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## ***Citrullus lanatus* Extract Reverses Oxidative and Haematological Dysfunction in Carbon Tetrachloride Induced Liver Damaged Rats**

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**Abstract:** The study was carried out to evaluate the oxidative and haematologic effects of extract of *Citrullus lanatus* in carbon tetrachloride (CCl<sub>4</sub>) induced liver damaged rats. Thirty male albino rats were divided into five groups of six animals per group. The normal group was administered with distilled water for seven days. CCl<sub>4</sub> treated group was given vehicle on the first four days and with the vehicle and hepatotoxin on the fifth, sixth and seventh day. The animals in the treatment category were respectively administered with 500, 1,000 and 1,500 mg kg<sup>-1</sup> b.wt. of extract for the first four days and with extract and CCl<sub>4</sub> on the last three days. There was significantly reduced (p<0.05) levels of catalase (CAT), superoxide dismutase (SOD), Glucose 6-Phosphate Dehydrogenase (G6PD), Packed Cell Volume (PCV) and haemoglobin (Hb) concentration in CCl<sub>4</sub> treated rats but were however, normalized by the extract. Furthermore, the activities of Gluthathione s-transferase (GST) and malondialdehyde (MDA) in plasma were significantly increased (p<0.05) in CCl<sub>4</sub> treated group when compared with normal control rats, these were stabilized by the groups treated with the plant extract. Our findings suggest that *C. lanatus* exhibited reversal effects on these selected oxidative stress and haematologic markers in rats which were previously damaged by CCl<sub>4</sub>.

**Key words:** *Citrullus lanatus*, oxidative stress markers, carbon tetra chloride, haematological indices

### **INTRODUCTION**

Free radicals can be defined as atoms or group of atoms with unpaired electrons and capable of interacting with molecules thereby causing serious damage to living tissues. Free radicals have been implicated in the pathogenesis of a number of disorders such as: Liver diseases, inflammatory disorder, carcinomas, diabetes mellitus and cardiovascular diseases (Adebayo *et al.*, 2011a). Antioxidants are substances that can safely interact with these free radicals and significantly delay, scavenge or inhibit these radicals from their deleterious effects on the tissues. Antioxidants can either be localized intracellularly or extracellularly. Those located intracellularly are known to scavenge aberrant free radical species while the extracellular antioxidants can intercept free radical reactions and remove delocalized metal ions and decompartmentalized haem proteins (Rice-Evans, 1995). The intracellular antioxidants include: Catalase (Ogura and Yamazaki, 1983), glutathione peroxidase (Cohen and Hochstein, 1963) superoxide dismutases (Fridovich, 1978) and other peroxidases. Natural product

such as plants is beginning to serve as veritable source of antioxidant and blood supplement. Thus, *Citrullus lanatus* (Thunb.) Matsum plant belongs to the family of Cucurbitaceae and it is found in the tropical and subtropical climates worldwide. There are two cultivated watermelons: *C. lanatus* var. *lanatus* and *C. lanatus* var. *citroides* (Bailey) Mansf. *C. lanatus* is native to West Africa (Nigeria), Egypt, Middle-East, China and North America (Levi *et al.*, 2001; Gichimu *et al.*, 2009). *Citrullus lanatus* seeds are rich sources of oil. For instance, high oil content is produced from egusi-type of watermelon seeds in Nigeria (Dane *et al.*, 2004). Watermelons are relatively rich in protein, lipids, carbohydrates, vitamin A,  $\beta$ -carotene and lycopenes. The search for medicinal plants with excellent antioxidant and haemopoietic properties which would serve as natural therapies for the treatment of diseases associated with oxidative stress while boosting the systemic blood flow is of paramount concern to natural product scientists. Thus, the study investigated the effects of *C. lanatus* extract on some antioxidant and haematological parameters in carbon tetrachloride induced liver damaged rats.

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## MATERIALS AND METHODS

**Plant material:** The leaves of *Citrullus lanatus* were obtained from a farmland at Owode, Ogun State, Nigeria on December 16, 2010 and the plant was authenticated at the Department of Biological Sciences, Covenant University, Ogun State, Nigeria by Dr. A.C. Omonhinmin.

**Preparation of extracts:** The procedure described by Adebayo *et al.* (2010) was adopted. The leaves of *Citrullus lanatus* were obtained and dried under room temperature for about three days and then smoothly grinded using a dry blender and thus prepared for extraction. The powdered leaves of *C. lanatus* (588 g) were soaked in 7 L of 95% ethanol for four days, after which the extract was filtered with filter paper and a cotton wool. It was further concentrated at 50°C using rotary evaporator. A yield of 88.2 g representing 15% was obtained upon concentration of the extract.

