

Long Term Consumption of Photoxidised Palm Oil Diet Impairs Reproductive Function in Male Wistar Rats

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Abstract: Photoxidation of palm oil, like other forms of oils/lipids oxidation has adverse effects on the physicochemical qualities of the oil including depletion of essential antioxidants and formation of free radicals which could be injurious to tissues. Unfortunately, this widely consumed edible vegetable oil is usually stored or displayed for sale in plastic containers under direct sun light and unknowingly subjecting the oil to photoxidation. This work therefore sought to evaluate the possible effect of long term consumption of palm oil exposed to light on some reproductive parameters in male wistar rats. Male wistar rats weighing 85g to 120g and aged 19 to 23 weeks were randomly divided into a control and photoxidised palm oil (PPO)-fed groups of five rats each. The control group was fed on normal rat chow while the PPO diet group was fed on photoxidized palm oil diet. The duration of feeding was 13 weeks after which the rats were euthanized, blood samples collected while testes were harvested from them for determination of relevant parameters. The result showed a significantly reduced seminal pH in the PPO-fed group compared with control ($P<0.01$). Sperm motility (%) and viability (%) were significantly decreased ($P<0.01$ and $P<0.01$ respectively) in the PPO-fed group compared with control. The PPO-fed group had significantly decreased ($P<0.001$) sperm count and a significantly increased percentage of morphologically defective sperm cells ($p<0.05$) compared to the control. Serum testosterone, follicle stimulating hormone (FSH) and Luteinizing hormone (LH) were significantly decreased ($P<0.001$, $P<0.01$ and $P<0.001$ respectively) in the PPO-fed rats compared with control. In conclusion, long term consumption of PPO diet impairs reproductive function in male wistar rats.

Keyword: Photoxidation, Palm oil, Luteinizing Hormone, Follicle stimulating Hormone.

INTRODUCTION

Palm oil is an edible vegetable oil extracted from the mesocarp of the ripe fruits of the oil palm especially the African variety, *Elaeis guineensis*. Raw palm oil is known to have several health and nutritional benefits including being a rich source of antioxidants, vitamins A, C and E [1-4]. Raw palm oil is said to protect tissues against oxidative damage [5], attenuates oxidative stress-induced sperm damage [2], protects against atherogenesis and tumorigenesis [6, 7]. It is widely consumed in Nigeria and other tropical countries. It is a common practice in these regions to leave palm oil in plastic containers under the sunlight during storage or displayed for sale in shops and markets. Even in the homes palm oil is constantly left exposed to light. This strongly suggests that most of the palm oil consumed including the so called "fresh" palm oil sold in the market must have undergone various degrees of photoxidation.

Oils undergo oxidation, being made susceptible by their polyunsaturated fatty acid content and results in increased primary and secondary oxidation products like hydroperoxides, aldehydes,

ketones and alcohols [8] as well as free radicals [9] which are known to have damaging effects on cells [10]. The rate of photoxidation is affected by intensity of radiation, degree of unsaturation and presence of oxygen as well as sensitizers [11]. Light of shorter wave length is shown to have more adverse effects than that of longer wave length [12].

Photoxidation of palm oil like other forms of lipid oxidation example thermoxidation, is associated with negative alterations in the physicochemical properties of the oil including elaboration of harmful end-products [13, 14]. During photoxidation (oxidation of lipids in the presence of light) sunlight oxygen species (1O_2) react with the oil setting up a chain reaction leading to the production of free radicals and toxic metabolites which could be injurious to tissues [14]. Reactive oxygen species, free radicals and other oxidants from the oxidative process set up peroxidation chain reactions in the oil or lipid structures in cells when consumed, forming more radicals and damaging cellular elements [9]. Peroxidation of oils may reduce the level of antioxidant compounds, while increasing the levels of end products of peroxidation like

malondialdehyde (MDA) and free radicals in tissues/serum [15]. Lipid peroxidation of testis is known to cause testicular injury with consequent impairment of function [16].

