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Assessment of Renal Sodium-Glucose Cotransporter 2 (SGLUT2) Expression and Glucose Levels in Blood and Urine after Chronic Consumption of Oxidized Palm Oil Diets

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Abstract **Original Research Article**

This study investigates the influence of consumption of oxidised palm oil on kidney tissue concentration of Sodium-Glucose Cotransporter 2 (SGLUT2) and glucose levels in blood and urine. A total of twenty (20) apparently healthy adult male wistar rats weighting between 140-160g were used in this study. 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% photo-oxidised palm oil (PPO) diet and 15% thermo-oxidised palm oil (TPO) diet respectively. The experiment lasted for 90 days. After the period of experiment, the animals were sacrificed under urethane anesthesia and blood samples were collected via cardiac puncture for determination of blood glucose level, while the kidneys were harvested for kidney tissue SLUT2 concentration. SGLUT2 was determined using immunohistochemistry method. The results revealed that there was a significant increase in blood and urine glucose levels of TPO-diet fed group when compared with the control group. Also, there was a significant decrease in the mean percentage expression of SGLUT2 in both thermally oxidized palm oil (TPO)-diet fed and photo-oxidized palm oil (PPO)-diet fed groups when compared with the control and fresh palm oil (FPO)-diet fed groups. These results suggest that oxidative modifications in palm oil may have an adverse impact on renal SGLUT2 expression. In conclusion, this study demonstrates that consumption of thermo-oxidized and photo-oxidized palm oil significantly decreases renal SGLUT2 expression, likely as a response to oxidative stress.

Keywords: Glucose, SGLUT2, Photo-Oxidised Palm Oil, Thermo-Oxidised Palm Oil.

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INTRODUCTION

Palm oil is an edible oil derived from the fruits of the oil palm and it's scientifically known as Elaeis guineensis (Siew, 2002). Crude palm oil is considered to be the richest natural source of carotenoids (about 15 times more than in carrots). The major carotenoids in palm oil are a- and b-carotene, which account for about 90% of the total carotenoids. It also contains tocopherols, and especially rich in *Y*- tocotrienol which are physiologically active as vitamin E and are useful antioxidants (Mukherjee S, Mitra, 2009). Palm oil is one of the major palm oil products that is domestically and industrially used as cooking/frying oil. The major advantage of palm oil is its high stability during frying that produced minimum amount of breakdown products in an acceptable level. A study conducted by Azmil and Siew, (2008) shows single-fractionated and double fractionated palm oil were more stable than high oleic sunflower oil after 80 hours of heating at 180°C. Palm oil tends to crystallize at low temperature that limits its usage in temperate countries. Thus, palm oil is not well considered as a recommended choice due to its higher

saturation content. However, removal of saturation is difficult due to the difficulty in controlling the crystallization (Calliauw *et al*., 2010). Other than that, blending palm oil with other soft vegetable oils such as canola oil, cottonseed oil, rice bran oil, sunflower oil, soybean oil etc. is implemented to reduce the saturation level of palm oil and for frying purposes in temperate countries (Razali and Noraini, 1994).

Thermo-oxidized palm oil (TPO) is a product that arises from the combination of heat and oxygen exposure, leading to chemical reactions that result in changes to the oil's composition, flavor, aroma, nutritional profile and consequently causing potential implications for human health and food quality (Jaarin *et al*., 2006). Studies have shown that 70% of the carotenes may be maintained after one deep fry, but after four deep fries, there may be virtually no carotenes left (Miglio *et al*., 2008; Demasse *et al*., 2007) and consuming products fried with it could be detrimental to health. Adam *et al*., (2008) showed that consumption of repeatedly heated palm oil (thermoxidized) increases lipid per oxidation generation of free radicals and reactive oxygen species (ROS) that are deleterious to health thus, causing oxidative stress. Oxidative stress is an imbalance between the systemic manifestation of reactive oxygen species (ROS) and the biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage (Weidinger and Kozlov, 2015). In other words, it is an imbalance between free radical and antioxidants in the body (2016). High oxidants level can cause damage to organ and tissues resulting in various abnormalities. Famurewa *et al*., (2017) have shown that chronic consumption of this form of palm oil causes damage to the tissues of the liver, lungs and kidney. It has also been shown to reduce Glomerular filtration rate (GFR) (Beshel *et al*., 2014) and alter the kidneys' ability to handle electrolytes (Beshel *et al*., 2018).

