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Hepatoprotective effect of methanol seed extract of *Sphenostylis stenocarpa* (Hoschst ex. A. Rich, Harms) against carbon tetrachloride induced liver toxicity in wistar rats

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ABSTRACT

The present study was carried out to evaluate the effect of methanol seed extract of Sphenostylis stenocarpa (African yam bean) on some antioxidant and liver marker enzymes of rats treated with CCl_4 as well as the acute toxicity test of the extract. Twenty four (24) Wistar rats were randomly distributed into 6 groups of 4 rats each. Group 1 rats were not induced with CCl_4 and were given normal rat feed and water (normal control). Groups 2-6 were induced with CCl_4. Group 2 rats received no treatment (negative control) and those in group 3 received 300 mg/kg body weight of ascorbic acid (standard drug). Groups 4, 5 and 6 were treated respectively with 200, 400 and 800 mg/kg body weight of S. stenocarpa methanol seed extract. The acute toxicity test (LD_{50}) of the extract showed no toxicity up to 5000 mg/kg body weight. This showed that the seed of the plant is safe for human and animal consumption. The glutathione concentration, catalase and superoxide dismutase activity of the rats increased significantly ($p<0.05$) in a dose dependent manner in all the treatment groups. The methanol seed extract had a positive effect on the antioxidant enzymes of rats treated with CCl_4. There were no significant ($p>0.05$) increase in the alanine aminotransferase, Aspartate aminotransferase and alkaline phosphatase activity of rats in all the treatment groups. These suggest that S. stenocarpa seeds possess some antioxidant and non-hapatotoxic properties.

Keywords: *Sphenostylis stenocarpa*, carbon tetrachloride, catalase, hepatotoxicity

INTRODUCTION

Antioxidants are compounds that are capable of delaying or inhibiting the oxidation of lipids or other biomolecules by inhibiting the initiation or propagation of oxidizing chain reactions (Wang and Ballington, 2007). There is an increasing interest in the biochemical basis of and on the mechanism of action of protective effect of naturally occurring antioxidants in biological systems (Omale *et al*., 2009). A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials.

Carbon tetrachloride (CCl_4) is a hepatotoxic solvent that produces several biochemical effects on animal organs. The toxicity of CCl_4 requires activation by the liver microsomal mixed function oxidase (CYP 2EI) to produce trichloromethyl radical ($\bullet CCl_3$) (Albano *et al*., 1982), which is subsequently converted into a trichloromethyl peroxyl radical ($\bullet CCl_3OO.$) in the presence of oxygen (Khan and Younus, 2011; Halliwell and Gutteridge, 2007). The trichloromethyl peroxyl radical oxidizes membrane polyunsaturated fatty acids via lipid peroxidation (Slater, 1982), and the membranes of liver cells are vulnerable to be attacked by both endogenously produced toxic substances and those consumed exogenously (Olajide *et al*., 2009). Free radical scavenging mechanisms and antioxidant substances play important role in the protection against CCl_4–induced hepatic damage (Xiong *et al*., 1998).

Several agents such as polyphenols, flavonoids and phenolic compounds, a-tocopherol (Vitamin E), ascorbic acids (Vitamin C) that are found in various herbs spices and seeds have been reported to exhibit antioxidant activity (Aqil *et al*., 2006) and have also been found to decrease and prevent CCl_4 toxicity in animals (Ajiboye *et al*., 2010). Also reduced glutathione, superoxide dismutase and catalase have also been implicated to play a role in detoxification of toxic metabolites in animals produced during the metabolism of CCl_4 (Ajiboye *et al*., 2010). Seeds, spices and herbs are recognized as sources of natural antioxidants and thus play an important role in the chemoprevention of diseases and aging (Khalaf *et al*., 2009). Natural antioxidants that are present in herbs and spices are responsible for inhibiting or preventing the deleterious consequences of oxidative stress. African yam bean (*Sphenostylis stenocarpa*) seeds have been shown to contain numerous antioxidants. Spices and herbs contain free radical scavengers like polyphenols, flavonoids and phenolic compounds. This necessitated the investigation of the antioxidant and liver function enzymes of rats induced with CCl_4 and treated with the methanol seed extract of *S. stenocarpa*.

