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Gastroprotective Role of the Combined Effects of Vitamins C and E Following Chronic Exposure to Thermoxidized palm oil in Albino Rats

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ABSTRACT

The effect of vitamins C and E on gastric acid secretion and cytoprotection on rats fed with thermally oxidized palm oil (TPO) was studied. Forty five albino wistar rats weighing 180 – 210g were randomly assigned into 3 groups (n = 15). Group 1 served as control and received normal rat feed. Groups 2 and 3 were fed with TPO diet. TPO diet was formulated by mixing rat feed with TPO in the ratio of 85g:15g. Group 3 rats received vitamins C (10mg/100g body weight) & E (400IU/100g body weight) in addition. The drugs were administered orally. All animals had access to water *ad libitum.* The feeding lasted for 28 days, after which gastric acid output, gastric mucus output and ulcer scores were assessed, using standard methods. Results showed that animals in the TPO group had significant increase in gastric acid secretion ($p<0.05$), decrease in mucus output ($p<0.05$) and increase in ulcer scores ($p<0.001$), compared with control. However, there was a significant decrease in gastric acid secretion ($p<0.001$), increase in gastric mucus output ($p<0.001$), and decreased gastric ulcer score ($p<0.001$) following treatment with vitamins C and E, compared with control and TPO group. Vitamins C and E reduced gastric ulceration occasioned by thermally oxidized palm oil diet consumption by reducing gastric acid secretion and increasing gastric mucus output. This study has shown that Vitamins C and E may be useful in ameliorating gastric ulcers presented by chronic consumption of thermoxidized palm oil diets.

INTRODUCTION

Palm oil is gotten from the oil palm tree (*Elaeis guineenis*). It is commonly used as cooking oil in either it fresh or thermoxidized form. It contains many phytochemical constituents that confers it health benefits when consumed in its fresh form. Amongst such constituents are vitamins A, K, C and E, flavonoids, as well as carotenoids (Al-Saqer *et al.*, 2004). When palm oil is subjected to prolonged heating, it is oxidized. Consumption of such oil has been documented to cause tissue damage, decrease biliary secretion and alter biliary electrolytes (Obembe *et al.*, 2010). Previous reports have documented distortion in villus morphology, with an attendant malabsorption of fluid and glucose (Obembe *et al.*, 2011), altered intestinal motility (Obembe *et al.*, 2008), growth retardation (Isong *et al.*, 1992), increased faecal electrolytes (Obembe *et al.*, 2014), alteration in haematological parameters and serum lipid profile (Ani *et al.*, 2015a, b), atherosclerosis (Jaarin *et al.*, 2006), altered serum electrolyte balance (Ani *et al.*, 2015c), deactivation of key enzymes required to enhance the process of metabolism, leading to essential fatty acid deficiency, nucleic acid deficiency, growth retardation, fatty liver, thrombosis and micronutrients malnutrition (Izaki *et al.*, 1984; Isong *et al.*, 1992; Osim *et al.*, 1994), among others. Boots *et al.*, (2003) described this effect as systemic, leading to a variety of disease symptoms (Isong *et al*, 1992). Vitamins C and E are among the non-enzymatic antioxidants. Vitamin C is a water soluble vitamin which provides intracellular and extracellular aqueous phase antioxidant capacity by scavenging oxygen free radicals. It is a cofactor in enzyme dependent reactions, such as synthesis of collagen, and its deficiency often manifest as scurvy (Padayatty *et al.*, 2003). Vitamin E on the other hand, is a lipid soluble vitamin which is concentrated in the hydrophobic interior site of cell membranes,

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hence, acting as the principal defense against oxidants – induced membrane injury. It also plays a role in inhibition of platelet aggregation as well as prevention of polyunsaturated fatty acids from oxidation (Traber and Atkinson, 2007). Cytoprotection is the means by which gastric mucosa is protected against injury (Wallace, 1992).

The gastric mucosa is exposed daily not only to the cruel conditions of low pH and pepsin, but also to a wide array of potentially damaging substances, such as; bacterial toxins, foods, temperature and even chemical toxins from regurgitated bile acids and pancreatic juice from the small intestine. In the mist of all these conditions, the gastric mucosa usually finds a way to build resistance.

