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COMPARATIVE EFFECTS OF FRESH PALM OIL AND VITAMIN E ON SOME HEMATOLOGICAL INDICES IN THERMO-OXIDIZED PALM OIL FED ALBINO RAT

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ABSTRACT

This study we aimed to compare the effects of consumption of fresh palm oil (FPO) and vitamin E on hematological indices of thermo-oxidized palm oil (TPO) fed rat. Sixty male albino rats weighing 140-160g were grouped into six (n=10): Control, TPO, FPO, Vit. E, TPO+FPO and TPO+Vit. E. 15g of TPO and FPO was mixed with 85g rat chow to prepare TPO and FPO diet respectively. Vit. E was administered 200mg/kg/day orally. Group 1-4 were fed for 4 weeks, while TPO+FPO and TPO+Vit. E were fed with FPO and Vit. E for another 4 weeks. Blood samples was collected via cardiac puncture for analysis. From the result, TPO significantly ($p<0.05$) reduce RBC, PCV, Hb, MCH and monocyte, but increase ($p<0.05$) MCV, WBC and lymphocyte compared to control. FPO and Vit. E significantly ($p<0.05$) increase RBC, PCV, platelet and Hb compared to control and TPO, increase ($p<0.05$) lymphocyte and reduce monocyte compared to control, increase ($p<0.05$) MCH and monocyte but reduce ($p<0.05$) MCV, WBC and lymphocyte compared to TPO. Vit. E significantly ($p<0.05$) increase MCH compared to FPO, and FPO significantly ($p<0.05$) increase monocyte compared to Vit. E. TPO+FPO and TPO+Vit. E significantly ($p<0.05$) increase RBC, Hb, MCH, platelet and monocyte, but reduce ($p<0.05$) WBC and lymphocyte compared to TPO. TPO+Vit. E significantly ($p<0.05$) increase PCV compared to TPO, increase ($p<0.05$) RBC, PCV, Hb, platelet and monocyte, but reduce ($p<0.05$) lymphocyte compared to TPO+FPO. Conclusion, Vit. E showed better therapeutic efficacy than FPO in ameliorating hematological derangements caused by TPO consumption.

KEYWORDS: Vitamin E, Fresh palm oil, TPO, RBC, PCV, MCV.

INTRODUCTION

Palm oil is the most edible vegetable oil obtained from the fruit of *Elaeis guineensis.*[1,2] It is widely consumed either in its fresh form or thermally oxidized form.[3] Palm oil is fresh when processed at low temperature to remove debris.[4] Fresh palm oil is a rich source of natural antioxidants such as vitamin E, vitamin C and carotenoids.[2] Consumption of fresh palm oil has been associated with improve immunity, possesses anti-tumorogenic and antiatherogenic effects[5], reduce occurrence of coronary heart diseases and reduce the effect of oxidative stress on rat spermatozoa.[2] Thermo-oxidization occurs when fresh palm oil is subjected to series of heating at high temperatures.[2] Ingestion of thermo-oxidized palm oil has been reported to generate free radicals which may be susceptible to diseases such as cancer, diabetes, arthritis and cataract formation.[1,6] It has also been reported that thermo-oxidation of palm oil deplete vitamin A and E in the body, causes damage to the liver and the kidneys[3], and affects haemostatic process, as well as haematological indices.[7] Since the discovery of vitamin E in 1922 in green leafy vegetables many studies have been performed to explore its therapeutic effects.[8] Vitamin E is essential for physiological activities and is required in averting or lessening free-radical damage associated with specific diseases, lifestyle patterns and processes.[9] Vitamin E have been reported to ameliorate the cellular damage caused by free radicals formations[10], protect hematopoietic stem and progenitor cells (HSPCs) from lipid peroxidation and ferroptosis[11], ameliorates membrane fragility of red blood cells and thus prevents hemolysis[11] and shield bone marrow erythroid colony-forming units from toxicity.[12] The hematological system is of physiological and environmental importance to help understand the underlying blood characteristics to the environment[13], and alterations in haematological indices are of importance in ascertaining various healthy and pathological state of an individual.[14] Several studies have reported the detrimental effects that consumption of thermo-oxidized palm oil has in hematological indices[3,4,7], and Aletan,[15] reported that increased mortality and morbidity have been directly linked with abnormal hematological findings. Despite these observations, much of palm oil is still being consumed in the thermo-oxidized form by majority of the populace not minding it adverse effect, partly because it is said to improve the taste of food. Hence, this study seeks to establish a possible protective role of fresh palm oil and vitamin E against thermo-oxidized oil induced changes on hematological indices.

MATERIALS AND METHODS

Experimental animals

Sixty male albino Wistar rats weighing 140-160 grams were used for this study, and they were purchased from Department of Agriculture, University of Calabar, Calabar, Cross River State, Nigeria. The animals were housed at room temperature in the animal house of the Department of Physiology, University of Calabar, Calabar, Nigeria under a 12-hour light and 12-hour dark cycle. The Ethics Committee of the Faculty of Basic Medical Sciences, University of Calabar gave the ethical approval for the study.

Preparation of palm oil diets

Twenty liters of fresh palm oil obtained from the palm tree *Elaesis guinnensis* was purchased from Marian market, in Calabar, Cross River State, Nigeria. The palm oil was shared into two black ten liters container to avoid oxidation; one container was fresh palm oil, the other container was thermally oxidized to yield thermo-oxidized palm oil (TPO). Thermo-oxidation of oil was done as described by Beshel et al.[16] The process involved heating FPO at 150^{o}c for 20 minutes at an interval of five and a cooling period of 5 hours before the commencement of the next round of heating in a stainless-steel pot. The FPO and TPO diet was prepared by mixing 15g of the respective oils with 85g of rat feed as used by Obembe et al.[17]

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

Experimental design

The animals were randomly divided into six groups of 10 rats each. Group 1 served as the control and received normal rat chow and water. Group 2 were fed fresh palm oil (FPO) diet and water. Group 3 were fed with thermo-oxidized palm oil (TPO) diet. Group 4 were fed with normal rat chow and water in addition to oral administration of Vitamin E (Vit. E). Group 5 were fed with TPO diet in addition to FPO diet (TPO + FPO) and Group 6, were fed with TPO diet in addition to oral administration of Vitamin E (TPO + Vit. E). All groups had free access to tap water. This feeding and treatment lasted for 4 weeks for Group 1-4, while TPO fed rats in Group 5-6 were fed for another 4 weeks with fresh palm oil diet (FPO) and Vitamin E respectively.

