potravinárstvo®

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 13, 2019, no. 1, p. 815-822
https://doi.org/10.5219/1180
Received: 25 August 2019. Accepted: 3 October 2019.
Available online: 28 November 2019 at www.potravinarstvo.com

ISSN 1337-0960 (online)

ORYCTES RHINOCEROS LARVA OIL SUPPLEMENTATION IMPROVES TISSUE ANTIOXIDANT STATUS IN CHOLESTEROL-FED RATS

Olarewaju Oluba

ABSTRACT

Experimental evidence from previous study has demonstrated the hypolipidemic effects of *Oryctes rhinoceros* oil (ORO) when fed as a supplement to a cholesterol-based diet. Due to renew interest in the consumption of insect derived oil, the present study was designed to elucidate the effect of *Oryctes rhinoceros* oil (ORO) supplementation in comparison to vitamin E on oxidative status in some tissues of rats fed a cholesterol-based diet. Forty (40) Swiss albino rats were divided into 4 groups (n = 10) and maintained on a basal diet (cholesterol free as control), a cholesterol-based diet (5% cholesterol as cholesterol), a cholesterol-based diet supplemented with ORO (cholesterol + ORO) and a cholesterol-based diet supplemented with vitamin E (Cholesterol + vit E) for 10 weeks. Animals in the cholesterol group had a significantly (p <0.05) higher malondialdehyde (MDA), conjugated diene and nitric oxide concentrations in the serum, liver, heart, kidney and lung compared to control, cholesterol + ORO and cholesterol + vit E groups. Tissue glutathione (GSH) concentration was significantly (p <0.05) higher in rats fed cholesterol-based diet supplemented with ORO and vitamin E compared to those fed cholesterol-based diet alone. Xanthine oxidase activity was significantly (p <0.05) reduced in tissues of rats fed ORO and vitamin E supplemented diets compared to cholesterol rat group. In addition, catalase and superoxide dismutase activities in the various tissues examined were significantly (p <0.05) higher in both ORO and vitamin E supplemented groups compared to the cholesterol group. No significant difference was observed between animals fed ORO and vitamin E supplemented diets. These results showed that *Oryctes rhinoceros* larva oil exhibited similar protective effects to vitamin E against diet-induced oxidative stress in rats. In addition, data from this study showed that *Oryctes rhinoceros* larva oil possessed antioxidant property. Overall, the potential nutritional benefit of *Oryctes rhincoceros* larva oil consumption on cardiovascular health could possibly involve its ability to upregulation of cellular antioxidant defense mechanisms.

Keywords: edible insect; *Oryctes rhinoceros* larva oil; cholesterol-based diet; oxidative stress; antioxidant

INTRODUCTION

Palm oil remains the most traded vegetable oil globally as it constitutes over 50% packaged products in supermarkets. However, in order to sustain the increase demand for palm oil or palm oil-based products several hectares of land are cleared yearly for palm plantations. The world population is estimated to exceed 9 billion by 2050 **(Teoh, 2010)**. This no doubt portends a serious challenge in terms of food security and environmental issues. Specifically, an astronomical increase in the demand for palm oil or palm-based product is expected. Thus, there is an urgent need to evaluate other nutrient sources for feed production. The use of edible insects for feed is widely viewed as a healthier and more sustainable potential solutions to overcoming the challenge of food security **(Van Huis, 2013)**. Insects are rich sources of valuable nutrients such proteins, fat, vitamins, minerals and energy. Compared to oil palm trees insects have better yield per hectare due to their high rate of proliferation and short life cycle, requires less space and can be reared on agro-waste stream.

Thus far, research on the utilization of edible insects for animal feed production has focused on their protein content. Nevertheless, insects contain between 5% to 40% (% dry matter, DM) oil with a better fatty acid profile compared to palm oil **(Womeni et al., 2009)**. In Europe, the use of animal-based protein with the exception of fish for animal feed production is restricted. However, there exist no prohibition on the use insect oil. Research on the use of insect oil in animal feeding trial is scanty. A recent study by **Belghit et al. (2019)** demonstrated that liver triacylglycerol level was reduced in freshwater Atlantic salmon fed insect meal and insect oil compared fish fed the control diet. Similarly, **Oluba et al. (2008a)** reported that the supplementation of *O. rhinoceros* oil with a cholesterol-based diet in rats resulted in improvement in serum lipid profile and reduced susceptibility to atherosclerosis.

The highly lipid soluble radical, nitric oxide ($NO^{\cdot}$) diffuses readily through cellular membranes, interacting with other radicals including superoxide, peroxide etc thus potentiating their actions **(Pacher, Beckman and Liaudet, 2007; Birben et al., 2012)**. Nitric oxide radical is also capable of simultaneously reacting with superoxide radical to form peroxinitrite radical **(Pacher, Beckman and Liaudet, 2007)**. Peroxinitrite radical is a very reactive 1thiolesters group of cysteine and methionine residues in peptides and proteins are potential oxidizing targets for peroxinitrite. Unregulated generation of free radicals in the body has been implicated in the pathogenesis of tissue damage in several diseased conditions such as ageing, diabetes, cardiovascular diseases, etc **(Uttara et al., 2009; Valko et al., 2007)**. Dietary fats in the form of cholesterol are considered to be important in the initiation of free radical production in these clinical disorders **(Nevin and Rajamohan, 2006)**. These free radicals subsequently attack and breakdown membrane phospholipids and thus trigger lipid peroxidation **(Sevanian and Hochstein, 1985; Thomas et al., 1990)**. Several dietary modifications including the inclusion of antioxidant such as vitamin E have been shown to ameliorate the attendant susceptibility of biological molecules to lipid peroxidation in humans and laboratory animals **(Farombi and Nwaokeafor, 2005; Adefegha et al., 2014)**. Reports from several studies have demonstrated that dietary oils improve plasma lipid profile as well as affect lipid peroxidation and antioxidant parameters in rats **(Oluba et al., 2008a; Oluba et al., 2008b; Celebi and Utlu, 2006)**.