The sodium–glucose cotransporters (SGLUT) are a family of transporters from the SLC5 gene family that couple sodium to the transport of sugars most often glucose, galactose, and mannose and, in addition, myoinositol and short-chain fatty acids. Unlike the GLUTs that are facilitative glucose transporters and simply assist in moving glucose down a concentration gradient, the SGLUTs are capable of collecting and transporting glucose against a concentration gradient, using the electrochemical force of the sodium gradient to accomplish this. SGLUTs work in either proximal tubule cells or intestinal cells transporting glucose into the requisite cell at the luminal surface followed by GLUT-mediated transport of glucose into the bloodstream at the basolateral membrane (Hermansen and Mortensen, 2007). In the kidney, SGLUT2, and to a lesser extent SGLUT1, account for more than 90% and nearly 3%, respectively, of glucose reabsorption from the glomerular ultrafiltrate. Substrates bind in an ordered fashion with Na^+ binding first, followed by a conformational change that opens up the site for glucose to bind (Wright, 2001).

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*., (2018), 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. Kidneys were

harvested for weighing and immunochemistry.

Kidney Biochemicals Analysis

Preparation of kidney tissues for biochemical assays were carried out after excising the harvested tissues. The kidney tissues were rinsed using ice-cold 0.1M phosphate buffer and blotted with filter paper before weighing on electrical weighing scale to determine the weight. The kidney tissues were then minced with scissors in 10 volumes of ice-cold homogenizing buffer (0.1M phosphate buffer, pH 7.4 prepared from 35.822g Di-Sodium hydrogen phosphate (Na_2HPO_4) and 15.603g of NaH_2PO_4 in 1000ml of distilled water), and homogenized using a Tefflon homogenizer. The resulting homogenates were centrifuged using cold centrifuge at 4°C at 10,000g for 15minutes. The supernatants were collected and used for tissue biochemical estimations.

SGLUT2 Immunohistochemistry Study

Immunohistochemistry staining study was carried out using (SLC5A2; Cat no. E-AB-93255) monoclonal antibodies *in immunoperoxidase techniques as described by Hsu et al.,* (1981) with few modifications as in Oyagbemi *et al.,*(2017). *Briefly, t*o determine the expression of SLC5A2 protein expressions in the kidney tissues, fixed tissues were embedded in paraffin and 5µm thickness of it were sectioned on charged slides. These were subsequently deparaffinized in xylene and rehydrated with varying grades of alcohol (100% to 70%). Antigen retrieval was carried out by immersing the slides in citrate buffer at 95-100 ^{0}C for 25 minutes with subsequent peroxidase quenching in 3% H_2O_2/methanol solution. The sections were blocked in goat serum followed by a 2 hours incubation at 4^{0}C in the SLC5A2 primary antibodies. Detection of bound antibody was carried out using biotinylated (goat anti-rabbit, 2.0 µg/mL) secondary antibody and subsequently, streptavidin peroxidase (HRP-streptavidin) according to manufacturer's protocol (Elascience Biotechnology Inc., Houston, Teas). Reaction product was enhanced with DAB for 1-3 minutes and counter-stained with high-definition hematoxylin (Enzo, New York). The sections were subsequently dehydrated in ethanol, cleared in xylene. The slides were covered with coverslips and sealed with resinous solution. The immunoreactive positive expressions of SLC5A2 anti-rabbit intensive regions were viewed starting from low magnification on each slice then with 400× magnifications using a photo microscope (Olympus, Tokyo, Japan) and a digital camera (Toupcam, Touptek Photonics, Zhejiang, China). The immunoreactivity was quantified with ImageJ software version 1.51.