Animal sacrifice and collection of blood samples

At the end of the feeding period, the animals were anaesthetised with 60 mg kg^{-1} of ketamine-hydrochloride (#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 haematological parameters.

Hematological indices

The following hematological indices were evaluated using automated blood cell analyzer (Model PCE 210, Japan); red blood cells (RBC) count, white blood cell (WBC) count, differential WBC counts, platelet count, packed cell volume (PCV), hemoglobin concentration, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration.

Statistical analysis

Data are presented as mean ± SEM and analyzed by one way analysis of variance (ANOVA) followed with a post hoc multiple comparison test. Values of $p< 0.05$ was accepted as significant. Microsoft Excel 2010 and SPSS 16.0 software were employed for the statistical analysis.

RESULTS

Red blood cell count in the different experimental groups

The red blood cell count (x1million cells/uL) was 6.36 ± 0.14, 9.72 ± 0.11, 4.90 ± 0.09, 9.86 ± 0.02, 6.87 ± 0.09 and 7.86 ± 0.11 for control, FPO, TPO, Vit. E, TPO + FPO and TPO + Vit. E respectively. The result presented a significant increase ($P<0.05$) in RBC count in FPO, Vit. E, TPO + FPO and TPO + Vit. E compared to control. However, RBC count in TPO reduced significantly ($P<0.05$) compared to control, Vit. E, TPO + Vit. E and TPO + FPO. RBC count for FPO increased significantly ($P<0.05$) compared to TPO, TPO + FPO and TPO + Vit. E. However, Vit. E was seen to cause a significant increase ($P<0.05$) in RBC count compared to TPO + FPO and TPO + Vit. E. Moreso, TPO + Vit. E showed a significant increased ($P<0.05$) in RBC count compared to TPO + FPO (Figure 1).

Packed cell volume

The PCV (%) was 42.18 ± 0.16, 42.82 ± 1.18, 33.94 ± 0.87, 44.01 ± 1.02, 38.24 ± 0.19 and 42.24 ± 0.43 for control, FPO, TPO, Vit. E, TPO + FPO and TPO + Vit. E respectively. The result showed that TPO was significantly reduced ($p<0.05$), while Vit. E increased ($P<0.05$)

when compared to control. There was no significant difference in FPO, TPO + FPO and TPO + Vit. E when compared to control. FPO, Vit. E and TPO + Vit. E were significantly increased (P<0.05) when compared to TPO. PCV was reduced significantly (P<0.05) in TPO + FPO when compared to Vit. E. TPO + Vit. E increased significantly (P<0.05) compared to TPO + FPO (Figure 2).

Haemoglobin concentration

The haemoglobin concentration (g/dl) was 14.40 ± 0.16, 14.78 ± 0.11, 10.60 ± 0.17, 15.28 ± 0.12, 12.32 ± 0.09 and 4.92 ± 0.21 for control, FPO, TPO, Vit. E, TPO + FPO and TPO + Vit. E respectively. The results showed that Vit. E was significantly (P<0.05) increased, while TPO and TPO + FPO were significantly (P<0.05) reduced compared to control. FPO, and TPO + Vit. E was not significant when compared to control. TPO and TPO + FPO were significantly (P<0.05) reduced when compared to FPO. Vit. E, TPO + FPO and TPO + Vit, E were significantly (P<0.05) increased when compared to TPO. TPO + FPO was significantly reduced (P<0.05) when compared to Vit. E, while TPO + VE was significantly (P<0.05) increased when compared to TPO + FPO (Figure 3).

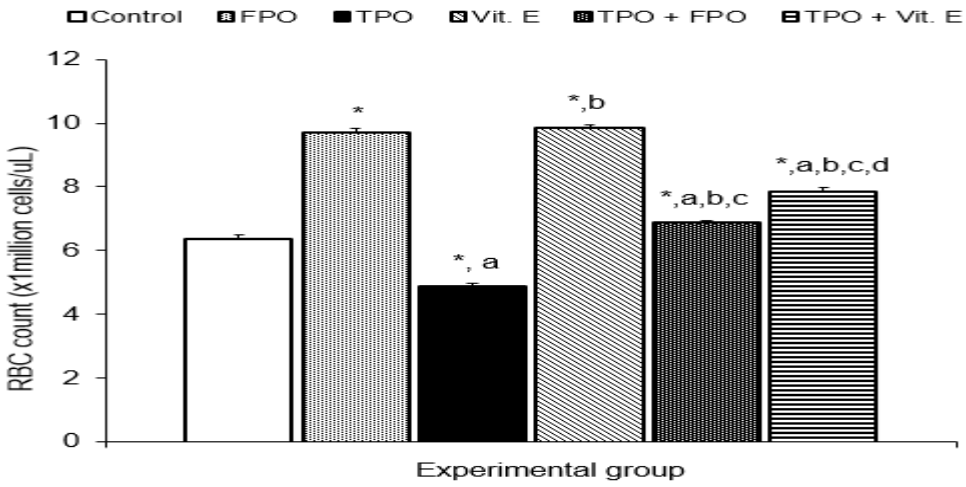

FIGURE 1: Red blood cell count comparison among groups.

