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Therioxidized palm oil diet (TPO)-induced haematological derangements in rats is ameliorated by *Aloe vera* and Garlic

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ABSTRACT

Objective(s): Therapeutic effects of garlic/*Aloe vera* on TPO-induced derangements in some haematological parameters and cytoarchitecture of the bone marrow in rats were studied. **Materials and Methods:** 30 male wistar rats weighing 200-250g were grouped into five (n=6): Control, TPO (TPO diet), TPO+G (TPO/Garlic), TPO+A (TPO/Aloe) and TPO+G+A (TPO/Garlic/Aloe). 15g of thermoxidized palm oil was mixed with 85g of rat chow to prepare TPO. Aloe gel and garlic juice were orally administered at doses of 6ml/kg and 2ml/kg respectively following lethality studies. After 3months, rats were sacrificed and blood collected via cardiac puncture for analysis. **Results:** From the result, TPO/Aloe showed a significant increase in food/water intake compared to control. TPO, TPO/Garlic and TPO/Garlic/Aloe had a significantly reduced body weight when compared to control but significantly higher in TPO/Aloe compared to control and other three groups. RBC, PCV and Hb was significantly decreased in TPO compared to control but was significantly higher in TPO/Garlic, TPO/Aloe and TPO/Garlic/Aloe compared to TPO. Total White blood cell was significantly lower in TPO/Garlic, TPO/Garlic/Aloe when compared to TPO but significantly higher in TPO/Aloe when compared to TPO/Garlic and TPO/Garlic/Aloe. MCV, MCH and MCHC showed a significant increase in TPO compared to control but decreased in TPO/Garlic, TPO/Aloe, TPO/Garlic/Aloe when compared to TPO. Histology of the bone marrow showed normal morphology in Control, TPO/Garlic, TPO/Aloe and TPO/Garlic/Aloe while some histological derangements were seen in TPO. **Conclusion:** Garlic and *Aloe vera* showed therapeutic efficacy in ameliorating haematological derangements caused by TPO consumption.

Keywords: Garlic, *Aloe vera*, Thermoxidized Palm oil, Haematological parameters, Bone marrow.

INTRODUCTION

In Nigeria, it is noted that *Aloe vera* is grown in every home mostly in the Southern Western regions while garlic is richly abundant in the North. There are many folklore beliefs on the efficacy of these two plants in the treatment of many ailments locally. Scientific approaches have confirmed the potency of *Aloe vera* and garlic in the management of several health complications. Fresh palm oil derived from *Elaeis guineensis* fruit is widely used as cooking oil mostly in its thermoxidized form in Nigeria. Thermoxidization occurs when its fresh form undergoes many rounds of heating at high temperatures [1]. Most of this thermoxidized palm oil is kept for re-use after frying, hence making it more thermoxidized. As a consequence of thermoxidation, several by-products which are harmful to cells, tissues and organs are generated [2]. Reports have shown that chronic consumption of oxidized oils cause fatty livers, thrombosis, and micro nutrient malnutrition leading to deactivation of key metabolic enzymes [3], alters liver function and histology [4], affects haemostatic process [5] as well as haematological indices [6, 7]. The chronic consumption of thermoxidized oil is very common in Nigeria as well as other countries and several reported effects exist in literature. The human population suffering from thermoxidized oil associated diseases is increasing and conventional treatments are not readily available or affordable by the majority of Nigerians in management or treatment of these ailments [8]. Therefore, research on medicinal plants which are potent and easily accessible in tackling these ailments is necessary, hence this research.

MATERIALS AND METHODS

Thirty male albino wistar rats weighing between 200-250g were used for this study after ethical approval was obtained from animal research ethical committee, Faculty of Basic Medical Sciences, University of Calabar with approval number 09/16. The animals were divided into five groups of six animals each

after acclimatization for one week. Animals in group one was designated as Control and were allowed free access to normal rat chow and water. Animals in the second group were fed TPO diet and water. The third group were fed with TPO in addition to oral administration of garlic juice. The fourth group were fed TPO in addition *Aloe vera* gel orally administered while the fifth group was fed with TPO in addition to oral administration of both garlic juice and *Aloe vera* gel. This feeding and treatment lasted for 90 days. The gel and juice were orally administered at doses of 6 ml/kg and 2 ml/kg respectively [4,5].

Preparation of garlic juice

Garlic bulbs were obtained from watt market in Calabar-Nigeria. The garlic bulbs were washed and cloves separated from the bulbs. The cloves were thinly sliced with a knife and homogenised to a paste form using an electric blender then filtered with a filter paper to obtain the juice as reported by Chowethdury *et al.* [9]. The juice was preserved in the physiology departmental refrigerator, university of Calabar.

