

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

^{1,2}Abiodun Humphrey Adebayo, ²Fu Hang Song, ²Xue Ting Liu, ²Qin Huan Dai, ²Pei Huang, ²Ji Yu Zhang and ²Li Xin Zhang

¹Department of Biological Sciences, College of Science and Technology, Covenant University, PMB 1023, Canaan Land, Ota, Ogun State, Nigeria

²CASKey Laboratory of Pathogenic Microbiology and Immunology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China

Abstract: The study was carried out to evaluate the oxidative and haematologic effects of extract of *Citrullus lanatus* in carbon tetrachloride (CCl₄) 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. CCl₄ 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⁻¹ b.wt. of extract for the first four days and with extract and CCl₄ 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 CCl₄ 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 CCl₄ 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 CCl₄.

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, β -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.

Corresponding Authors: Li Xin Zhang and Abiodun Humphrey Adebayo,
CASKey Laboratory of Pathogenic Microbiology and Immunology, Institute of Microbiology,
Chinese Academy of Sciences, Beijing, China

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⁻¹ body weight) for seven days. Animals in the positive control group were administered orally with vehicle for the first four days and vehicle +CCl₄ (50% solution of CCl₄ in olive oil, i.p., 2 mL kg⁻¹ 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⁻¹ b. wt. of distilled water and ethanolic leaf extract for the first four days and distilled water+ethanolic extract+CCl₄ 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 CCl₄ 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

