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Effect of crude methanol leaf extract of *Combretum racemosum* on histamine-stimulated gastric secretion in rats

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ABSTRACT

Objective: To investigate the effect of crude methanolic extract of *Combretum racemosum* (*C. racemosum*) leaves on histamine-stimulated gastric secretion in rats.
Methods: Phytochemical and acute toxicity tests were performed. Anti-secretory activity of *C. racemosum* extract was investigated in pyloric ligated rats administered histamine. Gastric juice was collected from all the animals and the volume, titratable acidity, pH and mucus content were measured. The effect of *C. racemosum* extract on calcium chloride induced contractions of the guinea-pig ileum suspended in high potassium, calcium-deficient depolarizing solution was investigated. The H_2 receptor antagonistic potency was also evaluated using the isolated non-gravid rat uterus.
Results: Phytochemistry revealed the presence of abundant amounts of saponins and moderate amounts of glycosides, terpenoids, proteins, reducing sugar, resins, alkaloids, flavonoids and carbohydrates. The oral LD_{50} of the extract was greater than 8 000 mg/kg body weight in mice. Pretreatment of pyloric ligated rats with *C. racemosum* prior to histamine administration significantly reduced ($P < 0.001$) the volume of gastric juice and titratable acidity, and significantly increased ($P < 0.001$) gastric pH and gastric mucus when compared to the negative control. Both doses of *C. racemosum* protected rats significantly ($P < 0.001$) from histamine-induced ulceration. *C. racemosum* potently inhibited contractions evoked by calcium chloride in a dose-dependent and reversible manner with an IC_{50} of 1 132 µg/mL. It also antagonized the relaxant effect of histamine on the isolated rat uterus in a manner comparable to cimetidine.
Conclusions: The leaves of *C. racemosum* possess gastric anti-secretory and anti-ulcer effects and justify its use in traditional medicine in South-East Nigeria for the treatment of peptic ulcer disease.

1. Introduction

Peptic ulcer disease (PUD) is widespread and is a common clinical ailment. It is estimated that one out of every 10 persons will develop an ulcer at some time[1]. PUD is believed to be due to an imbalance between destructive and defensive factors in the gastrointestinal tract[2]. The gastric mucosa is continuously exposed to conceivably damaging agents such as acid, pepsin, bile acids and drugs. The goals of ulcer treatment are to relieve pain, heal the ulcer, and prevent regularity. The high prevalence in peptic ulcer disease and related gastrointestinal complaints created enormous interest in the therapeutic potential of H_2 receptor antagonists (H_2RAs). The development of this kind of drugs increases our understanding of gastric physiology and the pathophysiology of acid related disorders. Histamine has been completely implicated in ulcer pathogenesis[3]. H_2RAs are reversible, competitive antagonists of the actions of histamine on H_2 receptors thereby decreasing the production of acid from the parietal cell[1].

However, the attention of anti-secretory therapy has moved away from H_2RAs to proton pump inhibitors (PPIs) which are potent acid suppressing agents[4]. PPIs include drugs like omeprazole, rabeprazole and pantoprazole. These drugs are acid-dependent prodrugs. These active inhibitors prevent acid secretion by

irreversibly binding to the sulphydryl group of cysteine from the extracellular domain of the H^+, K^+-adenosine triphosphatase, the energy source of the proton pump and the final intermediary of gastric acid secretion by the parietal cell[5]. Despite the success recorded with the use of H_2RAs and PPIs, treatment of PUD was fraught with high incidence of recurrence. Moreover, these anti-secretory agents are not devoid of side effects[6,7]. Also, drugs with imidazole nucleus may cause significant drug interaction by inhibition of hepatic microsomal enzyme-cytochrome P450. Cimetidine is an example of a drug with such nucleus. PPIs are substituted with benzimidazoles and consequently also have the potential to interact with drugs by this mechanism. Pharmaco-economic considerations and unbearable side effects of the conventional anti-ulcer drugs lead to poor compliance and consequential treatment failures. For these reasons, efforts are being made to find suitable treatment from natural products. An appreciable percentage of the world population relies on natural products to treat a variety of illnesses. The cost-effectiveness of these natural medicaments as well as faith and ancestral experience could account for the interest in natural remedies. Moreover, a number of plants have been scientifically validated to possess anti-ulcer effects[2,8-16].

