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Consumption of Heated Palm Oil and its Effect on Kidney in Rats

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Abstract: This research studied the effects of heated palm oil on serum creatinine and kidney histological changes in rats. Forty female Sprague-Dawley rats were divided into four groups (I to IV). Group I was fed with normal rats chaw (control). The groups II, III and IV were fed with rat chaw fortified with 15% weight/weight (w/w) fresh palm oil (FPO), heated five times palm oil (5HPO) and heated ten times palm oil (10HPO), respectively for a period of 16 weeks. Blood for serum creatinine was taken and determined at baseline and at the end of the study period using commercial kit and Cobas integra analyzer. The rats were sacrificed and the kidneys were weighed and examined histologically. The tissues were stained with haematoxylin and eosin. There was a significant reduction in the kidney's weight in 5HPO and 10HPO group compared to fresh and control group. At the end of 16 weeks there was a significant increase in serum creatinine in all study groups compared to the respective baseline. However, there was no significant difference in serum creatinine in the palm oil fed groups compared with control. Histologically, localized congestion was noted in the tubular region of the kidney in the group fed with FPO, while diffuse congestion was observed in the glomerulus and tubular region of the group fed with 5HPO and 10HPO. There was also evidence of chronic inflammation with lymphocytes infiltration located in the tubular region of the groups fed with 5HPO and 10HPO. The inflammatory changes were more severe in 10HPO compared to 5HPO group. In conclusion; heated palm oil diet reduced kidney's weight, causes tubular congestion and inflammation which were more severe in 10HPO compared to 5HPO. The histological changes were not accompanied by an increase in serum creatinine level.

Key words: Heated, palm oil, kidney, histology, inflammation

INTRODUCTION

The use of repeatedly heated frying oil is common in food industry. It takes place daily in domestic households or amongst the food vendors (Azman *et al*., 2012). Deep frying causes a series of chemical reaction which produces various products that include polymers and reactive oxygen species (ROS). Polyunsaturated fats are more susceptible to this reaction compared to mono-unsaturated or saturated fats. The fried food may absorb the harmful products and be apart of our diet. For example, reactive oxygen species (ROS) has been implicated in pathogenesis of cardiovascular disease (Chun *et al*., 2014; Adam *et al*., 2009; Leong *et al*., 2010), hepatotoxicity (Kamsiah *et al*., 2010, 2015), kidney disease (Totani and Ojiri, 2007), cancer (Dutta and Dutta, 2003) and bone (Shuid *et al*., 2007).

This study was conducted to see if the intake of heated palm oil has any detrimental effects on kidneys.

MATERIALS AND METHODS

Study design: Forty male rats of Sprague-Dawley species (200-250 g) were equally divided into four groups. The rats were given the following prescribed course of food: Group I-fed normal rats chaw diet (without any oil) as control; Group II, III and IV were fed rats chaw diet fortified with 15% weight/weight fresh palm oil (FPO), or heated 5 times palm oil (5-HPO) or heated 10 times palm oil (10H-PO), respectively for 16 weeks. The study was approved by the Institution Research Committee. The animals were kept in stainless steel cages (temperature of 27±2°C) and were acclimatized prior to the experiment. The animals had free access to food and tap water for 16 weeks. The food intake and body weight were taken four-weekly for 16 weeks. Blood was taken at the start of experiment and four weekly for measurement of serum creatinine. At the end of 16 weeks, the rats were killed and their kidneys were harvested and weighed. The organs were sectioned and formalin fixed. The sections were stained with haematoxylin-eosin. Histological examination was carried out using light microscopy.

Source and preparation of heated oil diets: The vegetable oils used were palm oil which was purchased from Lam Soon Edible Oils, Malaysia. The oils were used fresh, heated once or heated five times (as described by an earlier protocol by Owu *et al*. (1998). The heating process involved using 2500 ml of the

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vegetable oil to fry 1 kg of sweet potatoes in a metal wok. The temperature of the heated oil reached about 180°C and the cooking period lasted about 10 min. The oil was cooled for 5 h. The whole frying process was repeated with a new batch of sweet potatoes for five times or ten times to produce 5 times (5HPO) or ten times heated palm oil (10HPO) respectively. The normal rats chaw was obtained from Gold Coin (Malaysia). Fifteen percent (15%) weight/weight of the respective oils were mixed with grounded rat pellets. The pellets were reformed, dried in an oven at 70-90°C before using.