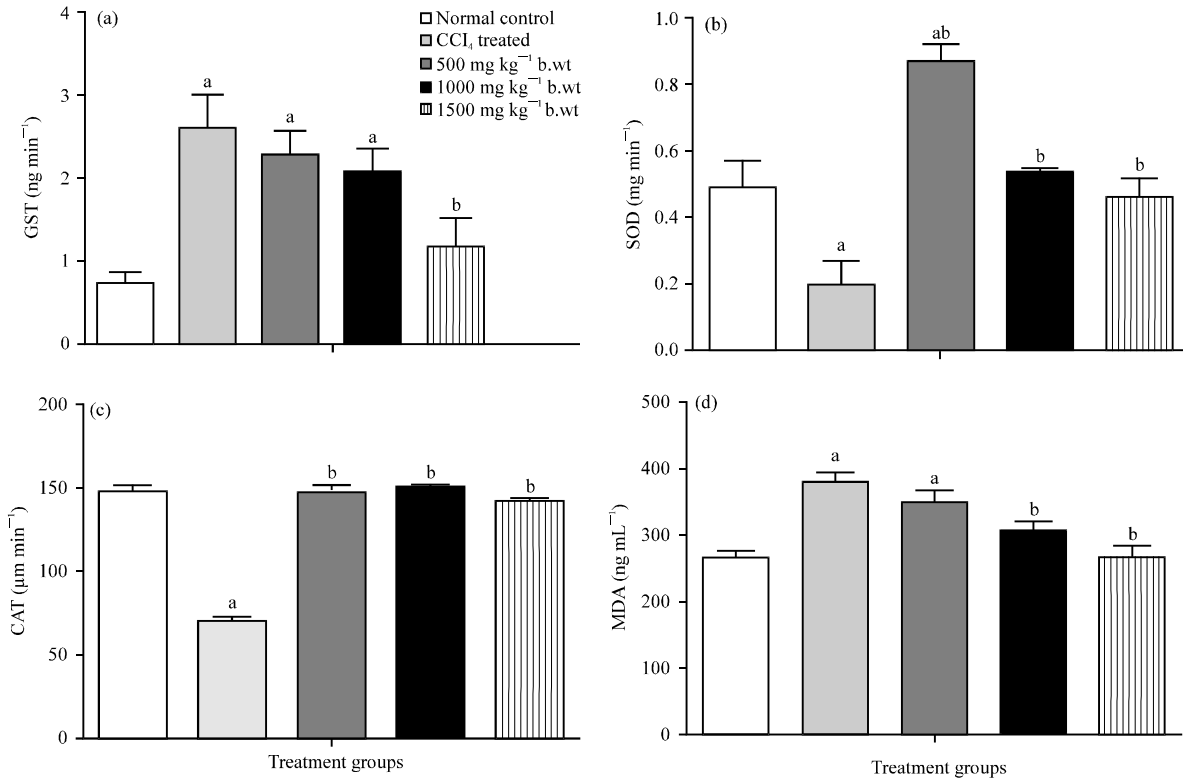


Fig. 1(a-d): Effect of *Citrullus lanatus* on selected plasma oxidative stress parameters in CCl₄ treated rats (a) GST, glutathione-S-transferase activity, (b) SOD, superoxide dismutase activity, (c) CAT, catalase activity and (d) MDA, malondialdehyde level (TBARS, thiobarbituric acid was measured as MDA) in plasma of rats. Values represent mean±SEM of 6 replicates. ^aSignificantly different from normal control: p<0.05 ^bSignificantly different from positive control: p<0.05

Table 1: Effect of *Citrullus lanatus* on the weight of organs of CCl₄ induced rats

Organs (g)	Normal control	CCl ₄ induced	500 mg kg ⁻¹ b. wt.	1000 mg kg ⁻¹ b.wt	1500 mg kg ⁻¹ 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, ^aSignificantly different from normal control: p<0.05, ^bSignificantly different from positive control: p<0.05

compared with the CCl₄ treated and the negative control groups. Figure 1 shows the effect of *C. lanatus* on selected plasma oxidative stress parameters in CCl₄ treated rats. CCl₄ 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 CCl₄ 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⁻¹ bw when compared with the normal and CCl₄ treated groups, whereas the animals treated with the higher doses of the extract significantly increase the activity of SOD only in the CCl₄ 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

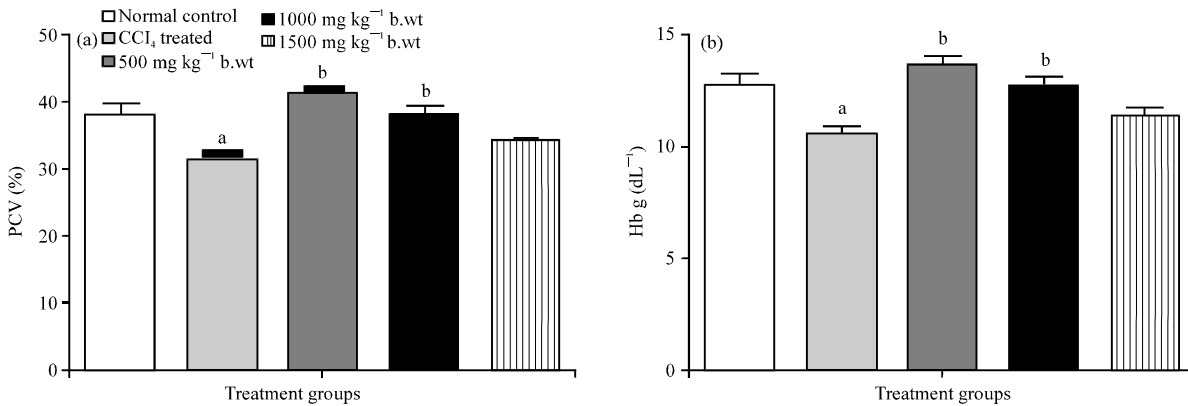


Fig. 2(a-b): Effect of *Citrullus lanatus* on packed cell volume and haemoglobin concentration in CCl₄ treated rats (a) PCV, packed cell volume, (b) Hb, haemoglobin concentration in whole blood of rats, Values represent mean±SEM of 6 replicates. ^aSignificantly different from normal control: p<0.05, ^bSignificantly different from positive control: p<0.05

