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Chronic Consumption of Thermoxidized *Palm Oil* Diet (TPO) Adversely Affects Haemostatic Status and Histology of Some Organs in Rabbit

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Original Research Article

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Abstract: This study seeks to investigate the effects of chronic consumption of fresh palm oil (FPO) and thermoxidized palm oil (TPO) diets on indices of haemostasis and related organs. Eighteen male rabbits weighing 750–1000g were randomly divided into 3 groups (n=6), namely; Control, FPO fed group and TPO fed group. The control group received normal feed+water, FPO fed group received normal feed mixed with FPO in the ratio 85:15g respectively, while TPO received normal feed and TPO in the ratio 85:15g respectively. The feeding lasted for 6months, after which indices of haemostasis were assessed. Platelet count for TPO group was significantly lower compared with control ($p<0.05$) and FPO group ($p<0.001$). Bleeding time in the TPO group was significantly higher ($p<0.05$, $p<0.001$) when compared with control and FPO respectively. Clotting time for TPO group was significantly higher ($p<0.01$) compared with control, but significantly lower ($p<0.05$) in FPO group compared with control. Prothrombin time for TPO group was significantly higher ($p<0.001$) compared with FPO group, whereas PT was significantly lower ($p<0.05$) in FPO group compared with control. Photomicrograph of the liver in the TPO group showed extensive diffuse steatosis and progressive inflammation with hepatocellular necrosis, while the bone marrow in TPO group showed infiltration with adipose tissue and reduction in the number of blood forming cells. Chronic consumption of thermally oxidized *palm oil* predisposes to thrombocytopenia secondary to infiltration of the bone marrow with adipose tissue and reduction in the number of blood forming cells. It also increases bleeding time, clotting time and prothrombin time and hence may predispose to prolonged haemorrhage.

Keywords: Bleeding time, Bone marrow, Clotting time, Hemostasis, Liver, Prothrombin time.

INTRODUCTION

Oil palm is a tropical plant that grows in warm climates at altitudes below 500 meters above sea level. It comes from the gulf of Guinea in West Africa, hence its scientific name *Elaesis guineensis*. It is popularly called the African oil palm and *palm oil* is obtained from the pulp of the fruits of this tropical tree [1]. *Palm oil* is obtained from the fruit of the palm tree [2, 3]. Researchers have documented a lot about the nutritional and health attributes of palm oil. However, some of these findings have been contradictory as some say it is beneficial to health while others see it as harmful. Overall, the beneficial effects appear to be skewed in favour of fresh *palm oil* over thermally oxidized palm oil. Fresh palm oil, which some refer to as red *palm oil* [4] is the largest natural source of tocotrienol which is the most potent form of vitamin E [5]. The combination of *tocotrienols*, carotenes and other antioxidants makes *palm oil* a super antioxidant [6] that is relatively stable to oxidation [7, 8]. In addition to its antioxidant action, *tocotrienols* strengthen the immune system and protect skin cells from toxins and ultraviolet radiation [9]. They have also demonstrated remarkable anticancer properties, far superior to most other antioxidants. They do not only prevent cancer from taking hold, but actively block its growth and initiate apoptosis [6].

Oils are generally thermally oxidised to render them highly palatable. However, this thermoxidation is said to have deteriorative effects on the body [10, 11]. Some researchers have previously shown that uncontrolled thermal oxidation of fats and oils leads to formation of peroxides and other products which are known to be very reactive, cytotoxic and destructive to tissues [12, 13]. The oxygen derived free radicals and dihydroxy esters contained in thermally oxidised *palm oil* have been demonstrated to cause injury to cells [14]. Long term consumption of oxidised oils and fats have been said to cause growth retardation, anaemia, thrombosis, fatty liver, essential fatty acid deficiency and nucleic acid deactivation of key metabolic enzymes [12, 15, 16]. In addition, the very reactive free radical species that are generated have been implicated in the

aetiology of diseases such as cancer, atherosclerosis, diabetes, arthritis and cataract formation [17].