There are mechanisms deployed by the body to build this resistance without which the acid content of the gastric secretion would excoriate and perforate the stomach wall, a condition referred to as ulcers (Davenport, 1972). Some of the mechanisms deployed to build resistance against ulcerations include: increase in gastric secretion of mucus and bicarbonate, presence of trefoil peptides which are acid resistant in the mucosa, presence of tight junctions between cells, and the ability of the mucosal cells to regenerate rapidly (Davenport, 1972). Cytoprotection can also be achieved by increased gastric mucosal blood flow, formation of prostaglandins, scavenging of free radicals and stimulation of cellular growth repairs and the process of cell restitution (Wallace, 1992).

Thermoxidized palm oil has been reported to potentiate various health complications, such as, destruction of tissues and organs like; the kidney, liver, among others. But before these effects are exerted in these parts of the body, it goes through the gastrointestinal tract where it is absorbed and distributed. This study therefore seeks to ascertain the combined effects of vitamins C and E on gastric acid secretion and cytoprotection on rats fed with thermally oxidized palm oil diets.

METHODOLOGY

Purchase of Palm oil

Ten liters of palm oil was purchased from Marian market in Calabar, Nigeria, and was taken to the department of Physiology for study. The palm oil was divided into two equal quantities, one of which remained in the fresh form and the other was thermoxidized. The thermoxidized palm oil was used to formulate thermoxidized palm oil diet.

Thermoxidation of Palm oil and Feed Formulation

The fresh palm oil was thermally oxidized as previously described by (Obembe *et al.*, 2008). Fresh palm oil was heated in a stainless steel pot over a heating mantle at a temperature of 190^{o}C for five consecutive times. Each round of heating lasted for about twenty minutes and then allowed to cool for five hours before commencement of the next round of heating. The experimental diet was formulated by mixing 15g of thermoxidized palm oil with 85g of rat feed as previously used (Obembe *et al.*, 2008; Obembe *et al.*, 2010; Ani *et al.*, 2015 a, b, c).

Experimental Drugs

The experimental drugs used in the research were vitamins C (Emzor Pharmaceuticals, Nigeria) and E (International health supplies and services, Baltimore Maryland). The drugs were purchased from BEZ Pharmacy in Calabar, Nigeria. Vitamin C tablet (100 mg) was dissolved in 1 ml of distilled water to 100 mg/ml stock solution. One capsule of vitamin E (400IU) which was 0.4 ml was drawn directly from the capsule with needles and 1 ml syringes. The drugs were administered orally to the animals at a dose of 10mg/100g body weight and 400IU/100g body weight, for vitamins C and E, respectively.

Experimental Animals

Forty five (45) albino wistar rats of both sexes weighing 180-210 g were obtained from the animal house of the department of Pharmacology, University of Calabar, Nigeria. The animals were housed in the animal house of the department of Physiology, University of Calabar, Nigeria. The animals were allowed to acclimatize for 1 week after which they were randomly divided into 3 groups (n = 15) thus; control group, TPO – fed group and TPO + vitamins C & E treated group. The control rats were fed with normal rat feed and water only. The rats in the TPO and TPO + vitamins C & E groups were fed with TPO diets for a period of 28 days. In addition, rats in TPO + vitamins C & E group were orally gavage with vitamins C (10 mg/100g b.w.) and E (400IU/100g b.w.) for 28 days. The test groups, like the control group, had access to water *ad libitum* and also had access to their modified diet *ad libitum*. Indeed, the principles guiding animal handling in laboratory research were strictly adhered to. Approval was sort and obtained from the Institutional Animal Ethics Committee of the University of Calabar (UC/CMS/PHS/01345).

Measurement of Gastric Acid Secretion

At the end of 28 days of administration, all animals were fasted for 14 hours, but were allowed access to water *ad libitum*. This was to ensure the bowel was cleared of all food particles. Using the method of Gosh and Schild (1958), as modified by Osim *et al.*, (1991) and used by (Akpan *et al.*, 2014; Oka *et al.*, 2014), gastric acid secretion was measured in 5 rats per group under 25% urethane anesthesia, 5 rats per group were used for measurement of gastric mucus output, while the remaining rats (n = 5) were used for ulcer scores assessment.