Preparation of *Aloe vera* gel

Aloe vera plants were bought from botanical garden, Calabar. The leaves were washed with tap water to remove dirt. The leaves were divided with knife into two parts in the margin to expose the gel. The gel was scooped into a bowl and were homogenised using an electric blender for 20-25 minutes to a fine juice [6]. Both *Aloe vera* and garlic were identified by a Botanist in the Department of Botany, University of Calabar, Calabar.

Preparation of thermoxidised palm oil and TPO

Forty litres of fresh palm oil were thermoxidized as described by Ime *et al.* [5] after the oil was certified fresh by a biochemist. 15g of thermoxidized palm oil was mixed with 85g of rat chow to prepare TPO [3].

34g TPO was given to each animal on a daily basis. The diet was stored in a black container at 4 °C to prevent further oxidation of the oil components.

Animal sacrifice and collection of blood samples

At the end of the feeding period, the animals were anaesthetised with chloroform after an overnight fast. 2ml of blood samples were collected by cardiac puncture into EDTA sample bottles for determination haematological parameters.

Measurement of blood parameters

Automated blood cell analyser (Model PCE 210, Japan) was used. The blood sample after being collected into EDTA sample bottles as described above was thoroughly mixed but gently to avoid clotting. The machine was switched on with its specific diluents and haemolysate and allowed to boot. When the machine indicated readiness, each sample bottle was raised for the probe of the machine to suck the required volume of the blood for the test. The machine automatically began to measure the blood parameters. The results were displayed on the screen and were subsequently printed out. Some of the results were confirmed by manual method of

haemocytometry and no significant difference was observed between the manual and automated methods.

Histological Analysis of the bone marrow

Permanent preparations using routine biopsy method was used as described by Karadeniz *et al.* [10].

Statistical analysis

One-way analysis of variance of SPSS statistical tool was used in data analysis followed by post-HOC test and hypothesis were tested at 95% level of significance. P value (<0.05) were considered significant.

RESULTS

Food Intake and water intake (FIG. 1 and 2 respectively)

After 90 days of administration, food intake (in g) was 69.57± 1.43; 78.29 ± 1.31; 70.22±1.09; 92.22±1.61; and 79.44±1.67 for control, TPO, TPO/Garlic, TPO/Aloe and TPO/Garlic/Aloe respectively. The result presented a significant increase (P<0.001) in food intake of TPO+Aloe group in comparison with control.

Water intake (in ml) were 87.00± 1.66; 89.44± 1.72; 85.00± 2.02; 96.06± 1.24 and 89.33± 1.55 for control, TPO, TPO/Garlic, TPO/Aloe, TPO/Garlic/Aloe respectively. The result showed a significant increase (P<0.001) in water intake of TPO/Aloe group compared to control and all test groups. TPO/Garlic had a significantly decreased (P<0.001) water intake compared to other groups. TPO/Garlic/Aloe showed a significant increase (P<0.001) in daily water intake compared to TPO/Garlic. Water intake was significantly decreased (P<0.001) in TPO/Garlic and TPO/Garlic/Aloe compared to TPO/Aloe.

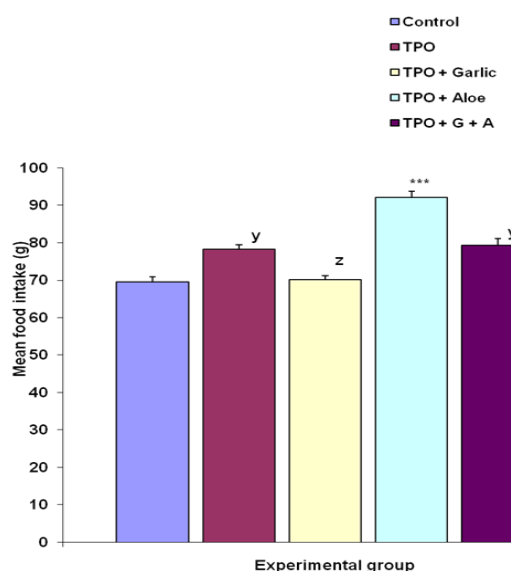


Figure 1: Comparison of mean food intake. Values are expressed as mean + SEM, n = 6.

*** = significantly different from control at p<0.001;
y = significantly different from TPO+Aloe at p<0.01;
z = significantly different from TPO+Aloe at P<0.001

