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Original Research Article

Increased Levels of Renal Inflammatory Cytokines (IL-1β and IL-6) Affect ADH Concentration in Wistar Rats Fed Oxidised Palm Oil Diets

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Abstract

The aim of this study is to find out the effect of phenylhydrazine on creatinine clearance, hence GFR and the relationship between GFR and aldosterone. Sixteen 16 male Wistar rats weighing 200 – 250 grams were randomly divided into four groups namely: Group 1 – Normal control Group 2 - Hematinic group (Fes): fed normal rat chow + tap water + ferrous sulphate (using an oral gavage at 75mg/kg bw); Group 3 - Anemic -treated group (AFes): administered Phenylhydrazine (PHZ) intraperitoneally for two consecutive days to induce anemia at a dose of 40mg/kg bw + normal rat chow + tap water + ferrous sulphate at 75mg/kg bw. Group 4 (Anu) – Anemic control group: administered Phenlyhydrazine (PHZ) intraperitoneally at a dose of 40mg/kg of bw + normal rat chow + tap water (as in group one). After 15 days, blood and urine samples were collected into sterile sample bottles for analysis. There was a significant ($P<0.01$, $P<0.01$, $P<0.05$) increase in aldosterone levels between Anu, control, Fes and AFes respectively. There was a significant ($P<0.001$) decrease in control compared with Anu. There was also a significant ($P<0.01$, $P<0.001$) decrease in Fes with AFes and Anu. Anu creatinine clearance was also significantly ($P<0.001$) lower than AFes. Phenylhydrazine intoxication led to a reduction in creatinine clearance and an increase in aldosterone levels, confirming a negative correlation ($r= 0.9956$, $P<0.01$) between aldosterone and creatinine clearance. Also, ferrous sulphate tends to reduce the extent to which aldosterone levels increased hence narrowing the margine and or reducing the significance of the correlation.

Keywords: Creatinine Clearance, Aldosterone, Phenylhydrazine, Glomerular Filtration Rate, Oxidative Stress, Kidney.

INTRODUCTION

Palm oil is an edible vegetable oil obtained from the mesocarp (reddish pulp) of oil palm fruit (Elaeis guineensis). It contains essential nutrients such as Vitamin E, carotenoids, and fatty acids, making it a nutritious choice for cooking and various food products (Ani *et al.*, 2015). Apart from being used as an ingredient in a variety of products, it is also used in the making of margarine and shortening (Mukherjee *et al.*, 2009). Food producers choose palm oil because it has a distinct quality, requires little or no hydrogenation and prolongs the shelf-life of different products (Mukherjee *et al.*, 2009). Like all fats, palm oil is made up of fatty acids that have been esterified with glycerol. It contains a particularly high percentage of saturated fat, primarily the 16-carbon saturated fatty acid palmitic acid, after which it is named (Ahsan *et al.*, 2015). A lot of monounsaturated oleic acid and tocotrienol, the most potent type of vitamin E is also found in abundance unrefined palm oil. It is also rich in carotenoids and is known to have about 15 times more carotenoids than carrots and 300 times more than fresh tomatoes (Mukherjee, *et al.*, 2009). Tocotrienol's therapeutic effects are extensively reviewed and are reported to have antioxidant, anti-diabetic, nephroprotective, neuroprotective, anti-cancer, anti-osteoporosis, gastroprotective, hepatoprotective, cardioprotective, immunoregulatory, lipid-lowering and anti-inflammatory effects (Medvedev and Medvedeva, 2018; Ranasinghe, *et al.*, 2022; Zainal *et al.*, 2022).

