

## Comparative Effects of Long Term Consumption of Thermo- and Photo-Oxidised Palm Oil Diets on some Reproductive Parameters in Male Wistar Rats

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**Abstract:** Consumption of thermoxidised and photooxidised palm oil diets is known to be harmful to tissues in the body including the reproductive system. It is not known which of these two forms of the commonly consumed vegetable oils is less harmful to reproductive function. Fifteen male Wistar rats weighing 80-125g and aged 19 to 23 weeks were randomly divided into control, thermoxidised palm oil (TPO) and photooxidised palm oil (PPO) diets-fed groups of five rats per group. Control group was fed on plain rat feeds while the other two groups were fed on TPO diet or PPO diet accordingly for 13 weeks. Results demonstrated a significantly lower seminal pH in the PPO-fed than in the TPO-fed group ( $P<0.01$ ) and control ( $P<0.05$ ). Sperm motility was significantly reduced in the PPO-fed compared with TPO-fed groups ( $P<0.05$ ). The viability of sperms was significantly decreased in PPO-fed rats compared with the TPO-fed group ( $P<0.05$ ) and control ( $P<0.01$ ). Sperm count was significantly lower in PPO-fed compared with TPO-fed group ( $P<0.01$ ). Percentage of sperms with morphological defects was significantly higher in the PPO- than in the TPO-fed groups ( $P<0.01$ ). Serum testosterone concentration was significantly decreased in PPO-fed compared with TPO-fed group ( $P<0.001$ ). Serum luteinizing hormone (LH) level was significantly decreased ( $p<0.001$ ) in PPO-fed group compared with control and TPO-fed groups. Serum concentration of follicle stimulating hormone (FSH) was significantly decreased ( $p<0.001$ ) in PPO-fed group compared with control and TPO-fed groups. In conclusion, chronic consumption of PPO diet has greater deleterious effects on male reproductive function than TPO diet.

**Keywords:** Hormone, Palm oil, Photooxidation, Thermoxidation, Seminal pH, Sperm count, Motility, Viability, Morphology, Testosterone.

### INTRODUCTION

Palm oil is an edible vegetable oil produced from the mesocarp of the ripe palm fruit of the African oil palm tree (*Elaeis guineensis*). It is widely consumed in Nigeria and many parts of the tropics. In its raw form, palm oil contains 50% saturated, 40% unsaturated and 10% polyunsaturated fatty acids [1]. It is a rich source of natural antioxidants, vitamin E, vitamin C and carotenoids which give it its characteristic red colour [2]. Vitamin E, vitamin C and carotenoids are strong biological antioxidants. Palm oil also contains enzymes, proteins, sterols, phosphatides, chlorophyll, phospholipids, phenolic acid and flavonoids [3]. Consumption of fresh or red palm oil has been associated with antitumorigenic effect [4] as well as several other positive health benefits like lowering incidence of coronary heart diseases [5] and reduction in the effect of oxidative stress on rat spermatozoa [6]. Palm oil tocopherol-rich fraction has been used to attenuate testicular toxicity induced by fenitrothin [7]. Unfortunately, this oil is not consumed much in its raw form but rather in its thermoxidised and photooxidised forms [8] which are believed to be toxic to tissues.

Thermoxidation of lipids/oils (oxidation of lipids following application of heat) is known to have deleterious effects on the quality of oils [9] like induction of increases in free fatty acid content, peroxide value and acid value, decrease in iodine value and linoleic acid concentration as well as destruction of its rich antioxidants like  $\beta$ -carotenes, ascorbic acid and tocotrienol [10]. Like other forms of lipid oxidation, thermoxidation of palm oil occurs via free radical process and releasing volatile aldehydes, reactive oxygen species, free radicals and other substances that may adversely affect cellular function [11]. Long term consumption of thermoxidised palm oil diet has been associated with impairment of several organ functions including reduction in red blood cell count [12], peptic ulceration [13], increase in serum liver enzymes [14], reproductive dysfunction [15], gestational reabsorption syndrome in rats, discoloration of uteri, ovaries and testes of male rats [16] as well as delay in gestation and embryo fetal toxicity. Despite these observations, much of palm oil is still being consumed in the thermoxidised form for economic reasons and partly because it is said to improve the taste of food.