The consumption of *Oryctes rhinoceros* (palm beetle) larva as a delicacy is a common practice in Nigeria especially in the Southern part of the country where palms are cultivated on a commercial scale. *O. rhinoceros* larvae feeds on decaying organic matter (palm logs, manure and rubbish dumps). These larvae are either eaten raw, boiled, fried or roasted and are sometimes used as meat substitute in the preparation of stews and soups. Reports on the proximate composition of O. *rhinoceros* larva from Nigeria by several investigators have shown that it contains as much as 38% oil (by dry weight). with high level (65%) of unsaturated fatty acids. The oil being majorly composed of unsaturated fatty acids has been shown to be hypercholestrolemic in action **(Oluba, Josiah and Fagbohunka, 2014)**. Presently, insect oil is gaining prominence in the scientific field as well as increased acceptability among consumers. Hence, there is urgent need to intensify scientific efforts on the possible nutritional and health benefits of insect oil. Therefore, this study was carried out to investigate the effects of *Oryctes rhinoceros* oil (ORO) supplementation in comparison to vitamin E on tissue oxidative status in rats fed a cholesterol-based diet.

Scientific hypothesis

Oryctes rhinoceros oil protect rat tissue against lipid peroxidation.

Oryctes rhinoceros oil possesses antioxidant activity.

MATERIAL AND METHODOLOGY

Insect material

Live *Oryctes rhinoceros* larvae were collected from decaying palm trees at Igoba village near Akure (Nigeria). The larvae were transported to the laboratory in an open plate within 2 h of collection. They were authenticated and identified at the Department of by a zoologist at the Department of Biology, University of Benin (Nigeria). The larvae were rinsed with distilled water before being anaesthetized by freezing. The frozen larvae were thaw at 37 °C and oven dried at 50 °C for 72 h. The dried larvae sample was powdered using a mechanical grinder and the powdered sample kept in an air-tight container at 4 °C for further analysis.

Oil extraction

Oil from the powdered larvae sample (100 g) was extracted using Soxhlet apparatus using hexane as solvent. The extracted oil was dried and stored in a dried dark air-tight container and refrigerated until required for further analysis.

Animal care and ethical consideration

This study was approved by the Animal Ethics Committee of the Department of Chemical Sciences, Joseph Ayo Babalola University, Ikeji-Arakeji, Nigeria and was conducted in compliance to NIH guidelines for the care and use of laboratory animals **(ILAR, 1985)**. Forty (40) male Swiss albino rats (weighing between 50.5 – 55.1 g) aged 6 weeks were purchased from the Department of Biochemistry, University of Ibadan (Nigeria) and used for the study. The animals were housed in wooden cages with raised wire-gauze floors at a temperature of 25.7 ±2.3 °C and a relative humidity of 45% – 60%, with 12 h light/dark cycles.

Experimental diet

Diet formulation and preparation followed the prescription of the American Institute of Nutrition. The formulated diets were designated: control, cholesterol, cholesterol + ORO and cholesterol + vit E. The composition of the respective diet is as shown in Table 1. Prior to the feeding experiment animals were conditioned to the laboratory environment for a period of 2 weeks. All through the 10 weeks feeding trial, animals in each group were given food and water *ad libitum.* Food intake and body weight were recorded daily. At the end of the 10-week feeding experiment, the animals were fasted overnight, weighed and euthanized with chloroform and sacrificed by cervical dislocation. The blood from the rats was rapidly collected by direct heart puncture into plain sample bottles, and the serum was prepared and stored at -4 °C until it was required for analysis. The liver, heart, kidneys and lung were quickly excised, washed with ice-cold phosphate buffered saline, freed of fat, weighed and stored separately at -4 °C until required for further analysis.

Biochemical analyses

Weighed portions of liver, heart, kidney and lung were separately minced with scissors and homogenized in solution (1:2 w/v) containing 0.15 M KCl and 3 mM EDTA, pH 7.4 in ice. The homogenates were diluted 4-folds and centrifuged at 10,000 x *g* at 4 °C for 15 min. The supernatant was decanted and used for the various analyses. Lipid peroxidation was determined by the method described by **Buege and Aust (1978)** while conjugated diene level was determined spectrophotometrically following the method of **Recknagel and Glende Jr. (1984)**. Nitric oxide (NO) concentration was estimated in terms of its stable metabolic product, nitrite, using Griess reaction as described by **Kang, Bansal and Mehta (1998)**. Xanthine oxidase (XOD) was determined according to the method described by **Litwack et al. (1953)**. Reduced glutathione (GSH) level was estimated according to the method of **Moron, Depierre and Mannervik (1979)**. Catalase (CAT) activity was assayed according to the methods described by **Aebi (1984)**. Superoxide dismutase (SOD) activity was determined following the method of **McCord and Fridovich (1969)**. The method of **Gornall, Bardawill and David (1949)** was adopted in protein determination.

Statistic analysis

Results are mean ± SEM of 10 determinations. Statistical comparison of means was by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test using SPSS version 20. p <0.05 was considered significant.

RESULTS AND DISCUSSION

Food intake and body weight

Rats fed cholesterol-based diet consumed significantly (p <0.05) higher amount of food compared to control rats fed normal rat diet (Table 2). Mean weekly body weight gain was significantly (p <0.05) in rats fed cholesterol-based diet only compared to control and those fed cholesterol-based diet supplemented with ORO and vit E. No observable significant (p >0.05) difference was seen in rats fed ORO supplemented diet and vit E supplemented diet in terms of their mean weekly body weight gain (Table 2).

