

Gastric acid anti-secretory, anti-ulcerogenic and mucogenic effects of aqueous leaves extract of *Ocimum gratissimum* in rats

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Summary: In this study, albino Wistar rats were placed on normal rats chow + drinking water and/or 500mg/kg, 1000mg/kg body weight of the *Ocimum gratissimum* extract orally, once daily for 28 days and gastric acid, mucus and ulcers determined. All the rats received normal rat chow + drinking water *ad libitum* for 28 days. Gastric acid, mucus secretion and ulcer scores were determined with standard procedures. Results showed that the mean basal gastric acid output for control, low dose and high dose groups were 11.28 ± 0.70 , 8.04 ± 0.57 and 6.14 ± 0.67 $\mu\text{mol/hr}$ respectively. The high dose extract recipients had a significantly ($P < 0.05$) reduced gastric acid output compared with control and low dose. Increase in gastric acid output as induced by histamine was highest in high dose (599.02%), followed by low dose 426.28%, then control (221.28%). Administration of ranitidine was observed to attenuate the effect of histamine in all the groups. The high dose group also had a significantly ($P < 0.05$) higher mean gastric mucus and lower ($P < 0.05$) ulcer levels compared with other groups. In conclusion, the aqueous leaves extract of *Ocimum gratissimum* decrease gastric acid secretion and ulceration, it also produced an increase in the gastric mucus secretion. If these results are applied to man, it could be beneficial in the management of peptic ulcers and other related complications.

Keywords: *Ocimum gratissimum*, gastric acid, gastric ulcer, mucus, rat

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INTRODUCTION

Ocimum gratissimum belongs to the family Lamiaceae, It is commonly called 'alfavaca' and is widely planted in many gardens around village huts in Nigeria for its medicinal and culinary uses (Aguiyi *et al.*, 2000). It originates from Central Africa and South East Asia (Simon and James, 1995).

Phytochemical screening of this plant reveals the presence of some active ingredients like eugenol, linalol, methyl cinnamate, camphor and thymol (Craveiro *et al.*, 1981; Janine de Aquino Lemos *et al.*, 2005). Eugenol, an isolate from *Ocimum gratissimum* has been observed to possess anti-helminthic properties (Pessoa *et al.*, 2002), nematicidal properties (Chatterje *et al.*, 1982) and insecticidal properties (Chavan and Nikam, 1982).

Several species and varieties of plant of the genus *Ocimum* have been reported to yield oil of diverse nature, commonly called basilica oils. The oils produced from *O. gratissimum* are active against several bacteria (*Staphylococcus aureus*, *Listeria monocytogenes*, *E. coli* etc) and fungi (*Trichophyton rubrum*, *T. mentagrophytes* etc) as reported by El-said *et al.*, (1969), Begum *et al.*, (1993), Nwosu and Okafor (1995), Akinyemi *et al.*, (2004), Lopez *et al.*, (2005).

It is also used in the treatment of other myriad ailments: upper respiratory tract infections, diarrhea, headache, fever, eye problems, skin diseases, and pneumonia (Correa, 1932; Onajobi, 1986 and Ilori *et al.*, 1996). It is also a potent anti-diabetic agent (Mohammed *et al.*, 2007; Egesie *et al.*, 2006) while Mbata and Saikia (2009) reported the use of *O. gratissimum* in flavouring foods and that it possesses some antimicrobial properties, but the effects of *O. gratissimum* on gastrointestinal functions has not been reported.

The stomach is a hollow organ situated just below the diaphragm and is concerned with storage of food, digestion, protection, excretion and in hemopoiesis. Excessive secretion of gastric acid can cause ulcerations of the stomach and adjoining tissues (Sembulingam and Sembulingam, 2006). Several substances taken into the body have been known to modify the physiological functions of the stomach. In the face of paucity in scientific literature on the effect of *O. gratissimum* on gastric acid, gastric ulceration and mucus secretion, it is therefore the aim of this study to investigate the effect of oral administration of *O. gratissimum* on gastric acid, gastric ulceration and mucus secretion.

MATERIALS AND METHODS

Experimental Animals

Thirty male albino Wistar rats were obtained from the animal house of the Department of Physiology, University of Calabar, Nigeria. The rats weighed between 220-250g at the time of sacrifice. They were weighed before commencement of the feeding experiment and thereafter were weighed daily. They were nursed under control environmental condition. The research was conducted in accordance with the internationally accepted principles for laboratory animal use and care, as found in for example the European Community guidelines (EEC Directive of 1986; 86/609/EEC)

Experimental Plant

Four kilogrammes of fresh leaves of '*ntong*' (*Scent leaves* as locally called) were purchased from a local market in Calabar-South Local Government of Cross River State, Nigeria, during the rainy season and were identified and authenticated as *Ocimum gratissimum* by a botanist (Mr. Frank Adepoju) in the Department of Biological Sciences, University of Calabar, Nigeria.

