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Acid buffering effects of aqueous leaf extract of Ocimum gratissimum L. in the rabbit stomach

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Background/aims: *Although the gastroprotective properties of Ocimum gratissimum L. have been mentioned, the exact mechanism is yet to be explored. Since acid output plays a significant role in the pathogenesis of gastric ulceration, the present study was aimed at investigating the effect of leaf extract of Ocimum gratissimum on gastric luminal pH, acid output, parietal cell mass and gastric mucous cell population in rabbits.* ***Materials and Methods:*** *The model of pyloric ligation for acid secretion and ulcer study was employed. Prior to the 4 h ligation, male New Zealand rabbits were treated orally with 75 mg/kg, 150 mg/kg and 250 mg/kg b.w aqueous leaf extract of Ocimum gratissimum twice daily for three weeks. The antisecretory and antiulcer effect of Ocimum gratissimum was compared with omeprazole (20 mg/kg p.o). Parietal cell mass and gastric mucous cell population were determined in the gastric samples by histometry.* ***Results:*** *Aqueous leaf extract of Ocimum gratissimum caused significant reduction in ulcer formation, gastric secretion volume and acid output in a dose dependent manner ($p<0.05$). Percentage inhibition was recorded as 29%, 46.2%, 52.9% for ulcer; and 16.2%, 35.9%, 52.1% for acid output upon pretreatment with 75 mg/kg, 150 mg/kg and 250 mg/kg b.w respectively. Parietal cell mass was also reduced while gastric mucous cell population and luminal pH increased accordingly when compared to the control group. Data were comparable with the antisecretory effect of omeprazole.* ***Conclusion:*** *The results indicate that the anti-secretory activity of Ocimum gratissimum may be the anti-ulcer mechanism of this plant.*

Key words: Ocimum gratissimum, acid output, parietal cell mass, mucous cell population

Ocimum gratissimum L.'nin sıvı yaprak ekstresinin tavşan midesindeki asit tamponlayıcı etkileri

Giriş ve Amaç: *Ocimum gratissimum L.'nin gastroprotektif özellikleri bildirilmiş olsa da, kesin mekanizma henüz tanımlanmamıştır. Gastrik ülserasyonun patogenezinde asit salgısı belirgin bir rol oynadığı için, bu çalışmada Ocimum gratissimum L.'nin yaprak ekstresinin gastrik lüminal pH, asit salınımı, parietal hücre kütlesi ve gastrik mukus hücre popülasyonu üzerine olan etkilerinin değerlendirilmesi amaçlanmıştır.* ***Gereç ve Yöntem:*** *Asit sekresyonu için pilorik ligasyon ve ülser çalışması uygulanmıştır. 4 saatlik ligasyondan önce, erkek Yeni Zelanda tavşanları günde iki kez üç hafta boyunca 75 mg/kg, 150 mg/kg ve 250 mg/kg b.w Ocimum gratissimum sıvı yaprak ekstresiyle muamele edildi. Ocimum gratissimum'un anti-sekretuvar ve antiülser etkisi, 20 mg/kg p.o. omeprazolle karşılaştırıldı. Parietal hücre kütlesi ve gastrik mukus hücre popülasyonu mide örneklerinden histometrik olarak değerlendirildi.* ***Bulgular:*** *Ocimum gratissimum'un sıvılaştırılmış yaprak ekstresi, ülser oluşumu, gastrik salgı hacmi ve asit salgısını doz bağımlı olarak anlamlı şekilde azalttı ($p<0.05$). Ülser ve asit salgısı için azalma yüzdesi 75 mg/kg, 150 mg/kg ve 250 mg/kg b.w tedaviyle sırasıyla 29%, 46,2%, 52,9%; ve 16,2%, 35,9%, 52,1% oldu. Parietal hücre kütlesi de azalırken, gastrik mukus hücre popülasyonu ve luminal pH kontrol grubuna göre uygun şekilde arttı. Veriler çalışmada kullanılan standart ilaçla elde edilen antisekretuvar etkiyle karşılaştırılabilir düzeydeydi.* ***Sonuç:*** *Sonuçlara göre Ocimum gratissimum'un antisekretuvar etkisi, bitkinin anti-ülser mekanizması olabilir.*

Anahtar kelimeler: Ocimum gratissimum, asit salgısı, parietal hücre kütlesi, mukus hücre popülasyonu

