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Vitamin E protects against biochemical alterations in testes of rats fed with photoxidised palm oil diet

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Abstract
Palm oil is a widely consumed vegetable oil in Nigeria and many parts of the tropics. Photoxidized palm oil (PPO) diet has been linked with testicular dysfunction and systemic expression of oxidative stress markers. Effect of vitamin E on testicular biochemical changes in those rats were not evaluated in previous studies and hence this research. Male wistar rats weighing 100-150g were equally divided into 3 groups: control (group 1), PPO diet-only (group 2)and PPO diet + vitamin E(group 3).Feeding and vitamin E administration were done for 13 weeks. Animals were sacrificed and their testes harvested for evaluation of relevant parameters. Result shows malondialdehyde was significantly increased in group 2 compared with control ($P<0.001$) but reduced in group 3 compared with group 2 ($P<0.01$). Glutathione peroxidase was significantly increased in group 3 compared with control ($P<0.01$) and group 2 ($P<0.01$). Tumor necrosis factor-α was significantly increased in group 2 compared with control ($P<0.001$) but decreased in group 3compared with group 2 ($P<0.001$). C- reactive protein in the different groups was insignificant. We conclude that vitamin E protects against PPO-induced biochemical alterations and improves oxidative status in testes of rats.

Keywords: PPO, biochemical alterations, vitamin E, testes, protects

Introduction

Palm oil is a vegetable oil obtained from the mesocarp of the ripe fruits of the oil palm tree especially *Elaeis guineesis* (Reeves and Weichrauch, 1979) [1]. Palm oil is widely consumed in Nigeria and many other tropical countries. It is the second most consumed vegetable oil in the world (Edem, 2002) [2], being used in domestic and commercial food industries because of its lower cost and high oxidative stability (Cheman *et al*, 1999) [3]. It is a common practice in these regions to allow palm oil in transparent plastic containers exposed to light in market places, shops, storage sites and even homes as there are no standard methods of protecting it from effects of light. Exposure of palm oil to light in these ways predisposes it to photoxidation, a process which causes deterioration in the quality of the oil (Przybylski, 2005) [4].

Photoxidation occurs rapidly in palm oil because of the presence of photosensitizers like chlorophyll, one of the minor constituents of palm oil (Ayu *et al*, 2016) [5]. Photoxidation results in the formation of hydroperoxides, superoxide radicals and other reactive oxygen species which can trigger free radical chain reactions with formation of more free radicals (Dongho *et al*, 2014) [6]. Upon consumption of the oil, these radicals and reactive oxygen species initiate cycles of lipid peroxidation which also generates reactive oxygen species, non-radicals species and other free radicals (Yin *et al*, 2011) [7]. This process is considered a major molecular mechanism involved in oxidative damage to cell structure and death (Dianzani and Berrera, 2008) [8]. Peroxidation process results in the elaboration of peroxyl radicals, alcoxyl radicals, hydroxy peroxides, hydroxyl radicals and aldehydes which may affect cellular structure and function (Ayala *et al* 2014) [9]. Lipid peroxidation plays a role in pathogenesis of several pathologies including neurodegenerative disease, inflammation and nutritional diseases (Farooqui and Farooqui, 2011) [10].

A stable biochemical environment is essential for optimal cellular function, alteration of which may cause tissue dysfunction (Rhoades and Bell, 2013) [11]. Aprevious study shows that long term consumption of photoxidized palm oil (PPO) diet is associated with testicular dysfunction (Aribo *et al*, 2018a) [12] and systemic expression of oxidative stress biomarkers in rats (Aribo *et al*, 2018b) [13]. Possible biochemical changes in the testes of those rats fed with photoxidized palm oil diet which could explain the mechanism of injury were not evaluated and hence the need for this study. Biochemical parameters evaluated in testes were tumor necrosis factor (TNF)–α, malondialdehyde (MDA), glutathione peroxidase (GPx) and C-reactive protein (CRP).

Tumor necrosis factor–α is a cell signalling cytokine produced mainly by activated macrophages, T-lymphocytes and natural killer cells (Olszewski *et al* 2007; Swadfager *et al* 2010) [14, 15]. Its physiological roles include induction of insulin resistance, chemoattractant, stimulation of phagocytosis and apoptosis (Feng *et al* 2015) [16]. Its levels are increased in response to sepsis (Pederson *et al*, 2009),[17] during immune responses, inflammation, neurodegenerative disorders etc. It has been showed to modulate multiple signalling pathways leading to apoptosis and anti-tumorogenicity (Pfeffer, 2003) [18], inflammation (Holbrook and Lare-Reyna, 2019) [19], endothelial injury (Cai *et al*, 2020) [20] etc.

