

## EFFECT OF FRESH PALM OIL AND SHORT TIME REPEATEDLY HEATED PALM OIL ON HEPATO-RENAL FUNCTIONS IN WISTAR RATS

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### ABSTRACT

Palm oil is a commonly consumed vegetable oil in most Nigerian and African homes, consumed either fresh or in thermo-oxidized form. This study investigated the effects of fresh and thermo-oxidized palm oil (TPO) on some biochemical enzymes of liver and kidney function biomarkers in Wistar rats. Forty-nine Wistar rats weighing between 80-140 grammes were randomly assigned into seven groups of 6 animals each. The control group received standard animal feed and clean water only. The fresh palm oil groups received animal feed mixed with fresh palm oil in the ratio 98:2g; 96:4g and 94:6g respectively for each animal group. The TPO-fed groups received animal feed mixed with thermo-oxidized palm oil at the same ratios. The animals were fed *ad libitum* for 90 days. At the end of the feeding period, the animals were euthanized using ketamine vapour and blood was collected for assessment of some biochemical indices of liver and kidney function using standard methods. Results showed no significant differences ( $p>0.05$ ) were observed in the serum AST levels across experimental groups compared to the controls. In conclusion, thermoxidation of palm oil and subsequent formulation in diets at 2-6% showed probable hepatoprotective action in Wistar rats.

**Keywords:** Palm oil, thermo-oxidation, liver function, kidney function, biomarkers, Wistar rats

### INTRODUCTION

Palm oil is a widely consumed globally [1, 2]. Palm oil is either eaten fresh or at different thermal oxidation levels[3]. The overall benefit of palm oil is linked to fresh palm oil over thermally oxidised palm oil. Palm oil has both benefits and potential drawbacks

depending on its processing and use. Fresh palm oil has been shown to have positive effects on lipid profiles, potentially reducing cardiovascular disease risk [4, 5]. It contains beneficial compounds like carotenoids, vitamin E, and phenolics, which contribute to

its stability and potential health benefits [6]. However, thermally oxidized palm oil can have negative impacts on health, including decreased growth performance, altered lipid profiles, and increased oxidative stress in animal studies [5, 7]. The sustainability of palm oil production is a concern, but efforts are being made to develop sustainable practices [7]. Overall, moderate consumption of fresh palm oil as part of a balanced diet appears to be safe and potentially beneficial, while the use of thermally oxidized palm oil should be limited[8]. The intake of thermally oxidised oil has been said to bring about the creation of peroxides, and other reactive and cytotoxic products[9, 10]. The free radicals and dihydroxy esters derived from reaction with oxygen contained in thermally oxidised palm oil have been proven to bring about cells injury[11]. Moreover, the generated free radical species which are more reactive are described to cause arthritis, atherosclerosis, diabetes, cancer and synthesis of cataract[12]. The intake of oxidised fats and oils in a long term basis has been stated to lead to thrombosis, growth retardation, lack of essential fatty acid, anaemia, fatty liver and deactivation of nucleic acid of vital metabolic enzymes [13, 14]. Amsalu *et al.* [15] revealed an increase in the actions of plasma markers for liver function for

instance, alkaline phosphatase, alanine transaminase and aspartate transaminase, in rats nurtured with thermally oxidised palm oil diet.

The aim of the study was to compare the impacts of fresh palm oil (FPO) and short-time thermo-oxidized palm oil (TPO) on liver and kidney biomarkers of functions on Wistar rats. This observation shows that thermal oxidation can change the fatty acid content of palm oil and degrade its antioxidant content yet short times of heating but with medium concentrations of 2 and 6 percent showed no significant hepatotoxicity or nephrotoxicity. Interestingly, serum ALT and ALP were increased in FPO-fed rats and thus fresh palm oil may have mild effects on the hepatocellular. On the contrary, TPO presented very little or no significant effect on these liver enzymes and no significant effect on kidney biomarkers urea and creatinine except at certain concentrations. Notably, these findings imply that the use of thermally oxidized palm oil in moderate amounts (not heated to an extreme limit and repeatedly) may be of negligible risk to the health of both the liver and kidneys, at least in the short-term. Moreover, the changes in the biochemical indices were usually in the

physiological range, so there was no overt toxicity as it was studied.

