ejpmr, 2021,8(4), 11-18

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

THERMOXIDIZED AND PHOTOXIDIZED PALM OIL-INDUCED OXIDATIVE STRESS: EFFECTS ON SOME REPRODUCTIVE PARAMETERS IN FEMALE WISTAR RATS

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Article Received on 25/01/2021 **Article Revised on 14/02/2021** **Article Accepted on 06/03/2021**

ABSTRACT

Thermoxidized palm oil (TPO) and photoxidized palm oil (PPO) have been shown to impact negatively on reproductive function in male Wistar rats. The current studies evaluated effects of these oils on some female reproductive parameters and oxidative status considering variations in hormonal and reproductive functions. Fifteen female Wistar rats weighing 80 - 127 g were divided into 3 groups of 5 rats each. Groups 1 (control), 2 and 3 were fed liberally on plain rat's feeds, TPO and PPO diets respectively for thirteen weeks. Rats being 19-22 weeks old, were anaesthetized and necessary samples collected for estimation of relevant parameters. Results showed significant reduction in utero-ovarian weight in PPO- ($P<0.001$) and TPO-fed ($P<0.001$) groups compared with control. Serum malondialdehyde (MDA) concentration was significantly increased in TPO- ($P<0.001$) and PPO-fed ($P<0.001$) groups compared with control and in PPO-fed compared with TPO-fed ($P<0.01$) groups. Serum catalase (CAT) concentration was significantly reduced in TPO- ($P<0.001$) and PPO-fed ($P<0.001$) groups compared with control and in PPO- than TPO-fed ($P<0.01$) groups. Serum glutathione peroxidase (GPx) concentration was significantly lower in TPO- ($P<0.001$) and PPO-fed ($P<0.001$) groups compared with control and in PPO- compared with TPO-fed groups ($P<0.01$). Serum progesterone was significantly reduced in TPO- ($P<0.001$) and PPO-fed ($P<0.001$) groups compared with control. Serum estrogen was significantly higher in TPO- ($P<0.001$) and PPO- fed ($P<0.01$) rats compared with control but significantly lower in PPO-fed than TPO-fed ($P<0.05$) groups. We conclude that TPO and PPO induce oxidative stress and alterations in some reproductive parameters in female Wistar rats.

KEYWORDS: Thermoxidized, photoxidized, palm oil, oxidative stress, reproductive, parameters.

INTRODUCTION

Palm oil, a common vegetable oil is derived from the mesocarp of the ripe fruits of the oil palm tree, primarily *Elaeis guineensis*. The oil is consumed in large quantities in Nigeria and many tropical and subtropical countries not only due to its nutritional value but also its low cost and oxidative stability.[1] In its fresh form, the oil which is red in color is rich in tocopherols, tocotrienols, carotenoids, sterols, flavonoids, phenolic acids and coenzyme Q.[1,2]

Unfortunately, most of the palm oil is not consumed in its raw or fresh form. It is consumed mainly in the thermoxidized form. Thermoxidation of the oil is believed to improve the flavor and taste of the oil. The oil is also often subjected to repeated cycles of heating for economic reasons in the food frying industries of local cuisines.[3] Thermoxidation of the oil occurs following application of heat or allowing it to undergo cycles of repeated heating. It is a common practice in these regions to allow palm oil after extraction permanently exposed to light in shops, market places and even homes as there are no standard methods of shielding it from possible effect of light. Exposure of palm oil to light predisposes it to photoxidation.[4]

Oxidation of palm oil whether by thermoxidation or photoxidation alters its physicochemical properties[5] and results in the formation of reactive oxygen species like peroxides, hydroperoxides, aldehydes, hydroxyl radicals, peroxyl radicals and other free radicals.[6,7] On ingestion of the oil the free radicals and other reactive oxygen species initiate lipid peroxidation which is a chain reaction to produce more aldehydes, reactive oxygen species and other free radicals which again produce cycles of peroxidation.[8] These products of peroxidation process or reactive oxygen species are said to act as oxidants and may cause damage to cellular structures.[9,10] Increased production of reactive oxygen species above physiological limits may lead to oxidative stress and consequent damage to organelles, cell membrane, proteins, DNA etc.[11,12]

Oxidative stress is a disturbance in the balance between production and accumulation of reactive oxygen species (ROS) or oxidants and antioxidant defenses.[13] Cells

deploy a wide range of antioxidant defense systems utilizing enzymes (glutathione peroxidase, catalase, superoxide dismutase), metals and biomolecules to protect themselves from ROS-induced cellular damage. Oxidative stress is been implicated in the pathogenesis of several diseases including cardio-vascular diseases,[14] cancers,[15] neurological diseases,[16] respiratory diseases,[17] diabetes mellitus[13] etc.

