JOURNAL OF VETERINARY AND APPLIED SCIENCES
2020 VOL. 10 (1): 1 - 8
Manuscript No. JVAS/2018/058; Received: 03/12/2018; Accepted: 01/01/2020
Published by: Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria

ANTIOXIDANT POTENTIALS OF D-3-O-METHYLCHIROINOSITOL AND GARCINIA HYDROXYBIFLAVANONOL IN ATTENUATING OXIDATIVE STRESS-INDUCED BY CHRONIC CADMIUM CHLORIDE TOXICITY IN MALE WISTAR RATS

Edwin A. Uwagie-Ero[1], Clifford N. Abiaezute[2], Chinaka O. Nwaehujor[3], Ifeanyichukwu C. Egbuniwe[4]*

[1]Department of Surgery, Faculty of Veterinary Medicine, University of Benin, Benin City, Nigeria; [2]Department of Anatomy, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria;[3]Department of Biochemistry, Faculty of BasicMedical Sciences, University of Calabar, Calabar, Nigeria and [4]*Department of Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Benin, Benin City, Nigeria.

==========

ABSTRACT

The ameliorative effects of D-3-O-methylchiroinositol (D-3-O) and Garcinia hydroxybiflavanonol (GB1) administration to male Wistar rats exposed to cadmium chloride ($CdCl_2$) toxicity was investigated. Forty-eight (48) adult male albino Wistar rats were used for the study. The rats were randomly assigned to 4 groups of 12 rats each. Group A was used as normal control and was given distilled water. Groups B, C and D were each challenged with $CdCl_2$ in drinking water (2.5 mg/kg/ b.w. day) while groups C and D, in addition received ; Group C: D-3-O-methylchiroinositol (2 mg/kg/ b.w ./daily) and Garcinia hydroxybiflavanonol (2mg/kg b.w./daily) respectively. All treatments lasted for 90 days. On days 30, 60 and 90, four (4) animals from each group were humanely sacrificed and blood samples collected from the media canthus of the eye. Serum samples were assayed for antioxidants including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and total antioxidant enzyme (TAC). The levels of SOD, CAT, GPx and TAC in both D-3-O- and GB1-treated groups were significantly (P< 0.05) lower than values recorded in the normal control (GroupA) but significantly (P< 0.05) higher than corresponding values recorded in $CdCl_2$-challenged untreated group (Group B). The activities of SOD, CAT, GPx increased in the D-3-O- and GB1-treated groups respectively, but decreased progressively in $CdCl_2$challenged untreated group, from days 30 to 90. It was concluded that, D-3-O-methylchiroinositol (D-3-O) and Garcinia hydroxybiflavanonol (GB1) improved antioxidant capacity in male rats chronically challenged with toxic doses of cadmium chloride. This suggests that D-3-O and GB1 may be

***Correspondence: E-mail: ifeanyiegbuniwe@gmail.com; Tel: +234 8065308980**
ISSN: 2315 – 6856; e-ISSN: 2636 – 5553; *Website: www.jvasonline.com*

useful in the management of toxicities from drinking water contaminated with toxic heavy metals.

Keywords: *Salmonella enterica serovar enteritidis*, *Proteus mirabilis*, offal, cattle, antibiogram.

==========

INTRODUCTION

D-3-O-methylchiroinositol (D-3-O), an isomer of inositol, is a six-carbon polyol described as an insulin sensitizer [1,2,3] and exerts beneficial effects at metabolic, hormonal and ovarian levels [4]. This D-3-O improves glucose tolerance in normal rats and increased gluconeogenesis in the diaphragm [5]. *Garcinia kola* (Guttiferae) seed, also known as "bitter kola", is widely used in African ethnomedicine and traditional hospitality; especially in the South east region of Nigeria. Valuable phytochemicals and bioactive pure compounds have been isolated from the seeds and most prominent amongst them is the *Garcinia hydroxylbiflavanonol* (GB1). The extracted GB1 possesses an array of biological activities which includes antioxidant, antidiabetic, antigenotoxic and hepatoprotective properties [6]. This GB1 is atropisomeric in nature[7]and is the major antihepatotoxic component of *Garcinia kola*[8].The GB1 stimulates the synthesis of ribonucleic acids andproteins in primary cultured rat hepatocytes [8].