This study is important in providing knowledge on the increasing cases of health issues related to the consumption of oxidized foods obtained through dietary consumption of cooking oils, particularly in areas such as Nigeria where palm oil is material cooking oil, and is commonly used to replenish frying. In the study, the aspect of how the thermal processing influences the nutritional and biochemical safety of palm oil is important. The research added to the body of evidence that the public health recommends to ensure safe palm oil consumption. It raises the possible dangers of excessive heating though it implies that hepatogenic and renal functions could be incapacitated at short term low percentages of thermal oxidation.

## MATERIALS AND METHODS

### *Materials and Reagents*

Fresh palm oil fruits were purchased from Marian Market in Calabar Municipality, Cross River State, Nigeria. The fruits authentication was carried out by a Botanist in the Department of Botany, University of Calabar; a specimen was deposited in the herbarium of the same Department for future reference.

### *Oil samples preparation*

The palm oil was processed to following the method of Alhaji *et al.* [16]. Ripe fresh unbruised palm fruits (1 kg) were carefully selected, boiled for thirty (30) minutes in a steel pot and mashed in a mortar (wooden) while hot. The mashed mesocarp was manually squeezed to extract palm oil. The extracted palm oil was boiled with one-third its volume of water to clarify the crude palm oil. next, the upper oil layer was separated and dried by reheating for five (5) minutes at 105°C. The oil was subsequently filtered to remove suspended particles. No additional refining was performed prior to analysis. The oil was stored in amber-stoppered plastic container and kept in a refrigerator at 4°C until required. For thermo-oxidation, 1.5 litres of the oil was heated using an electric hot plate at 160°C in a stainless steel frying pan for five (5) minutes in five consecutive rounds, with 2- hour cooling intervals between heating cycles. Some of oil samples were stored in airtight bottles at room temperature for physico-chemical analyses, while remaining thermo-oxidized and fresh palm oil samples were used for diet formulation for the animal studies.

***Diet formulation***

Pelleted rat chow was purchased from Pfizer Livestock Feeds, Abia State, Nigeria. Diet formulation followed the procedures described by Ani *et al.* [17] with modifications. Two grammes (2g), four grammes (4g) and six grammes (6g) of oils were used instead of fifteen grammes (15g) of oil. The formulated diets were stored in sealed containers at 4°C to prevent further oxidation.

***Experimental Animal and design***

Forty-nine (49) male and female Wistar albino rats weighing 80-140g were obtained from the Department of Biochemistry animal house, University of Calabar, Nigeria. Animals were housed in well-ventilated wire-mesh cages with wooden bottoms under

controlled environmental conditions (temperature:  $28 \pm 2^\circ \text{C}$ , relative humidity: 46%, 12- hour light-dark cycle). The experimental procedures followed ethical guidelines approved by the Faculty of Basic Medical Sciences Animal Ethics Committee. The animals were allowed a 2-week acclimatization period with the laboratory surroundings throughout the period standard animal feeds and water was fed *ad libitum* prior to the beginning of the experiment. Subsequently, the rats were randomly shared into seven groups of seven animals each according to the experimental design presented in table 1. The feed oil ratio was following an earlier described report by Ratnayake *et al.* [18] on usage of oxidized oils with minor modifications in diets

