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Antioxidant Activity of the Aqueous Crude Extract of *Ocimum gratissimum* LINN. Leaf on Basal and Cadmium-induced Serum Levels of Phosphatases in Male Guinea-pigs.

APRIOKU, J.S.; OBIANIME, A.W.

Department of Pharmacology, Faculty of Basic Medical Sciences. University of Port Harcourt
Email Address- sydaprio@yahoo.com. Phone No: 08035082379

ABSTRACT: The antioxidant activity of the aqueous crude extract of *Ocimum gratissimum* Linn. leaf on the basal and traumatized (cadmium-induced) serum levels of alkaline phosphatase (ALP), total acid phosphatase (ACP_T) and prostatic acid phosphatase (ACP_P) of the male guinea-pig (GP) were evaluated. Preliminary experimentation showed that the effects of the aqueous crude extract of *O. gratissimum* on basal serum phosphatases were slightly more in the oral than the intraperitoneal (i.p) route. Oral administration of 20mg of *O. gratissimum* caused a time-dependent decrease in the basal serum levels of ACP_T and ACP_P without an effect on ALP values. The inhibitory effects compared to the control were maximum at 4 hours. Furthermore, *O. gratissimum* given orally, caused significant dose-dependent decreases ($p<0.05$) of the basal serum levels of ACP_T and ACP_P at $p<0.05$. Basal serum concentrations of ACP_T and ACP_P changed from 23.50±1.04 and 7.50±0.29 to 8.25±0.75 and 2.25±0.29 IU at 20mg, representing 65 and 70 % decreases respectively. In contrast, 0.25-8mg/kg of cadmium (Cd) given intraperitoneally, caused significant dose-dependent increases ($p<0.05$) in the phosphatase enzymes. However pretreatment with 5mg of the crude extract, which on its own had little effect on basal serum phosphatase levels, followed by i.p administration of Cd, caused a reversal of the Cd-induced dose-response curves on the various phosphatase levels to negative values. These results may be due to the oxidative and the antioxidative biochemical antagonistic properties of the agents used in these experiments. They may also be due to enzyme conformational changes and effects of eugenols and flavonoids in the crude extract of *O. gratissimum*. @ JASEM

Oxidation is an essential process in all organisms which involves redox reactions. These reactions normally generate reactive oxygen species (ROS) or highly reactive free radicals that can react virtually with all cell components to cause tissue injury (Collier *et al*., 1992). Exogenous sources of free radicals include tobacco smoke, ionization radiation, certain pollutants such as cadmium, vanadium, crude oil, organic solvents and pesticides (Robinson *et al*., 1997). The resulting oxidative free radicals are obligate intermediates of many metabolic reactions but may also cause pathological damage (Floyd *et al*., 1990). Similarly, antioxidation is the reversal of the above described processes mediated through scavenging of the free radicals, by dismutase enzyme systems, and phenolic-rich compounds such as flavonoids (Sardesai 1995; Rice-Evans *et al*., 1996). The body has evolved a number of interrelated antioxidant mechanisms to maintain redox homeostasis (Toyokuni *et al*., 1999). These antioxidant mechanisms include antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT), gluthathione peroxidase (GSH-Px), heme oygenase (HO) etc (Ames *et al*., 1993; Sardesai 1995; Sies, 1997; Vertuani *et al*., 2004). A disturbance in the balance between the production of reactive oxygen species or free radicals and antioxidant defense, which may lead to tissue injury, is termed '*Oxidative Stress'*. *Ocimum gratissimum* Linn. locally referred to as "Scent Leaf", is a vegetable commonly used as a spice in its unprocessed form in most West African and Nigerian dishes because of its very intense and dominant flavour of cloves (Odukoya *et al*., 2005). It is also an antioxidant (Odukoya *et al*., 2005; Leal *et al*., 2006; Afolabi *et al*., 2007) with volatile oils which contain phenolic-eugenols, thymol, flavonoids, phenylpropanoids, linalool, citral etc as pharmacologically active agents (Jedlickova *et al*., 1992; Leal *et al*., 2006). These active agents confer on it its anti-oxidative, chemotherapeutic, antispasmodic and analgesic actions (Ilori *et al*., 1996; Nakamura *et al*., 1999; Aziba *et al*., 1999; Rabelo *et al*., 2003). Most of the studies/investigations carried out with *O. gratissimum* did not show a qualitative (dose-and-time-dependent) effect of the agent. Furthermore, none of the studies reveal whether this agent will inhibit both basal and traumatized levels of phosphatases in the body's pathology. It is therefore in that light that we seek to answer these questions in this study, using cadmium to induce oxidative stress by stimulating serum phosphatase activities.