Cadmium (Cd) is a known environmental and industrial pollutant which generates free radicals known to cause oxidative stress in humans and animals [9]. Although the body has natural antioxidant system to detoxify free radicals, exposure to several exogenous chemicals and toxic heavy metals can cause generation of free radicals to exceed the protective capacity of the body's antioxidants system, thereby resulting in oxidative stress [10].

Information is lacking in available literature on the effects of D-3-O and GB1 on oxidative stress and antioxidant *in vivo.* The current study evaluated the antioxidant potential of D-3-O and GB1 on oxidative stress induced by chronic cadmium chloride toxicity in Wister rats.

MATERIALS AND METHODS

Experimental Protocol

D-3-O-methylchiroinositol (D-3-O) and *Garcinia hydroxybiflavanonol* (GB1) Isolation

D-3-O-methylchiroinositol was isolated from the stem bark of *Piliostigma thonningii* as described by Asuzu *et al.* [11]. The pure compound was isolated using column and TLC, lyophilized and stored in the fridge at 4°C until used for the experiments [3].

Mature, fresh seeds of *Garcinia kola* were purchased from Watt Market in Calabar, Cross River State and identified by Dr. Michael Ekpoof the Department of Botany, University of Calabar, Nigeria. A voucher specimen was deposited in the herbarium of the Department of Biochemistry, University of Calabar, Nigeria. The extraction of *Garcinia hydroxybiflavanonol* 1 was done as described bySonnenbichler*et al*[6] and Iwu *et al*[12] with slight modifications. Briefly, the fresh seeds of *G. kola* were dried at room temperature and reduced to coarse powder by grinding with a laboratory mill. One (1) kg of the pulverized plant material was defatted with 3 L of n-hexane in a Soxhlet apparatus for 48h. The fat-free plant material was then extracted with80% methanol for 72 h and later concentrated with a rotary evaporator (Büchi, Switzerland). This procedure produced 180.4 g of methanol extract (brown sticky gum). A total of 100 g of the methanol extract was suspended in distilled water and subjected to liquid-liquid partitioning with ethyl acetate (EA) to give 64.3 g, and further isolations of pure compounds, described and identified previously by Madubunyi [7].

Experimental design

A total of 48 rats were randomly assigned to 4 groups (each group having 12 rats), namely: Group A: normal control, given distilled water only; Group B: $CdCl_2$ only in drinking water (2.5 mg/kg/ b.w. day)

only; Group C: $CdCl_2$ in drinking water (2.5 mg/kg b.w. day) + D-3-O-methylchiroinositol (2 mg/kg/ b.w./daily) and Group D: $CdCl_2$ in drinking water (2.5 mg/kg b.w. day) + *Garcinia hydroxybiflavanonol* (2mg/kg b.w./daily) Cadmium chloride ($CdCl_2$) was dissolved in the drinking water at a dose of 2.5 mg/kg, while D-3-O-methylchiroinositol (D-3-O) and *Garcinia* hydroxybiflavanonol(GB1) were each dissolved in 0.5% Tween20 and administered, individually, to each rat *per os*. The dose for $CdCl_2$ was determined from previous studies by [3] and [13]. The chosen dose for cadmium chloride was shown to cause significant oxidative stress in various tissues of the body [13, 14]. Also, the doses of D-3-O-methylchiroinositol (D-3-O) and *Garcinia hydroxybiflavanonol*(GB1) were determined from previous studies that showed that these doses were effective as anthelmintic and in reproductive distress [11,15]. After every 4 weeks, 4 animals from each group were humanely sacrificed for blood collection. The experiment lasted for 3 months (90 days). The experimental animals were handled humanely all through the experiment.

Sample collection

Blood samples were collected from media canthus of the eye. Serum was separated from blood clot by centrifugation at 3,000× *g* for 10 min.