**Table 1: Experimental Design**

Group	Treatment	Number of Rats	Feed Composition
1	Control	6	100% rat chow only
2	2% Fresh Palm Oil (FPO)	6	98% rat chow + 2% fresh palm oil
3	4% Fresh Palm Oil (FPO)	6	96% rat chow + 4% fresh palm oil
4	6% Fresh Palm Oil (FPO)	6	94% rat chow + 6% fresh palm oil
5	2% Thermo-oxidized Palm Oil (TPO)	6	98% rat chow + 2% thermo-oxidized palm oil
6	4% Thermo-oxidized Palm Oil (TPO)	6	96% rat chow + 4% thermo-oxidized palm oil
7	6% Thermo-oxidized Palm Oil (TPO)	6	94% rat chow + 6% thermo-oxidized palm oil

***Animal Sacrifice and Sample Collection***

After 90 days of feeding, animals were fasted for 12 hours before sacrifice. Euthanasia was performed using ketamine intraperitoneal at 2  $\mu\text{L/kg}$  body weight following Uti et al [19]. Blood samples were collected via cardiac puncture into sterile plain tubes, allowed to clot for 4 hours, and centrifuged at 1000 rpm for 15 minutes to obtain serum for biochemical analyses.

### ***Biochemical Analyses***

#### ***Liver Function Enzymes***

Aspartate Aminotransferase (AST): AST activity was determined using the Randox kit assay method based on Reitman and Frankel [20]. Briefly, in this colorimetric procedure, AST enzymatic reaction with its substrate creates a certain colour change that is also proportional to the enzyme activity. Absorbance of the resulting product is measured spectrophotometrically giving a quantitative indication of the levels of AST in sample. The method involves the reaction between  $\alpha$ -oxoglutarate and L-alanine to form oxaloacetate and L-glutamate, with oxaloacetate production proportional to AST activity. The analysis is done following the protocol of the manufacturer. The process is common in clinical and experimental research to ascertain the functioning of the liver alongside the tissue destruction through

transamination reaction. Alanine Aminotransferase (ALT): ALT activity was determined using the Randox kit assay method according to Reitman and Frankel [20]. Alkaline Phosphatase (ALP): Serum ALP activity was determined using the Randox kit assay method based on Reitman and Frankel [20]. The principle involves hydrolysis of p-nitrophenylphosphate by ALP to form phosphate and p-nitrophenol, with color development proportional to enzyme activity.

#### ***Kidney Function Parameters***

Serum Urea: Urea concentration was determined using the Randox assay kit employing the urease-Berthelot method as described by Weatherburn [21]. Serum Creatinine: Creatinine concentration was determined using the Randox diagnostic kit based on Akesson [22] and Cabaniss *et al.* [23]. The method involves reaction of creatinine with picric acid to produce a colored creatinine-alkaline picrate complex.

#### ***Statistical Analysis***

Data were analyzed using one-way analysis of variance (ANOVA) with post-hoc multiple comparisons for between-group differences. Results are expressed as mean  $\pm$  standard

error of the mean (SEM). Statistical significance was set at  $p < 0.05$ . Analyses were performed using SPSS version 17.0 and Microsoft Excel 2010. Image J software was used for gene expression fold-change calculations.

## RESULTS AND DISCUSSION

### Liver function biomarkers

Effect of fresh palm oil (FPO) and thermo-oxidized (TPO) diets on serum aspartate aminotransferase (AST), and alanine transaminase (ALT) activity were studied in Wistar rats. Serum AST activities across all palm oil treatment groups showed no significant differences ( $p > 0.05$ ) compared to controls or between treatment groups (Figure 1). All the fresh palm oil diet groups (2%, 4% and 6% FPO) and 2% TPO group showed

significant increases in the activity level of ALT ( $p < 0.05$ ) compared to the control. The 4% and 6% TPO group were not significantly different from the control. However, the 4% and 6% TPO diet groups showed a significantly higher ALT activity ( $p < 0.05$ ) compared to 2%, 4%, 6% FPO groups and 2% TPO diet group (Figure 2). The 2%, 4% FPO diet groups results showed a significant increase ( $p < 0.05$ ) in the alkaline phosphatase (ALP) activity of the rats compared to the control while the other test groups presented no significant changes in ALP activity compared to the control. Moreover, the 2%, 4% and 6% TPO diet groups demonstrated significantly lower ALP activity ( $p < 0.05$ ) compared to the 2% FPO group. Additionally, the 4% TPO diet group showed significantly lower ALP activity at  $p < 0.05$  compared to the 4% FPO diet group (FIG. 3).

