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Thermoxidized palm oil diet (TPO) induced protein derangements in rats is ameliorated by fresh palm oil (FPO) and Vitamin E

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ABSTRACT

Objective(s): The therapeutic efficacy of FPO and vitamin E were studied against TPO induced derangements in some proteins in rats. **Materials and Methods:** 60 male albino rats (140-160g) weight range, grouped (n=10) as: Control, TPO, FPO, Vitamin E, TPO treated with FPO (TPO+FPO) and Vitamin E (TPO+ Vitamin E). TPO and FPO (15g each) mixed with 85g rat feed was used to prepare FPO and TPO diet. Vitamin E was administered 200mg/kg daily by oral gavage. Animals were fed for 4 weeks, while group 5 and 6 were further treated with FPO and Vitamin E for another 4 weeks. At the end of the experiment blood were collected from sacrificed rats via cardiac puncture and protein indices were analyzed using serum. **Results:** Sero-protein analysis revealed a significant reduction in total protein, albumin, globulin and albumin-globulin ratio in TPO compared to control, FPO and Vitamin E, but was significantly increased in TPO+FPO and TPO+ Vitamin E compared to TPO. Total protein was significantly increased in TPO+FPO compared to TPO+ Vitamin E, while globulin was significantly increased in TPO+ Vitamin E compared to TPO+FPO. Total bilirubin and unconjugated bilirubin were significantly increased in TPO compared to control, FPO and Vitamin E, but was significantly reduced in TPO+FPO and TPO+ Vitamin E compared to TPO. Total bilirubin was significantly reduced in TPO+FPO compared to TPO+ Vitamin E. Conjugated bilirubin was significantly reduced in TPO compared to control, FPO and Vitamin E, but was significantly increased in TPO groups treated with FPO and Vitamin E in relation to TPO. **Conclusion:** FPO and vitamin E displayed therapeutic efficacy in ameliorating protein derangements instigated by TPO consumption.

Keywords: Fresh palm oil, Vitamin E, Protein indices, Derangement.

INTRODUCTION

In Nigeria, palm oil obtained from the fruit of *Elaeis guineensis* is the most widely used vegetable oil for cooking. It is used either in its fresh form or after its thermal degradation following series of heating [1]. Fresh palm oil is thermally degraded when it is exposed to heat at varying time intervals and at high temperatures. Generally, the essence of thermal oxidation of palm oil is to increase the taste of food [2]. Palm oil in its fresh form is made of fatty acids such as saturated (50%), polysaturated (10%) and unsaturated (40%). Triglycerides and little quantities of di- and mono- glycerides constitute it main components whereas free fatty acids as well as phytonutrients form its minor components [3]. The main phytonutrients comprise of carotenoids, vitamin C and E. Beta-carotene gives palm oil its seemingly reddish black radiance [4]. The constituent vitamins serve as network of natural antioxidants converting vastly reactive radicals to fewer vigorous species thereby protecting tissues against oxidative harm [3]. Studies proved that the benefits of FPO include lessening of stress with anti-inflammatory and cholesterol reducing property [4], it inhibits cholesterol biosynthesis and platelets aggregation, increase immunity, is an anti-tumorogenic agents, lessens the ratio of total cholesterol to HDL levels [4], prevent or converses the buildup of plaques in brain blood vessels which lessens the risk of ischaemic stroke [4]. The antioxidants in FPO was also proven to improve the state of the heart, soften vascular plaques, reduce peripheral resistance of blood flows as well as the risk of hypertension and myocardial infarction [4]. The beneficial effect of vitamin E has been widely reported in various conditions to be due to its antioxidant potential [5]. It has been reported that Vit E prevents the production of toxic by-products formed during metabolism in biological membrane such as lipid peroxides [5,6]. Nevertheless, thermal oxidation of fresh palm oil has a depreciating impact on the quality of edible oils because it modifies the physicochemical properties of the oil leading to destruction of many of its valuable constituents [3], resulting in formation of cytotoxic as well as destructive by-products which are injurious to cells, tissues as well as organs [2]. We earlier reported that TPO negatively alters hematological indices [1] and lipid profiles in rats [2] but consumption of FPO and vitamin E improved hematological indices [1] and lipid