Lipid peroxidation parameters

Lipid peroxide concentration as determined by tissue malondialdehyde concentration was significantly (p <0.05) elevated following feeding with cholesterol. However, when rats were fed cholesterol + ORO and cholesterol + Vit E diets, serum, liver, heart, kidney and lung MDA concentrations were significantly (p <0.05) reduced compared to its level in rats fed cholesterol diet only. Serum, heart, kidney and lung MDA concentrations in cholesterol + ORO was not significantly (p >0.05) different from that in cholesterol + vit E fed rats (Figure 1a). Conjugated dienes concentration in the serum, liver, heart, kidney and lung in rats fed cholesterol diet was significantly (p <0.05) higher compared to rats fed cholesterol + ORO and cholesterol + vitamin E diets (Figure 1b). Nitric oxide (NO) level in the serum and lung of rats fed cholesterol diet supplemented with ORO and vitamin E was significantly (p <0.05) lower compared to the observed level in rats fed cholesterol diet alone (Figure 1c).

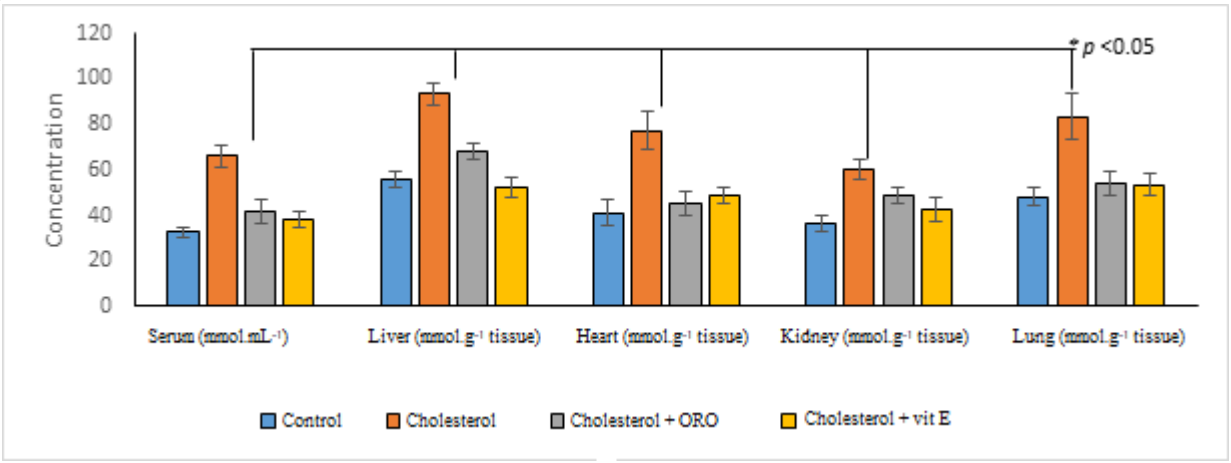
Antioxidant parameters

Xanthine oxidase activity was significantly (p <0.05) lower in rats fed ORO and vitamin E supplemented diets compared to those fed cholesterol diet without supplementation (Figure 2a). Reduced glutathione (GSH) concentration reduced significantly (p <0.05) in tissues of rats fed cholesterol diet compared to rats fed normal diet. However, in rats fed cholesterol diet supplemented with *Oryctes rhinoceros* oil (Cholesterol + ORO) and vitamin E (Cholesterol + Vit E) significant improvement was observed in serum, liver, heart, kidney and lung GSH concentrations compared rats fed cholesterol diet only (Figure 2b). Catalase activity was observed to be significantly (p <0.05) lower in serum, liver, heart, kidney and lung of rats fed cholesterol diet compared to rats fed normal diet. On the other hand, in rats fed cholesterol diet supplemented with *Oryctes rhinoceros* oil (Cholesterol + ORO) and vitamin E (Cholesterol + Vit E) CAT activity was significant (p <0.05) higher in serum, liver, heart, kidney and lung compared rats fed cholesterol diet only (Figure 2c). Tissues (serum, liver, heart, kidney and lung) SOD activities were significantly (p <0.05) lower in rats fed cholesterol diet compared to rats fed normal diet. However, in rats fed cholesterol diet supplemented with *Oryctes rhinoceros* oil (Cholesterol + ORO) and vitamin E (Cholesterol + Vit E) SOD activity was significant higher in these tissues compared rats fed cholesterol diet only (Figure 2d).

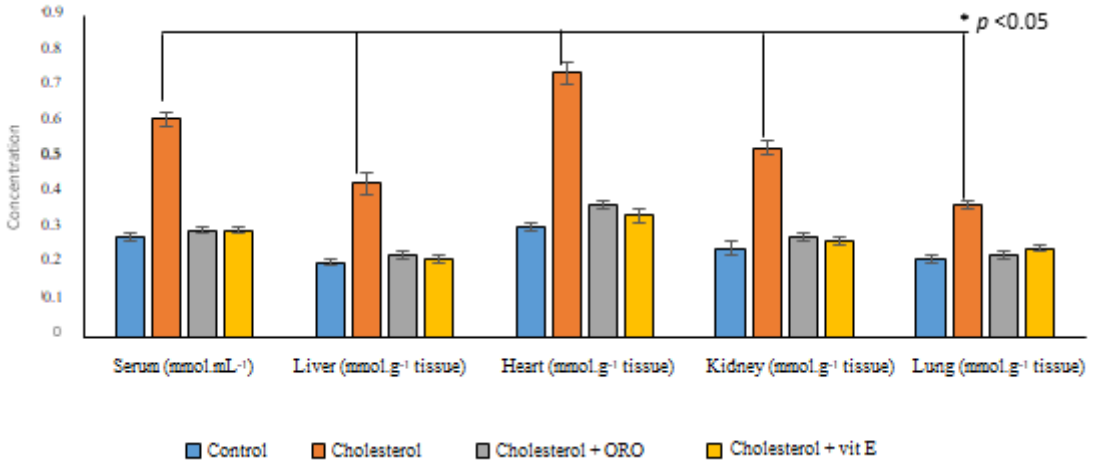
Cholesterol-based diets have been implicated to be particularly damaging to the vascular membrane. Thus, hypercholesterolemia and related cardiovascular syndrome are predominant in regions of the world, where fat-rich foods constitute the bulk of their daily meals. A recent report from a study by **Oluba, Josiah and Fagbohunka (2014)** showed that consumption of a cholesterol-based diet led to imbalance in serum lipid profile and release of proinflammatory cytokines. The inflammation resulting from tissue damage that most often accompany high fat consumption is viewed as a consequence of oxidative stress.