MATERIALS AND METHODS

MATERIALS

Electrical centrifuge (Hamilton Bell Co Inc, Montvale NJ); analytical weighing balance, atomic mass spectrophotometer (Humalyzer Junor, Human GmBH); incubator (Phillip Harris Ltd, England); fresh *O. gratissimum* L. leaves; test tubes; dissecting sets, pipettes, oral canular; sterile injection needles and syringes; sterile specimen bottles.

Chemicals: Absolute alcohol (BDH Chemicals Ltd, England); Urethane m.p 47^0C-50^0C (BDH Chemicals Ltd, England,); 40% formalin (M&B, England);

* Corresponding author: Aprioku, J.S.

Cadmium Chloride 99.5% (Chadwell Heath Essex, England); commercially available Alkaline Phosphatase test kit (QCA, S.A, Amposta / Spain); Acid Phosphatase test kit (Randox Lab Ltd, UK); distilled water (obtained from the Department of Chemistry, University of Port Harcourt, Nigeria); all other chemicals were of analar grades.

Animals: Adult male guinea-pigs of average weight 450±5g were obtained from the animal house in the University of Port Harcourt, Nigeria and allowed to acclimatize for 14 days. The guinea-pigs were fed with fresh elephant grass daily *ad libitum* at a room temperature of 22^0C with 12-hr light/dark cycle.

METHODS

Extraction of *Ocimum gratissimum* (Lamiaceae) Linn. leaf: The plant, *O. gratissimum* L. was identified by a senior botanist in the botanical garden of the University of Port Harcourt and some fresh leaves were collected, and dried in an oven at 60^0C to a constant weight and ground to fine powder. Forty grams (40g) weight of the powdered herb was added to 400ml of boiling distilled water and allowed to boil for five minutes. The mixture was allowed to cool for 45 minutes and filtered to obtain a solution of 200mg/ml of the crude extract. The extract was stored in the refrigerator at 4^0C and used for the experiments.

Effects of the aqueous crude extracts of *Ocimum gratissimum* L. leaf on basal serum phosphatase levels of the male GP through different routes of administration: In this study, twenty (20) adult male GPs were divided into four groups (A, B, C and D) of five animals each. The animals in group A were given 20mg of *O. gratissimum* crude extracts orally and those in group B (serving as control) were orally administered with distilled water. Furthermore, the animals in group C were given 20mg of the aqueous crude extract of *O. gratissimum* intraperitoneally, while animals in the last group D (which were used as control for the i.p study) were administered with distilled water through the i.p route. At the end of four hours, the animals were anaesthetized with 25% urethane solution at a dose of 6ml/kg and dissected carefully to collect blood samples through the major artery and the heart to analyze serum levels of ALP, ACP_T and ACP_P using commercially available kits from Randox Laboratories Ltd, Uk and QCA, Spain.

Time-dependent effect of the aqueous crude extracts of *O. gratissimum* L. leaf on basal serum phosphatase level: In n=40 animals, adult male guinea-pigs were divided into 7 groups and a control group of five animals each. While the control animals were orally administered with distilled water, the test animals were given 20mg of aqueous crude *O. gratissimum* L. extract orally and observed over 0.5, 1, 2, 4, 8, 18, and 24 hours. The blood samples were respectively collected later for biochemical assay.

Dose-dependent effects of the aqueous crude extract of *O. gratissimum* L. leaf on basal serum phosphatase level: In n=40 animals, guinea-pigs were orally administered with the crude aqueous extract of *O. gratissimum* L. in the dose range of 5-320mg. Blood was then collected 4 hours later and analyzed to measure the serum levels of ALP, ACP_T, and ACP_P as above.

Effects of cadmium on basal serum phosphatase levels of the male GP: In n=35 animals, guinea-pigs were divided into seven groups of five each and were administered single doses of 0, 0.25, 0.5, 1, 2, 4, and 8mg/kg of cadmium as $CdCl_2$ intraperitoneally. After 24 hours, blood samples were collected and analyzed for ALP, ACP_T and ACP_P.