Consumption of thermoxidised palm oil has been linked with several organs malfunction including growth retardation, fatty liver [17], increase serum liver enzymes [18], anemia [19], reduced glomerular filtration rate and reduced renal blood flow [20] and peptic ulceration [21]. Photoxidation of palm oil like other forms of oil oxidation (thermoxydation) results in the formation of toxic substances. If consumption of thermally oxidized palm oil is associated with the above mentioned tissues dysfunction, there is probability that consumption of photoxidised palm oil may also disrupt tissue function.

Infertility is globally of a serious concern not only because of the need for continuity of the species but also due to its social, economic, and psychological implications [22]. Nearly half of these cases is said to be due to male factor in infertility [23]. The main clinical manifestations of male infertility include reduced sperm count (oligozoospermia), reduced sperm motility (asthenozoospermia) and sperm morphological abnormalities (teratozoospermia) [24]. Palm is constantly exposed to light and consumed in that form and during which time varying degrees of photoxidation could have taken place. Unfortunately, there is paucity of information on the effect of long term consumption of photoxidised palm oil diet on reproductive function and hence the need to evaluate the effects of PPO diets on this essential function in male wistar rats.

METHODOLOGY

Experimental animals

Ten male wistar rats aged 19 to 23 weeks and weighing 85-120g were acclimatized in metal cages for two weeks in the animal house of the Department of Physiology, University of Calabar, Calabar. They were housed at room temperature under a 12 hour day and 12 hour night cycle.

Experimental design

The ten male Albino wistar rats were randomly divided into two groups viz a control and a photoxidised palm oil (PPO) diet-fed groups of five rats per group. The control group was fed on normal rat feeds while the test group was fed on PPO diet. All rats had free access to drinking water and their respective feeds. The feeding period lasted for thirteen weeks (at a time rats were 19-23 weeks old) after which the rats were euthanized and blood samples taken for assay of testosterone, follicle stimulating hormone and luteinizing hormone, while the testes and epididymis were dissected out for epididymal seminal fluid analysis.

Preparation of photoxidizedpalm oil diet

Photoxidation of palm oil was achieved by exposing the oil covered in transparent plastic containers to light (including sun light on sunny days) and stored unprotected from light mimicking the way the oil is normally handled for the duration of feeding. Photoxidized palm oil diet was prepared following the method used by Obembe *et al* [25] by mixing 15g of photoxidised palm oil with 85g of rat feed.

Determination of seminal fluid parameters

Seminal fluid parameters were evaluated according to World Health Organization (WHO) standard [26]. A summary is given below.

pH

Semen was aspirated from the epididymis with a sterile syringe and its pH measured using a hand-held pH meter.

Sperm motility

One drop of well mixed semen was placed on glass slide and covered with a cover slip. Using x10 and x40 objective lenses, motility of the sperm cells was observed and expressed in percentage.

Viability

One drop of semen was mixed with a drop of 0.5% Eosin solution on a slide. The preparation was left for 2minutes. A x10 objective lens was used to focus the sample while a x40 objective lens was used to count the percentage of viable (unstained) and non-viable (stained) spermatozoa.

Sperm count

The semen was diluted in 1 in 20ml sodium bicarbonate-formalin diluting fluid and mixed. An improved Neubauer-ruled chamber was used for the counting with x10 objective with condenser. Number of spermatozoa in an area of 2 square mm noted and number of spermatozoa in 1ml of semen calculated by multiplying the number counted by 100,000.

Morphology

A thin smear of well mixed semen was made on a slide and while still wet fixed with 95% v/v ethanol for 10 minutes and allowed to air-dry. The smear was then covered with carbolfuchsin (1 in 20) and allowed to stain for 3minutes. The stain was washed with water. The smear was counter stained with diluted Loeffler methylene blue for 2 minutes. Using x40 objective lens, morphology of sperm cells was assessed for abnormalities.

