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Effect of *Aloe vera* gel on thermoxidized palm oil-induced derangements in some haematological and biochemical parameters

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ABSTRACT

Thermoxidized palm oil has been shown to generate free radicals in the body, and constitute a major risk factor for developing varying degree of disorders. Following the widely celebrated medicinal uses of *Aloe vera* gel, this study therefore seeks to examine the effects of its administration on various haematological parameters of rats chronically fed with thermoxidized palm oil. Fifteen albino Wistar rats weighing 200 – 250 g were randomly assigned 1 of 3 groups ($n = 5$), thus; control, thermoxidized palm oil (TPO) fed group - untreated and TPO fed group – treated with *Aloe vera* gel (TPO + AV) at a dose of 0.2ml/100g body weight per oral route. The administration of *Aloe vera* gel lasted for 4 weeks, after which the animals were sacrificed and blood samples collected for analysis. Results showed that TPO significantly ($p < 0.05$) increased mean daily water intake and decreased ($p < 0.001$) white blood cell count, compared with control. White blood cell count was significantly ($p < 0.05$) reduced in TPO + AV group compared with control, and significantly ($p < 0.05$) higher in TPO + AV group, compared with TPO group. Platelet count was significantly ($p < 0.05$) increased in TPO + AV group, compared with control and TPO groups. Low density lipoprotein (LDL-c) was significantly ($p < 0.05$) reduced in TPO + AV group, compared with control. Very low density lipoprotein (VLDL-c) was significant ($p < 0.05$) reduced in TPO + AV group, compared with TPO group. Thermoxidized palm oil consumption did not significantly alter red blood cell count, total serum cholesterol, and serum electrolytes in this study. However, *Aloe vera* gel administration appeared to be beneficial in reducing LDL-c and VLDL-c, hence, a possible anti - hyperlipidemic effect.

Keywords: Blood cell count, body weight, lipid profile, thermoxidized palm oil, serum electrolytes

INTRODUCTION

Palm oil is an edible vegetable oil obtained from the mesocarp of the fruits of the tropical *Elaeis guineensis* [1]. It is the cheapest and most commonly used oil in Nigeria. It is used either in its fresh form or in its thermally oxidized (bleached) form. Thermally oxidized palm oil is different from fresh palm oil owing to the presence of free radicals generated as a result of heating [2, 3]. Thermally oxidized palm oil has been reported to decrease body weight [4], deplete vitamins A and E in the body [5]. Osim *et al*, [2] had previously reported that consumption of thermally oxidized palm oil diet causes damage to the liver and the kidneys, and blood vessels are not spared [6].

Aloe is a cactus-like, succulent perennial plant with over 360 species. It belongs to family Liliaceae (sub-family of the Asphodelaceae), cultivated in warm climatic areas and native to North Africa [7]. *Aloe vera* barbadensis has been named the most therapeutically effective species among the over 360 identified species [7]. *Aloe vera* gel has been shown to contain 75 nutrients, 20 minerals, 12 vitamins and 18 amino acids (including 7 essential amino acids)

[8, 9]. *Aloe vera* is reputed for a number of therapeutic benefits. They include; alleviating respiratory tract disorders [10], cardiovascular system disorders [11–13], endocrine system disorders [13–15], blood and immune system disorders [13–15], and gastrointestinal system disorders associated with peptic ulcer and type 1 diabetes mellitus [16–21].

Following the widely reported beneficial use of *Aloe vera* gel in the management of varying degrees of health disorders, this study seeks to determine the effect of *Aloe vera* gel on possible derangements in haematological and biochemical indices secondary to chronic consumption of thermally oxidized palm oil.

MATERIALS AND METHODS

2.1 Experimental Animals

Fifteen adult male albino Wistar rats weighing 200 – 250 g were purchased from the animal house of the Department of Physiology, College of Medical Sciences, University of Calabar, Nigeria. The animals were randomly assigned into 3 groups of 5 animals each. The animals were fed with normal food and water for 2 weeks during the acclimatization period. Group 1 served as control, group 2 and 3 served as thermoxidized palm oil (TPO) and TPO + *Aloe vera* fed group, respectively.