In the present study, inclusion of ORO as a supplement to a cholesterol-based diet led to normalization of body weight. Rats fed cholesterol-based diet tend to be overweight which could have been a consequence of increased fat deposit in the body. The observed higher relative organ (liver, heart, kidney and lung) weight further gave credence to this assumption. The development of fatty liver has been reported in high-fat fed rats.

(a)



(b)



(c)

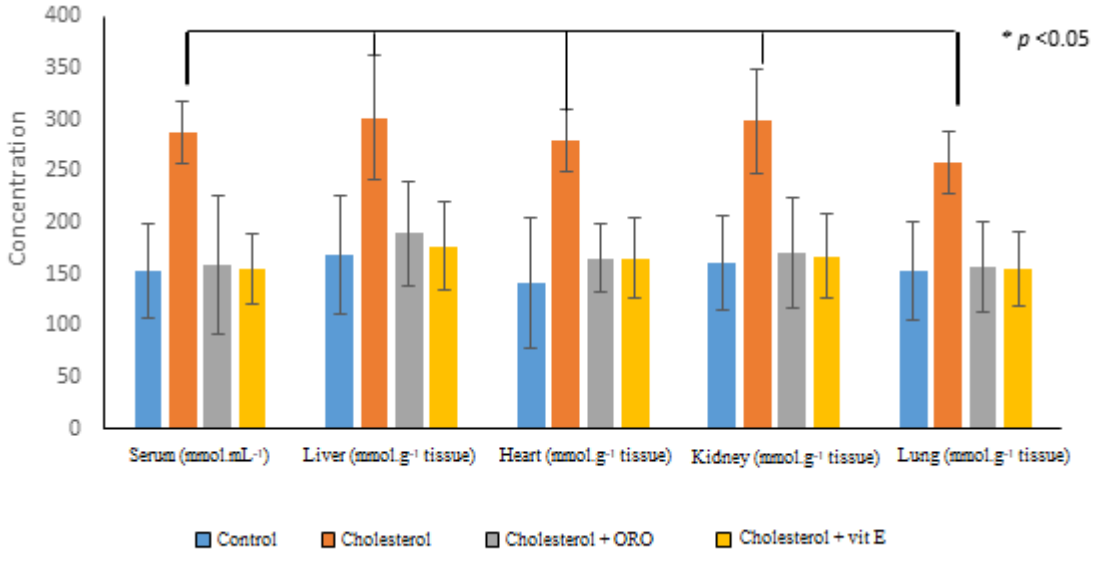
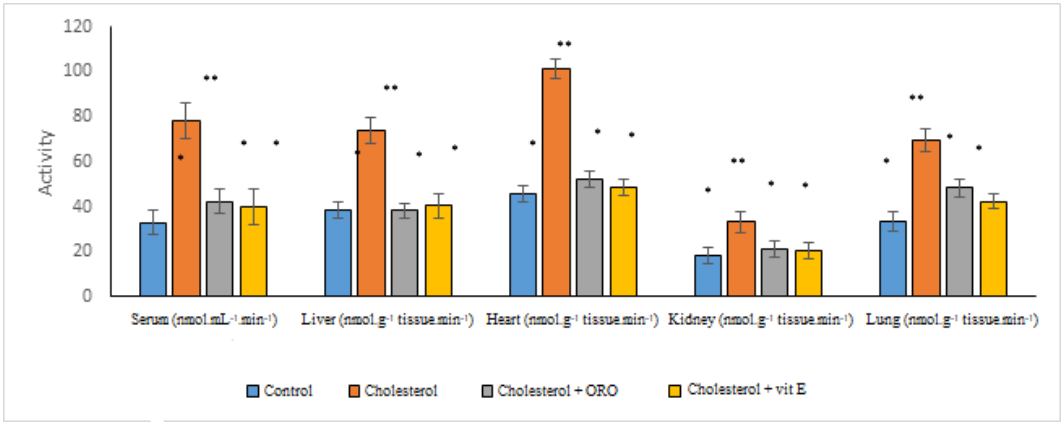
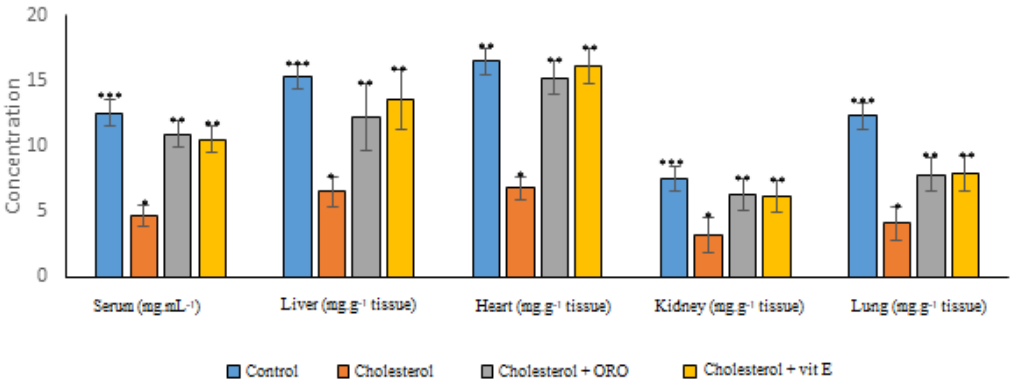


Figure 1 Tissue (a) MDA and (b) conjugated dienes and (c) nitric oxide (NO) concentrations in rats fed *Oryctes rhinoceros* oil supplemented diet over a period of 10 weeks. Note: Values are means ± SEM of 10 determinations. * Significant (p <0.05) different from control. ORO: *Oryctes rhinoceros* oil; vit.: vitamin.