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

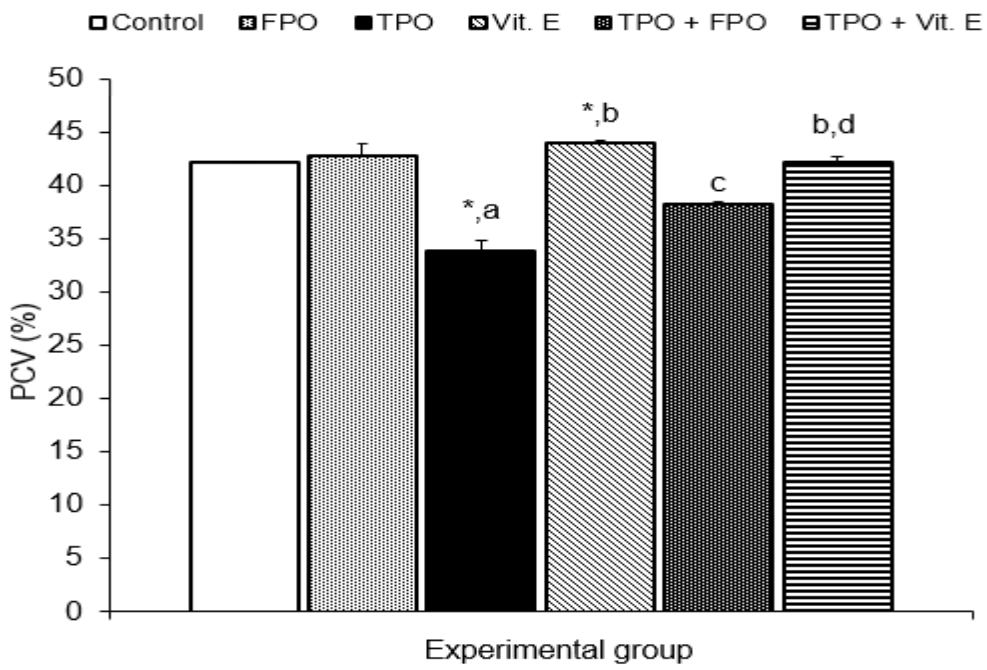

FIGURE 2: packed cell volume comparison among groups.

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

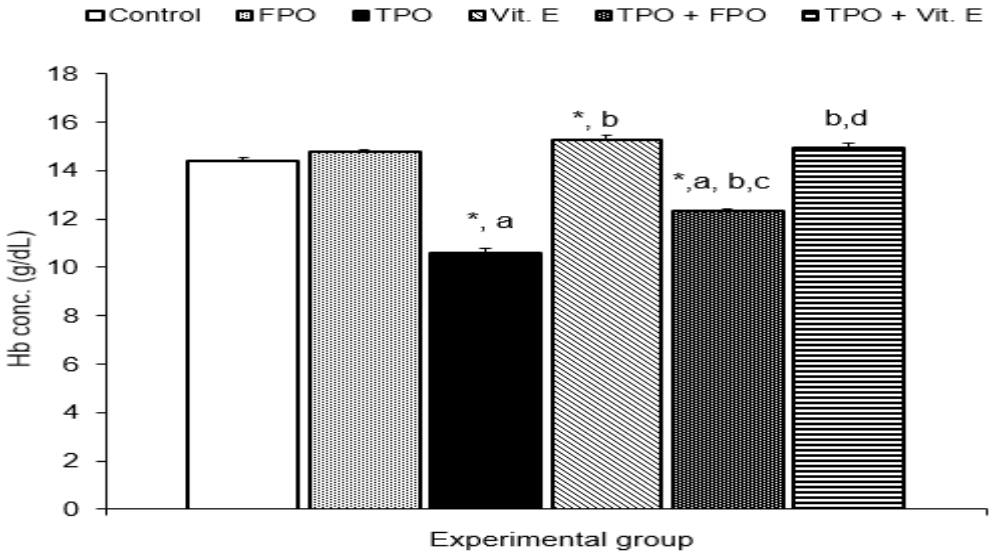

FIGURE 3: Haemoglobin concentration comparison among groups.

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

Mean corpuscular volume

The MCV (fL) for control, FPO, TPO, Vit. E, TPO + FPO and TPO + Vit. E was 64.39 ± 1.42, 62.93 ± 0.86, 73.71 ± 0.72, 61.87 ± 2.16, 65.37 ± 0.51 and 63.29 ± 0.99 respectively. The results for Vit. E, TPO + FPO and TPO + Vit. E were not significant when compared to control. However, TPO was significantly increased ($P<0.05$) when compared to FPO and Vit. E (Figure 4).

Mean corpuscular hemoglobin

The MCH (pg) for control, FPO, TPO, Vit. E, TPO + FPO and TPO + Vit. E was 22.70 ± 0.68, 21.75 ±0.22, 14.67 ± 0.43, 23.20 ± 0.26, 17.46 ± 0.11 and 17.94 ± 0.25, respectively. The results for FPO and Vit. E were not significant when compared to control. However, TPO, TPO + FPO and TPO + Vit. E were significantly ($P<0.05$) reduced when compared to control. Vit. E was significantly ($P<0.05$) increased, while TPO, TPO + Vit. E and TPO + FPO were significantly ($P<0.05$) reduced when compared to FPO. TPO, TPO + Vit. E and TPO + FPO were significantly ($P<0.05$) reduced when compared to Vit. E. TPO + Vit. E and TPO + FPO were significantly increased ($P<0.05$) when compared to TPO (Figure 5).

Mean corpuscular haemoglobin concentration

The MCHC (g/dl) for control, FPO, TPO, Vit. E, TPO + FPO and TPO + Vit. E was 34.14 ± 0.35, 34.60 ± 0.75, 34.20 ± 0.78, 33.58 ±1.00, 35.32 ± 0.22 and 36.00 ± 0.65, respectively. The results for FPO, TPO, Vit. E, TPO + FPO and TPO + Vit. E were not significant when compared to control. However, TPO + Vit. E was significantly ($P<0.05$) increased when compared to Vit. E (Figure 6).