2.2 Diet Formulation

Ten litres of palm oil was purchased from Okuku market in Okuku, Yala local government area, Cross River State, Nigeria. The palm oil was thermally oxidized as earlier described by Isong, [4] and Osim *et al.*, [2]. Fresh palm oil was heated at 150°C in a stainless steel pot intermittently for five times with each lasting twenty minutes. At the end of each heating session, the oil was allowed to cool for five hours. Thermoxidized palm oil was mixed with normal rat feed in the ratio 15g : 85g respectively.

2.3 Preparation of Crude *Aloe vera* gel

Aloe vera plant with leaves 40 - 60 cm in length was purchased from the University of Calabar, botanical garden and was authenticated by the Chief Herbarium Officer of Botany Department of University of Calabar, Calabar, Nigeria as *Aloe barbadensis miller*. The leaves were plucked and rinsed using clean water, then dried with a clean piece of cloth. Using a knife, the leaf was sliced longitudinally to expose the gel. The gel was gently scraped into an electric blender to shatter the block. This preparation was done daily, throughout the administration period, and the *Aloe vera* gel was administered to the animals without storage, to prevent loss of phytoconstituents secondary to prolonged storage [15]. *Aloe vera* gel was orally administered to group 3 animals (TPO + *Aloe vera* gel) at a dose of 0.2ml/100g body weight [15,22] for 4 weeks.

2.4 Collection of Blood Sample

After 28 days of administration of *Aloe vera* gel, the animals were anesthetized using chloroform (3.8%) anaesthesia which was soaked in cotton wool and enclosed in a desiccator. The animals were introduced into the desiccator, one at a time. On observation of loss of reflexes, the animal was removed and blood was collected immediately into EDTA coated vials and plain capped bottles for whole blood and serum analysis, respectively. Blood was collected via cardiac puncture using a 5 ml syringe and a 21G needle.

2.5 Blood Cell Count

Blood samples were analysed using an automated cell counter (Coulter Electronics, Luton, Bedfordshire, UK) having standard calibrations, according to the manufacturer's instructions. The parameters measured were; red blood cell (RBC) count, white blood cell (WBC) count and platelet count.

2.6 Serum Lipid Profile Estimation

Serum lipid profile was assessed using the enzymatic colorimetric test kit method of Siedel *et al.*, [23] for serum total cholesterol (TC) and high density lipoprotein (HDL-c) estimation. Serum triglyceride (TG) concentration was determined by method of Negele *et al.*, [24]. Very low density lipoprotein (VLDL-C) concentration was obtained by dividing the serum TG by 5. This factor of 5 is based on the fact that in fasting subjects with triglyceride concentration of 400 mg/dl, the VLDL to total plasma triglyceride ratio is fixed at 1:5.

$$\text{VLDL-C (mg/dl)} = \frac{\text{Triglyceride (TG)}}{5}$$

Using the method of Friedewald *et al.*, [25], low density lipoprotein (LDL-c) was derived from the difference between serum TC and sum of HDL-c and VLDL-c, thus;

$$\text{LDL-c} = \text{TC} - (\text{HDL-c} + \text{VLDL-c})$$

2.7 Determination of Serum Electrolytes Concentration

Serum sodium and potassium ion concentrations were determined using a flame photometer (Model 410C, Petracourt Ltd, England). Serum chloride ion concentration was determined by end point calorimetric titration as described by Kolthoff *et al*, [26]. Serum bicarbonate ion concentration was measured by the modified method of Forrester *et al*, [27].

2.8 Statistical Analysis

All results in this study are presented as Mean \pm SEM. The one – way analysis of variance (ANOVA) was used to analyse the results obtained in this study, followed by a Post HOC multiple comparison – the least significant difference procedure (LSD). The level of significance was fixed at $p < 0.05$. Computer software - SPSS version 17.0 and Excel analyser (Microsoft corporation) 2007 version were used for the analysis.

RESULTS

Mean daily water intake was 24.1 ± 1.19 , 28.5 ± 1.15 and 28.0 ± 1.29 ml, for control, TPO and TPO + AV respectively. Mean water intake was significantly higher in TPO group, compared with control. Mean daily food intake was 28.1 ± 0.85 , 30.1 ± 0.52 and 29.3 ± 1.23 g, for control, TPO and TPO + AV respectively. There was no significant difference in mean daily food intake across the groups measured. There was no significant difference in body weight change among the groups – control (16.0 ± 4.0 g), TPO (24.0 ± 2.45 g) and TPO + AV (31.0 ± 6.96 g), (Table 1).

