

The Effect of Ethanol Extract of *Dennettia tripetala* (Pepper fruit) on Gastric Acid Secretion in Wistar Rats

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ABSTRACT

The aim of this experiment was to ascertain the effect of *Dennettia tripetala* (pepper fruit) on gastric acid secretion in Wistar rats. Twenty one rats weighing 160-180 g were used in this research work. The rats were divided into three groups of seven rats each and designated A, B and C. Group A served as control, whereas groups B and C served as the experimental groups. *Dennettia tripetala* fruits extract was administered twice daily at high dose of 0.0017 ml/g body weight to group B and low dose of 0.00085 ml/g body weight to group C. The control group received 0.5 ml of normal saline. The administration lasted for 28 days. All the animals were given feed and water freely during this period. At the end of the 28 days the rats were anaesthetized with 25% urethane (0.6 ml/100 g). Gastric acid was collected using Shay technique, in which pyloric ligation is performed in the unconscious rat immediately before the collection of gastric juice. The results obtained showed Basal Acid Output (BAO) levels of 4.35 ± 0.25 mM/L/h, 6.1 ± 0.41 mMol/L/h and 6.75 ± 0.7 mMol/L/h across groups A, B and C respectively. The significant variations in results obtained among control and experimental groups revealed that the effect of the seeds of *Dennettia tripetala* on gastric acid secretion is dose-dependent, with increasing action observed when consumed at a higher dose.

INTRODUCTION

Healing with medicinal plants is as old as mankind itself. Most of these herbs are also noted for their nutritional and ceremonial values ^[1]. *Dennettia tripetala* (also known as Pepper fruit) is one of such plants. It belongs to the family, Annonaceae and class magnoliopsida. It is found in the tropical rainforest region of Nigeria and sometimes in Savana areas ^[2]. It is also used traditionally as a remedy for cough, fever, toothache, diabetes, and nausea. The fruits are rich in protein, carbohydrates, as well as the antioxidant, vitamins A, C and E. The plant possesses phytochemicals that have been shown to elicit antimicrobial, insecticidal, analgesic and anti-inflammatory properties. The plant has also been shown to possess chemotherapeutic, ant hyperglycemic, and antioxidant properties. In addition, *D. tripetala* finds application in food preservation and seasoning. The fruits are applied to the food meant for pregnant women and are important in the diets of postpartum women, during which time it is claimed that spices and herbs aid uterine contraction. Kelly ^[1], Okwu et al. ^[2], Achinewhu et al. ^[3] and Okwu et al. ^[4] also reported that *D. tripetala* fruits contain important nutritive substances such as vitamins, minerals and fibre. *D. tripetala* tree grows as a small woody shrub to a height of 12-15 m and have a girth of 0.6 m. The wood is white in color and soft. The bark of DT possesses a very strong characteristic scent. The fruits are green when developing, but start to turn red with ripening ^[5]. The moisture content also increases with ripening. The fruits possess a very strong characteristic smell. The leaves are 3-6 inches long and 1.5-2.5 inches broad. They are elliptic in shape. The fruits are mainly made up of the seeds and a bit of hard, spicy flesh. The fruit and seeds are edible and are consumed because of the spicy nature. The wood is used as fuel. The plant usually produces fruit between the months of March and May. For this reason, local traders preserve the seeds of pepper fruit by drying it under the sun in order to ensure continuous availability until the next harvest ^[2].

Gastric acid is one of the main secretions of the stomach, together with several enzymes and intrinsic factor. It is produced

by parietal cells of the gastric gland influenced by some factors like alcohol and caffeine, which increase its secretion. Hormonal factors such as gastrin, histamine, somatostatin and vagal stimulation also stimulate its secretion while secretin, gastric inhibitory polypeptide and peptide inhibit secretion. A typical adult human stomach will secrete about 1.5 L of gastric acid daily^[6].

Although several authors have already researched on the effect of different products on stomach secretion^[7-13] and *D. tripetala* fruits have also being extensively used but, much work has not been done to study its gastrointestinal implications. Motivated by this, the aim of this study was to investigate the effect of *Dennettia tripetala* seeds extract on gastric acid secretion of Wistar rats.