The effects of *O. gratissimum* pretreatments on Cd-induced phosphatase serum levels: In n=35 animals, guinea-pigs were divided into seven (7) groups of five animals each and pretreated with 5 mg of *O. gratissimum* L., given orally for four hours, before administering 0.25, 0.5, 1, 2, 4, and 8mg/kg of Cd intraperitoneally. Blood samples were also collected and analyzed for ALP, ACP_T and ACP_P after 24 hours as above.

Blood Enzyme Assays: Sample serum was separated from the cells, centrifuged at 3400r for 10 minutes and used for the assays. Serum alkaline phosphatase was assayed using the phenolphthalein method (Babson *et al*., 1966), while the colourimetric method was used to assay total and prostatic acid phosphatases (Fishman and Davidson, 2006)

Statistical Analysis: Data were expressed as means ± standard errors of mean. Comparisons between control and treated groups of guinea-pigs were performed with one-way analysis of variance (ANOVA), followed by Duncan's multiple comparison test. Statistical significance was set at $p<0.05$.

RESULTS AND DISCUSSION

In this study, the effects of *O. gratissimum* on the basal and traumatized (cadmium-induced) serum phosphatase (ALP, ACP_T and ACP_P) levels in male guinea-pigs were investigated. Also investigated were the dose- and-time-dependent effects of the crude extracts of *O. gratissimum* on basal phosphatase levels. In n=20, preliminary experiment in this study, the activity of the aqueous crude extract was observed to be dependent on the route of

* Corresponding author: Aprioku, J.S.

administration. 20mg of the aqueous crude extract of *O. gratissimum* given through the oral and intraperitoneal routes over 4 hours showed that oral administration of *O. gratissimum* caused slightly higher levels of ACP_T and ACP_P inhibitions (Fig. 1a). Thus, the oral route was used in all subsequent investigations. Time-based studies also showed that *O. gratissimum* caused time-dependent effects on basal serum phosphatase levels (Fig. 2a). In this study, using a single dose of *O. gratissimum* (20mg), the basal concentrations of ACP_T and ACP_P were decreased sharply from 22.50±1.04 and 7.25±0.25 to 18.75±1.94 and 6.50±0.29 units (25.6% and 41.4% decreases) respectively in the first hour, maximizing at the fourth hour (Fig. 2a). In the dose-response study, *O. gratissimum* caused significant decreases ($p<0.05$) in the basal serum levels of ACP_T and ACP_P over the dose-range of 0.5-20mg, with little or no effect on ALP (Fig. 2b). The maximal decrease in basal values was obtained at 20mg of the crude extract, decreasing basal serum levels of ACP_T and ACP_P from 24.50±1.04 and 6.50±0.29 to 9.75±1.25 and 2.50±0.29 units (60.20 and 61.54% decreases) respectively. However, further increases in the dose of *O. gratissimum* from 20-320mg caused a reversal of these effects (Fig 2b).

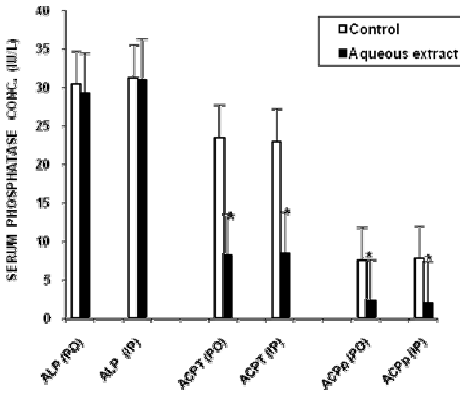

Fig. 1. The effects of aqueous crude extract of *O.gratissimum* on basal serum phosphatase levels of the male GP through different routes- (PO- oral and IP- intraperitoneal). Data are mean ± SEM, n=5. *Treated GPs significantly different from control GPs at P<0.05 ANOVA.

