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LONG TERM CONSUMPTION OF THERMOXIDISED OR PHOTOXIDISED PALM OIL DIETS IS ASSOCIATED WITH SYSTEMIC EXPRESSION OF OXIDATVE STRESS BIOMARKERS IN WISTAR RATS

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ABSTRACT

Several researches have demonstrated that consumption of thermoxidised palm oil (TPO) or photoxidised palm oil (PPO) diets impairs tissue functions in many organ systems suggestive of a systemic toxicity. It is also known that lipid peroxidation of tissues results in tissue damage and consequent dysfunction from oxidative stress, a common pathway in many pathological conditions. But evidence on the general peroxidative status of the animals following long term ingestion of these forms of palm oil is lacking. Fifteen male wistar rats weighing 80 -122g and aged 20-23 weeks were divided into control, PPO-fed and TPO-fed groups of five rats each. Control group was fed on normal rat feed while the PPO-fed and TPO-fed groups were fed on PPO and TPO diets respectively for 13 weeks and their blood samples evaluated for serum catalase (CAT), glutathione peroxidase (GPx) and malondialdehyde (MDA) levels as biomarkers of oxidative stress. Serum MDA concentration in TPO-fed and PPO-fed groups was significantly increased ($p<0.001$) compared with control. It was also significantly increased in the PPO-fed group $p<0.01$) compared with the TPO-fed group. Serum concentration of CAT in the TPO-fed and PPO-fed groups was significantly decreased ($P<0.01$ and $p<0.001$ respectively) compared with control. Serum GPx concentration in TPO-fed and PPO-fed groups was also significantly decreased ($P<0.05$ and $p<0.001$ respectively) compared with control. In conclusion, long term ingestion of TPO or PPO diets is associated with systemic expression of oxidative stress biomarkers in wistar rats.

KEYWORDS: Diet; Oxidative stress; Palm oil; Peroxidation; Photoxidation; Thermoxidation.

INTRODUCTION

Palm oil is a vegetable oil derived from the mesocarp of the ripe fruits of the oil palm tree especially *Elaesis guineensis* and is widely consumed in the tropical and subtropical regions.[1] Red palm oil contains tocopherols, carotenoids, ascorbic acid and several phytonutrients which are potent antioxidants but which can be destroyed following photoxidation[2] or thermoxidation.[3] Unfortunately, most of the palm oil consumed must have undergone some degree of photoxidation as there is no standard storage method of shielding it from effect of light during storage. From the oil mills to the market and then to the homes, palm oil is constantly exposed to light. Palm oil is often displayed for sale in the market, shops and in storage sites in plastic containers under direct sun light. Even in the homes, it is stored unprotected from light. Much of the palm oil is also consumed in the thermoxidised form for economic reasons and for the improvement of the taste of food.[4]

Oxidative degradation of oils/lipids by light (photoxidation) or by application of heat (thermoxidation) results in the formation of malondialdehyde, peroxides, hydroperoxides, reactive oxygen species and free radicals.[5] This process is quickly followed by lipid peroxidation in the oil or in cellular lipids if the oil is consumed resulting in the formation of more free radicals and other toxic substances which could have damaging effects on cell function.[6,7] Biological systems combat the effects of these oxidants with the help of natural molecules or antioxidants to limit the production and effects of reactive oxygen species and ensure tissue repair. These biomolecules include tocopherols, tocotrienols, carotenoids, zinc, ascorbic acid and proteins like albumin, catalase, glutathione peroxidase, superoxide dismutase etc. Combating-effect is done by scavenging free radicals, quenching singlet oxygen, catalytically neutralizing oxidants or inactivating sensitizers.