INTRODUCTION

Ocimum gratissimum is a plant widely used in ethnomedicine throughout the tropical countries (1, 2). This shrubbery plant belongs to the family of Lamiaceae, and is popularly known as teabush or scent

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leaf because of its pleasant smell. In naturopathic medicine, it is used for a number of therapeutic purposes which are now being validated scientifically including anti-bacterial (3), antihelminthic and antifungal (4), antiprotozoal (5), hypoglycemic (6), anti-diarrheal (7), antimutagenic (8), insecticidal (9), and anticonvulsant activities (10). The plant is reported to contain terpenoids such as eugenol and thymol, saponins, and alkaloids which in no small measure have contributed to its multipurpose usage (11). Nakamura et al. (12) has also reported that the plant possesses antimicrobial properties, and it is used for the treatment of upper respiratory tract infections, headache, fever, ophthalmic conditions, skin diseases and pneumonia.

Gastroprotective effects of the leaf extract of *Ocimum gratissimum* have also been validated following the claim in folklore of its high efficacy against gastric ulceration (13). However, the role of acid output which plays a very significant role in the pathogenesis of gastrointestinal ulceration was ignored. In the intact animal, gastric acid secretion is influenced by the interplay of stimulatory and inhibitory factors arising from both the central nervous system and within the gastrointestinal system (14). In addition to these regulatory processes are factors arising from daily assaults on the gastrointestinal tract such as food and drugs. Hydrochloric acid, produced by the parietal cells in the stomach, is produced by the activity of H^+/K^+- ATPase in the apical membrane (15). It is finely regulated by overlapping neural, hormonal, paracrine pathways (16), and when levels of acid and proteolytic enzymes overwhelm the mucosal defense mechanisms, ulcers occur. This forms the basis of action for many anti-ulcerogenic drugs, and several lines of evidence provide support for the potential of gastric acid-inhibitory therapy in the management of gastroduodenal injury (17, 18). In this study we aimed to investigate the possible antisecretory mechanism of *Ocimum gratissimum*'s gastroprotective potential since there is a paucity of documented information on its effect on gastric secretion volume, pH, acid output, parietal cell mass and mucous cell population.

MATERIALS AND METHODS

Plant material

Fresh *Ocimum gratissimum* leaves were obtained between June and July 2011 from Okada village, Ovia North-East L.G.A of Edo state, identified and authenticated at the Department of Botany, University of Benin, Nigeria. They were air-dried at room temperature and pulverized into fine powder. The extraction was carried out using a Soxhlet extractor with water as solvent. The extract was then concentrated to approximately 10% of the original volume using a rotary evaporator (BUCHI, type RE111, Rotavapor).

Animals

Twenty-five male New Zealand rabbits weighing between 1.1-1.5 kg were used for this study. The animals were obtained from the Central Animal Facility of Igbinedion University, Okada, and then separated randomly into five large wooden cages of five rabbits each where they were kept for two weeks before the start of the experiment. The animals were housed under standard conditions of temperature (23±2°C); humidity (55±15%) and a 12-hour light cycle (7.00 am - 7.00 pm). The cages were routinely cleaned in order to prevent diseases. They were fed with standard commercial rat pellets (Ladokun Feeds Limited, Nigeria) and fasted for 48 hours except for water before the commencement of experiments. The Animal Care Committee of Igbinedion University, Okada approved the experimental protocols.

Experimental Design

Grouping

The animals were divided into five groups of five rabbits each.

- *Group one:* Animals were treated with normal saline. They served as the negative control group.
- *Group two:* Animals were pre-treated with aqueous leaf extract of *Ocimum gratissimum* (75 mg/kg b.w) for three weeks.
- *Group three:* Animals were pre-treated with aqueous leaf extract of *Ocimum gratissimum* (150 mg/kg b.w) for three weeks.
- *Group four:* Animals were pre-treated with aqueous leaf extract of *Ocimum gratissimum* (250 mg/kg b.w) for three weeks.
- *Group five:* Animals were treated with omeprazole (20 mg/kg b.w). They served as the positive control group.

The route of administration for both extract and reference drug was oral. Omeprazole was dissolved in normal saline. All the animals were sacrificed under sodium pentobarbitone anaesthesia.