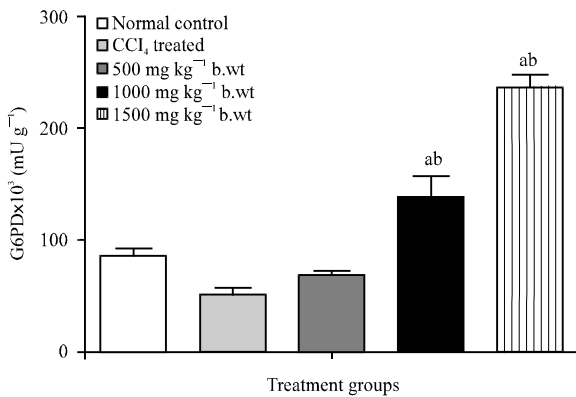


Fig. 3: Effect of *Citrullus lanatus* on glucose 6 phosphate dehydrogenase activity in CCl₄ treated rats. Values represent Mean±SEM of 6 replicates. ^aSignificantly different from normal control: p<0.05, ^bSignificantly different from positive control: p<0.05

MDA (296.83±19.56) was increased significantly in the CCl₄ treated animals indicating cellular damaged caused by CCl₄ (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⁺) 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 CCl₄ 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 CCl₄ supports the work of Kujawska *et al.* (2007). The rise in the activity of G6PD observed in treated group after induction with CCl₄ 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 CCl₄ 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