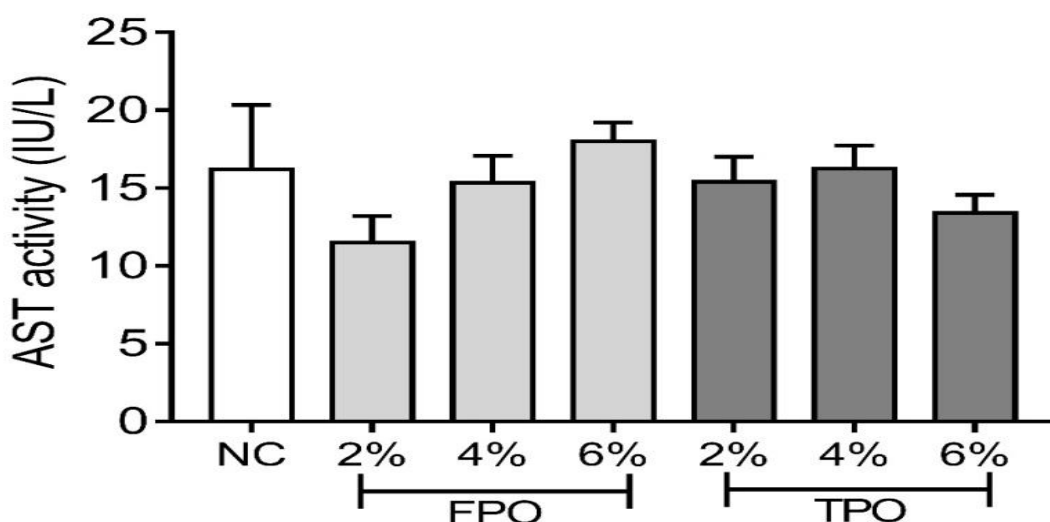


FIG 1: Comparison of serum AST level between the different experimental groups. Values are mean  $\pm$  SEM,  $n = 5$ . Serum AST level did not differ across the groups ( $p > 0.05$ ).