From the oil mills to shops, markets and even homes, palm oil is constantly exposed to light. Palm oil is stored and sold in the market in plastic containers under direct sunlight. Even in shops and homes it is stored unprotected from light. Exposure of palm oil to light in these manners allows the oil like any other lipid to undergo photooxidation [17]. This means that most palm oil consumed generally must have undergone some level of photooxidation, a process known to have adverse effect on the oil quality and also have negative effects on body tissues. During photooxidation of lipids/oil (oxidation of oils in the presence of light), single oxygen species react directly with the double bonds to produce both conjugated and unconjugated hydroperoxides [18]. Light is said to accelerate oxidation of oils with the production of reactive oxygen species or free radicals [19] which are capable of inducing oxidative stress; an important mediator of damage to cellular structures [20]. Consumption of thermoxidised and photooxidised forms of palm oil is a common practice in Nigeria and many tropical countries [8]. Consumption of the two forms of palm oil is known to have deleterious effects on health. But information on the safer of the two forms of palm oil is not found. A comparison of the effects of long term consumption of thermoxidised or photooxidised forms of palm oil on some reproductive parameters namely seminal pH, sperm count, motility, viability and morphology as well as serum concentrations of testosterone, follicle stimulating hormone and luteinizing hormone in male wistar rats was therefore carried out.

## MATERIALS AND METHODS

Fifteen male Wistar rats weighing 80 to 125g used for the experiment were housed at room temperature in the animal house of the Department of Physiology, University of Calabar, Calabar, Nigeria under a 12-hour light and 12-hour dark cycle. The Ethics Committee of the Faculty of Basic Medical Sciences, University of Calabar gave the ethical approval for the study.

### Experimental design

The animals were randomly divided into three groups of five rats per group. Group 1 served as the control, group 2, thermoxidised palm oil diet-fed group (TPO) while group 3 was photooxidised palm oil (PPO)-diet fed group. Group 1 was fed on only animals feed, group 2 received animal feed mixed with thermoxidised palm oil while group 3 was fed with photooxidised palm oil diet. Animals were fed for thirteen weeks (at which time rats were 19-23 weeks old) at the end of which they were euthanized and blood samples taken for assay of follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone. The testes were dissected out for seminal fluid analysis.

### Preparation of palm oil diets

Five litres of palm oil bought from Marian market, Calabar, Nigeria were used for the study. A

portion of the oil was thermally oxidized to yield thermoxidised palm oil (TPO) while the other portion was left in transparent plastic containers and permanently exposed to light (including sun light on sunny days) to form the photooxidised palm oil (PPO). Photooxidation of the oil was done in this manner to mimic the way palm oil is normally handled. Thermoxidation of oil was done as described by Isong [21]. Briefly, the process involved the oil undergoing four rounds of heating at about 150°C and allowing it to cool in between heating. The two forms of palm oil diets i.e. thermoxidised palm oil (TPO) and photooxidised palm oil (PPO) diets were prepared by mixing 85g of rat feeds with 15g of the respective oils as used by Obembe *et al.* [13].

### Determination of epididymal seminal fluid parameters

Seminal fluid parameters were evaluated according to World Health Organization (WHO) standard [22]. 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 2 minutes. A x 10 objective lens was used to focus the sample while a x 40 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 carbol fuchsin (1 in 20) and allowed to stain for 3 minutes. 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 for abnormalities was assessed.

### Determination of serum testosterone

Serum testosterone concentration was assayed using enzyme-immunoassay (EIA) technique with rats EIA kits (Immunometrics, London, UK) and reading the absorbance at 492-550nm wave length with a Jenway 6300 spectrophotometer [23].

### Determination of serum luteinizing hormone (LH)

Serum was assayed for LH by enzyme-immunoassay methods using rats EIA kit from Immunometrics, London, UK, Optical density was read at 492-550nm using a Jenway 6300 spectrophotometer [23].

### Determination of serum follicle stimulating hormone

Serum concentration of FSH was determined in triplicated samples by radio immunoassay (RIA) technique using rats FSH kits obtained from Biocode Company, Belgium, according to the protocol provided in the kit [24].

### Statistical analysis

Data were expressed as mean  $\pm$  SEM and analyzed by one way ANOVA followed with a post hoc test of least significant difference. P-values of  $p < 0.05$  were considered statistically significant.

## RESULTS AND DISCUSSION

### Comparison of Seminal Fluid Parameters in the Different Experimental Groups

#### Epididymal seminal pH

The mean pH values (%) in control, TPO- and PPO- fed rats were  $6.62 \pm 0.18$ ,  $6.74 \pm 0.24$  and  $6.00 \pm 0.00$  respectively showing a significant decrease in pH in the PPO-fed group compared with TPO fed group ( $P < 0.05$ ) and control ( $P < 0.01$ ) as shown in table 1.