Measurement of serum creatinine: Serum creatinine was analyzed using commercialized kits by colorimetric assay technique using Cobas Integra 800 General Chemistry Analyzer (Mannheim Germany). This technique is based on Jaffe kinetic reaction which produces color. The color intensity is directly proportional to concentration of creatinine in the sample. The absorbance was measured at 512 nm.

Data analysis: The data was presented as the mean±S.E.M. Normally distributed data were analyzed using parametric tests, i.e., Student's t-test and ANOVA. Data which were not normally distributed were analyzed using non-parametric test, i.e., the Kruskal-Wallis, Mann-Whitney and Wilcoxon Signed Rank tests. A value of $p<0.05$ was considered to be significant. All statistical analyses were conducted using the Statistical Package for Social Sciences (SPSS) software.

RESULTS

Body weight: In the Fig. 1 there was a significant increase in body weight in all groups at the end of 16 weeks of study compared to baseline ($p<0.05$). In Fig. 2, the increase in body weight appeared to be significantly lower in 5HPO and 10HPO groups compared to control ($p<0.05$).

Weight of the kidney: Figure 3 illustrates that there was a significant reduction in the weight of the kidneys for 5HPO and 10HPO groups compared to FSO and control ($p<0.05$).

Serum creatinine: As shown in Fig. 4, there was a significant increase in serum creatinine at the end of 16 weeks of study in all groups compared to their respective baseline value ($p<0.05$). However, the was no significant difference in serum creatinine among the groups at the end of the study period. In Figures 5-8, there was a significant positive correlation between body weight and serum creatinine for control, FPO, 5HPO and 10HPO. There r values were 0.82, 0.86, 0.81 and 0.86, respectively with $p<0.05$.

