

# Research and Reviews: Journal of Medical and Health Sciences

## Effect of Methanolic Extract of *Xylopi aethiopia* Fruits on Cytoprotection in Cold Stress - Induced Gastric Ulcer in Albino Wistar Rats.

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### Research Article

Received: 03/03/2014

Revised: 22/03/2014

Accepted: 28/03/2014

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**Keywords:** Cold stress,  
cytoprotection, gastric ulcer,  
*Xylopi aethiopia*

#### ABSTRACT

Following the wide spread consumption of fruits of *Xylopi aethiopia* in Nigeria, this study seeks to examine the effects of the fruit on gastric ulcer. Twelve male albino wistar rats weighting 150 - 200 g were randomly assigned one of two groups (n = 6). After 7 days of acclimatization, the test group was administered methanolic extract of *Xylopi aethiopia* at a daily oral dose of 10 mg/100g body weight for 14 days. All animals had access to food and water *ad libitum*. Animals were sacrificed and gastric acid secretion, ulcer score and adherent mucus was determined using standard methods. Histamine, an acid secretagogue and cimetidine, a blocker were used to aggravate and reduce acid secretion respectively. The mean basal gastric acid output was significantly ( $P < 0.05$ ) lower in the test group compared with control. After administration of histamine, the mean gastric acid output was significantly ( $P < 0.001$ ) lower in the test group compared with control. There was no significant difference in gastric acid output in the different groups after cimetidine administration. The mean ulcer score was significantly ( $P < 0.001$ ) lower in the test group compared with control. The mean gastric mucus output was significantly ( $P < 0.01$ ) lower in the test group compared with control. Fruit extract of *xylopi aethiopia* may be beneficial in treating gastric ulcers, since it reduces gastric acid secretion and increases gastric mucous output.

#### INTRODUCTION

Gastric ulcer refers to perforations or simply injuries in the gastric mucosa. Occurrence of gastric or stomach ulcer depends on the balance between gastro - aggressive and gastro - protective factors [1]. When there is a compromise, such that there is a sustained increase in gastro - aggressive factors (gastric acid secretion, abnormal motility, *Helicobacter pylori* infection), without a corresponding increase in gastro - protective factors (mucus secretion, proliferation of mucus secreting cells and mucosal cells of the stomach, etc), gastric ulcer results. The incidence of gastric ulcer is determined by factors such as life style, age, environment among others [2,3].

*Xylopi aethiopia* is a widely used medicinal plant, belonging to family Annonaceae (the custard family) [4]. It is commonly found in lowland forest and moist fringe forest in the savannah zone of Africa but is largely located in the West, Central and Southern Africa [4,5,6]. It is a green aromatic tree which grows up to 20 m high with peppery fruits [6].

In most parts of Nigeria, fruits of *Xylopi aethiopia* is being consumed as spice in food. Fruit extract of *Xylopi aethiopia* have been demonstrated to be beneficial in treatment of various medical conditions. *Xylopi aethiopia* have been named beneficial in treatment of dysentery [7] and malaria [8]. Essential oils of *X. aethiopia* have been used together with cosmetic products like creams and perfumes, they have also been used as insecticide [9] and as a preservative [10] Woode1 (2011) reported that extract of *X. aethiopia* increased steroid hormones and sperm count, and also have analgesic effect [11,12].

Following the widely promoted use of *X. aethiopica* in the treatment of various medical conditions, and its frequent use as spice in meals, it became important to ascertain its effect on gastric ulcers with a view to better inform the public on its use.

## MATERIAL AND METHODS

### Plant Material and Preparation of Extract

Dried fruits of *Xylopia aethiopica* were bought from Watt market in Calabar, Cross River State, Nigeria. It was identified by the Chief herbarium officer of Botany Department, University of Calabar. The dried fruit were washed and milled to a coarse powder using electric blender. 1 g of *Xylopia aethiopica* was then macerated in 10 ml of methanol and stirred to mix evenly. The mixture was kept at air free room temperature to allow for evaporation of methanol. A stock of 1 g in 10 ml of olive oil was prepared from the residue. The median lethal dose of the extract was carried out by method of Lorke, (1983) <sup>[12]</sup>. A daily oral dose of 10 mg/100g was adopted for this study.

### Animal Preparation and Grouping

Twelve male albino wistar rats weighing 150 - 200 g were obtained from the animal house of Department of Physiology, College of Medical Sciences, University of Calabar, Nigeria. All animals had access to food and water *ad libitum*, and were kept in well ventilated cages, exposed to room temperature and 12/12 hours light/dark cycle. The animals were allowed to acclimatize for seven days after which they were randomly divided into 2 groups (n = 6). Group 1 served as control and received 0.2 ml distil water orally while group 2 served as the treated group and received methanolic extract of *Xylopia aethiopica* fruit at a daily oral dose of 10 mg/100g body weight, for 14 days.