White blood cell count for control, TPO and TPO + AV was 9.72 ± 0.49 , 4.14 ± 0.44 and $5.94 \pm 0.37 \times 10^3$ Cell/ μL , respectively. White blood cell count was significantly ($p < 0.001$) reduced in TPO and TPO + AV groups, compared with control. White blood cell count was significantly ($p < 0.05$) higher in TPO + AV group, compared with TPO group. Red blood cell count was 7.88 ± 0.44 , 6.75 ± 0.78 and $6.18 \pm 0.74 \times 10^6$ Cell/ μL , for control, TPO and TPO + AV, respectively. There was no significant difference in RBC count among the groups in this study. Platelet count was 1014.6 ± 65.0 , 998.0 ± 25.8 and $1277.0 \pm 103.9 \times 10^3$ Cell/ μL , for control, TPO and TPO + AV, respectively. Platelet count was significantly ($p < 0.05$) higher in TPO + AV group, compared with control and TPO groups (Table 1).

Table 2 shows results of the various serum electrolytes assessed in this study. Sodium, potassium, chloride and bicarbonate ions were not significantly altered in this study.

Table 1: Comparison of mean water and food intake, and body weight change among the groups

Parameter	Control	TPO	TPO + <i>Aloe vera</i>
Daily Water Intake (ml)	24.1 ± 1.19	$28.5 \pm 1.15^*$	28.0 ± 1.29
Daily Food Intake (g)	28.1 ± 0.85	30.1 ± 0.52	29.3 ± 1.23
Body Weight Change (g)	16.0 ± 4.00	24.0 ± 2.45	31.0 ± 6.96
WBC Count ($\times 10^3$ Cell/ μL)	9.72 ± 0.49	4.14 ± 0.44^a	$5.94 \pm 0.37^{a,b}$
RBC Count ($\times 10^6$ Cell/ μL)	7.88 ± 0.44	6.75 ± 0.78	6.18 ± 0.74
Platelet Count ($\times 10^3$ Cell/ μL)	1014.6 ± 65.0	998.0 ± 25.8	$1277.0 \pm 103.9^{*b}$

* $p < 0.05$, $a = p < 0.001$ vs Control; $b = p < 0.05$ vs TPO

Table 2: Comparison of serum electrolytes in the different experimental groups

Parameter	Control	TPO	TPO + <i>Aloe vera</i>
Sodium (mmol/L)	128.2 ± 3.51	135.0 ± 1.51	127.2 ± 4.65
Potassium (mmol/L)	4.36 ± 0.23	4.75 ± 0.18	4.16 ± 0.33
Chloride (mmol/L)	98.0 ± 2.36	104.4 ± 2.04	95.6 ± 3.70
Bicarbonate (mmol/L)	19.0 ± 1.51	20.4 ± 1.21	19.0 ± 0.89

Serum total cholesterol concentration for control, TPO and TPO + AV was 3.38 ± 0.04 , 3.68 ± 0.17 and 3.77 ± 0.37 mmol/L, respectively. Serum TG concentration was 0.47 ± 0.01 , 0.48 ± 0.00 and 0.44 ± 0.01 mmol/L for control, TPO and TPO + AV group, respectively. Serum HDL-c for control, TPO and TPO + AV group was 1.60 ± 0.12 , 1.68 ± 0.01 and 1.71 ± 0.16 mmol/L, respectively. There was no significant difference in TC, TG and HDL-c between the groups in this study. Serum concentration of LDL-c for control, TPO and TPO + AV group was 1.79 ± 0.05 , 1.80 ± 0.14 and 1.33 ± 0.17 mmol/L, respectively. Serum LDL-c concentration was significantly ($p < 0.05$) lower in TPO + AV group, compared with control. Serum concentration of VLDL-c for control, TPO and TPO + AV group was 0.21 ± 0.00 , 0.22 ± 0.00 and 0.17 ± 0.02 mmol/L, respectively. Serum VLDL-c concentration was significantly ($p < 0.05$) lower in TPO + AV group, compared with TPO group (Table 3).

