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

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Abstract **Original Research Article**

This study investigated the impact of photo-oxidized palm oil (PPO) and thermo-oxidized palm oil (TPO) diets on the liver of male wistar rats. The experimental animals were randomly divided into four (4) groups of five (5) animals each. Group 1 served as the control group and were fed with normal rat chow and water *ad libitum*, group 2, 3 and 4 were fed with 15% fresh palm oil (FPO) diet, 15% PPO diet and 15% TPO diet respectively. The experiment lasted for 90 days. After the period of experiment, the animals were sacrificed under chloroform anesthesia and blood samples were collected via cardiac puncture for biochemical analyses while the livers were harvested for histological analysis. The results of the study showed that there was significant increase ($p<0.05$) in Aspartate transaminase or Aspartate Aminotransferase (AST), Alanine transaminase (ALT) and Alkaline phosphatase (ALP) levels in TPO and PPO-diets fed groups when compared with the control and FPO diet fed group. Histopathological studies revealed the presence of mild and moderate cytoplasmic destruction in PPO and TPO-diet fed groups respectively. In conclusion, the findings of this study showed that chronic consumption of PPO and TPO is toxic to the liver.

Keywords: AST, ALT, ALP, Photo, Thermo-Oxidised Palm Oil.

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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). It is naturally reddish in colour because it contains a high amount of beta-carotene and has been used in food preparation for over 5,000 years (Chandrasekharan *et al*., 2000).

The liver is the largest organ in the mammalian body and has metabolic functions that deal with very essential processes such as detoxification, deamination, transamination, removal of ammonia in the form of urea, biosynthesis and release of the non-essential amino acids and plasma proteins with the exception of immuno-gamma globulins, gluconeogenesis, storage of glycogen, conversion of carbohydrates and proteins into lipids etc. Several functional tests have been formulated to explore hepatic status (Johnson, 1995; Nelson and Cox, 2000), and numerous enzymes have been determined to explore hepatic status such as alanine amino transferase (ALT), aspartate amino transferase (AST) and alkaline phosphatases (ALP) (Burtis and Ashwood, 1999).

Palm oil, as cooking oil, is generally consumed in its oxidized 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, 2015). 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).

It is therefore hypothesized in this study that liver functions could negatively be affected by the consumption of oxidized palm oil. Since the effect of oxidized palm oil on the liver has

not been fully investigated, this study therefore sought to investigated its impact on the liver.

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-oxidized 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-oxidized 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. Livers were harvested for weighing and liver 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. Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), and alkaline phosphatases (ALP) 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.

