Niger. J. Physiol. Sci. 39 (June 2024): 87 – 90
www.njps.physiologicalsociety.com

Niger. J. Physiol. Sci.

Full length Research Article

The Effect of Long-term Consumption of Calabash Chalk on Peptic Ulcer Scores in Wistar Rats

***Okpo-Ene A, Obembe A., Osim E.E.**

Department of Physiology, College of Medical Sciences, University of Calabar, Calabar PMB 1115, Nigeria

Summary: This study was aimed at investigating the effects of long-term consumption of calabash chalk on ulcer scores in Wistar rats. Fifty (50) male adult Albino Wistar rats weighing between 160g - 180g were used. The rats were randomly divided into ten (10) groups of 5 rats each consisting of five (5) control and five (5) test rats. Group 1 was the control group and was administered 1ml of distilled water daily while group 2 was the test group and was administered 1ml of calabash chalk suspension orally daily. They were allowed food and water ad libitum. The experiment lasted for 28 days, thereafter, basal gastric acid secretion, gastric pepsin secretion, gastric pH, gastric mucous output, and gastric ulcer scores were measured. The mean basal gastric acid secretion for the control and the test group was 0.58± 0.22 and 0.61 ± 0.37uMol/10min respectively. Basal gastric acid secretion was not significantly higher in the test group when compared with the control group. The mean gastric pepsin secretion for the control and the test group was 0.46 ± 0.02 and 0.57 ± 0.02mg/100ml respectively. The mean gastric pepsin secretion in the test group was significantly higher ($P<0.01$) when compared with the control. The mean gastric pH for the control and the test group was 4.10 ± 0.37 and 2.94 ± 0.14 respectively. The pH of the test group was significantly higher ($p<0.05$) when compared with that of the control group. The mean gastric mucous output in the control and the test group was 0.14 ± 0.014g and 0.08 ± 0.01g respectively. The results showed that the gastric mucous output in the test groups was significantly reduced ($p<0.01$) when compared with the control group. The mean gastric ulcer score in the control and the test group was 3.70 ± 0.30 and 7.50 ± 1.25 respectively. The results showed that the ulcer score in the test group was significantly higher ($p<0.05$) when compared to the control group. In conclusion, the long-term consumption of calabash chalk predisposed to peptic ulceration in Albino Wistar rats.

Keywords*: calabash chalk, ulcer scores, gastric secretions, mucous, Pepsin, pH*

**Authors for correspondence:* aokpoene@gmail.com*;*

Manuscript received- March 2024; Accepted: May 2024

DOI: https://doi.org/10.54548/njps.v38i2.11

INTRODUCTION

Calabash chalk is a naturally occurring substance that is common in West African countries. It is referred to as kaolin, edible clay, and marble chalk in English. It is also known by different names in different parts of the world; "Shile" in Ghana, "La-Craie" in France, "Umcako" in Zulu, "Nzu in Ibo Nigeria, and "Mabele" in Congo (Reilly & Henry 2000). Chalk is not a food substance but it is readily consumed by numerous people in different parts of the world (Halstead, 1986). This is a form of geophagy and a form of pica.

Calabash chalk is consumed by both males and females, but more by females especially during pregnancy and in the postpartum phase. It is said to prevent morning sickness, excessive salivation, postpartum depression, anxiety, and feelings of alienation (Reilly & Henry, 2000). Tests on calabash chalk done by the Foods and Standards Agency of the United Kingdom have shown high levels of lead, aluminium, arsenic, and alpha lindane which are known to be toxic to the body (Campbell & Weir, 2002).

Calabash chalk is known to cause low birth weight, iron deficiency anemia, reduced red blood cell count and platelet count, and reduced zinc and iron absorption in babies in utero and in breastfeeding infants. It has also been demonstrated to cause histomorphological changes to the architecture of the liver, stomach, and esophagus including, ulceration of the gastrointestinal mucosa and dilatation of the liver sinusoids (Ekong *et al*., 2009). Since there was no attempt to score the gastric ulceration and elucidate the causative factors that could provoke the ulcer if any; this study was therefore carried out to investigate the effect of chronic consumption of calash chalk on gastric ulcer scores, and to elucidate some factors that could provoke the ulcers notably gastric acid secretion, gastric pepsin secretion and gastric mucous output.

MATERIALS AND METHODS

Preparation of calabash chalk suspension: Blocks of non-salted calabash chalk were purchased from Watt market in Calabar, south-south of Nigeria, and ground into a powder using a manual grinder. Forty grams of the powder was dissolved in1liter of water to get a concentration of 40mg/ml. This concentrate was filtered with Whatman filter paper to remove all impurities. The suspension was stored in a plastic jug in a cool dry place.

