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Menthol Ameliorates Secretagogue-Induced Gastric Acid Secretion in the Rat

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Abstract

Gastric ulcer, diarrhoea, irritable bowel syndrome (IBS) and constipation are some of the prevalent gastrointestinal diseases in global health problems today. The present work aimed to evaluate the gastric acid secretion effects of (-)-Menthol (ME). Male Wistar rats (n=6) weighing 200-230 g were used in all bioassays. Rats were orally treated with menthol (20, 40 and 60 mg/kg). After one hour, the rats were anaesthetized, cannulated and infused with either pentagastrin, carbachol or histamine. Gastric acid secretion (GAS) was assessed by continuous perfusion and its acidity determined by titration. Data were express as Mean± SEM. Student's t-test and one-way ANOVA were used to determine levels of significance ($p<0.05$) with the aid of Graphpad Prism 5. There were significant decreases in pH over time (min) in all the menthol treated groups with the pentagastrin, carbacol and histamine stimulated GAS (mEq/l) compared to the basal normal saline groups.

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Keywords

Menthol, gastric acid, gastric ulcer, titration.

Introduction

The gastric mucosa is continuously exposed to damaging agents that are either endogenous such as hydrochloric acid, pepsin, refluxed bile, and reactive oxygen species, or exogenous such as alcohol consumption, excessive coffee ingestion, and administration of non-steroidal anti-inflammatory drugs (Rozza *et al.*, 2013). The basic physiopathology of gastric ulcers results from an imbalance between damaging factors and cytoprotective factors, which include an intact mucus barrier, prostaglandins, adequate mucosal blood flow, activity of antioxidant compounds, and other mediators, although the exact ulcer etiology is complex and multifactorial (Laine *et al.*, 2008). Menthol is a naturally occurring cyclic terpene alcohol of plant origin with a residual minty smell used since antiquity for medicinal purposes such in topical anti-pruritic, antiseptic, analgesic, and cooling formulations (Tejesh *et al.*, 2007). It is also one of the most important flavouring additives used in a variety of consumer products ranging from confections such as chocolate and chewing gum to oral-care products such as toothpaste as well as in over-the-counter medicinal products for its cooling effects. Its cooling effects are not exclusive to medicinal use (Guy *et al.*, 2013). Menthol, a key active component of the peppermint oil has been used for a variety of health conditions such as nausea, indigestion, cold symptoms, headaches, muscle and nerve pain, and irritable bowel syndrome. Peppermint has been described as an excellent remedy for chills, colic, fevers, dizziness, flatulence, nausea, vomiting, diarrhoea, dysentery and heart trouble (Blumenthal, 1998). In another study, the gastroprotective effect of the cyclic terpene (-)-menthol

has been demonstrated to include mucus secretion and prostaglandin E2 (PGE_2) production (Rozza *et al.*, 2013). The present study was undertaken to further investigate the possible mechanism of menthol gastroprotective action by analyzing its effect on gastric acid secretion.

Materials and Methods

Chemicals

(-)-Menthol (99% purity) was purchased from Sigma-Aldrich. (St. Louis, MO, USA), 0.0025N Sodium hydroxide (Sigma- St Louis, MO), 1% phenolphthalein solution, Histamine acid phosphate (Sigma- Aldrich, St Louis MO), Ketamine Hydrochloride (Rotexmedica, Trittau, Germany), Carbamylcholine chloride (Carbacol: Sigma- Aldrich MO), Pentagastrin (Sigma Aldrich). Chemicals and reagents were of analytical grade.

Animals

Male Wistar rats weighing 200-230 g obtained from the Central Animal House of College of Medicine, University of Ibadan, Ibadan Nigeria were used for the studies. They were fed standard rat pellet with free access to tap water under standard light-dark cycles (12 h dark/12 h light) and room temperature (23- 28^{o}C). Prior to the experiments, all rats were fasted for 16 hours and housed in cages with a raised meshed floor to prevent coprophagy. Oral administrations were carried out without any anaesthetic procedure. After the experiment, the rats were euthanized with excess dose of anaesthesia. All efforts were done to minimize animal suffering. Male Wistar rats that had been fasted for 16 h were distributed into four groups (n= 6). The rats were then orally dosed with the vehicle (0.9% normal saline, menthol (20, 40, 60 mg/kg) (Rozza *et al.*, 2014). The study was conducted in accordance with the Organization for Economic Development (OECD) guidelines on good laboratory practice (2001) and the "Principles of laboratory animal care" (NIH, 1985) were followed.

