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Assessment of Some Kidney Function Indices and Kidney Histopathology of Wistar Rats Exposed to Crude Palm Oil of Varied Free Fatty Acid Levels

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ABSTRACT: Palm oil is a major source of dietary fats and oils in many regions of the world. In this study, the effect of crude palm oil (CPO) of varied free fatty acid (FFA) levels on some kidney function indices and kidney histopathology in Wistar rats was investigated. Thirty-six female Wistar rats were grouped into six categories: a control group with no palm oil intake and five experimental groups receiving crude palm oil with FFA levels of 0.4 %, 4.8 %, 8.4 %, 21.9 % and 42.7 %, respectively. The rats were administered a dosage of 480 mg/kg body weight of CPO for four weeks. Body and kidney weights, as well as serum creatinine, urea, sodium, potassium and chloride levels were determined. Furthermore, histopathological examination of kidney tissues was performed to assess structural changes. A reduction in serum creatinine and urea levels was observed across all experimental groups compared to the control. A significant reduction ($p < 0.05$) in creatinine levels from 5.98 mg/dL in the control to 4.00 mg/dL in the 42.7 % FFA group was observed, while urea decreased from 9.58 mg/dL to 9.23 mg/dL in the 8.4% FFA group. Sodium reduced from 140.76 meq/L in the control to 105.00 meq/L in the 8.4 % FFA group, and potassium declined from 8.67 mmol/L to 6.23 mmol/L in the 42.7 % FFA group, these changes were not statistically significant ($p > 0.05$). Chloride concentrations remained stable across all groups. Histopathological analyses showed no observable damage to renal features across all groups, indicating preserved kidney integrity. The findings suggest that FFA levels up to 42.7 % in palm oil does not impair kidney function in Wistar rats.

Keywords: Palm oil, Kidney function indices, Histopathology, Wistar rats

Introduction

Palm oil is the most widely consumed vegetable oil globally, particularly in tropical regions where it is a staple in both dietary and industrial applications (Ismail *et al.*, 2018; Echioda *et al.*, 2018).
Palm oil's distinctive composition of fatty acids and bioactive compounds offers a range of health benefits when consumed. The oil is also rich in lipophilic vitamins and antioxidants, such as carotenoids and tocopherols, which are easily absorbed by the body, supporting its nutritional value (Nagendran *et al*., 2015). The antioxidants help combat oxidative stress and protect cells from damage. These antioxidants may reduce the risk of chronic diseases such as cancer and cardiovascular diseases (Goh *et al*., 2020). Tocotrienols, particularly, have shown promise in protecting neurons and may prevent neurodegenerative diseases like Alzheimer's and Parkinson's (Aggarwal *et al*., 2018). Palm oil's balance of saturated and unsaturated fatty acids helps regulate lipid metabolism and support thermogenesis, which can aid in weight management and prevent obesity-related disorders (Nagappan *et al*., 2019).
Emerging research suggests that when consumed in moderation, palm oil can positively influence cardiovascular health and modulates immune response by enhancing interleukin (IL)-10 production and suppressing IL-6 levels in preclinical studies (Choo *et al*., 2021). However, concerns have been raised regarding the potential health effects of oxidized palm oil, particularly on renal functions. Some studies have explored the

impact of different forms of palm oil such as fresh, oxidized and thermally processed on kidney function indices and histopathology in Wistar rats, a common model for biomedical research.

Experimental studies in Wistar rats have highlighted the nephrotoxic potential of oxidized palm oil, which has been linked to disruptions in kidney function indices like serum creatinine and urea levels (Promise *et al.*, 2019). Moreover, histopathological examinations reveal structural alterations in renal tissues, including glomerular atrophy and tubular degeneration, suggesting potential long-term renal damage (Kola-Ajibade *et al.,* 2024). Conversely, some studies indicate that fresh palm oil may exert protective effects against nephrotoxicity by enhancing antioxidant defenses (Emmanuel *et al.,* 2021; Achuba and Ogwumu, 2014). Therefore, this study investigated the effects of palm oil on some kidney function indices and renal histopathology in Wistar rats, thereby contributing to the broader discourse on dietary fats and kidney health.

Materials and methods

Preparation and collection of the palm oil samples: Freshly milled palm oil sample (A) and palm oil samples (B-E) that were stored for sixteen months at room temperature, containing free fatty acid (FFA) levels of 0.4 %, 4.8 %, 8.4 %, 21.9 % and 42.7 %, respectively, were obtained from the Nigerian Institute for Oil Palm Research (NIFOR), Benin City, Edo State.