Oxidative stress is a disturbance in the balance between production of reactive oxygen species (ROS) and other oxidants (free radicals) and antioxidant defenses.[8,9] The different oxygen metabolites superoxide radicals, hydrogen peroxide, hydroxyl radicals, nitric oxide, singlet oxygen etc. are formed in cells during cellular activities. These radicals in normal concentrations play important role in several physiological processes but if produced in excess, antioxidant forces are overwhelmed and results in tissue dysfunction[10,11] via oxidative stress. To protect tissues from the harmful effects of these metabolites, biological systems are endowed with antioxidant mechanisms that utilize vitamins, metals, proteins etc. to combat the formation and effects of oxidants that would have resulted in oxidative stress and tissue dysfunction when antioxidants are overwhelmed. Oxidative stress is a common pathway for several pathological conditions and is produced by oxidation and peroxidation of cellular lipids, proteins, carbohydrates and amino acids.[12] Functional oxidative modification of cellular biomolecules induces post-translational changes that alter the function of important cellular proteins and signaling pathways and so are causal steps in cellular dysfunction.[13]

Biomarkers of oxidative stress are molecules that are modified by interactions with reactive oxygen species in the microenvironment and molecules of antioxidant system or are produced as intermediates or end-products of such reactions. Such biomarkers include malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) etc.[14] Malondialdehyde is a highly reactive compound[15] and one of the final products of polyunsaturated fatty acid/lipid peroxidation in cells. Reactive oxygen species degrade the lipids into MDA which causes toxic stress in cells.[16] MDA is therefore used as a biomarker of oxidative stress in organisms, its level in tissue being directly proportional to the degree of tissue peroxidation.[17] Catalase is a common enzyme found in all organisms exposed to oxygen. It catalyzes the breakdown of hydrogen peroxide to water and oxygen and reducing the formation of free radicals in the process. Its concentration or activity is inversely proportional to the degree of oxidative stress in the tissue.[18]

Glutathione peroxidase (GPx), a selenoenzyme, functions in protecting the organism from oxidative damage by catalysing the reduction of harmful hydroperoxides with thiol cofactors[19], its level being low in high oxidative stress states.[20]

Long term consumption of thermally oxidized palm oil has been associated with damage/dysfunction of several body tissues including the reproductive system. Long term consumption of thermoxidised palm oil diet is said to cause growth retardation, fatty liver[21], reduction in both red and white blood cells count[22], peptic ulceration[23], distortion of villi morphology and concomitant malabsorption of fluid and glucose[24] as well as hepatotoxicity.[25] Consumption of this form of palm oil has also been associated with impaired carotid sensitivity[26], reduced glomerular filtration rate and renal plasma flow[27] and reproductive toxicity.[28] Similarly, chronic consumption of photoxidised palm oil diet has been linked with tissue dysfunction including reproductive impairment in wistar rats.[29] Though peroxidation of cellular lipids is known to induce tissue dysfunction via oxidative stress, the systemic peroxidative status following chronic consumption of photoxidised or thermoxidised palm oil diets said to cause dysfunction in several body systems has not been ascertained, hence this study using serum CAT, MDA and GPx as biomarkers.

MATERIALS AND METHODS

Preparation of TPO and PPO diets

Red or fresh palm oil was purchased from Marian Market, Calabar. Thermoxidised palm oil (TPO) was prepared as described by Isong.[30] In summary, the oil in a steel pot over a heating mantle underwent four rounds of heating at high temperatures (150^0C) and allowed to cool in between heating sessions with each session lasting for 20 minutes. Photoxidised palm oil was prepared by leaving a portion of the fresh palm oil constantly exposed to light including sunlight mimicking the way the oil is always handled. The TPO and PPO diets were prepared by mixing 15g of TPO or PPO respectively with 85g of rat chow as used by Ani *et al.*[26] and Obembe *et al.*[23]

Experimental design

Fifteen male Wistar rats weighing 80-120g and aged 20-23 weeks used for the research were housed in metallic cages in the animal house of the Department of Physiology, University of Calabar, Calabar, under a 12-hour night and 12-hour day cycle. They were randomly divided into control, thermoxidised palm oil diet (TPO)-fed and photoxidised palm oil diet (PPO)-fed groups of five rats each. The control was fed on normal rat chow while the TPO-fed and PPO-fed groups were fed on their respective diets. All animals were given free access to potable water and their respective diets for 13 weeks after which they were anaesthetised and their blood collected for determination of serum CAT, MDA and GPx concentrations.