C-reactive protein is a homopentameric acute phase protein primarily of hepatic origin but also of smooth muscles,

macrophages and lymphocytes (Sproston and Ashworth, 2018) [21]. It exhibits elevated expression during inflammation and infectious conditions (Du Clos and Mold, 2004).[22] Its release is triggered by a rise in IL6 which promotes its synthesis and is the principal downstreammediator of acute phase response following an inflammatory event (Pradham *et al*, 2001) [23]. It up-regulates production of pro-inflammatory cytokines including TNF (Pasceri *et al*, 2000) [24].

Malondialdehyde (MDA) is one of the last or secondary products of polyunsaturated fatty acid peroxidation and is therefore used as a marker of the degree of peroxidation and oxidative stress (Gawel *et al*, 2004) [25]. Consumption of oxidized vegetable oils has been associated with various tissue dysfunctions or toxicity (Fernandez-Duenas and Demian, 2009; Jiang *et al*, 2015; Aribo *et al*, 2018a) [26, 27, 12]. Malondialdehyde is known to be mutagenic and toxic to tissues (Esterbauer *et al*, 1982) [28] acting like a signalling molecule to regulate gene expression (Ayala *et al*, 2014) [9].

Glutathione peroxidase (GPx) an enzyme family with peroxidase activity plays its main biological role in the protection of cells against oxidative damage (Nachiappam and Murthukumar, 2010) [29]. Biochemically, its function is to reduce lipid hydroperoxides to their corresponding alcohols and free hydrogen peroxides to water (Murthukumar *et al*, 2011) [30]. It is therefore used as oxidative stress marker. It is consumed in high peroxidative states and oxidative stress (Sullivan-Gunn and Lewandowski, 2013) [31].

The testes or male gonads have both gamatogenic and endocrine functions and require optimal pH and normal biochemical environment to function maximally (Sembulingam and Sembulingam, 2014) [32]. The role of normal functioning testes in fertility cannot be over emphasized.

Vitamin E is a group of 8 fat-soluble compounds with antioxidant effects (Jiang, 2014) [27]. Vitamin E has been shown to ameliorate the effects of cytotoxic agents on cellular function (Schneider, 2005; Niki and Traber, 2012) [33, 34]. It acts as a peroxyl radical scavenger to maintain integrity of long chain polyunsaturated fatty acids in cell membranes (Traber and Atkinson, 2008) [35].

Materials and methods

Experimental animals

Fifteen male wistar rats were used for the research. The animals were housed in metal cages in the animal house of the Department of Physiology, University of Calabar, Calabar and acclimatized for one week before experimentation. They were kept at room temperature under a 12 hour day and 12 hour night cycle and standard laboratory conditions. Ethical approval was obtained from the Animal Research and Ethics Committee of the Faculty of Basic Medical Sciences (No: 83PHY10220) University of Calabar, Calabar.

Experimental design

The fifteen male wistar rats weighing 100-150g and aged 9-10 weeks were randomly divided into 3 groups of five rats each. Group 1 served as the control and was fed with plain rat chow. Group 2 was fed with PPO diet-only while group 3 was fed with PPO diet and vitamin E. All animals were fed daily and had free access to water for 13 weeks.

Formulation of PPO diet and administration of Vitamin E

Fresh palm oil was purchased from a local oil mill and photoxidised by permanently leaving it in transparent plastic bottles exposed to light including sunlight on sunny days for the duration of feeding. This method of photoxidation was chosen to mimic the way palm oil is kept for use traditionally since there are no standard methods of shielding it from light. Photoxidized palm oil diet was prepared by mixing 85g of rat feed with 15g of the oil. Vitamin E (Mega Life Sciences Public Company Ltd, Samutprakan, Thailand) was given by gavaging once a day at a dose of 268mg/kg/day for the duration of experimentation.

Harvesting and homogenizaton of testes

At the end of the feeding period (13weeks), animals were euthanized and left testis of each rat dissected out clean of connective tissues. Each testis was mechanically homogenised in ice water bath using a Dounce homogenizer. The homogenized tissue was then centrifuged at 3500rpm for 10 minutes and the supernatant used for determination of various biochemical parameters.