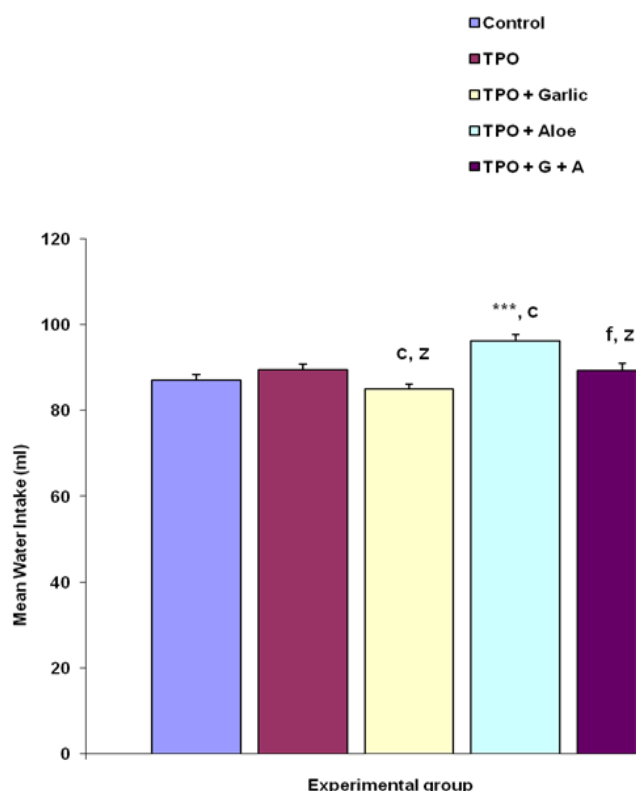


Figure 2: Comparison of mean daily water intake in the different experimental groups. Values are mean \pm SEM, n = 7.

***p<0.001 vs Control;
 c = p<0.001 vs TPO;
 f = p<0.001 vs TPO+Garlic;
 z = p<0.001 vs TPO+Aloe.

Mean Body Weight Change (FIG. 3)

The mean body weight change (g) was 41.43 \pm 2.82; 14.29 \pm 3.21; -35.71 \pm 5.06; 67.14 \pm 6.55 and -21.43 \pm 3.67 for control, TPO, TPO/Garlic, TPO/Aloe, TPO/Garlic/Aloe respectively. This result presented a decrease (P<0.001) in body weight change of TPO group compared to control. There was a negative decrease (P<0.001) in body weight change in TPO/Garlic and TPO/Garlic/Aloe when compared to control. TPO/Garlic/Aloe had a lesser negative decrease (P<0.05) in body weight change than that of TPO/Garlic. The mean body weight change in TPO/Garlic and TPO/Garlic/Aloe were significantly lower (P<0.05, P<0.001) than that of TPO respectively. TPO/Garlic/Aloe had a negative decrease (P<0.001) in mean body weight change than that of TPO/Aloe. TPO/Aloe caused a significant increase (P<0.01) in mean body weight change compared to control. TPO/Aloe resulted in a significant increase (P<0.001) in mean body weight change compared to TPO and TPO/Garlic respectively.

Red blood cell count in the different experimental groups (FIG. 4)

The red blood cell count (x1million cells/uL) was 7.54 \pm 0.22; 8.35 \pm 0.33; 8.43 \pm 0.29; 8.19 \pm 0.23; and 8.27 \pm 0.22 for Control, TPO, TPO/Garlic, TPO/Aloe and TPO/Garlic/Aloe respectively. The result presented a significant decrease (P<0.05) in mean RBC count in TPO group compared to Control. However, TPO/Garlic was seen to cause a significant increase (P<0.05) in mean RBC count compared to control and TPO. More so, TPO+A and TPO/Garlic/Aloe showed a significant increase in RBC count compared to TPO.

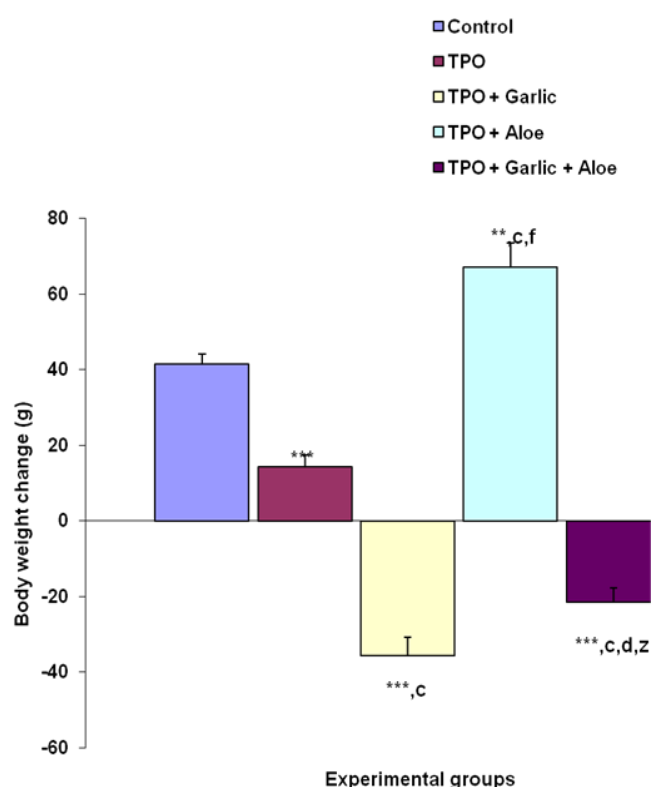


Figure 3: Mean body weight change of the different experimental groups.