MATERIALS AND METHODS

Experimental Animals

A total of twenty one male Wistar albino rats weighing 160-180 g were used for this experiment. The rats were purchased from the central Animal House of the Department of Physiology, Faculty of Basic Medical Sciences, University of Calabar, Cross River State, Nigeria. West Africa. The rats were kept in iron cages under standard laboratory conditions at room temperature with 12 h light/dark cycle with access to standard laboratory diet and water *ad libitum*.

Experimental Fruits

Fresh mature fruits of *Dennettia tripetala* were purchased from the nearby bush market in Calabar, Nigeria and were authenticated by the chief botanist of the Department of Biological Science, University of Calabar, Calabar, Nigeria. West Africa, where voucher specimens were deposited in their herbarium.

Drugs and Chemicals

The effect of *D. tripetala* on gastric acid secretion was further provoked by histamine and treated by ranitidine.

Extraction and Isolation

Ripe fruits of *Dennettia tripetala* were obtained from the bush, washed to be free from debris and sundried for two days and later dried with Astell Hearson oven at a temperature range of 45°C-50°C. The dried sample was milled with an electric blender and finally pulverized into powder with a manual blender. 200 g of the powder sample was extracted with 95% ethanol (500 ml) in a soxhlet for 24 h. The ethanol extract was concentrated using a rotator evaporator at 45°C and hot air circulating oven to obtain dark brown oil (15 g; 7.5% yield). The oil was left overnight at laboratory temperature for complete evaporation of remaining ethanol. The extract was stored in dark air tight bottles and refrigerated at 4°C temperature for usage.

Experimental Protocol and Administration of Extract

Twenty one wistar rats were assigned into three groups of seven rats each. The groups were A (control group), B (Low dose group) and C (High dose group). 1 g of *D. tripetala* extract was dissolved in 10 ml distilled water and administration was done orally according to weight. The experimental groups (B and C) took 0.0017 ml and 0.00085 ml of extract respectively whereas the control group received 0.5 ml of distilled water every day.

Preparation of Animals for Collection of Gastric Acid

The animals were kept in cages in a well-ventilated room and fed with normal rat pellets. Water was also given *ad libitum*. The cages were sanitized daily; food and water were also changed regularly.

The Experiment (Gastric Acid Secretion)

After an 18 h fast, each animal was anaesthetized with 25% w/v ethylcarbamate (urithane) at a dose of 0.6 ml/100 body weight, given intraperitoneally. The neck of each of the animals was incised to insert the trachea cannula. The stomach cannula was also inserted at the level of the pyloro-duodenal junction (2 cm below the pyloric ring). An orophageal cannula was also run from the aspirator bottle containing normal saline into the stomach through the mouth. A heating lamp was kept at about 30 cm above the animal to maintain environmental temperature. The stomach was perfused with 0.9% normal (pH 7.00) at the rate of 1 ml/min. before the collection of aliquots.

Collection of Aliquot

A 10 min basal aliquot was first collected across the groups. The experimental animals were again challenged with 2 ml of *Dennettia tripetala* fruit extract infused directly into their stomach through an incision made at the pyloric end. After 1 h, their stomachs were flushed free from the extract, then gastric acid was obtained for 10 min using the continuous perfusion method by Gosh and Schild modified by Ibu et al.^[14].

RESULTS

4.35 ± 0.25 mMol/L h mean basal acid output (MBAO) was obtained. This value increased significantly (p<0.01) across

the experimental groups. Animals treated with low dose and high dose of *Dinnettia T.* extract recorded a mean acid output (MAO) of 6.1 ± 0.41 mMol/L h and 6.75 ± 0.71 mMol/L h, respectively. The three groups (control, low dose treated and high dose treated) were also challenged with Histamine and Ranitidine and at each point the MAO was obtained. With Histamine, the MAO values were; 7.9 ± 1.32 mMol/L h, 9.2 ± 1.32 mMol/L h and 10.8 ± 1.87 mMol/L h in the respective groups. When treated with Ranitidine, there was a significant ($p < 0.001$) reduction in MAO across the respect groups, 3.3 ± 1.99 mMol/L h, 5.45 ± 0.34 mMol/L h and 3.7 ± 0.06 mMol/L h (**Figures 1-5**).