Urine Collection

Urine samples were collected through intravenous fluid administration method. This involves cannulating the jugular vein and urinary bladder of the rats and administering normal saline through the vein. This method is commonly used to provide fluids directly into the bloodstream, leading to increased urination as the excess fluid is processed by the kidneys. A small plain container is kept at the end of the cannulated bladder to collect the formed urine. One (1) ml of normal saline is infused per 1 hour.

Determination of Blood and Urine Glucose levels

The urine and blood samples were kept in a plain and Ethylene diamine Tetra fluoride (EDTA) containers. Blood and urine glucose was determined immediately after collection 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.

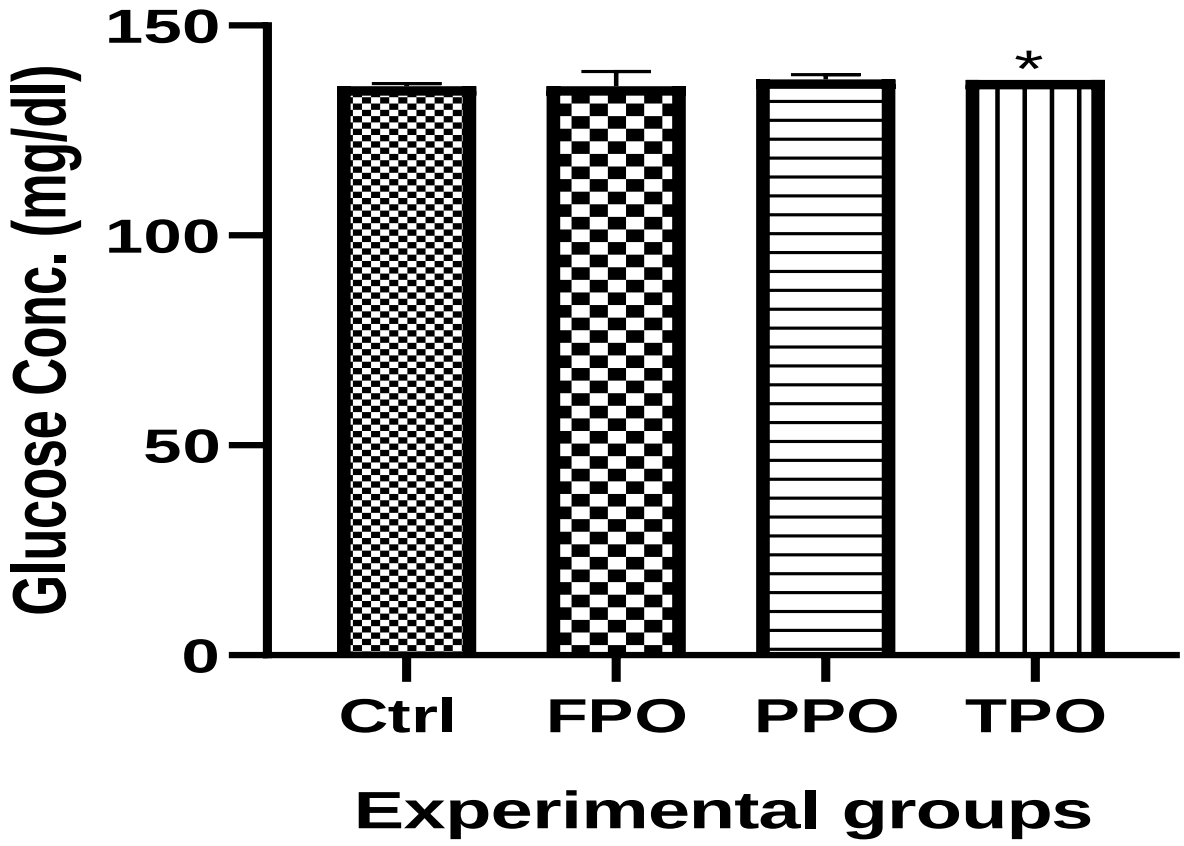
RESULTS

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

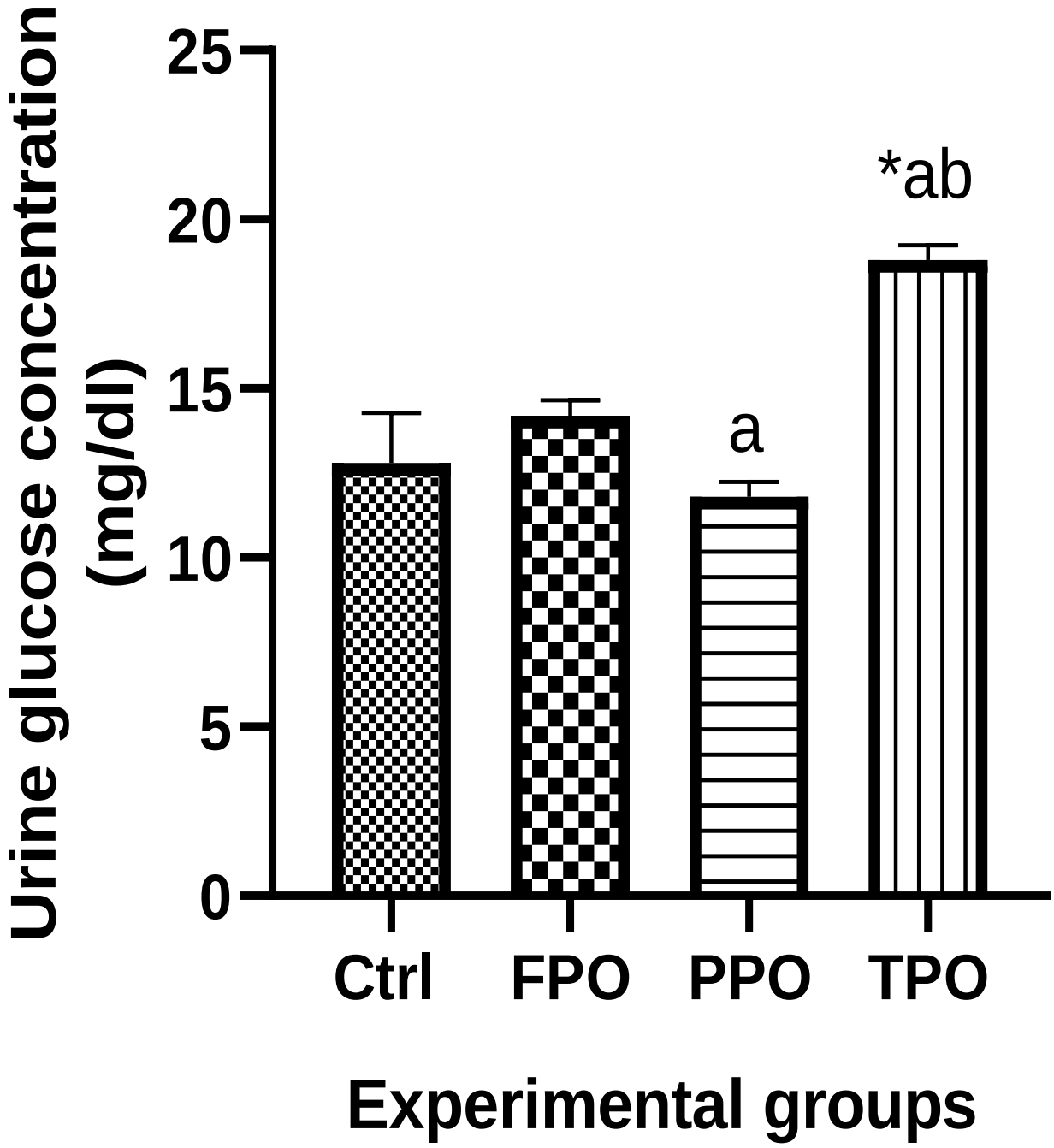
Accepted peroxide values for edible oils are between 10-20mEq O_2/kg. (Connell, 1975)