Values are expressed as mean \pm SEM, n = 6.
 ***significantly different from control at p<0.001;
 **significantly different from control at p<0.01;
 c = significantly different from TPO at p<0.001;
 d = significantly different from TPO + Garlic at p<0.05;
 f = significantly different from TPO + Garlic at p<0.001;
 z = significantly different from TPO + Aloe at p<0.001.

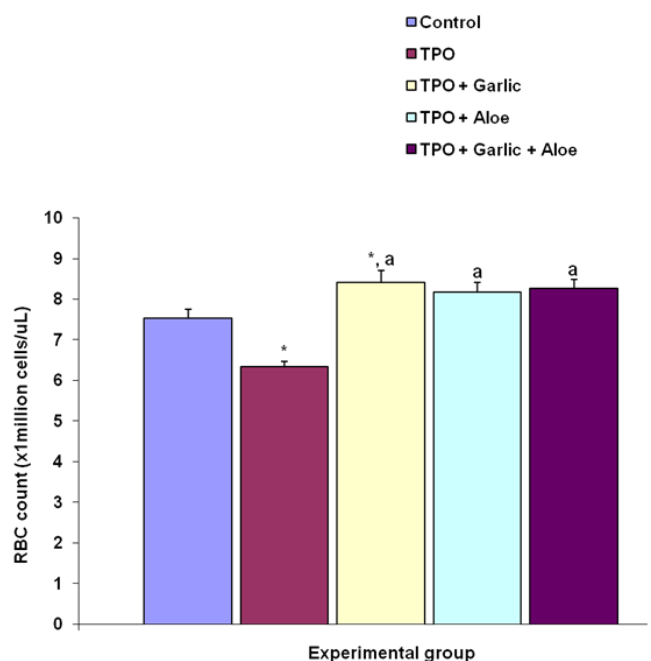


Figure 4: Comparison of red blood cell count in the different experimental groups.

Values are expressed as mean + SEM, n = 6.
 *significantly different from control at p<0.05
 a = significantly different from TPO at p<0.05

Total White Blood Cell Count (FIG. 5)

The white blood cell count (x1000 cells/ μ L) was 11.12 ± 1.13 ; 12.93 ± 1.09 , 9.63 ± 0.79 ; 13.00 ± 0.90 ; 9.00 ± 1.32 for control, TPO, TPO/Garlic, TPO/Aloe and TPO/Garlic/Aloe respectively. The result showed a significant decrease ($P < 0.05$) in total white blood cell count for TPO/Garlic when compared to TPO. Total white blood cell count for TPO/Aloe was significantly higher ($P < 0.05$) when compared to TPO/Garlic. Total white blood cell count for TPO/Garlic/Aloe was significantly lower ($P < 0.05$) when compared to TPO and TPO/Aloe respectively.

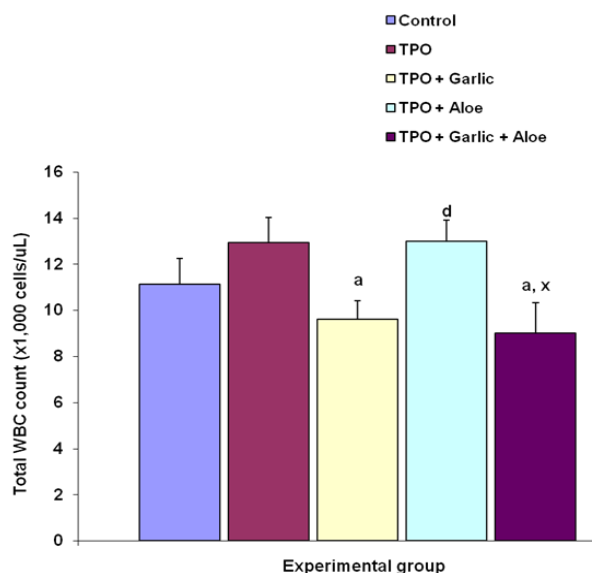


Figure 5: Comparison of total white blood cell count in the different experimental groups.

Values are expressed as mean \pm SEM, n = 6.

a = significantly different from TPO at $p < 0.05$;

d = significantly different from TPO + Garlic at $p < 0.05$;

x = significantly different from TPO + Aloe at $p < 0.05$

Packed cell volume (FIG. 6)

The packed cell volume in (%) was 44.70 ± 0.63 ; 41.88 ± 0.45 , 45.95 ± 1.51 , 46.35 ± 1.95 and 46.85 ± 1.40 for Control, TPO, TPO/Garlic, TPO/Aloe, TPO/Garlic/Aloe respectively. The result showed a significant decrease ($P < 0.05$) for TPO when compared to Control. The result for TPO/Garlic, TPO/Aloe and TPO/Garlic/Aloe was not significant when compared to control.