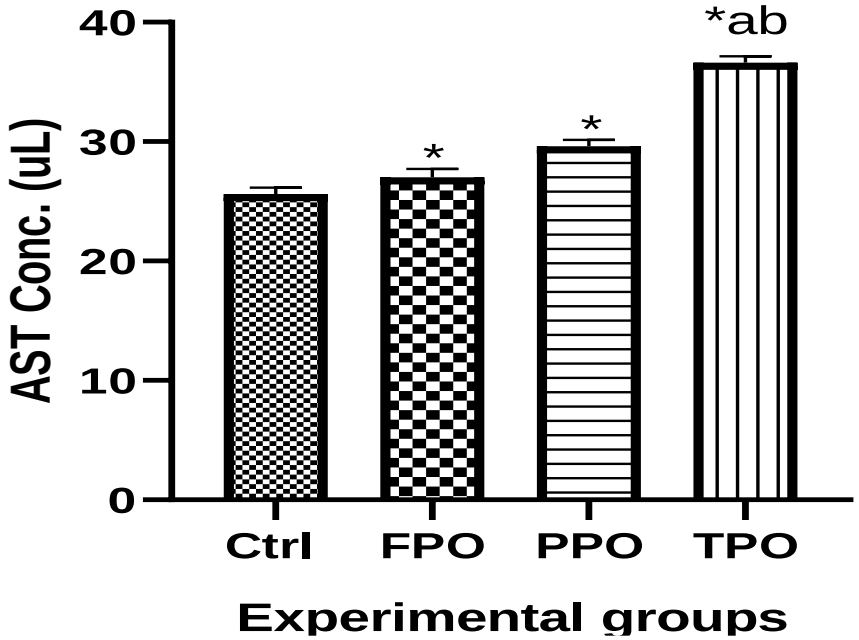
RESULTS

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

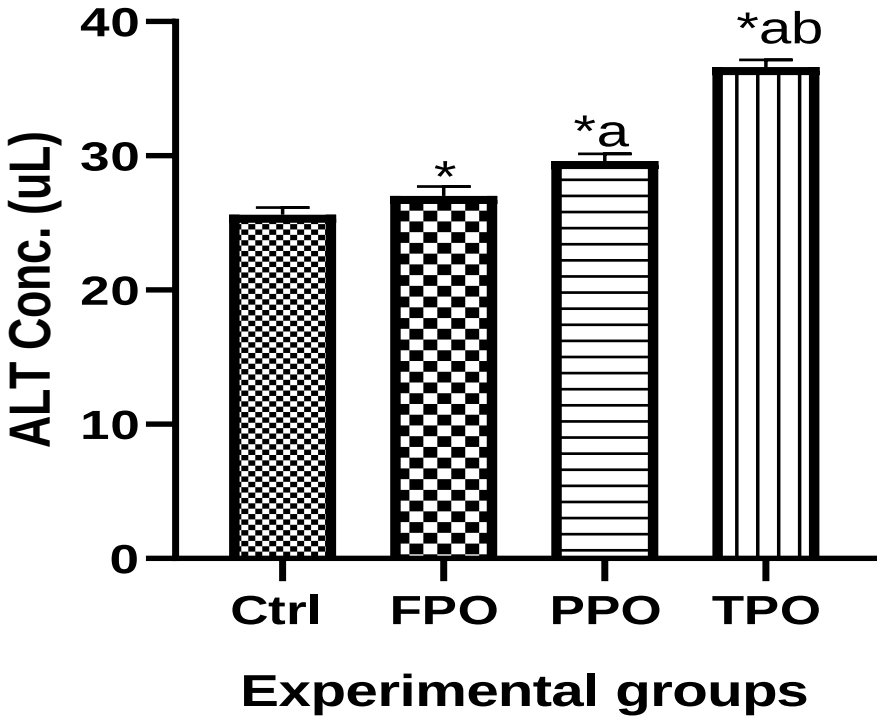
Accepted peroxide values for edible oils are between 10-20mEq O_2/kg (Connell, 1975).

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



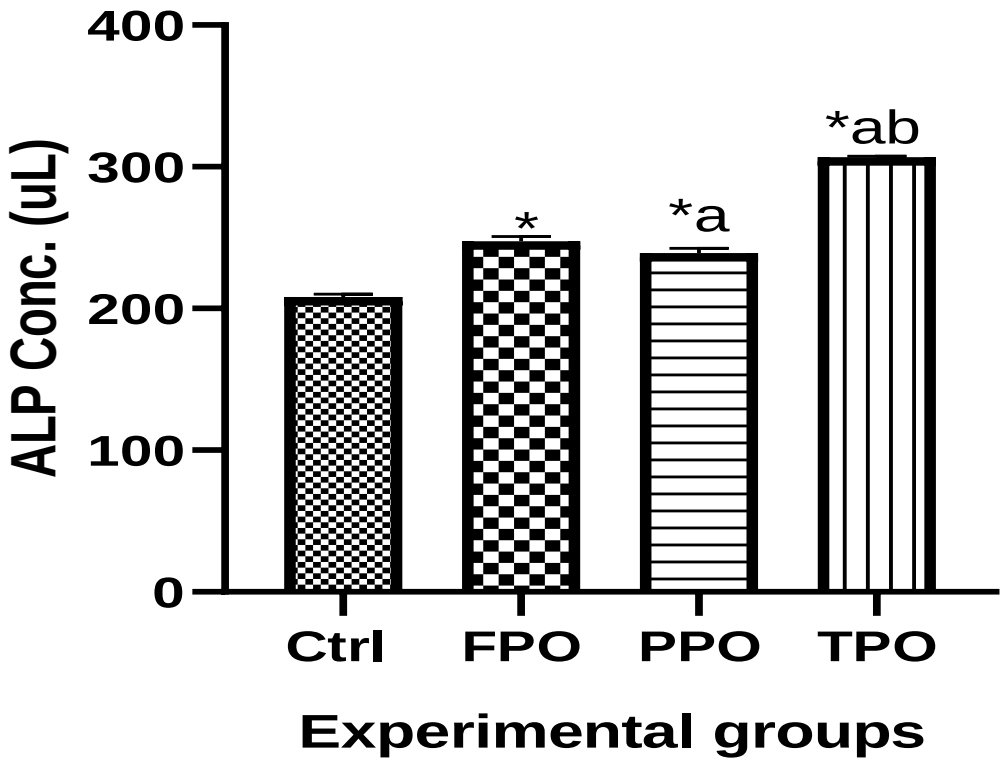
Aspartate Transaminase concentration in the different experimental groups
Values are expressed as mean +SEM, n = 5.
* = p<0.05 vs control
a = p<0.05 vs FPO
b = p<0.05 vs PPO

