

## Effect Of Chronic Consumption Of Calabash Chalk On Intestinal Motility, Absorption And Histo-Architecture

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### Abstract

Calabash chalk is a natural substance consumed by both males and females for pleasure. Its consumption has been reported to cause various systemic disorders and to change the histology of both the small and large intestines and so, may affect various gastrointestinal parameters. This study was therefore aimed at investigating the possible effect of chronic consumption of calabash chalk on small intestinal motility and absorption. Ten (10) adult Albino Wistar rats weighing between 160-180g were used for the experiments. The rats were randomly divided into 2 groups of 10 rats each. Group 1 was the control group while group 2 was the test group and was given a daily oral suspension of Calabash chalk for 28 days. At the end of the experimentation period, the rats were sacrificed and sections of the intestines dissected out for evaluation of intestinal motility and fluid absorption studies while the stomach was dissected out for histological staining. Results showed that serosal fluid transfer was significantly lower ( $p < 0.01$ ) in the test group ( $0.13 \pm 0.01$ ) when compared with the control ( $0.23 \pm 0.01$ ). Mucosal fluid transfer was significantly lower ( $p < 0.01$ ) in the test group ( $0.20 \pm 0.19$ ) when compared with the control ( $0.36 \pm 0.15$ ). Gut fluid uptake in the control and test groups showed no significant difference between the 2 groups. Transit time was significantly lower ( $p < 0.001$ ) in the test group ( $40.1 \pm 2.51$ ) when compared with the control ( $56.4 \pm 2.70$ ). The basal height of contraction of intestinal smooth muscle was significantly lower ( $p < 0.01$ ) in the test group ( $2.31 \pm 0.39$ ) when compared with the control ( $2.58 \pm 0.39$ ). The effect of atropine sulphate on intestinal smooth muscle motility was significantly lower ( $p < 0.01$ ) in the test ( $160.15 \pm 31.10$ ) when compared with the control ( $200.10 \pm 20.02$ ). In the comparison of the maximum contraction of ileal smooth muscle to graded concentrations of acetylcholine (Ach), the result showed that from  $-8M$  to  $-5M$  Ach concentration, the maximum contraction for the test group was significantly higher ( $p < 0.001$ ) when compared with the control group. At  $-4M$  Ach concentration, the maximum contraction of the test group was significantly lower ( $p < 0.05$ ) when compared with the control. Histology showed signs of inflammation, ulceration/ patchy erosion of the epithelium of both the small and large intestines in the test group. There was also evidence of submucosal edema. In conclusion, chronic consumption of calabash chalk resulted in the impairment of intestinal motility and fluid absorption in rats.

**Keywords:** Intestinal, Motility, Absorption, Gastrointestinal, Contraction

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### I. Introduction

Calabash Chalk is a naturally occurring substance common in West African countries. It is also known as Kaolin, Edible Clay, and Marble Chalk. Due to migration, this West African substance has been carried worldwide and can be found in African/ethnic stores and markets in different parts of the Western world. It is known as “La-Craie” in French, “White dirt” or “White clay” in the United States of America, “Mabele” in Congo, and “Nzu” in Nigeria, to mention a few<sup>1</sup>.

Chalk is not a food substance but is readily consumed by numerous people worldwide<sup>1</sup>. This is a form of geophagy and Pica. Pica is a phenomenon associated with a deep craving for non-food substances like sand, soap, powder, dust, paint, money, calabash chalk, and fuel. Pica has been observed in animals and humans of both sexes and all races<sup>2</sup>.

The practice of geophagy is usually associated with pregnant women. The migration of people from countries where geophagy is especially prevalent results in a cultural transfer of the practice to countries not typically associated with geophagy. Thus, in the United Kingdom, geophagy is known to be associated with immigrants from South Asia<sup>3</sup>, and West Africa<sup>4</sup>, with the latter consuming Calabash chalk that has been imported from Africa and sold in ethnic shops<sup>5</sup>. In Nigeria, Calabash chalk is usually consumed by women and children<sup>6</sup>. It helps to prevent morning sickness and excessive salivation in pregnant and breastfeeding women<sup>7</sup>.

The gastrointestinal tract is a coiled tube extending the length of the trunk<sup>8</sup>. The tract can be divided into five segments: mouth and pharynx, esophagus, stomach, and small and large intestines. The food is put in the mouth and moved into the pharynx by the activity of the skeletal muscles, and then propelled along the rest of the tract by the activity of smooth muscles. The food substance is broken down to an appropriate semi-fluid consistency, and the nutrients in it are dissolved and digested by secretions coming into the tract at various locations. Muscular contractions help to mix the secretions with the food<sup>8</sup>.

The associated organs located outside the gastrointestinal tract are essential for digestive processes and include exocrine glands, which secrete vital digestive juices. The associated organs include three pairs of salivary glands producing saliva which has a spectrum of functions, but specifically to lubricate the upper gastrointestinal tract to allow movement of food along it; the exocrine pancreas which secrete pancreatic juice that contains most of the essential digestive enzymes required to breakdown the food into smaller molecules which can be absorbed; and the exocrine liver which provides bile, a secretion that has a vital role in digestion and absorption of fats<sup>9</sup>. The small intestine lies between the pyloric sphincter of the stomach and the ileocaecal valve, which opens into the large intestine. It consists of three parts- duodenum, jejunum, and ileum. It has a length of between four and six meters, the walls made up of several layers, including a mucous layer, a submucous layer, a muscular layer that contains two layers of smooth muscles, an outer circular layer made of an inner longitudinal layer, and an outer serous layer<sup>8</sup>.