**Experimental animals:** Thirty male albino rats with an average weight of 150 g used for this study were obtained from the Animal House of the Department of Biochemistry, University of Agriculture, Abeokuta, Nigeria. The animals were maintained at constant room temperature and fed with standard feed (grower's mash). The experimental animals were handled and used in accordance with the international guide for the care and use of laboratory animals (NIH, 1985). They were kept in standard laboratory conditions under natural light-dark cycle. The rats also had access to clean drinking water and variable factors such as light, temperature and humidity were maintained.

**Experimental design:** Experimental protocol as described by Adebayo *et al.* (2011b) was adopted for the study. Thirty male albino Wistar rats were used for the study. The rats were divided into five groups, each group consisting of six animals. The first two groups served as negative and positive control groups, respectively while three groups served as the treatment groups. Animals in the normal control group were given only vehicle (distilled water, 1 mL kg<sup>-1</sup> body weight) for seven days. Animals in the positive control group were administered orally with vehicle for the first four days and vehicle +CCl<sub>4</sub> (50% solution of CCl<sub>4</sub> in olive oil, i.p., 2 mL kg<sup>-1</sup> b. wt.) on the 5th, 6th and 7th day. Similarly, animals in the extract treatment category were treated with 500, 1,000 and 1,500 mg kg<sup>-1</sup> b. wt. of distilled water and ethanolic leaf extract for the first four days and distilled water+ethanolic extract+CCl<sub>4</sub> on the last three days. They were all administered according to their body weight and after

seven days, the rats were subjected to overnight fasting and were sacrificed the following day. The rats were anaesthetized with diethylether.

**Blood collection and preparation of sample for assays:** At the end of the experimental period, venous blood was collected from the experimental animals by cardiac puncture into EDTA heparinized and plain tubes for the determination of haematological and serum enzyme levels, respectively. The serum was prepared by centrifuging the heparinized blood samples at 3000 rpm for 15 min (Ogbu and Okechukwu, 2001) and collected by pipetting. The animals were quickly dissected and the liver organs removed. The livers were removed, perfused with ice-cold 1.15% KCl and homogenized in buffered saccharose solution (Tris, pH 7.55). The homogenates were maintained at -20°C overnight to ensure maximum release of the enzymes (Ngaha *et al.*, 1989).

**Assays of antioxidant enzyme activities and haematological parameters:** The blood and homogenate samples were collected for catalase (CAT), malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione-S-transferase (GST) assays. Glucose 6-Phosphate Dehydrogenase (G6PD) was determined by the method described by Kornberg *et al.* (1955). The haemoglobin (Hb) concentration and Packed Cell Volume (PCV) were analyzed according to the standard techniques described by Baker *et al.* (1998) and Cheesbrough (2000).

**Analysis of biochemical parameters:** Commercial test kits obtained from Randox Laboratories, United Kingdom were used for all biochemical parameters measured. Standard methods were used to estimate GST (Habig and Jakoby, 1981) TBARS was analyzed and expressed as the amount of MDA formed (Niehaus and Samuelson, 1968). Superoxide dismutase was assayed utilizing the technique of Misra and Fridovich (1972) while catalase activity was performed by the methods of Sinha (1972).

**Statistical analysis:** All data are expressed as Mean±SEM and Tukey's *post hoc* test was carried to analyze significance of difference between different groups using the Graph pad prism version 5 Software Program. Values with p<0.05 are considered as significant.

## RESULTS

The effect of *C. lanatus* on the weight of organs of CCl<sub>4</sub> induced liver damaged rats is as shown in Table 1. There was no significant (p>0.05) difference in the lungs, liver, brain, heart, spleen and kidney organs when