Haemoglobin Concentration (FIG. 7)

The haemoglobin concentration (g/dL) was 13.72 ± 0.21 ; 14.17 ± 0.72 ; 14.00 ± 0.41 ; 13.68 ± 0.59 ; 14.05 ± 0.28 for Control, TPO, TPO/Garlic, TPO/Aloe, TPO/Garlic/Aloe respectively. The result showed a significant decrease ($P < 0.05$) for TPO when compared to Control. TPO/Garlic, TPO/Aloe, TPO/Garlic/Aloe were significantly higher ($P < 0.05$) when compared to TPO.

Mean corpuscular volume (FIG. 8)

The mean corpuscular volume (fL) was 59.45 ± 1.25 ; 58.11 ± 0.57 ; 54.54 ± 0.20 ; 56.49 ± 0.96 ; 56.63 ± 0.36 for control, TPO, TPO/Garlic, TPO/Aloe, TPO/Garlic/Aloe respectively. TPO was significantly

higher ($P < 0.05$) when compared to Control. TPO/Garlic was significantly lower ($P < 0.001$) when compared to control and also significantly lower ($P < 0.05$) when compared to TPO. TPO/Aloe and TPO/Garlic/Aloe showed significant decrease ($P < 0.05$) when compared to control and also showed significant decrease ($P < 0.05$) when compared to TPO.

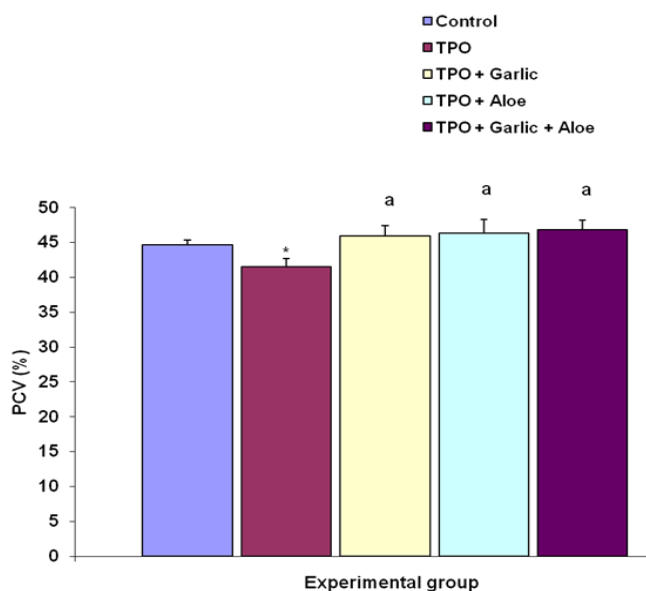


Figure 6: Comparison of packed cell volume in the different experimental groups.

Values are expressed as mean \pm SEM, n = 6.

*significantly different from control at $p < 0.05$

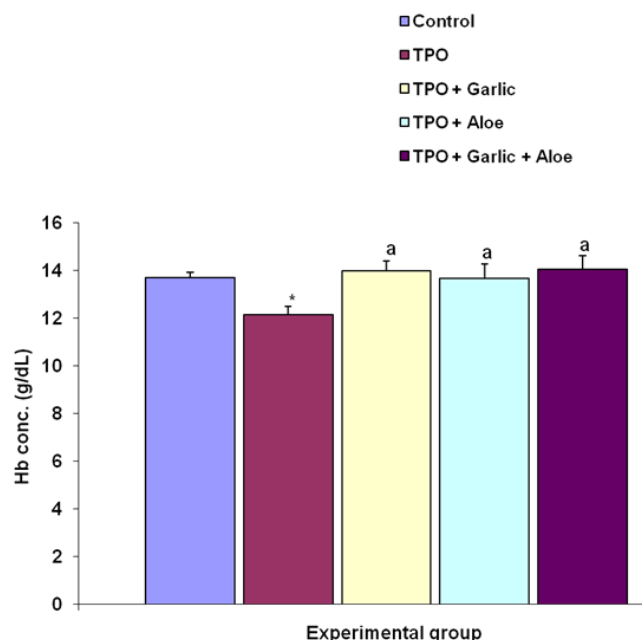


Figure 7: Comparison of haemoglobin concentration in the different experimental groups.

Values are expressed as mean \pm SEM, n = 6.