Determination of serum hormones concentration FSH and LH

Serum follicle stimulating hormone (FSH) level was determined in triplicated samples by radioimmunoassay (RIA) technique using rats FSH kits obtained from Biocode Company Belgium according to

the protocol provided with the kit [27]. Serum luteinizing hormone (LH) concentration was assessed by enzyme-immunoassay (EIA) technique using rat LH kit from Immunometrics, London, UK. Optical density was done using Jenway 6300 Spectrophotometer at a wave length between 492-550nm [28].

Testosterone

Serum testosterone levels were measured by enzyme-immunoassay (EIA) using rat testosterone kit from Immunometrics, London, UK. Using a Jenway6300 Spectrophotometer, the optical density was read at 492-550nm [28].

STATISTICAL ANALYSIS

Data were presented as mean± standard error of mean (SEM) and analyzed using the Student's t-test. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Epididymal seminal fluid analyses

The epididymal seminal pH in the PPO-fed rats (6.00 ± 0.00) was significantly lower ($P < 0.01$) than that of the control (6.62 ± 0.18) as in table 1. A significant reduction ($P < 0.01$) in sperm motility (%) was observed in PPO-fed group (20.00 ± 15.8) compared with control (58.00 ± 7.65) as shown in table 1. The viability (%) of sperm cells was significantly reduced

($P < 0.01$) in the PPO-fed group (80.00 ± 1.58) compared with control (41.00 ± 7.65) as in table 1. Sperm count (x1 million/ml) was significantly lower ($P < 0.001$) in the PPO-fed rats (1.60 ± 0.00) compared with control (5.78 ± 0.24) as in figure 1. The percentage of sperms with total morphological defects was significantly higher ($P < 0.001$) in the PPO-fed group (25 ± 2.27) compared with control (14.00 ± 1.87) as in table 2. Similarly, control rats significant increase in sperm cells with head defects ($P < 0.001$) was obtained in the PPO-fed group (8.00 ± 0.32) compared with (4.40 ± 0.40). The percentage of sperm cells with tail defects was significantly higher ($P < 0.01$) in the PPO-fed group (25.00 ± 2.74) compared with control (14.00 ± 1.87) as in table 2.

Serum hormones

Serum testosterone level (ng/ml) was significantly lower ($P < 0.001$) in PPO-fed (3.06 ± 0.05) compared with control (5.04 ± 0.11) rats as in figure 2. Serum level of luteinizing hormone ($\mu\text{l/ml}$) in the PPO-fed rats (2.62 ± 0.12) was significantly lower ($P < 0.001$) compared with that of control group (4.42 ± 0.10) as in figure 3. Serum concentration of follicle stimulating hormone (ng/ml) was significantly lower ($P < 0.01$) in the PPO-fed (11.20 ± 0.22) compared with control (12.28 ± 0.15) rats as in figure 4.

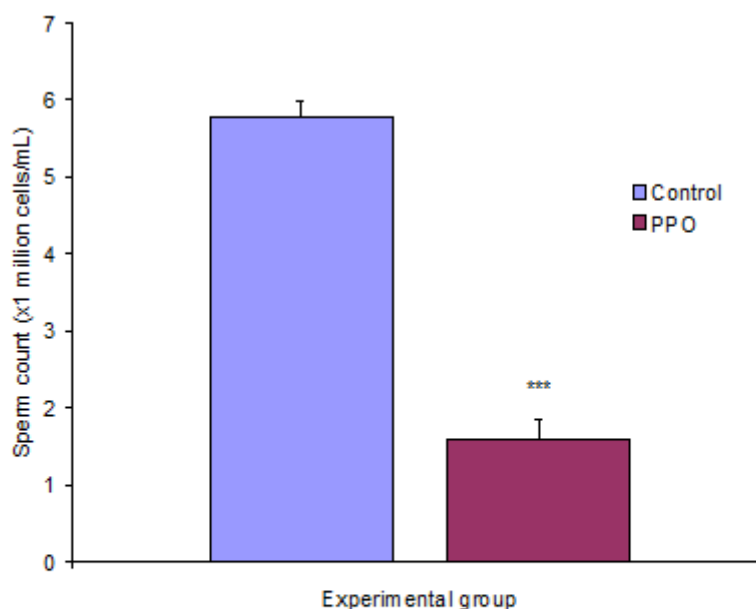


FIG. 1: Comparison of sperm count in control and photoxidized palm oil fed male rats.

