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Kidney Function Assessment after Chronic Consumption of Oxidised Palm Oil Diets in Male Wistar Rats

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Abstract **Original Research Article**

This study investigates the impact of photo-oxidised palm oil (PPO) and thermo-oxidised palm oil (TPO) diets on the kidneys of male wistar rats. A total of twenty (20) male Wistar rats were used in this study. The animals were divided into 4 groups (n=5) namely control (CT), fresh palm oil diet (FPO), thermo-oxidised palm oil diet (TPO) and photo-oxidised palm oil diet (PPO). The experiment lasted for 90 days. The animals were sacrificed under urethane anaesthesia and blood samples were collected via cardiac puncture for biochemical assay using the serum. The kidneys were harvested for histopathological studies. The results showed that there was a significant increase ($p<0.05$) in serum creatinine in TPO group when compared with the control, FPO and PPO groups. There was a significant increase ($p<0.05$) in blood urea nitrogen (BUN) levels in TPO group when compared with the control, FPO and PPO groups. There was also a significant increase ($p<0.05$) in BUN levels in PPO group when compared with the control group. Histopathological findings revealed the presence of mild and moderate lesion and atrophy of tubular epithelium as well as vacuolation of renal corpuscle, tubular necrosis in the PPO and TPO groups. The findings of this study revealed that extreme consumption of PPO and TPO is toxic to the kidney.

Keywords: Photo-Oxidised Palm Oil, Thermo-Oxidised Palm Oil, Serum Creatinine, Blood Urea Nitrogen (BUN).

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INTRODUCTION

Today, palm oil is the second most abundant edible oil after soya bean oil and is in universal use. By definition palm oil is a type of vegetable oil derived from the fresh mesocarp of the fruits of palm tree (Elaies guineensis) of African origin (tropical rain forest of Western Africa) (Mohd *et al*., 2013).

Palm oil has been a vital resource in the majority of Nigerians diet. The global need for palm oil is fast increasing because of an increase in population globally. Palm oil also serves as a source of income for the majority of individuals in developing countries (Bassey, 2016). Nigeria was the world's leading producer and exporter of palm oil as at 1965 and has since 1974 ceased to contribute to the export trade in the 'commodity'. Due to the decline in agricultural productivity in Nigeria, the country's ability to increase production that can match the domestic demand and consumption became a challenge. The outcome of the West African Agricultural conferences held between 1927 and 1930 adduced the decline in palm oil export from Nigeria to 'inefficiency, inability to develop plantation to sizeable level and poor quality of oil produced (Bassey, 2016). Palm oil is naturally reddish in colour because it contains a high amount of beta-carotene. Palm oil has been used in food preparation for over 5,000 years. Palm oil is the most widely produced edible vegetable oil in the world and its nutritional and health attributes have been well documented (Chandrasekharan *et al*., 2000).

The kidneys are bean shaped organs that serve several essential

regulatory roles in vertebrate animals. They are essential in the urinary system and also serve homeostatic functions such as the regulation of electrolytes, maintenance of acid-base balance, and regulation of blood pressure (via maintaining salt and water balance). They serve the body as a natural filter of the blood, and remove wastes, which are diverted to the urinary bladder. Common clinical conditions involving the kidney include the nephritic and nephrotic syndromes, renal cysts, acute kidney injury, chronic kidney disease, urinary tract infection, nephrolithiasis, and urinary tract obstruction (Cotran *et al*., 2005).

Palm oil, as cooking oil, is generally consumed in its oxidised forms (thermo-oxidised and photo-oxidised). However, this oxidation changes the physical appearance and chemical nature of the oil. Some of the chemical reactions that occurred during the frying of oils are hydrolysis, oxidation, and polymerization (Falade and Oboh, 2005). Hydrolysis reactions that occurred during frying of foods with oil result in the increment of free fatty acids, reactive oxygen species (ROS), and transfatty acids. These foods can induce organ failure (AlAam *et al*., 2012; Boniface and Ejimofor, 2014). Correspondingly, oxidation of oils during frying alters the nature of enzymes and the status of antioxidants and causes the formation of lipid peroxidation and transfatty acids (Patsioura *et al*., 2017; Perumalla, 2016).
Hence, consumption of oxidised palm oil has shown to have adverse effects on different organs and thus affecting their functions negatively.