There are some anatomical, functional and biochemical differences between males and females which may determine their responses to foods and drugs. Estrogen which is predominantly found in females can act as a free radical scavenger due to its phenol ring and so may reduce oxidative damage to molecules[18] while testosterone has a pro-oxidant effect.[19] Hormone replacement therapy is reported to reduce DNA lipid oxidation.[20] It is also shown that there is a differential response or susceptibility of tissues to lipid peroxidation and oxidative stress.[21]

Previous studies have linked consumption of TPO diet with development of anemia,[22] hepatotoxicity,[23] distortion of villi morphology,[24] increased gastric acid secretion as well as reproductive toxicity[3] in male rat models. Also, photoxidized palm oil diet has been demonstrated to impair male reproductive function.[25] Systemic expression of oxidative stress markers was reported in male rats following ingestion of photoxidized or thermoxdized palm oil diets.[26] However, information on the effects of these diets on oxidative stress and reproductive parameters in female rats is lacking considering hormonal and structural differences between males and females, and hence this study.

MATERIALS AND METHODS

Experimental animals and protocol

Fifteen female Wistar rats weighing 80 – 127 g were used for the study. The rats were kept in metallic cages at room temperature and standard laboratory conditions under 12 hour light and 12 hour dark cycles. Experimental feeding commenced after one week of adaptation. The rats were divided into 3 groups of 5 rats each. Group 1 was used as control and fed with plain rat chow only. Group 2 was fed with TPO diet while group 3 was fed with PPO diet. All animals had daily free access to their respective diets and potable water. At the end of the feeding period i.e. thirteen weeks, when rats were 19 - 20 weeks old, their estrous phases were determined and staggered sacrifices conducted during the estrous phase of each rat. Their blood samples were collected via cardiac puncture into labelled plain sample bottles for determination of serum parameters while uteri and ovaries were dissected out and weighed.

Preparation of thermo- and photoxidized palm oil diets

Thermoxidation of palm oil was done as described by Isong[27] and used by Ani et al[22] In brief, the oil was heated in stainless steel pot over a heating mantle at about 150^0C. The heating was done for five times, the oil being allowed to cool in between heating sessions. TPO diet was prepared by mixing 15 g of TPO with 85 g of rat feed. Another portion of the purchased oil was left in transparent plastic bottles exposed permanently to light including sunlight on sunny days to produce photoxidized palm oil (PPO). The PPO diet was prepared by also mixing 15 g of PPO with 85 g of rat feed.

Determination of estrous phase of the rat's estrous cycle

This was done described by Marcondes et al,[28] In brief, using a plastic pipette with its tip in the rat's vagina, 0.05 ml of 0.9% normal saline was released into the vagina and aspirated back into the pipette. A drop of the fluid so aspirated was placed on labelled glass slides and viewed under light microscope (Olympus) with x10 and x40 objective lenses. Three types of cells could be identified namely round nucleated cells (epithelial cells), irregularly shaped anucleated cells (cornified epithelial cells) and little roundish cells (leucocytes). The proportion among the three types of cells in a smear was used to decide the phase of the estrous cycle. The estrous phase consisted overwhelmingly of large irregularly shaped anucleated cells. Rats were sacrificed in a staggered manner at estrous phase of their estrous cycle because of uterine, ovarian and hormonal changes in the different phases of the cycle. Microscopy was done under low illumination without condenser to ensure a good contrast between 9 am – 12 noon each day.

Determination of utero-ovarian weights

Following euthanization of the rats, each rat in supine position was opened up via a midline incision. The uteri and ovaries were identified and dissected out completely. They were then cleared of adherent areolar tissues and weighed (g) using an electronic weighing scale.

Estimation of serum progesterone concentration (mg/mL)

This was estimated using ELISA method with Progesterone Kits (Cayman Chemicals, USA) and following manufacturer's protocol.