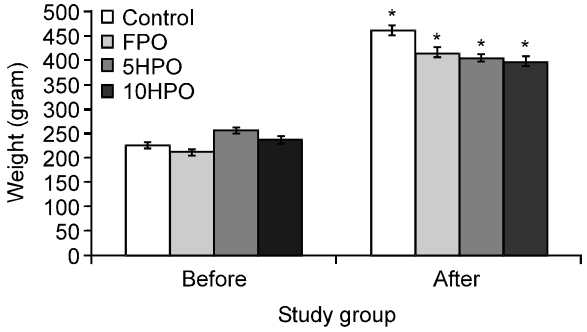


Fig. 1: Changes in body weight

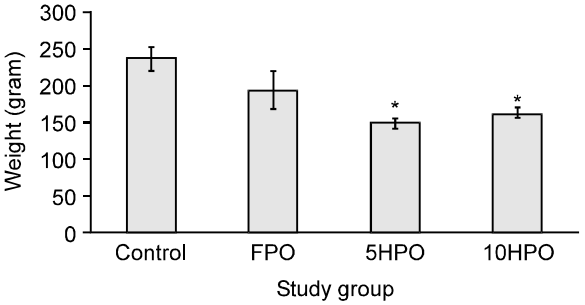


Fig. 2: Body weight

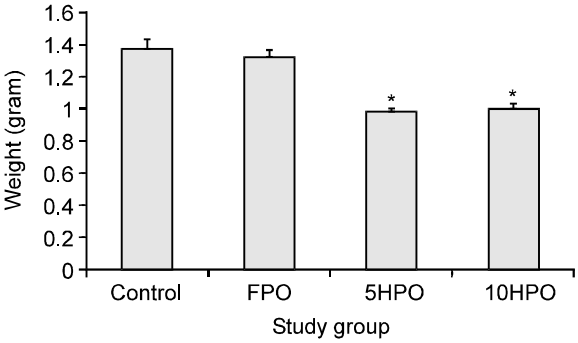


Fig. 3: Weight of the kidney

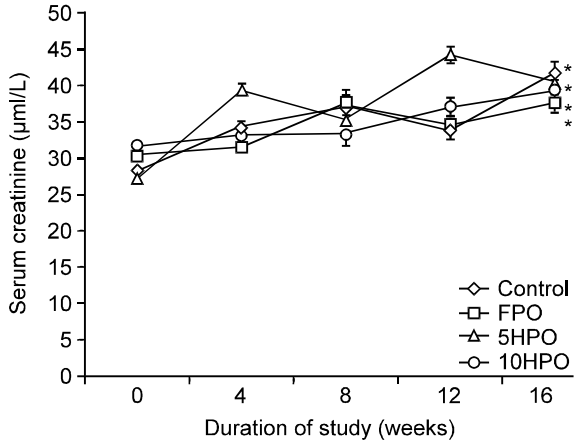


Fig. 4: Changes in serum creatinine with heated palm oil

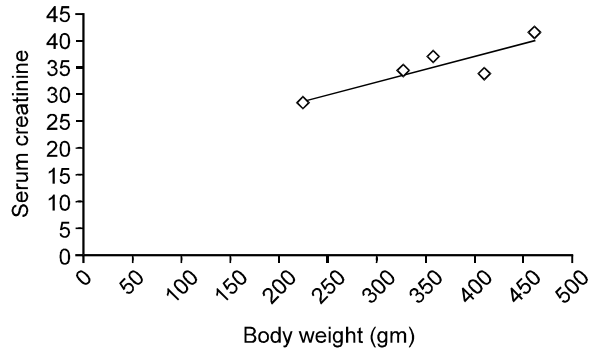


Fig. 5: Correlation of body weight and serum creatinine with control

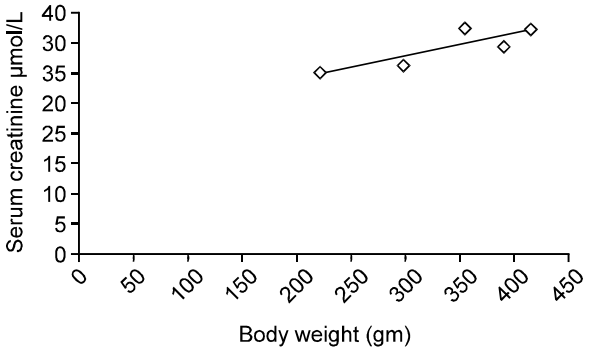


Fig. 6: Correlation of body weight and serum creatinine with FPO

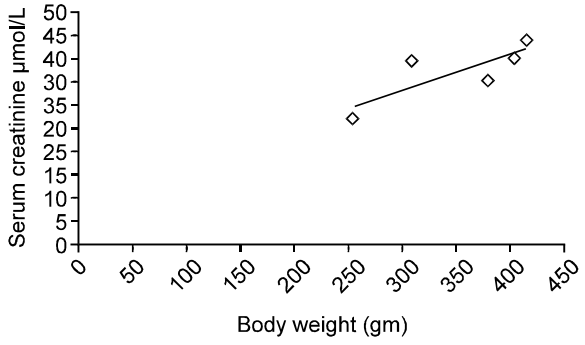


Fig. 7: Correlation of body weight and serum creatinine with 5HPO

Microscopic appearance of the kidney (Photomicrograph 1-4): There were congestion and inflammation noted in the glomerulus and the tubules of the 5HPO and 10HPO group compared to control. The degree of congestion and inflammation were more severe in 10HPO compared to 5HPO (Photomicrograph 2-4). There was minor congestion noted in FPO group (Photomicrograph 2-3).