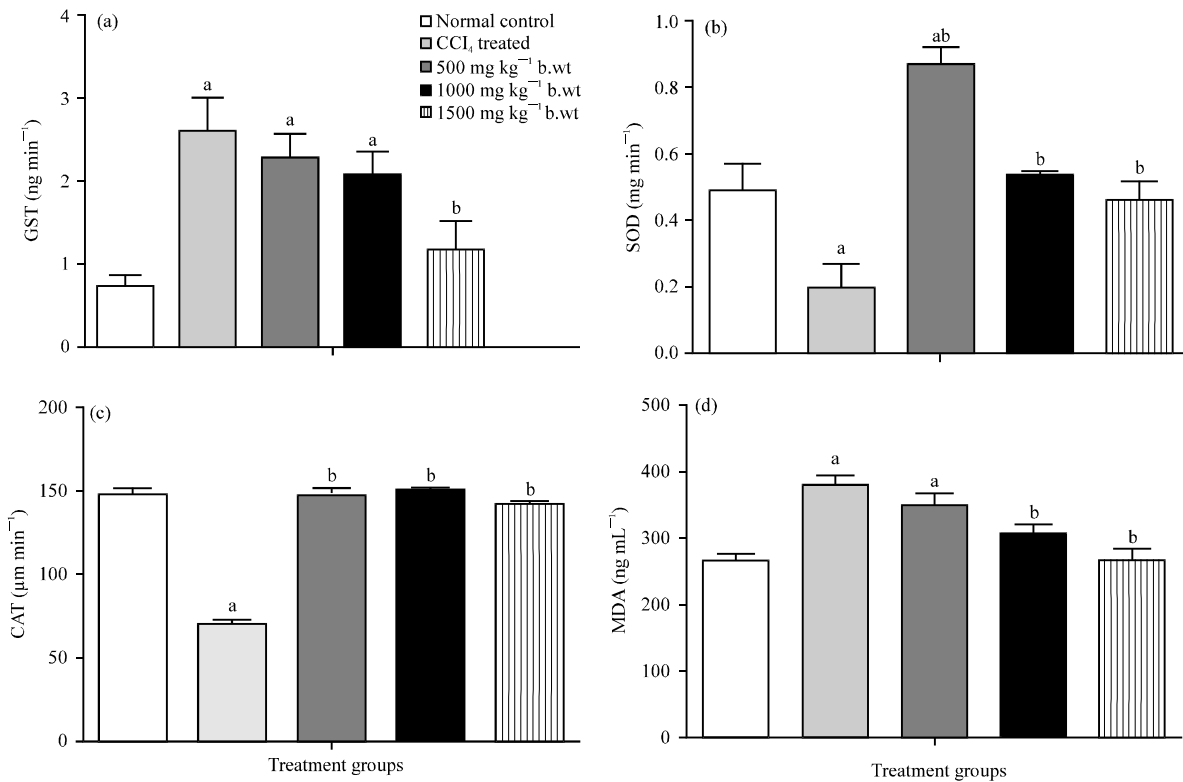


Fig. 1(a-d): Effect of *Citrullus lanatus* on selected plasma oxidative stress parameters in CCl<sub>4</sub> treated rats (a) GST, glutathione-S-transferase activity, (b) SOD, superoxide dismutase activity, (c) CAT, catalase activity and (d) MDA, malondialdehyde level (TBARS, thiobarbituric acid was measured as MDA) in plasma of rats. Values represent mean±SEM of 6 replicates. <sup>a</sup>Significantly different from normal control: p<0.05 <sup>b</sup>Significantly different from positive control: p<0.05

Table 1: Effect of *Citrullus lanatus* on the weight of organs of CCl<sub>4</sub> induced rats

Organs (g)	Normal control	CCl <sub>4</sub> induced	500 mg kg <sup>-1</sup> b. wt.	1000 mg kg <sup>-1</sup> b.wt	1500 mg kg <sup>-1</sup> b.wt
Lungs	1.84±0.04	1.79±0.35	2.07±0.07	1.795±0.09	2.27±0.35
Liver	7.49±0.31	8.68±0.45	9.06±0.44	8.61±0.50	8.08±0.59
Brain	2.70±0.07	2.19±0.23	2.84±0.54	3.77±0.03	2.78±0.03
Heart	1.63±0.05	1.58±0.071	1.53±0.13	2.05±0.19	1.92±0.21
Spleen	1.89±0.44	1.55±0.074	1.89±0.11	1.83±0.11	1.56±0.07
Kidney	1.86±0.02	2.03±0.02	1.93±0.59	1.98±0.11	2.30±0.07