Evaluation of oxidative stress marker

Superoxide dismutase (SOD) activity was evaluated using the Northwest Life Science Specialties SOD kit (NWLSS™ NWK-SOD02), based on the method of monitoring the auto-oxidation rate of hematoxylin originally described by [16] and with modifications to enhance reliability [17]. The activity of catalase (CAT) was determined by spectrophotometry according to the method of [18], with modifications to increase robustness and convenience using the NWLSS™ Catalase activity assay kits protocol NWK-CAT01. Glutathione peroxidase activity (GPx) was evaluated using the Northwest Life Science Specialties (NWLSS™) glutathione peroxidase assay kits protocol NWK-GPX01, adapted from the method described by [19]. Briefly, GPx catalyzed the reduction of hydrogen peroxide and oxidized the reduced glutathione to form oxidized glutathione (GSSG). Oxidized glutathione was then reduced by glutathione reductase and β-nicotinamide adenine dinucleotide phosphate (NADPH) forming NADP+. This resulted in decreased absorbance at 340 nm and the recycling of GSH. The decrease in absorbance at 340 nm was directly proportional to the GPx concentration.

Measurement of serum total anti-oxidant capacity (TAC) was performed with a commercial kit (Randox Laboratories, Crumlin, UK). The suppression of the color was compared with that of Trolox, which is widely used as a standard for TAC measurements and the assay results are expressed as Trolox equivalents (in nmol/mL) [20].

Statistical analysis

Data obtained were expressed as mean ± standard error of the mean (mean ± SEM). Group means for antioxidant enzymes and total antioxidant capacity were compared using analysis of variance, followed by Turkey's *post-hoc* test. Values of $P < 0.05$ were considered significant [21].

RESULTS

Figure 1 shows the changes in superoxide (SOD) activity at days 30, 60 and 90 of the study period in cadmium chloride-challenged Wistar rats treated with either D-3-O or GB. The SOD activity was significantly ($P<0.05$) lower in Wistar rats challenged with cadmium chloride (untreated control) when compared with those of normal control (group A) at days 30, 60 and 90, respectively. Similarly, SOD activity in Wistar rats treated with D-3-O was significantly ($P < 0.01$) lower than the values obtained in normal control group at days 30, 60 and 90, respectively. There was no significant difference in SOD activity of GB1 treated Wistar rats and the normal control group at days 30 and 90.

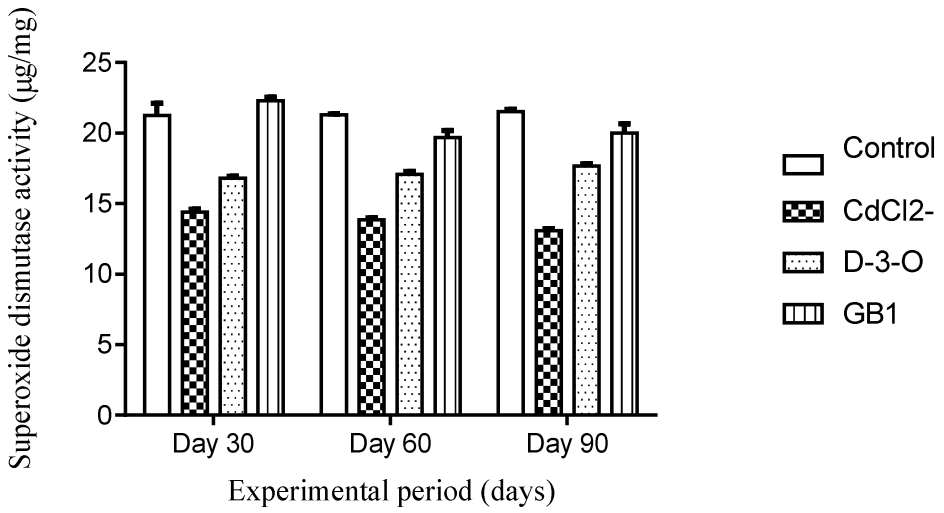

Figure 1: Superoxide dismutase activity in cadmium chloride challenged Wister rats treated with D-3-O and GB 1 (n = 3).