Platelet count comparison among groups

The platelet count (x1,000cells/µL) for control, FPO, TPO, Vit. E, TPO + FPO and TPO + Vit. E was 592.60 ± 3.97, 751.60 ± 4.70, 603.00 ± 3.86, 750.40 ± 5.35, 712.20 ± 5.95 and 862.60 ± 5.20, respectively. The result showed a significant increased ($P<0.05$) in FPO, Vit. E, TPO + FPO and TPO + Vit. E when compared to control. TPO and TPO + FPO were significantly reduced ($P<0.05$) compared to FPO, while TPO + Vit. E increased significantly ($P<0.05$) when compared to FPO. TPO and TPO + FPO were significantly decreased ($P<0.05$) compared to Vit. E group, while TPO + Vit. E increased significantly ($p<0.05$) when compared to Vit. E. TPO + Vit. E increased significantly ($P<0.05$) when compared to TPO + FPO (Figure 7).

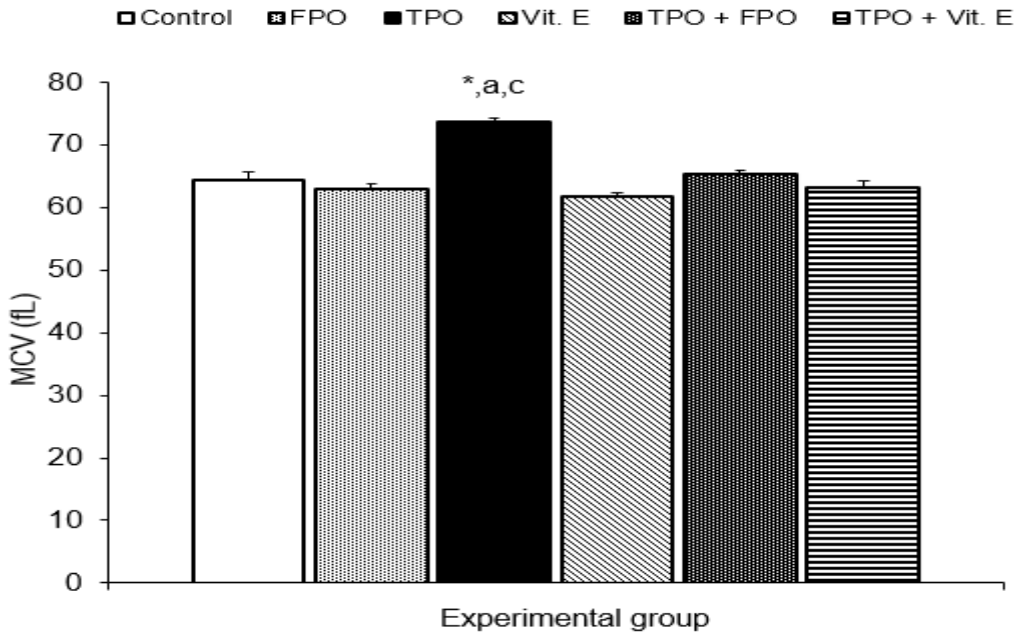

FIGURE 4: Mean corpuscular volume comparison among groups.

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
c = significantly different from Vit. E at p<0.05

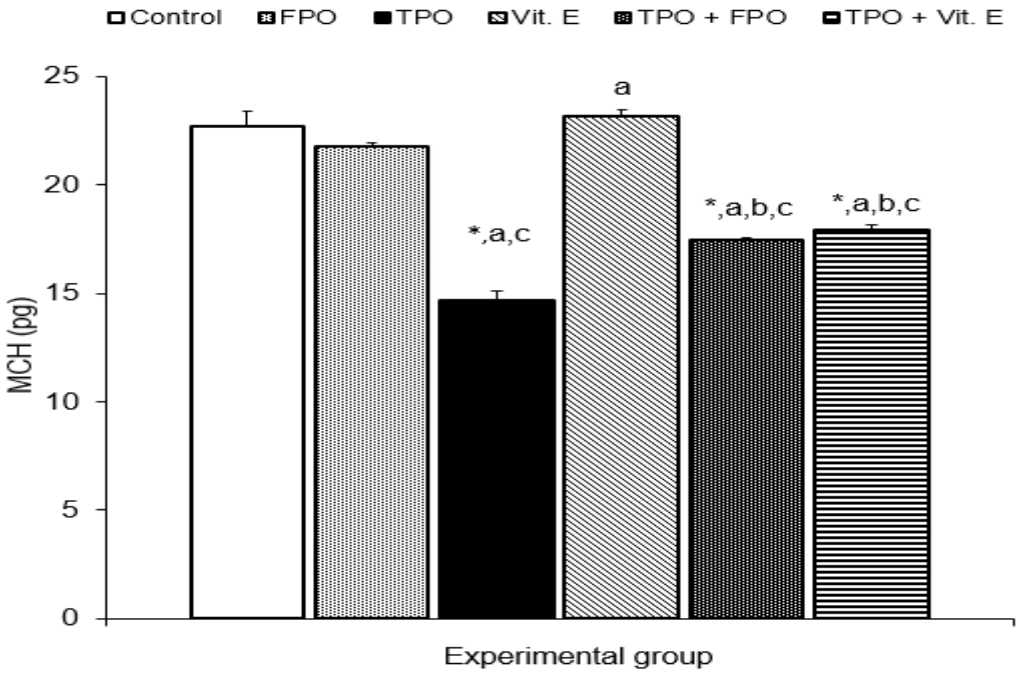

FIGURE 5: Mean corpuscular haemoglobin comparison among groups.