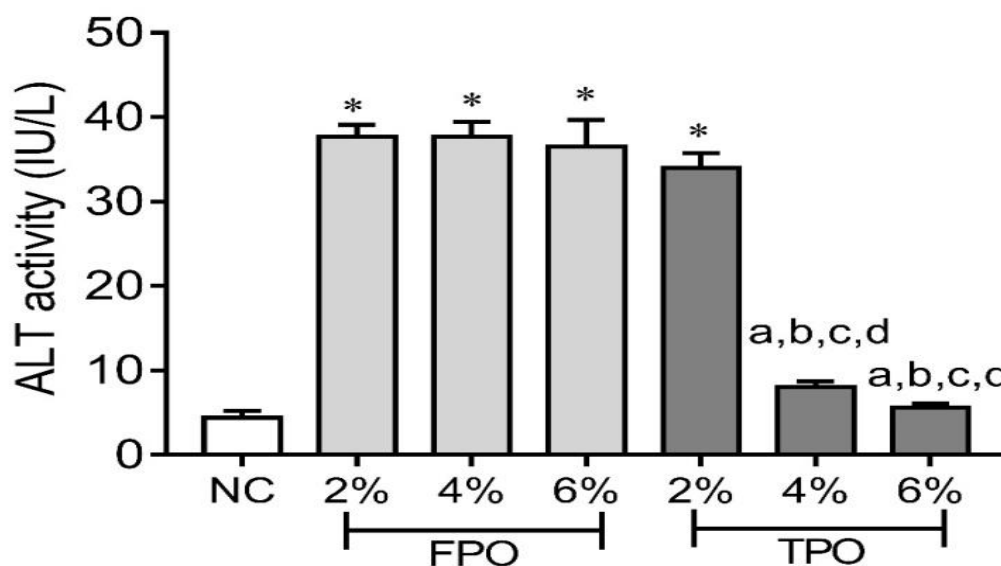


FIG.2: Comparison of serum ALT level between the different experimental groups. Values are mean  $\pm$  SEM,  $n = 5$ . \*  $p < 0.05$  vs NC; <sup>a</sup> $p < 0.05$  vs FPO 2%; <sup>b</sup> $p < 0.05$  vs FPO 4%; <sup>c</sup> $p < 0.05$  vs FPO 6%; <sup>d</sup> $p < 0.05$  vs TPO 2%.

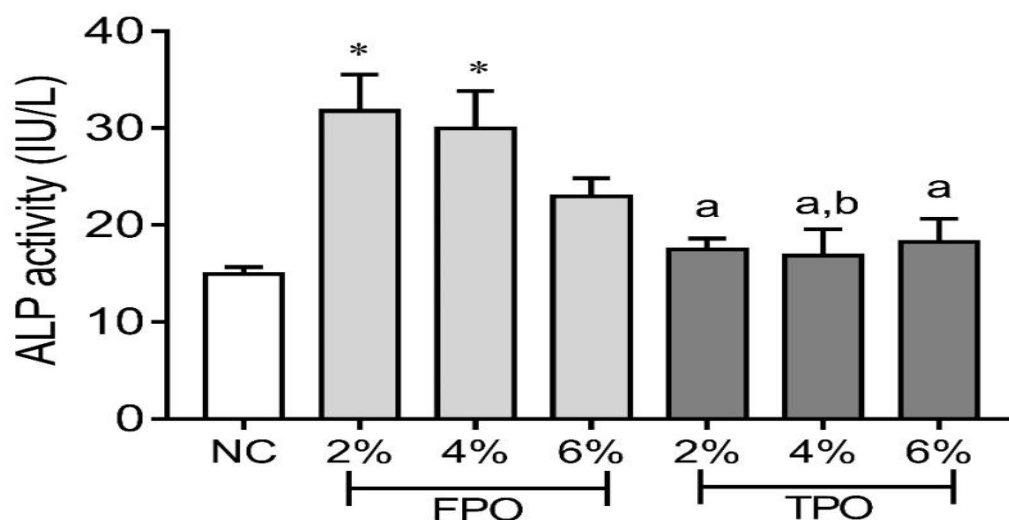


FIG.3: Comparison of serum ALP level between the different experimental groups. Values are mean  $\pm$  SEM,  $n = 5$ . \*  $p < 0.05$  vs NC; <sup>a</sup> $p < 0.05$  vs FPO 2%; <sup>b</sup> $p < 0.05$  vs FPO 4%.



### ***Kidney Function Parameters.***

Effect of fresh palm oil (FPO) and thermo-oxidized (TPO) diets on serum urea, serum creatinine level, and urea/ creatinine ratio level of Wistar rats is presented in Figures (4, 5, and 6). The serum urea level of the experimental rat administered with fresh and thermoxidized palm oil at all the percentage showed no significance differences ( $p > 0.05$ ) compared to normal control and amongst the various animal groups (FIG. 4). The 2% FPO group showed significantly lower serum

creatinine ( $p < 0.05$ ) compared to the controls. The other groups showed no significant differences ( $P > 0.05$ ) from the control. However, the 6% FPO and 4% TPO groups showed significantly higher creatinine levels ( $p < 0.05$ ) compared to the 2% FPO animal group (FIG 5). Serum urea/creatinine ratios showed no significant differences ( $p > 0.05$ ) across all experimental groups compared to controls or between groups (Figure 6).