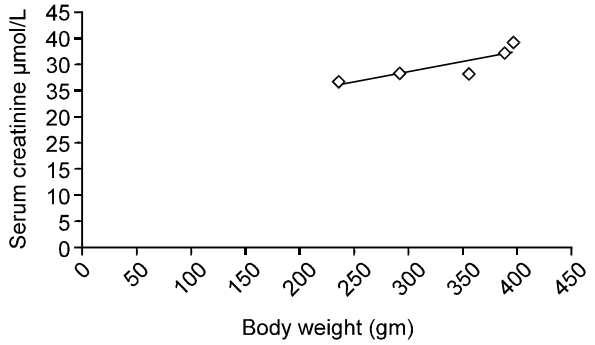
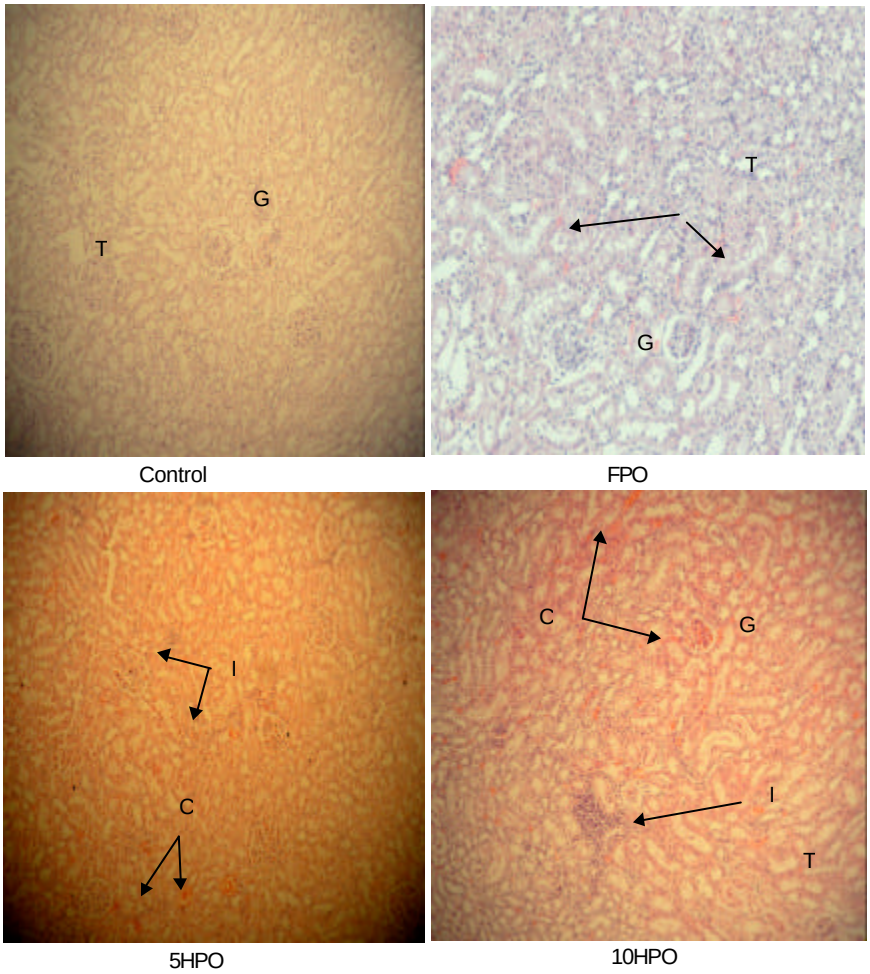


Fig. 8: Correlation of body weight and serum creatinine with 10HPO

DISCUSSION

In this study, there was significant increase in body weight at the end of the experimental period suggesting that heated oil did not interfere with the animal's growth. The increase in the body weight was smaller with heated palm oil groups compared to control. This finding was in accordance with Eder *et al.* (1999), who reported that oxidized oil reduced body weight. However, it was in contrast to Leong *et al.* (2010) who reported that heated palm increase body weight, while heated soy oil had no significant effect on body weight (Leong *et al.*, 2010). The reason for the discrepancy in the result was not clear. It thus appears that the type of oil used may be attributable for the difference observed. The smaller increase in body weight observed in the heated palm oil group in this study was likely due to smaller food intake. The smaller increase in food intake with heated oils were also reported by Adam *et al.* (2008a, b); Badlishah *et al.* (2013) and Hamsi *et al.* (2015). There is a possibility that oxidized oil may affect food quality and make it less palatable to the animals. Heated 5 times and ten times palm oil reduces kidney's weight. This finding was comparable to Isong *et al.* (2000) who reported heated palm oil reduced the weight of the kidney and lung. The effect of heated oil on organ weight in this study was in contrast to Totani and Ojiri (2007), Gabriel *et al.* (1976) that reported oxidized soy oil and rapeseed oil had no significant effect on kidney's weight, respectively. Similarly, the reason for the discrepancy in the result may be due to the difference in types of oil and animal used in this study. We used Sprague-Dawley rats while Totani and Ojiri (2007) and Gabriel *et al.* (1976) used Windstar rats. Fresh palm oil had minimal effect of renal histology with minimal inflammation and congestion. In contrast, heated 5HPO and 10HPO caused marked glomerular and tubular congestion and inflammation. The degree of congestion and inflammation were more severe with 10HPO compared to 5HPO. However, there was no obvious tubular destruction or atrophy noted in this study. This finding was not in accordance with