Haemostasis is the arrest of bleeding from an injured blood vessel. It involves the combined inputs of vascular, platelet and plasma factors. The vascular factor reduces blood loss from trauma through local vasoconstriction (an immediate reaction to injury). Various mechanisms, including endothelial cell nitric oxide and prostacyclin, promote blood fluidity by preventing platelet stasis and dilating intact blood vessels. These mediators are lost when the vascular endothelium is disrupted. Under these conditions, platelets adhere to the damaged tunica intima of the blood vessel and form aggregates (platelet plug). Plasma coagulation factors interact to produce thrombin, which converts fibrinogen to fibrin. The fibrin produced helps to strengthen the clot [18]. Haemostasis therefore, is essential for survival. Alteration in platelet count, blood clotting factors and distortion in the integrity of blood vessels could lead to abnormal haemostasis.

Following the potential health hazards associated with consumption of thermally oxidized oils, this study seeks to compare the effects of chronic consumption of fresh *palm oil* (FPO) and thermally oxidized *palm oil* (TPO) diets on indices of haemostasis.

MATERIALS AND METHODS

Experimental Animals

The design of this study involved the use of eighteen, male, five months old New Zealand rabbits weighing between 750 to 1000g at the onset. The animals were obtained from the animal house of the Department of Pharmacology, University of Calabar, and kept in the animal house of the Department of Physiology, University of Calabar. The animals were divided into three (3) groups of six (6) rabbits each. The animals were kept in separate cages and allowed to acclimatize for 2 weeks. They had access to food and water *ad libitum,* and exposed to normal room temperature.

Preparation of *Palm Oil* Diets

Palm oil was purchased from a local oil mill in Odukpani, Cross River State, Nigeria. The oil was divided into two equal parts. One part was used as fresh *palm oil* (FPO) while the other part was subjected to heat to give thermally oxidised *palm oil* (TPO). Thermoxidation of the *palm oil* was done as described Osim *et al.* [13], and used by Ani *et al.*, [16, 19, 20] Fresh *palm oil* diet was prepared by mixing 15 g of fresh *palm oil* with 85 g of feed while thermoxidised *palm oil* diet was prepared by mixing 15 g of thermoxidised *palm oil* with 85 g of feed [19, 20]. The feeding period lasted for 6 months, after which the animals were used for the various experiments. Same experiments were done on the life animals while some were done after sacrificing the animals as described below.

Collection of Blood Sample

After 6 months of feeding, the animals were anaesthetized with chloroform after an overnight fast. Blood was collected into heparinized bottles for estimation of platelet count. A full automatic blood cell counter (Model PCE 210, Japan) was used for estimation of platelet count.

Determination of Bleeding Time

Bleeding time was measured by the method of Duke [21], as described by Ghai [22]. This method takes the bleeding time as the time elapsed between the moment of escape of blood outside the vessel and the moment of cessation of blood flow. A quick deep prick was made with a lancet on the shaved, dorsal aspect of the animal's ear. Before the prick, the area was cleaned gently with cotton wool and methylated spirit to ensure asepsis. The time the prick was made was noted. A filter paper was used to touch the puncture site every 30 seconds, without pressing or squeezing the wound. A different part of the filter paper was used to dab the blood each time. This process was continued as the blood spots gradually got smaller until they disappeared altogether. This point was noted and it corresponded to when bleeding stopped. The blood spots were then counted and divided by two. This gave the bleeding time in minutes.

Determination of Clotting Time

The clotting time was measured by the capillary method of Wright [23], as described by Ghai [22]. The principle here is to observe the time taken for a fibrin thread to develop in a standard capillary tube filled with blood. The method involved cleaning a shaved area on the dorsum of the animal's ear with methylated spirit and making a deep prick with a lancet. Immediately blood oozed out, the time was noted and recorded. The blood was immediately drawn into a capillary tube by placing one end of the tube on the drop of blood. The tube was placed tilted downwards so that the blood could easily flow in. When the tube was almost filled (about 10 cm), it was removed. After two minutes, small lengths of the tube were snapped off at 30 seconds intervals. Initially, the blood column broke cleanly but after sometime, a thick strand or coagulated blood column was seen stretching between the broken ends. The time when this strand appeared was noted. The total time from when blood started to flow to when the fibrin strand appeared was taken as the clotting time.