Combretum racemosum (C. racemosum) is a climbing shrub and its leaves are used in traditional medicine by inhabitants of the eastern parts of Nigeria for the treatment of ulcers, diarrhoea, sexually transmitted diseases, cholera and menorrhagia. The anti-ulcer effects of C. racemosum in various experimental ulcer models have been evaluated in rats[15]. The present study was, therefore, undertaken to evaluate the anti-secretory effect of the crude methanol extract (CME) of C. racemosum against histamine-induced gastric secretion in rats.

2. Materials and methods

2.1. Plant collection and taxonomy

The plant was collected from Ire village, Ogidi in Idemili North local government area of Anambra State in the month of August, 2014. The plant was identified by a taxonomist at the Department of Plant Science and Biotechnology, University of Nigeria, Enugu, Nigeria. A specimen of the plant was deposited at the herbarium of the same department for future reference (UNH/47b). The leaves of C. racemosum were dried under the shade to a constant weight and powdered using a mill grater III (model MS 223, Taiwan).

2.2. Plant material extraction

The pulverized leaves (5kg) were macerated with methylene chloride: methanol (1:1) for 48 h. The filtrate was concentrated using rotary evaporator (model type 349/2 Corning Ltd, England) under reduced pressure to get the CME. The yield of the methanol extract was 14.5% (w/w). The solid residue obtained was stored at (4 ± 2) °C. The residue (20 g) was dissolved in physiological saline and made up to 100 mL with the same solvent. From this, appropriate dilutions were made for the pharmacological screenings.

2.3. Drugs and chemicals

Histamine was obtained from Sigma Chemical Co., USA, stilboestrol was obtained from Bristol Pharmaceuticals, United Kingdom and cimetidine was obtained from Taj Pharma, India.

2.4. Experimental animals and maintenance

Albino Wistar rats (180–200 g) and albino mice (28–39 g) and a rabbit (1 kg) were obtained from the Animal House of the Faculty of Veterinary Medicine, University of Nigeria, Enugu, Nigeria. Guinea-pigs (480–550 g) were obtained from the Animal House of the College of Medicine, University of Nigeria, Enugu Campus. These animals were housed in clean and steel cages at the Animal House of the College of Medicine, University of Nigeria under standard conditions [temperature of (25 ± 4) °C and light/dark periodicity of 12/12 h]. The rats, mice, and rabbit were fed with standard chow (Guinea feed® Nigeria Plc.) while the guinea-pigs were fed with local grass *Panicum maximum* L. Clean water was given to all the animals *ad libitum* and the period of acclimatization was 2 weeks. All animals were handled in this study according to institutional and international guidelines for experiments involving the use of animals[17].

2.5. Phytochemical screening

Preliminary phytochemical screening for the presence of glycosides, flavonoids, saponins, steroids, tannins, carbohydrates, proteins and terpenoids was carried out. Procedures outlined by Trease and Evans were employed for the analyses[18].

2.6. Whole animal experiments

2.6.1. Acute toxicity test (LD_{50})
The method described by Lorke was used for the determination of the oral LD_{50} using adult Albino mice (weighing 20–25 g)[19]. Different doses of the extract were administered to respective groups of mice after an overnight fast. All the animals were observed for 24 h and mortality and/or other signs of toxicity were noted.