Figure 1: Effect of the different forms of palm oil on blood glucose level



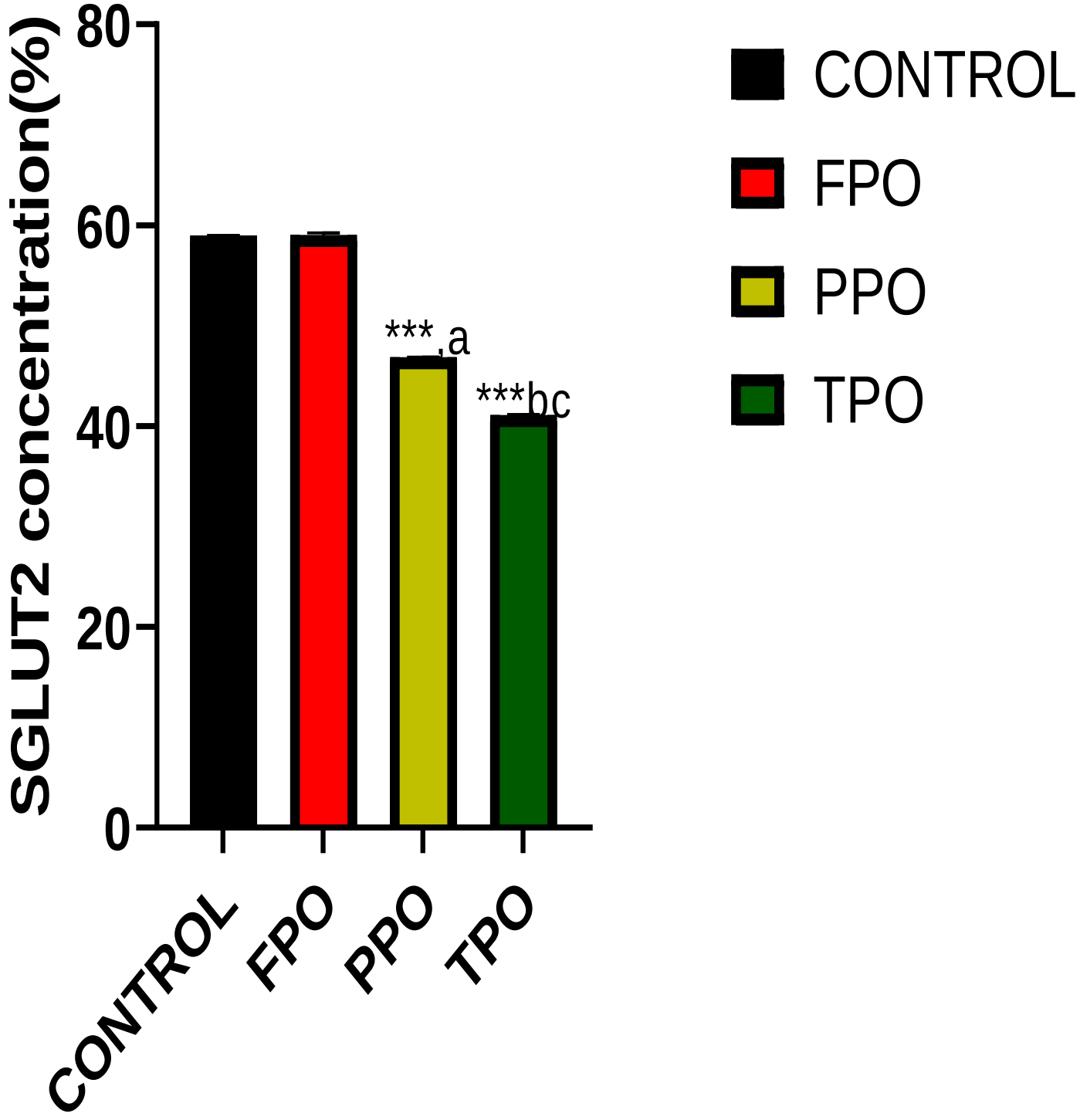
Glucose concentration in the different experimental groups
Values are expressed as mean +SEM, n = 5.
* = p<0.05 vs control

Figure 2: Effect of the different forms of palm oil on urine glucose level



Urine glucose 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 different forms of palm oil on percentage expression of SGLUT2



