Effects of Ethanolic Root Bark Extract of *Chrysophyllum albidum* on Serum Superoxide Dismutase, Catalase and Malondialdehyde in Rat

Onyeka C.A*, Aligwekwe A.U, Nwakanma A.A, Bakare A.A^, Ofoego U.C

Department of Anatomy. Faculty of Basic Medical Sciences. Madonna University Okija, Elele Campus. River State. Nigeria.

^Department of Anatomy. College of Medicine of the University of Lagos. Idi-Araba. Lagos State. Nigeria

Abstract:

Purpose: The ethanolic root bark of *Chrysophyllum albidum* were evaluated for their effect on antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and lipid peroxidation (malondialdehyde) activities in rats.
Methods: Eighteen male Wister rats weighing 100-200g were divided into three groups of six animals each. Two treated groups received a daily dose of ethanolic root bark of *Chrysophyllum albidum* at 100mg/kg and 200mg/kg body weight respectively via gastric gavage, while equal volume of normal saline was administered to the control group for 3 weeks.
Result: All the *in vitro* models showed dose dependent activity. However the weight gain was dose related with a decrease activity of SOD and CAT ($p<0.05$) in 100mg/kg treated rats, indicating an altered oxidative statues in the animals but as the dose increase, SOD and CAT activity increase. MDA concentration was reduced ($p<0.05$) in the treated rats.
Conclusion: The result suggests that the root bark extract of *C. albidum* has evidence of scavenging and antiperodative properties.

Keywords: *Chrysophyllum albidum*, superoxide dismutase, catalase, MDA

Introduction

Plants are generally believed to be rich in wide variety of secondary metabolites such as alkaloids, flavonoids, terpenoids and saponins of these metabolite plant antioxidants such as numerous phenolic compounds have received increased attention as useful nutraceutical in the management of diseases [1]. Oxidative stress and its related biological damage have been proposed to be involved in the development and maintenance of disease [2]. Consequently; the use of medicinal plants exhibiting antioxidative activity in the treatment and management of disease has been on the increase in recent times [3]. In the living organisms the first line of defense against free radical is the oxidative stress enzymes superoxide dismutase (SOD) [4].

Chrysophyllum albidum (Linn), also known as African star apple, belongs to the family *Sapotaceae*. It is widely distributed in the low land rain forest zones and frequently found in villages [5]. It is often called the white star apple and distributed throughout the southern part of Nigeria [6]. It is known by several local names and is generally regarded as a plant with diverse ethno-medicinal uses [7]. In South-western Nigeria, the fruit is called “agbalumo” and popularly referred to as “udara” in South-eastern Nigeria. It is a plant which has been used in traditional/alternative medicine in Nigeria to treat health problem, various parts of this herb have been proved to have a wide range of therapeutic effects. Phytochemical profile shows it contains an array of biologically active substances that include alkaloids, tannin, saponin, phenol and flavonoid [8]. It is rich sources of natural antioxidants have been established to promote health by acting against oxidative stress related disease such infections as; diabetics, cancer and coronary heart diseases [9]. Several other components of the tree including the roots and leaves are used for medicinal purposes [10]. The bark is used for the treatment of yellow fever and malaria while the leaf is used as an emollient and for the treatment of skin eruption, stomachache and diarrhea [11; 6]. Eleagnine, an alkaloid isolated from *C. albidum* seed cotyledon has been reported to have anti-nociceptive, anti- inflammatory and antioxidant activities [6] and antiplatelet effect [12]. The stem bark has

antimicrobial activity [13]. The study therefore is aimed at providing information on the effects of the ethanolic root bark extract on the antioxidant enzymes and lipid peroxidation in albino Wistar rats.

Material and Method

Collection of plant materials:

The root bark of the *Chrysophyllum albidum* was obtained from Ekeakpara Village in Osisioma Ngwa LGA, Abia State in July 2011. It was authenticated in the Department of Botany, University of Nigeria. Nsukka. A voucher specimen has been preserved in the laboratory for future reference. The ethanolic extraction process was carried out in the Pharmacognosy Department of the Faculty of Pharmacy, Madonna University. Elele. River State.

Preparation of the plant extract

The method of [14] was used. Root bark of *C. albidum* was separated from the root of plant collected. The sample was washed in tap water and was chopped into bits with a knife on a chopping board. The bits were dried in an uninhabited room for four weeks at room temperature. Dried samples were ground into powder mechanically, using manual grinder. The fine powdered root bark was kept in airtight containers at room temperature until the time of use. 281.6g of plant powder was soaked in 1400ml of 99% ethanol and kept in refrigerator at 4°c for 48 hours. The mixture was then vigorously shaken intermittently for additional 2 hours, to allow for complete extraction. The resulting mixture was rapidly filtered through Whatman No 1 filter paper and later with cotton wool to obtain a homogenous filtrate. These filtrates were then concentrated *in vacuo* at low temperature (37- 40°C) to about one tenth the original volume using a rotary evaporator. The concentrates were allowed open in a water bath (40°C) for complete dryness yielding 67g (8.6%) of brown gummy substance. The extract was later reconstituted in normal saline (0.90% NaCl) at a concentration of 1 g/ml before administration. The extract was then refrigerated at 2- 8°C until use.