2.6.2. Anti-secretory activity
To determine anti-secretory activity, 24 rats were grouped into four groups [A to D (n = 6)]. The rats were fasted for 36 h prior to the test but had access to portable water which was, however, withdrawn 1 h before the experiment. At 30 min before surgery, the rats in Groups B and C received 200 and 400 mg/kg CME respectively, while those in D and A received 100 mg/kg cimetidine, and 5 mL/kg saline respectively through the oral route. The concentration of stock CME (200 mg/mL) was made in such a way that the highest volume of extract administered was not more than 0.38 mL. The

fasted rats for 36 h were anesthetized 30 min later under diazepam/ketamine hydrochloride and the abdomen was cut open, the stomach was brought out and the pylorus was ligated. Care was taken to avoid bleeding or occlusion of the blood vessels. The incisions were carefully sutured with a silk thread immediately after pyloric ligation[20]. Histamine (2 mg/kg) was administered intra-peritoneally 30 min after pyloric ligation. The animals were sacrificed at 4 h after the pyloric ligation. The stomachs were removed and the contents were collected, measured, centrifuged, and subjected to the analysis of pH and titratable acidity against 0.01 mol/L NaOH at pH 7 with neutral red indicator[21]. The pH was measured using a digital pH meter (Consort P 107, Belgium) since gastric juice with pH > 7.0 did not need to be titrated.

2.6.3. Determination of anti-ulcer effect of the CME in pyloric ligated rats

The stomach was cut open along the greater curvature, rinsed with normal saline and pinned on a cork board for observation. Erosions formed in the animals were counted and each given a severity rating on a one to three scale based on the diameter of the ulcer and the ulcer index (UI) was calculated. The ulcers were viewed with the aid of a magnifying lens (×10). The severity rating was as follows:

$\leqslant$ 1 mm = 1

> 1 mm and $\leqslant$ 2 mm = 2

> 2 mm = 3

The overall total was divided by a factor of 10 to derive the UI for each animal[22].

$$\text{Ulcer protection (\%)} = \frac{(\text{UI}_{\text{control}} - \text{UI}_{\text{test}})}{\text{UI}_{\text{control}}} \times 100$$

2.6.4. Determination of the effect of crude methanolic extract of C. racemosum on gastric mucus of pyloric-ligated rats

The glandular segments of the stomach were removed and weighed using the method of Corne *et al.*[23]. Each segment was transferred to 1% alcian blue solution in 10% sucrose. Glandular mucus was allowed to complex with alcian blue for 30 min. The excess dye of each segment was removed by rinsing with sucrose solution. The complexed dye was extracted in 5 mL of 5% magnesium chloride solution which was then shaken with equal volume of diethyl ether. The resulting emulsion was centrifuged at 4000 r/min for 15 min and absorbencies of the aqueous layers were measured at 580 nm. The quantity of alcian blue extracted per gram of glandular tissue was then calculated from a standard curve of alcian blue.

2.7. In vitro pharmacological experiments

2.7.1. Effect of the methanol extracts on Ca^{2+} channels

The effect of the crude methanolic extracts on calcium chloride induced contraction of the guinea-pig ileum suspended in high potassium and calcium-deficient depolarizing solution was investigated to ascertain their effect on intracellular calcium ion mobilization[24,25]. A guinea-pig was starved of food for 24 h but allowed access to clean water. The animal was euthanized and the abdomen was cut open. The ileum was excised. The experiments

were set up as described previously[26]. Segment of the ileum of about 2 cm long was suspended in an 30 mL aerated organ bath filled with a high potassium ion, calcium ion-free depolarizing solution of the following composition: NaCl of 1.58 g/L, $NaHCO_3$ of 1.26 g/L, KCl of 7.46 g/L, $MgCl_2.6H_2O$ of 0.25 g/L, and glucose of 1.98 g/L. The tissue was allowed to equilibrate for 60 min. During this time, the tissue was constantly washed with the depolarizing solution to remove intracellular and extracellular calcium ions. Two concentration-response curves for $CaCl_2$ (80 µg) was obtained using 30 s contact time. A third curve was then established in the presence of verapamil (0.50 µg/mL) added 1 min earlier. In the same manner, the effect of different doses of CME (160 µg–5.12 mg) on calcium chloride induced contractions was recorded. The procedure was carried out in triplicate.