*significantly different from control at $p < 0.05$

a = significantly different from TPO at $p < 0.05$

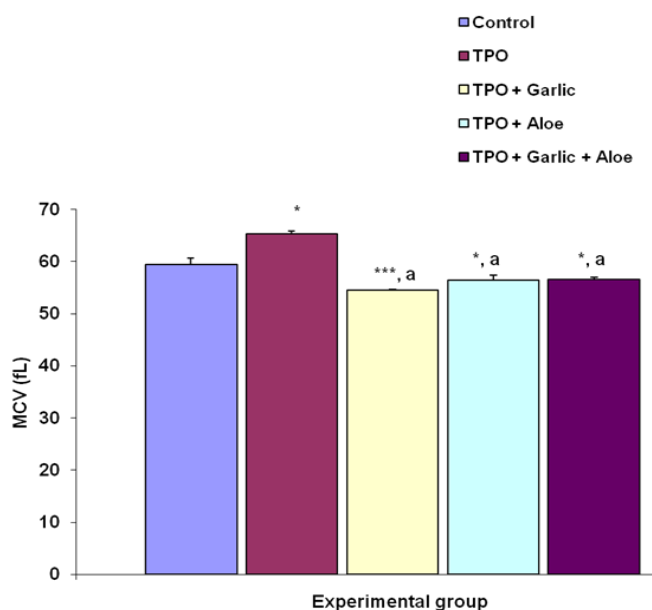


Figure 8: Comparison of mean corpuscular volume in the different experimental groups.

Values are expressed as mean \pm SEM, n = 6.

*p<0.05, ***p<0.001 vs Control;

a = significantly different from TPO at p<0.05

Mean corpuscular haemoglobin (FIG. 9)

The mean corpuscular haemoglobin (pg) was 18.25 \pm 0.41; 16.95 \pm 0.52; 16.63 \pm 0.24; 16.69 \pm 0.44; 17.01 \pm 0.18 for control, TPO, TPO/Garlic, TPO/Aloe, TPO/Garlic/Aloe respectively. TPO showed a significant increase (P<0.05) when compared to control. TPO/Garlic, TPO/Aloe, TPO/Garlic/Aloe showed a significant decrease (P<0.05) in mean corpuscular haemoglobin when compared to Control and also significantly lower (P<0.05) when compared to TPO.

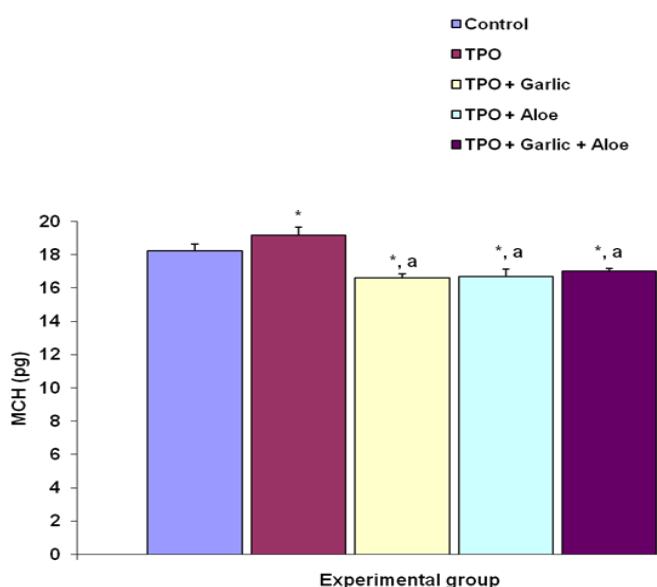


Figure 9: Comparison of mean corpuscular haemoglobin in the different experimental groups.

Values are expressed as mean \pm SEM, n = 6.

*significantly different from control at p<0.05

a = significantly different from TPO at p<0.05

Mean corpuscular haemoglobin concentration (FIG. 10)

The mean corpuscular haemoglobin concentration (%) was 30.69 \pm 0.15; 29.18 \pm 0.93; 30.50 \pm 0.45; 29.54 \pm 0.53; 30.04 \pm 0.39 for control, TPO, TPO/Garlic, TPO/Aloe, TPO/Garlic/Aloe respectively. The mean corpuscular haemoglobin concentration for TPO was significantly higher (P<0.05) when compared to Control. TPO/Garlic, TPO/Aloe, TPO/Garlic/Aloe showed a significant decrease (P<0.05) in mean corpuscular haemoglobin concentration when compared to TPO.