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African yam bean, (*Sphenostylis stenocarpa* Hoschst ex. A. Rich, Harms, Fabaceae) is an herbaceous leguminous plant occurring throughout tropical Africa and some parts of the world (Porter and Doyle, 1992). It is among the minor legumes in Nigeria and other surrounding countries because it is underutilized (Umerie *et al*., 2009). African yam bean seeds have recently attracted research interest because it possesses antioxidant properties against a variety of physiologically relevant free radicals (Ajibolu *et al*., 2011). Therefore, there is need to continue the investigation of the mechanism by which plants and plant products protect and prevent tissues from damage by chemical compounds such as CCl_4, H_2O_2 etc. This potential could enable its protective efficiency to the liver against the hepatotoxic effect of carbon tetrachloride (CCl_4).

MATERIALS AND METHODS

Plant material

Dried seeds of *Sphenostylis stenocarpa* were purchased from Orba market in Nsukka Local Government Area, Enugu State. The seeds were authenticated by Mr Alfred Ozioko at the Biodiversity Centre, Nsukka, Enugu State.

Preparation of Methanol Seed Extract of *S. stenocarpa*.

The seeds were pulverized to powder with a hand Mill. About 500g of the pulverized seeds was macerated in 1.5 L of methanol with thorough shaking at regular intervals for 24 h at room temperature (28 ± 2 ^{0} C). The resulting solution was then filtered using Whatman No. 1 filter paper. A constant weight of the extract was obtained by evaporating the solvent at 60 ^{0}C in an oven. The extractive weight of the extract is 18 g.

Experimental Animals and administration of compounds.

Twenty (20) Wistar rats (*Rattus novergicus*) weighing between 180-220 g were obtained from the Animal House of the Faculty of Biological Sciences, University of Nigeria, Nsukka and were used in the study. The animals were housed individually in steel metabolic cages of dimensions 33 x 20.5 x 19 cm with cleaning of the cages done once daily. They were acclimatized for one week before the commencement of the study. All through the study, they were fed *ad libitum* on standard pellet feed (Grand Cereals and Oil Mills Ltd, Jos, Nigeria) and freely provided drinking water. The rats were divided into five groups (n= 4). Group 1 received 0.5 ml of paraffin oil once daily without treatment with CCl_4 (normal control). Group 2 rats were intraperitoneally treated with 0.5 ml of CCl_4 solution only (prepared by mixing 0.5 ml of CCl_4 with 1.5 ml of paraffin oil). Groups 3, 4 and 5 were simultaneously treated with 300 mg/kg body weight of ascorbic acids (positive control), 400 mg/kg and 800 mg/kg body weight of methanol seed extract of *S. stenocarpa* respectively through oral tubation with intraperitoneal treatment with 0.5 ml of CCl_4.

Preparation of serum and tissue homogenate

The rats were sacrificed 24 h after their last daily doses using the anaesthetic method described by Yakubu *et al*., 2005. The rats were made to bleed under diethyl ether anesthesia, through their cut jugular veins (slightly displaced to prevent blood from being contaminated with interstitial fluid) into centrifuge tubes. The blood samples were allowed to clot for 15 min and centrifuged at 33.5 g to obtain the sera. The sera were stored frozen and used within 12 h of preparation for the biochemical assay.

Biochemical assay

Biochemical parameters were assayed as decribed for alkaline phosphatase (ALP) (Wright *et al*., 1972), alanine aminotransferase (ALT) (Bergmeyer *et al*., 1986a), aspartate aminostransferase (AST) (Bergmeyer *et al*., 1986b), superoxide dismutase (SOD) (Misra and Fridovich, 1972), catalase (CAT) (Beers and Sizer, 1952) and reduced glutathione (Ellman, 1959).