Collection of Blood Samples

The rats were anaesthetised using 3.5% chloroform and blood samples collected via cardiac puncture using 5mL syringe attached to 21G needle into plain capped bottles.[31] The samples were left for two hours to clot after which they were centrifuged at 10,000 rpm for 10 minutes and the serum collected for analysis of serum concentrations of MDA, CAT and GPx.

Determination of serum malondialdehyde (MDA) concentration

This was determined as described by Buege and Aust.[32] In brief, 0.1ml of serum (Tris-HCL buffer, pH 7.5) was treated with 2ml (1:1:1 ratio) of thiobarbituric acid (TBA)-trichloroacetic acid (TCA) –HCL reagent (TBA 037%, 0.25N HCL and 15% TCA). The mixture was placed in water bath for 15 minutes and allowed to cool. The absorbance of the resultant clear supernatant was measured against reference blank at 535nm. The concentration was expressed as nmol/ml.

Determination of serum catalase (CAT) concentration

This was determined according to the method of Aebi.[33] Briefly 0.1ml of serum was pipetted into a cuvette containing 1.9ml of 50 mM phosphate buffer of pH 7.0. Reaction was started by adding 1.0ml of freshly prepared 30% (v/v) hydrogen peroxide. The rate of decomposition of hydrogen peroxide was measured spectrophotometrically from changes in absorbance at 240nm. Enzyme activity was expressed as IU/ml protein.

Determination of serum GPx

Glutathione peroxidase activity was measured using the method described by Rotruck *et al.*[34] The reaction mixture contained 2.0ml of 0.4M Tris-HCL buffer, pH 7.0 and 0.01ml of 10mM sodium azide, 0.2ml of enzyme, 0.2ml of 10mM glutathione and 0.5ml of 0.2mM H_20_2. The content was incubated at 37^0C for 10 minutes followed by termination of the reaction by addition of 0.4ml 10% v/v TCA, and centrifuged at 3000rpm for 5 minutes. The absorbance of the product was read at 430nm. Result was expressed as nmol/mg protein.

Statistical analysis

Results are expressed as mean ± standard error of mean (SEM) and analyzed by one-way analysis of variance (ANOVA) followed with a post hoc test of least significant difference using Statistical Package for Social Science (SPSS) (Version 20). $p<0.05$ was considered statistically significant.

RESULTS

Comparison of serum concentrations of oxidative stress biomarkers in the different groups

Serum malondialdehyde (MDA) concentration

Table 1 shows serum MDA concentrations for control, TPO-fed and PPO-fed groups. Serum MDA concentration was significantly ($p<0.001$) increased in the TPO-fed and PPO-fed groups compared with control. It was also significantly ($p<0.01$) increased in the PPO-fed group compared with the TPO-fed group.

Serum catalase (CAT) concentration

The serum concentrations of CAT for control, TPO-fed and PPO-fed groups are presented in table 1. Serum CAT concentration was significantly decreased in TPO-fed ($p<0.01$) and PPO-fed ($p<0.001$) groups compared with control. CAT concentration was also significantly ($p<0.01$) decreased in PPO-fed group compared with TPO-fed group.

Serum glutathione peroxidase (GPx) concentration

Table 1 shows serum GPx concentrations for control, TPO-fed and PPO-fed groups. Serum GPx concentration was significantly decreased in TPO-fed ($p<0.05$) and PPO-fed ($p<0.001$) groups compared with control. It was also significantly ($p<0.01$) decreased in PPO-fed group compared with TPO-fed group.

Table 1: Comparison of serum concentrations of oxidative stress biomarkers in the different experimental groups.

Parameter	Control	TPO	PPO
MDA (nmol/mL protein)	2.42±0.10	3.40±0.11***	3.92±0.08***,b
CAT (IU/mL protein)	0.77±0.02	0.68±0.02**	0.54±0.02***,b
GPx (nmol/mg protein)	85.88±1.07	72.50±5.30*	63.56±1.04***,b

values are expressed as mean ± SEM, n = 5.
**p<0.05, **p<0.01, ***p<0.001 vs control*
b = p<0.01 vs TPO

DISCUSSION

Serum levels of the biomarkers of oxidative stress (MDA, CAT and GPx) in this study were significantly deranged in both TPO-fed and PPO-fed rats compared with the control, suggesting a systemic affectation.