The mucosa of the small intestine is covered by folds called plicae circulares, from which project microscopic finger-like projections called villi. Plicae circulares, villi, and microvilli function to increase the surface area of the small intestine available for absorption of nutrients (the main function of the small intestine). The small intestine also secretes intestinal juice called Succus entericus. This juice consists of 99.5% water and 0.5% solids. Its alkaline pH of 8.3 protects the duodenum from being destroyed by chyme, which is acidic in nature. It contains enzymes, mucin, intrinsic factor, and electrolytes.

The small intestine carries out several functions, which include digestion and absorption of 90% of ingested food and mechanical function which helps mix the chyme. It also has hormonal function by secreting Secretin and Cholecystokinin<sup>9</sup>.

The large intestine is about 1.5m in length, making up about 1/5th of the whole length of the gastrointestinal tract and is made up of several parts. Functions of the large intestine include absorption of food, water, electrolytes, drugs, alcohol, etc; secretory function, excretory function as well as formation of feces<sup>9</sup>.

Gastrointestinal motility focuses on it's digestive motor function and the transit of ingested materials within the gastrointestinal tract. Motility involves the coordination of smooth muscle and nerve function to mix, triturate, and propel the products of digestion<sup>10</sup>. The movement is accomplished by coordinating contractions and relaxations of the smooth muscles in the gut. The regulation of gut motility is complicated, involving the enteric nervous system, interstitial cells of Cajal (ICC), hormones, paracrine substances, and inflammatory mediators. The enteric nervous system includes intrinsic neural plexuses and autonomic extrinsic neural pathways, which are of fundamental importance for generating major motor patterns and regulating the amplitudes of contractions<sup>11</sup>.

Dysfunctions, such as slow transit and hypo- or hypercontractility in one of the anatomical regions of the gastrointestinal tract can lead to various symptoms. Gastrointestinal motility disorders, often chronic, are an ultimate result of neuromuscular dysfunction and are associated with a severe impact on the quality of life and an increased healthcare burden<sup>11</sup>.

The absorption of water and solutes occurs predominantly in the small intestine<sup>12</sup>. Because sodium is the main electrolyte in extracellular fluid, it is closely involved in the homeostatic control of the body's water. Consequently, many of the sensory signals and mechanisms that control water intake also influence sodium<sup>13</sup>. Potassium, the main osmotically active cation in the intracellular space, does not play as pivotal a role as sodium in the maintenance of water balance<sup>14</sup>.

Consumption of calabash chalk causes histomorphological changes to the stomach, liver, and esophagus, which may lead to other path-physiological changes and neoplasms of the gastrointestinal tract<sup>15</sup>; this has made it necessary to carry out further studies into the effects of Calabash chalk on the gastrointestinal tract. Carrying out this research here at the University of Calabar is especially important because Calabar, Cross River State, falls within the geographical area in Nigeria where Calabash chalk is commonly ingested<sup>16</sup>.

## **II. Materials And Methods**

### **Ethical approval**

Approval for the use of the animals was obtained from the Ethical Committee of the Faculty of Basic Medical Sciences sciences, University of Calabar, Calabar.

### **Preparation of calabash chalk suspension**

Blocks of non-salted Calabash chalk were purchased from Watt Market in Calabar South, Calabar. The calabash chalk was ground to a powder using a manual grinder. 40g of the ground powder was dissolved in 1 liter of distilled water to get a concentration of 40mg/ml. This concentrate/suspension was filtered with Whatman filter paper to remove all impurities. The suspension was stored in a plastic jug in a cool, dry place.

### **Lethal effect of Calabash chalk**

A report of previous work showed that no mortality was recorded after oral treatment with calabash chalk, even at a concentration of 5000 mg/kg<sup>17</sup>.

### **Experimental animals**

Ten (10) adult albino male rats of the Wistar strain weighing between 160 and 180g were used for these experiments. They were housed in the animal facility of the Physiology Department, University of Calabar, at a temperature of 28±2°C and 12/12 hours of light and dark cycles. The animals were allowed to acclimatize for two weeks before the onset of the experiments. The animals were fed with standard feed pellets and were allowed access to food and water ad libitum. Five animals were kept in each cage (wooden cages with wire net covers), and the cages were always kept clean.

### **Experimental design**

The animals were randomly divided into 2 groups of 5 rats each. Group 1 served as the control group and was administered 1 ml of distilled water every morning for 28 days while group 2 served as the test group and was administered 1 ml of calabash chalk suspension every morning for 28 days<sup>18</sup>. The calabash chalk and distilled water were administered through the oro-gastric route. Food and water intake were administered daily, their body weight measured daily using an animal weighing balance.

### **Measurement of intestinal motility**

Rats were fasted for eighteen hours before experimentation to ensure that the small intestine was completely emptied of food particles. The cervical dislocation method was used to render the rats unconscious. A midline incision was made in the abdomen along the linea alba to expose the small intestine. The distal ileum was identified, isolated, and quickly incised and put in a beaker that contained an aerated Tyrode solution. The solution in the tissue bath was bubbled with air and maintained at 37°C. The ileum was cut into segments of about 2cm long and mounted vertically in a 10ml organ bath (Searle Instruments, England) containing Tyrode solution. An end of the ileum was tied to a fixed support of the oxygen tube inside the bath while the other end was tied utilizing a thread to a lever placed tangentially to a kymograph drum (Searle instrument, England) exerting a load of 0.5g for recording isotonic contractions. The segment of the ileum was allowed for forty-five minutes, and the bathing fluid (Tyrode solution) was changed every fifteen minutes during this period to prevent metabolites from accumulating. Acetylcholine and Atropine were tested on the isolated rat ileum as previously described by<sup>19</sup>.