MATERIALS AND METHODS

Experimental Animals

A total of twenty (20) apparently healthy Adult Male Wistar rats weighting between 120-160g were used for this study. The animals were housed at a room temperature of 29 ± 20C temperature, and a relative humidity of 40-55%, and had free access to water and normal rat chow. They were acclimatized for two weeks (14 days) before the commencement of the experiments.

Purchase of Fresh Palm Oil

Ten litres of fresh palm oil were purchased directly from the palm oil mill at Odukpani Palm Oil Mill in Obudu Local Government Area of Cross River State, Nigeria and immediately stored inside a black container. The container was kept in a cool dry room and not exposed to sunlight or heat.

Preparation of Thermo and Photo-oxidised Palm Oil

The photo-oxidised palm oil was prepared by exposing fresh palm oil to sun light for 5 hours daily for 15 days to mimic what happens in the open market. This is according to Beshel *et al*., (2014), with slight modification.
The thermo-oxidised palm oil was prepared by exposing another portion of fresh palm oil to 5 rounds of heating for 10 minutes each. After each round of heating, the palm oil was allowed to cool down before reheating at 190°C. This was done to mimic what is used in frying akara, yam, etc.

Formulation of Palm Oil (Fresh Palm Oil, Photo-oxidised and Thermo-oxidised Palm Oil)

The palm oil (fresh palm oil, photo-oxidised and thermo-oxidised palm oil) diet was formulated as previously described by Beshel *et al.*, (2018). This formulation entails mixing 15g of the palm oil with 85g of rat chow, making 15% palm oil diet, as this is the usual composition of a typical Black African diet as reported by Umoh, 1972.

Ethical Approval

Ethical approval was obtained from the Faculty of Basic Medical Sciences Research Animal Ethical Committee with approval number *296PHY3724.*

Experimental Protocol

The animals were randomly divided into four (4) groups, each containing five (5) animals.
Group 1 served as the control group
Group 2 were fed with 15% fresh palm oil (FPO) diet
Group 3 were fed with 15% photo-oxidised palm oil (PPO) diet
Group 4 were fed with 15% thermo-oxidised palm oil (TPO) diet
The experiment lasted for 90 days.

Collection of Blood and Tissue Samples

Twenty-four hours (day 91) after the last administration, blood samples were collected through cardiac puncture and the blood dispensed into containers. The animals were sacrificed under urethane anesthesia. Kidneys were harvested for weighing and kidney histology.

Biochemical Analysis

The blood samples in the plain containers were allowed to clot and then centrifuge at 2000rpm for 10minutes. The serum was separated from the blood. Blood urea nitrogen (BUN) and serum creatinine were estimated using standard laboratory procedures.

Method of Determination of Peroxide Values (PV) of the Different Forms of Palm Oil

$$PV = \frac{S \times N \times 103}{W}$$

Where S = Sulphur
N = Nitrogen
W = Weight

$$\text{Sample FPO} = \frac{2.9 \times 0.025 \times 103}{1} = 7.467$$

$$\text{Sample PPO} = \frac{5.5 \times 0.025 \times 103}{1} = 14.162$$

$$\text{Sample TPO} = \frac{7.6 \times 0.025 \times 103}{1} = 19.570$$

Statistical Analysis

The results were expressed as mean ± standard error of mean (SEM). The results were analyzed using GraphPad prism software version 8.02 (GraphPad Software, SanDiego, CA). One-way analysis of variance (ANOVA) was used to compare means followed by a post hoc Turkey's multiple comparison test where p values of 0.05 was considered significant.