Statistical Analysis

Data were mean of 3 replicates ± SD. Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS) version 19. One way analysis of variance was adopted for comparison, and the results were subject to post hoc test using least square deviation (LSD). The data were expressed as mean ± standard deviation. $P< 0.05$ was considered significant.

RESULTS

The effect of methanol seed extract on Catalase

The catalase activity of the animals were seen to increase significantly ($p<0.05$) in all the groups treated with the methanol seed extract of *S. Stenocarpa*. The increase was found to be in a dose dependent manner with the group treated with 800 mg/kg body weight having the highest activity (Figure 1).

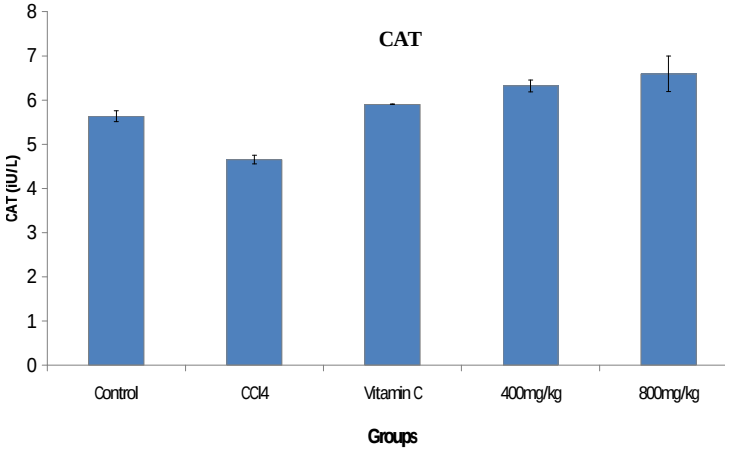

Fig 1. The effect of methanol seed extract of *Sphenostylis stenocarpa* on catalase activity in rats.

The effect of methanol seed extract on Superoxide dismutase

The administration of CCl_4 alone resulted in a reduction of the activity of superoxide dismutase in group 2. The administration of methanol seed extract produced a significant increase in the activity of superoxide dismutase in the treatment groups (4 and 5) which com-

pared favourably with the group 3 treated with ascorbic acid (Figure 2).

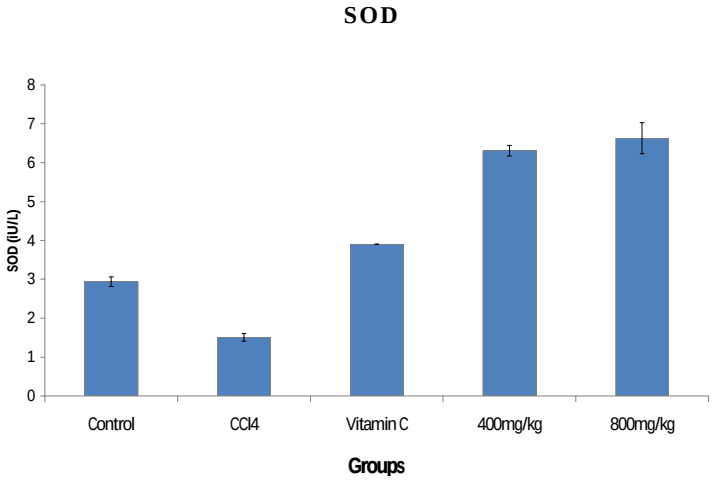

Fig 2. The effect of methanol seed extract of *Sphenostylis stenocarpa* on superoxide dismutase activity in rats.