### Induction of Ulcer

Stress - induced ulcer was instituted by method of Senay and Levine, 1997 <sup>[14]</sup> and modified by Wong *et al.*<sup>[15]</sup> After 24 hours fast, animals were placed in an ice chamber containing block at a temperature of 4 - 10 °C for 4 hours, after which they were removed and anaesthetized.

### Measurement of Gastric Acid

Gastric acid measurement was carried out by Gosh and Schild <sup>[16]</sup> continuous perfusion method modified by Osim *et al.* <sup>[17]</sup>. All animals were fasted for 24 hour prior to measurement of gastric acid. Each animal received urethane (6ml/kg of 25 per cent (v/v) solution; Sigman, UK) intra - peritonally as anesthesia. The trachea was exposed through a semicircular cut and cannulated. Another cannula was passed through the mouth and esophagus until it reached the stomach. It was then tied firmly in place with a ligature around the oesophagus in the neck. The abdomen was then cut opened along the linea alba to minimize bleeding. The stomach was exposed and the pyloric end cannulated at the pyloric sphincter. Isotonic (0.9 per cent) saline was introduced gently via the esophageal cannula to wash out the stomach content. The perfusate was allowed to flow freely after clearing the stomach of its contents. The abdominal incision was then covered with a moist cotton wool soaked in normal saline. The stomach was continuously perfuse with normal saline at the rate of 1ml/min. The pH of the saline was maintained at 7.0 and the body temperature of the rat was maintained at 37°C by a heating lamp. This was monitored using a rectal thermometer. The flow was adjusted to give an effluent volume of about 1ml per minute. The effluent was collected at 10 minutes interval and care was taken not to ligate the blood vessels to avoid collecting a stained perfusate. At 10 minutes interval, the perfusate was titrated against 0.01N NaOH (May and Baker, UK) to determine its total acidity, using phenolphthalein as indicator. The experiments were repeated using histamine (100mg/kg body weight) and cimetidine (100mg/kg body weight) as acid secretagogue and blocker respectively.

### Cytoprotection Studies

Each animal's stomach was isolated, washed and cut open along the greater curvature and rinsed with normal saline. Pins were used to fasten the tissue in place for proper visualization. Magnifying lens and vernier caliper were used to measure the extent of ulceration. Ulcer scoring was done by the method of Alpin and Wards <sup>[18]</sup> and Adeniji and Olowokorun <sup>[19]</sup>.

### Measurement of Gastric Mucus

Adherent gastric mucus was measured by method of Tan *et al.*<sup>[20]</sup> The animals were fasted for 24 hours prior to commencing the experimental procedure, after which they were sacrificed and their stomachs were removed. Each stomach was cut open along the greater curvature and spread out on a dissecting board and using

pins to hold the edges. Using a spatula, gastric mucus was scraped off the surface of the mucosa and introduced into a 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 a sensitive electronic balance. Mucus output was then obtained as the difference in weight between the sample bottle containing water and sample bottle containing water and mucus. Values were recorded.

### Statistical Analysis

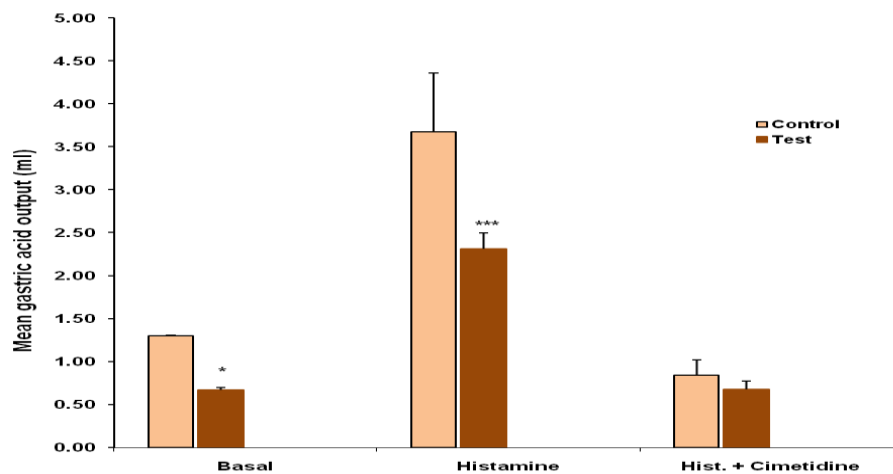
Results are presented as mean  $\pm$  standard error of mean (SEM). The data was analyzed using student's t-test.  $P = .05$  was considered significant. Computer software SPSS and Excel Analyzer was used for the analysis.