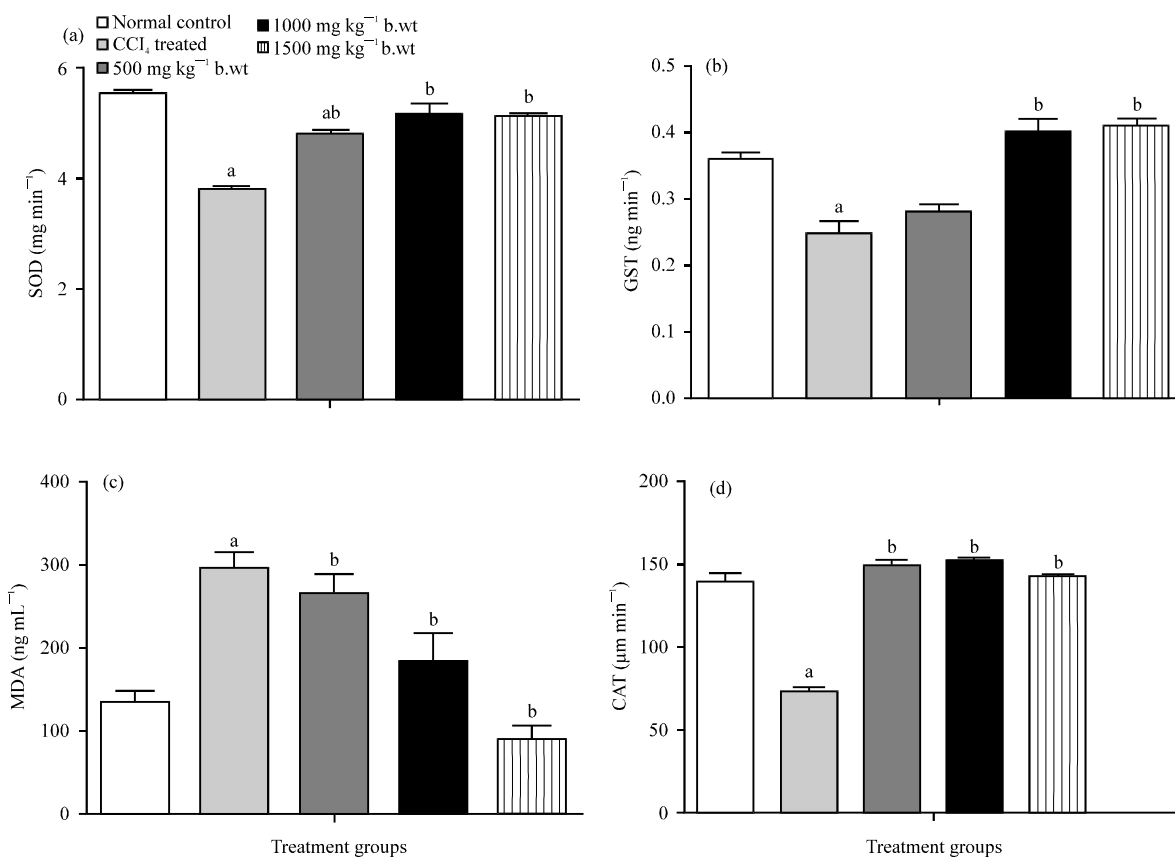


Fig. 4(a-d): Effect of *Citrullus lanatus* on selected oxidative stress parameters of liver homogenate in CCl₄ treated rats (a) SOD, superoxide dismutase activity, (b) GST, glutathione-S-transferase activity, (c) MDA, malondialdehyde level (TBARS, thiobarbituric acid was measured as MDA) and (d) CAT, catalase activity of liver homogenate tissue of rats, Values represent mean±SEM of 6 replicates. ^aSignificantly different from normal control: p<0.05, ^bSignificantly different from positive control: p<0.05

concentration was also observed in the liver homogenate in CCl₄ treated rats, this may result in a number of harmful effects due to the accumulation of superoxide radicals and hydrogen peroxide (H₂O₂). 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 O₂⁻. SOD which converts superoxide radicals to H₂O₂ 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 H₂O₂ to H₂O and O₂ and thus protecting the cell from oxidative damage by H₂O₂ and OH. The main function of catalase is to detoxify H₂O₂ (Naganuma *et al.*, 1990). A decrease in the activities

of SOD and CAT with CCl₄ probably results in the accumulation of O₂ and H₂O₂ 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 CCl₄ 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 O₂[•] and OH[•] (Raghavan and Kumari, 2006). This action could involve the mechanism of O₂[•] 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 CCl₄ 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 CCl₄, 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.

ACKNOWLEDGMENTS

We wish to thank Chinese Academy of Sciences (CAS) and The World Academy of Sciences (TWAS) for the Post Doctoral Research Fellowship granted to AHA.