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



Alanine Transaminase 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 3: Effect of the different forms of palm oil on ALP levels



Alkaline Phosphatase 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 liver

Groups	Normal Liver	Mild Cytoplasmic Destruction	Moderate Cytoplasmic Destruction
A: CONTROL	Present	Absent	Absent
B: FPO	Present	Absent	Absent
C: PPO	Absent	Present	Absent
D: TPO	Absent	Absent	Present

Photomicrograph of Slides

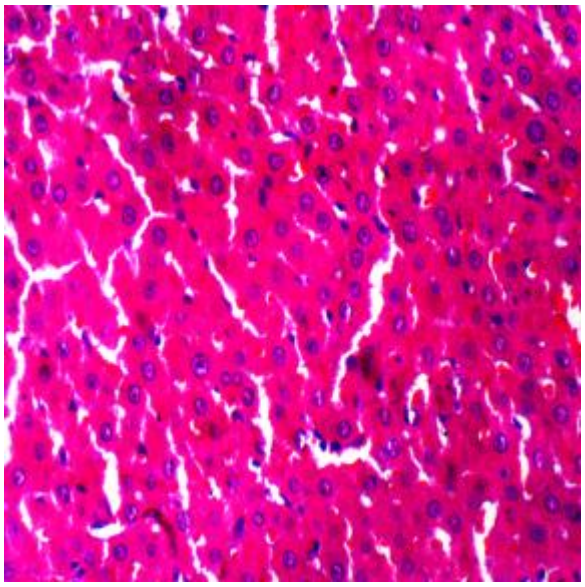


Plate 1 Control Group: Showing Normal Liver

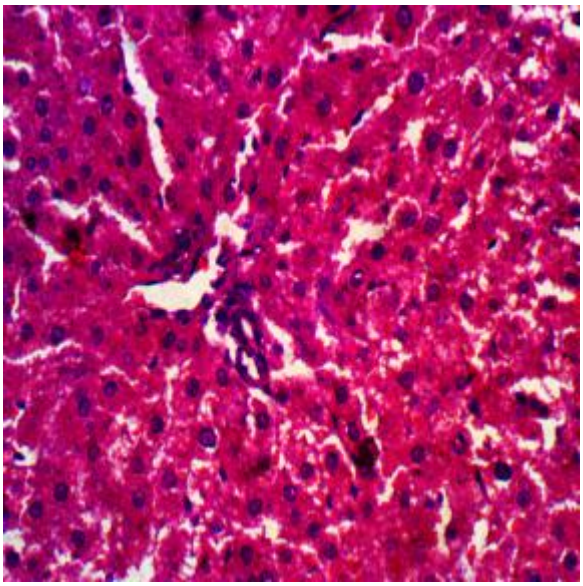


Plate 2 FPO Group: Showing Normal Liver

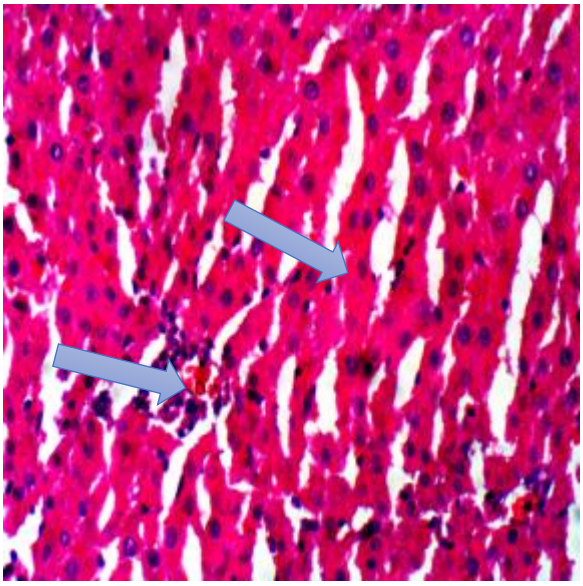


Plate 3 PPO Group: Showing Cytoplasmic Destruction

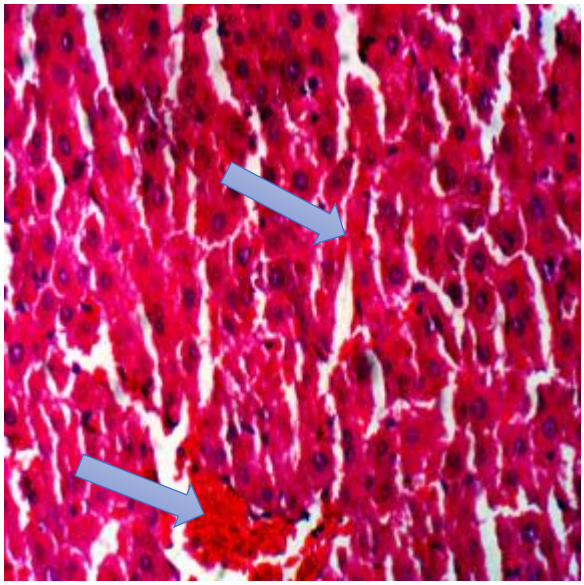


Plate 4 TPO Group: Showing Moderate Cytoplasmic Destruction

DISCUSSION

Palm oil is majorly consumed in its oxidized forms (thermo-oxidised and photo-oxidised). This oxidation changes the physical appearance and chemical nature of the oil. Liver plays a major role in the detoxification and excretion of many endogenous and exogenous compounds. Any injury or impairment of its function may lead to several implications on one's health. Management of liver diseases is still a challenge to modern medicine.

In this study, we examined how diets containing photo-oxidised palm oil (PPO) and thermo-oxidised palm oil (TPO) affect the liver of male Wistar rats, focusing specifically on the activities

of hepatic enzymes and any histopathological alterations. Based on the peroxide value (PV) results, the degree of rancidity due to oxidation was significantly affected by both photo and thermo-oxidation. The peroxide value serves as an indicator of the level of oil rancidity or deterioration, reflecting the quantity of peroxides created in the cooking oil during the oxidation process. Reheating the oil more often or exposing it to sunlight results in a higher peroxide index. However, in comparison to the earlier report, soya oil exhibited a greater peroxide value when it was heated multiple times under identical frying conditions (Leong *et al*., 2010).

The significant increase ($p<0.05$) in the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) found in rats that consumed PPO and TPO diets, in contrast to those fed control and fresh palm oil (FPO), suggests liver damage and impaired liver function.