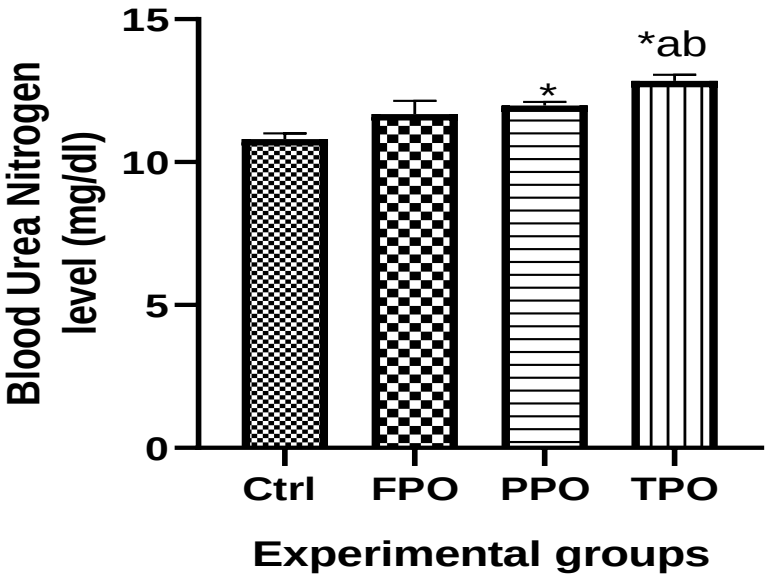
RESULTS

Table 1: Showing the results of peroxide values of the different forms of palm oil

Samples	Peroxide Value (mEq O_2/kg)
TPO	19.570
PPO	14.162
FPO	7.467

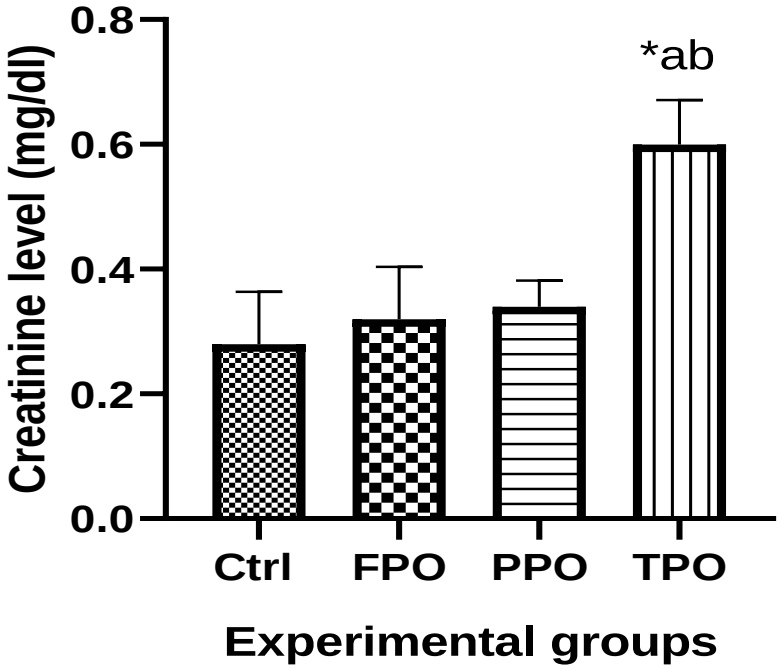
Accepted peroxide values for edible oils are between 10-20mEq O_2/kg (Connell, 1975).

Figure 1: Effect of the different forms of palm oil on BUN levels



Blood Urea Nitrogen concentration in the different experimental groups
Values are expressed as mean +SEM, n = 5.
* = p<0.05 vs control
a = p<0.05 vs FPO
b = p<0.05 vs PPO

Figure 2: Effect of the different forms of palm oil on serum creatinine levels



Serum Creatinine concentration in the different experimental groups
Values are expressed as mean +SEM, n = 5.
* = p<0.05 vs control
a = p<0.05 vs FPO
b = p<0.05 vs PPO

Table 2: Effect of different forms of palm oil on the histology of the kidney

Groups	Normal Kidney	Mild Lesion/Atrophy of Tubular Epithelium	Moderate Lesion/Atrophy of Tubular Epithelium
A: CONTROL	Present	Absent	Absent
B: FPO	Present	Absent	Absent
C: PPO	Absent	Present	Absent
D: TPO	Absent	Absent	Present