Pylorus-ligated gastric secretion and ulceration

Oral administration of 75 mg/kg, 150 mg/kg and

250 mg/kg b.w of the leaf extract of *Ocimum gratissimum* and normal saline was done for three weeks. After treatments, all the animals were subjected to surgery under light ether anaesthesia for gastric secretion collection according to Olaleye et al. (19). Briefly, an abdominal incision through the *linea alba* was made to expose the stomach and a ligation was made at the junction of the pylorus with the duodenum. The 4 h gastric secretion collection was drained into a graduated test tube and centrifuged at 2000 rpm for 10 min. The supernatant volume and pH were recorded. The total acid content of the gastric secretions was also determined by titration to end point (pH 7.0) with 0.1 N NaOH, using phenolphthalein as an indicator. Ulcers formed in the glandular portion of the stomachs were scored using the method of Rao et al. (20) as follows: 0=no ulcers (normal stomach); 1=up to 5 petechial hemorrhages, 2=up to 5 petechial hemorrhages with erosions of depth 1 mm, 3=up to 10 petechial hemorrhages with erosions of depth 1 mm; 4=up to 10 petechial hemorrhages with erosion of depth above 1 mm. The index of ulceration was calculated as the total lesion lengths divided by the number in each group (21, 22). The percentage inhibition was calculated according to Ibironke et al. (23) as $(1 - It/Ic) \times 100$, where It is the ulcer index for the treatment group and Ic is the ulcer index for the untreated group.

Determination of parietal cell mass and mucous cell populations

The stomach was opened along the greater curvature, washed and transferred into a beaker containing 10% formalin. Sections were prepared from strips removed from the fundic area of the stomach and stained as explained by Oluwole et al. (24), using Hematoxylin and Eosin. The various gastric mucosal secretory cells were clearly differentiated under staining. The nuclei of the parietal cells were stained deep blue while the mucous cells were foamy and clearly vacuolated. Parietal cell mass index was calculated as described by Perraso et al. (25). The number of cells per mm^2 was multiplied by the thickness of the glandular layer. Five counts from randomly selected fields were made on each section and the average count per unit area was calculated for each stomach by dividing the number of cells seen by the number of counts made.

Statistical analysis

Data were analyzed by Microsoft Excel's XL Toolbox statistical package (2.60 version) using descriptive statistics, one-way ANOVA and the Bonferroni-Holm posthoc test with statistical significance at $p<0.05$ (26).

RESULTS

Effects of aqueous leaf extract of *Ocimum gratissimum* on pylorus-ligated acid secretion and ulceration

Pyloric ligation for 4 h resulted in the accumulation of gastric secretions and an increase in the titratable acidity. Administration of the leaf extract produced a significant dose-dependent decrease in the volume of gastric secretions, titratable acidity and ulcer index, while pH increased when compared to control (Table 1). Percent inhibition was 29%, 46,2%, 52,9% for ulcer; and 16,2%, 35,9%,

Table 1. Effects of *Ocimum gratissimum* on gastric juice volume, pH, acid output and ulcer index in pylorus ligated rabbit stomach.

Group	Treatment (mL/4 h)	Volume	pH ($X10^4$ mmol/4 h)	Acid output (mm)	Ulcer index
1	Normal saline + 4 h pyloric ligation	20,2±1.30	1,38±0,10	23,4±2,1	18,6±0,9
2	75 mg/kg OG + 4 h pyloric ligation	15±0,70*	1.9±0,10	19,6±1,70*	13,2±0,5*
3	150 mg/kg OG + 4 h pyloric ligation	11,8±0.85*	2,6±0,20*	15±1,10*	10±0,45**
4	250 mg/kg OG + 4 h pyloric ligation	9±0,30**	3.48±0,16**	11,2±0,80**	8,8±0,20**
5	Omeprazole (20 mg/kg) + 4 h pyloric ligation	8,4±0,20**	3,72±0,27**	10,2±0,70**	5,4±0,20**

*p<0.05, **p<0.001. Treated groups are significant when compared with control. (n=5). *OG: Ocimum gratissimum.*

52,1% for acid output upon pretreatment with 75 mg/kg, 150 mg/kg and 250 mg/kg b.w respectively (Figure 1). Also, administration of the extract (250 mg/kg) and omeprazole produced 152% and 170% increases in luminal pH respectively when compared with the normal saline treated controls.