#### Sperm motility

The mean values (%) of sperm motility in the control, TPO- and PPO-fed groups were  $59.00 \pm 7.65$ ,  $49.00 \pm 10.17$  and  $20.00 \pm 15.8$  respectively demonstrating a significant reduction in motility in PPO-fed group compared with control ( $P < 0.01$ ) and TPO- fed group ( $P < 0.05$ ) as shown in table-1. In the PPO fed-group, there was significant reduction in spermatozoa with rapid progressive forward movement (RPFM) ( $p < 0.01$ ), slow progressive forward movement (SPFM) ( $p < 0.01$ ) and residual movement (RSM) ( $p < 0.05$ ) compared with control. There was significant increase in non-motile spermatozoa (NMS) in the PPO group compared with control ( $p < 0.01$ ) and TPO-fed ( $p < 0.05$ ) groups. In the PPO-fed group, spermatozoa with RPFM ( $p < 0.01$ ) and RSM ( $p < 0.01$ ) were significantly decreased compared with the TPO-fed group (Table-1).

#### Sperm count

The mean values of this index (x1 million/ml) for control, TPO-fed and PPO-fed groups were  $5.78 \pm 0.24$ ,  $3.18 \pm 0.24$  and  $1.60 \pm 0.00$  respectively. This showed a significantly ( $p < 0.001$ ) reduced sperm count in both PPO- and TPO-fed groups compared with control. Sperm count was significantly ( $p < 0.01$ ) decreased in the PPO-fed group compared with the TPO-fed group (Table-1).

#### Sperm viability

The mean percentages of non-motile (non-viable) sperms in the control, TPO-fed and PPO fed groups were  $41.00 \pm 7.65$ ,  $51.00 \pm 10.17$  and  $80.00 \pm 1.58$  respectively. This showed a significant increase of this index in both PPO-fed and TPO-fed rats but with a more significantly increased percentage of non-motile sperms in the PPO-fed compared with the TPO-fed group ( $p < 0.05$ ) as in table 1.

#### Percentage of sperms with morphological defects

The percentages of sperms with total morphological defects in control, TPO- and PPO-fed groups were  $14.00 \pm 1.87$ ,  $11.75 \pm 1.78$  and  $25 \pm 2.74$  respectively showing a significant increase in this parameter in the PPO-fed group compared with TPO-fed group ( $P < 0.01$ ) and also with control ( $P < 0.05$ ) (Table-2). In the PPO-fed group, there was significant increase in spermatozoa with head and tail defects compared with control ( $p < 0.01$ ) and FPO-fed ( $p < 0.05$ ;  $p < 0.001$ ) groups.

### Comparison of Serum Reproductive Hormones Concentrations in the Different Experimental Groups

#### Testosterone

Serum testosterone concentrations (ng/ml) in control, TPO- and PPO-fed groups were  $5.04 \pm 0.11$ ,  $3.76 \pm 0.09$  and  $3.06 \pm 0.05$  respectively being significantly reduced in the TPO-fed and PPO-fed groups compared with control ( $P < 0.001$  each) but also significantly decreased in the PPO-fed group compared with the TPO-fed group ( $P < 0.001$ ) (Figure-1).

#### Luteinizing hormone (LH)

The serum levels of luteinizing hormone (IU/mL) in control, TPO- and PPO- fed groups were  $4.42 \pm 0.10$ ,  $3.32 \pm 0.06$  and  $2.62 \pm 0.12$  respectively. The levels were significantly lower in both TPO-fed ( $P < 0.001$ ) and PPO-fed ( $P < 0.001$ ) groups compared with control. Serum LH level was also significantly reduced in the PPO-fed group compared with the TPO-fed group ( $P < 0.001$ ) (Figure-2).

#### Follicle stimulating hormone (FSH)

The serum levels of FSH (ng/ml) in control, TPO- and PPO- fed groups were  $12.28 \pm 0.15$ ,  $12.20 \pm 0.11$  and  $12.30 \pm 0.22$  respectively. There was no significant difference in serum FSH level between the

TPO and control groups. However, serum FSH level was significantly decreased ( $p < 0.001$ ) in the PPO-fed group compared with control and TPO-fed groups (figure-3).