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

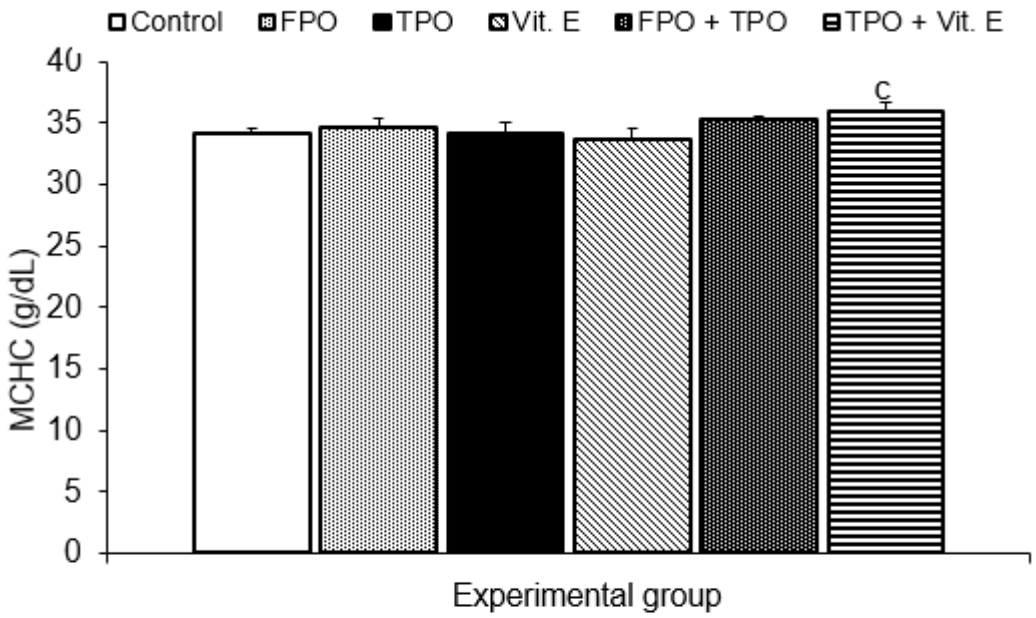

FIGURE 6: Mean corpuscular haemoglobin concentration comparison among groups.

Values are expressed as mean ±SEM, n = 10.
c = significantly different from Vit. E at p<0.05

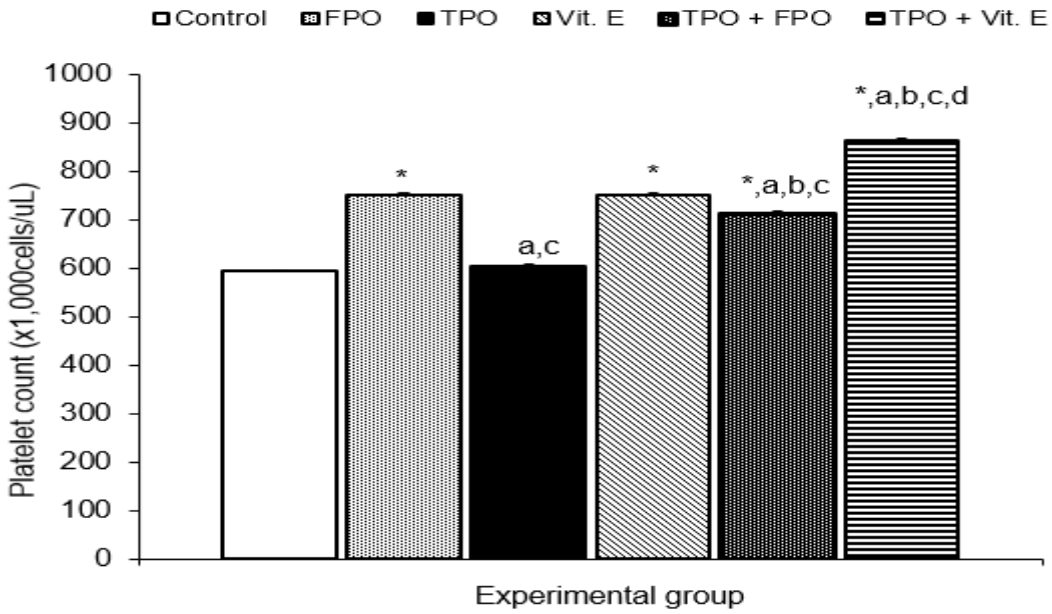

FIGURE 7: Platelet count comparison among groups.

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

Total white blood cell count comparison among groups

The total WBC count (x1,000cells/µL) for control, FPO, TPO, Vit. E, TPO + FPO and TPO + Vit. E was 6.40 ± 0.19, 6.17 ± 0.14, 8.60 ± 0.07, 6.25 ± 0.11, 7.30 ± 0.11 and 7.45 ± 0.14, respectively. The result showed a significant increase ($P<0.05$) in TPO, TPO + FPO and TPO + Vit. E when compared to control. The results for FPO, Vit. E, TPO + FPO and TPO + Vit. E were significantly decreased ($P<0.05$) when compared to TPO. TPO + FPO and TPO + Vit. E were significantly increased ($P<0.05$) compared to FPO and Vit. E respectively (Figure 8).

Lymphocyte count

The lymphocyte count (%) for control, FPO, TPO, Vit. E, TPO + FPO and TPO + Vit. E was 69.20 ± 0.86, 78.80 ± 0.06, 89.80 ± 0.80, 76.40 ± 2.04, 77.40 ± 1.08 and 70.00 ± 0.89 respectively. The result showed a significant increase ($P<0.05$) in FPO, Vit. E, TPO and TPO + FPO compared to control. The results for FPO, Vit. E, TPO + FPO and TPO + Vit E were significantly reduced ($P<0.05$) when compared to TPO. TPO + Vit. E reduced significantly ($P<0.05$) compared to FPO, Vit. E and TPO + FPO respectively (Figure 9).