Determination of Prothrombin Time

This was done by the one-stage method of Quick [24], as described by Ghai [22]. Plasma samples from the animals were collected and emptied into bottles containing sodium citrate. The citrated blood was centrifuged without delay. The supernatant plasma

was then removed and placed in a clean glass tube after which 0.1ml was delivered into the bottom of a tube measuring 75x10 mm. This tube was already in a water bath at 37°C. A volume of 0.1 ml of rabbit brain thromboplastin was then added to it. After one minute, 0.1 ml of warmed 0.025 M calcium chloride was added and the contents of the tube gently mixed. A stop watch was started and the tube was held with its lower end submerged. The tube was continuously but gently inclined from the vertical to just short of the horizontal so that its contents could be observed for the first signs of clotting. Development of a fibrin clot marked the end point. The test was repeated three times and the average reading taken as the prothrombin time.

Histological Studies

Permanent preparations using routine biopsy method was employed. Tissue sections were treated with haematoxylin and eosin stains. The tissue blocks from the liver and bone marrow were fixed in 10% neutral formalin after which they were dehydrated using alcohol and then cleaned in xylene. They were then embedded in paraffin wax and thin sections cut at five microns. The sections were then stained with haematoxylin for 15 minutes, differentiated with 1% acid alcohol, counter stained in eosin for two minutes and mounted with DP X biological mounter. The sections were then viewed under the microscope (X 100) and photomicrographs taken.

Statistical Analysis

The results are presented as mean ± SEM. The data was analysed using one–way analysis of variance (ANOVA), followed by the post hoc multiple comparison test (LSD). Values of $p<0.05$ were considered significant. Computer software SPSS (version 17.0) and excel analyser (Microsoft office, 2010 version) were used for the analysis.

RESULTS

Comparison of platelet count in the different experimental groups

Figure 1 shows platelet count in the control, FPO and TPO fed groups to be 371.67 ± 25.13, 437.67 ± 14.09 and 283 ± 31.74x10^3/mm^3, respectively. Platelet count for TPO group was significantly lower compared with control ($p<0.05$) and FPO group ($p<0.001$). Platelet count was significantly lower ($p<0.001$) in TPO group, compared with FPO group (Figure 1).

Comparison of bleeding time in the different experimental groups

Bleeding time for control, FPO and TPO group was 1.62 ± 0.22, 1.08 ± 0.05 and 2.25 ± 0.18 minutes, respectively. Bleeding time in the TPO group was significantly higher ($p<0.05$) compared with control. Bleeding time was significantly reduced in FPO ($p<0.05$) group compared with control. Bleeding time in TPO group was significantly higher ($p<0.001$) compared with FPO group (Figure-2).

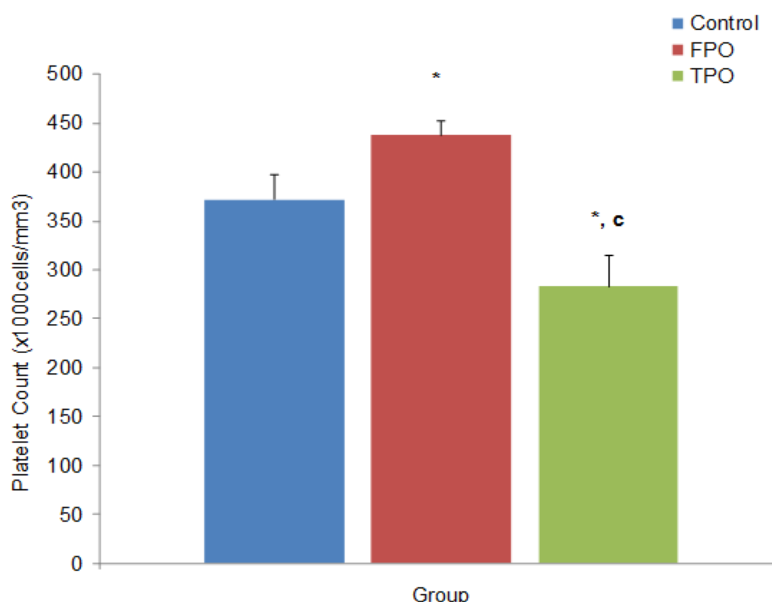

Fig-1: Comparison of platelet count in the different experimental groups
Values are mean ± SEM, n = 6
*p<0.05 vs Control; c = p<0.001 vs FPO