### **Intestinal transit**

The intestinal transit experiment was done following a modified method of<sup>20</sup>. The marker was prepared fresh by suspending 10% charcoal in 1g gum Arabic. The animals were deprived of food for twenty-four hours but were allowed free access to water. All the rats were later administered 1.5 ml of the marker and kept without food and water for one hour before the determination of intestinal transit. At the end of one hour, each rat was sacrificed by cervical dislocation, the intestine was quickly isolated, and the distance (in cm) traveled by the charcoal meal from the pylorus was measured. This distance was then expressed as the percentage of the total length of the small intestine (cm) from the gastro-pyloric to the ileocaecal junction<sup>19</sup>. The intestinal transit (distance traveled by charcoal meal expressed as a percentage of the total length of the small intestine) was calculated as follows:

Intestinal transit =  $\frac{\text{length traveled by the black marker}}{\text{Total length of the small intestine}} \times 100$

### **Determination of fluid absorption**

Fluid absorption was determined using the everted sac technique<sup>21</sup>. Four portions (I, II, III, and IV), each 10cm in length (two from jejunum and two from ileum), were cut out as shown below for making the sacs.

### **Fluid transfer**

Each sac was made by tying the distal end of the segment with a dry thread having a standard length. From the ligated end, a rod was placed to push the end inwards, thereby inverting the sac (Mucosal end out, serosal end in). The sac was then filled with 1 ml of Krebs solution (serosal fluid), and the free end was tied afterward with a

similar thread. Forty milliliters (40ml) of the Krebs solution was put in incubating flasks labeled (I, II, III, and IV) respectively, and each flask was aerated using 95% oxygen and 5% carbon dioxide gas mixture in a Gallen Lamp shaker bath for thirty minutes. The sacs were immersed in the aerated fluid and aerated for 2 minutes, after which they were incubated for another twenty-eight minutes. After incubation, the sacs were bottled and weighed as follows:

W1= Weight of empty dish + 2 ligatures  
W2 = Weight of empty dish + 2 ligatures + empty sac  
W3 = Weight of empty dish + 2 ligatures + initial weight of full sac  
W4 = Weight of empty dish + 2 ligatures + final weight of full sac  
W5 = Weight of empty dish + 2 ligatures + final weight of empty sac

The units for fluid transfer employed in this study are those of<sup>22</sup>, where fluid transfer was determined as a measure of volume transferred by a unit of wet weight of the intestine for a given period. The mucosal fluid transfer (MFT), serosal fluid transfer (SFT), and gut fluid uptake (GFU) were determined by using the results from the weighing in the following formulae:

Initial wet weight (IWW) = W2 – W1

Initial serosal V (ISV) = W3 – W2

Final V (FSV) = W4 – W5

1) Serosal Fluid transfer (SFT) = (W4 – W5) – (W3 – W2)

2) Gut Fluid Uptake (GFU) = W5 – W2

3) Mucosal Fluid Transfer (MFT) = SFT + GFT

SFT, GFU, and MFT were given as volume/g/sac/30 minutes, where serosal fluid transfer (SFT) is defined as a change in serosal lumen during incubation. Gut fluid uptake (GFU) is defined as an increase in the fluid content of the intestinal tissue owing to the increased water content of the intestinal tissues and the swelling of the epithelial cells. Mucosal fluid transfer (MFT) is the decrease in the volume of fluid on the mucosal side during absorption.

### **Histology**

Sections of the stomach and small and large intestines were made using normal histological processing<sup>23</sup>. Staining was done using hematoxylin and eosin stains. After staining, the photomicrographs were captured at x100 magnification and x400 magnifications using a Presto Image Folio package.

### **Statistical analysis**

The results were presented as Mean  $\pm$  SEM. Independent t-test analysis was used to compare the two means of the groups. P<0.05 was taken to be statistically significant. Computer Software –Graph Pad Prism, SPSS, and Excel Analyzer were used for the analysis.

## **III. Results**

### **Comparison of fluid transfer between the control and the test group**

#### **Comparison of serosal fluid transfer between the control and the test groups**

The mean values obtained in the serosal fluid transfer in the control and the test groups were  $0.23 \pm 0.01$  and  $0.13 \pm 0.01$  mg/gsac/30min, respectively. The result shows that the serosal fluid transfer was significantly reduced ( $p < 0.01$ ) in the test group when compared with the control group. (FIG 1).

#### **Comparison of mucosal fluid transfer between the control and the test groups**

The mean values of mucosal fluid transfer in the control and the test group were  $0.36 \pm 0.15$  and  $0.20 \pm 0.19$ , respectively. The result shows that the mucosal fluid transfer was significantly reduced ( $p < 0.01$ ) in the test group when compared with the control group. (FIG 2).

#### **Comparison of gut fluid uptake between the control and the test groups**

The mean values of gut fluid uptake in the control and the test group were  $0.16 \pm 0.02$  and  $0.14 \pm 0.03$ , respectively. The result shows no significant difference between the control and the test group (FIG 3).

### Comparison of small intestinal motility

#### Comparison of small intestinal transit between the control and the test groups

The mean values of the small intestinal transit in the control and the test group were;  $56.4 \pm 2.70$  and  $40.1 \pm 2.51$ , respectively. The result shows that the small intestinal transit in the test group was significantly reduced ( $p < 0.001$ ) when compared with the control group (FIG 4).

#### Comparison of maximum contraction of the ileal smooth muscle to graded concentrations of acetylcholine (Ach) in the control and the test groups.