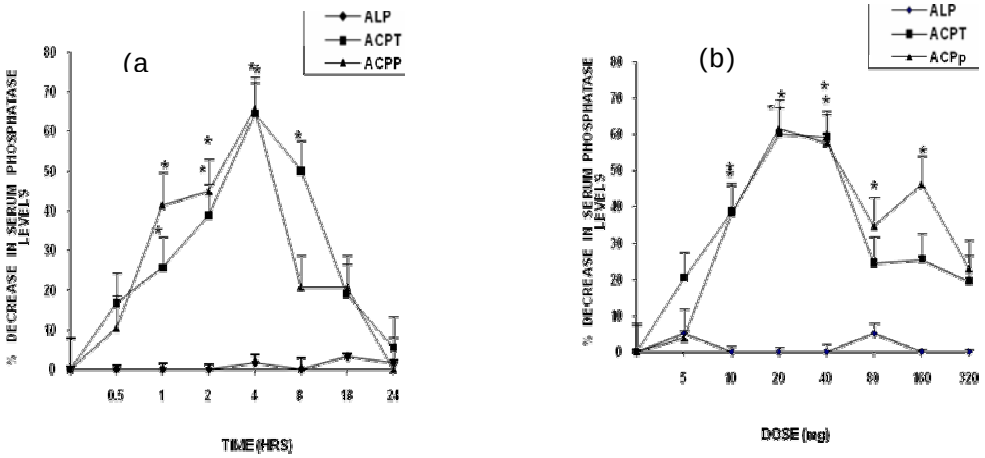

Fig. 2. Graphs showing (a)- Time-dependent and (b)- Dose-dependent (0.5-320mg) effects of *O. gratissimum* on the serum phosphatase levels of the male GP, (represented as % decrease). Data are mean ± SEM, n=5. *Treated GPs significantly different from control GPs at p<0.05 ANOVA.

In contrast, Cd over the dose range of 0.25-8mg/kg caused significant dose-dependent increases ($P<0.05$) in the basal serum phosphatases (Fig. 3). Maximal effects were observed at 2mg/kg of cadmium causing 77, 85 and 71% increases in ALP, ACP_T and ACP_P respectively (Fig. 3). The actual serum values obtained for ALP, ACP_T and ACP_P were 54.00±2.16, 40.75±2.93 and 12.00±1.08 units, compared to baseline values of 30.50±0.65, 22.00±0.71 and 7.00±0.41 to 33.75±1.31, 23.00±0.71 and 7.75±0.25 units respectively. Oral pretreatment of the animals with 5mg of *O. gratissimum*, with subsequent intraperitoneal administration of Cd reversed the effects of Cd on basal serum phosphatase levels,

* Corresponding author: Aprioku, J.S.

causing the dose-response curves of Cd to have negative values (Figs. 4a, b and c). *O. gratissimum* (5mg) caused a very significant inhibition ($P<0.05$) of cadmium's activity on phosphatase enzymes, by decreasing Cd-induced levels of ALP, ACP_T and ACP_P from 54.00±2.16, 40.75±2.93 and 12.00±1.08 to 30.25±2.56, 11.75±1.65 and 4.25±0.25 units respectively at 2mg/kg. These values were less than the control basal serum levels, hence causing negative % increases: -0.82%, -46.59 and –39.29 respectively (Figs. 4a, b and c).

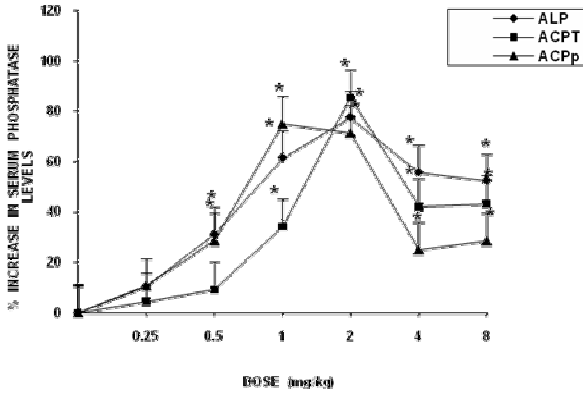

Fig. 3. The dose-dependent effects of cadmium on the serum phosphatase levels of male GPs. Data are mean ± SEM, n=5. *Treated GPs significant different from control GPs at $P<0.05$ ANOVA.