Values are expressed as mean ± SEM, n = 5.
 *** = significantly different from control at $p < 0.001$

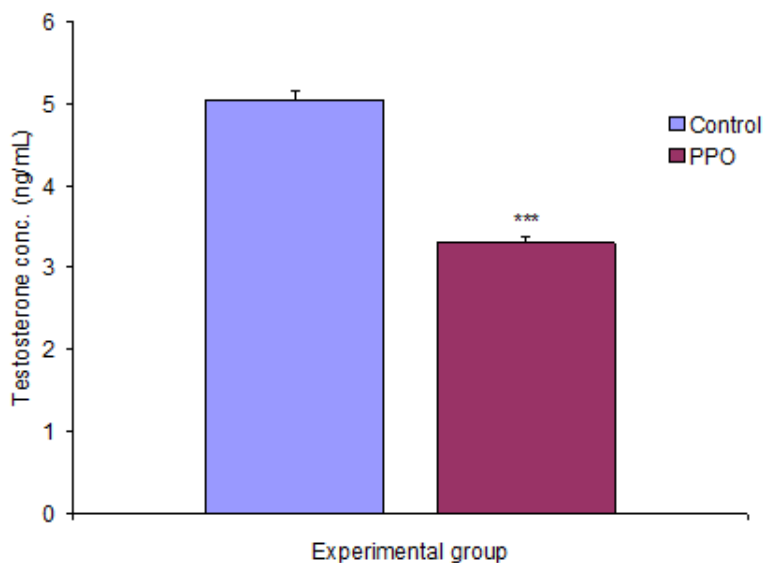


FIG. 2: Comparison of testosterone level in control and photoxidized palm oil fed male rats.

Values are expressed as mean \pm SEM, n = 5.
*** = significantly different from control at $p < 0.001$

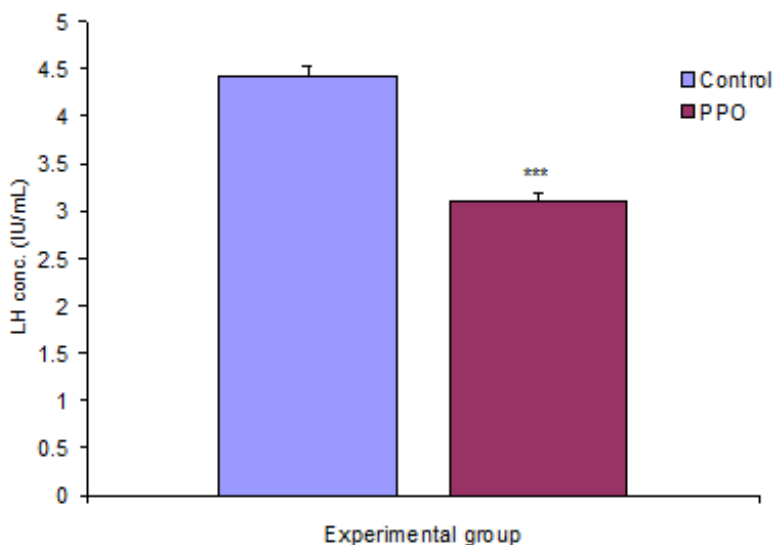


FIG. 3: Comparison of luteinizing hormone level in control and photoxidized palm oil fed male rats.