## RESULTS

### Comparison of Acid Secretion in the Different Groups

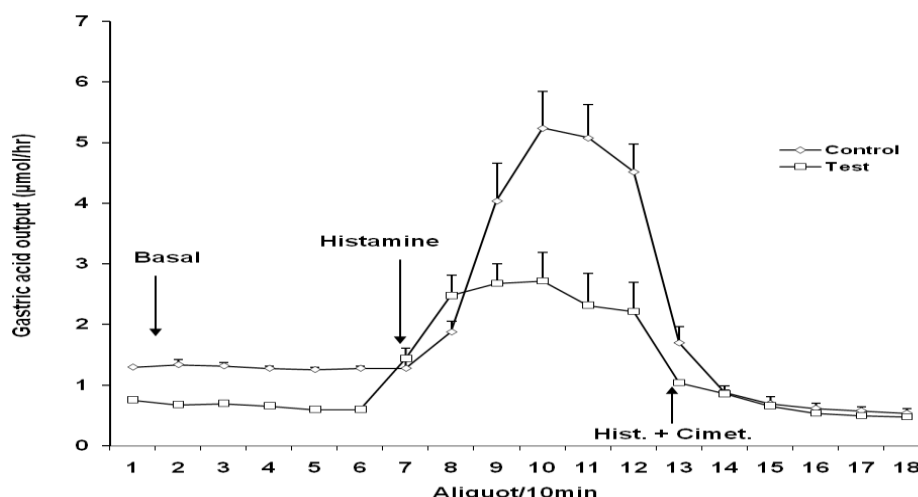
The mean basal gastric acid output was  $1.30 \pm 0.01$  and  $0.67 \pm 0.03$   $\mu\text{mol}/10\text{minutes}$  for control and test group respectively. Basal gastric acid output was significantly ( $P < 0.05$ ) lower in the test group compared with control. After administration of histamine, the mean gastric acid output was  $3.67 \pm 0.69$  and  $2.31 \pm 0.19$   $\mu\text{mol}/10\text{minutes}$  for control and test group respectively. In response to histamine, acid output was significantly ( $P < 0.001$ ) lower in the test group compared with control. Gastric acid output after administration of cimetidine was  $0.84 \pm 0.18$   $\mu\text{mol}/10\text{minutes}$  for control group and  $0.68 \pm 0.09$   $\mu\text{mol}/10\text{minutes}$  for the test group. There was no significant difference in gastric acid output in the different groups after cimetidine administration. (Fig. 1 and 2).

Figure 1: Comparison of mean gastric acid output in the different experimental groups.



Values are mean  $\pm$  SEM, n = 6. \* $p < 0.05$  vs control; \*\*\* $p < 0.001$  vs Test

Figure 2: Comparison of mean gastric acid output in the different experimental groups.



Values are mean  $\pm$  SEM, n = 6.

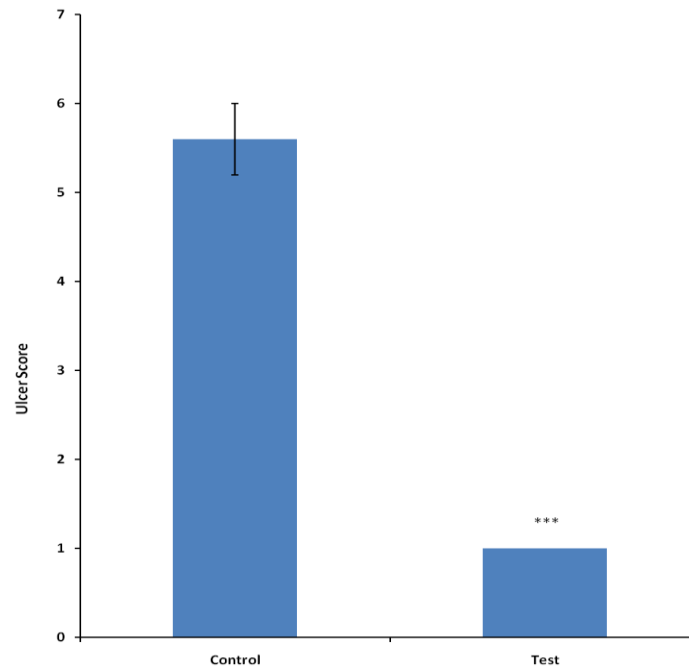
### Comparison of Stress - Induced Ulcer in the Different Experimental Groups

The mean ulcer score for control group was  $5.6 \pm 0.4$ , while that of the test group was  $1.0 \pm 0.0$ . The mean ulcer score was significantly ( $P < 0.001$ ) lower in the test group compared with control. (Fig. 3).