The mean values of Ach concentration in the control and the test group at  $-8M$  were  $11.05 \pm 0.53$  and  $80.00 \pm 1.48$ , respectively. At  $-7M$ , the mean values were  $26.84 \pm 0.53$  and  $87.04 \pm 1.85$  for the control and the test group, respectively. At  $-6M$ , the mean values were  $75.00 \pm 1.32$  and  $94.81 \pm 1.48$  for the control and the test group, respectively. At  $-5M$ , the values were  $95.53 \pm 0.79$  and  $99.81 \pm 0.1,9$  respectively. At  $-4M$ , the mean values for the control and the test group were  $100.00 \pm 0.00$  and  $86.30 \pm 2.59$ , respectively. From the results obtained, from  $-8M$  to  $-5M$  Ach concentrations, the maximum contractions for the test group were significantly higher when compared with the control group ( $p < 0.001$ ). At  $-4M$  Ach concentration, the maximum contraction of the test group was significantly lower when compared with the control group ( $p < 0.05$ ) (FIG 5).

#### Comparison of basal height of contraction of the intestinal smooth muscle in the control and the test group

The mean values of basal height of contraction of the intestinal smooth muscle in the control and the test group were  $2.58 \pm 0.39$  and  $2.31 \pm 0.39$ , respectively. The result shows that there was a significant reduction ( $p < 0.01$ ) in the basal height of contraction of the small intestinal smooth muscle in the test group when compared with the control group (FIG 6).

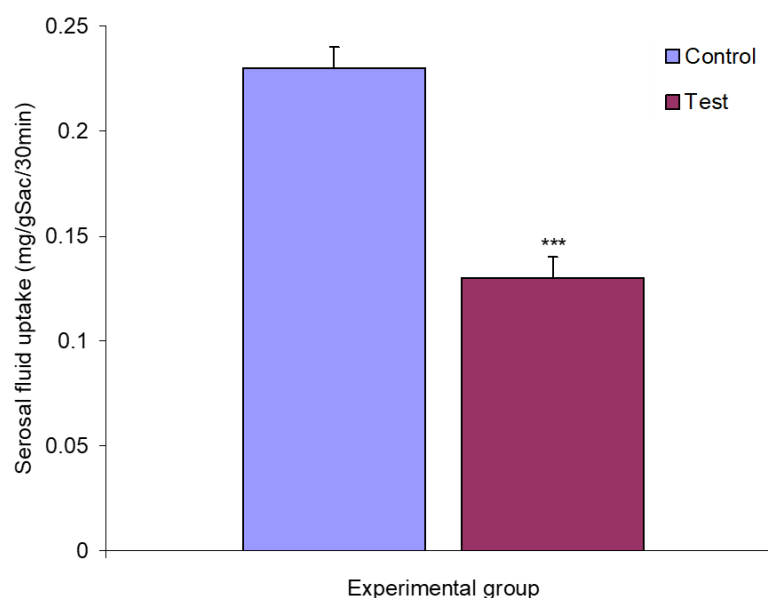


Fig. 1: Comparison of serosal fluid transfer between the control and test group.

Values are expressed as mean  $\pm$  SEM,  $n = 5$ .

\*\*\* =  $p < 0.001$  vs control

Cg

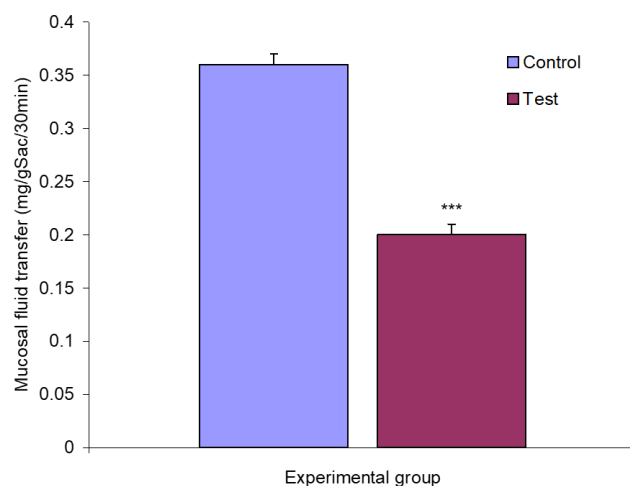


Fig. 2: Comparison of mucosal fluid transfer between the control and test group.

Values are expressed as mean +SEM, n = 5.  
\*\*\* =  $p < 0.001$  vs control

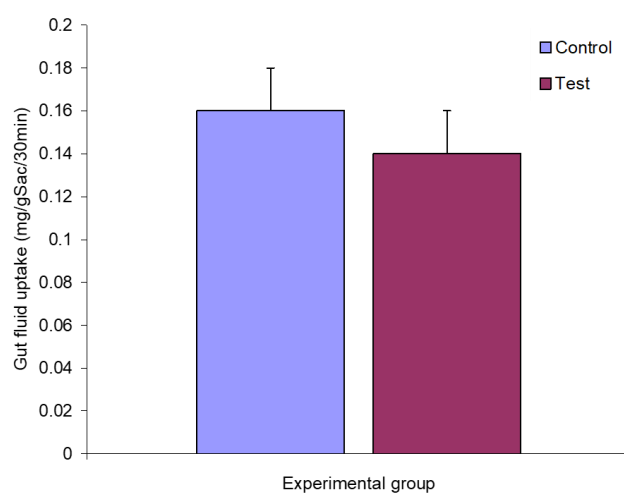


Fig. 3: Comparison of gut fluid uptake between the control and test group.

Values are expressed as mean +SEM, n = 5.  
No significant difference between groups

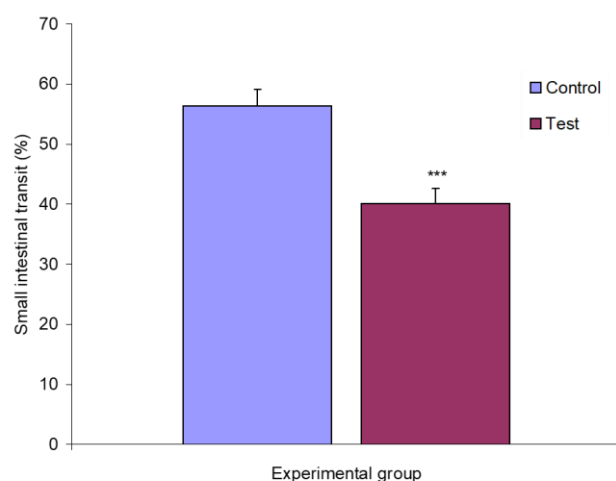


Fig. 4: Comparison of small intestinal transit between the control and test group.