Monocyte count

The monocyte count (%) for control, FPO, TPO, Vit. E, TPO + FPO and TPO + Vit. E was 25.20 ± 1.24, 20.00 ± 0.49, 5.80 ± 0.58, 16.40 ± 0.75, 21.60 ± 2.66 and 28.00 ± 1.41, respectively. The results showed a significant decrease ($P<0.05$) in FPO, TPO, Vit. E and TPO+FPO when compared to control, while TPO+Vit. E was significantly increased ($P<0.05$) when compared to control. The results for FPO, Vit. E, TPO+FPO and TPO+Vit. E were significantly increased ($P<0.05$) when compared to TPO. Vit. E was significantly reduced ($P<0.05$) compared to FPO, while TPO+Vit. E and TPO+FPO were significantly increased ($P<0.05$) when compared to FPO. TPO+Vit. E significantly increased ($P<0.05$) when compared to Vit. E and TPO+FPO respectively (Figure 10).

Eosinophil count

The eosinophil count (%) for control, FPO, TPO, Vit. E, TPO + FPO and TPO + Vit. E was 2.00 ± 0.00, 0.00 ± 0.00, 1.00 ± 0.00, 3.00 ± 0.00, 1.00 ± 0.00 and 2.00 ± 0.00, respectively. The eosinophil count didn't differ significantly among groups (Figure 11).

Basophil count comparison among groups

The basophil count (%) for control, FPO, TPO, Vit. E, TPO + FPO and TPO + Vit. E was 3.60 ± 0.24, 2.00 ± 0.00, 3.40 ± 0.60, 4.20 ± 1.37, 0.00 ± 0.00 and 0.00 + 0.00, respectively. Basophil count didn't differ significantly among groups (Figure 12).

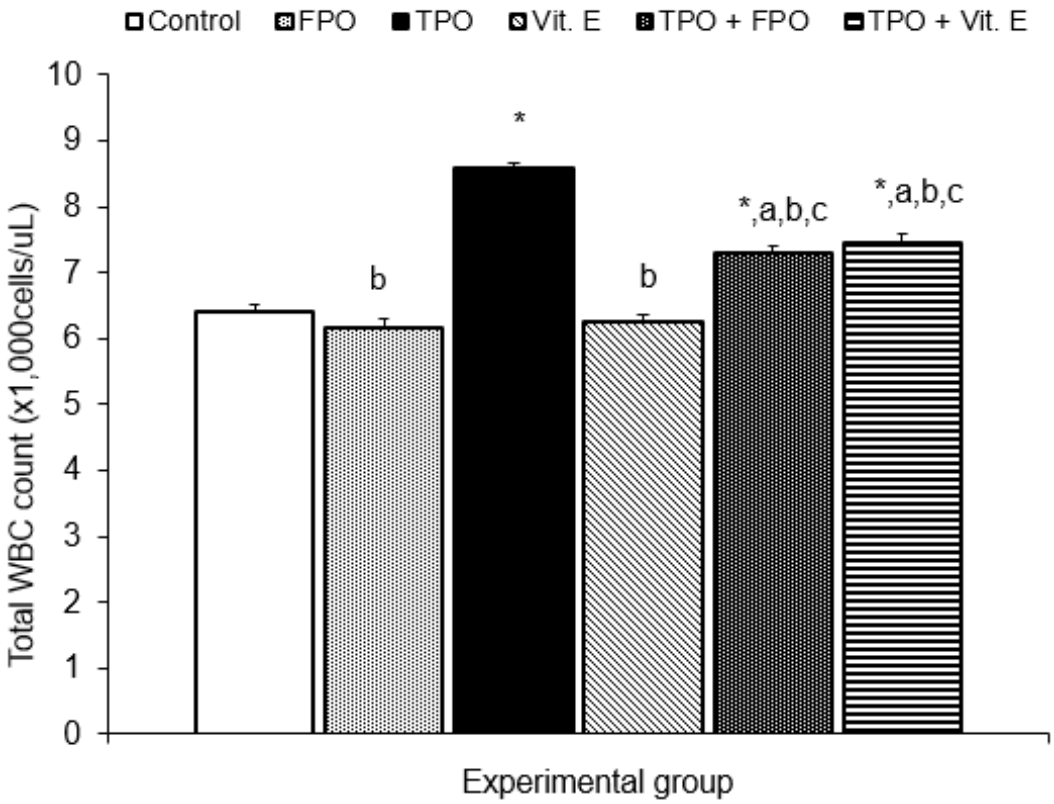

FIGURE 8: Total white blood cell count comparison among groups.

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

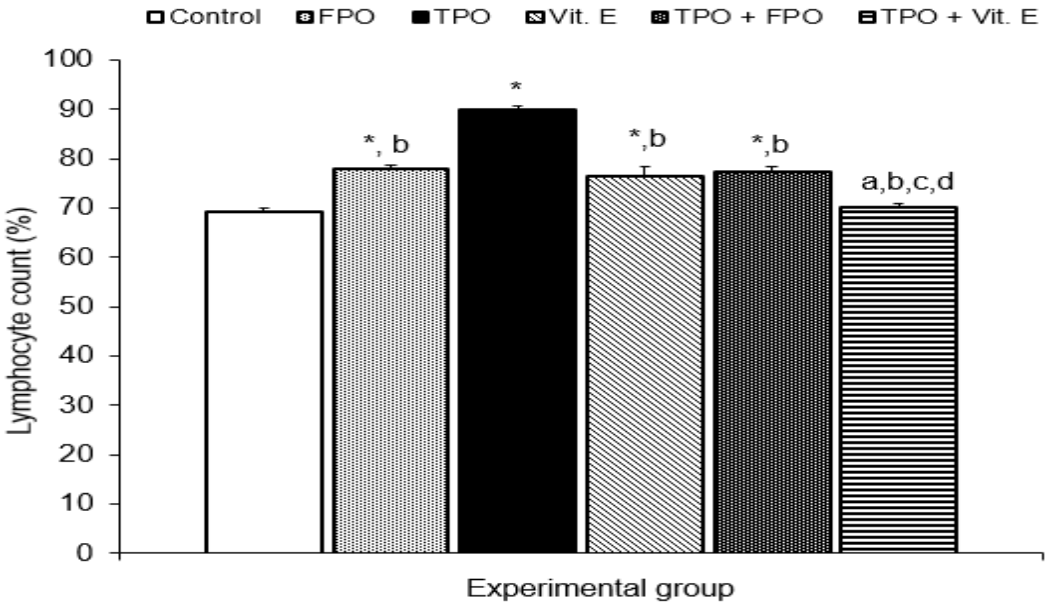

FIGURE 9: Lymphocyte count comparison among groups.