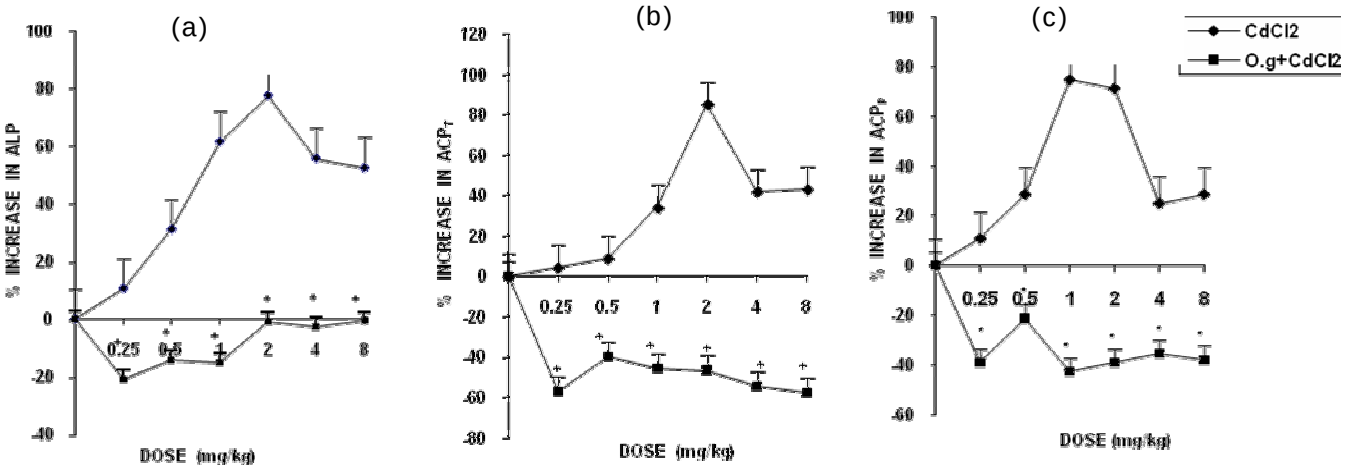

Fig. 4. The effects of *O. gratissimum* pretreatment on Cd-induced increases in serum phosphatase levels of male the GP (a- ALP; b- ACP_P and c- ACP_T). Data are mean ± SEM, n=5; $^+$Pretreated GPs significantly different from Cd-treated GPs at $P<0.05$ ANOVA

The antioxidant activity (AA) of *O. gratissimum* has been assessed using various assay techniques, including the coupled reaction of beta-carotenoid linoleic acid (Leal *et al*., 2006); ferric thiocyanate method (Odukoya *et al*., 2003) DPPH scavenging activity (Afolabi *et al*., 2007) and reducing power assay (Odukoya *et al*., 2005; Afolabi *et al*., 2007). In this study, the effects of *O. gratissimum* on basal serum phosphatases and under traumatized or raised tones were evaluated to assess its AA. Phosphatase enzymes are produced by various organs of the body, but mainly in the liver, testis and the prostate gland. The serum activity of an intracellular enzyme rises as it is released from the damaged cells that contain it. Increase in the levels of serum *phosphatase enzymes* and other hepatic enzymes such as alanine aminotransferase (ALA), aspartate aminotranferase (APT) and sorbitol dehydrogenase (SDH) are good indicators of oxidative stress in several studies (Habeebu *et al*., 2000; Obianime and Aprioku, 2008), which also indicates possible damage of the affected tissues. Phosphatases can therefore be considered as oxidative enzymes, which are stimulated under oxidative stress. Oxidative stress results from the generation of oxidative free radicals during normal or abnormal metabolic processes, which are capable of causing damage to cellular components of the body. In this study, *O. gratissimum* caused a significant

* Corresponding author: Aprioku, J.S.