In Nigeria and most parts of West Africa, palm oil is a major constituent of most traditional dishes because it is not only the most widespread vegetable oil, but also cheap and readily affordable (Akusu, *et al.*, 2000; Frank *et al.*, 2011; Almeida *et al.*, 2013). Unfortunately, though, it is eaten in its oxidized form. This is because most of the open market oil which is erroneously referred to as "fresh" palm oil, is photoxidized (Beshel *et al.*, 2019). Apart from this, it is usually used in its thermoxidized form to fry several rounds of various roadside foods which are patronized by people from all works of life (Ebong *et al.*, 1999). It is thermally oxidized when the fresh form is subjected to heating at high temperatures and at different time intervals. Generally, palm oil is thermally oxidized to increase its palatability, and this has been a usual practice in most homes. (Ani *et al.*, 2015) However, thermal oxidation has a deteriorative effect on dietary oils. Oil oxidation, for example, can produce free radicals and reactive oxygen species, which contribute to oxidative stress in the body. Oxidative stress refers to elevated intracellular levels of reactive oxygen species (ROS) that cause damage to lipids, proteins and DNA. Oxidative stress has been linked to a myriad of pathologies. However, elevated ROS are also signaling molecules i.e. redox biology that maintain physiological functions. (Scheiber and Chandel, 2015) This has been associated with several chronic diseases (del Campo *et al.*, 2023). Palm oil has been known to destroy tissues (Osim *et al.*, 1994), cause hemolysis (Mesembe *et al.*, 2004), decrease biliary secretion, alter biliary electrolyte concentrations (Obembe *et al.*, 2011), increase fecal electrolyte concentrations (Obembe *et al.*, 2012) reduce GFR and increase blood pressure (Beshel *et al.*, 2014) alteration of some hematological parameters (Ani *et al.*, 2015 derangement of potassium levels (Beshel *et al.*, 2018, Beshel and Beshel, 2019). So, when these oxidized oils are consumed, they lead to the accumulation of reactive oxygen species in the body thereby causing oxidative stress.

Inflammation is one of the end products of oxidative stress. It is part of a complex biological response of vascular tissues to harmful organisms (Soomro, 2019). Inflammatory cells release several reactive oxygen species (ROS) at the site of inflammation leading to exaggerated oxidative stress (Biswas, 2016). On the other hand, an increase in the number of reactive oxygen species can initiate intracellular signalling cascades that enhance proinflammatory gene expression (Flohe, *et al.*, 1997). It must be noted that ROS are not always dangerous. This is one of the natural ways in which phagocytic cells fight microorganism invasion. The phagocytic cells themselves release these reactive oxygen species to kill invading agents. (Fialkow, *et al.*, 2007). We have scavengers in the body that can rid the body of these ROS after they have served their purpose. However, when external sources of ROS combine with the body's own, such as is the case with chronic consumption of oxidised palm oil, it overwhelms the body's ability to scavenge leading to oxidative stress.

It has already been established that the disorders listed above are because of oxidative stress because of chronic consumption of oxidised palm oil. But is there also inflammation, considering the already explained relationship between inflammation and ROS? Does long term consumption of oxidised palm oil cause inflammation? If yes, how does it affect the kidneys? What happens to the levels of renal inflammation markers with prolonged consumption of oxidised palm oil?

The aim of this study therefore is to find out what happens to renal inflammation markers such as IL6, IL-1β and c-reactive protein during prolonged consumption of oxidised palm oil diets, and how this may affect anti diuretic hormone levels in circulation.

MATERIALS AND METHODS

Acquisition and Preparation of Different Forms of Palm Oil

Palm oil was 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. This was done to prevent oxidation. The palm oil was divided into three portions. The first palm oil portion was kept fresh and kept in a dark container. The photo-oxidized palm oil was prepared by exposing the second portion of fresh palm oil to sun light for 5 hours daily for 15 days. (Beshel *et al.*, 2018), The thermo-oxidized palm oil was prepared by exposing the third portion of fresh palm oil to five rounds of heating for 10 minutes each. After each round of heating, the palm oil was made to cool down before reheating at 190°C. This was to mimic what is used in frying akara, yam, etc.

Formulation of Palm Oil diets

The palm oil (fresh palm oil, photo-oxidised and thermo-oxidised palm oil) diets were formulated as previously described by Beshel *et al.*, (2018). Briefly, 15g of fresh, photoxidised and thermoxidized palm oil were mixed with 85g of rat chow to make 15% fresh, photoxidised and thermoxidised palm oil diets. This is the average percentage palm oil composition of a typical palm oil diet in Nigeria (Osim, *et al.*, 1996).

Animals

A total of twenty (20) healthy adult male Wistar rats weighting between 140-160g were used in this study. The animals were kept 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 allowed to acclimatize for two weeks before the

commencement of the experiments. All the animal experiments were done in accordance with the guidelines of the Faculty of Basic Medical Sciences Ethical Committee on the use of laboratory animals. (approval no. 296PHY3724). Animals were housed in separate labelled metabolic cages. They 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-oxidized palm oil (PPO) diet. Group 4 were fed with 15% thermo-oxidized palm oil (TPO) for 90 days.

Collection of Samples

Tissue Samples

The animals anaesthetised with 60mg/kg pentobarbital and sacrificed. Blood samples of the animals were collected via cardiac puncture using 5ml syringes attached to 21G needles. A middle abdominal incision was made anteriorly, and the kidneys were excised. The kidneys were trimmed of connective tissues and rinsed with normal saline to remove excess blood.