Serum malondialdehyde levels were significantly increased in both TPO-fed and PPO-fed rats compared with control. Malondialdehyde is one of the final products of polyunsaturated fatty acid peroxidation in cells. Reactive oxygen species degrade polyunsaturated fatty acids and lipids into MDA which could cause toxic stress in cells.[16] It is a highly reactive compound.[15] It reacts with deoxyadenosine and deoxyguanosine in DNA to form DNA adducts which are mutagenic.[35] Thermoxidation and photoxidation accelerate oxidation/peroxidation of lipids with consequent elaboration of toxic products like MDA, reactive oxygen

species and free radicals.[36] Consumption of the TPO rich in MDA, reactive oxygen species and toxic products of lipid oxidation may initiate cellular peroxidation, damaging the tissues and at the same time causing more cycles of lipid peroxidation with further production of peroxidation products. The increase in these peroxidation products could overwhelm the natural antioxidant defenses leading to oxidative stress.[13]

Biological effects of free radicals and other reactive oxygen metabolites are controlled by a system of antioxidants in the body. Part of this natural defense system against oxidants includes catalase, glutathione peroxidase and superoxide dismutase.[14] Catalase (CAT) is a common enzyme present in nearly every living organism exposed to oxygen. As an antioxidant it catalyzes the breakdown of hydrogen peroxide into water and oxygen.[18] Thermoxidation and photoxidation accelerate oxidation/peroxidation of lipids with consequent elaboration of toxic products like reactive oxygen species and free radicals.[36] An increase in peroxidation is associated with a rise in hydrogen peroxide formation[5] requiring more catalase for its decomposition and in the process, catalase becomes consumed. The observed decrease in the concentration in serum CAT in the TPO-fed and PPO-fed rats in our study could have been the result of increased peroxidation with consequent elaboration of hydrogen peroxide which consumed CAT for its decomposition to water and oxygen. This process depletes CAT concentration and creates an imbalance in the oxidants/antioxidants status in the tissues which may result in tissue dysfunction.

Our research demonstrated a significantly reduced serum level of GPx in both TPO-fed and PPO-fed rats. The possible explanation for this observation and its implication is provided here. Glutathione peroxidase's main biological function is the protection of cells against oxidative damage by reducing lipid hydroperoxides to their corresponding alcohols and hydrogen peroxides to water and oxygen. It therefore interrupts free radicals chain mechanism, functions by being preferentially oxidized and as a reducing agent. Its concentration is therefore depleted in high peroxidative states as much of it is used to mop up the free radicals so generated during peroxidation.[20] Thermoxidation and photoxidation accelerate oxidation/peroxidation of lipids with consequent elaboration of toxic products like reactive oxygen species and free radicals.[36] The observed significant reduction in serum GPx concentration in both TPO-fed and PPO-fed rats may be attributed to a high consumptive anti-oxidative processes caused by excessive formation of reactive oxygen species or free radicals in these rats. The depletion of GPx could result in oxidants/antioxidants imbalance (oxidative stress) which may potentiate toxicity by the unmatched free radicals to cause tissue damage/dysfunction.

CONCLUSION

Chronic consumption of thermoxidised or photoxidised palm oil diets is demonstrated to have deleterious effects on several body functions as earlier enumerated. Though there is no documentation on the precise mechanism(s) responsible for the multiple systems affectation following long term ingestion of TPO or PPO diets, our study showed a systemic expression of oxidative stress biomarkers (increase in the concentration of serum MDA and decreases in serum CAT and GPx levels) with a pattern observed in oxidative stress conditions. Therefore, the multiple organ dysfunction reported following long term consumption of TPO or PPO diets might be oxidative stress-mediated.

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