### Gastric Mucus Output

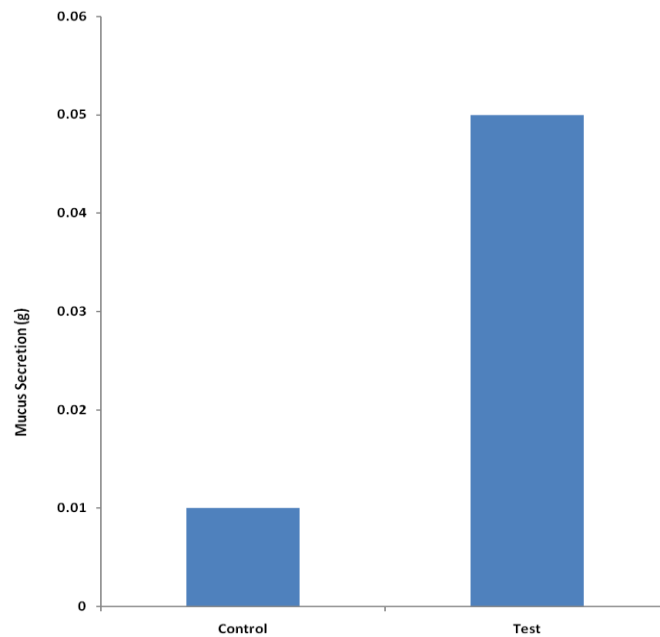
Gastric mucus output in the control group was  $0.01 \pm 0.00g$  and that of the test group was  $0.03 \pm 0.00g$ . The mean gastric mucous output was significantly ( $P < 0.01$ ) lower in the test group compared with control. (Fig. 4).

Figure 3: Comparison of mucus secretion in the different groups.



Values are mean  $\pm$  SEM, n = 6. \*\*\*P < 0.001 vs control.

Figure 4: Comparison of adherent mucus in the different experimental groups.



Values are mean  $\pm$  SEM, n = 6.

## DISCUSSION

Gastric ulceration is a matter of great concern the world over, owing to its high mortality and morbidity. This study was carried out to investigate the cytoprotective property of *Xylopi aethiopia* using cold stress - induced ulcer model in albino wistar rats.

Increased acid secretion is a major determinant of the degree of gastric ulceration. Mean basal gastric acid output was significantly lower in the *Xylopi aethiopia* treated group compared with control. In response to histamine, a secretagogue, mean acid secretion in the extract treated group was significantly lower compared with control. Administration of cimetidine (histamine H<sub>2</sub> receptor blocker), reduced the gastric acid output, though not significant compared with control. Fruits of *Xylopi aethiopia* have been reported to contain several fatty acids and precursors of prostaglandin [21]. Prostaglandins have been reported to reduce gastric acid secretion. *Xylopi aethiopia* may improve synthesis of prostaglandins which in turn reduce gastric acid secretion [22,23,24].

Mean adherent mucus in the gastric mucosa was significantly higher in the *Xylopi aethiopia* treated group, compared with control. *Xylopi aethiopia* significantly reduced ulcer score in the treated group, compared with control. This gastric ulcer lowering effect can be directly correlated with the fact that acid secretion (gastric ulcer aggressive factor) was reduced and mucus secretion (gastric ulcer protective factor) was increased in the treated group.

Gastric mucus secreting cells located in the gastric antrum are known to secrete mucus which contain some amount of bicarbonate [25]. Increased mucus secretion in the treated group contributes to the degree of neutralization of gastric acid, thus, a gastric protective factor.

Aside gastric acid secretion, previous studies had also demonstrated that prostaglandins are important precursors for mucus production [26,27]. Barminas [21] showed that *Xylopi aethiopia* contains polyunsaturated lipid which are necessary for the formation of prostaglandins, implying that they may play the role in offering protection to the gastric mucosa and enhance mucosal blood flow.

## CONCLUSION

From the results obtained in this study, we therefore conclude that fruit extract of *Xylopi aethiopia* may be beneficial in treating gastric ulcers, since it reduces gastric acid secretion and increases gastric mucous output.'

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