profiles of TPO fed rat [2]. Other studies reported that TPO increases the content and density of free fatty acid [3], impoverishes β-carotenes as well as other phytonutrients and antioxidants in palm oil, making it vulnerable to peroxidation [7], it disables vital metabolic enzymes, it leads to fatty livers and alters the function of the liver and its histology [8]. Notwithstanding these findings, palm oil is still being eaten in the thermo-oxidized form. Consumption of TPO by the human populace is on the rise and no recognized therapy is identified for diseases associated with it. Hence, this present investigation was undertaken to assess the protective effect of FPO and vitamin E against TPO induced changes on total protein, albumin and globulin levels in albino wistar rat.

MATERIALS AND METHODS

Palm oil diets preparation

Fresh palm oil obtained from *Elaeis guineensis* was purchased (20 liters) from a market in Calabar (Marian market), Cross River State, Nigeria. The fresh oil was equally poured into two ten liters (black) container to prevent oxidation and labeled. The one labeled FPO contained fresh palm oil, while the container labeled TPO was thermally degraded to produce thermo-oxidized palm oil (TPO). The degradation of the oil by thermo-oxidation was as described by Beshel *et al*. [9]. This was done by heating FPO at 150^{o}c using a stainless-steel pot at an interval of five for 20 minutes each and 5 hours cooling period before commencements of the next interval. TPO and FPO (15g each) mixed with 85g rat feed was used to prepare FPO and TPO diet, as used by Obembe *et al*. [10].

The vitamin E supplement used for the study was obtained from a pharmacy store, Bez Pharmacy, Calabar, Cross River State. Vitamin E was administered 200mg/kg/day by oral gavage as used by [1].

Experimental animals

This study made used of male albino rats of Wistar strain weighing between 140 to 160g after obtaining ethical clearance from ethical research committee for use of animal with approval number 07/23 in the Faculty of Basic Medical Sciences, University of Calabar. Animal acclimatization was for seven days before the start of the experiment. Animal housing was at room temperature and under a 12-hour light and 12-hour dark cycle of the Department of Physiology animal house, University of Calabar, Calabar, Nigeria.

Experimental design

Animals were randomly divided into six groups of n=10. Group 1 was control and received rat chow and water only. Group 2 were served FPO diet and water. Group 3 were served TPO diet. Group 4 received rat chow and water as well as Vitamin E (orally). Group 5 were served TPO diet and later treated with FPO diet (TPO + FPO) and Group 6, were served TPO diet and later treated with Vitamin E (TPO + Vitamin E). All groups had free access to tap water. Feeding and treatment phase for Group 1-4 lasted for 4 weeks, while that of Group 5-6 was for 8 weeks.

Sacrifice and blood sample collection

At the end of the treatment, animals were anaesthetized using 60 mg kg^{-1} of ketamine-HCL (#50155, Rotex Medica, Trittau, Germany) after an overnight fast. Blood samples was collected via cardiac puncture and stored in heperinized screw cap bottles for estimation of the various protein indices. Serum was used for analysis.

Measurement of protein indices

Standard laboratory kits (Randox laboratory Ltd, Co. Antrim, UK) were used to spectrophotometrically ascertain total protein, albumin, globulin, total bilirubin and conjugated bilirubin levels. These were all estimated following adherence of manufacturers guidelines. Albumin-Globulin ratio was determined by dividing albumin by the concentration of globulin. Unconjugated bilirubin was estimated as (total bilirubin – conjugated bilirubin).