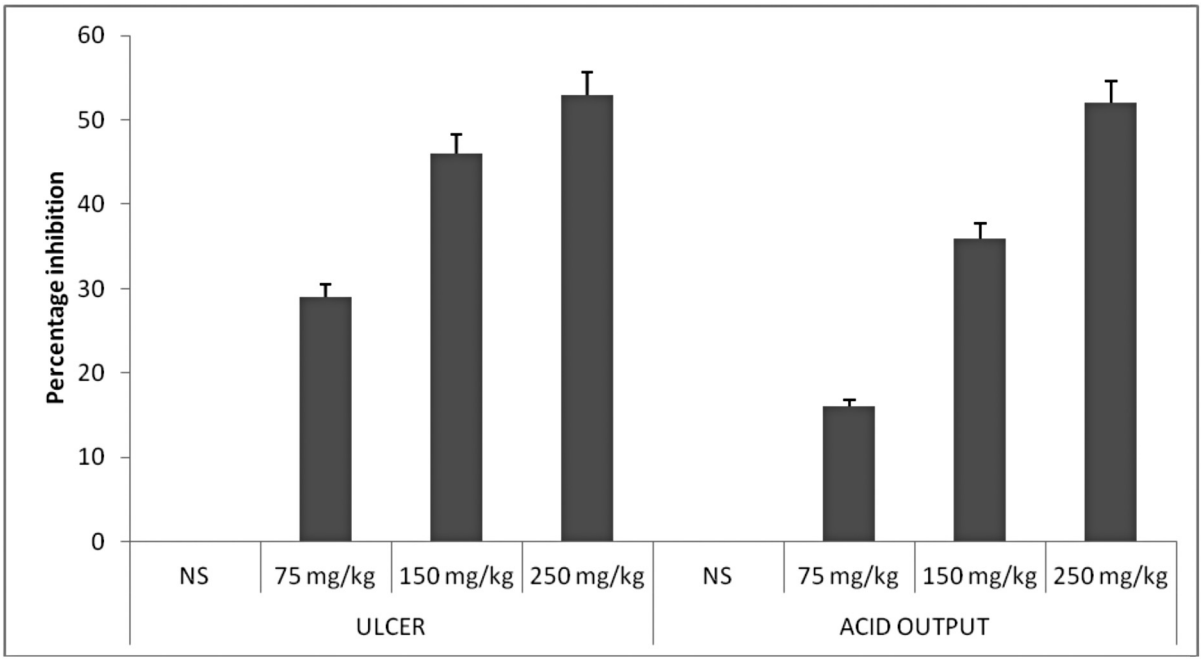

Figure 1. Percentage inhibition of ulcer formation and acid output by leaf extract of *Ocimum gratissimum* in the rabbit.