Experimental animals: Fifty (50) adult male Albino Wistaria rats weighing between 160-180g were used for these experiments. They were housed in a well-ventilated room in the animal facility of the Department of Physiology, University of Calabar. They were kept at a temperature of 28 ± 3° C and humidity of 85 ± 5%. They werekept in a 12/12, dark/ light cycle and were allowed to acclimatize for 2 weeks before the onset of the experiments. The rats were fed with standard feed pellets and were allowed access to water and food *adlibitum*.

Experimental design: The animals were randomly divided into 10 groups of 5 rats each. Group 1 served as the control group and was administered 1ml of distilled water while group 2 served as the test group and was administered1ml of the calabash chalk suspension. This administration was done orally, every morning for a period of 28 days. An initial body weight was taken at the start of the experiments and a final body weight wastaken at the end of the experiments. During the experiments, the animals were weighed daily using an animal weighing balance. The difference between the final and initial body weight of each animal was taken as the body weight change of the respective animal. Approval for the use of the animals was obtained from the ethical committee of the faculty of basic medical sciences, University of Calabar. This is in tandem with internationally accepted standards for laboratory animal care and their use.

Table 1:
Grading of ulcer points

GRADE	INTERPRETATION
0.0	No les
0.5	Pin size ulcer
1.0	Two or more small linear or hemorrhagic ulcer
2.0	Ulcer spots greater than 3mm

Ulcer index for each group = $\frac{\text{number of rats x number of grade}}{\text{Total number of rats in a group}}$

Ulcer incidence (%) = $\frac{\text{number of rats with ulcers x 100}}{\text{Total number of rats}}$

Measurement of various gastrointestinal parameters: Measurement of mean basal gastric acid was done using the continuous perfusion method (Ghosh and Schild, 1958; Osim *et al.*, 1991). Gastric acid output in the effluent sample was measured by titrimetricanalysis. The calculation of acid in millimoles per hour (mMol/Hr) was by the principle that states that a gram equivalent of acid balances a gram equivalent of the base at the neutralisation point. The determination of the proteolytic activity of gastric acid secretion (which is the basis for the measurementof pepsin) was done using Casein as a substrate (Tarique *et al.*, 2016). The weight of adherent gastric mucous was determined using the method of (Tan *et al.*, 2006). Ulcers were induced by gastric instillation of acid-ethanol. The stomach of each animal was spread and pinned to a dissecting board tohave a good view of the mucosa of the stomach (Joshii *et al.*, 2004). A careful macroscopic examinationfor the presence of any ulcerative lesion was carried out and scoring of ulcer spots was done (Obembe *et al.*, 2011). Ulcer scoring was done according to the following grading system (Table 1).

RESULTS

Comparison of mean basal gastric acid output between the control and the test group

The mean basal gastric output (BGAO) in the control group and the test group was 0.58 ± 0.22 uMOL /10min and 0.61 ± 0.37uMol/10min respectively. The mean histamine-mediated mean gastric acid output(Hist-GAO) in the control and the test group was 1.43 ± 0.144uMol/10min and 1.33 ± 0.08uMol/10min respectively. The mean Histamine + Cimetidine-mediated gastric acid output (Hist+Cim-GAO) in the control group and the test group was 0.66 ± 0.08uMol/10min and 0.65 ± 0.04uMol/10min respectively. There was no significant difference in the various mean gastric acid outputs between the control and thetest group (Figure 1).

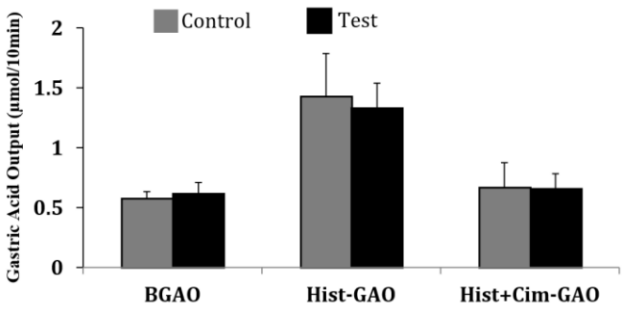

Figure 1
Gastric acid output between control and test groups.
Values are expressed as mean ± SD, n = 5.
No significant difference between groups