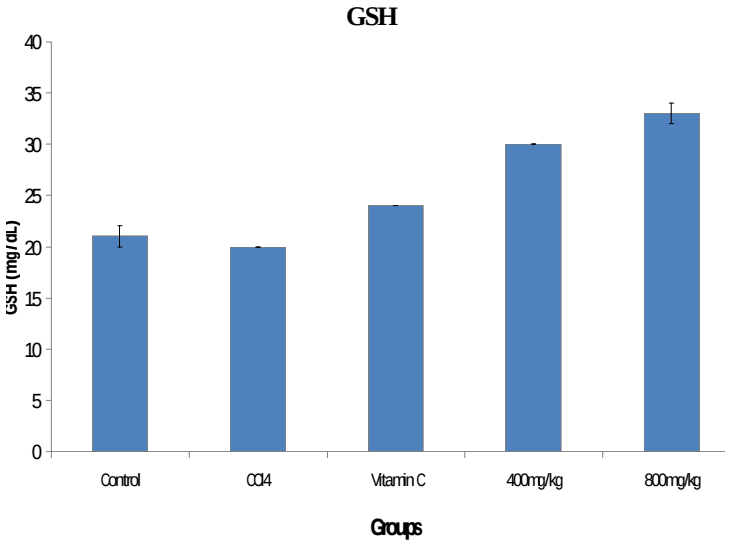

Fig 3. The effect of methanol seed extract of *Sphenostylis stenocarpa* on reduced glutathione in rats.

The effect of methanol seed extract on reduced glutathione

CCl_4 administration produced a decrease in the concentration of reduced glutathione (group 2). The extract administration produced a significant ($p<0.05$) increase in the concentration of reduced glutathione. The increase was also found to be in a dose dependent manner (Figure 3).

The effect of methanol seed extract on alanine aminostransferase

The activities of alanine aminotransferase in the serum of the animals were not significantly ($p>0.05$) increased in groups 3-5 (Figure 4). Also, a decrease was observed in the alanine aminotransferase activity of the animals in group 2 was found not to be significant ($p>0.05$).

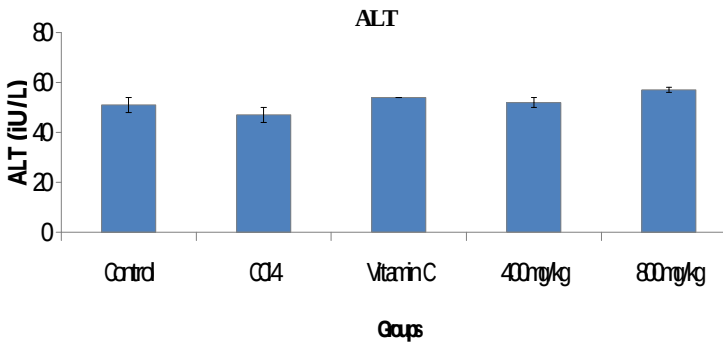

Fig 4. The effect of methanol seed extract of *Sphenostylis stenocarpa* on alanine aminotransferase activity in rats.

The effect of methanol seed extract on the aspartate aminotransferase

The administration of the CCl_4 alone resulted in no significant ($p>0.05$) in the aspartate aminotransferase activity of rats (group 2) (Figure 5). There was a non significant ($p>0.05$) increase in the activity of the enzyme after treatment with the extract.

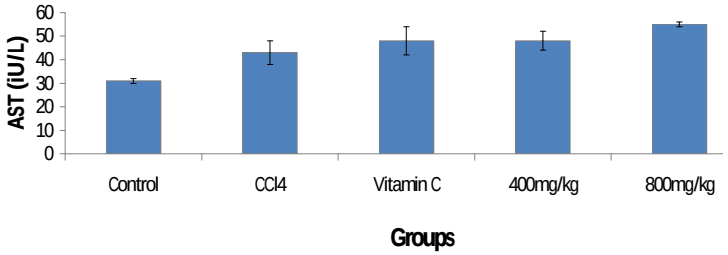

Fig 5. The effect of methanol seed extract of *Sphenostylis stenocarpa* on aspartate aminotransferase activity in rats.

The effect of methanol seed extract on the alkaline phosphatase

Administration of CCl_4 alone resulted in a significant ($p<0.05$) increase in the activity of alkaline phosphatase. This trend was reversed with the administration of the extract. The administration of 800 mg/kg body weight of the extract produced the highest reduction of the activity of alkaline phosphatase (Figure 6).