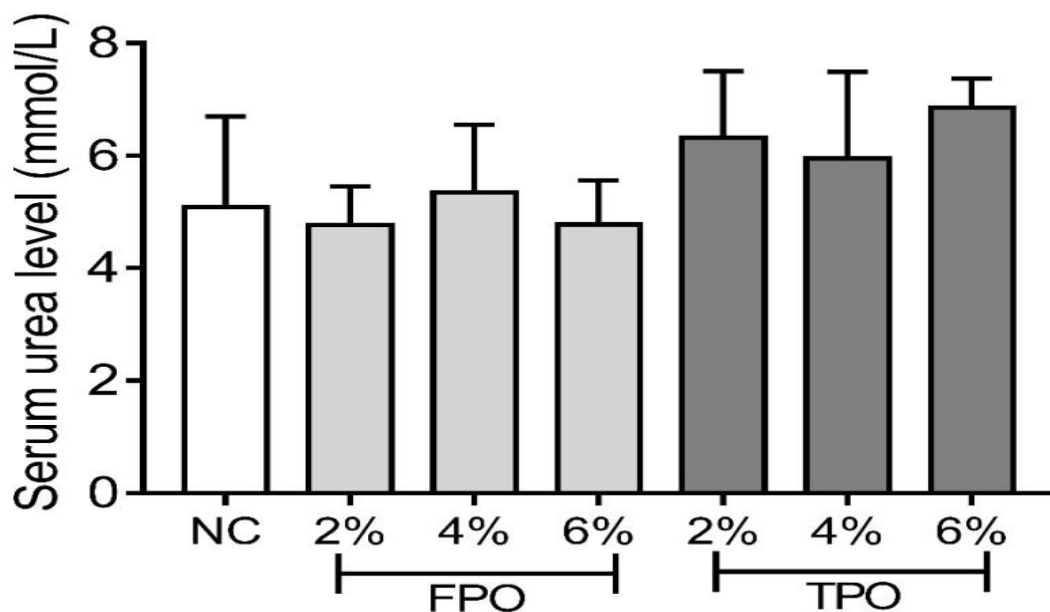


FIG. 4: Comparison of serum urea level between the different experimental groups. Values are mean  $\pm$  SEM,  $n = 5$ . Urea level did not differ across the groups ( $p > 0.05$ ).



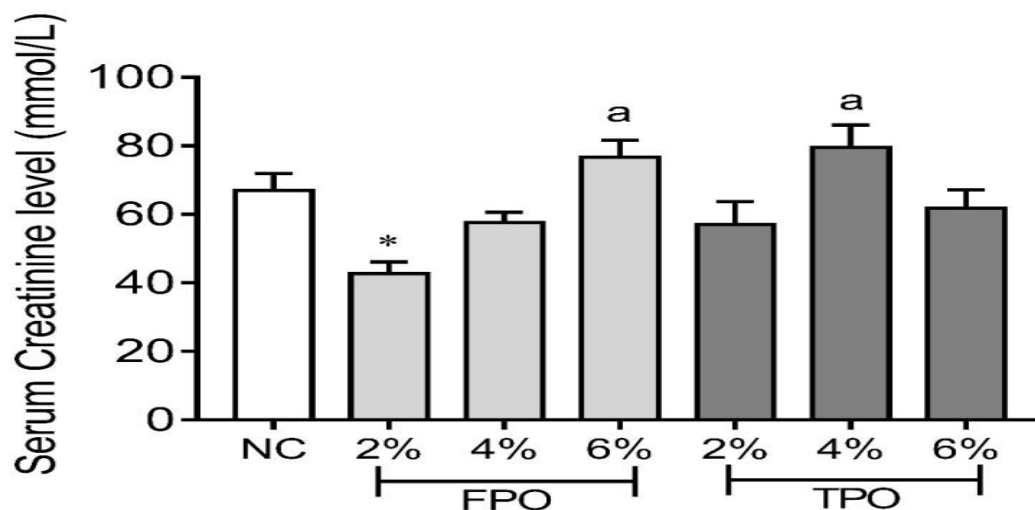


FIG.5: Comparison of serum creatinine level between the different experimental groups. Values are mean  $\pm$  SEM,  $n = 5$ . \*  $p < 0.05$  vs NC; <sup>a</sup> $p < 0.05$  vs FPO 2%.