Statistical Analysis

Data presentation (mean ± SEM); one way analysis of variance (ANOVA) for data analysis and post hoc multiple comparison test was done. Values of $p< 0.05$ were accepted as significant. Microsoft Excel 2010 and SPSS 16.0 software were statistical tools used.

RESULTS

Total protein concentration

Total protein in (g/dl) was as follows; control (7.66 ± 0.07), FPO (7.50 ± 0.04), TPO (5.44 ± 0.09), Vitamin E (7.10 ± 0.09), TPO+FPO (6.89 ±0.06) and TPO+ Vitamin E (6.45 ± 0.03). Total protein was reduced significantly in TPO ($P<0.05$), as well as in TPO groups treated with Vitamin E and FPO ($P<0.05$) in comparison to control, while FPO as well as Vitamin E group didn't differ significantly compared to control. Total protein concentration was reduced significantly in TPO ($P<0.05$), as well as in TPO groups treated with Vitamin E and FPO ($P<0.05$) compared to FPO. Total protein was increased significantly in Vitamin E ($P<0.05$), as well as in TPO groups treated with Vitamin E and FPO ($P<0.05$) in comparison to TPO. Total protein was significantly reduced in TPO+ Vitamin E ($P<0.05$) compared to Vitamin E. Total protein levels was decreased significantly in TPO+ Vitamin E ($P<0.05$) compared to TPO groups treated with FPO (FIG. 1).

Albumin concentration

Concentration of albumin for control, fresh palm oil, TPO, Vitamin E, TPO treated with FPO and Vitamin E was 3.46 ± 0.05, 3.78 ± 0.06, 2.68 ± 0.14, 3.52 ± 0.07, 3.01 ± 0.04 and 2.98 ± 0.05 (g/dl), respectively. The albumin level was higher in FPO significantly ($p<0.05$) in comparison to control, however was reduced in TPO, as well as in TPO groups treated with Vitamin E and FPO significantly ($p<0.05$) compared to control. Albumin concentration didn't differ significantly in Vitamin E control in comparison to control. Albumin was reduced in Vitamin E, TPO, as well as in TPO groups treated with Vitamin E and FPO significantly ($p<0.05$) in relation to fresh palm oil. Albumin levels was elevated in Vitamin E, as well as in TPO groups treated with Vitamin E and FPO significantly ($p<0.05$) in comparison to TPO. Albumin was reduced in TPO groups treated with Vitamin E and FPO significantly ($p<0.05$) compared to Vitamin E control (FIG. 2).

Globulin concentration

Concentration of globulin for control, fresh palm oil, TPO, Vitamin E, TPO treated with FPO and Vitamin E was 4.12 ± 0.04, 3.76 ± 0.09, 3.74 ± 0.04, 4.16 ±0.02, 4.62 ± 0.04 and 5.72 ± 0.07 (g/dl), respectively. The globulin concentration was decreased in FPO and TPO significantly ($p<0.05$) compared to control, and yet increased in TPO groups treated with Vitamin E and FPO significantly ($P<0.05$) compared to control. Vitamin E group did not differ significantly relative to control. Globulin was elevated in Vitamin E, as well as TPO groups treated with Vitamin E and FPO significantly ($p<0.05$) relative to FPO and TPO respectively. Globulin was elevated in TPO groups treated with Vitamin E and FPO significantly ($p<0.05$) relative to Vitamin E. Globulin increased in TPO groups treated with Vitamin E significantly ($p<0.05$) in comparison to TPO groups treated with FPO (FIG. 3).

Albumin-globulin ratio

Albumin-globulin ratio for control, fresh palm oil, TPO, Vitamin E, TPO treated with FPO and Vitamin E was 0.82±0.03, 1.02±0.04, 0.69±0.09, 0.99±0.02, 0.83±0.04 and 0.82±0.06 g/dl respectively.