Determination of testicular concentration of tumor necrosis factor – α.

This was done in duplicating samples using the Quantikine ELISA kit for TNF –α (R&D Systems Inc, USA) as described in the accompanied operating manual.

Evaluation of testicular C-reactive protein concentration

The testicular level of CRP was assayed in duplicates by ELISA method using a high sensitive CRP test system (ACCulite CLIA Microwells, MonobindInc, USA).

Assay of testicular glutathione peroxidase (GPx) concentration

This was determined as described by Wendel (1981) [36] and used by Luchese *et al* (2009) [37] with commercially available reagents.The test is based on the principle that glutathione peroxidase (GPx) catalyses the conversion of reduced glutathione (GSH) to oxidized glutathione (GSSG) during the process of the former reducing lipid hydroperoxide to their corresponding alcohols and hydrogen peroxides to water. In the reaction, GPx reduces cumene hydroperoxides while oxidizing GSHto GSSG. The generated GSSG is reduced to GSH by glutathione reductase with consumption of NADPH. The decrease in NADPH which is proportional to GPx activity can easily be measured colorimetrically at 340nm in wavelength. The concentration of GPx was calculated using standard formula.

Determination of testicular malondialdehyde (MDA)

The concentration of testicular MDA was evaluated using the method of Ohkawa *et al* (1979) [38] and used by Basak *et*

al (2010) [39] with commercially available reagents. This test is based on the fact that lipid peroxidation yields malondialdehyde and 4-hydroxynonenal (4-HNE) as secondary end products. MDA reacts with thiobarbituric acid (TBA) to generate an MDA-TBA adduct or thiobarbituric acid reactive substance (TBARS), which can be easily quantified colorimetrically at 532nm.In summary, 1.5ml of 0.8% TBA was added to 1ml of 0.2ml homogenates. 0.4ml of 8.1% sodium dodecyl sulphate and 1.5ml glacial acetic acid were added. The mixture was made up to 5ml with distilled water, then vortexed and kept in water bath at 95^{O}C for 1 hour before been cooled in ice bath. The mixture was then centrifuged at 4000rpm for 10minutes at room temperature. The resulting supernatant was then transferred to a microplate reader and the absorbance read at 532nm wavelength. MDA standard curve was plotted and its concentration calculated using standard formula.

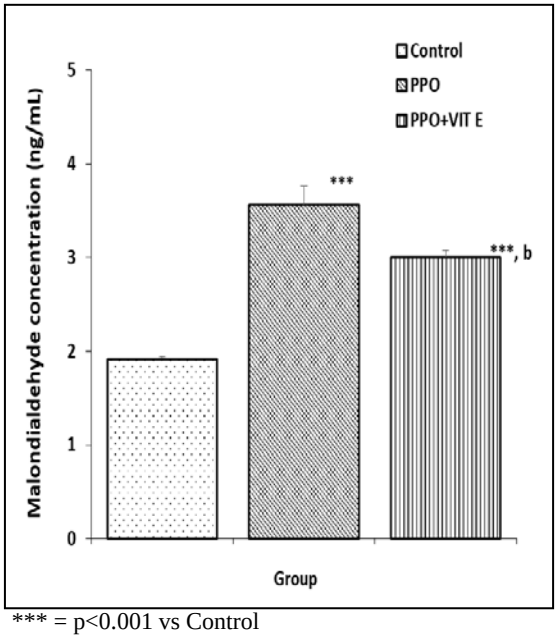
Statistical analysis

Data are expressed as mean ± standard error of mean (SEM) and analysed using one way analysis of variance (ANOVA) followed by a post hoc test of least significant difference. Probability level was tested at 95% level and a p-value of $p<0.05$ considered statistically significant.

Results

Concentration of malondialdehyde

Our results show malondialdehyde concentration was significantly higher in the PPO diet-only fed rats compared with control ($p<0.001$) but significantly reduced in the PPO diet + Vitamin E group when compared with the group that was fed with only PPO diet ($P<0.01$) as shown in Figure 1.