Surgical procedure for GAS collection

The animals were tied to the dissecting board after anaesthesia. An incision was made in the upper part of the trachea and cannulated. This was to ensure that the airway was clear. Mucus was removed from the airway using a moist cotton wool. A size 3-cannula from the modified Langerdoff apparatus was passed into the oesophagus, care being taken not to puncture the esophageal wall. The cannula was push until it could be felt in the cardiac region of the stomach. A ligature was tied around the oesophagus to secure the cannula. The fur on the lower abdominal portion was shaved and a midline incision was made through the skin so as to bring out the stomach. A incision was made an inch distal to the pyloro-duodenal junction and through it the stomach was washed by the normal saline fluid until clear effluent was observed. The duodenum was cannulated and tied. The stomach was put back into the peritoneum and the cut surface closed and covered with moist cotton wool. The end of the cannula was then put into a beaker to collect the effluent. The femoral vein was exposed by dissection, cut made in the upper thigh with the femoral sheath containing the femoral vein, artery and nerve exposed. The vein was isolated and ligated in two places. Blood flow through the vein was first occluded by holding the vein with a bulldog clip in the body-ward direction. The vein was made to distend fairly by pushing blood towards the clip. A femoral cannula was inserted in the femoral vein from the toe-ward direction and tied. After cannulating the femoral vein, the rate of flow of the perfusing fluid from the modified Langerdoff apparatus was adjusted. The rate of perfusion was regulated such that 10ml of gastric contents was collected from the stomach cannula at 10 minutes interval. This technique is known as the continuous perfusion technique method of Ghosh and Schild (1958). The collected effluent was titrated with 0.0025N NaOH after adding two drops of phenolphthalein to it.

Collection of maximal gastric acid secretion using different secretagogues

Carbachol-induced gastric acid secretion

The basal acid secretion and the maximal acid secretion were measured. Carbachol at a dose of 4 µg/kg body weight was administered intraperitoneally (i.p.) (Naseri *et al.*, 2007). Ketamine chloride (0.2 ml/100g body weight), *i.p.* was used as anesthesia. Then 10ml of gastric contents was collected after 10 minutes, after which the timing for 10ml collection was made every 10 minutes for one hour twenty minutes (80 minutes).

Histamine-induced gastric acid secretion

The basal acid secretion and the maximal acid secretion were measured. A dose of 2.5ml/100g body weight histamine acid phosphate was injected intravenously through the femoral vein into the rats after anaesthetized with ketamine chloride (0.2 ml/100g body weight) *i.p*.

10ml of gastric contents was collected at the rate of 1ml per minute for one hour twenty minutes (80 minutes) using gastroduodenal cannula.

Pentagastrin-induced gastric acid secretion

The basal acid secretion and the maximal acid secretion were measured. Pentagastrin at a dose of 25 µg/kg body weight was administered intraperitoneally (Fatema *et al.*, 2006) and Ketamine chloride (0.2 ml/100g body weight), *i.p.* was used as anesthesia. Then 10ml of gastric contents was collected at the rate of 1ml per minute for one hour twenty minutes (80 minutes) using gastroduodenal cannula.

Measurement of gastric acid concentration and volumetric analysis of samples

Titrations and calculations using the principle of volumetric analysis were used to measure the strength of the samples collected i.e. basal and maximal gastric secretions. The total acidity of the gastric contents was determined by using the titrating method with an initial drop of 1% phenolphthalein. This was titrated with 0.0025N NaOH from a burette. The end point was determined when the solution turns pink from the burette reading. At the end of the titration process, a multiplication factor of 0.25 V_B was used in calculating the concentration of the acid in the effluent in meq/litre, where V_B is titre volume of base.

Statistical analysis

The results were analyzed using a one-way analysis of variance (ANOVA) followed by Student's test. The results are presented as the mean± the standard error of the mean (SEM). All analyses were performed using GraphPad Prism 5 software. A value of p=0.05 was considered significant.