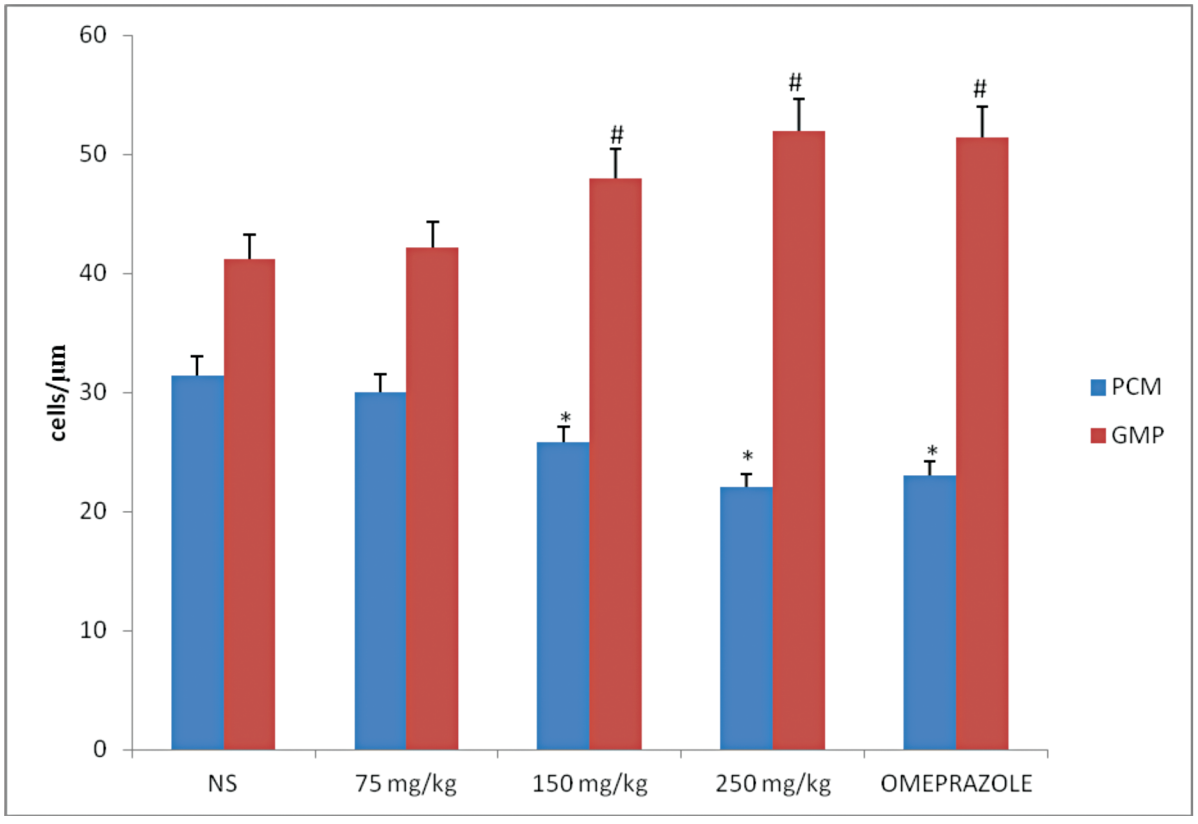

Figure 2. Effect of varying doses of *Ocimum gratissimum* and omeprazole on parietal cell mass (PCM) and gastric mucous cell population (GMP) in rabbit stomach. Each vertical bar represent Mean±SEM value of five microscopic views (*,# significant difference cf NS, $p<0.05$).

Effect of aqueous leaf extract of *Ocimum gratissimum* on parietal cell mass and gastric mucous cell population

Pretreatment of rabbits with leaf extract of *Ocimum gratissimum* caused a reduction in the parietal cell mass in a dose dependent fashion ($p<0.05$). The data was recorded as 30±1,2 cells/µm, 25,8±1,1 cells/µm and 22±1,2 cells/µm respectively versus 31,4±1,5 cells/µm control. On the other hand, gastric mucous cell population was enhanced with 42,2±2,0 cells/µm, 48±2,5 cells/µm, 52±3,1 cells/µm versus 41,2±1,8 cells/µm control. This is shown in Figure 2,3. Omeprazole increased gastric mucous cell population by 24,8% and reduced parietal cell mass by 27%.

DISCUSSION

Besides oxidative stress (27) and a number of other factors, gastric acid secretion still remains an important factor in the pathogenesis of inflammatory disorders of the gastrointestinal tract, especially peptic ulceration. This is evidenced by the action of