**Table-1: Comparison of Sperm pH, Motility, Count, RPFM, SPFM, RSM and Viability in Control, TPO - and PPO-fed groups**

	pH	Motility	Count	RPFM	SPFM	RSM	NMS
<b>Control</b>	6.62±0.14	59.00±7.65	5.78±0.24	7.00±1.22	50.00±6.12	5.75±1.34	41.00±7.65
<b>TPO</b>	6.74±0.24	49.00±10.17	3.18±0.24 <sup>***</sup>	10.00±1.58	34.00±7.81	9.25±1.34	51.00±10.17
<b>PPO</b>	6.00±0.00 <sup>**</sup> , a	20.00±1.58 <sup>**</sup> , a	1.60±0.00 <sup>***</sup> , b	0.00±0.00 <sup>**</sup> , b	15.00±1.58 <sup>*</sup> , *	1.00±1.00 <sup>*</sup> , b	80.00±1.58 <sup>**</sup> , a

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

\*, \*\* and \*\*\*=significantly different from control at  $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$  respectively

a = significantly different from TPO at  $p < 0.05$

b = significantly different from TPO at  $p < 0.01$

RPFM = Rapid progressive forward movement

SPFM = Slow progressive forward movement

RSM = Residual sperm movement

NMS = Non-motile sperm

**Table-2: Comparison of Sperm Morphology in the Control, TPO - and PPO-fed groups**

	Total defect	Head defect	Middle piece defect	Tail defect
<b>Control</b>	14.00	4.40	2.50	7.60
	±1.87	±0.40	±0.26	±1.21
<b>TPO</b>	11.75	5.50	2.00	4.75
	±1.18	±0.65	±0.58	±1.18
<b>PPO</b>	25.00	8.00	2.00	15.00
	±2.74 <sup>**</sup> , <sup>b</sup>	±0.32 <sup>**</sup> , <sup>a</sup>	±0.00	±1.58 <sup>**</sup> , <sup>c</sup>

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

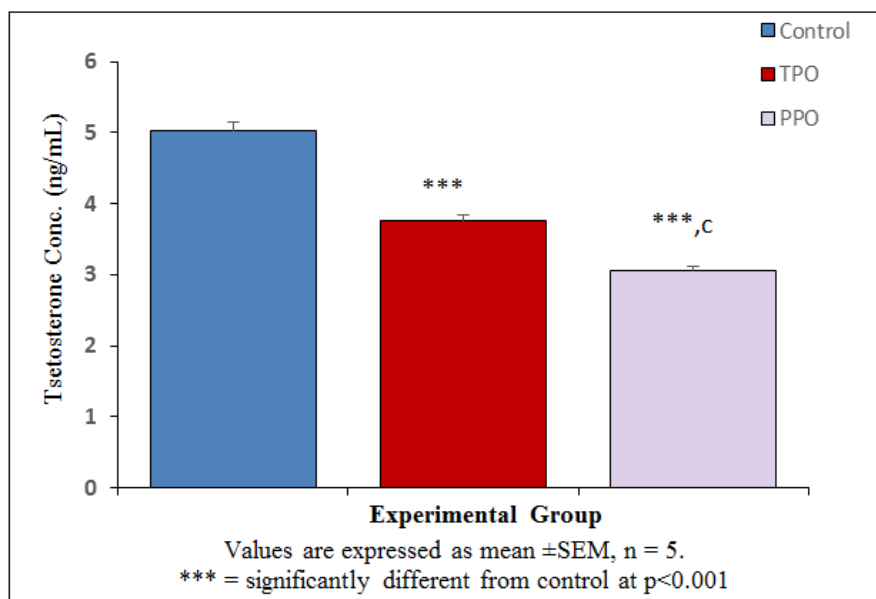
\*=significantly different from control at  $p < 0.05$