Results and Discussion

The treatment of rats with menthol resulted in a decrease in gastric acid secretion (GAS) in the presence of pentagastrin, carbachol and histamine. These decrease were all significant (p>0.05) in the treated animals compared to that of the control group treated with normal saline as shown in figures 1, 2 and 3. The decreases were all dose dependent in all the three secretagogue used. There were significant reductions in gastric acid secretion after pentagastrin (25 µg/kg, *i.p.*) administration when compared to the control (p≤0.05). Between 0-40 minutes, the basal secretion was rising fairly constant in their secretions in all the groups (Figures 1, 2 and 3). After the 40th minutes, pentagastrin a known secretagogue was administered intraperitoneally. After 10 minutes, there was a sharp increase in GAS in the control rats, while the menthol-treated groups showed a fall in gastric acid secretion that were all significantly different (p≤ 0.05) compared to those of the control groups. Analysis of the 50th – 80th minute's interval also showed a significant decrease in gastric acid secretion in the menthol treated rats compared to the control rats group.

The results obtained from the GAS showed that menthol affected the normal gastric acid secretory response to carbachol, histamine (H_2) and pentagastrin stimulation. With histamine (H_2) secretory response, there was no significant change in the basal secretion of gastric acid with the different doses of menthol (20 mg/kg, 40 mg/kg and 60 mg/kg). But when histamine (0.1 mg/g) was administered to these different menthol treated animals, there was an initial rise followed by a decrease in response respectively. On pentagastrin secretory response, there were steady rise in the basal secretion of gastric acid with the different doses of menthol (20 mg/kg, 40 mg/kg and 60 mg/kg). But, when pentagastrin (25 µg/kg) was injected, there was an initial increase in gastric acid secreted followed by significant decrease with the different doses of menthol used. From these observations, menthol appears to cause an inhibition, blocking H_2 receptors, but with pentagastrin, there was partial inhibition indicating partial inhibition of CCK-B receptors for gastrin. In all cases, GAS is significantly reduced, indicating that there may be a significant depression in intracellular free Ca^{2+}. It is generally accepted that gastrin acts mainly by releasing histamine from the enterochromaffin-like (ECL) cells in the oxyntic mucosa, so also from actions on parietal cells where they are equally distributed (Prinz, 1993, 1994a, b; Sandvik *et al.*, 1998). Furthermore, it has been reported that prostaglandins do exert inhibitory effects on parietal cells (Soll, 1986). From this study, apart from antagonizing H_2 and gastrin CCK-B receptors, menthol may also be potentiating the effect of endogenous prostaglandins leading to inhibition of gastric acid secretion. Acetylcholine from nerve endings have been shown to acts by two possible pathways. First is the release of histamine from the ECL cells, which then stimulate acid production and secondly, by interaction with muscarinic (M_3) receptors on the oxyntic cells resulting in increase in intracellular calcium concentration (Wilkes *et al.*, 1991). Thus, the ability of

menthol to decrease acid secretion after carbachol administration may be due to its effectively antagonizing the muscarinic receptors. The treatment of rats with menthol resulted in a significant decrease in gastric acid secretion in the presence of pentagastrin, carbachol and histamine in the treated animals compared to those of the control group (Figure 1, 2 and 3). Essential oils, such as peppermint containing menthol exerts a significant smooth muscle relaxant effect believed to relate to inhibition of calcium channels (Taylor *et al.*, 1985). Menthol is known to block the carbachol (acetylcholine-like) induced influx of calcium ions into cells.