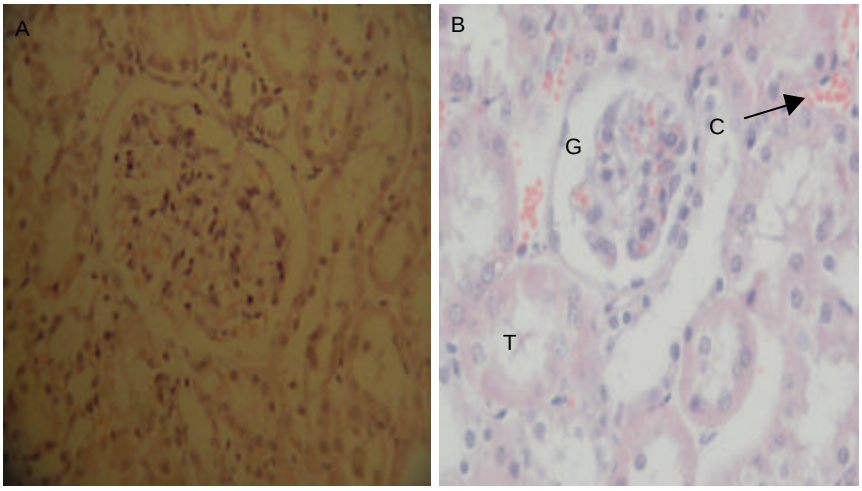


Control FPO

5HPO 10HPO

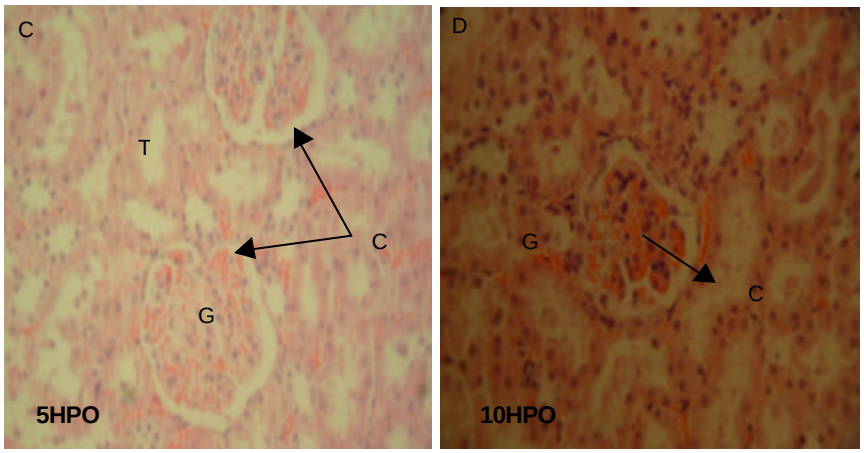
Photomicrograph 1: Cross sections of the rat's kidney with heated palm oil [Hematoxylin and Eosin (H and E), magnification x 100].

A-Control; B: Fresh Palm Oil (FPO); C: Five times heated palm oil (5HPO) glomerulus ➔ shows congestion (C) and inflammation (I); D: 10 times heated palm oil (10HPO) Tubular and glomerular sections ➔ show congestion (C) and inflammation (I). T: Tubule; G: Glomerulus, C: Congestion; I: inflammation.