Table 3: Lipid profile analysis in the different experimental groups

Parameter	Control	TPO	TPO + <i>Aloe vera</i>
TC (mmol/L)	3.38 ± 0.04	3.68 ± 0.17	3.77 ± 0.37
TG (mmol/L)	0.47 ± 0.01	0.48 ± 0.00	0.44 ± 0.01
HDL-c (mmol/L)	1.60 ± 0.12	1.68 ± 0.01	1.71 ± 0.16
LDL-c (mmol/L)	1.79 ± 0.05	1.80 ± 0.14	1.33 ± 0.17 ^{*a}
VLDL-c (mmol/L)	0.21 ± 0.00	0.22 ± 0.00	0.17 ± 0.02 ^a

^{*} $p < 0.05$ vs Control and TPO; ^a $p < 0.05$ vs TPO

DISCUSSION

In Nigeria today, several foods are being fried in the course of preparation. Quite disturbing is the fact that the palm oil used in frying is been collected and stored for re-use. Considering the fact that thermally oxidized palm oil increases levels of various oxidants in the body, this study was designed to assess the effects of TPO on some haematological and biochemical parameters. Water intake was significantly ($p < 0.05$) higher in TPO group, compared with control. Although food intake was higher in TPO group compared with control, this was not significant. Body weight change increased in the TPO and TPO + AV groups compared with control, but this also was not significant. Consistent with reports of Finnegan, [28] WBC count was significantly ($p < 0.05$) reduced in TPO group, compared with control. *Aloe vera* gel significantly ($p < 0.05$) increased WBC count in TPO + AV group, compared with TPO group. Although there was a reduction in RBC and platelet count in TPO group, this was not significant compared with control. Thermally oxidized palm oil is known to contain free radicals and may have been responsible for these observed effects on blood cell count.

Serum electrolytes are known to control or regulate neuromuscular activities and contribute in body fluid homeostasis [29]. Presence of kidney disease contributes a great deal to the likelihood of developing electrolyte imbalance in the body. Also, there has been strong positive correlation between the incidence of edema and hypertension with elevated levels of sodium chloride (NaCl) [30]. Studies have now suggested that controlling serum electrolyte levels have improved survival and prevented cardiovascular diseases associated with electrolyte imbalance [31]. It is now known that electrolyte imbalances are not solely associated with altered hormonal status, but could also be influenced by dietary habits [32]. Following the chronic administration of thermally oxidized palm oil in this study, there was no alarming deviation in serum electrolyte levels (table 2). Although sodium and chloride concentrations were elevated in the TPO fed group, the increase was not significant, compared with control group. However, *Aloe vera* gel reduced the concentrations of these electrolytes to levels lower than that of the control group, showing its potential beneficial effect.

Also assessed in this study was serum lipid profile, which is an important biochemical index that gives information on whether or not an individual is likely to develop cardiovascular disease. Serum lipids were only slightly elevated in TPO group, compared with control. This is consistent with other findings [33–35] that also reported an insignificant increase in serum lipid profile following the consumption of thermally oxidized palm oil. On the other hand, Gill *et al*, [33] observed that continuous feeding with thermally oxidized palm oil causes a decrease in triglyceride concentration. Following the reports that TPO has no significant negative effect on lipid profile, there is no cause for celebration yet as this wide range of variation may have been consequent upon the small duration of exposure to thermally oxidized palm oil and the quantity one is being exposed to. In our study, *Aloe vera* gel significantly ($p < 0.05$) reduced LDL-c concentration in the TPO + AV group, compared with control and TPO group. *Aloe* also significantly reduced VLDL-c concentration in the TPO + AV group, compared with TPO group. This shows the possible beneficial role of *Aloe vera* gel in preventing atherosclerosis.

CONCLUSION

Thermally oxidized palm oil consumption did not significantly alter red blood cell count, total serum cholesterol, and serum electrolytes in this study. However, *Aloe vera* gel administration appeared to be beneficial in reducing LDL-c and VLDL-c, hence, a possible anti - atherogenic effect.

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