3^-$ ).

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## 5. Conclusion

Pre-treatment with the ethanol extract of *C. dolichopentalum* dose dependently stabilized the altered perturbation of the test organisms by  $\text{CCl}_4$  intoxication. *C. dolichopentalum* could be employed as a pharmacological tool for the protection of liver owing to ingestion of carbon tetrachloride.

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## Compliance with ethical standards

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### *Disclosure of conflict of interest*

The authors declare no conflict of interest.

### *Statement of ethical approval*

This study was conducted in accordance with laws and regulations for handling experimental animals as was approved by the Department of Biochemistry, FUTO

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