dose-dependent decrease ($P<0.05$) in the basal serum levels of ACP_T and ACP_P, causing about 64 and 66% decreases in their activities respectively at 20mg. This result indicates that *O. gratissimum* inhibits the oxidative phosphatase enzyme activity, and can therefore prevent oxidative stress/damage that could occur during normal metabolic processes. In contrast, in this study, cadmium (0.25-8mg/kg) caused a significant dose-dependent increase ($P<0.05$) in the basal serum levels of ALP, ACP_T and ACP_P, thus inducing oxidative stress, which is consistent with previous works on Cd (Ikediobi *et al*., 2004; Kara *et al*., 2005, Obianime and Aprioku, 2008). The elevated levels of these are excellent indicators of Cd-induced hepatocellular, testicular and prostate damages (Jeong *et al*., 2000; Robert *et al*., 2002; Gary and Michael, 2002) through the generation of reactive oxygen species (ROS) especially H_2O_2 and O_2^- and oxidative damage to the protective antioxidant enzymes including Superoxide Dismutase (SOD), Glutathione Reductase (GR) and Glutathione Peroxidase (GPx) in the body (Ikediobi *et al*., 2004). Furthermore, the increase in the serum phosphatase levels is attributed to leakages from the membrane joining the binary canalicules and the sinusoidal border of parenchyma cells of these tissues/organs into the blood due to Cd-induced damage to their cell membranes (Gary and Michael, 2002). In this study, Cd-induced levels of serum phosphatases were reversed by *O. gratissimum*. It also showed that the inhibitions were independent of the dose of Cd, shifting the dose-response curves of Cd to the right and decreasing the maximum. This suggests that *O. gratissimum* non-competitively blocked the effects of Cd. This may be due to its inhibitory effects on some molecular mechanisms resulting in the inhibition of Ca^{2+} metabolism and utility. In this study, it has been shown that *O. gratissimum* decreases both basal and raised serum levels of oxidative phosphatase enzymes. Previous studies have reported that *O. gratissimum* L. has antioxidant properties (Afolabi *et al*., 2007) but no work has reported on its effect on basal and traumatized oxidative states, which makes this work novel and useful. Oxidative stress has been implicated in the etiology of a large number of human conditions including atherosclerosis (Haffner, 2000; Baynes and Thorpe 2000; Jialal and Devaraj, 2003), type II diabetes mellitus (Lipinski, 2001), rheumathoid arthritis (Blake *et al*., 1994), long-term neurodegenerative diseases and pancreatitis (Spector, 1995). *O. gratissimum* may be protective in these clinically- and environmentally-induced pathological conditions.

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REFERENCES

Afolabi, C; Akinmoladun, EO; Ibukun, EA; Obuotor, EM; Farombi, EO (2007). Phytochemical constituent and antioxidant activity of extract from the leaves of *O. gratissimum*, Scientific Research and Essay. 2 (5); 163-166

Ahmed, RG (2005). Is There A Balance Between Oxidative Stress And Antioxidant Defense System During Development? Med J of Islamic World Academy of Sciences. 15(2);55-63.

Akinyemi, KO; Oladapo, O; Okwara, CE; Ibe, CC; Fasure KA (2005). Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for anti-methicillin resistant *Staphylococcus aureus* activity. BMC Complement Altern Med. 5; 6.

Ames, BN; Shigenaga, MK; Hagen, TM (1993). Oxidants, antioxidants and the degenerative diseases of aging. Proc Natl Acad Sci, U.S.A. 90; 7915-7922

Aziba, PI; Bass, D; Elegbe, Y (1999). Pharmacological investigation of Ocimum gratissimum in rodents: Phytotherapy Research. 13(5); 427 – 429.

Babson, LA; Greeley, SJ; Coleman, CM; Phillips, GD (1966). Phenolphthalein Monophosphate as a Substrate for Serum Alkaline Phosphatase. Clin. Chem. 12; 482- 490

Baynes, TW; Thorpe, SR (2000). Glycoxidation an lipoxidation in atherogenesis. Free Radic Biol Med. 28; 1708-1716.

Blake, DR; Winyard, PG; Marok, R (1994). The contribution of hypoxia-reperfusion injury to inflammatory synovitis: the influence of reactive oxygen intermediates on the transcriptional control of inflammation. Ann N Y Acad Sci. 723; 308-317

Collier, A; Rumiey, A; Rumley, AG; John, RP; John, PL; Gordon, DO; Micheal, S (1992). Free radical activity and hemostatic factors in NIDDM patients with and without microalbuminuria. Diabetes. 41; 909-913.

* Corresponding author: Aprioku, J.S.

Fishman, HW; Davidson, MH (2006). Determination of Serum Acid Phosphatases Method Biochemical Analysis. 4**;** 257-284.

FIoyd, RA (1990). Role of oxygen free radicals in carcinogenesis & brain ischemia.FASEB J. 4; 2587-97

Gary, MW; Michael JL (2002). Alteration of Liver Cell Function and Proliferation: Differentiation between Adaptation and Toxicity. In: Taylor S, Francis D, eds. Toxicologic Pathology. 2nd ed. London, PA: Elsevier; 41

Habeebu, SS; Liu, J; Liu, Y; Klaassen, CD (2000). Metallothionein null mice are more sensitive than wild-type mice to liver injury induced by repeated exposure to Cd. Toxicol. Sci.55,223–232.

Haffner, SM (2000). Clinical relevance of oxidative stress concept. Metabolism. 49; 30-34.