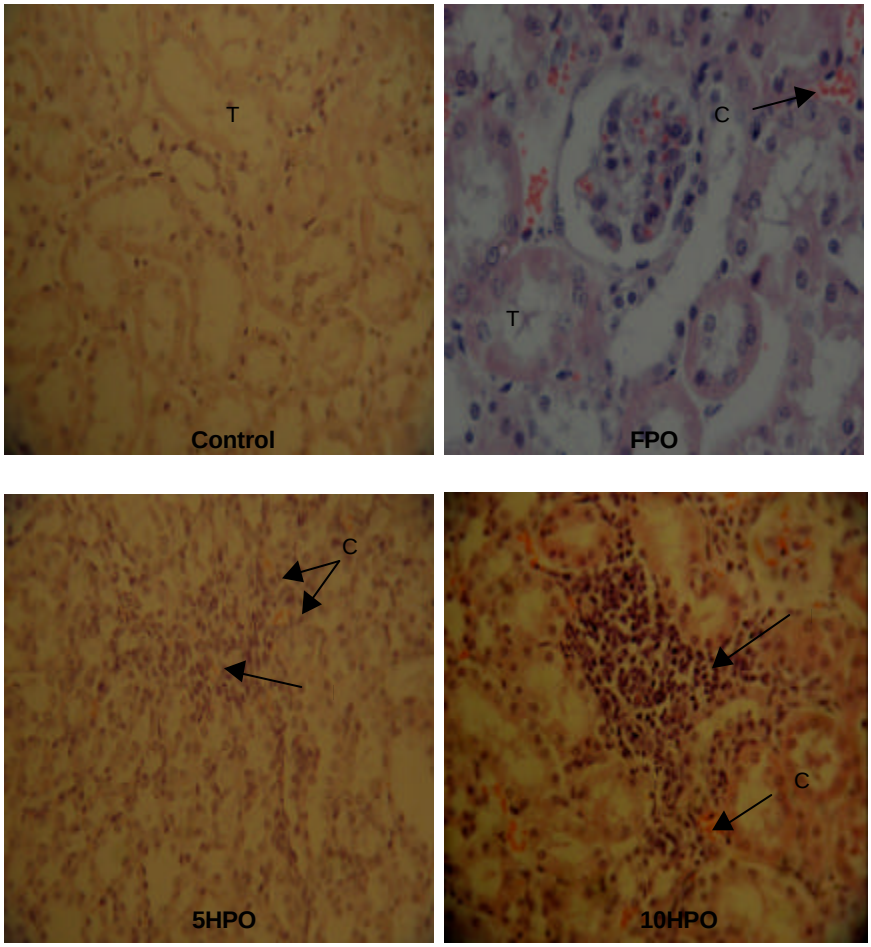


Control FPO

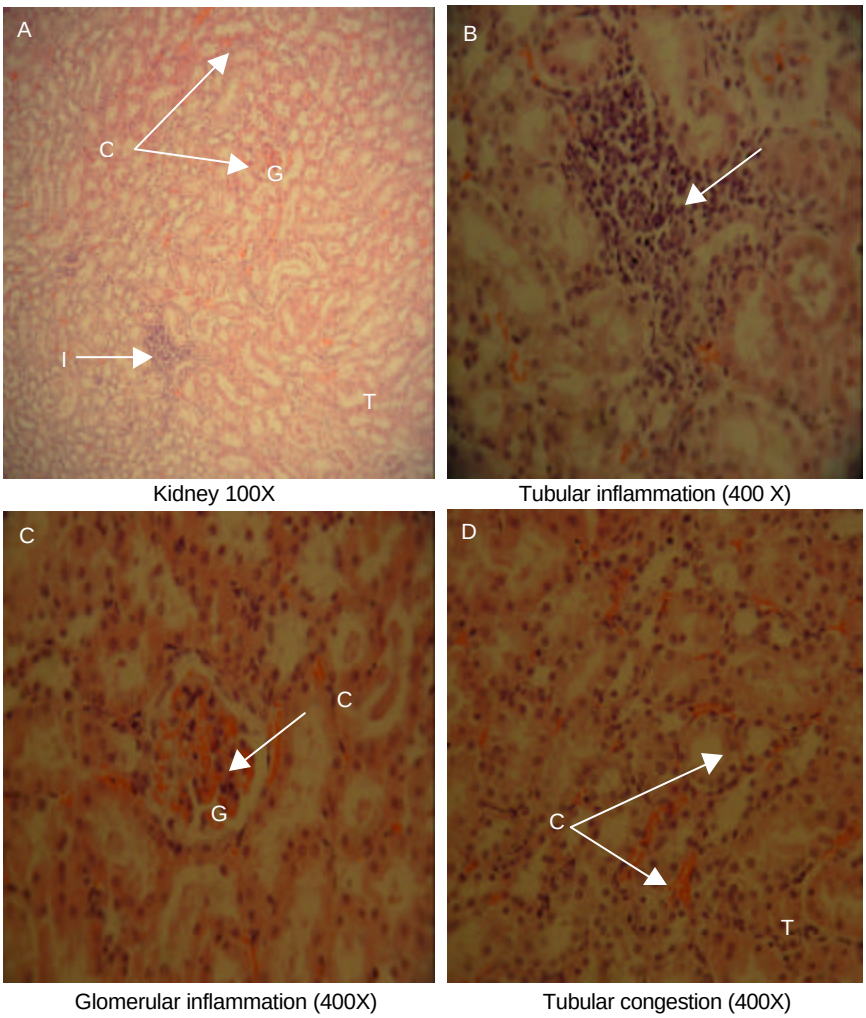
Photomicrograph 2 Continued:



Photomicrograph 2: Cross sections of the kidney with heated palm oil [Hematoxylin and Eosin (H and E), magnification 400]. A-Control (H&E, x100); B: Fresh Palm Oil (FPO (H & E, x400); C: Five times heated palm oil (5HPO) glomerulus ➔ shows congestion (C) [H & E, x400]; D: 10 times heated palm oil (10 HPO) Tubular section ➔ shows congestion (C) [H & E, x 400]. T: Tubule; G: Glomerulus, C: Congestion; I: inflammation.



Photomicrograph 3: Cross sections of the kidney with heated palm oil [Hematoxylin and Eosin (H and E), magnification 400]. A-Control (H&E, x100); B: Fresh Palm Oil (FPO (H & E, x400); C: Five times heated palm oil (5HPO) glomerulus ➔ shows congestion(C) and inflammation (I) [H & E, x400]; D: 10 times heated palm oil (10HPO) Tubular section ➔ shows congestion (C) and inflammation (I) [H & E, x 400]. T: Tubule; G: Glomerulus, C: Congestion; I: inflammation



Kidney 100X | Tubular inflammation (400 X)

Glomerular inflammation (400X) | Tubular congestion (400X)

Photomicrograph 4: Cross sections of the kidney for 10 times heated palm oil (10HPO): [Hematoxylin and Eosin (H and E), magnification x 100 and 400].
A-Cross section of the kidney (H&E, x100); B tubular section ➔ shows mark inflammation (I)(H & E, x400); C glomerulus ➔ shows congestion(C) [H & E, x400]; D Tubular section ➔ shows congestion (C) [H & E, x 400]. T: Tubule; G: Glomerulus, C: Congestion; I: inflammation

Gabriel *et al*. (1976), Alexander (1978), Totani and Ojiri (2007) and Ani *et al*. (2015) which reported that heated fat or oil were detrimental to kidney and caused renal damage. The reason for the discrepancy in this finding was unclear. Palm oil, being a mono-saturated oil may have less detrimental effects on the kidney compared to polyunsaturated soy oil as it produces less reactive oxygen species compared to unsaturated fat or oil. There was a significant increase in serum creatinine level at the end of 16 weeks of study in all groups compared to the respective baseline readings. However, there was no significant difference in serum creatinine among the groups at the end of study period. This finding suggests that heated palm oil had no detrimental effect on renal function. The increase in serum creatinine observed in this study was likely attributable to an increase in body mass as there was strong positive correlation between serum creatinine and body weight. The increase in body weight may indicate increase in muscle mass as protein intake and muscle mass affect serum creatinine as reported by Smith *et al*. (1998) and Tierny and Ojiri (2007). The effect of heated palm oil on serum creatinine and kidney in this study was in contrast with Ani *et al*. (2015), as they reported that heated palm oil for 6 months increase serum creatinine and sodium in rabbits. The reason for the differences in the results was unclear. It may appear that Winstar rats and rabbits were more susceptible to the harmful effect of heated oil compared to Sprague Dawley rats.

Conclusion: Heated palm oil causes glomerular, tubular inflammation and congestion of the kidney; however it has no detrimental effects on renal function.

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