Determination of LD_{50}

The LD_{50} was determined using the fixed-dose procedure described by [15]. Briefly, the *Chrysophyllum albidum* root bark was given at one of the three fixed doses at a time to 5 male wistar rats.

Experimental Animals

Eighteen albino rats (males only) of wistar strain weighing about 100-200g were procured from the animal house of University of Nigeria, Nsukka. The rats were housed in cages under standard conditions (12h light / 12 h dark, 25ºC ±2ºC) and were kept for 15 days for acclimation prior to experimentation. The animals were maintained on pelletized Growers feed obtained from Vital Feeds, Port Harcourt, Rivers State, Nigeria, and tap water *ad libitum*.

Experimental design

The rats were randomly divided into three groups (A to C) comprising of six rats each. The group A serve as the control while B and C constituted the treated groups. The group A animals received equal volume of 0.9% (w/v) normal saline daily while group B (low dose group) received ethanolic root bark extract of *C. albidum* at 100 mg/kg body weight/ day. However, group C (high dose group) animals received doses of 200 mg/kg body weight/day of ethanolic root bark extract of *C. albidum.*

All the doses administered via gastric gavage daily for 3 weeks. At the end of the experiments, the rats were sacrificed a day after the last dose of administration of the extract or distilled water. The weight of the animals were taken weekly and before the sacrifice. All sacrifices were done under mild anesthesia with intra-peritoneal ketamine hydrochloride at a dose titrated against consciousness starting with 0.01 ml. Blood samples were taken from the left ventricle, centrifuged at 1000 g, 25°C for 10 min in an angle head centrifuge. Blood sera were separated and immediately assayed for SOD, CAT and MDA levels.

General Protocol

All animals were observed for clinical signs of plant toxicity (such as tremors, weakness, death, loss of hair, behavioral abnormalities, salivation, refusal of feed, weight loss) throughout the duration of the experiment. All procedures in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the care and use of Animals and approved by the Department Committee on the Use and Care of Animals.

Determination of superoxide dismutase (SOD) activity

Superoxide Dismutase activity was determine by its ability to inhibit the auto-oxidation of epinephrine determined by the increase in absorbance at 480nm as described by [16]. The reaction mixture (3ml) contained 2.95ml, 0.05M sodium carbonate buffer initiate the reaction. The reference cuvette contained 2.95ml buffer, 0.03ml of substrate (epinephrine) and 0.02ml of water. Enzyme activity was calculated by measuring the change in absorbance at 480nm for 5 minutes.

Determination of catalase activity

Serum catalase activity was determined according to the method of Beers and Sizer as described by [17] by measuring the decrease in absorbance at 240nm due to the decomposition of H_20_2 in a UV recording spectrophotometer. The reaction mixture (3 ml) contained 0.1 ml of serum in phosphate buffer (50mM, pH 7.0) and 2.9ml of 30mM H2O2 in phosphate buffer pH 7.0. An extinction coefficient for H_2O_2 at 240nm of $40.0M^{-1}$ cm^{-1} was used for calculation. The specific activity of catalase was expressed as moles of H_2O_2 reduced per minute per mg protein.

Determination of MDA

Serum MDA was measured by a thiobarbituric acid assay procedure [18], which was calibrated using 1,1,3,3, -tetraethoxypropane (Sigma Chemicals, St. Louis, MO, USA) as a standard. Results were expressed as nanomoles of MDA per millimeter of serum.

Statistical Analysis

All biochemical results were expressed as Mean±SD significant differences among the groups were determined by one-way analysis of variance (ANOVA) followed by Bonferroni post-test or unpaired Student's t-test using the SPSS statistical analysis program. Statistical significance was considered at $p<0.05$.

RESULTS

No obvious signs of toxicity such as tremors, weakness, death, loss of hair, behavioral abnormalities, salivation, refusal of feed, weight loss were seen in any of the animals. However, most of the animals exhibited calmness; improve appetite for food and water and general sense of well-being, all through the duration of the study.

Body Weight

Significant body weight gain was observed in all the experimental groups mostly the group B when compared to the control group ($P<0.005$) (**Table i**).