2.7.2. H_2 receptor antagonistic activity

A multi-parous rat was used for this procedure. The non-gravid rat uteri were stimulated to be oestrous by pre-treating the animal with 0.1 mg/kg diethyl stilboesterol in arachis oil sub-cutaneously for 24 h before the experiment. The rat was euthanized, the abdomen was cut open and the uterus was obtained. The uterus was cut into smaller longitudinal pieces of about 2.0 cm and aerated in a shallow dish containing De-Jalon's solution. The tissue was set up using standard methods[26]. The effect of histamine (20 µg) and CME (640 and 1280 µg) on spontaneous uterine contractions was recorded. The effect of cimetidine (1 mg) on histamine induced relaxation of the rat uterus was recorded. Also, the effect of CME on histamine induced relaxation of the uterus was determined. The contractions were recorded using a writing lever connected to a kymograph and stimulator (Bioscience, Sheerness, Kent). This test was carried out three times.

2.8. Statistical analysis

Results were expressed as mean ± SD. The differences between the means were determined with the Student's *t*-test and results considered significant at $P < 0.05, 0.01, 0.001$.

3. Results

Preliminary analysis for active metabolites revealed the presence of abundant amounts of saponins and moderate amounts of glycosides, terpenoids, proteins, reducing sugars, resins, alkaloids, flavonoids, carbohydrates, and trace amounts of steroids. The oral LD_{50} of the extract was greater than 8000 mg/kg body weight in mice.

The results of the effects of the CME on gastric secretion were presented in Table 1. Pretreatment of pyloric ligated rats with methanol extracts of *C. racemosum* (200 and 400 mg/kg body weight) prior to histamine administration significantly reduced the volume of gastric juice, titratable acidity, and increased the pH ($P < 0.001$). These effects were comparable to those of the reference drug cimetidine. The CME also significantly increased gastric mucous content ($P < 0.001$) in pyloric-ligated rats with values of (298.50 ±

0.90) μg and (386.82 ± 1.70) μg alcian blue/g wet tissue for 200 and 400 mg/kg CME respectively when compared with negative control [(186.09 ± 0.64) μg alcian blue/g wet tissue]. Both doses of CME protected rats significantly ($P < 0.001$) with ulcer protective rates of 55.92% and 83.62% respectively when compared to the negative control (Table 2). The reference drug (cimetidine) protected rats significantly with percentage ulcer protective value of 90.42%.

Table 1

Effect of the crude methanolic extract of *C. racemosum* on histamine-induced gastric secretion in rats.

Groups	Dose (mg/kg)	Gastric juice (mL)	Titratable acidity (mmol/L)	Gastric mucus (μg alcian blue/g wet tissue)	Gastric pH
A (0.9% saline, mL/kg)	5	7.57 ± 0.32	220.67 ± 9.35	186.09 ± 0.64	2.42 ± 0.24
B (CME)	200	4.06 ± 0.70*	138.50 ± 11.82*	298.50 ± 0.90*	3.75 ± 0.15
C (CME)	400	2.18 ± 0.42*	88.45 ± 3.91*	386.82 ± 1.70*	4.21 ± 0.10*
D (cimetidine)	100	2.05 ± 0.47	40.54 ± 6.28	400.00 ± 0.80*	4.50 ± 0.27*

*: $P < 0.001$ compared with the negative control.

Table 2

Effect of *C. racemosum* on histamine-induced gastric ulcers in pyloric ligated rats.

Groups	UI	Ulcer protection (%)
A (5 mL/kg saline)	19.42 ± 0.84	-
B (CME of 200 mg/kg)	8.56 ± 0.41	55.92*
C (CME of 400 mg/kg)	3.18 ± 0.15	83.62*
D (cimetidine of 100 mg/kg)	1.86 ± 0.24	90.42*

*: $P < 0.001$ compared with the negative control.