REFERANCES

- Adebayo, A.H., R. Aliyu, D. Gatsing and I.H. Garba, 2006. The effects of ethanolic leaf extract of *Commiphora africana* (Buseraceae) on lipid profile in rats. *Int. J. Pharmacol.*, 2: 618-622.
- Adebayo, A.H., G.Z. Zeng, Y.M. Zhang, J.T. Fan and C.J. Ji *et al.*, 2010. Biochemical, haematological and histopathological studies of ethanolic leaf extract of *Ageratum conyzoides* in Sprague Dawley rats. *J. Med. Plant Res.*, 4: 2264-2272.
- Adebayo, A.H., A.O. Abolaji, R. Kela, O.O. Ayepola, T.B. Olorunfemi and O.S. Taiwo, 2011a. Antioxidant activities of the leaves of *Chrysophyllum albidum* G. Pak. *J. Pharm. Sci.*, 24: 545-551.
- Adebayo A.H., A.O. Abolaji, R. Kela, S.O. Oluremi, O.O. Owolabi and O.A. Ogungbe 2011b. Hepatoprotective activity of *Chrysophyllum albidum* against carbon tetrachloride induced hepatic damage in rats. *Can. J. Pure Applied Sci.*, 5: 1597-1602.
- Agarwal, R. and A. Rami, 2003. Redistribution of Glucose-6-phosphate dehydrogenase in response to cerebral ischemia in rat brain. *Indian J. Clin. Biochem.*, 18: 64-70.
- Baker, F.J., R.E. Silverton and C.J. Pallister, 1998. Baker and Silverton's Introduction to Medical Laboratory Technology. 7th Edn., Butterworth Heinmann, London, UK., pp: 356-360.
- Beutler, E., S. Duparc and G6PD Deficiency Working Group, 2007. Glucose-6-phosphate dehydrogenase deficiency and antimalarial drug development. *Am. J. Trop. Med. Hyg.*, 77: 779-789.
- Cheesbrough, M., 2000. District Laboratory Practices in Tropical Countries, Part 2. 2nd Edn., Cambridge University Press, London, UK., pp: 267-334.
- Cohen, G. and P. Hochstein, 1963. Glutathione peroxidase: The primary agent for the elimination of hydrogen peroxide in erythrocytes. *Biochemistry*, 172: 1420-1428.
- Dane, F., P. Lang and R. Bakhtiyarova, 2004. Comparative analysis of chloroplast DNA variability in wild and cultivated *Citrullus* species. *Theor. Applied Genet.*, 108: 958-966.
- Ekor, M., E.O. Farombi and G.O. Emerole, 2006. Modulation of gentamicin-induced renal dysfunction and injury by the phenolic extract of soybean (*Glycine max*). *Fundam. Clin. Pharmacol.*, 20: 263-271.
- Fridovich, I., 1978. The biology of oxygen radicals. *Science*, 201: 875-880.
- Gichimu, B.M., B.O. Owuor, G.N. Mwai and M.M. Dida, 2009. Morphological characterization of some wild and cultivated watermelon (*Citrullus* sp.) accessions in Kenya. *ARPN J. Agric. Biol. Sci.*, 4: 10-18.
- Habig, W.H. and W.B. Jakoby, 1981. Assays for differentiation of Glutathione S-transferases. *Methods Enzymol.*, 77: 398-405.
- Kornberg, A. and B.L. Horekar, 1955. *Methods in Enzymology*. Academic Press, New York, Pages: 323.
- Kujawska, M., J. Jodynis-Liebert, M. Ewertowska, T. Adamska, I. Matlawska and W. Bylka, 2007. Protective effect of *Aquilegia vulgaris* (L.) on carbon tetrachloride-induced oxidative stress in rats. *Indian J. Exp. Biol.*, 45: 702-711.
- Levi, A., C.E. Thomas, T.C. Welner and X. Zhang, 2001. Low genetic diversity indicates the need to broaden the genetic base of cultivated watermelon. *Hortscience*, 36: 1096-1101.
- Misra, H.P. and I. Fridovich, 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J. Biol. Chem.*, 247: 3170-3175.
- NIH, 1985. Guide for the Care and Use of Laboratory Animals. NIH Publication No. 85-23, National Institutes of Health, Bethesda, USA.
- Naganuma, A., M.E. Anderson and A. Meister, 1990. Cellular glutathione as a determinant of sensitivity to mercuric chloride toxicity: Prevention of toxicity by giving glutathione monoester. *Biochem. Pharmacol.*, 40: 693-697.
- Ngaha, E.O., M.A. Akanji and M.A. Madusolomo, 1989. Studies on correlation between chloroquine-induced tissue damage and serum changes in rats. *Experientia*, 45: 143-146.
- Niehaus, Jr. W.G. and B. Samuelsson, 1968. Formation of malonaldehyde from phospholipid arachidonate during microsomal lipid peroxidation. *Eur. J. Biochem.*, 6: 126-130.

- Ogura, Y. and I. Yamazaki, 1983. Steady-state kinetics of the catalase reaction in the presence of cyanide. *J. Biochem.*, 94: 403-408.
- Ogbu, S.I. and E.I. Okechukwu, 2001. The effect of storage temperature prior to separation on plasma and serum potassium. *J. Med. Lab. Sci.*, 10: 1-4.
- Ohta, Y., M. Kongo-Nishimura, T. Matsura, K. Yamada, A. Kitagawa and T. Kishikawa, 2004. Melatonin prevents disruption of hepatic reactive oxygen species metabolism in rats treated with carbon tetrachloride. *J. Pineal Res.*, 36: 10-17.
- Parvez, S., H. Tabassum, H. Rehman, B.D. Banerjee, M. Athar and S. Raisuddin, 2006. Catechin prevents tamoxifen-induced oxidative stress and biochemical perturbations in mice. *Toxicology*, 225: 109-118.
- Raghavan, B. and S.K. Kumari, 2006. Effect of *Terminalia arjuna* stem bark on antioxidant status in liver and kidney of alloxan diabetic rats. *Indian J. Physiol. Pharmacol.*, 50: 133-142.
- Rice-Evans, C., 1995. Free Radicals and Antioxidants in Artherosclerosis. In: *Immunopharmacology of Free Radical Species*, Blake, D. and P.G. Winyard (Eds.). Academic Press, Londonm, UK., pp: 39-52.
- Sinha, A.K., 1972. Colorimetric assay of catalase. *Anal. Biochem.*, 47: 389-394.