SOD activity

Results summarized in the (**Table ii**) shows a significant decrease ($p < 0.05$) in the serum activity of SOD in the rats treated with ethanolic root bark extract of *C. albidum* as compared to the untreated rats. This decrease was more significant ($p < 0.05$) in group B of *C. albidum;* however group C shows increase in SOD activity when compared to group B.

Catalase activity

Catalase activity was significantly lower ($p < 0.05$) in the rats treated with extracts (**Table ii**). Group B shows a significant ($p < 0.05$) decrease in CAT activity when compared to the control. However, group C shows a significant ($p < 0.05$) increase in the CAT activity from group B.

MDA concentration

Serum MDA concentration was significantly decrease ($p < 0.05$) in *C. albidum* ethanolic root bark extract treated rats when compared to the control (**Table ii**). Group C showed a significant ($p < 0.05$) increase in the serum MDA concentration when compared to Group B.

Discussion

The findings of the present study showed that the ethanolic root bark extract of *C. albidum* significantly increase the body weight ($p<0.05$) of treated animals. Cells maintain a variety of defenses against oxygen toxicity. Living systems are therefore protected from ROS by antioxidant enzymes (SOD, CAT, LPO etc.) and other endogenous antioxidant sources [19]. SOD has been reported as one of the most important enzymes in the enzymatic antioxidant defense system. It scavenges the superoxide anion to form hydrogen peroxide and thus diminishing the toxic effect caused by this radical [20]. Catalase (CAT) is an enzymatic antioxidant widely distributed in all animal tissues. CAT decomposes hydrogen peroxide and protects the tissues from highly reactive hydroxyl

radicals [21]. Exposure of rats to the extract shows decreased activities of SOD and CAT at a lower dose but with increase of the dose there was an increase of the activity of SOD and CAT. The decrease in SOD and CAT in the rats initially at a lower dose of the extract could be the protective response by the rats to counteract the peroxidative stress in the tissues. The relationship between SOD and CAT is buttressed in ethanol-treated rats of the extract which demonstrated similar response of the antioxidative enzymes to the plant.

MDA is a product of lipid peroxidation [22]. Extensive lipid peroxidation leads to disorganization of membrane by peroxidation of unsaturated fatty acids which also alters the ratio of polyunsaturated to other fatty acids. This would lead to a decrease in the membrane fluidity and the death of the cell [22]. Marked decrease in the levels of lipid peroxides was recorded in rats treated with *C. albidum*, from the result of our investigation; *C. albidum* has the potential to prevent lipid peroxidation by inhibiting the lipid peroxidation process.

The present study results address the scavenging ability of *C. albidum* which also showed promising dose-dependent antioxidant activity. Although the exact agent(s) responsible for all these effects is/ are not clear and might be substance(s) present in *C. albidum* extract. However, more specific research may still be done to ascertain the plant actual effect on the activity of SOD, catalase and MDA.

Conclusion

The observed antioxidant and decrease in the levels of malondialdehyde of the experimental animal, suggest the use of the plant as a remedy for oxidative stress or damage caused by free radicals in the body.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] J. Wan, D. Diaz-Sanchez. Antioxidant enzyme induction: A new protective approach against the adverse effects of diesel exhaust particles. Inhal Toxocol ., 2007, 19:177-182.

[2] M. G. Repetto, S. F. Llesuy. Antioxidant properties of natural compounds used in popular medicine for gastric ulcers. Braz J Med Bio Res., 2002, 35: 523-534.

[3] F. Poumorad, S. J. Hosseinmerhr, S. Shahabirmajd. Antioxidant activity, phenol and flavonoids content of some selected Iranian medicinal plants. Afr J Biotech., 2006, 5:1142-1145.

[4] Y. Aksoy, M. Balk, H. Ogus, N. Ozer. The mechanism of inhibition of human erythrocyte catalase by azide. Turk J. Biol., 2004, 28:65-70.

[5] F. N. Madubuike, O. Ogbonnaya. The Potential Use of White Star Apple Seed (*C .albidum*) and Physic Nut (*jatropha curcas*) as Feed Ingredients for Rats. J. Fac. Agric. Vet.Med., 2003, 1: 97-105.

[6] T. O. Idowu, E.O. Iwelewa, M. A. Aderogba, B. A. Akinpelu, A. O. Ogundaini. Biochemical and behavioral effects of eleagine from *C. albidum*. J. Biol. Sci., 2006, 6: 1029- 1034.

[7] N. A. Amusa, O. A. Ashaye, M. O. Oladapo. Biodeterioration of the African Star apple (*Chrysophyllum albidum*) in storage and the effect on its food value. Afr. J. Biotech., 2003, 2: 56 – 59.