Values are expressed as mean +SEM, n = 5.  
\*\*\* = p<0.001 vs control

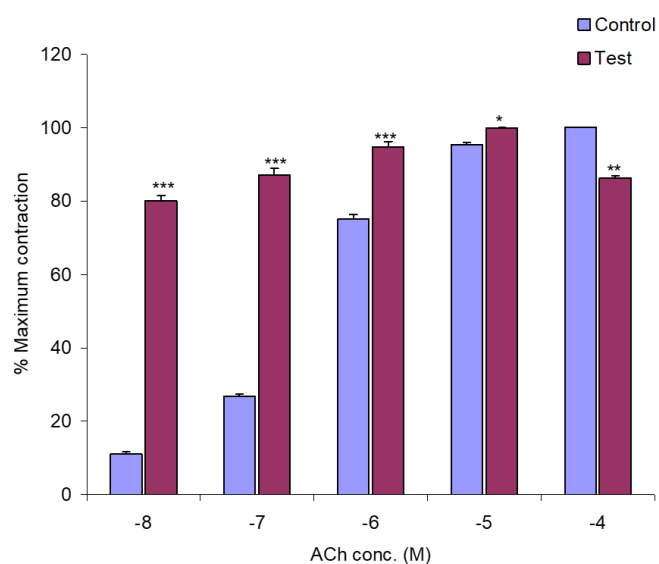


Fig. 5: Bar chart of percentage maximum contraction of the smooth muscle to graded contractions of ACh in the control and test groups.

Values are mean +SEM, n = 4.  
\* = p<0.05, \*\* = p<0.01, \*\*\* = 0.001 vs control.

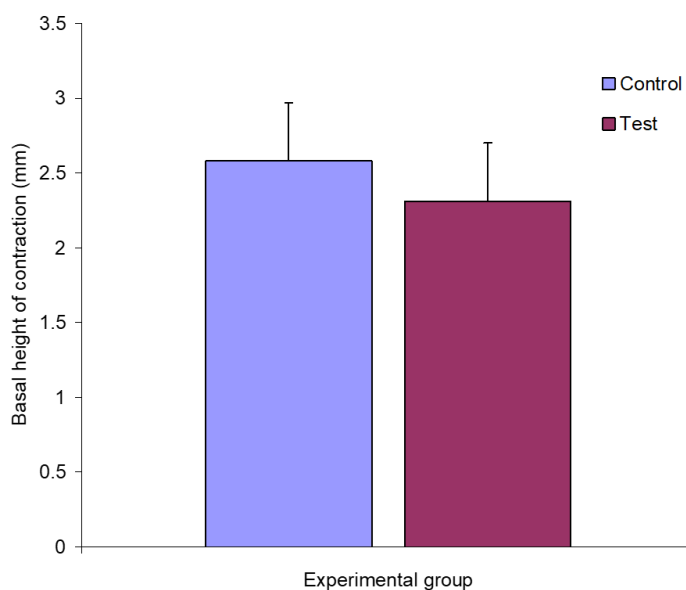


Fig. 6: Comparison of basal height of contraction between the control and test group.

Values are expressed as mean +SEM, n = 5.  
\*\*\* =  $p < 0.001$  vs control

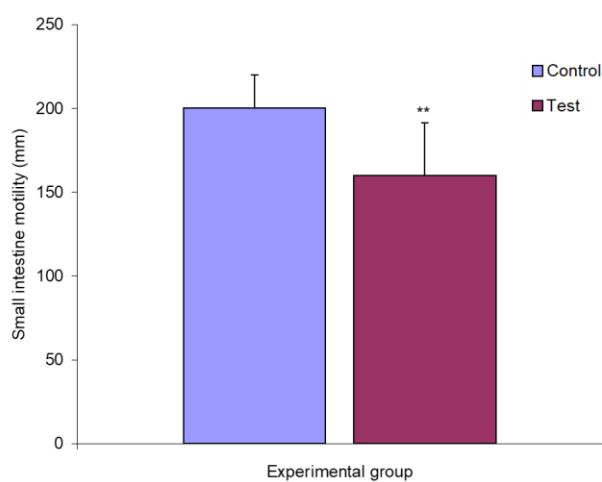


Fig. 7: Comparison of effect of atropine sulphate on intestinal contraction between the control and test group.