Evaluation of serum estrogen (ng/mL)

This was evaluated using ELISA method with estrogen EIA antisera (Cayman Chemicals, USA) and following protocol in the accompanying manual.

Estimation of serum malondialdehyde concentration

This was done using colorimetric (Model 260, Sherwood, USA) method described by Buege and Aust.[29] It is based on the principles that lipid peroxidation forms MDA as one of its natural bye-products which reacts with thiobarbituric acid (TBA) to generate MDA-TBA adduct which is quantified colorimetrically at 532 nm.[30]

Determination of glutathione peroxidase (GPx) concentration

This test was done according to the method described by Rotruck et al.[31] It is based on the principles that glutathione peroxidase reduces cumene hydroperoxide while oxidizing reduced glutathione to oxidized glutathione and the generated oxidized glutathione reduced to glutathione with consumption of NADPH, the decrease of which can be easily measured colorimetrically as a function of glutathione peroxidase activity.[32]

Estimation of serum catalase concentration (IU/ml)

This was assayed by colorimetry as described by Aebi[33] using commercially available reagents. The optical density was read against the blank at 570 nm using a Mindray Chemistry Analyzer, B5-120 (China) and the catalase concentration calculated using standard formula.

RESULTS

Utero-ovarian weights (g) in control, TPO- and PPO-fed groups

The weights for control, TPO-fed and PPO-fed groups were 0.78 ± 0.04, 0.30 ± 0.08 and 0.36 ± 0.07 respectively showing significantly reduced utero-ovarian weights in the TPO- and PPO- fed groups compared with control (P<0.001 in each case). This is shown in FIG 1.

MDA levels (nmol/mL) in control, TPO- and PPO-fed rats

The serum concentrations of MDA in control, TPO- and PPO-fed groups were 2.26 ± 0.15, 3.52 ± 0.06 and 4.40 ± 0.11 respectively with a significant elevation of MDA in the TPO-fed (P<0.01) and PPO-fed (P<0.001) groups compared with control and also significantly increased in PPO- compared with TPO-fed (P<0.001) groups as in FIG 2.

Concentration of glutathione peroxidase (mU/mg protein) in the experimental groups

The concentrations of this enzyme in control, TPO- and PPO-fed groups were 76.40 ± 0.34, 51.62 ± 0.69 and 41.24 ± 0.40 respectively. GPx was significantly decreased in TPO- (P<0.01) and PPO-fed (P<0.001) groups compared with control and also significantly reduced in the PPO-fed compared with the TPO-fed groups (P<0.05) as in FIG 3.

Comparison of serum catalase concentration (IU/ml) in the experimental groups

The mean values for control, TPO- and PPO-fed groups were 0.63 ± 0.01, 0.43 ± 0.02 and 0.34 ± 0.02 respectively. Catalase concentration was significantly reduced in the TPO- (P<0.001) and PPO-fed (P<0.001) groups compared with control and also lower in PPO- than in TPO-fed groups (P<0.01) as shown in FIG 4.

Serum progesterone concentration (ng/ml) in the various groups

Progesterone concentration in the control, TPO- and PPO-fed groups were 8.91 ± 0.06, 6.08 ± 0.06 and 5.03 ± 0.78 respectively. This showed a significant decrease in progesterone in the TPO-fed (P<0.01) and PPO-fed (P<0.001) groups compared with control as shown in FIG 5.

Comparison of serum estrogen concentration (µg/mL) in the various groups

Concentration of estrogen in control, TPO- and PPO- fed groups were 20.20 ± 0.48, 26.46 ± 0.60 and 23.80 ± 0.78 respectively. Estrogen concentration was significantly increased in the TPO-fed (P<0.001) and PPO-fed (P<0.01) groups compared with control and also significantly lower in the PPO-fed compared with the TPO-fed groups (P<0.05) as shown in FIG 6.