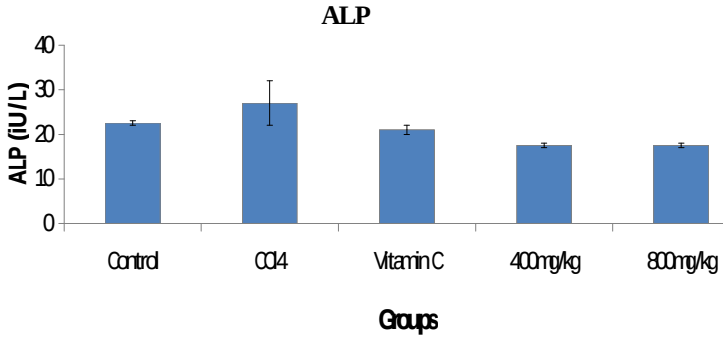

Fig 6. The effect of methanol seed extract of *Sphenostylis stenocarpa* on alkaline phosphatase activity in rats.

DISCUSSION

This research was undertaken to evaluate the effect of methanol seed extract of *Sphenostylis stenocarpa* on some antioxidants and liver function enzymes of rats induced with carbon tetrachloride. Carbon tetrachloride is one of the most commonly used hepatotoxin in experimental study in animal model, it is biotransformed by cytochrome P_{450} in liver to produce highly reactive trichloromethyl free radical (Das *et al*., 2008) which results in the destruction of the cell membrane and the intracellular organelles of the hepatocyte. The hepatotoxic effect of CCl_4 is thought to be initiated as a result of its reductive dehalogenation by cytochrome P450 2E1 to the highly reactive trichloromethyl radical ($\bullet CCl_3$), which is subsequently converted into a trichloromethyl peroxyl radical ($\bullet CCl_3OO.$) in the presence of oxygen (Khan and Younus, 2011; Halliwell and Gutteridge, 2007). Antioxidant enzymes such as CAT, SOD, GSH-Px, GST and GSR are the endogenous defenses; actively involved in scavenging of free radicals to maintain the steady state level and consequently integrity and functionality of cells. Superoxide dismutases are specific for catalytic removal of superoxides by converting them into H_2O_2 (Halliwell and Gutteridge, 2007). Hydrogen peroxide is catalytically dismutated by catalase into ground-state oxygen and water (Reiter *et al*., 2000). GSH is a predominant endogenous antioxidant and used as a cofactor to remove hydrogen peroxide and lipoperoxides by the GSH-Px family during which GSH is converted into oxidized form of glutathione (GSSG). Oxidized glutathione is converted back into GSH by another rate controlling enzyme the glutathione reductase (GSR) thereby maintain the intracellular GSH levels. This optimum level of GSH is an utmost criterion in maintaining the structural integrity and physiology of cell membranes. Dietary plants with proven antioxidant properties may function as a direct anti-radical chain breaking of free radical propagation, produced especially by CCl_4 and other free radical generating substances, interaction with transition metals, and inhibition of reactive oxygen species (ROS) generating enzymes as well as inducing specific antioxidant enzymes (Ajiboye *et al*., 2010). These antioxidant properties are made possible due to the phytochemicals including polyphenolic compounds and flavonoids that are present in plants.