Measurement of Gastric Mucus

The adherent gastric mucus was measured by the method of (Tan *et al.*, 2011). The animals were fasted for about 18 hours prior to the experiment, after which they were sacrificed and their stomachs removed. The stomachs were then opened along the greater curvature and pinned on a flat board. Using a spatula, the gastric mucus was scraped off the surface of the mucosa and introduced into pre-weighed sterilized sample bottle containing 3

ml of distilled water. The sample bottle containing distilled water and the collected mucus was then weighed on an electronic balance. Mucus output was calculated as the difference in weights of sample bottle containing water and sample bottle containing water and mucus.

Measurement of Ulcer Scores

Gastric ulcer score was assessed using the method of Alphin and Wards (1967) and used by Akpan *et al.,* (2014). The rats were anesthetized using 6 ml/kg of 25% urethane, after which an abdominal incision was made and the pylorus exposed. A pyloric incision was made and a cannula inserted and held in place by tying with a thread. The stomach was infused with 1.5 ml of acid alcohol. The infusion was made via the pyloric incision. The animals were allowed for an hour. The stomach was then surgically removed, washed, cut open along the greater curvature and rinsed with normal saline. Pins were used to hold the tissue to the dissecting board. A magnifying lens and a vernier caliper were used to measure the extent of ulceration. Ulcer score was done according to the grading system shown in the table below;

Grade 0.0 - No Lesion (normal stomach)
Grade 0.5 - Pin size ulcer
Grade 1.0 - 2 or more haemorrhagic or small linear ulcers
Grade 2.0 – Ulcer spots greater than 3 mm.

The ulcer score was calculated by multiplying each grade with its frequency of occurrence. The sum of all the values formed the ulcer score for each animal (Koike *et al.*, 2001).

Statistical Analysis

Results are presented as mean ± standard error of mean (SEM). The data were computed using Microsoft excel analyzer (Microsoft office version 2010). The One-way Analysis of Variance (ANOVA) was used to analyze the data. Values of $p<0.05$ was considered significant.

RESULTS

Gastric Acid Secretion

The mean basal gastric output in the control, TPO and TPO + Vitamin C and E groups were 1.86 ± 0.114, 2.14 ± 0.034 and 0.57 ± 0.039 µmol/10minutes respectively. Basal acid output was significantly ($p<0.001$) lower in the TPO + Vitamin C and E group compared to the control and TPO groups. Acid output was significantly ($p<0.05$) higher in TPO group compared to the control group (Figure 1 & 2). The mean histamine-induced gastric acid output in the control, TPO and TPO + Vitamin C and E groups were 2.70 ± 0.210, 3.42 ± 0.358, and 1.39 ± 0.260 µmol/10minutes respectively. Histamine – induced gastric acid output was significantly ($p<0.001$) lower in TPO + Vitamin C and E group compared to the control group. Gastric acid output was significantly ($p<0.001$) lower in TPO + Vitamin C and E group compared to TPO group (Figures 1 & 2).

When cimetidine was introduced, the mean gastric acid output for control, TPO and TPO + Vitamin C and E groups were 1.45 ± 0.322, 1.97 ± 0.563, 1.32 ± 0.178 µmol/10minutes respectively. Gastric acid output was non-significantly increased in the TPO group compared to control and TPO + Vitamin C and E groups (Figures 1 & 2).

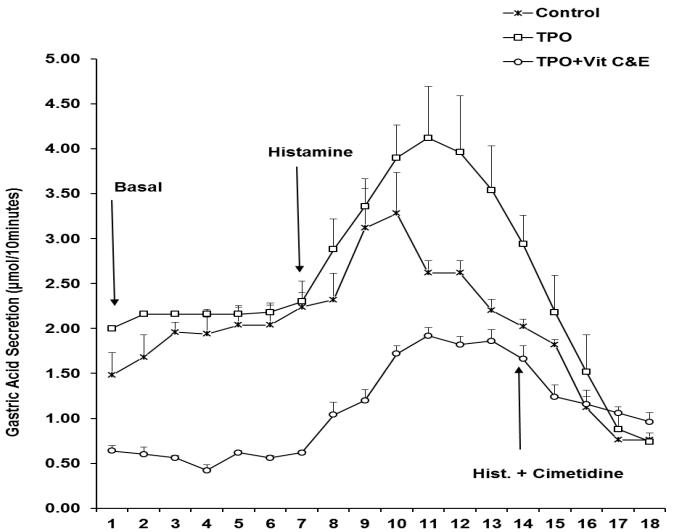

Fig. 1: Comparison of gastric acid secretion in the different experimental groups. Values are mean±SEM, n=5.