Values are expressed as mean \pm SEM, n = 5.
*** = significantly different from control at $p < 0.001$

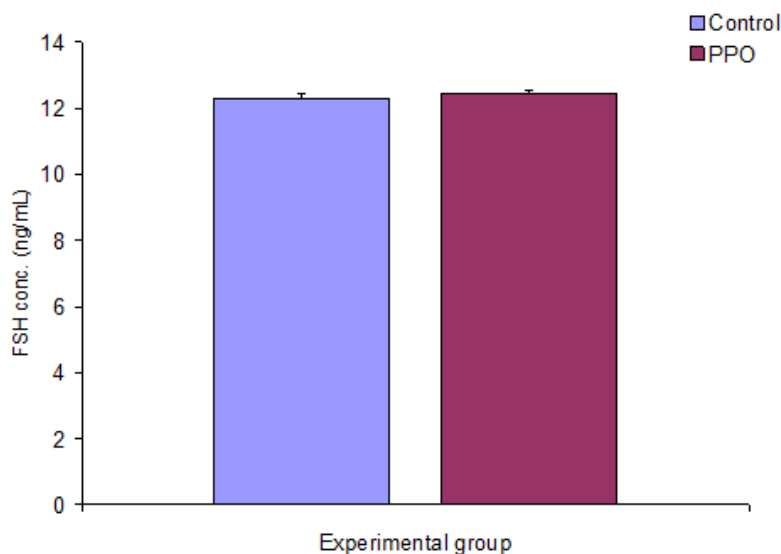


FIG. 4: Comparison of follicle stimulating hormone level in control and photoxidized palm oil fed male rats.

Values are expressed as mean ±SEM, n = 5.
No significant difference among groups

Table-1: Sperm pH, motility and viability in control and photoxidized palm oil fed rats

	pH	Motility	RPFM	SPFM	RSM	NMS
Control	6.62	59.00	7.00	50.00	5.75	41.00
	±0.14	±7.65	±1.22	±6.12	±1.34	±7.65
PPO	6.00	20.00	0.00	15.00	1.00	80.00
	±0.00**	±1.58**	±0.00**	±1.58**	±1.00*	±1.58**

Values are expressed as mean ±SEM, n = 5.

* = significantly different from control at p<0.05

** = significantly different from control at p<0.01

RPFM = Rapid progressive forward movement

SPFM = Slow progressive forward movement

RSM = Residual sperm movement

NMS = Non-motile sperm

Table-2: Sperm morphology in control and photoxidized palm oil fed rats

	Total defect	Head defect	Middle piece defect	Tail defect
Control	14.00	4.40	2.50	7.60
	±1.87	±0.40	±0.26	±1.21
PPO	25.00	8.00	2.00	15.00
	±2.74**	±0.32**	±0.00	±1.58**

Values are expressed as mean ±SEM, n = 5.

** = significantly different from control at p<0.01

*** = significantly different from control at p<0.001

DISCUSSION

Epididymal semen is slightly acidic [29]. The observed increase in acidity (lower pH) in the PPO-fed rats could be due to increased peroxidation in tissues or from enhanced local epididymal/testicular peroxidation with increase in the formation of acids [9]. It is well established that degree of tissue peroxidation varies from one tissue to another [30]. The testis is rich in polyenoic fatty acids that are prone to oxidative

decomposition with formation of acids [16]. This reduced pH could equally have been due to the decrease in serum testosterone, which also modulates seminal pH and osmolarity. The slightly acidic pH of the epididymal fluid of the control agrees with the finding of Pastor-Soleret *et al.*, [29]. A normal pH is essential for optimum function of sperms.

Sperm motility is an important factor in assessing sperm viability. The observed increase to the percentage of non-motile sperms (viability) and the decrease in sperm motility in the PPO-fed rats could be due to the reduced pH in the epididymal semen [31, 32] or possible abnormality in sperm DNA methylation following peroxidation. Abnormalities in sperm DNA methylation which results in DNA damage is known to affect sperm motility [33]. The reduced sperm motility in PPO-fed rats compared to control could also have been due to the increase in total morphological defects as well as increase in defective heads and tails of sperms in the PPO-fed rats. Returbation in the microenvironment surrounding the sperm cell also affects its motility or function [29].