Animal grouping and administration of palm oil sample: Adult albino rats (Wistar strain) of weights 130 – 150 g of the female sex were selected for this study. The animals were obtained from the Animal House of the Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria. Thirty-six (36) rats were divided into six groups of six rats per group and treated as follows: Group I (normal control) given feed and water only. Group II (Experimental Control) was given freshly-milled crude palm oil containing 0.4 % FFA (sample A) while Groups III – VI were given stored palm oil samples B-E (4.8 %, 8.4 %, 21.9 % and 42.7 % FFA), respectively. Palm oil was administered orally to the rats at a dosage of 480 mg/kg body weight for four weeks. The animals were kept in clean cages in a 12-hour light/dark cycle room with daily litter change. They were acclimatized for two weeks before the experiment commenced and were fed with grower's mash and water *ad libitum*. The weights of the rats were monitored throughout the duration of the experiment. During the study, rats were maintained under standard conditions. The animals were euthanized in mild anaesthesia at the end of the treatment period of four weeks after an overnight fast and a portion of the blood was collected by cardiac puncture into plain sample bottles for biochemical analyses while the kidney was excised and used for histopathological examination.

Kidney function indices

Determination of serum creatinine levels: Creatinine level in the serum was assayed by the procedure of Bartels and Bohmer (1971).

Urea estimation: The level of urea in the serum was evaluated according to the method of Urease-Berthelot (Weatherburn, 1967).

Assay of serum electrolytes: Sodium ion level in the serum was assayed using Teco kit according to a modified procedure of Trinder (1951) and Maruna (1958). Also, the level of potassium ion and chloride ion was assayed using Teco kits according to the procedure of Teeri and Sesin (1958) and Skeggs and Hochestrasser (1964), respectively.

Histopathological examination of organs: The kidney was excised, blot-dried on tissue paper before preserving in 10 % buffered formalin solution and processed 48 hours later, through graded ethanol, followed by xylene and embedded in paraffin wax. Tissue sections (6 µm thick) were prepared and stained with hematoxylin-eosin (H & E). The stained sections were analyzed and photographed under a light microscope using ×400 magnification to ascertain histopathological properties (Drury and Wallington, 1980).

Data analysis: Data analysis was carried out using the statistical package for social science (SPSS) version 21.0. Results were expressed as mean ± SEM of six replicates. The levels of homogeneity amongst groups were tested using one-way analysis of variance (ANOVA) with $p < 0.05$ considered significant. Duncan's multiple range test was used to separate homogenous groups.

Results

Effect of palm oil on body and kidney weights of Wistar rats: It was observed that the control group exhibited an 8.54 g increase in body weight, rats fed with 0.4 % FFA had the most significant body weight gain (11.10 g) while groups exposed to moderate FFA levels (4.8 % and 8.4 %) exhibited reduced weight gain and notably, the

8.4 % FFA group showed the least weight gain (3.13 g). Interestingly, the highest FFA group (42.7 %) showed a moderate weight gain (7.69 g). Also, the kidney weight was highest in the 4.8 % FFA group (0.95 g) and lowest in the 0.4 % FFA group (0.84 g), while the kidney-to-body weight ratio followed a similar trend, with the normal control and 42.7 % FFA groups showing the highest ratios. Significant differences were observed between groups ($p < 0.05$). The results are depicted in Table 1.

Table 1: Mean values of the body weight and kidney weight of rats

Groups	Body Weights before Treatment (g)	Body Weights after Treatment (g)	Difference in Weights (g)	Weight of Kidney (g)	Kidney:Body Weight Ratio (g)
I – Normal control	136.50±0.11^{a}	148.06±0.14^{b}	8.54±0.05^{e}	0.8900±0.00^{c}	0.006000±0.00^{d}
II – 0.4% FFA	141.30±0.11^{c}	157.00±0.11^{e}	11.10±0.08^{f}	0.8400±0.00^{b}	0.005300±0.00^{a}
III– 4.8% FFA	149.50±0.11^{f}	161.60±0.11^{f}	8.06±0.06^{d}	0.9500±0.00^{d}	0.005800±0.00^{c}
IV – 8.4% FFA	140.30±0.11^{b}	144.70±0.11^{a}	3.13±0.08^{a}	0.8100±0.00^{a}	0.005500±0.00^{b}
V– 21.9% FFA	142.30±0.11^{d}	150.70±0.11^{c}	5.90±0.08^{b}	0.9000±0.00^{c}	0.005900±0.00^{d}
VI– 42.7% FFA	143.00±0.11^{e}	154.00±0.11^{d}	7.69±0.08^{c}	0.9400±0.00^{d}	0.006067±0.00^{d}

Values are in mean ± SEM (n = 6). Differences in superscript letters within a column indicate significant differences ($p < 0.05$) between groups. FFA= Free fatty acid.