\*\* = significantly different from control at  $p < 0.01$

a = significantly different from TPO at  $p < 0.05$

b = significantly different from TPO at  $p < 0.01$

c = significantly different from TPO at  $p < 0.001$



**Fig-1: Comparison of testosterone level in control, thermoxidised and photoxidised palm oil fed male rats**

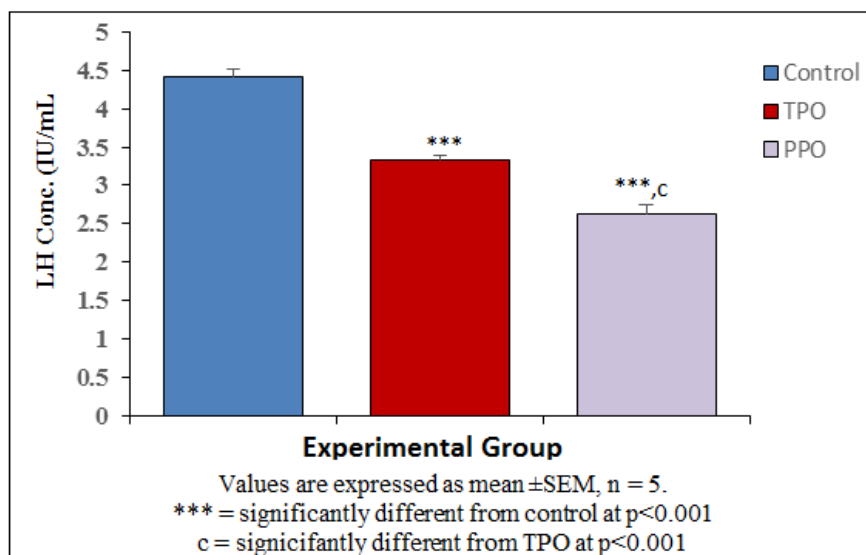


Fig-2: Comparison of Luteinizing hormone level in control, thermoxidised and photoxidised palm oil fed male rats

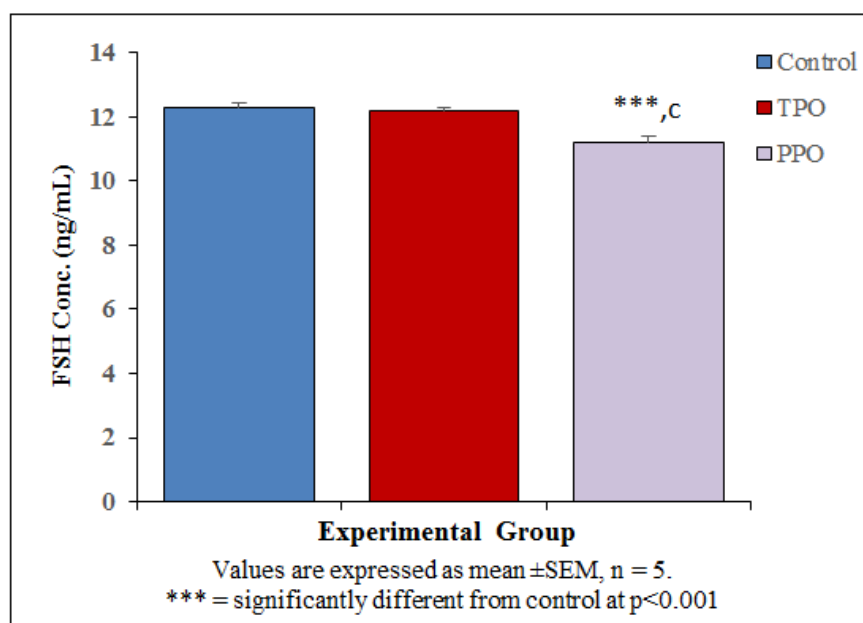


Fig-3: Comparison of follicle stimulating hormone level in control, thermoxidised and photoxidised palm oil fed male rats

Palm oil is a widely consumed vegetable oil, most of which is consumed in the thermo- or photo-oxidised forms [8]. Photooxidation and thermoxidation of palm oil are known to negatively alter the physicochemical properties of palm oil and lead to production of aldehydes, reactive oxygen species, peroxides and free radicals which may be injurious to tissues when consumed [25]. The results from this research showed significant derangement in reproductive parameters in both TPO- and PPO-fed rats compared to their control, but which was worse in the PPO-fed group compared with the TPO-fed rats.

A normal seminal fluid pH is an important factor in maturation and optimal functioning of

spermatozoa. Epididymal seminal pH is weakly acidic [26]. The epididymal seminal pH was observed to be significantly lower in the PPO-fed than in the TPO-fed rats. This difference might have been due to the greater reduction in serum testosterone observed in the PPO-fed group. Testosterone helps in the control of seminal pH and osmolarity [27]. It might have also reflected a differential higher peroxidation in the testis with increased production of acids [28]. There was a significant reduction in sperm motility in the PPO-fed group compared with the TPO-fed rats. Sperm motility is an important factor in normal sperm physiology. Factors which impair sperm motility include changes in seminal fluid pH and osmolarity [29], morphological defects in sperms as well as temperature changes [30].