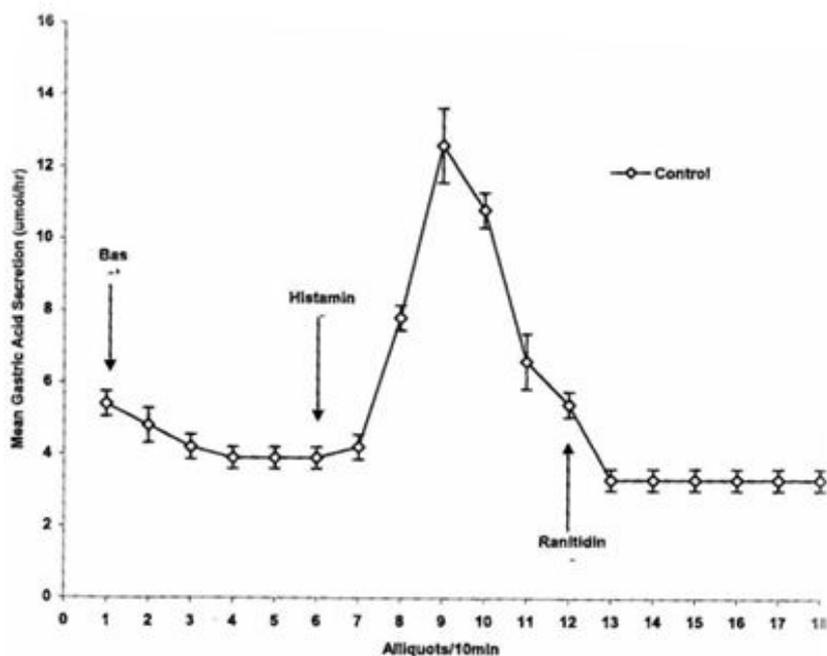


Figure 1. Mean gastric acid out in control group following administration of histamine and rantidine. Values are mean \pm SEM, n=4

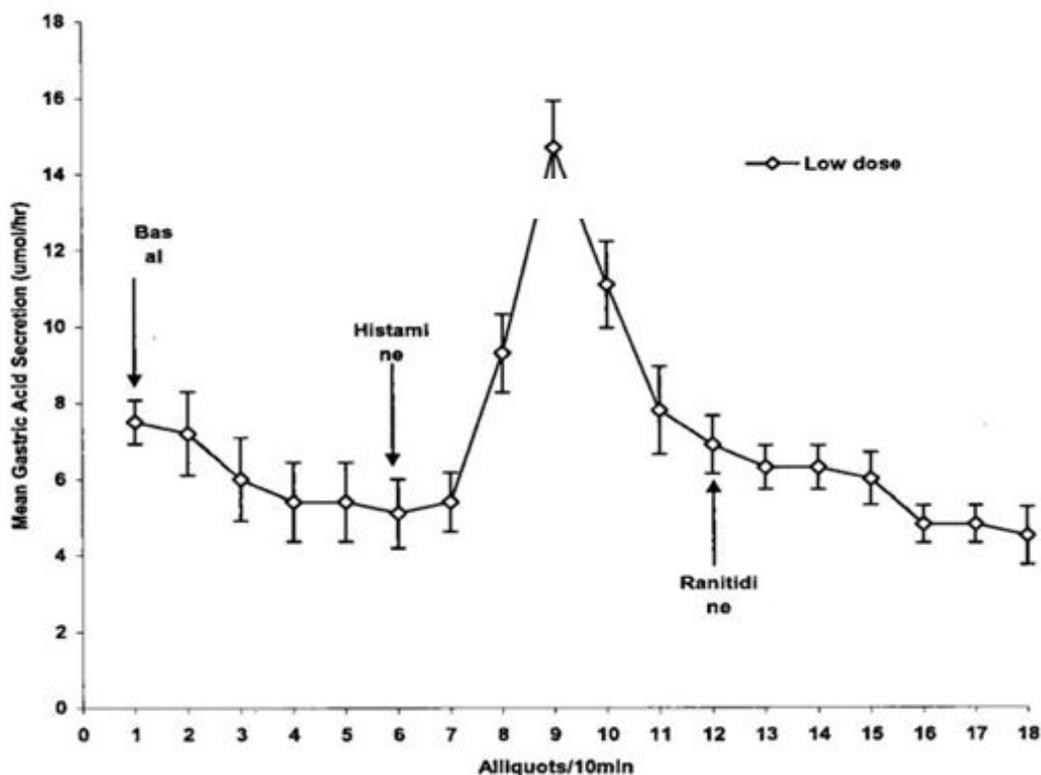


Figure 2. Mean gastric acid out in low dose extract treated group following administration of histamine and ranitidine. Values are mean \pm SEM, n=4