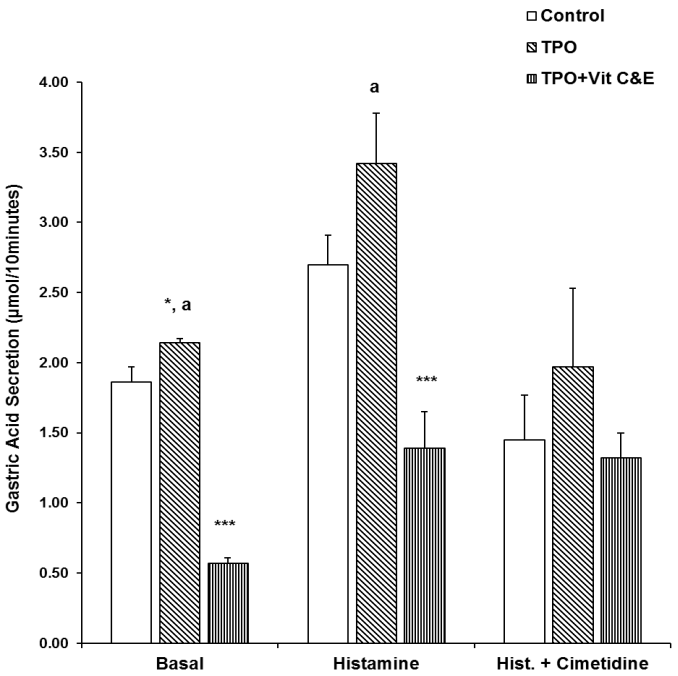

Fig. 2: Comparison of mean gastric acid output in the different experimental group. Values are mean±SEM, n=5.
***p<0.001, *p<0.05 vs control; a=p<0.001 vs TPO+Vit. C&E.

Gastric Mucus Output

Mean gastric mucus output in the control, TPO and TPO + Vitamin C and E groups were 0.12 ± 0.02, 0.05 ± 0.00 and 0.17 ± 0.03g respectively. There was a significant ($p<0.05$) decrease in the gastric mucus output of the TPO group compared to the control group. There was also a significant ($p<0.001$) decrease in the TPO group compared to the TPO + Vitamin C and E group (Figure 3).

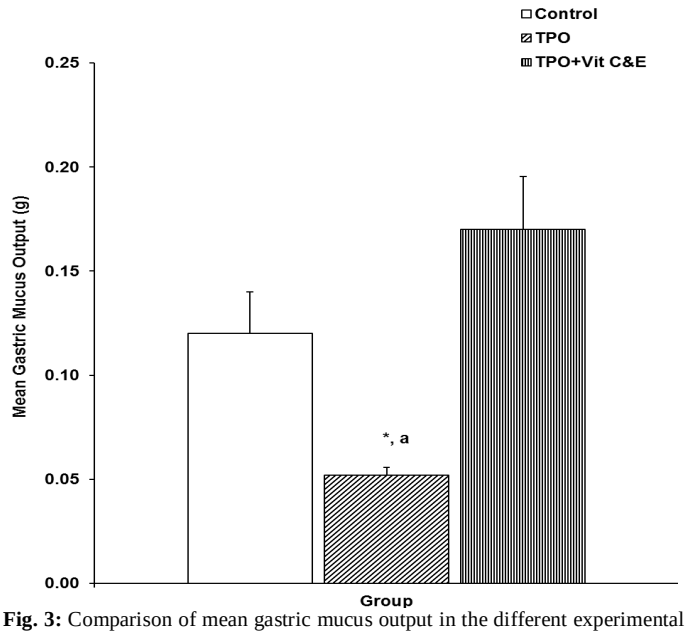

Fig. 3: Comparison of mean gastric mucus output in the different experimental groups. Values are mean±SEM, n=5.
*p<0.05 vs control; a=p<0.001 vs TPO+Vit. C&E

Gastric Ulcer Score

The mean ulcer scores for the control, TPO, and TPO + Vitamin C and E groups were 9.88 ± 0.89, 14.88 ± 0.35 and 6.98 ± 0.82 respectively. Gastric ulcer score was significantly (p<0.001) higher in the TPO group compared to the control.