CME potently inhibited contractions induced by calcium chloride on guinea-pig ileum exposed to high potassium ion, Ca^{2+}-deficient depolarizing solution in a dose dependent and reversible manner. The CME had an IC_{50} value of 1 132 μg/mL. Verapamil (0.5 μg/mL) completely abolished the contractions produced by calcium chloride (Table 3). The extract also antagonized the relaxant effect of histamine on the isolated rat uterus. This antagonism was moderately dose-dependent. Cimetidine (1 mg/mL) abolished the relaxant effect of the same dose of histamine completely (Figure 1).

Table 3

Effect of the methanol extracts of *C. racemosum* on calcium chloride (80 μg)-induced contractions of the guinea-pig ileum.

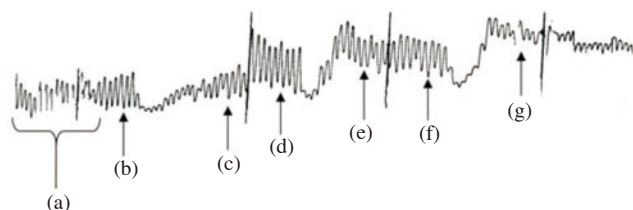| Doses of CME (μg)	% Inhibition of maximal response (mean ± SEM)
160	11.54 ± 1.07
320	19.23 ± 1.41
640	26.92 ± 1.67
1 280	57.69 ± 1.25
2 560	65.38 ± 1.52
5 120	88.46 ± 1.87
Verapamil (0.5 μg)	100

$IC_{50} = 1 132$ μg; $r^2 = 0.96$

Figure 1. The effect of CME of *C. racemosum* on histamine-induced relaxation of the rat uterus.
a: Spontaneous contractions of the uterus; b: 20 μg histamine; c: 20 μg histamine + 640 μg CME; d: 20 μg histamine; e: 20 μg histamine + 1.28 mg CME; f: 20 μg histamine; g: 20 μg histamine + 1 mg cimetidine.

4. Discussion

The extracts of *C. racemosum* are used in folklore for the treatment of ulcers, cholera, and uterine bleeding. Okwuosa *et al.* demonstrated that the extracts of the plant were protective against ulceration induced by stress, indomethacin, and histamine in rats[15]. In the present study, the methanol extracts significantly protected pyloric ligated rats from histamine-induced ulcers. The CME also reduced volume of gastric juice, gastric acid output and increased gastric pH and mucus content in the histamine stimulated pyloric-ligation model. The leaves of *C. racemosum* contain abundant amounts of saponins and moderate amounts of glycosides, terpenoids, proteins, reducing sugars, resins, alkaloids, flavonoids, carbohydrates, and trace amounts of steroids. One or two of these active principles could be responsible for the anti-secretory and anti-ulcer effects.

Acetylcholine and histamine have been implicated in ulcer pathogenesis[27]. Endocrine and paracrine cells in the oxyntic glands express receptors for both muscarinic agonists and gastrin and they secrete histamine in response to activation of these receptors[28]. Histamine is a powerful gastric secretagogue and evokes a profuse secretion of acid from parietal cells by acting on H_2 receptors[29]. Gastrin and acetylcholine stimulate the parietal cells to produce acid by stimulating Ca^{2+} dependent signaling pathway[30]. The inhibitory activity of the methanol extracts of *C. racemosum* on contractions induced by calcium chloride in high K^+, Ca^{2+}-deficient depolarized tissue indicates inhibition of calcium mobilization into the intracellular compartment by blockade of voltage-gated calcium channels. High K^+ depolarizes the membrane, opens the voltage-dependent Ca^{2+} channels, increases Ca^{2+} influx which elicits sustained contraction[31]. Additionally, inhibition of Ca^{2+}-evoked contractions of K^+ depolarized tissues is commonly accepted as a test for agents that act non-specifically by inhibiting calcium participation in the excitation-contraction coupling process[24]. Verapamil has been reported to inhibit gastric acid secretion and thereby offers protection against induced ulceration[32]. This shows the role of calcium mobilization in the control of gastric acidity and may explain, in part, the gastric anti-secretory potency of the methanol extract.