Albumin-globulin ratio was lower in TPO significantly ($p<0.05$) relative to control, and was increased in FPO and Vitamin E significantly ($p<0.05$) relative to control. Albumin-globulin ratio was lower in TPO, as well as in TPO groups treated with Vitamin E and FPO significantly ($p<0.05$) relative to fresh palm oil and Vitamin E. The albumin-globulin ratio was increased significantly in TPO groups treated with Vitamin E and FPO ($p<0.05$) relative to TPO (FIG. 4).

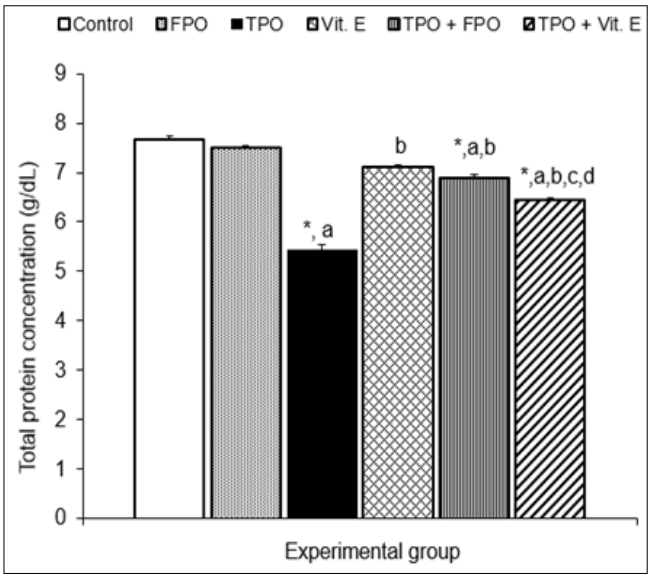

Figure 1: Total protein concentration comparison among groups

Values are expressed as mean ± SEM, n= 10
*= significantly different from control at p<0.05
a= significantly different from FPO at p<0.05
b= significantly different from TPO at p<0.05
c= significantly different from Vit. E at p<0.05
d= significantly different from FPO + TPO at p<0.05

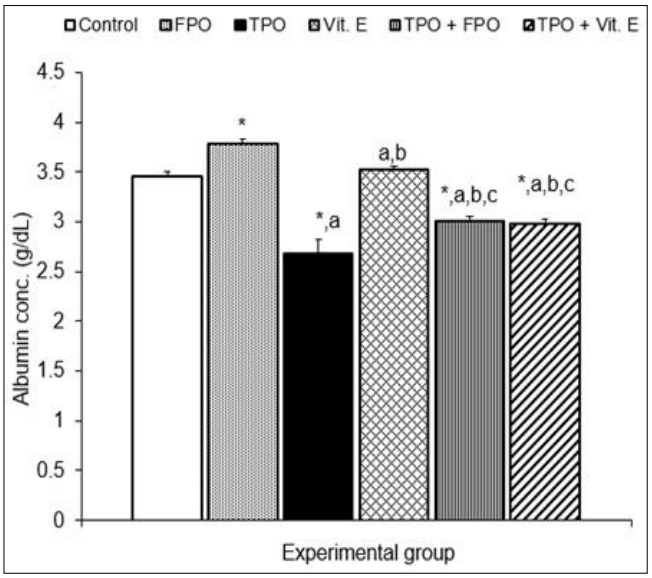

Figure 2: Albumin concentration comparison among groups

Values are expressed as mean ± SEM, n= 10
*= significantly different from control at p<0.05
a= significantly different from FPO at p<0.05
b= significantly different from TPO at p<0.05
c= significantly different from Vit. E at p<0.05