Determination of Cytokines

Laboratory Determination of Tissue Concentrated of C-Reactive Protein (C-RP)

The kidneys were properly rinse in ice cold PBS (0.01M, pH=7.4) to thoroughly remove excess blood and then weighed. The tissues were then minced to small pieces and homogenized with a micro tissue grinder in fresh PBS buffer (tissue weight (g): PBS (mL) volume=1:9). The homogenates were centrifuged for 5-10 minutes at 5000 x g at 2-8°C and the supernatant collected and assayed immediately using rat C-RP ELISA kit (Cat: E-EL-R0506). C-RP concentrations in the samples were calculated using the OD curve (www.elabscience.com). All procedures were done as described by Elabscience Biotechnology and following standard laboratory rules and precautions.

Laboratory Determination of Tissue Concentrated of Interleukin 6

The kidneys were properly rinsed in pre-cooled PBS to completely remove excess blood and then weighed. The tissues were then minced to small pieces and homogenized with a micro tissue grinder in fresh PBS lysis buffer (900 µL lysis buffer was added in 100 mg tissue sample). The homogenates were centrifuged for 5 minutes at 10000 x g and the supernatant collected and assay immediately using rat IL-6 ELISA kit (Cat: ELK1158). IL-6 concentration in the samples was calculated by comparing the OD of the samples to the standard curve (www.elkbiotech.com). procedures were done as described by ELK Biotechnology and following standard laboratory all principles.

Laboratory Determination of Tissue Concentrated of Interleukin-1 beta (IL-1B)

The kidneys were properly rinse in ice cold PBS (0.01M, pH=7.4) to thoroughly remove excess blood and then weighed. The tissues were then minced to small pieces and homogenized with a micro tissue grinder in fresh PBS buffer (tissue weight (g): PBS (mL) volume=1:9). The homogenates were centrifuged for 5-10 minutes at 5000 x g at 2-8°C and the supernatant collected and assay immediately using rat IL-1β ELISA kit (Cat: E-EL-R0012). IL-1β concentrations in the samples were calculated using the OD curve (www.elabscience.com). All procedures were done as described by Elabscience Biotechnology and following standard laboratory rules and precautions.

Determination of ADH

Blood samples were collected via cardiac puncture with a 10ml syringe and emptied into well labelled sample bottles and then allowed for 1 hour to clot at a room temperature of 2-8°C after which, they were centrifuged for 20 minutes at 1000 × g at 2-8°C. The supernatant was collected and immediately froze and stored at -80oC until when they were used for ADH assay. ADH was assayed using rat ADH ELIZA kit (Cat: E-EL-R0522).

Statistical Analyses

Statistical analysis: GraphPad prism version8.0.2(263) was used for the analysis of data. ANOVA was used for analysis of Data followed by Turkeys adhoc test. The results were expressed as mean ±SEM.

RESULTS AND DISCUSSION

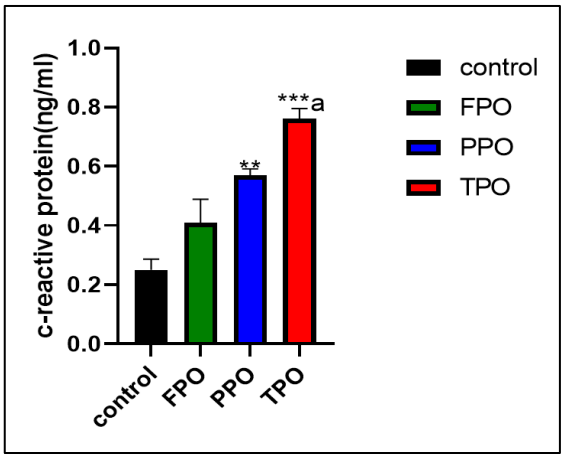

Fig. 1: The mean ±SEM concentrations of c-reactive protein in the control, FPO, PPO and TPO groups of animals

Fig. 1 shows the comparison of mean ±SEM concentrations of C- reactive proteins (C-RP) in the renal tissue of control, FPO, PPO and TPO animal as follows: 0.25±0.036; 0.41±0.071; 0.57±0.021; 0.76±0.035 respectively. There was no significant difference C-RP

between the control and FPO, but there was a significant (P<0.01, P<0.001) increase when the PPO and TPO were respectively compared with the control and a significant increase in C-RP when TPO was compared with FPO.