Values represent Mean±SEM of 6 replicates: p>0.05, <sup>a</sup>Significantly different from normal control: p<0.05, <sup>b</sup>Significantly different from positive control: p<0.05

compared with the CCl<sub>4</sub> treated and the negative control groups. Figure 1 shows the effect of *C. lanatus* on selected plasma oxidative stress parameters in CCl<sub>4</sub> treated rats. CCl<sub>4</sub> treated (positive control) animals showed significant (p<0.05) increase in the plasma levels of GST (2.59±0.41), MDA (378.83±16.77) and a significant decrease in CAT (71.26±0.77), PCV (31.6±0.76) and Hb (10.57±0.26) as compared with normal control group, indicating anomaly in the cells and tissues producing these markers caused by CCl<sub>4</sub> treatment (Fig. 1 and 2). Animals treated with the extract of *C. lanatus* exhibited dose dependent reversal effects with a significant (p<0.05) decrease in levels of GST and MDA, while significantly

increasing CAT, PCV and Hb levels (Fig. 2). There was a significant (p<0.05) reduction in the activity of G6PD when compared with the normal control group but this was however, restored with a significant (p<0.05) increase when the rats were treated with the extract of *C. lanatus* (Fig. 3). SOD activity was significantly (p<0.05) raised in the group treated with 500 mg kg<sup>-1</sup> bw when compared with the normal and CCl<sub>4</sub> treated groups, whereas the animals treated with the higher doses of the extract significantly increase the activity of SOD only in the CCl<sub>4</sub> treated group (Fig. 1). The activities of SOD (3.80±0.71), GST (0.25±0.02 and CAT (73.30±2.12) in liver homogenate were significantly (p<0.05) decreased while the level of

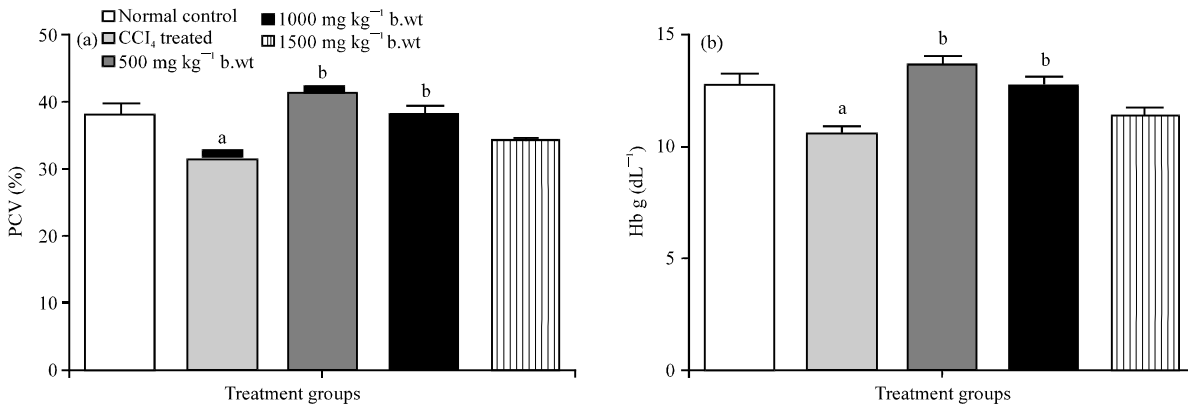


Fig. 2(a-b): Effect of *Citrullus lanatus* on packed cell volume and haemoglobin concentration in CCl<sub>4</sub> treated rats (a) PCV, packed cell volume, (b) Hb, haemoglobin concentration in whole blood of rats, Values represent mean±SEM of 6 replicates. <sup>a</sup>Significantly different from normal control: p<0.05, <sup>b</sup>Significantly different from positive control: p<0.05

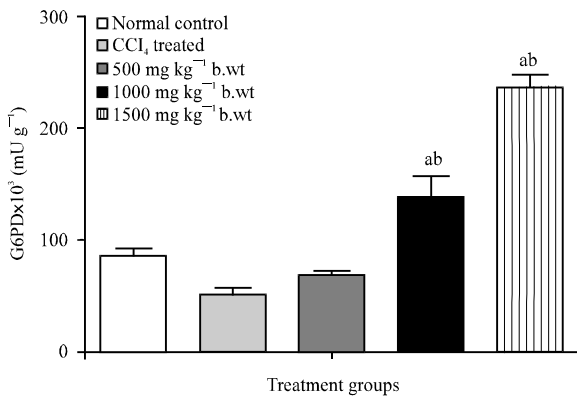


Fig. 3: Effect of *Citrullus lanatus* on glucose 6 phosphate dehydrogenase activity in CCl<sub>4</sub> treated rats. Values represent Mean±SEM of 6 replicates. <sup>a</sup>Significantly different from normal control: p<0.05, <sup>b</sup>Significantly different from positive control: p<0.05

MDA (296.83±19.56) was increased significantly in the CCl<sub>4</sub> treated animals indicating cellular damaged caused by CCl<sub>4</sub> (Fig. 4). However, as observed from all these parameters, these effects were reversed when the animals were treated with the extract of *C. lanatus* (Fig. 4).