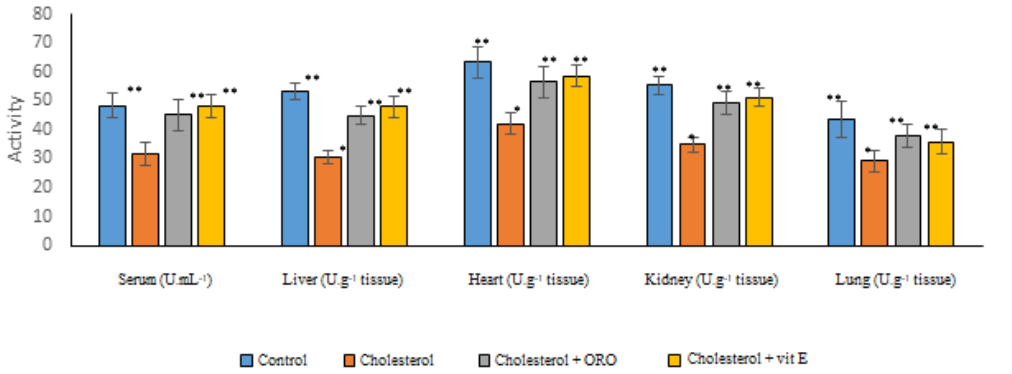
(a)

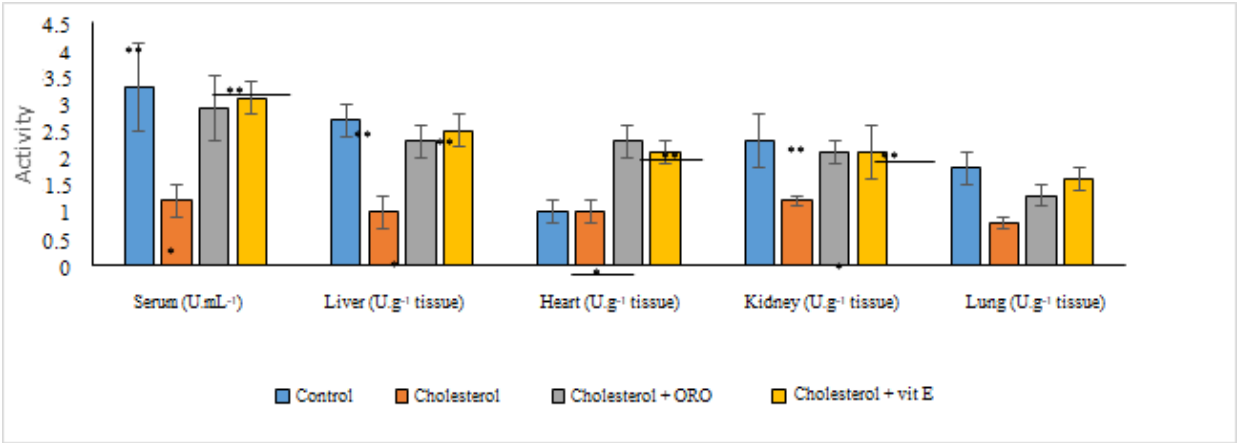


(b)



(c)





(d)

Figure 2 Tissue (a) Xanthine oxidase (b) GSH concentration (c) catalase activity (CAT) and (d) superoxide dismutase activity (SOD) in rats fed Oryctes rhinoceros oil supplemented diet over a period of 10 weeks. Note: Values are means ± SEM of 10 determinations. Bars carrying different alphabets are significant ($p < 0.05$). ORO: Oryctes rhinoceros oil; vit.: vitamin.

Table 1 Animal grouping and dietary regimen.

Feed composition (g.kg^{-1}, DM)	Control	Cholesterol	Cholesterol + ORO	Cholesterol + Vit E
Corn flour	600.0	600.0	600.0	600.0
Fish meal	200.0	200.0	200.0	200.0
Cholesterol	-	5.0	5.0	5.0
Mineral premix (AIN-76)[a]	30.0	30.0	30.0	30.0
Vitamin premix (AIN-76)[b]	10.0	10.0	10.0	10.0
Fiber	100.0	100.0	100.0	100.0
Groundnut cake	60.0	60.0	60.0	60.0
ORO	-	-	5.0	-
Vit E	-	-	-	5.0

Note: ORO, *Oryctes rhinoceros* larva oil.

[a] Mineral premix (AIN-76) composed of Ca as $CaSO_4$ (5.2 g.kg^{-1}), Ka as KCl (3.8 g.kg^{-1}), Na as NaCl (1.1 g.kg^{-1}), Mg as $MgSO_4$ (0.5 g.kg^{-1}), Fe as $FeSO_4$ (34.25 mg.kg^{-1}), Zn as $ZnSO_4$ (36.75 mg.kg^{-1}), Mn as $MnSO_4$ (59.34 mg.kg^{-1}), Cu as $CuSO_4$ (6.73 mg.kg^{-1}), Co as $CoCl_2$ (0.03 mg.kg^{-1}), and I as KI (0.21 mg.kg^{-1}).