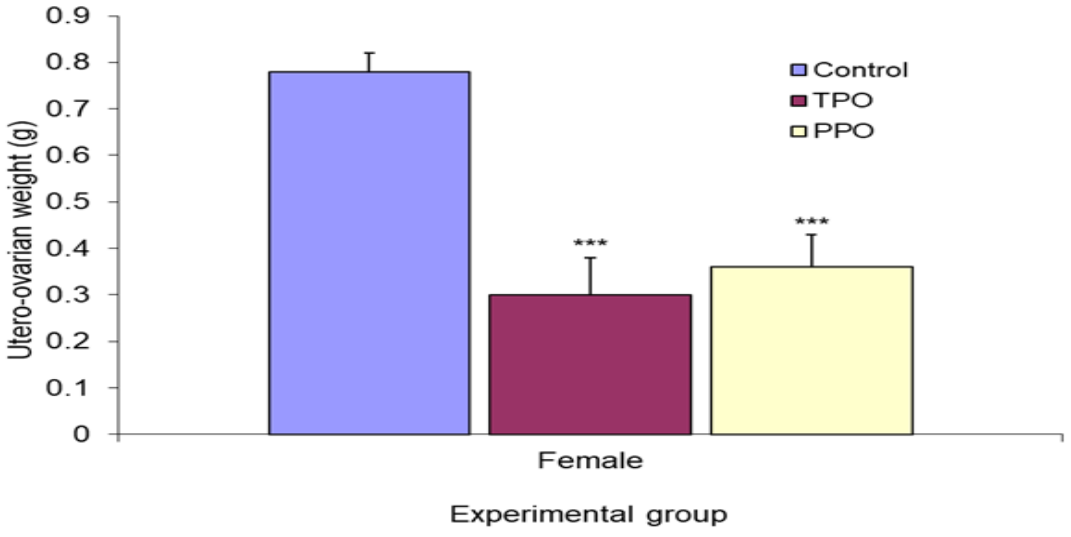

FIG. 1: Comparison of utero-ovarian weight of control thermoxidized and photoxidized palm oil-fed female rats.

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

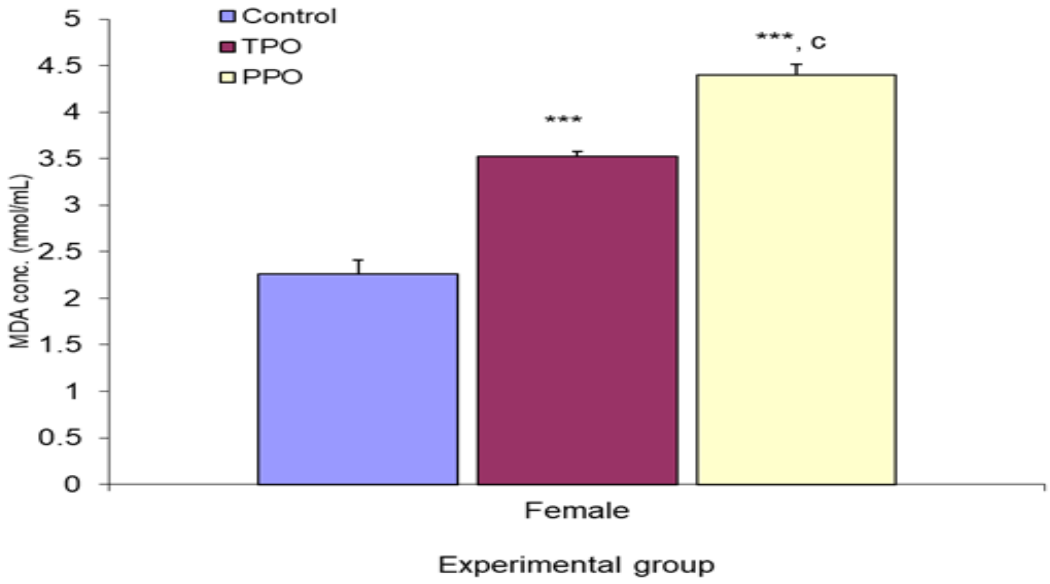

FIG. 2: Comparison of malondialdehyde level in control thermoxidized and photoxidized palm oil-fed female rats.