Figure 2 shows the activities of CAT of cadmium challenged rats and those treated with either D-3-O or GB1. The CAT activities of Wistar rats challenged with cadmium chloride at day 30, 60 and 90were significantly ($P< 0.05$) lower than the values recorded in normal control group. Wistar rats treated with D-3-O recorded significantly ($P < 0.05$) lowered CAT activities when compared with values in normal control group, but significantly ($P< 0.05$) higher values of CAT activities when compared with those untreated control challenged with cadmium chloride. In addition, CAT activities in GB1 treated Wistar rats differed significantly ($P< 0.05$) when compared with the values obtained on corresponding days in thenormal control group. However, the CAT activity was significantly ($P < 0.05$) higher in GB1 treated group when compared with the value obtained in cadmium chloride-challenged untreated group.

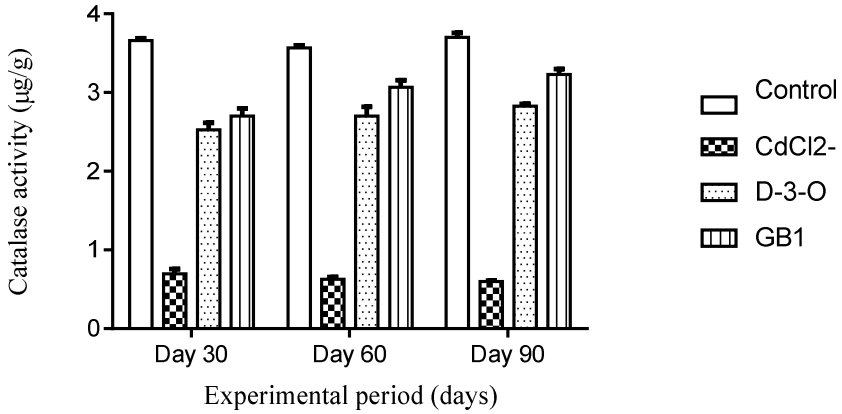

Figure 2: Catalase activity in cadmium chloride challenged Wister rats treated with D-3-O and GB 1 (n = 3).

Figure 3 shows variations in activities of glutathione peroxidase (GPx) of Wistar rats across groups at days 30, 60 and 90. Cadmium chloride– challenged untreated Wistar rats recorded significantly lower ($P < 0.001$) GPx activities on days 30, 60 and 90 of the study period; when compared with the corresponding

values obtained in the normal controls and treated grops. The values of GPx on days 30, 60 and 90 in D-3-O treated Wistar rats were significantly ($P < 0.05$) lower when compared with values obtained in the normal control group, but significantly higher ($P < 0.05$) than those recorded in the cadmium chloride–challenged untreated Wistar rats. Similarly, GPx activities in Wistar rats treated with GB1 on days 30, 60 and 90 were significantly ($P < 0.01$) lower when compared with values obtained in normal control group and significantly ($P<0.05$) higher than those recorded for the cadmium chloride challenged untreated rats.

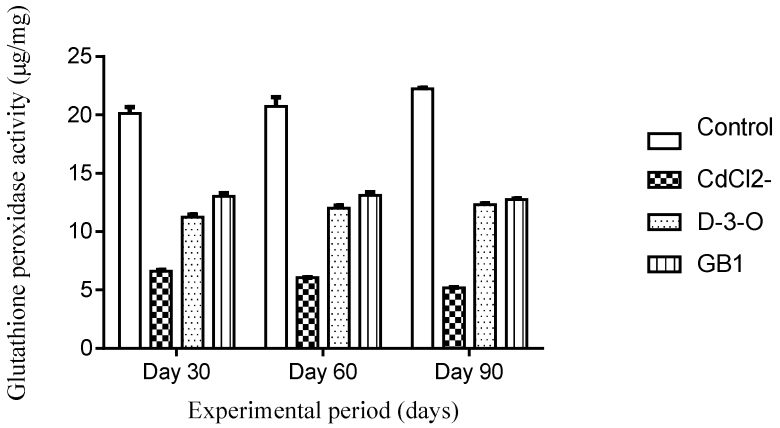

Figure 3: Gliutathione peroxidase activity in cadmium chloride challenged Wister rats treated with D-3-O and GB 1 (n = 3).