Preparation of Plant Extract

Fresh leaves of *O. gratissimum* were first washed free of sand and debris. Wash water was blotted off and the leaves ground to paste. A quantity of the ground sample (50g) was weighed and soxhlet extracted with 150ml distilled water at 100°C for 9hr. Where larger ground samples were used, extraction was done under reflux with an appropriate volume of distilled water. The extract was slowly evaporated to dryness in vacuum at 40°C using a rotary evaporator. A total yield of 33.2% was obtained. Weighed samples of the extract were then used to prepare the stock solution as previously described (Eno *et al.*, 2001)

Feeding Protocol

The thirty male albino Wistar rats were divided into 2 batches of 15 rats each. Batch 1 was used for gastric acid secretion and mucus secretion studies, while batch 2 was used for the ulcer study. Each batch was further sub-divided into 3 groups of 5 rats each. They were fed as follows: Group 1 (control) was fed on normal rat pellet + drinking water. Group 2 (LD) was fed on normal rat pellet + 500mg/kg of extract orally once daily. Group 3 (HD) was placed on normal rat pellet + 1000mg/kg of extract orally once daily. They were all allowed free access to drinking water. The feeding regimens lasted for four weeks.

Measurement of Gastric Acid Secretion:

Measurement of gastric acid secretion was done by the continuous perfusion method of Ghosh and Schild (1958) modified by Osim *et al.*, (1991). Rats from the control and test groups were fasted for 18-24 hours before the start of the experiment. The rats were anaesthetized with 0.6ml/100g body weight of 25%

(wt/v) solution of urethane (Sigma, UK) given intraperitoneally. The trachea was exposed and cannulated. An infusion tube 75cm length and 3mm diameter connected to 60ml syringe carried by a pump was passed to the stomach through the mouth and oesophagus. A ligature to stop back flow was made around the oesophagus in the neck. The abdomen was opened along the *linea alba* to minimise bleeding. The small intestine was reached and a semi-transection of 1-2cm away from the pylorus was made and a fistula 8cm long passed gently into the stomach through the pyloric sphincter and knotted.

Normal saline solution pH 7.00 placed in the pump was perfused through the stomach at 1ml/minute via a perfusor. After an initial wash, the perfusate was collected every 10 minutes interval and titrated with 0.01N NaOH solution in a 25ml burette using phenolphthalein as indicator with pink coloration indicating the end point.

The pH of the saline was maintained by passing the perfusion tube through a water bath maintained at temperature of 37°C. Also a low wattage bulb was placed above the animal to warm it and the body temperature monitored. A rectal thermometer was inserted via the anus to ensure that the body temperature was at 37°C, care had to be taken not to ligate the vagus nerve or other blood vessels. To each 10 minute perfusate was added 2 drops of phenolphthalein indicator before titration against 0.01N NaOH (Analar BOH, England) to determine total acidity.

Analysis of gastric acid.

Gastric acid output was measured by titrimetric analysis. The calculation of acid in millimole per litre per hour (mMol/L/hr) follows the principle that states that a gram equivalent of acid balances a gram equivalent of the base at neutralization point. This means that:

$$\begin{aligned} \text{Normality (N) of Acid} \times \text{Volume (V) of} \\ \text{Acid} &= \text{Normality of Base} \times \text{Volume of Base} \\ \text{i.e. } N_A \times V_A &= N_B \times V_B \end{aligned}$$

From the above equation since Normality (N) of base is known i.e. 0.01N and the volume of base needed for neutralization is known, the gram equivalent can be calculated thus: $N_B \times V_B$. This at the end points to the gram equivalent of the acid. If the volume is in mls, the acidity end point is in milli-equivalent of acid. For a small animal like the rat milliequivalent will be too small and is always converted to μeq or μmol .

Ulcer studies

Gastric ulceration was induced in rats as described by Tekeuchi *et al.*, (2001) by oral instillation of 1ml of 0.1N HCl + 70% ethanol through intubation after an overnight fast. One hour later, the animals were sacrificed using over dose of diethyl ether/chloroform

and the stomachs were removed and opened along the greater curvature. Haemorrhagic lesions were examined macroscopically and scored as described by Elegbe (1978).