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

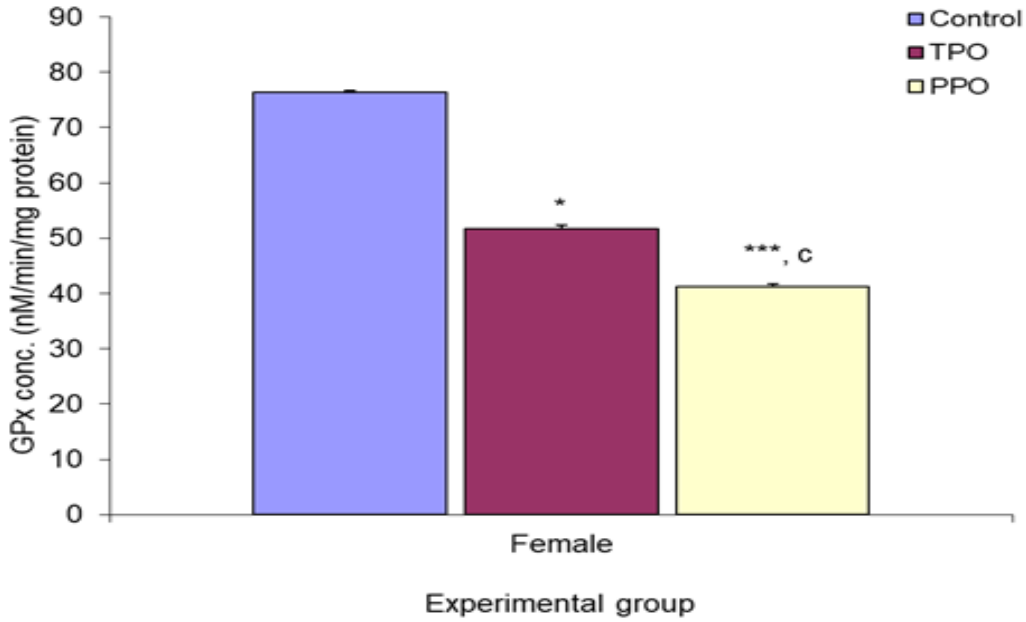

FIG. 3: Comparison of glutathione peroxidase concentration in control thermoxidized and photoxidized palm oil-fed female rats.

Values are expressed as mean ±SEM, n = 5.
*** = significantly different from control at p<0.001;
* = significantly different from control at p<0.01
c = significantly different from TPO at p<0.05

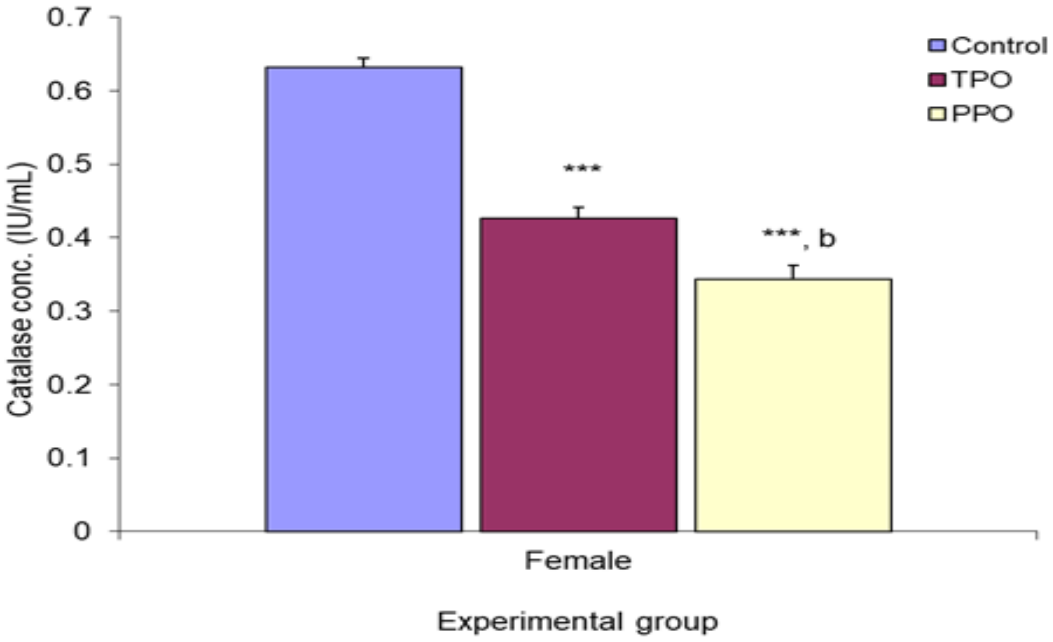

FIGURE 4: Comparison of catalase concentration in control thermoxidized and photoxidized palm oil-fed female rats.