Total antioxidant capacity (TAC) on day 30 did not differ ($P> 0.05$) between Wistar rats in normal control and GB1–treated group but were significantly ($P< 0.01$) lower in rats challenged with cadmium chloride and also treated with D-3-O (Figure 4). More so, the TAC were significantly lower in untreated rats challenged with cadmium chloride and those challenged with cadmium chloride and treated D-3-O and GB1 when compared with values obtained on days 60 and 90 in the normal control group(Figure 4). Total antioxidant capacity were significantly ($P< 0.05$) higher in the D-3-O and the GB1 treated groups when compared with values of obtained on day 30, 60 and 90 in the untreated cadmium challenged group (Figure 4).

DISCUSSION

The results of the present study indicate that $CdCl_2$-induced toxicity lowered serum antioxidant status in male Wistar rats. This was evident from the significantly ($P < 0.01$) lower concentrations of SOD, CAT and GPx in the challenged but untreated group (negative control group). The lowered antioxidant status implies that $CdCl_2$ is deleterious to antioxidant systems; consequently, subjecting them to adverse effects of free radicals especially reactive oxygen species (ROS) which in turn exposes them to oxidative stress. These reactive free molecules have been established to damage living tissues by reacting with lipids, carbohydrates and protein components of cells. The findings of the present study agrees with Stohs and Bagchi [23] who demonstrated that metal ions depletes glutathione and protein-bound sulfhydryl groups, resulting in the generation of reactive oxygen species (ROS) such as superoxide ion, hydrogen peroxide, and hydroxyl radical. The oxidative potential of $CdCl_2$ toxicity necessitates the counter-effects of antioxidants to ameliorate the adverse effects of ROS. This study showed that, although activities of SOD, CAT and GPx in the D-3-O-methylchiroinositol and *Garcinia hydroxybiflavanonol*-treated rats were lower ($P < 0.05$) compared with normal control, these activities were significantly higher than values obtained in the $CdCl_2$-challengeduntreated group. This suggests that D-3-O and GB1improved antioxidant enzyme activities of rats subjected to $CdCl_2$ toxicity. These agree with reports of De et al [24] which demonstrated that administration of D-chiro-inositol in patients with poly cystic ovarian cancer

reduced oxidative stress in women, and also the reports of Igado et al[25], who showed that *Garcinia* biflavonoids specifically up-regulated antioxidant defense capacity and reduced *in vivo* markers of oxidative damage to lipids, proteins and DNA.

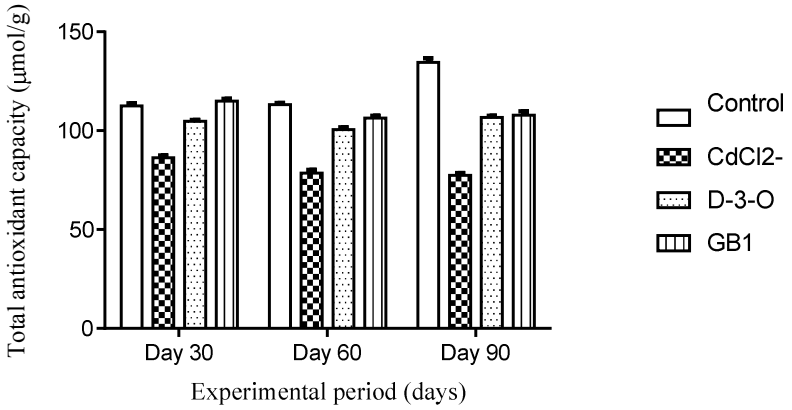

Figure 4: Total antioxidant capacity in cadmium chloride challenged Wister rats treated with D-3-O and GB 1 (n = 3).