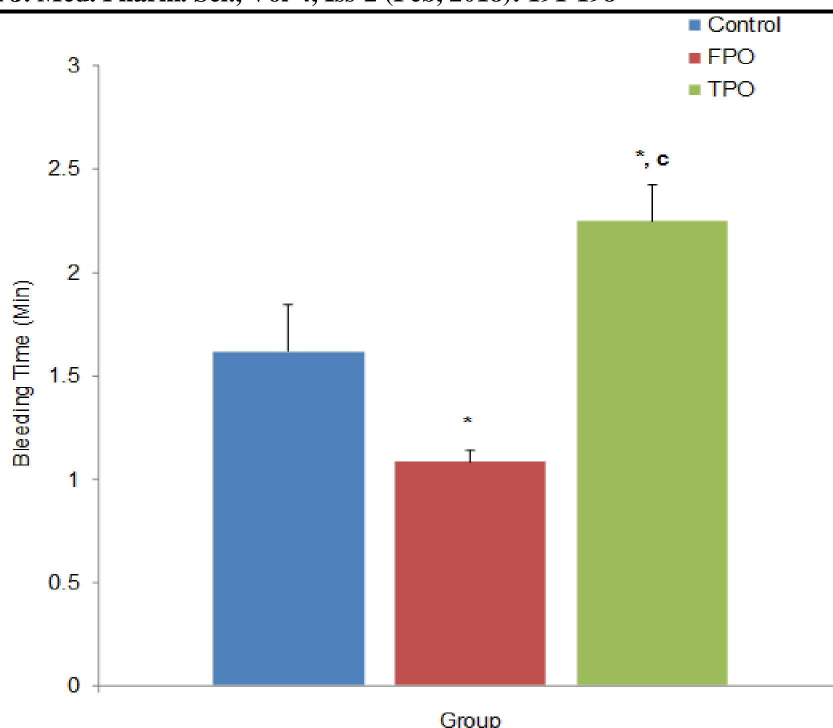

Fig-2: Comparison of bleeding time in the different experimental groups

Values are mean ± SEM, n = 6.

*p<0.05 vs Control; c = p<0.001 vs FPO.

Comparison of clotting time in the different experimental groups

Figure 3 shows that the clotting time for control, FPO and TPO group was 1.25 ± 0.07, 1.05 ± 0.05 and 2.45 ± 0.26 minutes, respectively. Clotting time for TPO group was significantly higher (p<0.01) compared with control, but significantly lower (p<0.05) in FPO group compared with control. Clotting time for TPO group was significantly higher (p<0.001), compared with FPO group (Figure 3).