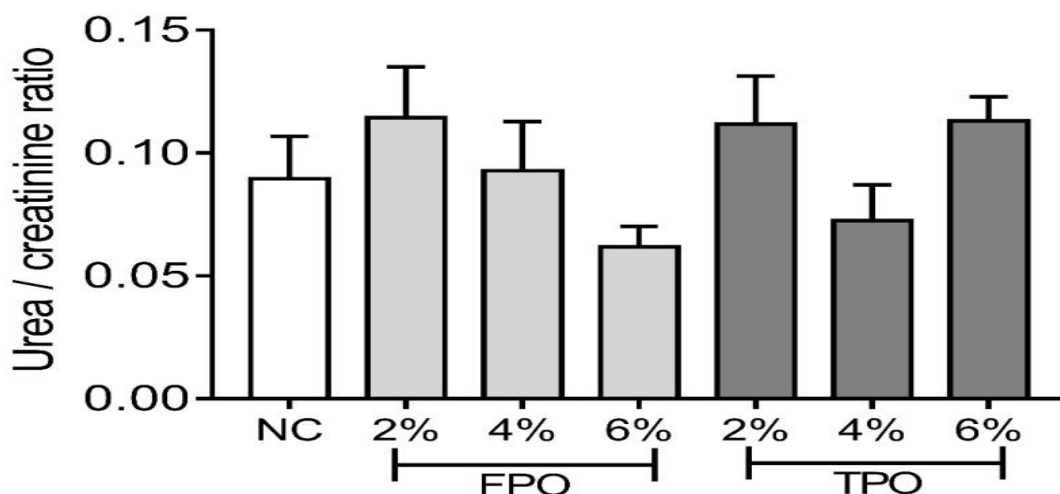


FIG. 6: Comparison of serum urea/creatinine ratio between the different experimental groups. Values are mean  $\pm$  SEM,  $n = 5$ . Urea/creatinine ratio did not differ across the groups ( $p > 0.05$ ).

### Discussion

This study investigated the effects of fresh and thermo-oxidized palm oil on liver and kidney function biomarkers in Wistar rats. Palm oil is widely consumed in tropical

African households, typically in fresh form or at various degrees of thermal oxidation, with treatment duration varying among households [24].

Serum enzymes including ALP, AST, and ALT serve as biological markers for monitoring liver structural integrity and damage [25, 26]. ALT and AST are intracellular liver enzymes that leak into circulation upon hepatocyte injury, resulting in elevated serum levels. ALT is considered more specific for liver inflammation, while AST levels may be elevated in other organ diseases [27].

The non-significant changes in serum AST levels across all experimental groups compared to controls affirm the non-toxicity of the palm oil treatments at the tested concentrations and suggest hepatoprotective properties. This finding aligns with Murwani *et al.* [28], who reported no significant changes in plasma AST activity in animals fed 10-minute and 15-minute thermally oxidized palm oil compared to fresh palm oil. However, this contrasts with Manorama *et al.* [29], who observed significant increases in AST with 10% and 20% oxidized palm oil. These discrepancies may be attributed to differences in experimental design and thermal oxidation duration.

The significant increase in ALT levels in all FPO groups and the 2% TPO group compared to controls may result from alterations in hepatocyte membrane

architecture [26]. This suggests that palm oil's influence on serum ALT is not substantially affected by the heating process. These findings are consistent with Adam *et al.* [30], who reported that both heated and fresh palm oil increase serum ALT enzyme activity. The significant increase in ALP activity observed in the 2% and 4% FPO groups may indicate large bile duct obstruction [31]. The significant reduction in ALP activity in TPO groups compared to fresh palm oil groups suggests that thermal oxidation may reduce bile duct function or impair liver metabolic activity.