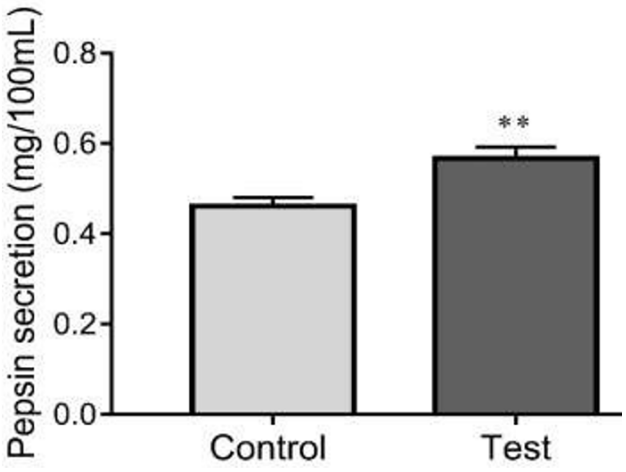

Figure 2:
Comparison of gastric pepsin level between the control and test groups.
*Values are mean ± SEM, n = 5. **p< 0.01 vs control.*

Gastric pepsin secretion in the control and the test group The mean concentration of gastric pepsin in the control and the test group was 0.46 ± 0.02mg/100ml and 0.57 ± 0.02mg/100ml respectively. The concentration of gastric pepsin in the test group was significantly higher ($p<0.01$). when compared with the control group (Figure 2).0.57 ± 0.02mg/100ml respectively. The concentration of gastric pepsin in the test group was significantly higher ($p<0.01$) when compared with the control group (Figure 2).

Gastric mucous output in the control and the test group The mean gastric mucous output in the control and the test group was 0.14 ± 0.014g and 0.08 ± 0.01g respectively. The gastric mucous output in the test group was significantly reduced ($p<0.01$) when compared to the control group (Figure 3).

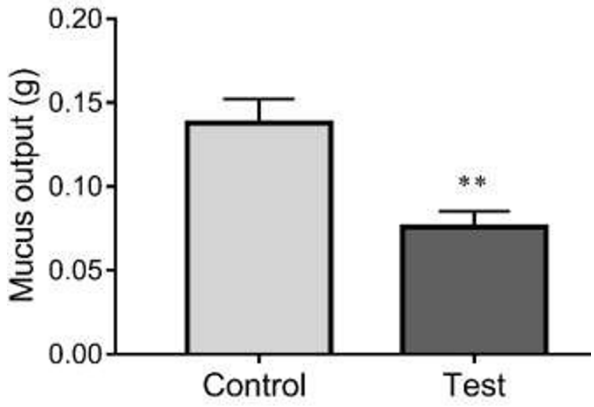

Figure 3:
Comparison of gastric mucus output between the control and test groups.
Values are mean ± SEM, n = 5. ** *p*< 0.01 vs control

Gastric ulcer scores in the control and the test group: The mean gastric ulcer score in the control and the test group was 3.70 ± 0.30 and 7.50 ± 1.25 respectively. The ulcer score in the test group was significantly higher ($p<0.05$) when compared with the control group (Figure 4).

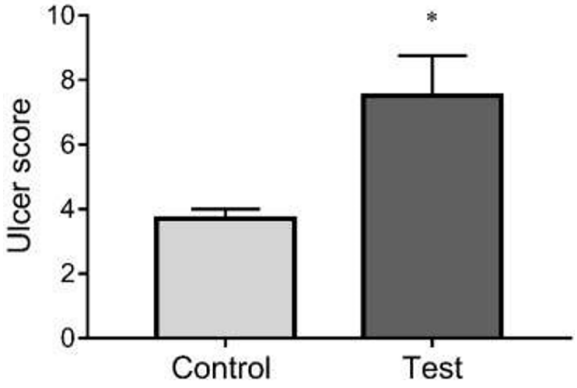

Figure 4:
Comparison of gastric ulcer score between the control and test groups. *Values are mean ± SEM, n = 5. * p< 0.05 vs control.*

Gastric pH in the control and the test group: The pH value for the control group was 4.10 = 0.37 while the pH value for the test group was 2.94 ± 0.14. The pH value of the test group was significantly higher than that of the control group (Figure 5).