Effect of palm oil on some kidney function indices of rats: There was a significant decrease ($p < 0.05$) in the serum creatinine and urea levels of rats fed palm oil of varied FFA levels (Groups II – VI) compared to the normal control group. Administration of palm oil led to a non-significant decrease in serum sodium and potassium levels of rats fed with stored palm oil (Groups III – VI) compared to the control rats. There was no significant difference ($p >0.05$) in the chloride levels of rats fed with palm oil of varied FFA levels (groups II-VI) compared to the normal control group. The results are depicted in Table 2.

Table 2: Kidney function indices of rats

Groups	Creatinine (mg/dL)	Urea (mg/dL)	Sodium (meq/L)	Potassium (mmol/L)	Chloride (mmol/L)
I (Normal Control)	5.98±0.38^{c}	9.58±0.00^{c}	140.76±11.32^{b}	8.67±0.28^{b}	98.17±3.64^{a}
II (0.4 % FFA)	5.67±0.19^{b}	9.37±0.04^{b}	132.40±8.49^{b}	7.90±0.28^{b}	98.78±11.63^{a}
III (4.8 % FFA)	5.00±0.57^{a}	9.51±0.04^{b}	117.40±7.15^{b}	7.92±0.12^{b}	98.58±3.99^{a}
IV (8.4 % FFA)	4.99±0.19^{a}	9.23±0.12^{a}	105.00±9.99^{a}	6.38±0.19^{a}	98.65±5.07^{a}
V (21.9 % FFA)	4.66±0.00^{a}	9.37±0.04^{b}	138.32±1.50^{b}	8.17±0.36^{b}	105.06±0.45^{a}
VI (42.7 % FFA)	4.00±0.00^{a}	9.30±0.08^{b}	123.74±10.49^{b}	6.23±0.47^{a}	100.11±1.87^{a}

Values are in mean ± SEM (n = 6). Data with the same superscript are not significantly different from each other ($p > 0.05$), while data with different superscript are significantly different from each other ($p < 0.05$). FFA= Free fatty acid.

Effect of palm oil on the histopathology of kidney: The effect of palm oil on the histopathology of the kidney of normal rat and those of rats fed with palm oil of varied FFA levels for a period of four (4) weeks is depicted in plates A - F. There was no observable change in the kidney glomeruli of rats fed with palm oil of varied FFA concentration (0.4 % - 42.7 %) compared to the normal control group.

Discussion

This study investigated the effects of different levels of free fatty acids (FFA) in crude palm oil on kidney function indices and renal histopathology in Wistar rats. The results from changes in body and kidney weights shows that the highest weight gain was recorded in the 0.4 % FFA group while the 8.4 % FFA group had the least weight gain. Also, kidney weight was highest in the 4.8 % FFA group and lowest in the 8.4 % FFA group. Nevertheless, kidney-to-body weight ratios remained stable. These outcomes suggest a potential threshold for FFA toxicity that could hinder normal metabolic processes. Notably, a significant reduction was observed in serum creatinine and urea levels across all palm oil fed rats compared to the control. The observed reductions in urea and creatinine may reflect enhanced renal clearance or reduced muscle catabolism probably due to the phytonutrients present in palm oil. These reductions suggest a potential adaptive or protective metabolic response. The findings stand in contrast to those of Promise *et al.* (2019), who linked thermally oxidized palm oil to elevated creatinine levels.