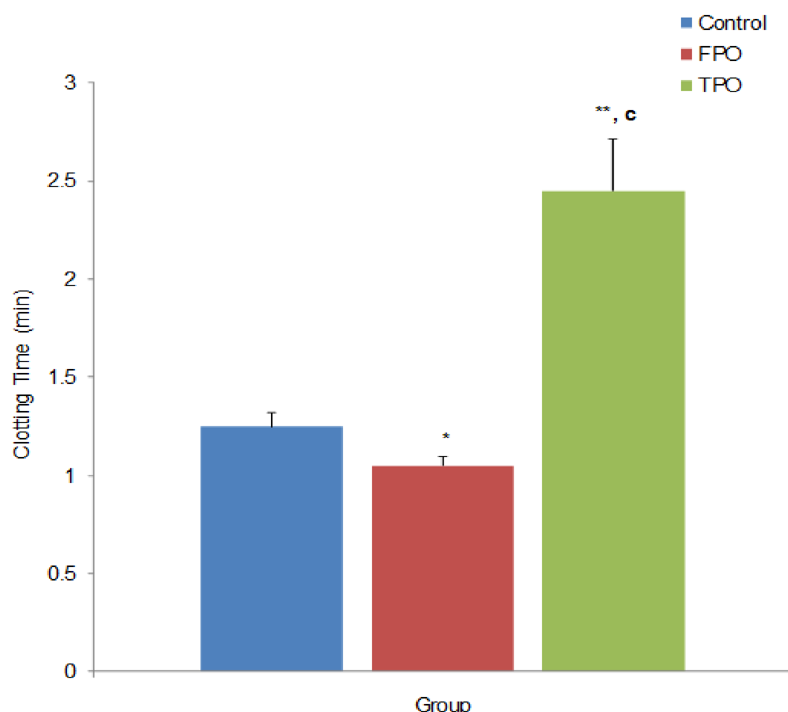

Fig-3: Comparison of clotting time in the different experimental groups

Values are mean ± SEM, n = 6.

*p<0.05; **p<0.01 vs control; c = p<0.001 vs FPO.

Comparison of prothrombin time in the different experimental groups

Figure 4 shows that prothrombin time for control, FPO and TPO group was 56.17 ± 4.14, 42.0 ± 3.19 and 69.67 ± 3.08 seconds, respectively. Prothrombin time for TPO group was significantly higher ($p<0.05$) compared with control, but significantly lower ($p<0.05$) in FPO group compared with control. Prothrombin time for TPO group was significantly higher ($p<0.001$) compared with FPO group (Figure-4).

Comparison of photomicrograph of the liver in the control, FPO and TPO group

Plate 1 show photomicrographs of the liver in (a) control (b) FPO and (c) TPO groups. The control group shows a normal liver architecture displaying the central vein (CV) and limiting plate (LP). The hepatic lobules and portal tracts are normal. The FPO group shows areas of diffuse steatosis (DS). There is no necroinflammation and the limiting plates are intact. The TPO group shows extensive diffuse steatosis (fatty liver). There is progressive inflammation with hepatocellular necrosis (HN). The limiting plates are destroyed in many places.

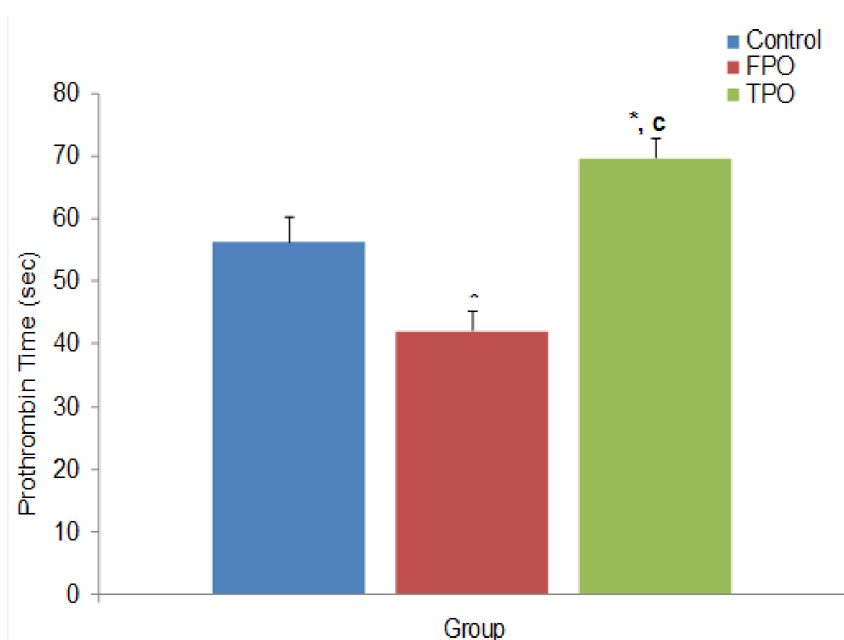

Fig-4: Comparison of prothrombin time in the different experimental groups

Values are mean ± SEM, n = 6.

*p<0.05 vs Control; c = p<0.001 vs FPO.

