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Ocimum grastissimum extract inhibits stimulated acid secretion by Carbachol and induces gastric mucus secretion

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Abstract

In this study, the effects of ethanol extract of *Ocimum gratissimum* (EEOG) on both basal and stimulated gastric acid secretion and gastric mucus secretion were investigated in Albino rats treated with the extract. Four groups of animals were used. Sub-group 1A serves as control. Animals in Group 2A, 3B and 4B were pretreated with 200mg/kg of (EEOG) for 1, 7 and 14 days respectively. Basal gastric effluents were collected from all the groups of animals at intervals of 10mins for 60mins. Thereafter, Subgroups 1A, 2A, 3A and 4A were administered with 50μ/kg b.w. of carbachol (i.p.) intraperitonialy and effluents collected. Animals in Sub-group B were used for gastric mucus study. Carbachol stimulates gastric acid secretion in animals pretreated with the extract for 1, 7 and 14 days. 50-400 mg/kg b.w. doses of the extract significantly increase gastric mucus secretion. These results indicate the mechanism of anti-ulcer activity of the extract may be due to stimulation of gastric mucus secretion amongst pathways.

Keywords: *Ocimum gratissimum; histamine receptors, gastric mucus secretion; carbachol*

Résumé

Dans cette étude, nous enquêtons sur l'effet de l'extrait d'éthanol de *Ocimum gratissimum* (EEOG) sur la sécrétion d'acide gastrique de base et stimulée de même que la sécrétion des mucosités gastriques chez les souris Albinos traités avec l'extrait. Quatre groupes d'animaux ont été utilisés. Le sous-groupe 1A représente le groupe pilote. Les animaux qui se trouvent dans les groupes 2A, 3B et 4B ont été traités en avance avec 200mg/kg de O. gratissiumum pendant 1, 7 et 14 jours respectivement. Des effluents gastriques de Base ont été recueillis de tous les groupes d'animaux à intervalles de 10mins pendant 60mins. Après cela, les Sous-groupes 1A, 2A, 3A et 4A ont été administrés de 50μ/kg p.c. de carbachol (i.p.) par voie intra-péritonéale et des effluents ont été recueillis. Les animaux du sous-groupe B ont été utilisés pour une étude de mucosités gastriques. Le Carbachol stimule la sécrétion d'acide gastrique chez les animaux traités en avance avec l'extrait pendant 1, 7 et 14 jours. Des doses de 50-400 mg/kg p.c. de l'extrait augmentent considérablement la sécrétion des mucosités gastriques. Ces résultats montrent que le mécanisme de l'activité contre l'ulcère peut être dû à la stimulation de la sécrétion des mucosités gastriques.

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Introduction

We have shown that the aqueous extract of *Ocimum gratisssimum* leaf is highly potent in inhibiting indomethacin-induced gastric ulceration and inhibiting gastric acid secretion, and that both the histamine and cholinergic receptors may be involved in *Ocimum gratissimum* inhibition of gastric acid secretion (Ibironke *et al.*, 2001). However, its mechanisms involving other defensive factors have not been implicated.or understood in the peptic ulcer disease.

Various pathophysiological mechanisms have been suggested to explain the development of peptic ulcer. Prominent among these is imbalance between the protective/defensive factors (like mucus, blood flow and prostanglandin) and aggressive factors (like acid, pepsin, *Helicobacter pylori*) (Soll; 1980, Bjourne *et al.*, 1997). Drugs that are employed in the treatment of peptic ulceration are majorly designed to inhibit gastric acid secretion or increase gastric mucus secretion.

This study investigated the mechanism of the antiulcer effects of the ethanolic extract of *Ocimum gratissimum* in the albino rats.

Materials and methods

Extract preparation

The leaves of *Ocimum gratissimum* were collected and identified by Mr Olufemi Sasonya, Forest Research Institute of Nigeria (FRIN), Ibadan with voucher no: FHI 107847. The leaves were dried under shade for a period of 4 weeks during the harmattan (November - December) and were hand-crushed into powdered form. About 3kg of the powdered form of the sample was soaked in ethanol at room temperature in 5L of ethanol for 48hours, and the same process was repeated 3times. The extract was concentrated

by removing the solvent under reduced pressure in a rotatory evaporator at 40°C. The solid sample was stored in the refrigerator at 4°C until when needed. The extractive was prepared with different dilution of the extract in distilled water.

Animals

Male Wistar Albino rats weighing between 180-200g raised on commercial stock diet obtained from Ladokun feeds, Ibadan were used in the studies. The animals were purchased from the Pre-Clinical Animal House, Department of Physiology, College of Medicine, University of Ibadan, Nigeria.

Experimental design

Two studies were carried out and these include gastric acid secretion study and gastric mucus secretion study. Four groups of six albino rats were used for gastric acid secretion study. The rats in group 1 served as control. Animals in Group 2, 3 and 4 were pretreated with 200mg/kg of *O. gratissimum* for 1, 7 and 14 days respectively. Basal gastric effluents were collected from all the groups of animals at intervals of 10mins for 60mins. Thereafter, all animals were administered 50μg/kg of carbachol (i.p.) and gasric effluents were collected. Then, 5 groups of 6 albino rats each were also used for gastric mucus secretion. The rats in group 1 served as control. Groups 2, 3, 4 and 5 were treated with the extract at doses 50mg/kg, 100mg/kg, 200mg/kg and 400mg/kg respectively for 14 days. At the end of the experiment, the rats' stomachs were removed and washed gently in normal saline, blotted and weighed before gastric mucus secretion.