Comparatively, the results of the present study indicate thatGB1showed better antioxidant potentials than D-3-O sinceGB1 was more effective in protecting the rats from the deleterious effects of ROS and therefore agrees with those of Igado et al[25] who also showed that kolaviron ameliorated oxidative stress induced by heavy metal toxicity in the brain of rats. The study showed that the effects of $CdCl_2$, D-3-O-methylchiroinositol and *Garcinia hydroxybiflavanonol*on antioxidant enzyme activities and TAC varied with the duration of administration. While, activities of SOD and CAT increased in both the D-3-O- or GB1-treated rats, the activities of these enzymes decreased, progressively from day 30 – 90, in the $CdCl_2$-only challenged rats.

The results of this study suggest that D-3-O methylchiroinositol and *Garcinia* hydroxylbiflavanolol 1 may be administered as adjuvant therapy in the management of chronic Cadmium chloride intoxication as oxidative stress underlies Cadmium Chloride toxicity and D-3-O methylchiroinositol and *Garcinia* hydroxylbifavanolol 1 improved the activities of antioxidant enzymes in Wistar rats. Also, these compounds may be useful in the management of toxicities from drinking water contaminated with toxic heavy metals.

REFERENCES

1. Baillargeon, J.P., Nestler, J. E., Ostlund, R.E., Apridonidze, T., Diamanti-Kandarakis, E. (2008). Greek hyperinsulinemicwomen, with or without polycystic ovary syndrome, display altered inositols metabolism. *Human Reproduction,* 23: 1439-1446. Doi: 10.1093/humrep/den097.
2. Dunaifa, A. (1995). Hyperandrogenic anovulation (PCOS): a unique disorder of insulin action associated withan increased risk of non-insulin-dependent diabetes mellitus. *The American Journal of Medicine*, 98: S33-S39.
3. Asuzu, I.U. and Nwaehujor, C.O. (2013). The anti-diabetic, hypolipidemic and anti-oxidant activities of D-3-O-methylchiroinositol in alloxan-induced diabetic rats. *Hygeia Journal for Drugs and Medicines*, 5(2): 27-33.
4. Nordio, M. and Proietti, E. (2012). The Combined therapy with myo-inositol and D-Chiro-inositol reduces the risk of metabolic disease in PCOS overweight patients compared to myo-inositol supplementation alone. *European Review for Medical and Pharmacological Science*, 16: 575-581.