[b] Vitamin premix (AIN-76) composed of vitamin A (4.0 IU.g^{-1}), vitamin D_3 (1.0 IU.g^{-1}), α-tocopherol (64.24 IU.kg^{-1}), thiamine (5.90 mg.kg^{-1}), riboflavin (6.29 mg.kg^{-1}), niacin (30.15 mg.kg^{-1}), pantothenic acid (15.26 mg.kg^{-1}), choline (1040.0 mg.kg^{-1}), pyridoxine (7.12 mg.kg^{-1}), folic acid (2.10 mg.kg^{-1}), biotin (0.21 mg.kg^{-1}), vitamin B_{12} (10.10 mg.kg^{-1}), and vitamin K (0.50 mg.kg^{-1}).

Table 2 Food intake and body weight of rats fed a cholesterol-based diet supplemented with *Oryctes rhinoceros* larva oil for 10 weeks.

Treatment group	Food intake (grat^{-1}.day^{-1})	Initial body weight (g)	Final body weight (g)	Mean body weight gain (grat^{-1}.week^{-1})
Control	10.9 ± 2.5^{a}	52.6 ± 5.8^{a}	158.5 ± 12.1^{a}	13.2 ± 2.1^{a}
Cholesterol	15.5 ± 1.7^{b}	50.5 ± 3.7^{a}	228.9 ± 15.3^{c}	22.3 ± 2.8^{b}
Cholesterol + ORO	15.5 ± 2.1^{b}	55.1 ± 2.5^{a}	171.3 ± 15.3^{b}	14.5 ± 1.0^{a}
Cholesterol + Vit E	14.8 ± 2.8^{b}	53.8 ± 3.2^{a}	168.8 ± 11.9$^{a.b}$	14.4 ± 1.3^{a}

Note: Values are means ± SEM of 10 determinations. Values in the same column carrying different alphabets are significant (p <0.05). ORO: *Oryctes rhinoceros* oil; vit.: vitamin.

The hyperlipidemia that develops following the consumption of a high-fat diet has been shown to substantially contributes to the depletion of both non-enzymic (GSH) and enzymic (SOD, catalase and GPx) **(Oluba et al., 2008b; Ojieh et al., 2009; Oluba et al., 2011)**. Findings from this study showed that increased dietary consumption of cholesterol could create of a pro-oxidant state and a depletion of cellular redox status. This observation agrees concur with the report of **Oluba et al. (2008b)** which showed a significant increase in plasma MDA concentration with a concomitant decrease in cellular antioxidant level in rats fed a high cholesterol diet.

The increased tissue MDA, conjugated dienes and nitric oxide concentrations in animals fed cholesterol-based diet alone in this study could be attributed to augmentation in the rate of cellular lipid peroxidation due to high fat (cholesterol) in the diet. This assertion is further reimbursed by the fact that xanthine oxidase activity was higher in rats fed cholesterol diet without supplementation compared to rats fed ORO and vitamin E supplemented diets. Xanthine oxidase has been demonstrated to be a primary source of superoxide radical **(Gomez-Cabrera et al., 2005)**. MDA and conjugated dienes are established markers of lipid peroxidation while nitric oxide through its free radical activity is capable of interacting with cellular components such proteins, DNA, and lipids within the cell in a process known as nitrosative stress leading to cytotoxicity **(Chang, Liao and Kuo, 2001)**. Thus, the reduction in tissue MDA, conjugated diene and nitric oxide levels in rats fed ORO and vitamin E supplemented diets in this study is beneficial and could give an indication of an anti-oxidative potential of ORO. The antioxidant activity of vitamin E is well established in literature **(Niki et al., 1985; Traber and Atkinson, 2007)**. In this study, ORO demonstrated a similar effect on tissue MDA, conjugated diene and nitric oxide concentrations to vitamin E showing that its potential antioxidant effect could be comparable to that of vitamin E.

An important mechanism of action of oxidative stress is via enhanced generation of ROS and/or RNS, which most often form conjugates or adducts with cellular components such as DNA, membrane lipids, proteins and carbohydrates. Thus, the increased serum and lung xanthine oxidase activities observed in rats fed cholesterol diet without supplementation could provide a justification that the pro-oxidant effect of dietary cholesterol could involve its effect on xanthine oxidase activity. In the coronary artery of hypercholesterolemic individuals, NADPH oxidases and xanthine oxidase have been reported to be the major sources of superoxide radicals. However, these reactive oxygen species in the cells are neutralized by cellular antioxidant defense system including GSH,

CAT, and SOD. Thus, oxidative stress is the resultant effect of a disequilibrium between cellular oxidants versus antioxidants **(Oluba, 2019)**. Several studies have shown that alteration of antioxidant enzyme activities in different kinds of stress was associated with a depletion of GSH, CAT and SOD and an increase of lipid peroxidation, all of which can lead to oxidative stress and finally cell death **(Bouayed and Bohn, 2010)**. Overexpression of the peroxisomal enzyme, catalase which catalyzes the reduction of hydrogen peroxide to water and molecular oxygen have been shown to reduce atherosclerosis in high-fat fed rats **(Yang et al., 2009)**. Results from the present study showed that ORO could act to normalize the attendant depletion of cellular antioxidant molecules when fed as a supplement to a cholesterol-based diet.

CONCLUSION

In conclusion, this study which was carried out to evaluate the effect of *Oryctes rhinoceros* larva oil in comparison with vitamin E on tissue lipid peroxidation and antioxidant defense systems in rats fed a cholesterol-based diet showed that *Oryctes rhinoceros* larva oil supplementation decreased tissue lipid peroxidation and increased the antioxidant defense molecules in rats. These results showed that consumption of *Oryctes rhinoceros* larva oil extracted from *Oryctes rhinoceros* larva, exhibited similar protective effects to vitamin E against diet induced oxidative stress in rats. Overall, the potential nutritional benefit of *Oryctes rhinoceros* larva oil on cardiovascular health could possibly involve its ability to upregulation of cellular antioxidant defense mechanisms.