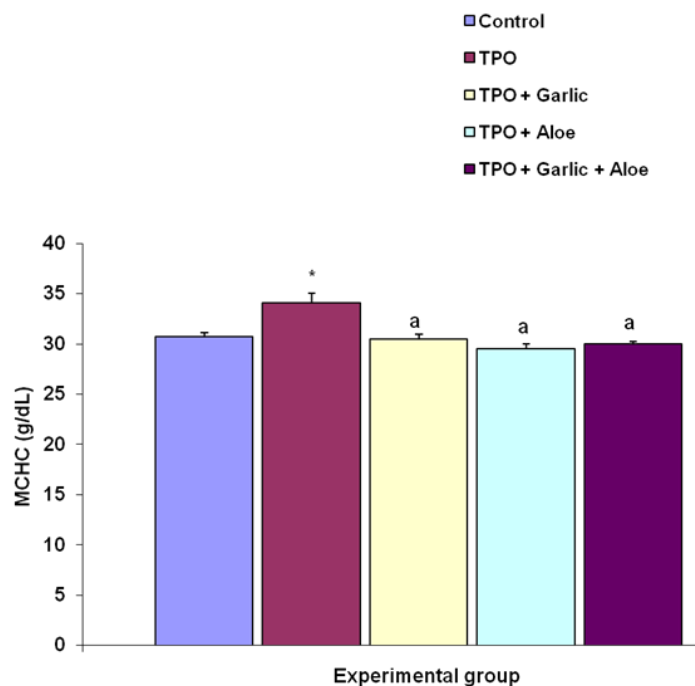


Figure 10: Comparison of mean corpuscular haemoglobin concentration in the different experimental groups.

Values are expressed as mean \pm SEM, n = 6.

*significantly different from control at p<0.05

a = significantly different from TPO at p<0.05

Histology of the bone marrow (Plate 1A-E)

A section of bone marrow from Control group shows hypercellular bone marrow with a myeloid to erythroid ratio of 5:1 (Normal range). The erythroid, granulopoietic, lymphopoietic and Megakaryopoietic cells are adequate with normal morphology. The plasma cells are normal and no foreign cells seen. (HSC-hypercellular smear, NES-Neutrophil series MEG-megakaryocytes NMB-normoblast OST-osteoblast).

Similar section from TPO fed rat shows a hypercellular bone marrow with a myeloid to erythroid ratio of normal range. There are numerous fat vacuoles and the megakaryoblasts have abnormal folded nuclei contours (MEG). The neutrophil lineage has u-shaped or kidney shaped nuclei and are adequate with normal morphology (NES).

Section from garlic treated group and TPO/Garlic/Aloe show hypercellular bone marrow with a myeloid to erythroid ratio of normal range. The megakaryoblasts are adequate with regular nuclei contours and few having abnormal folded nuclei contours (MEG). The neutrophil lineage has u-shaped or kidney shaped nuclei and are

adequate with normal morphology (NES). The normoblast lineage are adequate with normal morphology and no foreign cells noted

A Section from *Aloe vera* treated group shows a hypercellular bone marrow with a myeloid to erythroid ratio within normal range. The

megakaryoblasts are adequate with regular nuclei contours (MEG). The neutrophil lineage has u-shaped or kidney shaped nuclei and are adequate with normal morphology (NES). The normoblast lineage are adequate with normal morphology and the osteoid forming cells are adequate and no foreign cells noted.