The significant decrease in sperm motility in the PPO-fed compared to the TPO-fed rats could have been due to the greater reduction in seminal pH and high percentage of morphologically defective sperm cells in the PPO-fed group compared with the TPO-fed group. Reduction in seminal pH adversely affects sperm motility [31]. The motility of sperms is also adversely affected by morphological defects in sperm cells [32].

Viability of sperms (Percentage of non-motile sperms) was significantly higher in the PPO-fed than in the TPO-fed animals. Thermoxidation [11] and photooxidation [19] result in the formation of aldehydes, reactive oxygen species and free radicals which are capable of inducing oxidative stress, an important mediator of cellular damage [20]. Peroxidation of oils/lipid causes abnormalities of sperm DNA methylation. Oxidative stress is also known to cause sperm DNA damage [32]. The higher percentage of non-motile (dead) sperms in the PPO-fed group could have been due to higher DNA damage in these spermatozoa coupled with the altered seminal pH both of which may lead to reduced life span of the sperms [26, 29].

Our results showed a significant increase in total percentage of sperms with morphological defects in PPO-fed rats than in the TPO-fed group. The difference in percentage of structural abnormalities could have been the result of a greater PPO-induced DNA damage in the PPO-fed rats [32]. Lipid peroxidation causes DNA damage which may manifest as morphological defects in cells [33].

The significantly lower sperm count in the PPO-fed rats compared with the TPO-fed group could among other factors be due to the much reduced concentration of serum testosterone, [33], the reduced serum follicle stimulating hormone concentration [34] or the much lowered level of serum luteinizing hormone [27] in the PPO-fed rats observed in this study. Testosterone is essential for spermatogenesis especially for the growth and division of testicular germinal cells as well as regulation of seminal fluid osmolarity and pH [27]. Follicle stimulating hormone enhances production of androgen-binding protein by Sertoli cells and necessary for concentration of testosterone near sperm cells [35]. Luteinizing hormone modulates Leydig cell function to produce testosterone, an essential hormone in spermatogenesis. The greater reduction in sperm count in the PPO-fed rats could also have been due to a greater primary testicular damage by PPO. Reactive oxygen species, free radicals and other oxidative metabolites released in the processes of photooxidation and thermoxidation may cause tissue damage [20]. Consumption of thermally oxidized sun flower oil is known to increase the susceptibility of tissues to lipid oxidation [36] while testicular peroxidation reduces sperm quality [37].

The significant decrease in serum testosterone that was observed in the PPO-fed compared with the TPO-fed male rats could have resulted from a greater PPO-induced primary testicular toxicity in the PPO-fed group. Consumption of oxidized oils/fats is known to cause toxicity in several tissues including the testis [36]. Testosterone is synthesized by Leydig cells in the testis under the influence of LH. Testicular toxicity therefore is associated with a low serum testosterone level [27, 38]. It could also have resulted from the much greater decline in serum concentration of LH which normally stimulates Leydig cells to secrete testosterone [27].

Follicle stimulating hormone (FSH) is produced by gonadotropes in the anterior pituitary gland under stimulation by gonadotropin-releasing hormone and stimulates testicular growth and secretion of androgen binding hormone by Sertoli cells [39]. The significant decrease in serum concentration of follicle stimulating hormone in the PPO-fed compared to TPO-fed rats suggests a greater impairment of its secretion by PPO diet or failure of secretion of adequate gonadotropin releasing hormone [27]. This lower level of FSH could not have been due to the already lowered serum testosterone in the PPO-fed rats. High level of testosterone normally inhibits FSH secretion by a negative feedback [40].

Our findings demonstrated a significantly lower serum concentration of luteinizing hormone (LH) in the PPO-fed than in the TPO-fed rats. This difference could have been due to a possible greater impairment of gonadotropic function from primary pituitary toxicity or failure of gonadotropin releasing hormone secretion. It could not have been due to negative feedback from testosterone which was already very low [27]. Consumption of oxidized oils have been shown to cause functional impairment in many tissues [12, 13, 15, 41]. So, probably a similar primary effect on the gonadotropes might have occurred since the products of oil oxidation like aldehydes, peroxides, reactive oxygen species and free radicals have been demonstrated to cause varying tissues damage [11, 19, 20].

## **CONCLUSION**

In conclusion, long term consumption of thermoxidised or photoxidised palm oil diets causes impairment of male reproductive function with the impairment being worse with photoxidised palm oil consumption. This therefore suggests that photoxidised palm oil may be more cytotoxic than thermoxidised palm oil resulting in a greater impairment of reproductive function in male Wistar rats.

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