Semen quality is greatly influenced by the morphology of sperms and hence the probability for fertilization [34]. The observed increase in the number of sperms with morphological defects may be due to sperm DNA damage by oxidative processes [33]. Thermally oxidized fats cause morphological and functional changes in testes of rats [35]. Lipid peroxidation which the testis is already prone to because of its high content of polyenoic fatty acids, Roseblum *et al.*, [16] liberates DNA-damaging substances Lombardo *et al.*, [33] which could explain the increased defects in the PPO-fed rats. The reason for the differential affectation of heads and tails in the PPO-fed group compared with control is not clear.

Sperm count is affected by several factors including infection, increased intrascrotal temperature, irradiation, tubular defects, chromosomal abnormalities and hormonal deficiencies [36, 37]. The significant decrease in sperm count in the PPO-fed rats in this study could have resulted from primary testicular toxicity as well as sperm DNA damage by products of lipid peroxidation [33]. The testis is susceptible to lipid peroxidation due to its high content of polyenoic fatty acids [38]. The low concentration of serum testosterone in the PPO-fed rats could also have been responsible for the reduced sperm count in this group. Testosterone is an essential hormone in spermatogenesis [39]. The decrease might have also been secondary to the lower serum luteinizing hormone in the PPO-fed rats. Luteinizing hormone stimulates Leydig cells to synthesize testosterone, deficiency of which will lead to a low sperm count [40].

Testosterone is synthesized mainly from cholesterol by Leydig cells of testes and also from acetate [41]. A little amount of it is produced by the adrenal glands. The decrease in serum testosterone in the PPO-fed group observed in our work strongly suggests testicular toxicity with loss or deficiency of Leydig cell function. Krivenkova and Treschuk [35] had pointed out the possible testicular toxicity induced by inclusion of oxidized (thermally) fats in the diets of rats. Deficient gonadotropin release from the pituitary

as shown by the lower serum concentrations of FSH and LH, essential hormones in Leydig cell function could equally have been responsible for the low testosterone level in the PPO-fed group.

Low serum LH concentrations can be due to testicular, pituitary or hypothalamic abnormalities [42]. The significant decrease in serum luteinizing hormone level in the PPO-fed rats may suggest a PPO-induced pituitary toxicity, LH submit mutation, or the result of failure of release of gonadotropin-releasing hormone from hypothalamns [43]. Luteinizing hormone is a glycoprotein secreted by some gonadotropes in the anterior pituitary under the influence of hypothalamic gonadotropin-releasing hormone [41]. This lower level of LH could not have been the result of its regulation by a negative feedback from serum testosterone since testosterone level is even low [40]. LH stimulates Leydig cells to convert cholesterol to testosterone [40]. This lower LH level might have been responsible for the lower serum testosterone concentration observed in the PPO-fed group. LH stimulates testosterone secretion by Leydig cells through regulating of expression of 17 β hydroxi-steroid dehydrogenase, the enzyme required to convert androstenodione to testosterone [39].

Follicle stimulating hormone is secreted by gonadotropes in anterior pituitary under the effect of hypothalamic gonadotropin-releasing hormone [40]. The observed decrease in the serum level of this hormone in the PPO-fed rats suggests a toxic effect on the gonadotropes by photoxidised palm oil and/or probably defective gonadotropin-releasing hormone effect on anterior pituitary. FSH stimulates testicular growth and enhances production of androgen binding proteins by Serloli cells necessary for spermatogenesis [4]. The lower FSH level might have in part been responsible for the lower testosterone level in PPO-fed rats and not rather the reverse. In conclusion, long term consumption of photoxidised palm oil diet is associated with reproductive function impairment in male wistar rats. One would therefore recommend that long term consumption of PPO-should be done with caution. More so, effort should be made to develop means of protecting palm oil from the effect of photoxidation.

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