Photomicrograph of the bone marrow in the control, FPO and TPO group

Plate 2 show photomicrographs of the bone marrow in the (a) control, (b) FPO and (c) TPO group. The control group shows the normal blood forming cells (BFC) and adipose tissue (AT). The myeloid-erythroid (M.E) ratio is normal (i.e. 1:1). The FPO group is also normal with normal cytoarchitecture and myeloid-erythroid ratio of 1:1. The TPO group shows infiltration of the bone marrow with adipose tissue (AT) and reduction in the number of blood forming cells (BFC). The ratio of adipose tissue to blood forming cells is about 4:1.

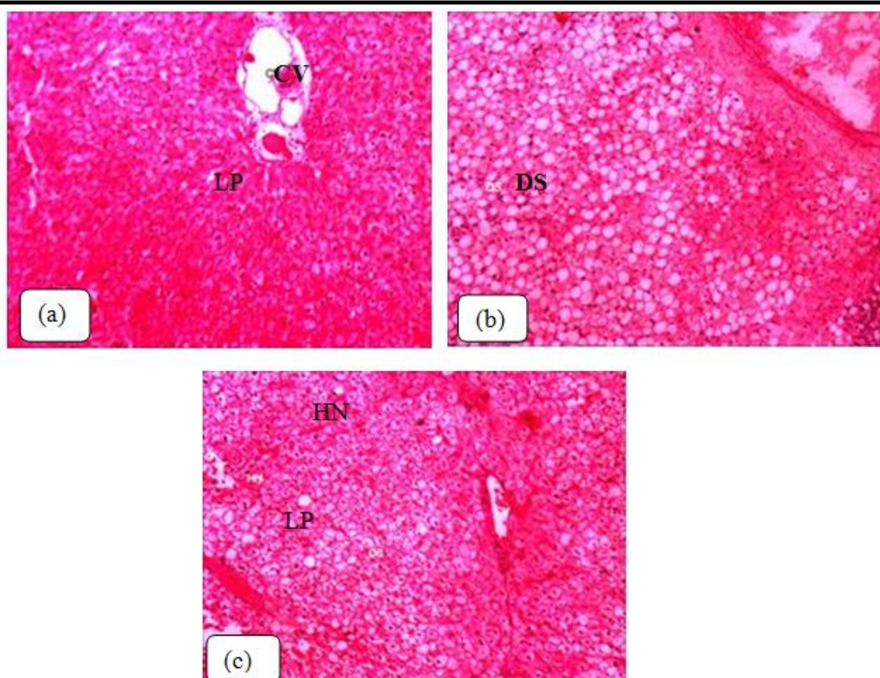

Plate-1: Photomicrograph of a cross section of the liver in the control (a) FPO (b) and TPO (c) group (magnification x100)

CV = central vein, LP = limiting plate, DS = diffused steatosis, HN = hepatocellular necrosis