Ulcer scoring:

Score	Description
0	Plan
0.5	0-6mm
1	2-3mm
2	>3mm

Determination mucous secretion:

The adherent gastric mucous was determined by the method described by Ettarh and Okwari (1999). The stomach was removed and washed in normal saline and then opened along the greater curvature. It was again rinsed in saline and pinned to a cork board with dissecting pins. Mucus was extracted using a spatula from the spread stomach into a known weight of beaker containing 4ml of water. The weight of mucus was derived from the difference in the initial and final weights of beaker + 4 ml of water as follows:

$$\text{Wt of beaker} + 4\text{ml of water} = x$$

$$\text{Wt of beaker} + 4\text{ml of water} + \text{mucus} = y$$

$$\text{Weight of Mucus} = (y-x) \text{ gm}$$

This procedure has also been described by Tan et al., (2002)

Statistical Analysis

Data are presented as mean ± SEM. Data were analysed using a one way analysis of variance (Anova) then followed with post hoc test (Least Square Deviation). P value of less than 0.05 was declared as significant statistically..

RESULTS

Comparison of mean basal gastric acid output in control and tests groups

The mean basal acid outputs (BAO) in the control, LD and HD extract recipients were 11.28 ± 0.70 , 8.04 ± 0.57 and $6.14 \pm 0.67 \mu\text{mol/hr}$ respectively. There was a significant ($P < 0.01$) decrease in the mean BAO of LD and HD groups compared with control. Fig. 1

Comparison of the effect of histamine on gastric acid secretion in control and tests groups.

Administration of histamine significantly ($P < 0.001$) increased the mean basal acid output in all the groups. The increase was highest in the test groups than in the control. In the control the increase in mean BAO was 221.28% (i.e. from $11.28 \pm 0.71 \mu\text{mol/hr}$ to $36.24 \pm 6.54 \mu\text{mol/hr}$), in the LD it was 426.28% (from $8.84 \pm 0.57 \mu\text{mol/hr}$ to $42.32 \pm 6.89 \mu\text{mol/hr}$) and 599.02% (from $6.14 \pm 0.67 \mu\text{mol/hr}$ to $42.92 \pm 4.10 \mu\text{mol/hr}$) in the HD. Fig. 2

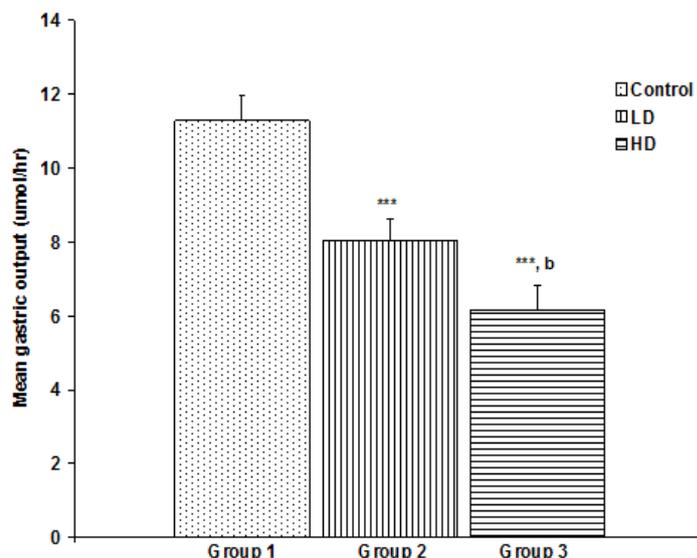


Figure1. Mean basal gastric output in the experimental groups, values are mean±SEM, n=5, ***P<0.001Vs control, b= P<0.01Vs LD

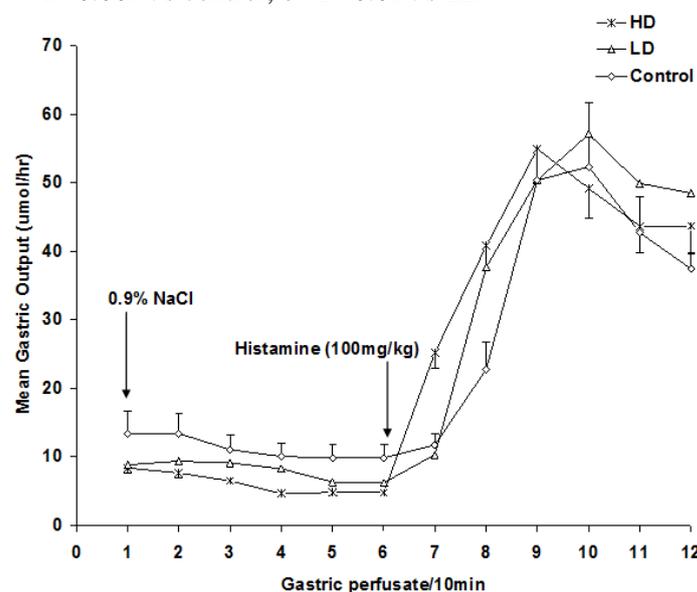


Figure 2. Effect of histamine on gastric output in the experimental groups, values are mean±SEM, n=5

Comparison of the effect of ranitidine on histamine-induced gastric acid secretion in control and tests groups.