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

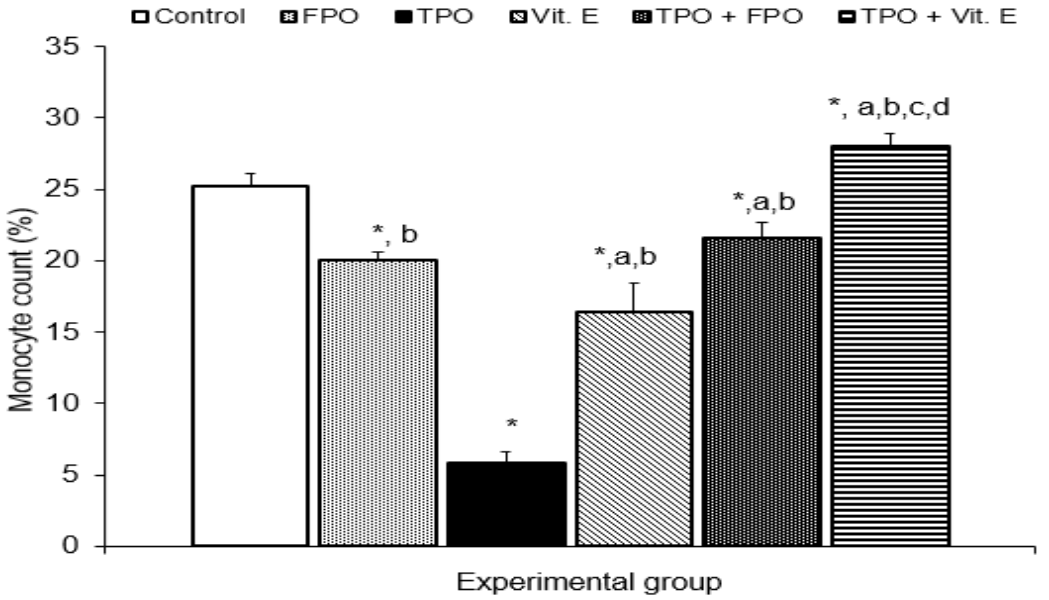

FIGURE 10: Monocyte count comparison among groups.

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

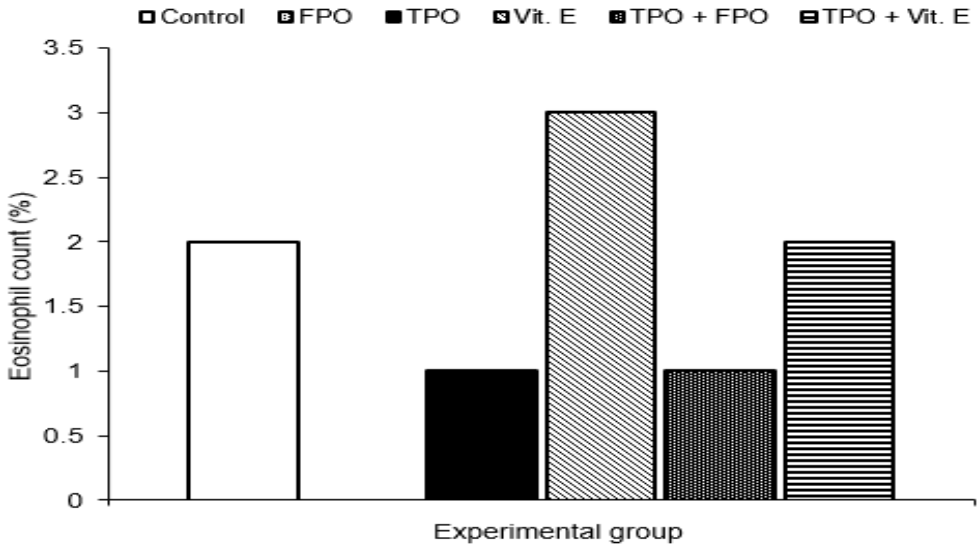

FIGURE 11: Eosinophil count comparison among groups.

Values are expressed as mean ±SEM, n = 10.
No significant differences among group

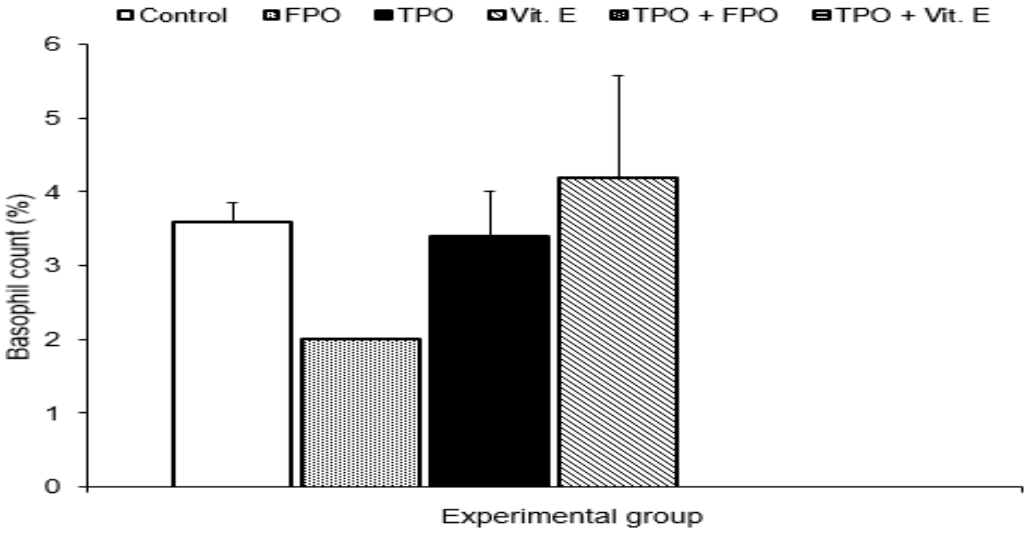

FIGURE 12: Basophil count comparison among groups.