Sodium Glucose transporter-2 Concentration in the different experimental groups
Values are expressed as mean +SEM, n = 5.
* = p<0.001 vs control
a = p<0.001 vs FPO
b = p<0.001 vs PPO

Photomicrograph of SGLUT2 immunohistochemistry

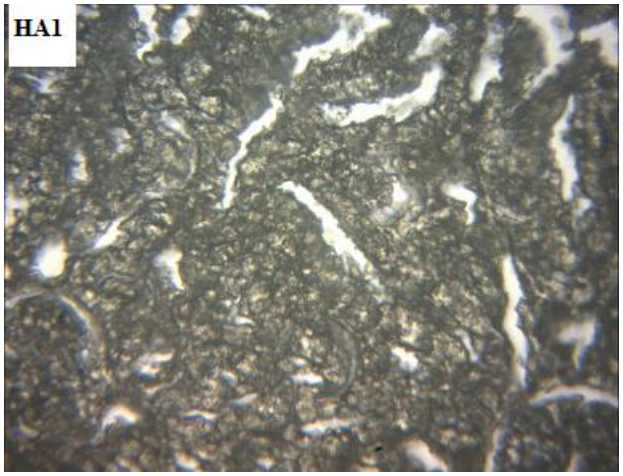


Plate 1: Control Group. HE x400

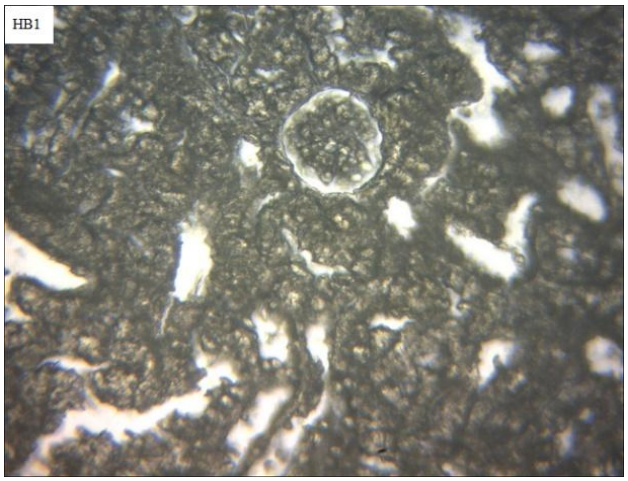


Plate 2: FPO Group. HE x400

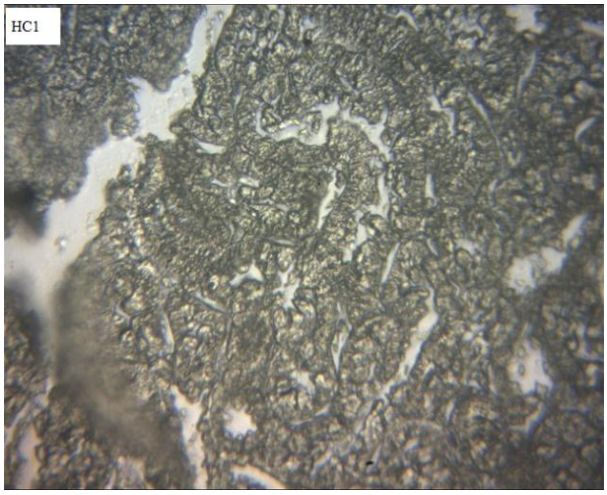


Plate 3: PPO Group. HE x400

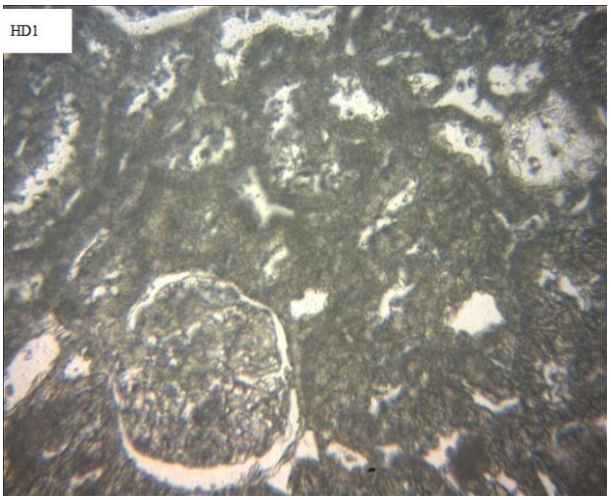


Plate 4: TPO Group. HE x400

DISCUSSION

This study examined the impact of consumption of thermo-oxidised palm oil (TPO) and photo-oxidised palm oil (PPO) diets on the renal expression of sodium glucose transporter 2 (SGLUT2), along with blood and urine glucose levels in Wistar rats. The results of the peroxide value (PV) of the different forms of palm oil showed 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 throughout the oxidation process. The frequency of oil reheating or its exposure to sunlight results in a higher peroxide index. However, in comparison to earlier studies, it was noted that soya oil exhibited a greater peroxide value when subjected to repeated heating under identical frying conditions (Leong *et al*., 2010).

A greater peroxide value signifies decreased chemical stability of the oil. Naghshineh *et al*., (2010) suggested that an elevated level of saturated fatty acids enhances the chemical stability of oils.

Our results indicate that the consumption of oxidized palm oil, especially TPO, leads to considerable changes in glucose handling and renal SGLUT2 expression, implying a possible mechanism for the impairment of renal glucose reabsorption.

Interestingly, rats that were fed TPO showed a notable increase in blood glucose levels when compared to the control group. This hyperglycemic reaction could be linked to reduced insulin sensitivity or dysfunction of the beta cells, conditions that are often related to damage caused by oxidative stress.

Additionally, the higher levels of urinary glucose found in the TPO group in comparison to the control, fresh palm oil (FPO), and PPO groups suggest a potential issue with the mechanisms responsible for renal glucose reabsorption.