Administration of ranitidine attenuated the effects of histamine in all the groups. In the control group, administration of histamine + ranitidine increased mean BAO from $11.28 \pm 0.71 \mu\text{mol/hr}$ to $20.88 \pm 6.27 \mu\text{mol/hr}$, in the LD and HD groups it was from $8.84 \pm 0.57 \mu\text{mol/hr}$ to $10.84 \pm 2.92 \mu\text{mol/hr}$ and from $6.14 \pm 0.67 \mu\text{mol/hr}$ to $11.96 \pm 2.46 \mu\text{mol/hr}$ respectively. This effect was most obvious in LD and least in control. Fig. 3

Comparison of ulcer scores in control and tests groups.

As shown in fig. 4, the mean gastric ulcers in the HD group (4.80 ± 1.05) was significantly ($P < 0.05$) lower

compared with the control (10.30 ± 2.00) and LD (8.80 ± 1.19) groups.

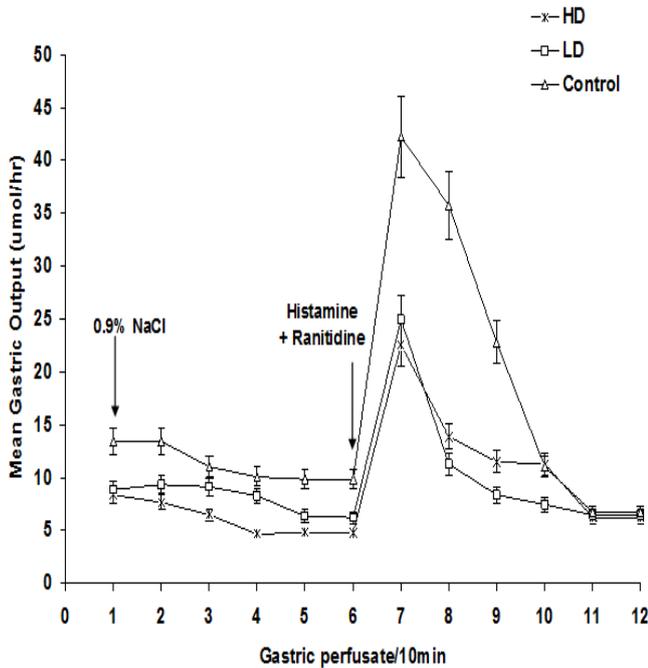


Figure 3. Effect of histamine+ranitidine on gastric output in the experimental groups, values are mean±SEM, n=5

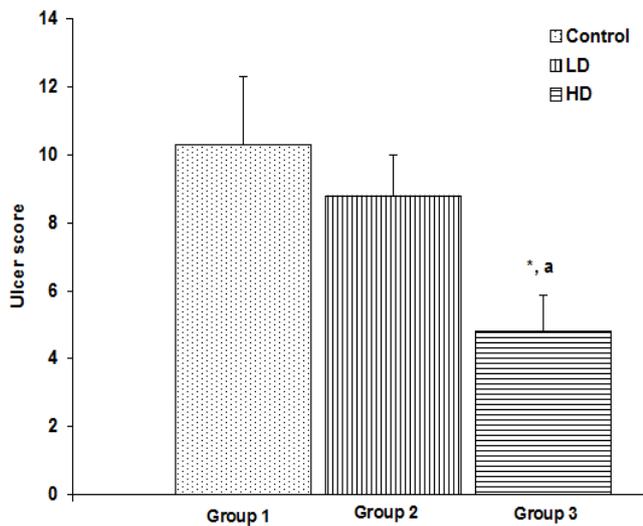


Figure 4. Ulcer scores in the experimental groups, values are mean±SEM, n=5, *P<0.05Vs control, a= P<0.05Vs LD

Comparison of mean gastric mucus levels in control and tests groups.