The effect of *Sphenostylis stenocarpa* seeds extract at different dose levels on antioxidants (catalase, superoxide dismutase, reduced glutathione) are shown in figures 1, 2, and 3. Catalase activity was increased significantly ($p<0.05$) in all the treatment groups and the increase was found to be in a dose dependent manner. The animals in group 2 (treated with CCl_4 alone) showed a reduced activity of catalase. This could be as a result of the high levels of ROS including lipid peroxides which could have been produced by the activity of CCl_4. ROS generated during metabolism can enter into reactions that, when uncontrolled, can affect certain processes leading to clinical manifestations of diseases (Winkler and Moser, 1992; Halliwell, 1993; Kesavulu, 1993) such as diabetes, arthritis, cancer, cardiovascular and liver diseases. The superoxide dismutase activity of the rats treated with carbon tetrachloride showed an increased activity of the enzyme in rats treated with the methanol seed extract of *S. stenocarpa*. The group induced with CCl_4 alone showed a decreased activity of the enzyme. The extract at 800 mg/kg body weight showed the highest activity of the enzyme. The extract was found to compete favourably with the standard drug, ascorbic acid (Figure 2). Superoxide dismutase is known to reduce superoxides to hydrogen peroxides and subsequently to hydroxyl radical. The reduced glutathione concentration of the animals treated with methanol seed extract of *S. stenocarpa* elevated significantly. This can be attributed to the possible antioxidant properties of the plant. Reduced glutathione is a power antioxidant that scavenges singlet oxygen, superoxides and hydroxyl radicals, and thus protect lipids and other macromolecules from the attack of electrophilic compounds by reacting the electrophilic center through the thiol group (-SH) (Singh and Ahluwalia, 2003). From the results, it is clear that the seed extract showed a dose-dependent protective activity against free radicals. The most profound antioxidant activity displayed by the highest dose of the extract suggests that it was more effective than the known standard drug, ascorbic acid used in the study.

Elevated levels of liver function enzymes such as AST, ALT and ALP in the serum indicates possible damage to the liver. Carbon tetrachloride-induced hepatocellular damage in rats could be attributed to the generation of trichloromethyl free radicals (Hassan *et al*., 2010) which in turn can cause cell membrane damage and mitochondrial damage in liver cells (Khalaf *et al*., 2009). ALT catalyses the conversion of alanine to pyruvate and glutamate is released in a similar manner. It is a specific marker of liver damage and also a better parameter for detect ing liver damage (Ferreira *et al*., 2010). When the liver cells are damaged, serum levels of ALP, AST and ALP are raised. This is so because the membranes of the liver cells are compromised and the enzymes are released into the blood stream. The results showed that there was no significant ($p>0.05$) increase in the ALT activity of groups 3, 4 and 5. But the alanine aminotransferase activity of the animals in group 5, treated with 800 mg/kg body weight of the extract increased significantly ($p<0.05$). Aspartate aminotransferase (AST) activity of rats treated with the extract showed a significant ($p<0.05$) increase in all the treatment groups. The activities of both AST and ALT are high in tissues especially liver, heart, and muscles. Any damage or injury to the cells of these tissues may cause release of these enzymes along with other intracellular proteins/enzymes into the circulation leading to increase activities of these enzymes in the blood. Alkaline phosphatase activity of the animals in all the experimental groups showed no significant ($p>0.05$) increase in the activity of ALP except group 2 (treated with CCl_4 alone) which was found to increase signifi cantly ($p<0.05$). ALP is a better marker enzyme for the assessment of the integrity of liver cells. (Akanji *et al.*,1993). The observed elevation of ALP in the group treated with CCl4 is an indication of a possible damage of the liver cells by the CCl4. The reduced activity of the enzyme in the experimental groups is a possible indication of hepatoprotective effect of the extract of *S. stenocarpa*. From this study, it shows that the extract had no adverse effect on ALT and AST activity of the rats but significantly decreased the activity of ALP.

CONCLUSION

The study revealed that *Sphenostylis stenocarpa* seeds possess significant antioxidant and hepatoprotective properties against liver damage caused by CCl_4 in rats**.** It provides evidence that the seeds have antioxidant properties and can protect against hepatotoxicity as shown in the catalase, superoxide dismutase, glutathione and the liver function enzyme activities. Therefore, this plant may find application in the prevention and treatment of diseases in which free radicals are implicated.