Photomicrograph of slides

Plate 1-Group A (Control Group): Showing Normal Kidney. HE x400

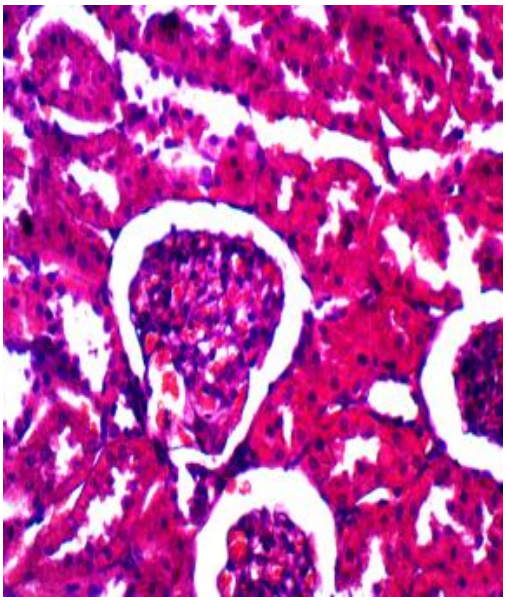


Plate 2-Group B (FPO Group): Showing Normal Kidney. HE x400

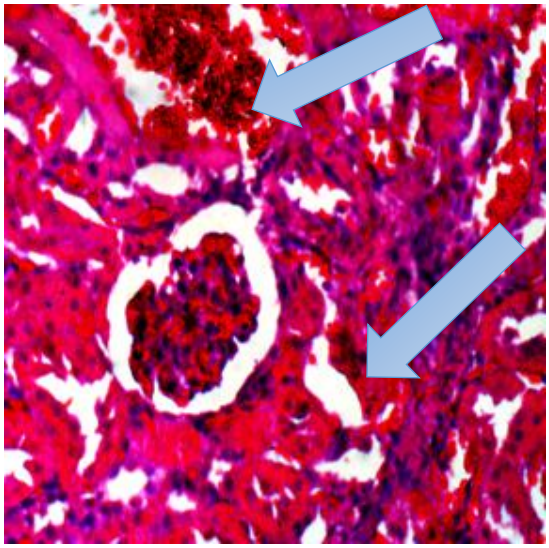


Plate 3-Group C (PPO Group): Showing Mild lesion and atrophy of tubular epithelium. HE x400

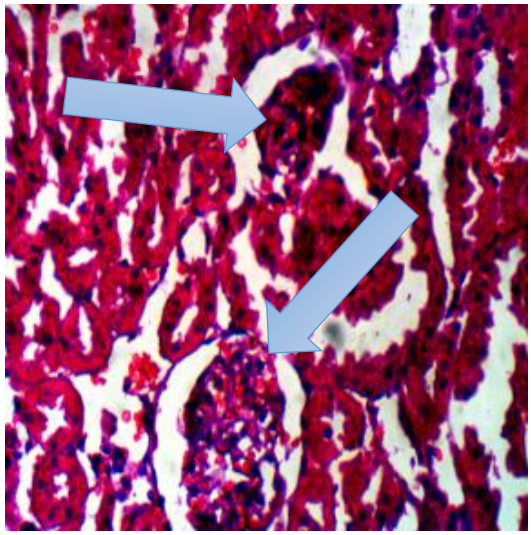


Plate 4-Group D (TPO Group): Showing Moderate lesion and atrophy of tubular epithelium. HE x400

DISCUSSION

Palm oil is mostly consumed in its oxidised forms (thermo-oxidised and photo-oxidised). This oxidation alters both the physical appearance and chemical composition of the oil. The kidneys play a vital role in the urinary system and perform important homeostatic functions, such as regulating electrolytes, maintaining acid-base equilibrium, and controlling blood pressure by managing salt and water levels. They act as the body's natural filter for blood, eliminating wastes, which are then directed to the urinary bladder. Typical medical conditions associated with the kidneys include nephritic and nephrotic syndromes, renal cysts, acute kidney injury, chronic kidney disease, urinary tract infections, nephrolithiasis, and urinary tract obstructions (Cotran *et al.*, 2005).