The mean gastric mucus levels for control, LD and HD groups were $0.092 \pm 0.01g$, $0.102 \pm 0.01g$ and $0.132 \pm 0.01g$ respectively. It was significantly ($P<0.05$) higher in the HD extract recipients than in control and LD. Fig. 5

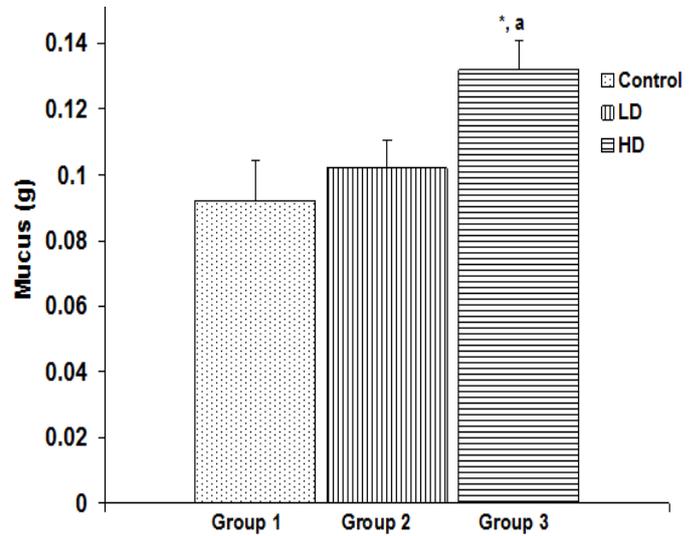


Figure 5. Mucus secretions in the experimental groups, values are mean±SEM, n=5, *P<0.05Vs control, = P<0.05Vs LD

DISCUSSION

This research work was designed to investigate the effect of aqueous leaves extract of *Ocimum gratissimum* on gastric acid, mucus secretion and ulceration in rats. Results obtained show that the mean basal acid output in the high dose extract recipients was significantly lower compared with control and low dose groups. The low dose recipients in turn were observed to have a lower acid output compared to the controls, although this decrease was not statistically significant. The extract also dose dependently decreased gastric ulcerations and increased the mean mucus secretion of the stomach when compared with control.

Gastric acid is continuously secreted from the parietal cells of the stomach as one of its basic physiological functions which helps in the digestion of especially proteins. The acid is prevented from damaging the mucosal walls because of the mucus cells that secretes very viscid and adherent mucus and the tight gap junctions between its adjacent epithelial cells. The formation and secretion of mucus by the stomach is enhanced by some prostaglandins (Bengmark, 1996). Ingestion of anti-inflammatory drugs like aspirin that interferes with the actions of prostaglandins, inflammation of the gastric mucosa, bacteria infection (*helicobacter pylori*), excessive production of gastric acid could break this barrier and disposes the mucosa to gastric injuries and ulcers (Guyton and Hall, 2006).

Leaves extract of *O. gratissimum* has been reported to contain phytochemical constituents like flavonoids (Ijeh et al, 2004) which are capable of promoting gastric mucosal formation, reduce gastric acid secretion and inhibit pepsinogen production,

thereby reducing gastric lesions and ulcers. Other components like alkaloids, oligosaccharides, phytates and tannins are also present in the leaf extract of *O. gratissimum* which tend to exhibit some anti-inflammatory and anti-oxidant properties (Ghosal, et al., 1996; Nwaogu, et al., 2007).

It is obvious that the low levels of acid secretion and ulceration produced by the root extract of *O. gratissimum* in this study could be due to the presence of these components or some other mechanisms yet unidentified.

The extract was also observed to potentiate the effect of administered histamine on gastric acid secretion. This inability of the extract to attenuate the effect of administered histamine on gastric acid secretion rules out the action of the extract on the H₂-histaminergic receptors. It has been suggested that subcutaneous histamine stimulates copious secretion of acid in rat's stomach through the H₂ receptor, and the cellular mechanism involves the activation of cyclic adenosine monophosphate (cAMP) a process that is driven by H⁺/K⁺ ATPase (Garrison, 1992). It is therefore possible that the extract could decrease gastric acid secretion by acting on and blocking the activity of other receptors rather than the histaminergic H₂-receptors. This evidence is supported by the fact that the extract recipient group produced a lower acid output when ranitidine + histamine were administered compared with control. Although, ranitidine was also observed to reduce acid output induced by histamine in all the groups studied. This is in consonance with reports that ranitidine is a pharmacological agent that blocks the action of histamine on the H₂ receptors (Boucher, 1977; Garrison, 1992).

We therefore conclude that the aqueous leaves extract of *O. gratissimum* decrease gastric acid secretion and ulceration, with concomitant increase in the gastric mucus secretion. This effect could probably be mediated by their actions on other receptor not investigated in this study, and not via the H₂-histaminergic receptors or probably by preventing gastric irritations.

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