REFERENCES

1. Ajibolu, F.C., Fashakin, B.J., Fagbemi, N.T. and Aluko, R.E. **(**2011**)**. Effect of Peptide Size on Antioxidant Properties of African Yam Bean (*Sphenostylis stenocarpa*) Protein Hydrosylate Functions. *International Journal of Molecular Sciences*, **12**:6685-6702.
2. Ajiboye, T.O., Yakubu, M.T., Salau, A.K., Oladiji, A.T., Akanji, M.A. and Okogun, J.I. (2010). Antioxidant and drug detoxification potential of aqueous extract of *Annona senegalensis* leaves in carbon tetrachloride-induced hepatocellular damage. *Pharmaceutical Biology*, **48**(12): 1361-1370.
3. Akanji, M.A., Olagoke, O.A. and Oloyede, O.B. (1993). Effect of chronic comsumption of metabisulphite on the
4. integrity of rat cellular system. *Toxicology*, **81**: 173-179.
5. Albano, E., Lott, K.A., Slater, T.F., Stier, A., Symons, M.C.R. and Tomasi, A. (1982). Spin trapping studies on the
6. free-radical products formed by metabolic activation of carbon tetrachloride in rat liver microsomal fractions isolated
hepatocytes and in vivo in the rat. *Biochemical Journal*, **204**:593-603.
7. Aqil, F., Ahmed, I. and Mehmood, Z. (2006). Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants. *Turkey Journal of Biology* **30**: 177-183.
8. Beers, R.F. J. and Sizer, I.W. (1952). A spectrophotometric method for measuring breakdown of hydrogen peroxide
9. by catalase. *Journal of Biological Chemistry*, **195**: 133-140.
10. Bergmeyer, H.U., Horder, M. and Rej, R. (1986a). Approved recommendation on IFCC methods for measurement
11. of catalytical concentration of enzymes. Parts 3. IFCC method for alanine aminotransferase. *Journal of Clinical Chemistry and Clinical Biochemistry* **24**: 481-489.
12. Bergmeyer, H.U., Horder, M. and Rej, R. (1986b). Approved recommendation on IFCC methods for the measurement of catalytical concentration of enzymes. Part 2.IFCC methods for aspatate aminotransferase. *Journal of Clinical Chemistry and Clinical Biochemistry* **24**: 497-508.
13. Das, S., Sarma, G., and Barman, S. (2008). Hepatoprotective Activity of Aqueous Extract of Fruit Pulp of *Cassia fistula* (AFCF) against CarbonTetrachloride (CCL_4) Induced Liver Damage in Albino Rats. *Journal of Clinical and Diagnostic Research*, **2**: 1133-1139.
14. Devasagayam, T.P.A, Tilak, J.C. and Boloor, K.K. (2004). Review: Free radical and antioxidants in human health.
15. *Curr Stat Fut Pros JAPI* **53**: 794-804.
16. Ferreira, E.A., Gris, E.F., Felipe, K.B., Correia, J.F., Cargnin-Ferreira, E., Filho, D.W. and Pedrosa, R.C.(2010). Potent Hepatoprotective Effect in CCL_4–Induced Hepatic Injury in Mice of Phloroacetophone from *Myrcia multiflora*. *Libyan Journal of Medicine*, **5**:4891-4900.
17. Ellman, G.L. (1959). Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics*, **82**: 70-77.
18. Halliwell, B. (1993).The role of oxygen radicals in human disease with particular reference to the vascular system. *Haemostasis* **23**: 118-126.
19. Halliwell,B. and Gutteridge, J.M. (2007). Cellular Responses to Oxidative Stress: Adaptation, Damage, Repair, Senescence and Death. In: Free Radicals in Biology and Medicine, Oxford University Press Inc., Oxford, pp.187-267.
20. Hassan, S.W., Salawu, K., Ladan, M.J., Hassan, L.G., Umar, R.A. and Fatihu,M.Y.,(2010). Hepatoprotective,Antioxidant and Phytochemical Properties of Leaf Extracts of *Newbouldia laevies*. *International Journal of Pharmtech Research*, **2**(1): 573-584.
21. Kesavulu, M.M., Giri, R., Kameswara, R. B, *et al.* (2000). Lipid peroxidation and antioxidant enzyme levels in type 2 diabetics with microvascular complications. *Diabetes Metab* **26**: 387-392.
22. Khalaf, A.A., Mekawy, E.M., Moawad, M.S. and Ahmed, A.M.(2009). Comparative Study on the Protective Effect of some Antioxidants against CCL_4 Hepatoxicity in Rats. *Egyptian Journal of Natural Toxins*, **6**(1): 59-82.
23. Khan, M.R. and Younus, T. (2011). Prevention of CCL_4 – Induced Oxidative Damage in Adrenal Gland of *Digera muricata* Extract in Rat. *Pakistan Journal of Pharmaceutical Science*, **24**(4): 469-473.
24. Misra, H.P. and Fridovich, I. (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple
25. assay for superoxide dismutase. *Journal of Biological Chemistry*, **247**: 3170-3175.
26. Olajide, J.E., Akanji, M.A., Omale, J. and Mohammad, N.O. (2009). Effect of administration of acetylsalicylic acid on phosphatase enzymes in liver of metabisulphite treated rats. *African Journal of Biochemistry Research*, **3** (4): 120-124.
27. Omale, J., Yahaya, A.K. and Okafor, P.N. (2009). Biological activities of stem and leaf extracts of *Occimum gratissimum. Nigerian Journal of Basic and Applied Sciences*, **17** (1): 13-18.
28. Potter, D. and Doyle, J. J. (1992). Origins of the African yam bean *(Sphenostylis stenocarpa* Leguminosae*)*; Evidence from Morphology, Isozymes and Chloroplast DNA. *Eco. Bot*. **46**: 276-292.
29. Reiter, R.J., Tan, D., Osuna, .C and Gitto, E. (2000). Actions of Melatonin in the Reduction of Oxidative Stress. *Journal of Biomedical Science*, **7**: 444-458.
30. Slater, T.F. (1982). Activation of carbon tetrachloride: Chemical principles and biological significance, in: McBrien, D.C.H. and Slater, T.F., eds., Free Radicals, Lipid Peroxidation