Enzymes like AST and ALT are generally found within hepatocytes, and their elevated levels in serum are well-recognised indicators of damage to hepatocellular membranes. In contrast, ALP is linked to the biliary system, and a rise in its levels may indicate cholestasis or injury to the biliary epithelium. These observations are consistent with earlier research conducted by Amsalu *et al*., (2020), which highlighted an increase in ALT and AST levels in Swiss albino mice after the administration of palm oil-fried street kokor.

The greater enzyme level increases observed in the TPO group compared to the PPO group can be linked to the intensified thermal degradation of fatty acids and the subsequent development of secondary oxidation products such as aldehydes, ketones, and polymeric substances during the heating process. Thermo-oxidation, particularly when occurring over extended or repeated heating, tends to create more intricate and harmful oxidation byproducts than photo-oxidation, which is more inclined to produce hydroperoxides and early-stage oxidation compounds.

Alongside the biochemical findings, histopathological analysis of liver samples showed different levels of cytoplasmic damage in both the PPO and TPO diets fed groups, with the PPO group displaying mild degeneration and the TPO group showing moderate degeneration. These morphological alterations further corroborate the enzyme data and suggest structural damage to hepatic tissue, probably resulting from oxidative stress and inflammation caused by harmful lipid peroxidation products.

The lack of notable enzyme increase or tissue damage in the FPO group emphasizes the safety associated with consuming fresh palm oil and points out the harmful effects of oil oxidation. These results are consistent with current research that highlights the dangers of consuming oxidized fats, which are often found in reused or improperly stored cooking oils, posing a considerable threat to liver health.

CONCLUSION

Having noted the above increase in liver enzymes and pathological damage to the liver, extreme consumption of PPO and TPO diets is toxic to the liver.

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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