Values are expressed as mean +SEM, n = 5.  
\*\* =  $p < 0.01$  vs control

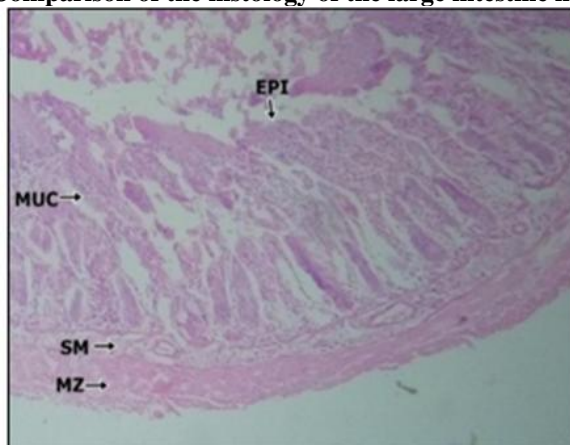


### Comparison of the effect of atropine sulphate on intestinal motility in the control and the test groups

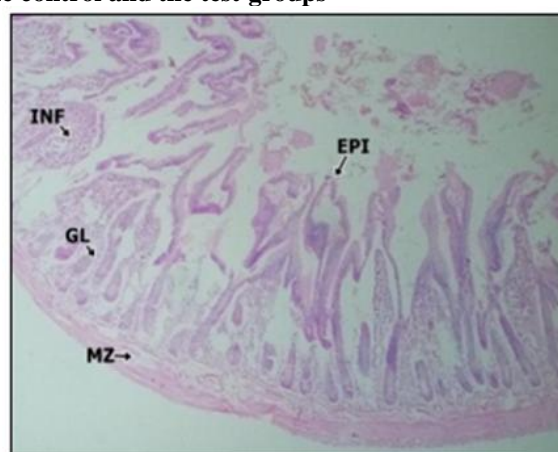
The mean values for the comparison of the effect of atropine sulphate on intestinal motility in the control and the test group were  $200.10 \pm 20.02$  and  $160.15 \pm 31.10$ , respectively. The value for the test group showed a significant decrease ( $p < 0.01$ ) when compared with the control group. (FIG 7).

### Histology

#### Comparison of the histology of the large intestine in the control and the test groups



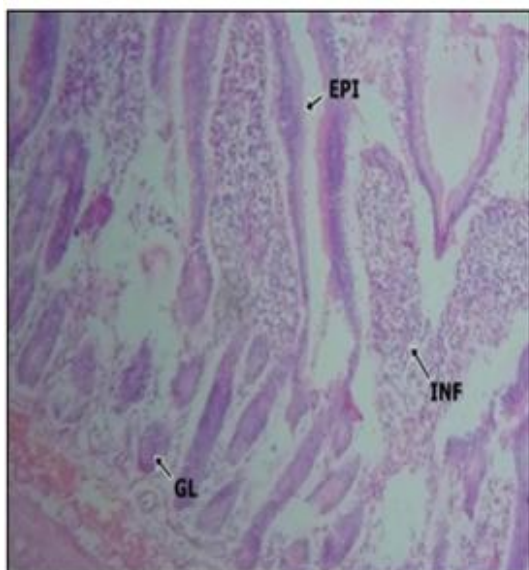
Large Intestine (control group) X100  
Plate 1



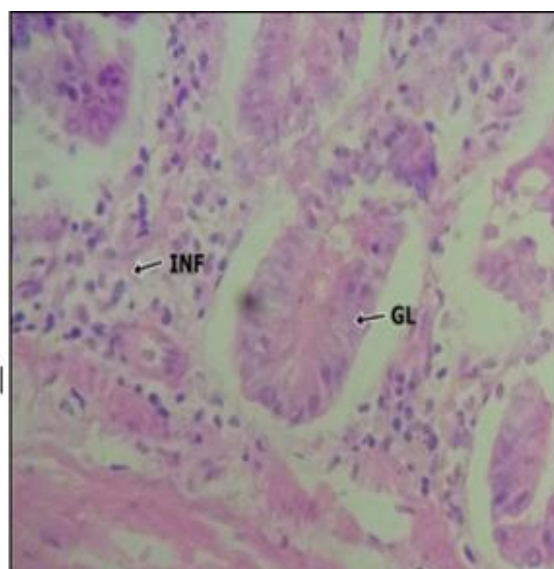
Large Intestine (test group) X100  
Plate 2

Plates 1 and 2: Sections of the large intestine of both the control and the test group. The result obtained showed that the lining surface epithelium is intact in the control group, and the glands are prominent and lined by benign epithelium. Mild inflammatory cells were seen within the lamina propria of the test group.

Keys: MZ - muscles, SM - smooth muscles, MUC - mucosa, EPI - epithelium, INF - inflammation



Large Intestine (control group) X400  
Plate 3

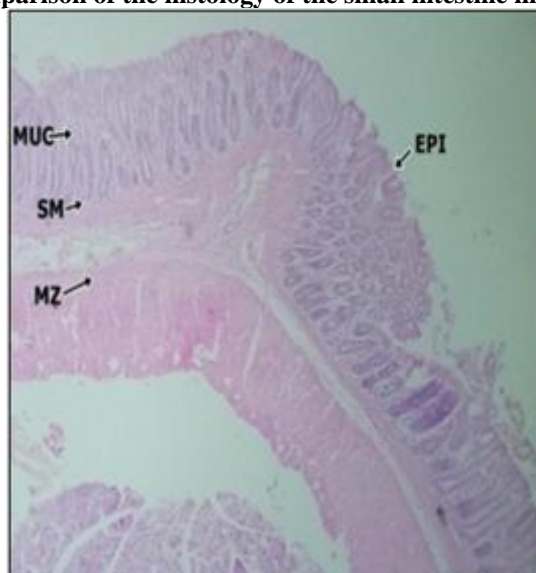


Large Intestine (test group) X400  
Plate 4

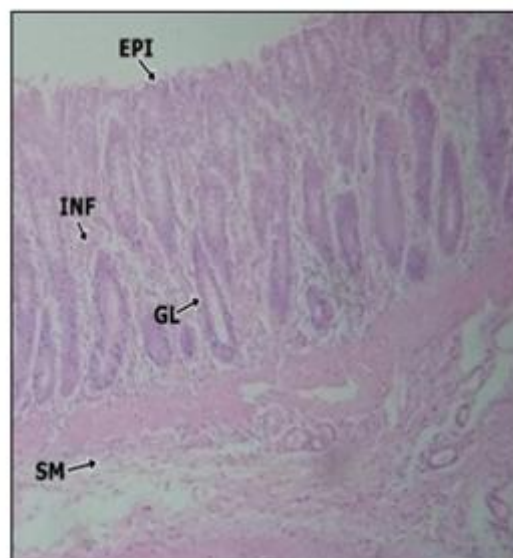
Plates 3 and 4: Sections of the large intestine of both the control and the test group. The lining simple columnar epithelium of the test group shows focal ulceration with mild inflammatory infiltration and edema. Also, the basally located glands are lined with columnar epithelium and show areas of focal hyperplasia.