### DISCUSSION

Glucose-6-Phosphate Dehydrogenase (G6PD) is an important enzyme involved in pentose phosphate pathway. It catalyzes Nicotinamide Adenine Dinucleotide Phosphate (NADP<sup>+</sup>) to a reduced form (NADPH). This process leads to the reduction of oxidative glutathione (GSSG) to reduced glutathione (GSH) which protects

human red blood cells from premature destruction caused by oxidative damage (Beutler *et al.*, 2007). The decrease in activity of G6PD in liver of the CCl<sub>4</sub> treated rats may be due to inhibition of protein synthesis and accelerated proteolysis and structural disintegration (Agarwal and Rami, 2003). This inhibition of the activity of G6PD with rats challenged with CCl<sub>4</sub> supports the work of Kujawska *et al.* (2007). The rise in the activity of G6PD observed in treated group after induction with CCl<sub>4</sub> is an indication that *C. lanatus* extract at the doses administered is capable of generating a more reduced form of GSH thus helping to increase the systemic antioxidant level. This present study is in consistent with the result of Parvez *et al.* (2006), where they investigated the modulatory and protective effects of catechin on the toxicity of tamoxifen, an anticancer drug. It was observed that the antioxidant significantly increased the activity of G6PD in both the liver and kidney of tamoxifen treated rats. The levels PCV and Hb of the CCl<sub>4</sub> treated group was significantly decreased and indicates the ability of this hepatotoxin to alter the haematopoietic elements. The extract showed a reversal effect with a significant increase in the PCV and Hb levels to a near normal level. PCV is a measure of erythrocytes in the blood, an increment connotes production of red blood cells and in turn an increase in blood volume (Adebayo *et al.*, 2006). From the results, it could be deduced that the plant has blood boosting effects. Free radicals play very important roles in various pathogenesis, inflammatory diseases and can result in necrosis of the liver (Adebayo *et al.*, 2011a). Increased activities of MDA and GST have been observed in the plasma while an elevation in MDA