Gastric mucus secretory Study

This was done by using the procedure described by Mojizis *et al,* (2000) The stomach of the rats used for this study were removed after sacrificing and each glandular portion of the stomachs was excised and opened along the lesser. The everted stomachs were soaked for 2 hours in 0.10 Alcian blue dissolved in 0.16M sucrose buffered with 0.05M sodium acetate, adjusted to pH with hydrochloric acid. Uncomplexed dry was removed by two successive washes at 15 and 45 mins in 0.25M sucrose. Dye complexes with mucus were diluted by immersion in 10ml aliquot of magnesium chloride for 2 hours. The resulting blue solutions were shaken briefly with equal amount of diethyl ether and the absorbance of aqueous phases was measured at 605nm with spectrophotometer.

The absorbance of each solution was then used to calculate the various concentration of dye (expressed in mg) deduced from a standard curve. The weight of dye was dye was then expressed in (mg) deducted from a standard curve. The weight of dye was then expressed over the stomach.

Weight of dye (mg) = Gastric mucus (mg/g)

Gastric acid secretory study

This was done by the method described by Ghosh and Schild, (1958) as modified by Osim *et al(1992)* Briefly, rats were fasted 24hours preceeding the start of an experiment. the animals were anaesthetized with 6ml/kg of a 25% (w/v) solution of urethane given intramuscularly. The trachea was exposed and cannulated. A cannula was passed into the oesophagus via the mouth and tied firmly in place with a ligature around the oesophagus in the neck. The abdomen was opened through a midline incision along the *linea alba*. The pyloric end of the stomach was cannulated at its junction with the duodenum. Isotonic (0.9%) saline was introduced gently via the oesophagus cannula to wash out any stomach contents. When the stomach was cleared of food particles and the normal saline perfusion was flowing freely, the abdominal incision was covered with moist cotton wool. The stomach was continuously perfused with normal saline (pH 7.0) at approx. 37°C and flow rate adjusted to a flow rate of 1ml/min at a 10minutes interval using the Watson Marlow flow induce after collection, volumes of each sample was measured and titrated against 0.01M NaOH using phenolphthalein as indicator to determine total acidity.

Statistical analysis

Results were expressed as mean±S.E.M. Statistical analysis was using t-test and significant difference was accepted at $p<0.05$.

Result

Table 1: Mean gastric outputs in control and animals treated with *Ocimum gratissimum* for 1, 7, 14 days before and 60mins after intraperitonial administration of carbachol

Treatment	No of animals	Mean basal acid output (mEq/L/10min)	Mean stimulated acid output (mEq/L/10min	% change in mean acid output
Control	6	0.22±0.10	0.54 ±0.10**	145.5
1 Day	6	0.24±-0.03	0.53±0.02**	120.8
7 Days	6	0.32±0.01	0.50±0.04**	56.3
14 Days	6	0.27±0.01	0.42±0.04**	55.5

p<0.01**: Significantly different from basal acid secretion. Control animals were not given extract. Unit of gastric acid secretion is meq/litre/10mins after 60mins

Table 2: Mean gastric mucus secretion in control and animals treated with ethanol extract of *Ocimum gratissimum*

Group	Dose (mg/kg)	Mean gastric mucus secretion(mg g^{-1} tissue $x10^{-2}$)
Control	10ml/kg	3.20±0.02
Group 1	50mg/kg	8.00±0.264**
Group2	100mg/kg	7.20±0.015**
Group3	200mg/kg	5.39±0.254**
Group4	400mg/kg	5.09±0.322**

*$P<0.01$**: Significantly (S) different from control. Control animals were not given extract. Unit of gastric mucus secretion is mg g^{-1} tissue $x10^{-2}$)*

Discussion

In the present study, a significant increase in gastric acid secretion following stimulation by carbachol in animals pre-treated with the extract for 1, 7 and 14 days when compared with values obtained for basal acid secretion in the same animals was observed. The observed result shows increase in gastric acid released by carbachol despite pre-treatment of the animals with Ethanol extract of Ocimum gasrissimum (EEOG). This could suggest the possibility that the extract could not block the cholinergic receptors. Therefore, this present result could rule out the possibility that the anti-secretory effect of the extract earlier reported by Ibironke *et al.*, (2001) is via competing with cholinergic pathway of gastric acid secretion, although aqueous extract was administered in that experiment. In contrast, decrease in percentage change in gastric acid secretion with increasing days of pre-treatment of the animals with EEOG following stimulation by carbachol was observed and may indicate the presence of anti-cholinergic properties at prolong days of treatment.

The mean gastric mucus secretion in control animals was low as compared to rats treated with various and increasing doses of EEOG which showed significant increase in gastric mucus secretion ($p<0.05$) (Table 2). The methanolic extract of *Ocimum gratissimum* was earlier reported to protect against indomethacin and ethanol-induced ulcers in experimental animals (Akah *et al.*, 2007).

In this study, animals pre-treated with the extract produced significant amount of gastric mucus when compared with values for control animals ($p<0.05$) (Table 2). The results indicate that the anti-ulcer activity of the extract is associated with its ability to cause stimulation of gastric mucus secretion. This is in addition to the fact that the drug has gastric anti-secretory potentials. This present study suggested the active stimulation of the mucus layer of the stomach by ethanol extract of *Ocimum gratissimum*, which remains the main factor protecting the gastric mucosa.

The significant increase in gastric mucus secretion however was at variance with the drug-dose response fashion. It appears that the extract in its crude form may possess a constituent that decrease gastric mucus which is more feasible at low secretion, long duration of treatment with the extract initiates desensitization of gastric mucus cells involved in the gastric mucus secretion.

Substance that increases gastric mucus secretion and decreases acid secretion may be a desired drug in the management of peptric ulcer. Hence, Ocimum gratissimum may be a perfect herbal tea preparation in the treatment of peptic ulcer disease.

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