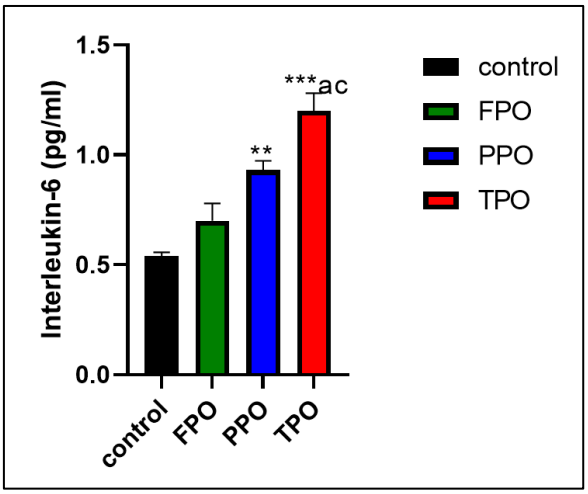

Fig. 2: The mean±SEM concentration of Interleukin-6(IL-6) in the control, FPO, PPO and TPO groups of animals

Fig. 2 shows the comparison of the mean±SEM of the IL-6 in the renal tissue of the control (0.54±0.017), FPO (0.70±0.037), PPO (0.93±0.043) and TPO (1.2±0.081) groups. When the FPO was compared with the control group, there was no significant difference, but when the PPO and TPO were compared to the control group, there was a significant (P<0.01, P<0.001respectively), increase in IL-6. FPO IL-6 was also significantly (P<0.001, P< 0.05) higher than FPO and PPO.

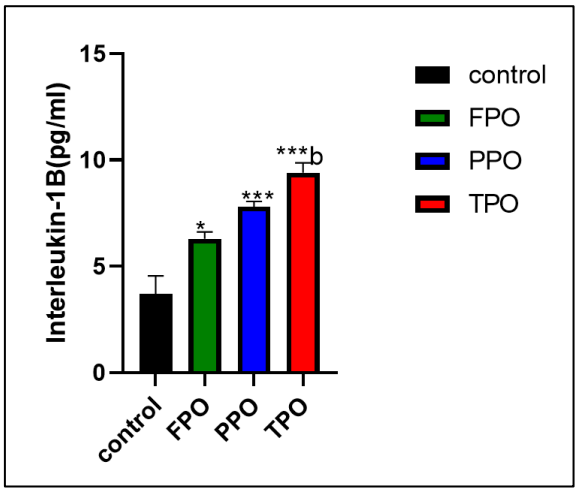

Fig. 3: The mean ±SEM concentration of Interleukin-1β (IL-1β) in the control, FPO, PPO and TPO groups of animals

Fig. 3 shows the comparison of the mean±SEM concentration of IL-1β between the control, FPO, PPO and TPO groups. They were: 3.7±0.86, 6.3±0.32, 7.8±0.2 and 9.4±0.47 respectively. There was a significant (P<0.05; 0.001, P<0.001) increase in FPO, PPO and FPO respecticely, when compared with the control. There was a significant (P<0.01) increase in IL-1β when TPO was compared with FPO, but no significant difference between TPO and PPO.

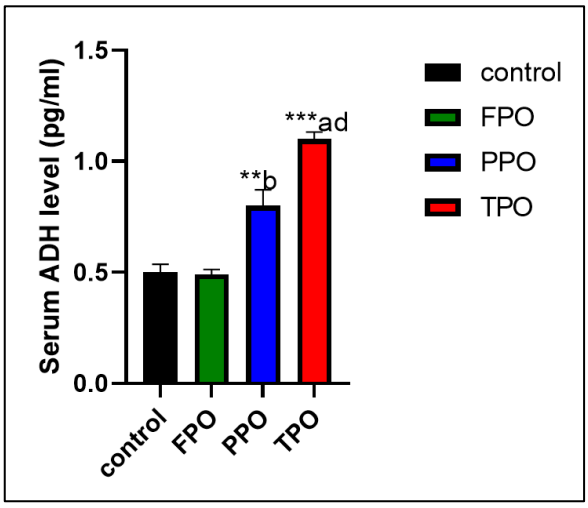

Fig. 4: The mean ± SEM ADH levels in the serum of control, FPO, PPO and TPO groups of animals

Fig.4 shows the comparison of the mean±SEM concentration of antidiuretic hormone (ADH) between the control, FPO, PPO and TPO groups. They were: 0.5±0.037, 0.49±0.023, 0.8±0.072` and 1.1±0.032 respectively. There was no significant between the control and FPO groups, while there was a significant (P<0.01; P<0.001) increase in PPO, TPO respectively when compared with the control. When PPO and TPO were compared with FPO, there was a significant (P<0.01, P<0.001 respectivelty) compared with the control. There was a significant (P<0.001, P<0.01) increase in ADH when TPO was compared with FPO and PPO respectively.