Ikediobi, OC; Badisa, LV; Ayuk-Takem, TL; Latinwo, ML; West, J (2004). Response of antioxidant enzymes and redox metabolites to cadmium-induced oxidative stress in CRL-1439 normal rat liver cells, International Journal of Molecular Medicine. 14; 87-92.

Ilori, M; Sheteolu, AO; Omonigbehin, EA; Adeneye, AA (1996). Antidiarrhoeal activities of *Ocimum gratissimum* (Lamiaceae). J Diarrhoeal Dis Res. 14 (4); 283-5.

Jedlicková, Z; Mottl, O; Serý, V (1992). Antibacterial properties of the Vietnamese cajeput oil and ocimum oil in combination with antibacterial agents. J. Hyg Epidemiol Microbiol Immunol. 36(3); 303-9.

Jialal, I; Devaraj, S (2003). Antioxidants and atheorosclerosis: don't throw out the baby with the bath water. Circulation. 107; 926-8.

Jeong, S; Habeebu, SS; Klaassen, CD (2000). Cadmium decreases gap junctional intercellular communication in mouse liver. Toxicological Sci. 57; 156-166

Kara, H; Karatas, F; Canatan, H; Serv, K (2005). Effects of exogenous metallothionein on acute cadmium toxicity in rats. Biological Trace Element Research. 104(3); 223-232

Leal, PF; Chaves, F; Celio, M; Ming, LC; Petenate, AJ; Meireles, M; Angela, A (2006). Global yeilds, chemical composotions and antioxidant activities of Clove basil (*O. gratissimum* L.) J. of Food Process Engineering. 29(5); 547-559

Lipinski, B (2001). Pathophysiology of oxidative stress in diabetes mellitus. J Diabetes Complications. 15; 203-210.

Nakamura, VC; Ueda-Nakamura, T; Bando, E; Melo, AFN; Cortez, DAG; Filho, PDB (1999). Antibacterial Activity of *Ocimum gratissimum* L. Essential Oil, Memorias do Instituto Oswaldo Cruz. 94(5); 675-678.

Obianine, AW; Aprioku, JS (2008). *Ocimum gratissimum* Linn. reverses cadmium-induced toxicity in the testicular structure and function of the male guinea-pig. West African Soc. for Pharmacol Book of Abstracts. ETP11, 17.

Oduksoya, AO; Ilori, OO; Sofidiya, MO; Aniunoh, OA; Lawal, BM; Tade, IO (2005). Antioxidant activity of Nigerian dietary spices; EJEAFChe. 4 (6); 1086-1093

Rabelo, M; Souza, EP; Soares, PMG; Miranda, AV; Matos, FJA; Criddle, DN (2003). Antinociceptive properties of the essential oil of *Ocimum gratissimumI* L. (Labiatae) in ice. Bra. *J. Med. Biol. Res*. 36;521-524.

Rice-Evans, CA; Miller, NJ; Paganga, G (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids Free Radical Biol. Med. 20; 933-956

Robert, KK; Michael, PW; Peter, L; Ben, L; Robert, SM; Glenn, SI (2002). Differential Hepatotoxicity Induced by Cadmium in Fischer 344 and Sprague-Dawley Rats. Toxicol. Sci. 65 (1); 151-59

* Corresponding author: Aprioku, J.S.

Robinson, EE; Maxwell, SRJ; Thorpe, GHG (1997). Free Rad. Res. 26; 291-302.

Sardesai, VM (1995). Role of antioxidants in health maintenance. Nutr Clin Pract. 10; 19-25, 1995.

Sies, H (1997). "Oxidative stress: oxidants and antioxidants". Exp Physiol. 82 (2); 291-5.

Somchit, MN; Reezal, I; Nur, EI; Mutalib, RR (2003). In vitro antimicrobial activity of ethanol & water extracts of Cassia alata. J. of ethnopharmacology. 84(1); 1-4.

Spector, A (1995). Oxidative stress-induced cataract: mechanism of action. FASEB J. 9;1173-1182.

Toyokuni, S (1999). Reactive oxygen species-induced molecular damage and its application in pathology. Pathol Int. 49; 91-102.

Vertuani, S; Angusti, A; Manfredini, S (2004). "The antioxidants and proantioxidants network: an overview". Curr Pharm Des. 10 (14); 1677–94.

* Corresponding author: Aprioku, J.S.