and Cancer. London, Academic Press, pp. 243-270.
31. Singh, K. and Ahluwalia, P. (2003). Studies on the effect of monosodium glutamate (MSG) administration on antioxidant enzymes in the arterial tissue of adult male mice. *Journal of Nutrition Science*, **49**: 145-148.
32. Umerie, S.C., Okonkwo, J.C. and Okorie, A.E. (2009). Utilization of African Yam Bean (*Sphenostylis stenocarpa*) and Guinea Corn (*Sorghum vulgare*) in Weaning Food. *Natural and Applied Sciences Journal*, **10**(2): 120-128.
33. Wang, S.Y. and Ballington, J.R .(2007). Free Radical Scavenging Capacity and Antioxidant Enzyme Activity in
34. Deerberry (*Vaccinium stamineum* L.). *Libyan Journal of Toxicology*. **40**: 1352-1361.
35. Wright,P.I., Leathwood, P.D, Plummer, D.T. (1972). Enzymes in rats urine. Alkaline phosphatase. *Enzymologia 42*: 317-327.
36. Winkler, R and Moser, M. (1992). Alterations of antioxidant tissue defense enzymes and related metabolic
37. parameters in streptozotocin-diabetic rats—effects of iodine treatment. *Wien Klin Wochenschr* **104**: 409-
38. 413.
39. Xiong, Q., Hase, K., Tezuke, Y., Tani, T., Namba, T. and Kadota, S. (1998). Hepatoprotective activity of phenylethanoids from *Cistanche deserticola*. *Planta Medica*, **64**: 120-125.
40. Yakubu, M.T. , Akanji, M.A. and Oladiji, A.T. (2005). Aphrodiasiac potentials of aqueous extract of *Fadogia agrestis* (Scheweinf. Ex Heim) stem in male albino rats. *Asian J. Andol* **7**: 399-404.

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