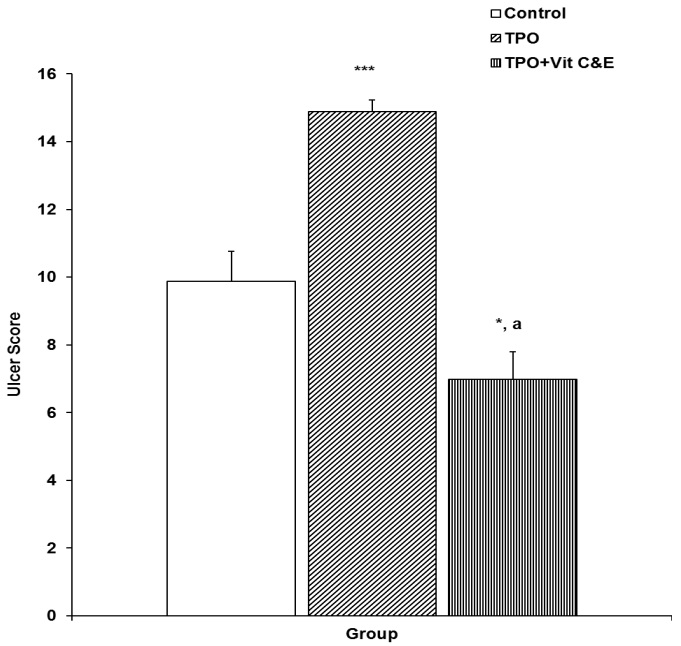

Fig. 4: Comparison of mean gastric ulcer score in the different experimental groups. Values are mean±SEM, n=5.
***p<0.001, *p<0.05 vs control; a=p<0.001 vs TPO.

It was significantly (p<0.05) lower in the TPO + Vitamin C and E group compared to control group. It was significantly (p<0.001) lower in the TPO + Vitamin C and E group compared to the TPO group (Figure 4).

DISCUSSION

Chronic exposure to TPO has been reported to potentiate various health complications through the generation of oxygen-derived free radicals, which are capable of damaging biologically important molecules and attacking significant macromolecules, leading to cell damage and homeostatic disturbance, making it harmful to tissues. Our study therefore looks at the effects of prolonged exposure to thermally oxidized palm oil on gastric cytoprotection, and the influence of the combined effects of vitamin C and E on gastric cytoprotection after prolonged exposure to TPO. Thermoxidized palm oil significantly increased (p<0.05) gastric acid secretion in the TPO fed group, while Vitamin C & E treated group had significant decrease (p<0.001) in gastric acid secretion, compared with control. Gastric mucus output significantly decreased in the TPO group compared with control and Vitamin C & E treated group (p<0.05), (p<0.001) respectively. An increase in mucus output was observed when treated with Vitamin C & E. The good physical shape of gastric mucosa maintains structural integrity and function in spite of continuous contact to harmful factors such as HCl and pepsin, and a lot of other exogenous insults that are capable of breaking off the gastric mucosa (Laine *et al.*, 2008). This ability of the gastric mucosa is maintained under normal circumstances by mucus, bicarbonate, and prostaglandins, and several other factors (Laine *et al.*, 2008). Ulceration of the gastric mucosa occurs when there is unevenness between gastric destructive factors and the defending factors giving room for both endogenous and exogenous aggressive substances to make their way through the mucosa, thereby altering cellular metabolism, leading to membrane lipid peroxidation, which results in ulceration. The increase in gastric acid secretion, decrease mucus output, and the resultant ulceration observed in this study could imaginably result from the harmful free radicals generated within the cell following prolonged exposure to TPO (Edem, 2002). However, treatments with Vitamins C & E following exposure to TPO, reduced gastric acid secretion, mucus output and ulcer scores. Vitamins C & E are important physiological antioxidants that aid in fighting against tissue lipid peroxidation, and a resultant oxidative stress, by donating its electrons and then preventing other compounds from being oxidized (Padayatty *et al.*, 2003).

CONCLUSION

In conclusion, vitamins C and E co-administration reduced gastric acid secretion and increased gastric mucus output, a possible mechanism for reduced gastric ulceration occasioned by thermally oxidized palm oil diet consumption. Therefore, this study has shown that vitamins C and E may be useful in preventing gastric ulcers.

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