DISCUSSION AND CONCLUSSION

The results from this study showed that there was generally no significant difference in C-RP, IL-6, IL-1β and ADH when FPO and control were compared while there were very significant increases in all the inflammatory cytokines and ADH in PPO and TPO when compared with the control and between TPO and PPO. However, there was a significant difference in IL-6 and ADH when TPO was compared with PPO.

These results show from first hand observation that the FPO had little or no oxidants while the PPO and TPO had oxidants in varying degrees with the composition in TPO being greater than that of PPO.

Many studies have reported that accumulation of reactive oxygen species can overwhelm the body's natural ability to fight these oxidants and the result is oxidative stress which leads to destruction of tissues.

Reactive oxygen species can also initiate intracellular signalling cascade that enhances pro-inflammatory gene expression (Biswas, 2016) to cause inflammation. Many authors have observed a relationship between oxidation and inflammation (Park and Shin, 2013; Soomro, 2019). There is also a link between inflammation and hyponatremia which is closely related to the levels of antidiuretic hormone levels (Swart, *et al.,* 2011). On the relationship between oxidative stress and inflammation, Biswas stated that inflammatory cells release several reactive oxygen species at the site of inflammation leading to exaggerated oxidative stress. On the other hand, reactive oxygen species can initiate intracellular signalling cascade that enhances pro-inflammatory gene expression and hence inflammation. So, one leads to a worsening of the other (Biswas, 2016). The release of reactive oxygen species can also lead to the secretion of inflammatory cytokines. The cytokines are tissue specific, even though some of them may overlap. In the kidneys, the cytokines released are IL-1β, IL-6, C-RP and tissue necrotic factor. In this study, however, we studied IL-1β, IL-6 and C-RP.

The increase in the concentrations of these cytokines in our study clearly shows that there was inflammation of the kidneys of PPO and TPO rats. Previous studies have shown a link between inflammation, hyponatremia and ADH. Swart, *et al.,* (2011) showed that IL-1β and IL-6 are involved in the development of hyponatremia associated with inflammatory conditions and that this process is related to ADH secretion. Meanwhile, Palin *et al.,* (2009) had earlier reported that IL-1β stimulated both central and peripheral release of vasopressin/ADH in rats. In our study however, we considered the relationship between inflammation and ADH. ADH values were seen to increase significantly in the groups of rats fed oxidised palm oil diets. And because IL-1β levels were also high in those animals, the rise or increase in ADH in the oxidised palm oil fed groups, may be attributed to IL-1β.

Many years ago, Mastorakos *et al.,* (1994) had shown that AVP levels rose two hours after injection of IL-6 into the brain, suggesting that it stimulates the magnocellular neurons responsible for AVP release. Also, circulating IL-6 can be transported across the blood brain barrier to stimulate ADH release. In our study, there was a rise in IL-6 levels in the oxidised palm oil fed groups of animals. And the rise in this cytokine was obvious especially in the TPO group of animals. This rise coincided with the extent to which ADH also rose in circulation. Since IL-6 can cross the blood brain barrier (Mastorakos *et al.,* 1993), there may be two explanations to this. It is either that the IL-6 released by the kidneys must have been released into the circulation or the same oxidative stress leading to inflammation that took place in the kidneys may have taken place in the brain tissues to cause the release of IL-6. This might be the case sine in earlier reports by Osim *et al.,* 1994), it was reported that there were structural deformities in several tissues owing to the chronic consumption of thermoxidized palm oil diets.

It would therefore appear that interleukin 6 played a greater role in the stimulation of ADH release than interleukin 1β and C-reactive proteins, even though Langraf *et al.,* (1995) IL-1β could stimulate both peripheral and central release of ADH.

CONCLUSION

We therefore conclude that prolonged consumption of oxidised palm oil diets causes inflammation of the kidneys and can lead to an increase in secretion of IL-1β, IL-6 and C-RP. The rise in ADH seen in our study must have been triggered by these cytokines.

This therefore further confirms earlier studies. The novelty in our study, however, is that all these complications can be caused by prolonged consumption of oxidised palm oil.

Therefore, prolonged consumption of palm oil should be discouraged especially in societies like ours where it is the most readily available vegetable oil.

RECOMMENDATION

If we must consume palm oil, we can combine it with foods and /or vegetables that are high in antioxidant content. This will help combat the deleterious effects of the oxidised palm oil.

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