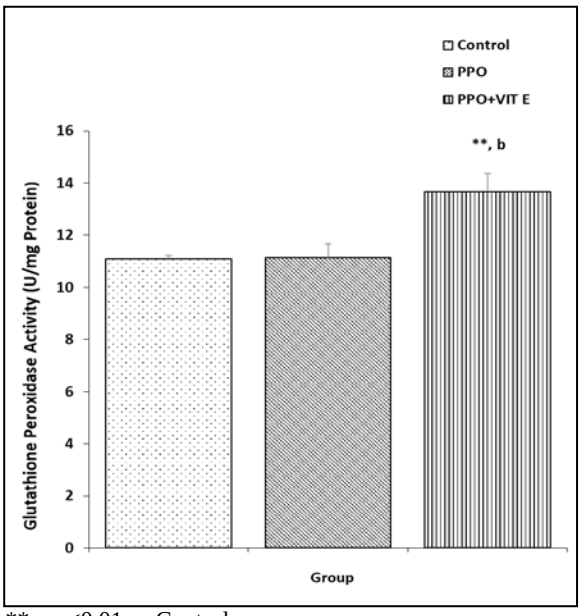


*** = p<0.001 vs Control
b = p<0.01 vs PPO

Fig 1: Comparison of malondialdehyde concentration in the different experimental groups. Values are mean ± SEM, n = 5.

Concentration of GPx

The testicular concentrations of GPx were not significantly different between control and PPO diet-only groups. GPx was however significantly increased in the PPO diet + vitamin E group compared with control ($P<0.01$) and PPO diet-only ($P<0.01$) groups as shown in Figure 2.

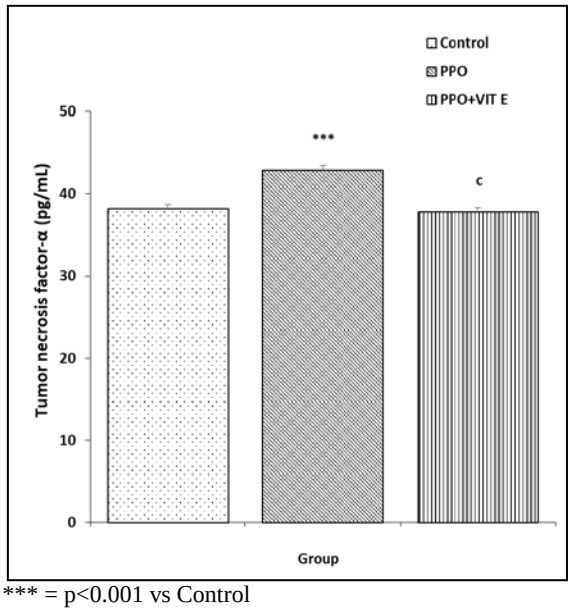


** = p<0.01 vs Control
b = p<0.01 vs PPO

Fig 2: Comparison of glutathione peroxidase activity in the different experimental groups. Values are mean ± SEM, n = 5.

TNF-α level in the testes

There was significant elevation in testicular concentration of TNF-α in the photoxidized palm oil (PPO) diet-only group compared with control ($P<0.001$) but a significantly reduced TNF–α in the PPO diet + Vitamin E group when compared with the PPO diet-only group ($P<0.001$) as shown in Figure 3.

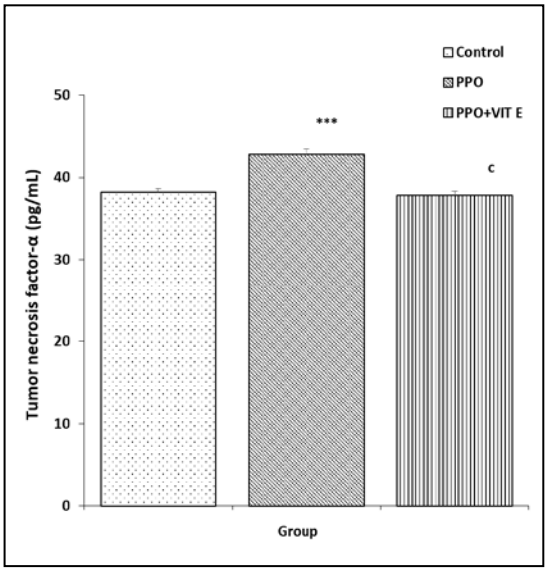


*** = p<0.001 vs Control
c = p<0.001 vs PPO

Fig 3: Comparison of tumor necrosis factor- α level in the different experimental groups. Values are mean ± SEM, n = 5.

Concentration of CRP

There were no significant differences in testicular concentrations of CRP among different groups as shown in Figure 4.