The notable downregulation of renal SGLUT2 expression found in the TPO-fed group implies that oxidized lipids hinder the renal proximal tubules' ability to retrieve filtered glucose. SGLUT2 is responsible for reabsorbing more than 90% of glucose in the proximal tubules, so its suppression can result in glucosuria even when hyperglycemia is not present, although both conditions were elevated in this instance.

Notably, the PPO-fed group also showed a decrease in SGLUT2 expression, but this decline was less pronounced than that in the TPO group and did not correspond with a significant rise in blood or urine glucose. This suggests that variations in the extent of oxidation and the nature of oxidative stress might uniquely affect the expression and function of renal transporters. TPO, likely due to the increased formation of reactive lipid peroxides and aldehydes from higher temperatures, may have more substantial cytotoxic and regulatory effects on the synthesis or stability of transporter proteins.

These results align with earlier studies by Beshel *et al*., (2019) who noted the occurrence of renal glycosuria after prolonged consumption of diets containing oxidized palm oil. The findings also support previous research connecting dietary oxidized lipids to renal oxidative stress, disrupted glucose metabolism, and dysfunction of transporters (Chae *et al*., 2023; Njeim *et al*., 2023). The observed downregulation of SGLUT2 may indicate either adaptive or maladaptive responses to oxidative damage, potentially mediated through pathways involving transcription factors sensitive to oxidative stress or inflammatory cytokines.

Conclusively, the consumption of oxidized palm oil, particularly in its thermally oxidized state, results in elevated blood sugar levels, the presence of glucose in urine, and a reduced level of renal SGLUT2 expression in Wistar rats. These findings may indicate early changes in renal and metabolic processes that could either predispose individuals to or worsen diabetic and kidney-related conditions. Further investigations focusing on the underlying mechanisms are necessary to clarify the specific molecular pathways involved and to assess the long-term effects of dietary oxidized lipid intake on renal glucose regulation.

CONCLUSION

In conclusion, this study demonstrates that the consumption of oxidized palm oil, especially in its thermo-oxidized form, leads to hyperglycemia, glucosuria, and decreased expression of renal SGLUT2 in Wistar rats.

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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