Fig.1

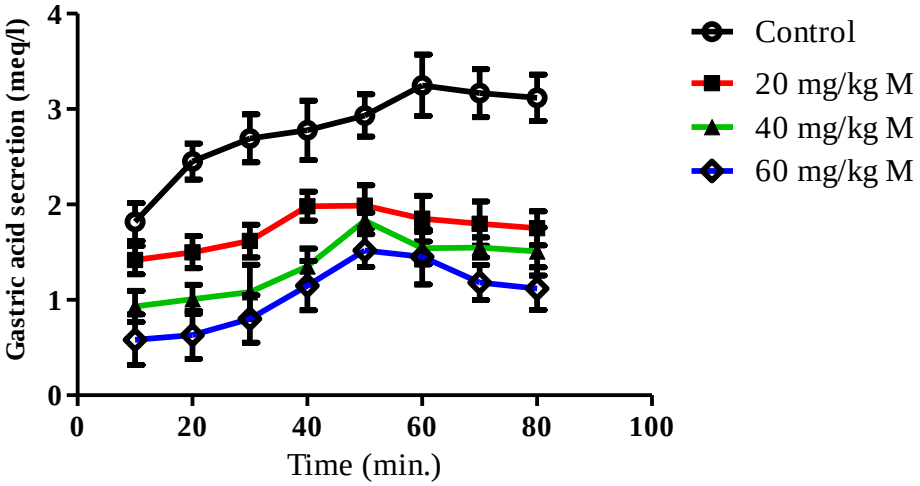

Fig. 1. Effect of pentagastrin on gastric acid secretion in menthol treated rats

Fig.2

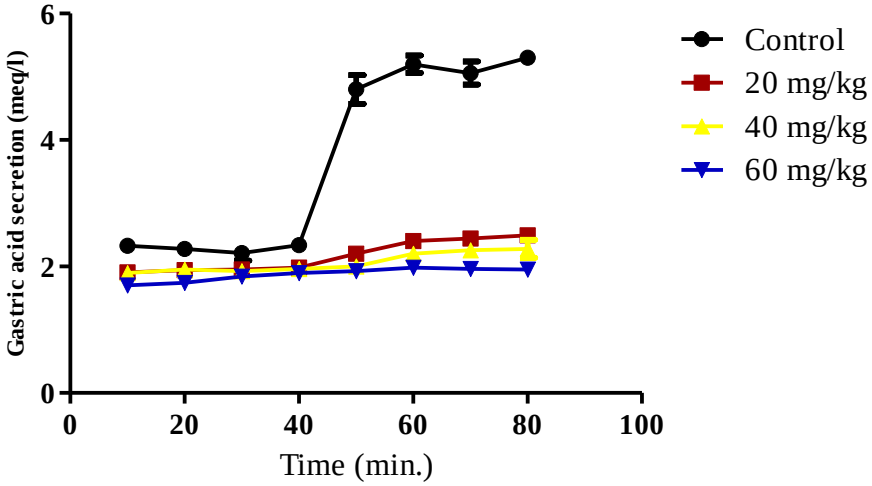

Fig. 2. Effect of carbachol on gastric acid secretion in menthol treated rats

Fig.3

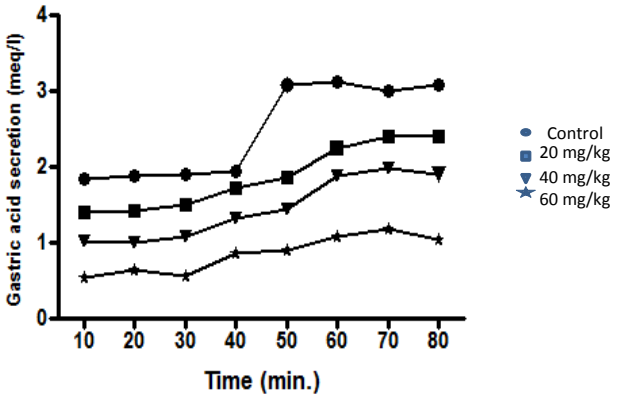

Fig.3.Effect of histamine on gastric acid secretion in menthol treated rats

This calcium channel blocker action of menthol exerts pharmacological effects similar to those observed with current prescription medications such as nifedepine or diltiazem, which are calcium channel antagonists (Blackwell *et al.*, 1981). Calcium channel blocking drugs, such as nifedepine or diltiazem exert effects on upper gastrointestinal motor function, inhibition of esophageal peristalsis and a reduction of the lower esophageal sphincter pressure in the absence of major effects on gastric motor function (Blackwell *et al.*, 1981). The effect of menthol on gastric acid secretion is in line with work of Rozza *et al.*, 2014 that reported oral treatment with menthol displaying a gastroprotective activity through anti-apoptotic, antioxidant and anti-inflammatory mechanisms.

Conclusion

The results obtained in this study suggest that menthol presents antiulcer activities. However, further clinical and toxicological studies must be conducted to support the use of menthol as a potential anti-ulcerogenic drug.

Conflict of interest: There are no conflicts of interest to disclose.

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