Values are expressed as mean ±SEM, n = 10.
No significant differences among group

DISCUSSION

The present study was design to compare the effect FPO and Vit. E in ameliorating the adverse effect of TPO fed diet in hematological indices of rats. We observed that consumption of FPO and Vit. E had a beneficial effect by normalizing the values of certain hematological indices of TPO fed rat. Consumption of vitamin E caused a significant improvement in recovery of hematological indices of TPO fed rats compared to FPO.

The RBC, PCV and Hb results in TPO fed group in this study is consistent with that reported by[4,7], while[6] reported a decrease PCV concentration in TPO fed group, but Hb concentration did not differ significantly. This could be due to the sample size use in this study. The decrease in RBC in TPO fed rats may be due to the suppressive effect of the toxic constituents of thermally oxidized palm oil on growth and differentiation in the bone marrow.[4] PCV represents the percentage of RBC in blood. Therefore, the decrease in PCV observed in this study agrees with the decrease RBC counts.[4] TPO diet was reported to damage intestinal mucosa resulting in a reduced bioavailability of iron in the system[4], it was also reported to damage the liver resulting in decrease storage of iron in the liver.[4] These could be the reason for the reduced Hb concentration in the TPO fed rat. In our study, FPO fed diet increase RBC compared to control, in contrasts with the study of Mesembe et al.[4] which reported that FPO fed group did not differ significantly compared to control. The observed inconsistency between this study may be due to the duration of exposure to TPO. This study employed 4 weeks, while the other study employed 14 weeks. As observed in this study, PCV and Hb concentration in FPO fed diet did not differ significantly compared to control. The increase in RBC, PCV and Hb concentration in vitamin E fed diet in this study is consistent with the study of[18] who reported that vitamin E increase RBC, PCV and Hb concentration at different dosage levels in rats fed with oxidized olive oil. The effect of vitamin E in RBC, PCV and Hb could be as reported by[11] that vitamin E protect hematopoietic stem and progenitor cells (HSPCs) from lipid peroxidation and ferroptosis, and by[12] that vitamin E shield the bone marrow erythroid colony-forming units from toxicity. As observe in this study treatment of TPO fed rat with vitamin E showed a marked increase in RBC, PCV and Hb concentration when compared to treatment with FPO. It is expected that FPO which is reportedly rich in vitamin E in addition to its other vital constituents should have been more effective than vitamin E alone in ameliorating the adverse effect of TPO fed diet, but Aribo et al.[2] reported that most fresh palm oil consumed generally must have undergone some level of photo-and-chemical oxidation, a process known to have adverse effect on the oil quality.

It has been reported that increased MCV, MCH and MCHC may indicate infection and fatigue.[7] In this study, only MCV was shown to increase in the TPO fed group and was consistent with that reported by.[7] It was then reduced significantly in TPO+FPO group and TPO+ Vit. E group compared to TPO alone. The decrease MCH count in TPO fed rats in this study is contrary to that reported by.[7] FPO and vitamin E were effective in restoring the

MCH count of TPO fed rats. MCHC was not significant in this study following TPO fed diet compared to control, while Obeten et al.[7] observed a significant increase compared to control.

FPO and vitamin E increased platelet count when compared to control and TPO. There was increase in platelet count following treatment of TPO fed rat with FPO and vitamin E. Zeb and Khan[18] reported that vitamin E normalized the low levels of platelet count in rats fed with oxidized olive oil. As observe in this study treatment with vitamin E showed a marked increase when compared to TPO fed rat treated with FPO. Malmir et al.[19] reported that platelet is one of the cells vitamin E concentrations is highest. This could be the reason for the increase platelet count in the vitamin E treated group observe in this study.

Total WBC and lymphocytes are mobilized in large numbers during chronic conditions. This probably explains the significantly increased total WBC and lymphocyte count observed in TPO fed diet.[6] The increase in total WBC was consistent with that of[4] but contrary to that reported by.[6] WBC was reduced significantly in TPO+FPO group and TPO+ Vit. E group compared to TPO. FPO and vitamin E increased lymphocyte count when compared to control. The increase in lymphocyte count in TPO fed diet was consistent with the report of.[6] Treatment of TPO fed rat with FPO and vitamin E showed a significant reduction in lymphocyte count. The reduction was more pronounced in TPO fed rat treated with vitamin E compared to FPO treated rat.

The decrease in monocytes count of TPO fed rat in this study was contrary to the report of[6] in which monocyte count did not differ significantly in TPO fed group compared to control. This could be due to the sample size use in this study. The monocyte count of FPO and vitamin E group was significantly reduced compared to control and that of vitamin E was also reduced compared to FPO. This study showed that treatment of TPO fed rat with vitamin E was more potent in restoring the monocyte count compared to control.

The non-significant changes in eosinophil and basophil in this study was consistent with that reported by.[6]

CONCLUSION

This study has demonstrated that vitamin E is more potent than FPO in reversing hematological changes due to consumption of TPO. This may be so because FPO, unless

consumed immediately after it is processed is not of the highest quality. Majority of the FPO are subjected to photo- and-chemical oxidation which reduces the potency of the oil.

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