Regarding kidney function parameters, the non-significant changes in serum urea levels across all groups indicate maintained kidney health and suggest that palm oils at 2-6% concentrations have no adverse effects on kidney function. This is consistent with Ani *et al.* [17], who reported non-significant changes in urea concentration in animals fed thermally oxidized palm oil compared to controls.

The non-significant change in serum creatinine levels in most groups (except 2% FPO) indicates the absence of acute kidney injury or considerable impairment of glomerular filtration functions of those animal groups. The significant decrease in

creatinine in the 2% FPO group may indicate increased glomerular filtration rate or enhanced excretion efficiency.

The outcomes of the present study provided information on the physicochemical parameters, fatty acids composition of thermoxidised and fresh palm oils and their effects on haematological indices, liver serum enzymes, renal function parameters and lipid metabolism enzyme genes in Wistar rats. The study showed that repeated heating of palm oil even at a relatively shorter period of time altered the fatty acid make-up of the palm oil by decreasing the unsaturated fatty acid constituents and also leads to the production of some toxic compound in the oil. It also showed that thermo-oxidation depletes the natural antioxidants in palm oil and increase the physicochemical parameters used for testing the undesirable oil quality.

Furthermore, changes in physiological functions based on the exposure of Wistar rats to the palm oils at 2%, 4% and 6% were estimated by evaluation of haematological parameters, creatinine, urea, urea/creatinine ratio, changes in serum alkaline phosphatase (ALP), aspartate amino-transferase (AST) and alanine aminotransferase (ALT) levels and gene expression of key enzymes of lipid metabolism. This study reported no

considerable damage brought about by usage of thermoxidised palm oil in Wistar rats at the percentage of exposure used. However, fresh palm oil had an effect on ALT and ALP serum liver enzyme and the thermoxidised palm oil had no influence on serum enzyme parameter except 2% TPO on ALT activity. The thermoxidised palm oil had no influence on the functions of the renal parameters but the fresh palm oil did have an influence on serum creatinine level at 2%FPO. Investigation on the impact of thermoxidised palm oil and fresh palm oil on gene expression indicated the only ACACA mRNA expression was affected by 6% FPO 2% and 4% TPO. The palm oils at this dose could be beneficial for individuals predisposed to renal and haematological diseases.

## **CONCLUSION**

This study demonstrates that short-term thermal oxidation of palm oil alters its fatty acid composition and produces toxic compounds while depleting natural antioxidants. However, consumption of thermally oxidized palm oil at low concentrations (2-6%) showed minimal harmful effects on liver and kidney function biomarkers in Wistar rats. Fresh palm oil affected ALT and ALP liver enzymes, while

thermally oxidized palm oil had limited effects on these parameters. Both oil types showed minimal impact on kidney function parameters.

The findings suggest that if palm oil must be thermally oxidized, consumption at low percentages may be relatively safer, showing potential ameliorative effects on liver, kidney, and hematological functions. However, prolonged heating of palm oil should be discouraged in households due to potential harmful effects on human health.

### **RECOMMENDATIONS**

1. Palm oil should be consumed preferentially in its fresh form
2. If thermal oxidation is necessary, minimize heating duration and temperature
3. Consumption should be limited to low percentages when using thermally oxidized palm oil

**Author contributions:** MAA & GEE conceived the idea. MAA, GEE & SOB designed the project, JEE carried out the study and wrote the manuscript, CIOU analysed data. All authors read and approved the final manuscript.

**Competing Interest:** No conflicts of interest.

**Data Availability:** Raw data is available on request

**Consent to participate:** Not applicable

**Ethics and Consent to Participate declarations:** Not applicable

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