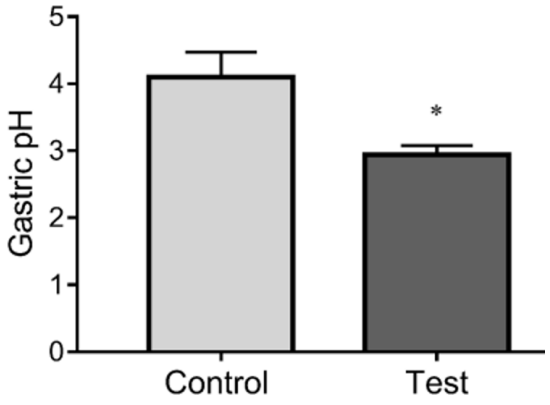

Figure 5:
Comparison of gastric pH between the control and test groups. *Values are mean ± SEM, n = 5. *p< 0.05 vs control.*

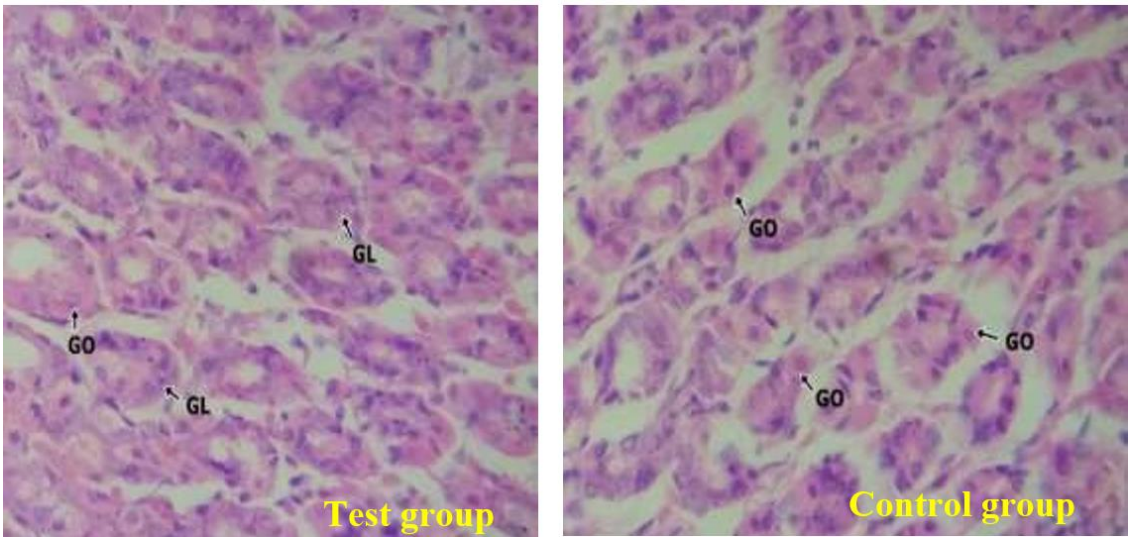

Plate 1:
Cross section of the stomach (test group and control group) X400

DISCUSSION

Gastric ulcer disease is common both in humans and animals. It occurs as a result of the imbalance between protective and aggressive factors present in the gastric mucosa. Every day, the gastric mucosa is exposed to factors that can cause injury such as gastric acid, certain types of food, pepsin, bacterial agents, bile acids, and certain drugs (Ezomike *et al*., 2020). Ekong *et al*., (2009) had shown some gastric lesions following the consumption of calabash chalk in rats. However, no attempt was made to elucidate the causative factors that could cause the gastric ulcer following the consumption of calabash chalk. This study therefore, investigated the aggressive factors that could provoke gastric ulceration, notably free hydrogen ions, low mucus secretion, and high pepsin secretion. There was also no significant difference in mean basal gastric acid secretion in the test group when compared to the control group. When Histamine was administered, the gastric acid output was significantly increased in both the control and the test group. When Cimetidine was administered, the gastric acid output of the animals in both groups was reduced as expected. However, the pH of the test group was significantly lower than the control group, suggesting a significant increase in free hydrogen ions in the test group than in the control. These free hydrogen ions may cause damage to the gastric mucosa.

The adherent mucous in the test group was significantly reduced when compared with the control group. The reduced mucous in the test group may be due to damage caused to the epithelial cells by the toxic elements and organic pollutants present therein (Ekong *et al*., 2009). Histology of the stomach from the test group showed edema, inflammation, erosion of the surface epithelium, and prominent goblet cells when compared to the control group. All these would have a negative effect on the ability of the epithelial cells to secrete mucous (Ekong *et al*. 2012).

Pepsin concentration in the test group was significantly higher when compared to the control group. Pepsin solubilises gastric mucous thus exposing the gastric mucosa to acid attack (Pearson *et al*., 1986). The significantly increased pepsin and pH and the significantly reduced gastric mucous worked synergistically to cause significantly increased gastric ulcerations observed in the test group when compared to the control group. The liberal consumption of calabash chalk should therefore be discouraged.

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