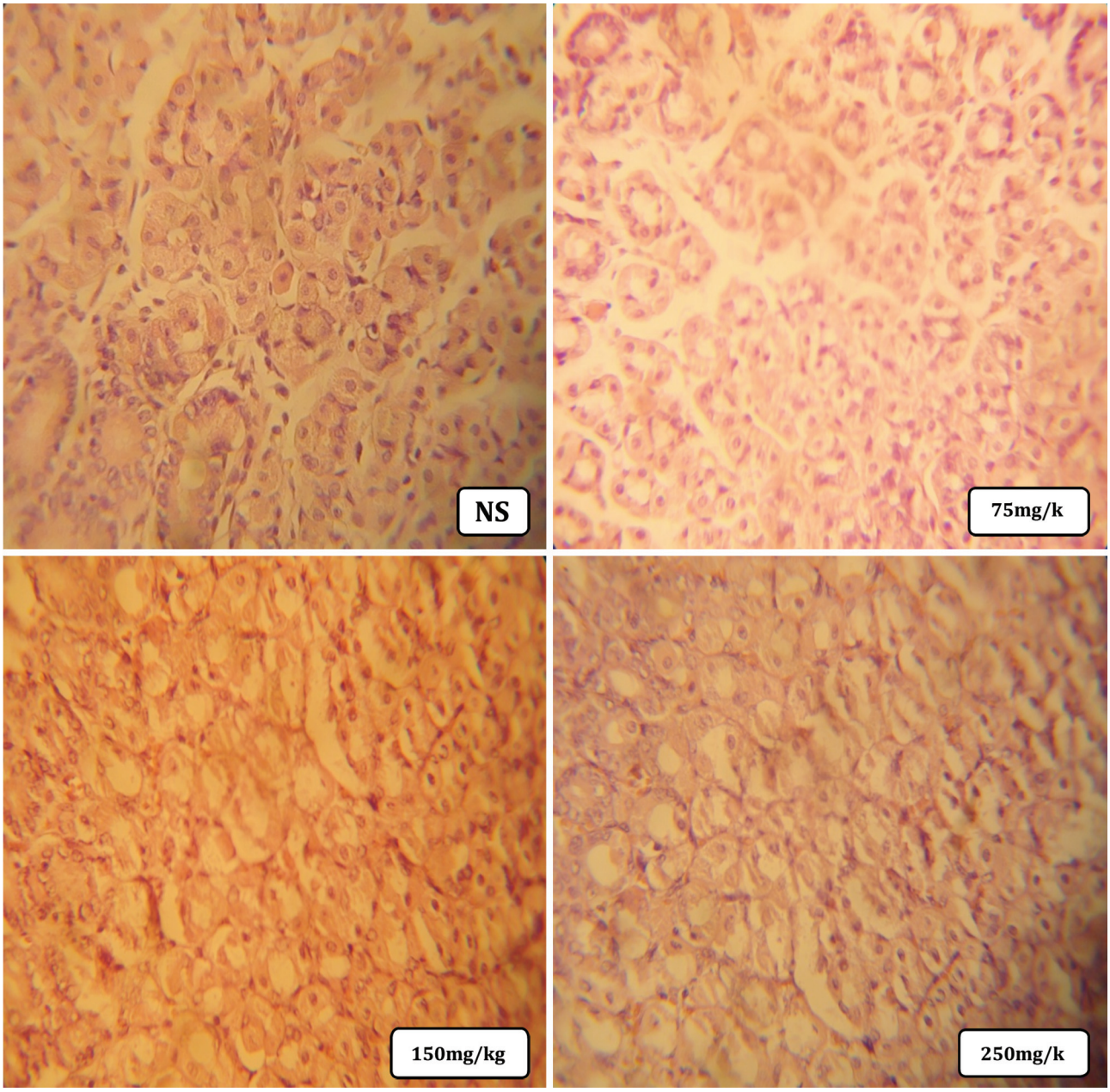

Figure 3. Photomicrographs of gastric mucosal sections from control rabbits (NS) and those treated with *Ocimum gratissimum* aqueous leaf extract showing relative densities of deep-blue-stained parietal cells.

many anti-ulcerogenic drugs which reduce acid secretion (28). For example, the proton pump enzyme is the target of proton pump inhibitors, used to increase gastric pH in diseases which feature excessive acid (17); H^2 antagonists indirectly decrease gastric acid production, and antacids neutralize existing acid. It has equally been shown that the anti-ulcer properties of some spices and herbs are mediated by antisecretory effects (18).

The results of the present study show that the aqueous extract of *Ocimum gratissimum* exhibits potent and dose-related anti-ulcerogenic activities via an antisecretory mechanism. This is observed by the increase in gastric luminal pH, reduced content and acid output.

Gastric acid is essential for the sterilization of food and water, and for digestion. Following its secretion there is a dramatic change in the morphology of the membranes of the parietal cell. Cytoplasmic tubulovesicular membranes which are abundant in the resting cell virtually disappear in concert with a large increase in the cannalicular membrane. The proton pump as well as potassium and chloride conductance channels which initially reside on intracellular membranes are transported to and fused into the cannalicular membrane just prior to acid secretion (29). The epithelium of the stomach is intrinsically resistant to the damaging effects of gastric acid and other insults. Nonetheless, excessive secretion of gastric acid is a major problem in humans, leading to gastritis, gastric ulcers and peptic acid disease. As a consequence, the parietal cell and the mechanisms it uses to secrete acid have been studied extensively, leading to development of several drugs useful for suppressing acid secretion.

The capacity of the stomach to secrete acid is almost linearly related to the number of parietal cells, and its deleterious effect on the mucosa known to be inversely related with the mucous cell population (30). In our study, we observed that aqueous extract of *Ocimum gratissimum* reduced the parietal cell mass, resulting in acid output inhibition. The plant also showed gastroprotective effects due to the increased mucous cell population observed, lending much credence to the early work on the plant's beneficial activities (13).

Further studies are ongoing in our laboratory to explore the gastroprotective tendencies of the plant. The results of the present study show that aqueous extract of *Ocimum gratissimum* reduces ulcer formation via antisecretory mechanisms.

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