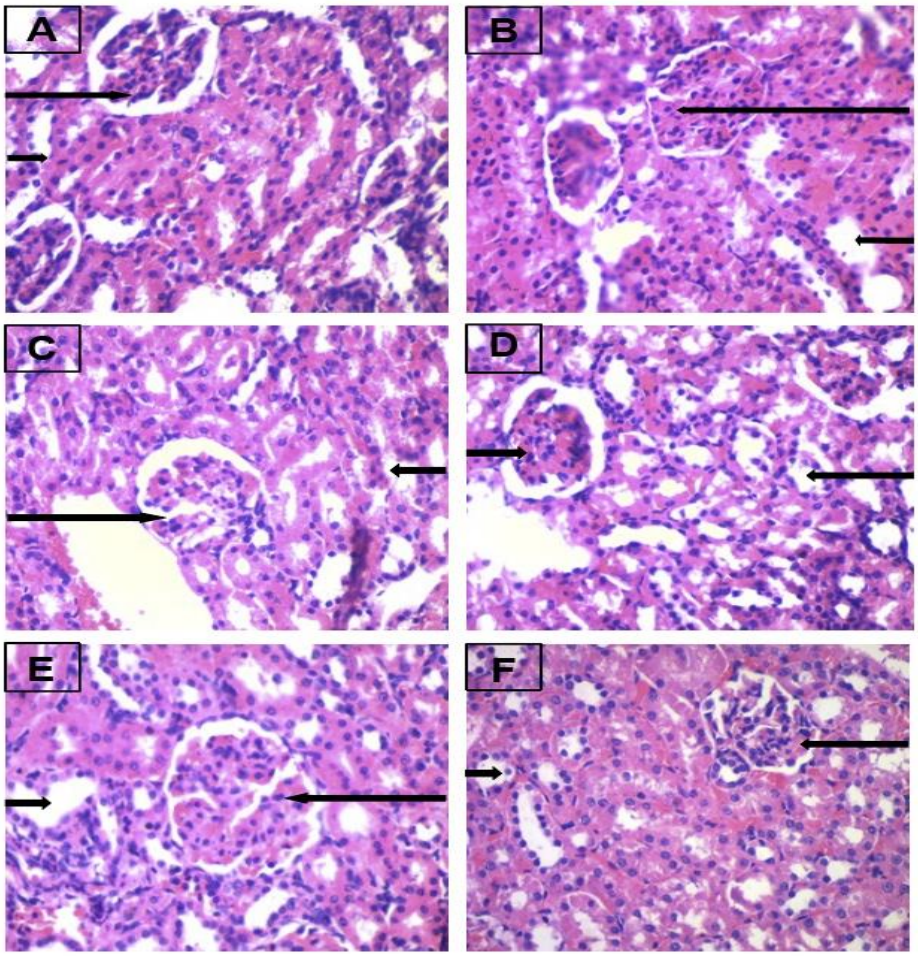

Figure 1: Sections of the kidney (A – F) representing Groups I – VI, respectively, showing normal glomeruli (long arrows) containing normal mesangium, blood vessels and epithelium. The tubules (short arrows) are oval shaped and lined by cuboidal epithelium with some tubules containing pale eosinophilic material. Features are in keeping with normal kidney, H&E ×400.

In addition, serum electrolyte levels were largely unchanged. Although sodium and potassium levels showed slight decreases, these changes were not statistically significant ($p > 0.05$). Similarly, chloride levels remained stable across all groups. This indicates that electrolyte regulation was not impaired by palm oil consumption, further supporting the idea that crude palm oil, even at high FFA concentrations (42.7 %), may not adversely perturb nephrotic function under short-term exposure.

Furthermore, histological examinations of kidney tissues across all groups revealed no structural alterations. There were no observable signs of glomerular distortion, tubular necrosis, or inflammatory infiltration. Sections stained with hematoxylin-eosin (H&E) showed well-preserved glomeruli and tubules, even at high (42.7 %) FFA levels. These results contrast prior findings that associated oxidized palm oil with renal structural damage (Kola-Ajibade *et al.*, 2024), thus, suggesting that unheated crude palm oil supports renal health. The difference in findings between this study and others may stem from the nature of the palm oil used. While previous works often utilized thermally oxidized or repeatedly heated oils which can degrade vital nutrients in the oil, this study employed naturally stored crude oil with beneficial bioactive compounds such as tocotrienols and carotenoids that may provide antioxidant defense (Achuba and Ogwumu, 2014; Emmanuel *et al*., 2021).

Conclusion

This study demonstrates that crude palm oil with FFA levels up to 42.7 %, does not impair kidney function or cause histopathological damage in Wistar rats. The findings suggest that freshly milled or stored palm oil may not pose immediate risks to renal health.

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Authors' Contribution

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. All authors have read and approved the manuscript.

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Conflict of Interest

No conflict of interest is associated with this work.

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