[8] B. J. Okoli, O. S. Okere. Antimicrobial Activity of the Phytochemical Constituents of *Chrysophyllum albidum* G.Don_Holl. (African Star apple) Plant. Journal of Research in National Development., 2010, 8(1).

[9] M. Burits, F. Bucar. Antioxidant activity of *Chrysophyllum albidum* essential oil. Phtother Res., 2002, 14:323-328.Institute of Pharmacognosy.

[10] H. A. Adewusi. The African Star Apple Chrysophyllum albidum Indigenous Knowledge from Ibadan, Southwestern Nigeria. In: Proceedings of a National Workshop on the Potentials of the Star Apple in Nigeria (Eds.)., 1997, pp: 25 – 33.

[11] S. A. Adisa. 2000. Vitamin C, protein and mineral content of Africa apple (*Chrysophllum albidum*) in proceedings of the 18th Annual Conference of NIST. (Eds)., 2000, pp: 141-146.

[12] A. H. Adebayo, A.O. Abolaji, T. K Opata, I. K. Adegbenro. Effects of ethanolic leaf extract of *Chrysophyllum albidum* G. on biochemical and hematological parameters of albino Wistar rats. African Journal of Biotechnology., 2010, 9(14): 2145-2150.

[13] E. O. Adewoye, A.T. Salami, T.O. Lawal and B.A. Adeniyi. The Antimicrobial and Kill kinetics of *Chrysophyllum Albidum* Stem Bark Extracts. European Journal of Scientific Research., 2011, 56 (3):434-444.

[14] I. J. Atangwho, P. E. Ebong, E. U. Eyong, M. U. Eteng. Combined administration of extracts of *Vernonia amygdalina* (Del) and *Azadirachta indica* (A. Juss) mimic insulin in time-course body weight and glucose regulation in diabetic and non diabetic rats. Nigerian Journal of Biochemistry and Molecular Biology., 2010, 25 (1): 44-49.

[15] E. Walum. 1998. Acute oral toxicity. Environ. Health Perspect., 106 (supp2): 497–503.

[16] [16] M. Sun, S. Zigman. An improved Spectrophotomeric assay for Superoxide dismutase based on epinephrine autoxidation. Analytical Biochemistry., 1978, 90 (1): 81-89

[17] [17] F. I. Usoh, E. J. Akpan, E. O. Etim, E. O. Farombi. Antioxidant actions of dried flower of Hibiscus sabidariffa L. on sodium arsenite- induced oxidative stress. Pak. J. Nutr., 2005, 4:135-141.

[18] [18] P. W Albro, J. T Corbelt, J. L Schroeder. Application of the thiobarbiturate assay to the measurement of lipid products in microsomes. *Chem. Biol. Interact.*, 1986, 86: 185-194.

[19] [19] P Nilesh, P Babre, S. Debnath, Y. S. Manjunath, P Parameshwar, S. V. Wankhede, K. Hariprasath. Antioxidant potential of hydroalcohoholic Extract of Barringtonia acutangula Linn roots on Streptozotocin induced diabetic rats. *Int J. Pharm Pharm Sci, 2010, 2(4): 201-203*.

[20] [20] J. J. Curtis and M. Mortiz. Serum enzymes derived from liver cell fraction and response to carbon tetrachloride intoxication in rats. Gastroenterol., 1972, 62: 84–92.

[21] [21] B. Chance and D. S. Greenstein. The mechanism of catalase actions-steady state analysis. Arch Biochem Biophys., 1992, 37: 301–339.
[22] [22]T. Devaki, H. R. B. Raghavendran and A. Sathivel. Hepathoprotective nature of seaweed alcoholic extract on acetaminophen-induced hepatic oxidative stress. J. Hlth. Sci., 2004, 50: 42-46.

TABLE i: Effect of ethanolic root bark extract of *C. albidum* on body weight of rats (Mean ±SEM).

Groups	Body weight (g)		WD (g)
(n=6)	Initial	Final	
A (control)	135±14.5	148±14.25	13
B (100mg)	138±15.26	184±5.84	46*
C (200mg)	137±17.37	171±13.61	34*

**(p<0.05) significantly different from the control*

WD: Difference between the final and initial body weight of the rats

TABLE ii: Measure of serum; SOD, CAT, MDA in all experimental and control group **(**Mean±SEM).

Groups	Group A (Control)	Group B (low dose *C. albidum*)	Group C (high dose *C .albidum*)
SOD (Units/mg) protein	50.32±0.18	44.58±0.05*	45.34±0.02*
CAT	18.10±0.05	14.45±0.05*	16.65±0.02*
MDA (nmol/ml)	3.21±0.12	1.54±0.02*	1.66±0.03*

**(p<0.05) significantly different from the control*

Figure X: Plant of *Chrysophyllum albidum*