REFERENCES

Adefegha, S. A., Oboh, G., Adefegha, O. M., Boligon, A. A., Athayde, M. L. 2014. Antihyperglycemic, hypolipidemic, hepatoprotective and antioxidative effects of dietary clove (Szyzgium aromaticum) bud powder in a high-fat diet/streptozotocin-induced diabetes rat model. *Journal of the Science of Food and Agriculture*, vol. 94, no. 13, p. 2726-2737. https://doi.org/10.1002/jsfa.6617

Aebi, H. 1984. Catalase in vitro. In Fleischer, S., Packer, L. *Methods in Enzymology*. Elsevier. vol. 105, p. 121-126. https://doi.org/10.1016/S0076-6879(84)05016-3

Belghit, I., Liland, N. S., Gjesdal, P., Biancarosa, I., Menchetti, E., Li, Y., Waagbø, R., Krogdahl, Å., Lock, E. J. 2019. Black soldier fly larvae meal can replace fish meal in diets of sea-water phase Atlantic salmon (Salmo salar). *Aquaculture*, vol. 503, p. 609-619. https://doi.org/10.1016/j.aquaculture.2018.12.032

Birben, E., Sahiner, U. M., Sackesen, C., Erzurum, S., Kalayci, O. 2012. Oxidative stress and antioxidant defense. *World Allergy Organization Journal*, vol. 5, no. 1, 9 p. https://doi.org/10.1097/WOX.0b013e3182439613

Bouayed, J., Bohn, T. 2010. Exogenous antioxidantsdouble-edged swords in cellular redox state: health beneficial effects at physiologic doses versus deleterious effects at high doses. *Oxidative Medicine and Cellular Longevity*, vol. 3, no. 4, p. 228-237. https://doi.org/10.4161/oxim.3.4.12858

Buege, J. A., Aust, S. D. 1978. Microsomal lipid peroxidation. In Fleischer, S., Packer, L. *Methods in Enzymology*. Elsevier. vol. 52, p. 302-310. https://doi.org/10.1016/S0076-6879(78)52032-6

Celebi, S., Utlu, N. 2006. Influence of animal and vegetable oil in layer diets on performance and serum lipid profile. *International Journal of Poultry Science*, vol. 5, no. 4, p. 370-373. https://doi.org/10.3923/ijps.2006.370.373

Chang, C. I., Liao, J. C., Kuo, L. 2001. Macrophage arginase promotes tumor cell growth and suppresses nitric oxide-mediated tumor cytotoxicity. *Cancer Research*, vol. 61, no. 3, p. 1100-1106. Available at: https://cancerres.aacrjournals.org/content/61/3/1100.short

Farombi, E. O., Nwaokeafor, I. A. 2005. Anti-oxidant mechanisms of kolaviron: studies on serum lipoprotein oxidation, metal chelation and oxidative membrane damage in rats. *Clinical and Experimental Pharmacoly and Physioogy*, vol. 32, no. 8, p. 667-674. https://doi.org/10.1111/j.0305-1870.2005.04248.x

Gomez-Cabrera, M. C., Borrás, C., Pallardó, F. V., Sastre, J., Ji, L. L., Viña, J. 2005. Decreasing xanthine oxidase-mediated oxidative stress prevents useful cellular adaptations to exercise in rats. *The Journal of Physiology*, vol. 567, no. 1, p. 113-120. https://doi.org/10.1113/jphysiol.2004.080564

Gornall, A. G., Bardawill, C. J., David, M. M. 1949. Determination of serum proteins by means of the biuret reaction. *Journal of Biological Chemistry*, vol. 177, no. 2, p. 751-766.

ILAR. 1985. Committee on Care, Use of Laboratory Animals, National Institutes of Health (US). Division of Research Resources. Guide for the care and use of laboratory animals. National Academies.

Kang, B. P., Bansal, M. P., Mehta, U. 1998. Selenium supplementation and diet induced hypercholesterolemia in the rat: changes in lipid levels, malonyldialdehyde production and the nitric oxide synthase activity. *General Physiology and Biophysics*, vol. 17, p. 71-78. Available at: http://www.gpb.sav.sk/1998/1998_01_71.pdf

Litwack, G., Bothwell, J. W., Williams, J. N., Elvehjem, C. A. 1953. A colorimetric assay for xanthine oxidase in rat liver homogenates. *Journal of Biological Chemistry*, vol. 200, no. 1, p. 303-310. Available at: https://www.ncbi.nlm.nih.gov/pubmed/13034787

McCord, J. M., Fridovich, I. 1969. Superoxide dismutase an enzymic function for erythrocuprein (hemocuprein). *Journal of Biological Chemistry*, vol. 244, no. 22, p. 6049-6055. Available at: http://www.jbc.org/content/244/22/6049.short

Moron, M. S., Depierre, J. W., Mannervik, B. 1979. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. *Biochimica et Biophysica Acta (BBA) - General Subjects*, vol. 582, no.1, p. 67-78. https://doi.org/10.1016/0304-4165(79)90289-7

Nevin, K. G., Rajamohan, T. 2006. Virgin coconut oil supplemented diet increases the antioxidant status in rats. *Food Chemistry*, vol. 99, no. 2, p. 260-266. https://doi.org/10.1016/j.foodchem.2005.06.056

Niki, E., Kawakami, A., Saito, M., Yamamoto, Y., Tsuchiya, J., Kamiya, Y. 1985. Effect of phytyl side chain of vitamin E on its antioxidant activity. *Journal of Biological Chemistry*, vol. 260, no. 4, p. 2191-2196. Available at: http://www.jbc.org/content/260/4/2191.short

Ojieh, G. C., Idokpesi, G. O., Eidangbe, G. O., Omage, K., Oluba, O. M. 2009. Hydrogenation impairs the hypolipidemic and antioxidant effects of palm oil in rats. *International Journal of Physical Sciences*, vol. 4, no. 7, p. 407-411. Available at: http://www.academicjournals.org/ijps/PDF/pdf2009/July/Ojieh%20et%20al.pdf

Oluba, O. M. 2019. Erythrocyte Lipid and Antioxidant Changes in Plasmodium falciparum-infected Children Attending Mother and Child Hospital in Akure, Nigeria.