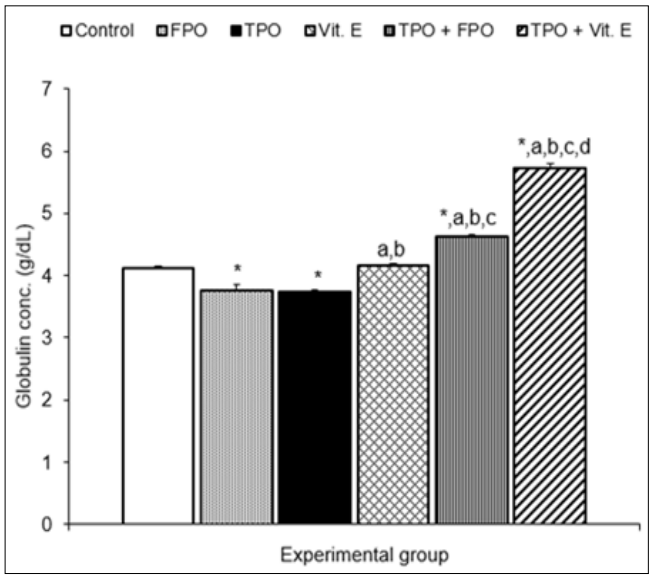

Figure 3: Comparison of globulin concentration in the different experimental groups

Values are expressed as mean ± SEM, n= 10
*= significantly different from control at p<0.05
a= significantly different from FPO at p<0.05
b= significantly different from TPO at p<0.05
c= significantly different from Vit. E at p<0.05
d= significantly different from FPO + TPO at p<0.05

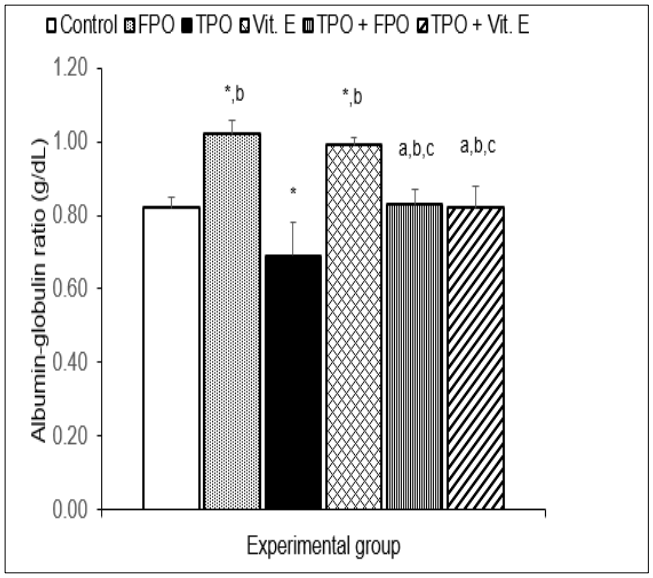

Figure 4: Comparison of albumin-globulin ratio in the different experimental groups

Values are expressed as mean ± SEM, n= 10
*= significantly different from control at p<0.05
a= significantly different from FPO at p<0.05
b= significantly different from TPO at p<0.05
c= significantly different from Vit. E at p<0.05

Total bilirubin concentration

Concentration of total bilirubin in the control, fresh palm oil, TPO, Vitamin E, TPO + FPO and TPO+ Vitamin E treated group was 1.52 ± 0.05, 1.12 ± 0.05, 3.22 ± 0.15, 1.34 ± 0.04, 1.45 ± 0.07 and 1.80 ± 0.09 (µmol/L), respectively. The total bilirubin was lower significantly in FPO and TPO group treated with FPO ($p<0.05$) relative to control, yet was increased in TPO and TPO group treated with Vitamin E significantly ($p<0.05$) relative to control, while Vitamin E group did not differ significantly from control. Total

bilirubin was higher in TPO and TPO group treated with Vitamin E and FPO significantly ($p<0.05$) relative to fresh palm oil. The concentration decreases in Vitamin E, as well as in TPO and TPO group treated with Vitamin E and FPO significantly ($p<0.05$) relative to TPO. Total bilirubin elevated significantly in TPO group treated with Vitamin E and FPO ($p<0.05$) relative to Vitamin E. Total bilirubin was increased significantly in TPO group treated with Vitamin E ($p<0.05$) in comparison to TPO group treated with FPO (FIG. 5).