Keys: MZ - muscles, SM - smooth muscles, MUC - mucosa, EPI - epithelium, INF - inflammation

**Comparison of the histology of the small intestine in the control and the test group**



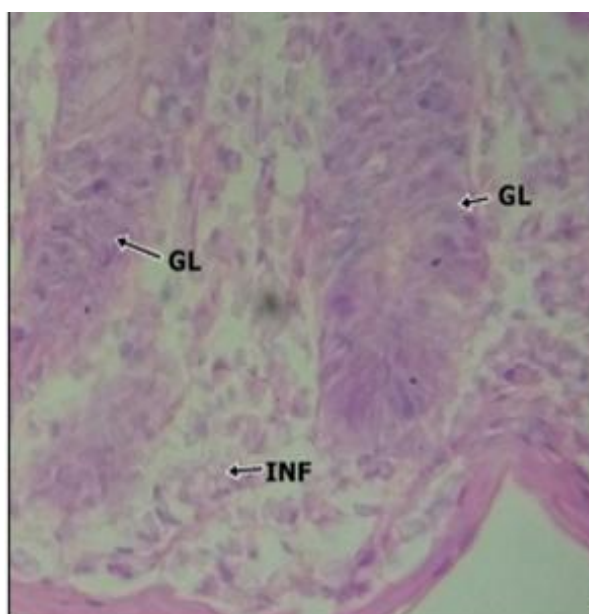
Small Intestine (control group) X100  
Plate 5



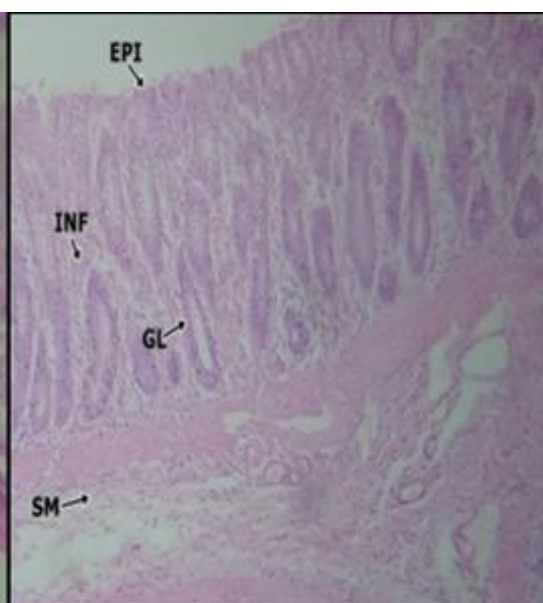
Small Intestine (test group) X100  
Plate 6

Plates 5 and 6: Sections of the small intestine of both the control (plate 5) and the test (plate 6) groups. The surface epithelium is intact in the control group. Prominent glands and inflammatory cells are observed within the lamina propria of the test group.

Keys: MZ - muscles, SM - smooth muscles, MUC - mucosa, EPI - epithelium, PT- portal tract, INF - inflammation



Small Intestine (control group) X400  
Plate 7



Small Intestine (test group) X400  
Plate 8

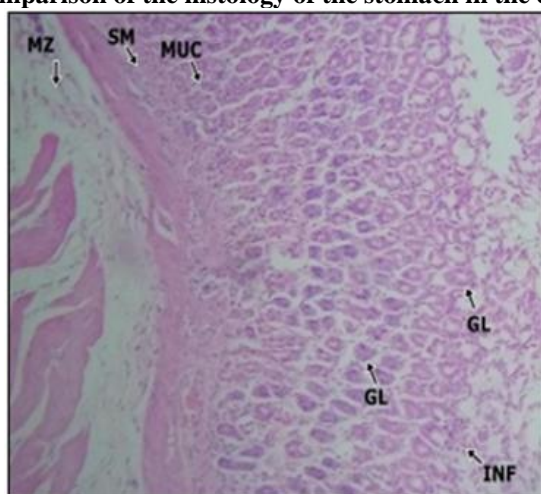
Plates 7 and 8: Sections of the small intestine of both the control (plate 7) and the test (plate 8) groups. There is patchy erosion of the surface epithelium of the test group. There are prominent mucosal glands lined by columnar epithelium with moderate mononuclear inflammation within the lamina propria of the two groups.

There is evidence of submucosal edema in the test group.

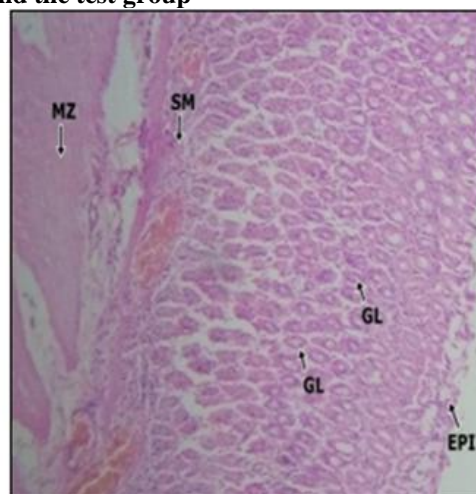
Keys: MZ - muscles, SM - smooth muscles, MUC - mucosa, EPI - epithelium, PT- portal tract, INF - inflammation