*Pakistan Journal of Biological Sci*ences, vol. 22, no. 6, p. 257-264. https://doi.org/10.3923/pjbs.2019.257.264

Oluba, O. M., Adeyemi, O., Adebisi, K. E., Isiosio, L. O., Aboluwoye, C. O. 2008b. Effects of dietary cholesterol on some serum enzymes. *Journal of Medical Sciences*, vol. 8, no. 4, p. 390-394. https://doi.org/10.3923/jms.2008.390.394

Oluba, O. M., Adeyemi, O., Ojieh, G. C., Aboluwoye, C. O., Eidangbe, G. O. 2008a. Comparative effect of soybean oil and palm oil on serum lipids and some serum enzymes in cholesterol-fed rats. *European Journal of Scientific Research*, vol. 23, no. 4, p. 559-566.

Oluba, O. M., Eidangbe, G. O., Ojieh, G. C., Idonije, B. O. 2011. Palm and Egusi melon oils lower serum and liver lipid profile and improve antioxidant activity in rats fed a high fat diet. *International Journal of Medicine and Medical Sciences,* vol. 3, no. 2, p. 47-51. Available at: http://www.academicjournals.org/app/webroot/article/article1378983845_Oluba%20et%20al.pdf

Oluba, O. M., Josiah, S. J., Fagbohunka, B. S. 2014. Effect of *Oryctes rhinoceros* larva oil supplementation on serum lipid profile and inflammatory markers in mice fed a cholesterol-based diet. *Current Research – Cardiology*, vol. 1, no. 2, p. 79-83. https://doi.org/10.4172/2368-0512.1000011

Pacher, P., Beckman, J. S., Liaudet, L. 2007. Nitric oxide and peroxynitrite in health and disease. *Physiological Reviews*, vol. 87, no. 1, p. 315-424. https://doi.org/10.1152/physrev.00029.2006

Recknagel, R. O., Glende Jr, E. A. 1984. Spectrophotometric detection of lipid conjugated dienes. In Fleischer, S., Packer, L. *Methods in Enzymology*. Elsevier. vol. 105, p. 331-337. https://doi.org/10.1016/S0076-6879(84)05043-6

Sevanian, A., Hochstein, P. 1985. Mechanisms and consequences of lipid peroxidation in biological systems. *Annual Review of Nutrition*, vol. 5, p. 365-390. https://doi.org/10.1146/annurev.nutr.5.1.365

Teoh, C. H. 2010. Key sustainability issues in the palm oil sector. documento de trabajo para las consultas con múltiples actores (encargado por el Grupo del Banco Mundial). 52 p. Available at: http://www.biofuelobservatory.org/Documentos/Otros/Palm-Oil-Discussion-Paper-FINAL.pdf

Thomas, J. P., Maiorino, M., Ursini, F., Girotti, A. W. 1990. Protective action of phospholipid hydroperoxide glutathione peroxidase against membrane-damaging lipid peroxidation. *Journal of Biological Chemistry*, vol. 265, p. 454-461. Available at: http://www.jbc.org/content/265/1/454.short

Traber, M. G., Atkinson, J. 2007. Vitamin E, antioxidant and nothing more. *Free Radical Biology and Medi*cine, vol. 43, no. 1, p. 4-15. https://doi.org/10.1016/j.freeradbiomed.2007.03.024

Uttara, B., Singh, A. V., Zamboni, P., Mahajan, R. T. 2009. Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. *Current Neuropharmacoy*, vol. 7, no. 1, p. 65-74. https://doi.org/10.2174/157015909787602823

Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T., Mazur, M., Telser, J. 2007. Free radicals and antioxidants in normal physiological functions and human disease. *The International Journal of Biochemistry and Cell Biology*, vol. 39, no. 1, p. 44-84. https://doi.org/10.1016/j.biocel.2006.07.001

Van Huis, A. 2013. Potential of insects as food and feed in assuring food security. *Annual Review of Entomology*, vol. 58, p. 563-583. https://doi.org/10.1146/annurev-ento-120811-153704

Womeni, H. M., Linder, M., Tiencheu, B., Mbiapo, F. T., Villeneuve, P., Fanni, J., Parmentier, M. 2009. Oils of insects and larvae consumed in Africa: potential sources of polyunsaturated fatty acids. *Oléagineux, Corps Gras, Lipides*, vol. 16, no. 4-5-6, p. 230-235. https://doi.org/10.1051/ocl.2009.0279

Yang, H., Zhou, L., Wang, Z., Roberts, L. J., Lin, X., Zhao, Y., Guo, Z. 2009. Overexpression of antioxidant enzymes in ApoE-deficient mice suppresses benzo(a)pyrene-accelerated atherosclerosis. *Atherosclerosis*, vol. 207, no. 1, p. 51-58. https://doi.org/10.1016/j.atherosclerosis.2009.03.052

Acknowledgments:

Technical assistance from the technical staff of the Department of Chemical Sciences, Joseph Ayo Babalola University, Ikeji-Arakeji, is acknowledged.

Contact address:

*Olarewaju Oluba, Landmark University, College of Pure & Applied Sciences, Department of Biochemistry, Omu-Aran, P.M.B. 1001, Omu-Aran, Nigeria, Tel.: +2347030496639,
E-mail: oluba.olarewaju@lmu.edu.ng
ORCID: https://orcid.org/0000-0002-5107-6959

Corresponding author: *