Conjugated bilirubin concentration

The concentration of conjugated bilirubin for control, fresh palm oil, TPO, Vitamin E, as well as TPO treated with FPO and Vitamin E was 0.76 ± 0.05, 0.70 ± 0.04, 0.11 ± 0.11, 0.74 ± 0.05, 0.54 ± 0.12 and 0.42 ± 0.10 (µmol/L), respectively. Conjugated bilirubin did not differ significantly in the fresh palm oil and Vitamin E compared to control, but was significantly reduced in TPO and TPO group treated with Vitamin E and FPO ($p<0.05$) relative to control. Conjugated bilirubin concentration was significantly reduced in TPO and TPO group treated with Vitamin E and FPO ($p<0.05$) relative to fresh palm oil. Conjugated bilirubin concentration was significantly reduced in TPO and TPO group treated with Vitamin E and FPO ($p<0.05$) relative to Vitamin E (FIG. 6).

Unconjugated bilirubin concentration

The concentration of unconjugated bilirubin for control, fresh palm oil, TPO, Vitamin E, as well as TPO treated with FPO and Vitamin E was 0.76 ± 0.02, 0.66 ± 0.04, 1.80 ± 0.09, 0.70 ± 0.07, 1.45 ± 0.07 and 1.52 ± 0.10 (µmol/L), respectively. Unconjugated bilirubin did not differ significantly in fresh palm oil and Vitamin E relative to control, but significantly ($p<0.05$) increased in TPO and TPO group treated with Vitamin E and FPO relative to control. Unconjugated bilirubin elevated significantly in TPO and TPO group treated with Vitamin E and FPO ($p<0.05$) relative to fresh palm oil and Vitamin E. Unconjugated bilirubin reduced significantly in TPO group treated with Vitamin E and FPO ($p<0.05$) relative to TPO (FIG. 7).

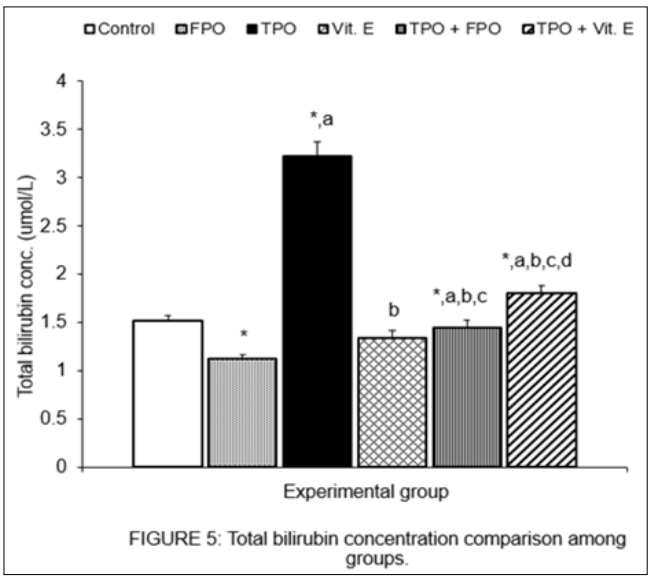

Figure 5: Total bilirubin concentration comparison among groups

Values are expressed as mean ± SEM, n= 10
*= significantly different from control at $p<0.05$
a= significantly different from FPO at $p<0.05$
b= significantly different from TPO at $p<0.05$
c= significantly different from Vit. E at $p<0.05$
d= significantly different from FPO + TPO at $p<0.05$