*** = p<0.001 vs Control
c = p<0.001 vs PPO

Fig 4: Comparison of C - reactive protein level in the different experimental groups. Values are mean ± SEM, n = 5.

Discussion

Previous studies have reported testicular dysfunction and systemic expression of oxidative stress biomarkers in rats following long term consumption of PPO diet but possible biochemical changes in the testes of those rats that may explain any involvement of local testicular factors in the dysfunction were not evaluated. Here we discuss our findings.

Among the ROS or radicals and non-radical oxidants produced as metabolites of lipid peroxidation in tissues is malondialdehyde (Dianzani and Barrera, 2008) [8]. The observed increase in MDA concentration in the group fed with only PPO diet could have resulted from an increased oxidative peroxidation induced by the diet. This is likely so because MDA is one of the last products of peroxidation (Dianzani and Barrera, 2008) [8] and so is used as a direct marker of lipid peroxidation or oxidative stress (Gawel *et al*, 2004) [25]. MDA is known to be toxic and mutagenic to tissues (Esterbauer *et al*, 1990) [28] acting like a signalling molecule to regulate gene expression (Ayala *et al*, 2014) [9]. It is also known to react readily with protein thiols resulting in the loss of protein function and homeostasis (Niki, 2015) [40]. The increase in the concentration of MDA in PPO-fed rats compared with control is similar to an earlier finding on systemic MDA expression by Aribo *et al*, (2018b) [13] supporting the fact that PPO diet elevates MDA concentration. Therefore the testicular toxicity and dysfunction previously reported by Aribo *et al* (2018a) [12] might have been in part MDA-induced. The significant reduction in MDA concentration following co-administration of vitamin E demonstrates the antioxidant effect of the vitamin and is in line with what other studies have shown (Sahin *et al*, 2002; Niki and Traber, 2012; Zare *et al*, 2018) [41, 34, 42].

Glutathione peroxidase enzyme family is a strong antioxidant enzyme system which is used as a co-substrate in the conversion of peroxides to their corresponding alcohols and water (Sarikaya and Dogan, 2020) [43]. The observed insignificant levels of GPx in control and PPO-only group do not suggest the absence of oxidative process in testes. This could rather be attributed to the large and elaborate array of antioxidant enzymes and free radical scavengers including GPx present in the testes (Aitken *et al*, 2008) [44]. These might have prevented early drop in the concentration of GPx in the group fed with PPO only. The increase in the concentration of GPx following co-administration of vitamin E could be due to the synergistic actions of the two antioxidants as was also observed by Hamid *et al* (2011) [45] and Tomoeda *et al* (2013) [46].

From our results, the testicular concentration of TNF-α was significantly increased in rats fed with only PPO dietwhen compared with control, a trend which was reversed following co-administration of vitamin E. TNF–α is a cell signalling cytokine produced by activated immune cells especially macrophages (Swadfager *et al*, 2010) [15] in response to infection or tissue damage (Walsh *et al* 1991) [47]. The observed increase in TNF–α could have been as a result of cellular response to testicular damage caused by the PPO diet. This increase in TNF-α might not have been associated with inflammation in the testes since the concentration of CRP was normal (Ryn *et al*, 2007) [48]. Also, though there is a cross-work between the two TNF-α receptors, the final outcome of stimulation by TNF-α is dependent on the particular receptor involved as well as the intracellular signalling pathway adopted (Aggarwal, 2003; Wajant and Siegmund, 2019) [49, 50]. The reversal of this trend following co-administration of vitamin E demonstrates the ability of the vitamin to ameliorate the TPO-induced testicular damage.

C-reactive protein is an acute phase reactant that is expressed in high levels especially in acute stage of inflammation (Pradham *et al*, 2001) [23]. The insignificant differences in the concentrations of CRP obtained in this study also support the fact that the possible mechanism of PPO-induced testicular injury might not have been inflammatory in origin. CRP is the principal downstream mediator of the acute phase response following an inflammatory event (Du Clos, 2000) [51] and can also increase the inflammatory process by modulating activities of pro-inflammatory agents like TNF-α, ROS and IL-1β (Ryn *et al*, 2007) [47].

Conclusion

We therefore conclude that photoxidised palm oil diet negatively alters biochemical parameters in testes of PPO-fed wistar rats but which was ameliorated by co-administration of vitamin E. Also vitamin E improves oxidative status of testes in wistar rats.

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