Values are expressed as mean ±SEM, n = 5.
*** = significantly different from control at p<0.001;
b = significantly different from TPO at p<0.01

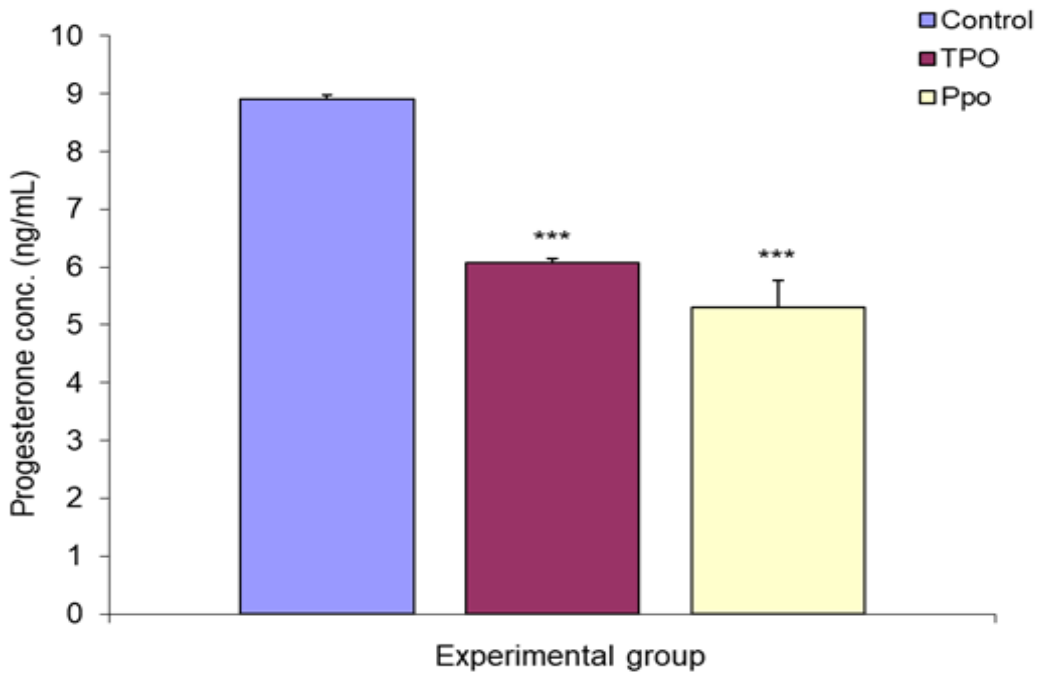

FIGURE 5: Comparison of progesterone level in control thermoxidized and photoxidized palm oil-fed female rats.

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

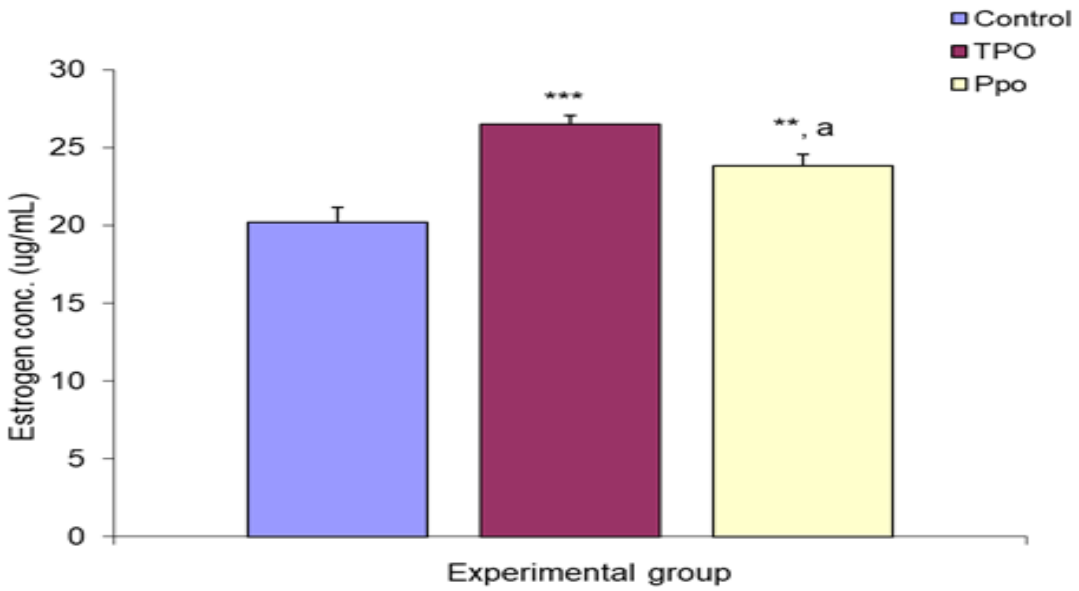

FIGURE 6: Comparison of estrogen level in control thermoxidized and photoxidized palm oil fed-female rats.