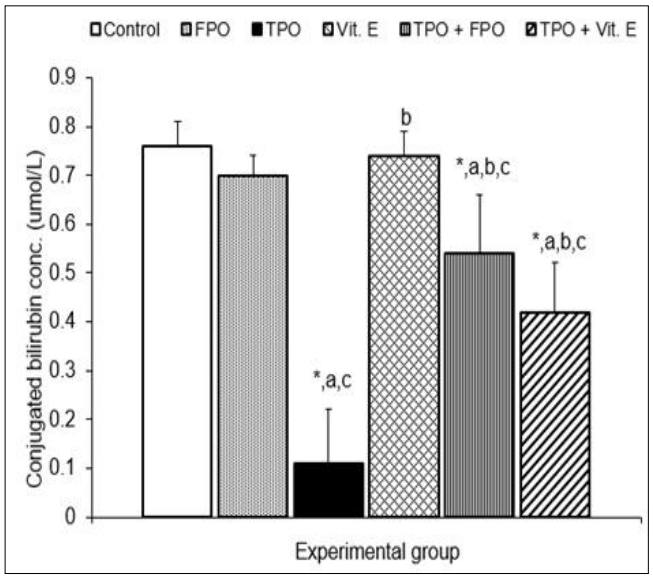

Figure 6: Conjugated bilirubin concentration comparison among groups

Values are expressed as mean ± SEM, n= 10
*= significantly different from control at $p<0.05$
a= significantly different from FPO at $p<0.05$
b= significantly different from TPO at $p<0.05$
c= significantly different from Vit. E at $p<0.05$

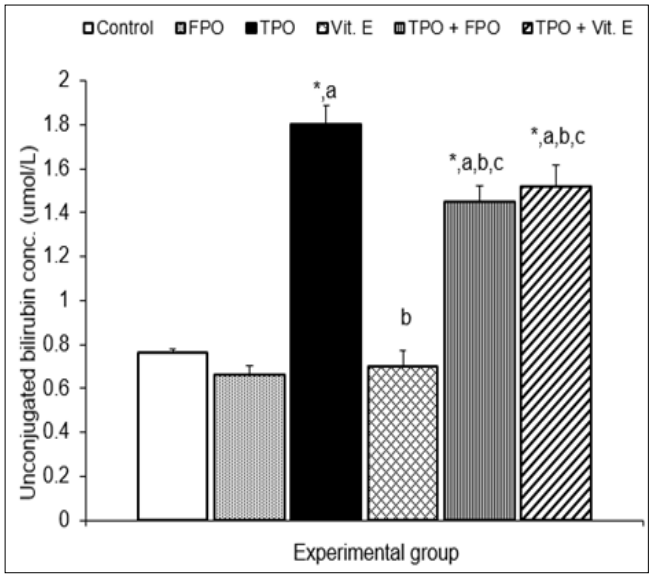

Figure 7: Unconjugated bilirubin concentration comparison among groups

Values are expressed as mean ± SEM, n= 10
*= significantly different from control at $p<0.05$
a= significantly different from FPO at $p<0.05$
b= significantly different from TPO at $p<0.05$
c= significantly different from Vit. E at $p<0.05$

DISCUSSION

This research work was designed to investigate the protective effect of FPO and vitamin E on serum proteins (total protein, albumin, globulin, albumin-globulin ratio, total bilirubin, conjugated and unconjugated bilirubin) profile of male albino rats fed with TPO. From the result of this study, consumption of FPO and vitamin E alone caused significant improvements in some of the mentioned parameters as well as recovery of the levels of these parameters despite TPO diet induced changes. TPO showed a significant reduction in total protein, albumin and globulin compared to control. The reduction in total protein and albumin is consistent with study of Ayodeji *et al*., [11] who earlier stated that TPO fed diet reduces the