Furthermore, classical H_2 receptor antagonists are used as therapy for peptic ulcer. Consequently, agents such as cimetidine inhibit acid secretion by H_2 receptor blockade[33]. Since histamine induces relaxation of the non-gravid uterus by H_2 histaminergic receptor stimulation[34]. An *in vitro* model of inhibiting histamine induced relaxation of uterine smooth muscle was adopted as a measure of H_2 receptor antagonistic potency. The methanol extracts of *C. racemosum* antagonized the histamine-induced uterine relaxation indicating H_2 receptor antagonistic potency and such agents are useful in the management of peptic ulcers.

In conclusion, the crude methanolic extract of *C. racemosum* possesses gastric anti-secretory activities due to possibly inhibition of calcium mobilization and H_2 receptor antagonistic potency which may be due to single or combined effect(s) of

the phytochemical constituents. Further research is going on in our laboratory using bioassay guided phytochemical and pharmacological studies in order to characterize the substance(s) responsible for the observed effects of the extract.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Snowden FM. Emerging and reemerging diseases: a historical perspective. *Immunol Rev* 2008; **225**(1): 9-26.

[2] Akah PA, Orisakwe OE, Gamaniel KS, Shittu A. Evaluation of Nigerian traditional medicines: II. Effects of some Nigerian folk remedies on peptic ulcer. *J Ethnopharmacol* 1998; **62**: 123-7.

[3] Abdul-Aziz, KK. Molecular pathogenesis of gastric ulcer's diseases and strategies for prevention. *Webmedcentral* 2011; **2**(5): 1-24.

[4] Zed PJ, Loewen PS, Slavik RS, Marra CA. Meta-analysis of proton pump inhibitors in treatment of bleeding peptic ulcers. *Ann Pharmacother* 2001; **35**: 1528-34.

[5] Sachs G, Shin JM, Briving C, Wallmark B, Hersey S. The pharmacology of the gastric acid pump: the H^+, K^+ ATPase. *Annu Rev Pharmacol Toxicol* 1995; **35**: 277-305.

[6] Collin-Jones DG. Safety of lansoprazole. *Aliment Pharmacol Ther* 1993; **7**(suppl 1): 56-66.

[7] Zlabek JA, Anderson CG. Lansoprazole-induced thrombocytopenia. *Ann Pharmacother* 2002; **36**(5): 809-11.

[8] Aguwa CN, Mittal GC. Study of antiulcer activity of aqueous extract of leaves of *Pyrenacantha standtii* (Family I cacinaceae) using various models of experimental gastric ulcer in rats. *Eur J Pharmacol* 1981; **74**: 215-9.

[9] Balogun ME, Nwachukwu DC, Salami SA, Besong EE, Obu DC Djobissie SFA. Assessment of anti-ulcer efficacy of stem bark extract of *Nauclea latifolia* (African peach) in rats. *Am J Biomed Res* 2016; **4**: 13-7.

[10] Zakaria ZA, Abdul Hisam EE, Norhafizah M, Rofiee MS, Othman F, Hasiah AH, et al. Methanol extract of *Bauhinia purpurea* leaf possesses anti-ulcer activity. *Med Princ Pract* 2012; **21**: 476-82.

[11] Noamesi BK, Mensah JF, Bogale M, Dagne E, Adotey J. Antiulcerative properties and acute toxicity profile of some African medicinal plant extracts. *J Ethnopharmacol* 1994; **42**: 13-8.

[12] De Pasquale R, Germano MP, Keita A, Sanogo R, Lauk L. Antiulcer activity of *Pteleopsis suberosa*. *J Ethnopharmacol* 1995; **47**: 55-8.

[13] Akah PA, Nwafor SV. Studies on anti-ulcer properties of *Cissampelos mucronata* leaf extract. *Indian J Exp Biol* 1998; **37**: 936-8.

[14] Al-Mofleh IA, Alhaider AA, Mossa JS, Al Sohaibani MD, Qureshi S, Rafatullah S. Pharmacological studies on "clove" *Eugenia caryophyllata*. *Pharmacogn Mag* 2005; **1**: 1055-9.