This research investigated the kidney-damaging effects of diets that included photo-oxidised palm oil (PPO) and thermo-oxidised palm oil (TPO) in male Wistar rats. The results of the peroxide value (PV) revealed that the degree of oxidative rancidity was affected by both photo and thermo-oxidation. The peroxide value serves as an indicator of oil degradation, measuring the quantity of peroxides produced in cooking oil during oxidation. The more often the oil is reheated or subjected to sunlight, the greater the peroxide index. However, in comparison to earlier findings, soya oil exhibited a higher peroxide value when repeatedly heated under identical frying conditions (Leong *et al*., 2010).

An elevated peroxide value suggests decreased chemical stability of the oil. According to Naghshineh *et al*. (2010), a greater concentration of saturated fatty acids enhances the chemical stability of oils.

The findings also indicated that the intake of both PPO and TPO resulted in considerable changes in kidney function and structure, as shown by increased levels of kidney biomarkers such as blood urea nitrogen (BUN) and serum creatinine, along with histopathological alterations in renal tissues. These results align with those reported by Shastry *et al*. in 2011, who noted that excessive consumption of thermally oxidised palm oil led to the presence of tubular cell vacuoles in the medullary region, affecting both the cytoplasm and the nucleus. Moreover, it was observed that the tubules occasionally contained eosinophilic substances within the lumen.

The increased concentrations of BUN and serum creatinine found in the PPO and TPO groups suggest compromised kidney function. These biomarkers are recognised indicators of glomerular filtration rate (GFR) and are frequently utilised to evaluate the extent of kidney impairment. Their rise indicates a reduction in the kidneys' capacity to effectively filter metabolic byproducts from the bloodstream, likely resulting from structural harm to the nephrons due to oxidised lipids.

Histopathological examination further corroborated this functional impairment, revealing mild to moderate lesions in the tubular epithelium, vacuolation within the renal corpuscles, tubular necrosis, and varying levels of atrophy. These structural injuries are likely due to the buildup of lipid peroxidation products and reactive oxygen species (ROS) produced during the oxidation of palm oil. Oxidised lipids are well-established inducers of oxidative stress, inflammation, and direct cytotoxicity, especially in organs with high metabolic rates like the kidneys. These observations align with the findings reported by Boniface and Ejimofor in 2014, who indicated that excessive intake of oxidised palm oil led to vacuolation of the renal corpuscles, tubular necrosis, degeneration of renal corpuscles, patchy or diffuse denudation of renal tubular cells accompanied by loss of brush border, and the buildup of homogeneous substances with eosinophilic debris. These effects could potentially result in kidney failure in the long term.

Interestingly, the extent of kidney damage seemed to differ between the two types of oxidised oil. Rats exposed to TPO displayed more significant histological damage than those treated with PPO, indicating that thermal oxidation might lead to the formation of more harmful lipid oxidation byproducts than photo-oxidation. This is consistent with earlier research suggesting that the thermal degradation of oils can yield a complex array of aldehydes, ketones, peroxides, and other reactive substances that harm cellular integrity (Dobarganes and Marquez-Rutz, 2003; Choe and Min, 2007).

The vacuolation observed in renal corpuscles and the occurrence of necrotic tubules can signify a disruption in cellular homeostasis and mitochondrial impairment, often resulting from prolonged oxidative stress. These observations strongly suggest that the intake of oxidised palm oil diets, especially those that have been thermally processed, significantly threatens kidney health and may lead to chronic kidney damage as time progresses.

Summarily, the results of this research emphasise the detrimental effects of oxidised palm oil on kidney health. The rise in blood urea nitrogen (BUN) and serum creatinine levels, along with changes in the structure of kidney tissues, indicates the importance of educating the public about the dangers of reusing or improperly storing cooking oils.

CONCLUSION

Having noted the above increase in kidney markers (BUN and serum creatinine) and pathological damages to the kidneys, extreme consumption of PPO and TPO diets is toxic to the kidney.

Conflict of Interest

All authors declare that they have no conflicts of interest.

Authors' Declaration

The authors affirm that the work presented is original, and will accept all liability for any claims about the content.

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