5. Ortmeyer, H.K., Huang, L.C., Zhang, L., Hansen, B.C. and Larner, J. (1993). Chiroinositol deficiency and insulin resistance. II. Acute effects of D-chiroinositol administration in streptozotocin diabetic rats, normal rats given a glucose load, and spontaneously insulin-resistant rhesus monkeys. *Endocrinology*,132: 646 - 651.
6. Sonnenbichler, J., Goldberg, M., Hane, L., Madubunyi, I., Vogl, S and Zetl, I. (1986). Stimulatory effect of silibinin on the DNA synthesis in partially hepatectomized rat livers: non-response in hepatoma and other malign cell lines. *Biochemical Pharmacology*, 35: 541 - 544.
7. Madubunyi, I. I. (1987). Isolierung und characterisierung antihepatotoxischerflavonoide aus *Garcinia kola* nussen und untersuhung ihres einflusses auf die protein- und nucleinsauresynthese von leberparenchymzellen so wie deren membranpermeabilitat. Ph.D Dissertation.
8. Farombi, E. O., and Owoeye, O. (2011). Antioxidative and chemopreventive properties of Vernonia amygdalina and Garcinia biflavonoid. *International Journal of Environmental Research and Public Health*, 8(6): 2533-2555.
9. Erboga, M., Kanter, M., Aktas, C., Sener, U., Erboga, Z. F., Donmez, Y. B., and Gurel, A. (2016). Thymoquinone ameliorates cadmium-induced nephrotoxicity, apoptosis, and oxidative stress in rats is based on its anti-apoptotic and anti-oxidant properties. *Biological Trace Element Research*, 170 (1): 165-172.
10. Wu, D. and Cederbaum, A. I. (2003). Alcohol, oxidative stress, and free radical damage. *Alcohol Research and Health*, 27: 277-284.
11. Asuzu, I. U., Gray, A. I. and Waterman, P. G. (1999). The anthelmintic activity of D-3-O-methylchiroinositol isolated from *Piliostigma thonningii* stem bark extract. *Fitoterapia*., 70: 77-79.
12. Iwu, M.M., Igboko, O.A., Elekwa, O.K. and Tempesta, M.S. (1990). Prevention of thioacetamide-induced hepatotoxicity by biflavanones of Garcinia kola. *Phytotherapy Research*, 4(4): 157-159.
13. El-Demerdash, F. M., Mokhtar, Y., Kedwany, F. S. and Baghdadi, H. H. (2004). Cadium-induced changes in lipid peroxidation, blood hematology, biochemical parameters and semen quality of male rats: protective role of vitamin E and β-carotene. *Food and Chemical Toxicology*, 42: 1563-1571.
14. Alkhedaide, A., Alshehhri, Z.S. and Sabry, T.A. (2016). Protective effect of grape seed extract against cadmium-induced testicular dysfunction. *Molecular Medicine Reports*, 13: 3101-3109.
15. Uwagie-Ero, E. A., Nwaehujor C.O, Abiaezute, Clifford N., Ocheja, O.B., Ekeolu, K. B. and Asuzu, S.I. (2018). D-3-O-methylchiroinositol (from *Pilostigma thonningii*) ameliorates cadmium chloride (CdCl2)-induced toxicity in male reproduction.*Journal of Applied Science and Environmental Management*, 22(6):853-856.
16. Martin Jr, J. P., Dailey, M. and Sugarman, E. (1987). Negative and positive assays of superoxide dismutase based on hematoxylin autoxidation. *Archives of Biochemistry and Biophysics*, 255(2): 329-336.
17. Aluwong, T., Kawu, M., Raji, M., Dzenda, T., Govwang, F., Sinkalu, V. and Ayo, J. (2013). Effect of yeast probiotic on growth, antioxidant enzyme activities and malondialdehyde concentration of broiler chickens. *Antioxidants*. 2(4): 326 - 339.
18. Beers, R. F. Jr. and Sizer, I. W. (1952). A spectrophotometric method for measuring the breakdown of hydrogen peroxide by Catalase. *Journal of Biological Chemistry*, 195: 133-140.
19. Paglia, D.E. and Valentine, W.N., (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *Journal of Laboratory and Clinical Medicine*, 70: 158-169.
20. Quintanilha, A.T., Packer, L., Davies, J.M., Racanelli, T.L. and Davies, K.J. (1982). Membrane effects of vitamin E deficiency: bioenergetics and surface charge density studies of skeletal muscle and liver mitochondria. *Annals of the New York Academy of Sciences*, 393: 32-47.
21. Snedecor, G.W., Cochran, W.G., (1994). *Statistical Methods*. Oxford and IBH Publishing Corporation, Calcutta, India, 509 pp.

22. Chen, N., Su, P., Wang, M. and Li, Y.M. (2018). Ascorbic acid inhibits cadmium-induced disruption of the blood-testis barrier by regulating oxidative stress-mediated p38 MAPK pathways. *Environmental Science and Pollution Research*, 25(22):21713-21720.
23. Stohs, S. J., and Bagchi, D. (1995). Oxidative mechanisms in the toxicity of metal ions. *Free Radical Biology and Medicine*, 18(2): 321-336.
24. De, V. L., La, A. M., Cappelli, V., Stendardi, A., Focarelli, R., Musacchio, M. C. and Piomboni, P. (2012). Evaluation of the treatment with D-chiro-inositol on levels of oxidative stress in PCOS patients. *Minerva Ginecologica*, 64(6):531 - 538.
25. Igado, O. O., Olopade, J. O., Adesida, A., Aina, O. O., and Farombi, E. O. (2012). Morphological and biochemical investigation into the possible neuroprotective effects of kolaviron (*Garcinia kolabioflavonoid*) on the brains of rats exposed to vanadium. *Drug and Chemical Toxicology*, 35 (4): 371 - 380.

.