[15] Okwuosa C, Unekwe P, Nwobodo E, Chilaka K. The anti-ulcer activities of leaf extracts of *Combretum racemosum* (Family: combracaceae). *J Biomed Investig* 2006; **4**(1): 9-14.

[16] Al Mofleh IA, Alhaider AA, Mossa JS, Al-Sohaibani MO, Al-Yahya MA, Rafatullah S, et al. Gastroprotective effect of an aqueous suspension of black cumin *Nigella sativa* on necrotizing agents-induced gastric injury in experimental animals. *Saudi J Gastroenterol* 2008; **14**(3): 128-34.

[17] World Medical Association; American Physiological Society. Guiding principles for research involving animals and human beings. *Am J Physiol Regul Integr Comp Physiol* 2002; **283**: R281-3.

[18] Trease GE, Evans WC. *Pharmacognosy*. 13th ed. Philadelphia: Bailliere Tindall; 1989.

[19] Lorke D. A new approach to practical acute toxicity testing. *Arch Toxicol* 1983; **54**: 275-87.

[20] Shay H, Komarov SA, Fels SS, Meranza D, Grunstein M, Siplet H. A simple method for assessing the extent of experimental erosion and ulcers. *Gastroenterology* 1945; **5**: 43-61

[21] Ochei J, Kolhatkar A. *Medical laboratory science: theory and practice*. 2nd ed. New Delhi: Tata Mcgraw-Hill Publishing Company Limited; 2000; p. 331-49.

[22] Main IH, Whittle BJ. Investigation of the vasodilator and antisecretory role of prostaglandins in the rat gastric mucosa by the use of non-steroidal anti-inflammatory drugs. *Br J Pharmacol* 1975; **53**(2): 217-24.

[23] Corne SJ, Morrissey SM, Woods RJ. Proceedings: a method for the quantitative estimation of gastric barrier mucus. *J Physiol* 1974; **242**(2): 116P-7P.

[24] Northover BJ. Indomethacin-a calcium antagonist. *Gen Pharmacol* 1977; **8**: 293-6.

[25] Quitana A. Effects of pimozide on the response of smooth muscle to non-dopamine agonists and calcium. *Eur J Pharmacol* 1978; **53**: 113-6.

[26] *Pharmacological experiment on isolated preparations*. 2nd ed. New York: Churchill Livingstone; 1971, p. 58-79.

[27] Cross LB, Justice LN. Combination drug therapy for gastroesophageal reflux disease. *Ann Pharmacother* 2002; **36**(5): 912-6.

[28] Wollin A. Regulation of gastric acid secretion at the cellular level. *Clin Invest Med* 1987; **10**(3): 209-14.

[29] Aihara T, Nakamura E, Amagase K, Tomita K, Fujishita T, Furutani K, et al. Pharmacological control of gastric acid secretion for the treatment of acid-related peptic disease: past, present, and future. *Pharm Ther* 2003; **98**: 109-27.

[30] Yao X, Forte JG. Cell biology of acid secretion by the parietal cell. *Annu Rev Physiol* 2003; **65**: 103-31

[31] Karaki H, Ozaki H, Hori M, Mitsui-Saito M, Amano K, Harada K, et al. Calcium movements, distribution, and functions in smooth muscle. *Pharmacol Rev* 1997; **49**(2): 157-230.

[32] Jain SM, Parmar NS, Santani DD. Gastric anti-ulcer activity of calcium channel blockers in rats. *Indian J Pharmacol* 1994; **26**: 29-34

[33] Feldman M, Burton ME. Histamine$_2$ receptor antagonist. Standard therapy for acid peptic diseases. 1. *N Engl J Med* 1990; **323**: 1672-80.

[34] Szeląg, Merwid-Lad A, Trocha M. Histamine receptors in the female reproductive system. Part II. The role of histamine in the placenta, histamine receptors and the uterus contractility. *Ginekol Pol* 2002; **73**(7): 636-44.