**Comparison of the histology of the stomach in the control and the test group**

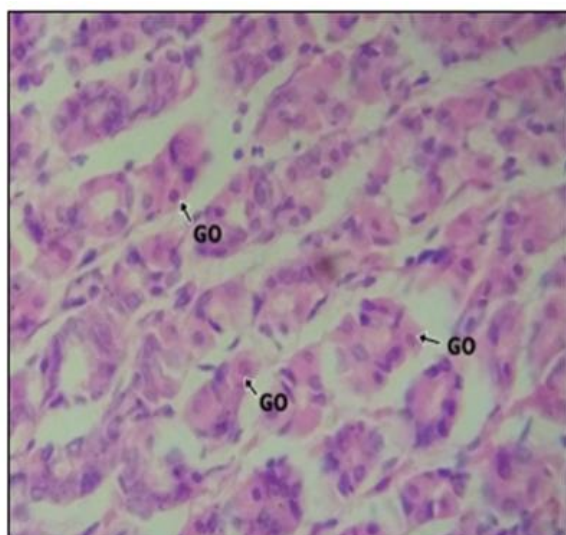


Stomach (control group) X100  
Plate 9

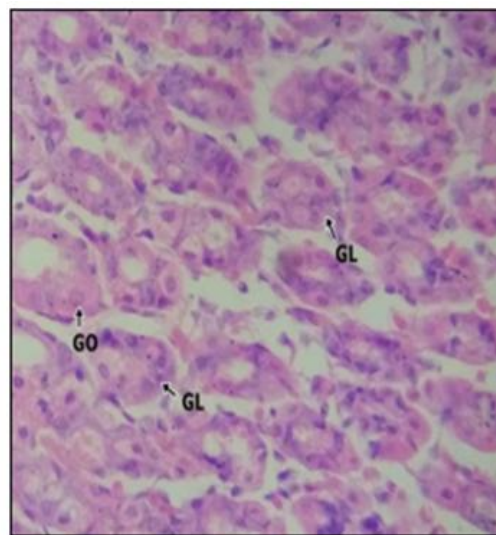


Stomach (test group) X100  
Plate 10

In Plate 9- the he layers are intact, consisting of the mucosa, submucosa, muscular, and adventitia layers. The surface epithelium is intact. Plate 10- shows proliferating glands lined with prominent goblet cells.



Stomach (control group) X400  
Plate 11



Stomach (test group) X400  
Plate 12

Plates 11 and 12 show sections of the stomach at X400. Plate 11- The surface epithelium is intact. The layers are intact, with prominent goblet cells in the mucosa. In Plate 12- the surface epithelium is patchy in appearance with evidence of cellular edema. Also, inflammatory mononuclear cells are seen within the lamina propria.

Keys: MZ - muscles, SM - smooth muscles, MUC - mucosa, EPI - epithelium, PT- portal tract, INF - inflammation.

#### **IV. Discussion**

The function of the gastrointestinal tract may be affected by its structural integrity. The rate of fluid absorption in the intestine is determined by the integrity of the lateral intercellular spaces, which in turn is determined by connective tissue and other factors. Absorption is disturbed when the morphology of the intestine is altered<sup>21</sup>.

The serosal, mucosal, and gut fluid transfers of the test group were significantly decreased compared with the control group. These decreases highlight delayed and interrupted absorption of glucose and fluid. This result implies that chronic consumption of calabash chalk disrupts the normal transportation of fluid across the viscera. Also, chronic consumption of calabash chalk impeded fluid uptake in the gut, as the results showed a

decrease in the gut fluid uptake of the test group. Chronic consumption of calabash chalk might have negatively interfered with the aquaporin water channels' functions in the visceral lining epithelia.

Intestinal motility is the result of complex gastrointestinal contractions that promote the aboral movement of intestinal chyme and indigestible solids<sup>24</sup>. Small intestinal transit is the time it takes for food to pass through the small intestine. Anterograde and retrograde movements of intestinal chyme occur in the jejunum and the ileum, with some areas progressing rapidly and others slowly. Specialized contractions serve a housekeeping role by sweeping undigested materials through the intestine. The function of the small intestine is to transport food as it empties from the stomach and to mix it with bile and pancreatic and intestinal secretions to facilitate absorption<sup>24</sup>. The small intestinal transit of the test group was significantly reduced. This implies that chronic consumption of calabash chalk delays the propagation of chyme through the small intestine. Altered small intestinal motility is thought to contribute to the development of small intestinal bacterial overgrowth (SIBO). Its manifestations include abdominal pain, bloating, malabsorption, nutritional deficiencies, and weight loss<sup>25</sup>. An earlier study by<sup>26</sup> on Calabash chalk demonstrated that it contains pollutants like lead, arsenic, and aluminium and causes constipation, malabsorption, nausea, anorexia, gastrointestinal irritation, and impaction in the gastrointestinal system.

In response to graded concentrations of Acetylcholine, the test group showed an increased percentage of maximum contraction compared to the control group. The difference in percentage of maximum contraction between the two groups was significant at lower concentrations of Acetylcholine (Ach), but the significance decreased gradually as the concentration of Ach increased. This is the norm; increased concentration of Ach leads to increased intestinal contraction<sup>9</sup>. This result infers that chronic consumption of calabash chalk does not significantly affect the maximum contraction of smooth muscles at high concentrations. The basal height of contraction in the test group was reduced, though not significantly, compared to the control group.

## V. Conclusion

Based on our findings, we conclude that chronic consumption of calabash chalk significantly reduces serosal, mucosal, and gut fluid transfers as well as reduced intestinal transit and motility.

## Conflict of interest

The authors declare no conflict of interest.

## Authors' declaration

Authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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