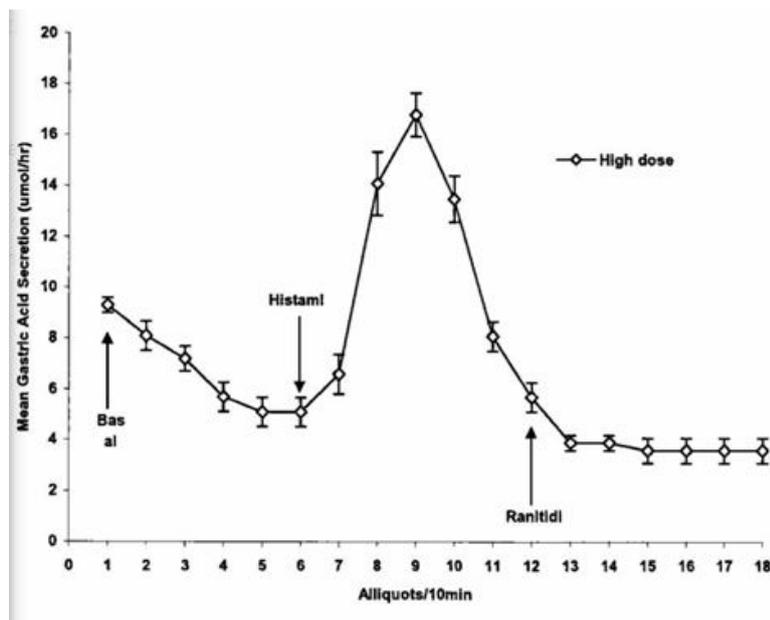


Figure 3. Mean gastric acid out in high dose extract treated group following administration of histamine and ranitidine. Values are mean ± SEM, n=4

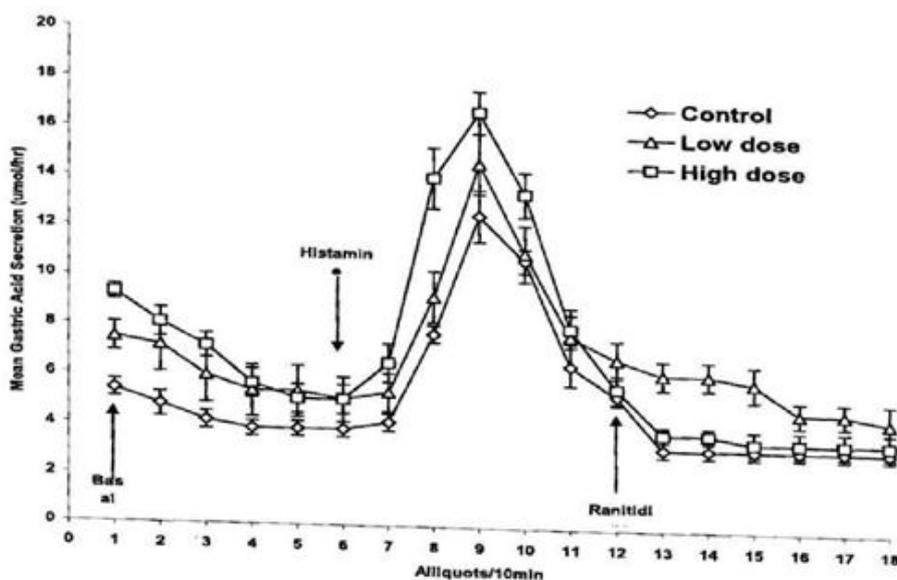


Figure 4. Mean gastric acid out in the different group following administration of histamine and ranitidine. Values are mean ± SEM, n=4