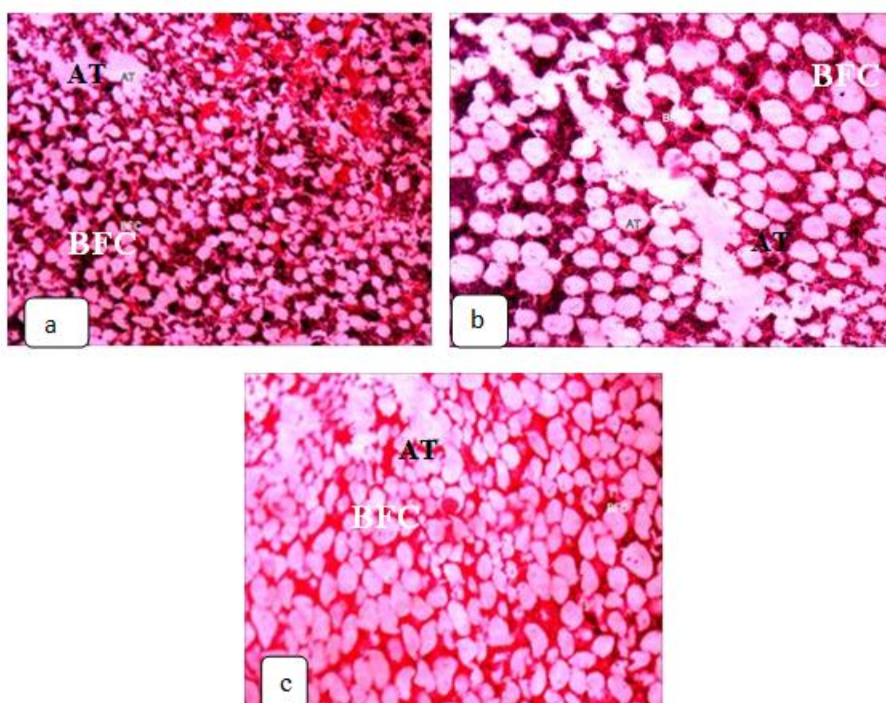

Plate-2: Photomicrograph of a cross section of the bone marrow in the (a) control (b) FPO and (c) TPO group (Magnification x100)

AT = adipose tissue, BFC = blood forming cells.

DISCUSSION

A decrease in platelet aggregation and vascular thrombus formation have been reported following consumption of polyunsaturated oil [25, 26], while the saturated fats have the opposite effect [27].

Among the indices of haemostasis assessed in this study were platelet counts, bleeding time, clotting time and prothrombin time. The platelet count was found to be significantly reduced in the thermoxidised *palm oil* diet fed group and increased in the fresh *palm oil* diet fed group, compared to control. Bleeding,

clotting and prothrombin times were significantly increased in the thermoxidised *palm oil* diet fed group and significantly decreased in the fresh palm oil diet fed group, when compared to control. These results indicate an adverse effect of thermally oxidized palm oil consumption on haemostasis. Prolongation of the bleeding, clotting and prothrombin times, with reduction in platelet count will definitely expose the animal to excessive, uncontrollable bleeding in the event of an injury, no matter how minor. Even without injuries, pathological decreases in platelet count can occur in conditions such as hereditary thrombocytopaenia and haemolytic disease of the new born [28]. The haemostatic picture from this study described above can lead to spontaneous bleeding in the skin, gums or into the joints and muscles [22].

The clotting and prothrombin times may have been prolonged because of the damage to the liver in the thermoxidised *palm oil* diet – fed group (plate 1c) seen in this study. The liver is essential for the production of clotting factors, which are necessary for normal blood coagulation to occur. Indeed, prothrombin time is already known to be prolonged in both early and end-stage liver failure, as well as congenital afibrinogenaemia [22].

A fibrinogenaemia is a condition where there is absence of factor I (fibrinogen). Fibrinogen is also a plasma protein and is produced in the liver, hence damage to the liver may also be responsible for the afibrinogenaemia, which is one of the conditions in which prothrombin time is prolonged. The decrease in platelet count agrees with a previous study by Osim *et al.*, [13], which reported enhanced platelet aggregability and thrombocytopaenia in rats fed on palm oil diets. The thrombocytopenia seen in TPO fed group in this study may result from the damage posed on the bone marrow (plate 2c) which contains the stem cells to go on to form blood cells. The significantly increased platelet count (thrombocytosis) seen in the fresh palm oil diet fed group may be beneficial for haemostasis. However, it harbours the potential danger of increasing the risk for vascular thrombosiss [29].

CONCLUSION

Chronic consumption of thermally oxidized palm oil predisposes to thrombocytopenia secondary to infiltration of the bone marrow with adipose tissue and reduction in the number of blood forming cells. Chronic consumption of thermally oxidized palm oil also causes extensive diffuse steatosis (fatty liver) in the liver and hepatocellular necrosis. This probably accounts for the prolonged clotting, bleeding and prothrombin times seen in TPO fed animals in this study, compared to FPO fed group whose haemostatic indices encouraged haemostasis. Therefore, TPO induces adverse changes in haemostatic status of rabbits.

CONFLICTS OF INTEREST

Nil

AUTHORS CONTRIBUTION

This study was carried out by four authors. EJA designed the study and did the laboratory work with the assistance of AUI. EJA wrote the initial draft which was corrected by EEO and prove read by DUO. The final manuscript was read and approved by all authors.

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