levels of total protein and albumin in rats. Albumin has the greatest composition in serum, hence reduction in it levels suggest protein deficiency in the body and indication of malnutrition [12]. Liver is the factory of the synthesis of these proteins [13], and they are needed in sufficient levels in the body to maintain adequate biochemical functions [14]. Ekam *et al*., [15] reported that total protein, albumin and globulin level may decrease due to liver dysfunction and acute hemolytic anemia. This suggests that TPO fed diet impairs the liver's capacity to synthesize proteins. Ukoh *et al*., [1] earlier observed reduced RBC and hemoglobin in rat fed with TPO diet. This signifies hemolytic anemia in rats fed with TPO diets. However, Vitamin E showed no significant difference in respective proteins compared to control, while FPO showed a significant increase in albumin, but a significant decrease in globulin compared to control. The increase in albumin concentration in FPO fed diet is consistent with that of Ayodeji *et al*., [11]. Albumin is a pointer to liver function to produce proteins [16]. The total protein results in FPO fed group compared to control agrees with Al-Amoudi [17] findings, who reported insignificant changes in fennel oil level relative to control. TPO fed rat treated with vitamin E and FPO heightened the levels of total protein, albumin and globulin relative to untreated TPO fed rats. This suggests complete protection of liver integrity. Also, treatment of TPO fed animals with FPO heightened total protein in relation to vitamin E. This may be due to the rich constituents in FPO in addition to vitamin E. While TPO fed animals treated with vitamin E heightened globulin in relation to FPO. This could be due to the observation of Ukoh *et al*., [1] who reported that most FPO are not consumed in its fresh form. That the oil quality must have been degraded due to photo-and-chemical oxidation.

From this study, TPO caused a significant reduction in Albumin Globulin Ratio (AGR). However, FPO and vitamin E fed diet significantly increased AGR compared to control and rat fed TPO. The lesser AGR seen in animal fed TPO may be due to disruption of protein synthesis in the liver. Ida [18] reported that when liver function is abnormal or damaged, it will disrupt the metabolic secretion of albumin and globulin. Treatment with FPO and vitamin E were effective in reversing the decreased AGR in TPO fed rat.

Total bilirubin and unconjugated bilirubin level was observed to increase in TPO fed diet compared to control, FPO and Vitamin E fed groups respectively. The increase total bilirubin in the TPO fed group compared to control is consistent with that reported by Ayodeji *et al*., [11]. This increase in both total bilirubin and unconjugated bilirubin may be due to haemolytic anaemia in the TPO fed rat as earlier observed by Ukoh *et al*., [1] who stated a lesser RBC and hemoglobin in rat consuming TPO diet. Bilirubin is a key breakdown product of hemoglobin. Hemoglobin is consequent of RBC that has exceed their lifespan and afterward been degraded by the spleen [11], resulting in separation of hemoglobin from iron [11]. While the level of conjugated bilirubin was observed to decrease in TPO fed diet compared to control, FPO and Vitamin E fed groups respectively. Total serum bilirubin is usually in the free (unconjugated) state, it is created from RBC breakdown in the recticulo-endothelial system and transported by albumin from the bloodstream to the liver where it is conjugated and transported to the intestines before it is excreted via the stool [19]. As observed in the TPO fed rat in this study, when the amount of albumin decreases, the level of unconjugated bilirubin in the blood serum increases [20], and the amount of bilirubin transported to the liver for conjugation decreases. These effects of TPO in bilirubin, unconjugated and conjugated bilirubin were seen to be improved following treatment with FPO and vitamin E. The significant reduction in the level of bilirubin and unconjugated bilirubin as well as the significant increase in conjugated bilirubin of TPO treated groups suggest hepato-protective and enhanced hematological replenishing of FPO and vitamin E against TPO induced damaged.

CONCLUSION

Although ingesting of foods containing TPO harmfully alters the proteins in this study, fresh palm oil and vitamin E were efficient in ameliorating most adverse deviations which are of health concern. Consequently, apart from shunning the copious consumption of TPO diets, ingestion of fresh palm oil and vitamin E should be encouraged since both shows therapeutic efficacy in ameliorating many of the health concern induced by TPO. If these outcomes are inferred to humans, this may be a less expensive way to avoid many diseases that plague humans.

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Conflict of interest

The authors declare that they have no conflict of interest.

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