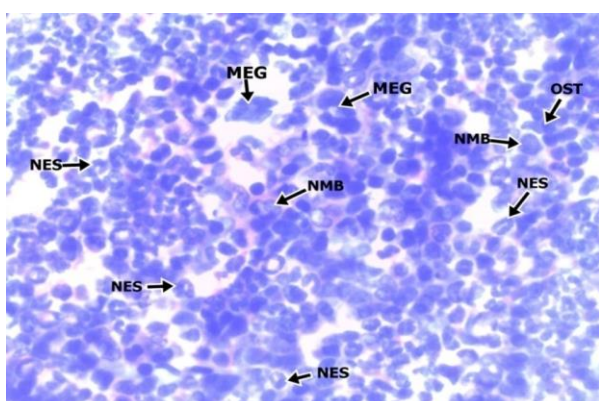


Plate 1A: Control Group

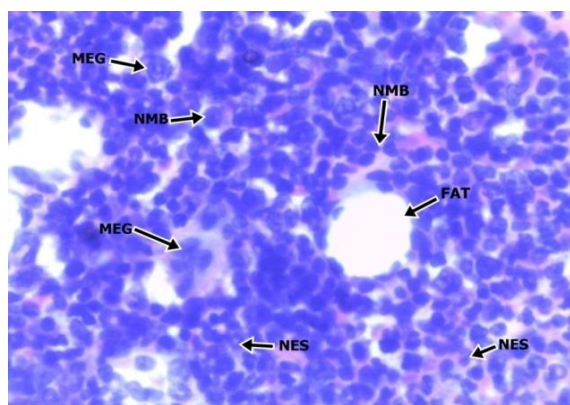


Plate 1B: TPOD Group

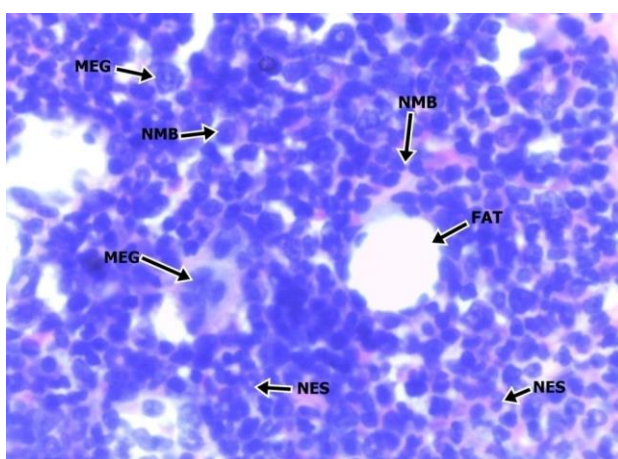


Plate 1C: TPOD+G Group

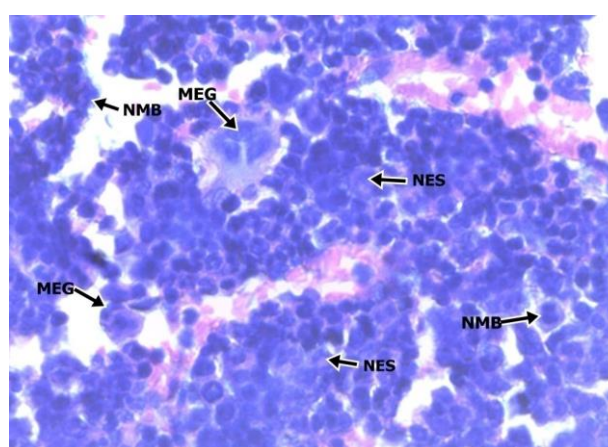


Plate 1D: TPOD+A Group

Plate 1: Photomicrograph of bone marrow tissue from TPO/Garlic/Aloe group Magnification: x1000.

DISCUSSION

From the result of this study, consumption of *Aloe vera* with little contribution of garlic caused significant improvements in some blood parameters, water/food intake and body weight as well as histology of the bone marrow despite thermoxidized palm oil diet induced cytotoxicity. TPO showed no significant decrease in food and water intake compared to control, however, *Aloe vera* treated group was seen to cause a significant increase in food, Water intake and body weight change compared to control as well as TPO untreated group. However, the mechanism by which *Aloe vera* induces these effects was not taken in this study. This result is in contrast with the work carried out by Nna *et al.* [11] who reported a decrease in food and water intake by *Aloe vera* following its treatment on Streptozocin-induced diabetes mellitus rats.

From this study, TPO caused a significant reduction in RBC count. However, *Aloe vera* and garlic treated group showed a marked increase in RBC when compared to TPO group. The decrease in red blood cell count seen in TPO group may be attributable to some derangements caused by TPO on the histology of bone marrow as seen in this study. More so, Osim *et al.* [3] reported thermoxidized

palm oil diet associated nephritic damage with partial tubular atrophy leading to anaemia. Therefore, it is possible that this affects the level of erythropoietin needed for erythropoiesis.

PCV and Hb concentration were found to be significantly lower in the thermoxidized palm oil diet fed group of rats compared to control. This reduction may be indicative of anaemia in the thermoxidized fed group of rats. It agrees with the work carried out by Ani *et al.* [6] who reported a decrease in PCV, RBC count and Hb concentration in rabbits fed TPO. This effect was seen to be improved by garlic and *Aloe vera*. MCV, MCH and MCHC were seen in this study to be significantly raised in the thermoxidized palm oil group in comparison with control. It was then reduced significantly in TPO+garlic group and TPO+ *Aloe vera* group compared to TPO alone. It has been reported that increased MCV, MCH and MCHC may indicate infection and fatigue and that the increase indicates variation in erythrocyte shape, size and haemoglobin content. This denotes the presence of hyperchromic macrocytic anaemia resulting from consumption of thermoxidized palm oil diet in male rats.

This study did not find any significant difference in total white blood cell count in the thermoxidized palm oil diet fed group of rats

compared to control. However, treatment with garlic and *Aloe vera* was seen to induce marked improvements in the histology of these organs as seen in reduction in the number and extent of inflammatory cells.

CONCLUSION

Though consumption of foods containing thermoxidized palm oil adversely affect the haematological system and distort histology of some organs like the bone marrow as seen in this study, garlic and *Aloe vera* have been found to ameliorate most of these negative changes which pose a health risk. Therefore, besides avoiding the liberal consumption of thermoxidized palm oil diets, intake of garlic and *Aloe vera* should be encouraged since they ameliorate many of the health risks posed by TPO. If these results are extrapolated to humans, this may be a cheap and effective way to prevent many ailments that plague humans.

Conflicts of Interest

Nil.

Contribution by Authors

Five authors carried out this research. The study was designed by EJA. CEO did the laboratory work with the assistance of AUI, AKN and OUE. CEO wrote the initial draft which was corrected by AUI and EJA. All authors read and approved the final manuscript before submission for publication.

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