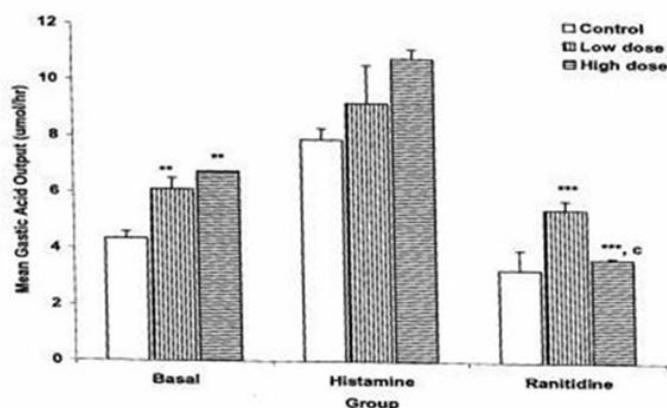


Figure 5. Mean gastric acid out in the different experimental groups following administration of histamine and ranitidine. Values are mean ± SEM, n=4; ***p<0.001 vs. control, c=P<0.001 low dose

DISCUSSION

The effect of acute administration of crude ethanol extract of *Dennettia tripetala* (pepper fruit) on gastric acid secretion was carried out in albino wistar rats. The results obtained showed that acute administration of *Dennettia tripetala* fruits extract in both low dose and high dose produced statistically significant increase in gastric acid secretion ($P < 0.01$) compared to control. This effect was greater in animals fed with high dose of the fruit extract compared to control.

This suggests that the extract has a dose-dependent effect on gastric acid secretion with a probable significant increase ($p < 0.001$) at high dose compared to control.

Although the mechanism at which *D. tripetala* exhibited such provocative effect on gastric acid secretion is not fully established but the extract tend to show some attribute of a H₂-receptor agonist. This observation was established when the different groups were further challenged with Histamine, a H₂-receptor agonist and ranitidine, a H₂-receptor blocker.

Histamine increased gastric output at a significant ($p < 0.01$) level compared with control. This effect was reduced at a significant ($p < 0.01$) level with the administration of ranitidine, hence, confirming previous reports by some researchers which states. that "subcutaneous histamine stimulates copious secretion of acid in rats stomach through the H₂-receptor and that ranitidine blocks Histamine action on H₂-Receptor.

There was further increased provocation in gastric acid secretion across the experimental groups when histamine was administered. The action of histamine on the experimental animals was more potent in the group of animals fed with high dose of the extract compared to control. Ranitidine also showed a significant ($p < 0.001$) action on high dose treated group compared to low dose group and control.

The reason why histamine caused such potent increase in gastric secretion in the experimental animals, especially with animals treated with high dose of the extract, was not fully investigated. But suggestively, it could be that *D. tripetala* may have some agents that potentiate histamine-stimulated acid secretion in the stomach. This action could have been mediated via H₂-receptors which are situated on the parietal cells. It is therefore probable that *D. tripetala* acts on the parietal cells directly in order to stimulate gastric acid secretion. This also could explain the significant ($p < 0.001$) inhibitory action of ranitidine, a potent H₂-blocker on histamine- induced gastric acid secretion in experimental groups. Especially in animals fed with high dose of *D. tripetala* seeds extract.

CONCLUSION

From the deductions gathered from this research, it is evident that:

1. *Dennettia tripetala* (pepper fruit) has a dose-dependent provocative influence on gastric acid secretion under normal physiological conditions, with greater potency recorded at high dose.
2. *Dennettia tripetala* follows the H₂-receptors pathway, stimulates the parietal cells and increase gastric acid output in the stomach. It also potentiates the activity of histamine on gastric acid secretion at all quantities.
3. The action of *Dennettia tripetala* at all dosage can be reduced by ranitidine or other H₂-receptor blockers.

RECOMMENDATIONS

The fruits of *Dennettia tripetala* can be recommended for consumption only by individuals who are not ulcer patients and those who are not in any way pre-dispose to ulcer unless otherwise taken with any antihistamine drug like ranitidine. In any case, it should only be taken at very little quantities.

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