Values are expressed as mean ±SEM, n = 5.
*** = significantly different from control at p<0.001;
** = significantly different from control at p<0.01;
a = significantly different from TPO at p<0.05

DISCUSSION

The results from this study show that both PPO and TPO diets cause significant alterations in some reproductive and biochemical parameters in female rats.

Malondialdehyde is one of the final products of lipid peroxidation and as a result is used as a direct measure of assessing the degree of lipid peroxidation and oxidative stress.[34] The observed increase in serum concentration of MDA in both PPO and TPO-fed groups is similar to our earlier findings on male rats fed with these diets.[26] This indicates that these diets are associated with an increase in the level of lipid peroxidation in tissues. Concentration of MDA was significantly higher in the PPO-fed group than in the TPO-fed group, suggesting there might have been a greater peroxidation process in the PPO-fed than in TPO-fed rats.

Glutathione peroxidase which is a general name for an enzyme family with peroxidase activity is a naturally occurring antioxidant which prevents oxidative damage by reducing hydroperoxides which are products of peroxidation, to their corresponding alcohols and the hydrogen peroxides to water.[35] In high consumption peroxidative states, its concentration is therefore reduced and so is used as a marker of oxidative stress.[36] The reduction in its concentration noted in the PPO- and TPO-fed groups therefore points to the fact that these diets induce peroxidation with consequent consumption of the antioxidant. This result is similar to previous findings on male rats fed with these diets.[3] lending a voice to the GPx-depleting tendency of PPO and TPO diets.

Catalase is a cytosolic heme-containing enzyme which prevents oxidative damage by promoting the reduction of hydrogen peroxide to water and oxygen. The concentration of the enzyme is therefore decreased during peroxidation states resulting because it is used to promote neutralizaton of hydrogen peroxide and other reactive oxygen species. The reduction in concentration in concentration of catalase in the TPO- and PPO-fed rats suggests an increase in peroxidation processes in the rats.[36] It is for this, that CAT is also used as a marker of oxidative stress.[37] A similar trend was observed in a previous study on male rats fed with these diets[26] supporting the fact that PPO and TPO deplete catalase in serum. From the foregoing (increased MDA, decreased CAT and reduced GPx concentrations), a state of oxidative stress can be said to have occurred in the PPO- and TPO-fed groups.[34, 36]

Several factors including period of sexual life span, pregnancy and phase of menstrual or estrous cycle influence the changes that occur in the uterus and ovaries at any time and consequently their weight. This is why all rats were sacrificed at same phase of estrous cycle (estrous phase). Malondialdehyde, ROS and several other products of peroxidation act as oxidants and may damage cellular structures including DNA following the developed state of oxidative stress.[11,12,10] This (direct tissue toxicity) might in part be responsible for the

decrease in utero-ovarian weight in PPO- and TPO-fed groups.

Uterine weight is maintained mainly by the activities of estrogen and progesterone. In non-gravid state, progesterone is produced mainly by corpus luteum but also in little quantity by theca interna of the ovaries and adrenal cortex and increases thickness of endometrium and deposition of lipids and glycogen in stromal cells.[38] The decrease in progesterone concentration in PPO- and TPO-fed groups might have been due to direct ovarian toxicity from oxidative stress which might also have contributed to the observed reduction in the utero-ovarian weight. The increase in estrogen concentration in PPO and TPO-fed groups might have been due to its reduced metabolism from toxicity of liver,[23] its main site of metabolism or due to plasticity in enzyme pathways making it possible for differential metabolism of steroid hormones.[39]

CONCLUSION

We therefore conclude that thermoxidized and photoxidized palm oil diets induce oxidative stress and may impair reproductive function in female wistar rats.

CONFLICT OF INTEREST AND FUNDNG: There are no conflicts of interest in this research. The funding for this work came through contributions from members of the team.

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