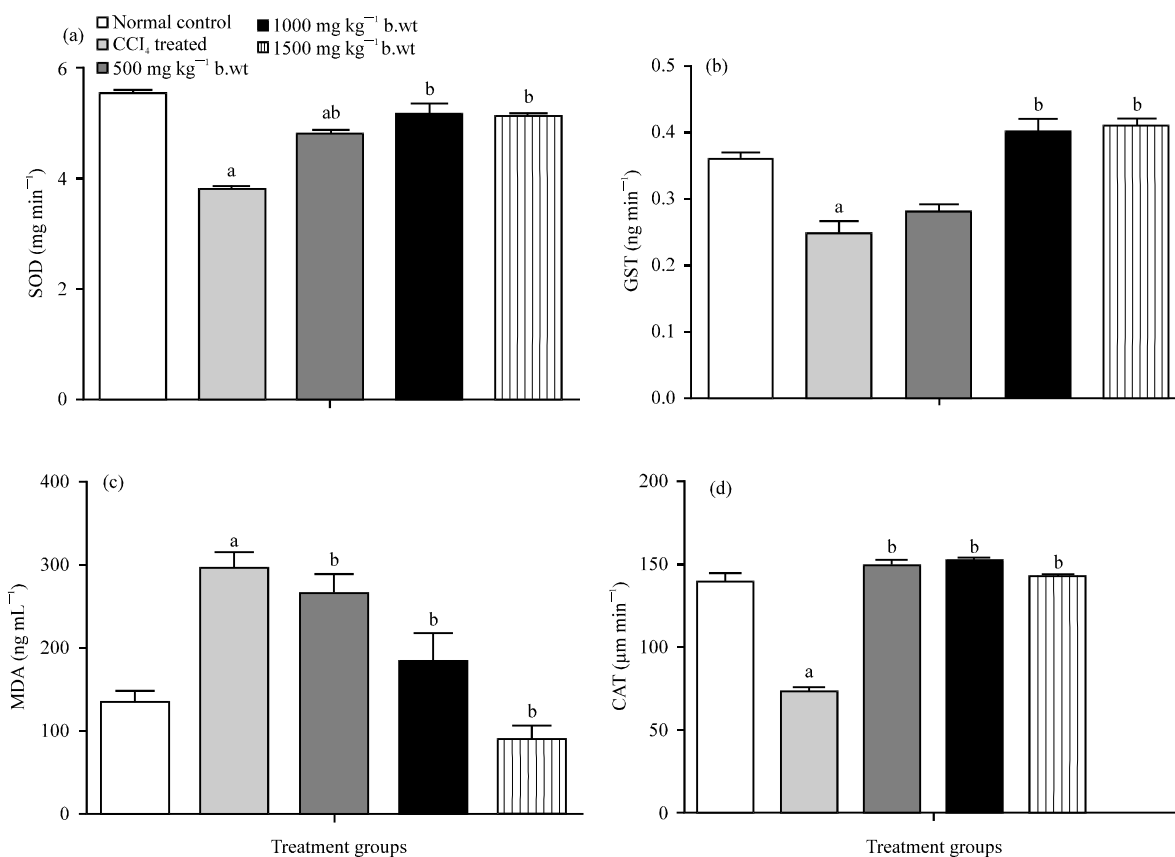


Fig. 4(a-d): Effect of *Citrullus lanatus* on selected oxidative stress parameters of liver homogenate in CCl<sub>4</sub> treated rats (a) SOD, superoxide dismutase activity, (b) GST, glutathione-S-transferase activity, (c) MDA, malondialdehyde level (TBARS, thiobarbituric acid was measured as MDA) and (d) CAT, catalase activity of liver homogenate tissue of rats, Values represent mean±SEM of 6 replicates. <sup>a</sup>Significantly different from normal control: p<0.05, <sup>b</sup>Significantly different from positive control: p<0.05

concentration was also observed in the liver homogenate in CCl<sub>4</sub> treated rats, this may result in a number of harmful effects due to the accumulation of superoxide radicals and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). The administration of the plant extract of *C. lanatus* normalizes the MDA and GST levels in the blood, thus indicating the ability of the extract to rapidly scavenge the free radicals. MDA is the end product of lipid peroxidation and a measure of free radical generation (Adebayo *et al.*, 2011a). SOD is a metalloprotein and is the first enzyme involved in the antioxidant defence by lowering the steady state level of O<sub>2</sub><sup>-</sup>. SOD which converts superoxide radicals to H<sub>2</sub>O<sub>2</sub> is widely distributed to protect such cells against the toxic effects of superoxide anion. CAT is a hemoprotein, localized in the peroxisomes or the microperoxisomes. This enzyme catalyses the decomposition of H<sub>2</sub>O<sub>2</sub> to H<sub>2</sub>O and O<sub>2</sub> and thus protecting the cell from oxidative damage by H<sub>2</sub>O<sub>2</sub> and OH. The main function of catalase is to detoxify H<sub>2</sub>O<sub>2</sub> (Naganuma *et al.*, 1990). A decrease in the activities

of SOD and CAT with CCl<sub>4</sub> probably results in the accumulation of O<sub>2</sub> and H<sub>2</sub>O<sub>2</sub> which react with metal ions to promote additional radical generation, with the release of the reactive hydroxyl radicals. Hydroxyl radicals react with lipids, DNA and proteins to cause loss of cellular integrity, enzyme function and genomic instability (Ohta *et al.*, 2004; Ekor *et al.*, 2006). *Citrullus lanatus* extract restored markedly both SOD and CAT activities reduced by CCl<sub>4</sub> pretreatment. The result of the SOD and CAT activities clearly shows that *C. lanatus* contains a free radical scavenging activity which could exert a beneficial action against pathological alteration caused by the presence of O<sub>2</sub><sup>•</sup> and OH<sup>•</sup> (Raghavan and Kumari, 2006). This action could involve the mechanism of O<sub>2</sub><sup>•</sup> in relation to the scavenging activity. In a similar experiment, a significant decrease was observed in CAT activity while a significant rise was noticed in the level of MDA in rats pretreated with CCl<sub>4</sub> and subsequently administered with *C. albidum* extract (Adebayo *et al.*, 2011a).

## CONCLUSION

Our findings suggest that *C. lanatus* exhibited reversal effects on oxidative stress and haematologic parameters in rats' liver tissues which were previously damaged by  $CCl_4$ , thus making the plant a potential source of natural antioxidant and blood boosters. Further study is however, required to determine the specific mechanism for initiating these properties.

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