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Original article

The Inhibitory Effect of *Ziziphora clinopodioides* Lam. on Gastric Acid Output at Basal, Vagotomized and Vagal Stimulated Conditions in Rat

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Abstract

Objective(s)

Ziziphora clinopodioides Lam. is a plant widely used in Iranian traditional medicine for gastrointestinal disorders. Several reports have demonstrated antibacterial (*Helicobacteria pylori*), antioxidant and anti-inflammatory properties of *Z. clinopodioides*. The aim of this study was to investigate the effects of aqueous-ethanol extract of *Z. clinopodioides* on rat's gastric acid output in basal, vagotomized (VX) and vagal stimulated conditions.

Materials and Methods

A total of 24 male Wistar rats weighed 200-250 g were randomly divided into two groups: control and test. Tracheostomy and gastroduodenostomy procedures were performed for each rat. In the vagotomized condition the vagus nerve in the cervical region was dissected and in the vagal stimulation condition the distal portion of the vogues nerve stimulated. Gastric content was collected for 15 min by wash out technique. A volume of 1 ml of three doses (0.5, 1 and 2 mg/kg) was introduced into the stomach (i.g.) of each rat in the test group and the same volume of saline was used in the control group. Total titratable acid was measured by a titrator.

Results

The extract inhibited acid secretion significantly at basal condition. At VX condition not only this inhibitory effect on acid secretion disappeared but also a stimulatory effect at the dose of 2 mg/kg was shown. In vagal stimulation condition the extract showed a significant inhibitory effect at 1 mg/kg dose.

Conclusion

Taking together our data resulted from comparison of three conditions showed that the extract exerted an inhibitory effect on acid secretion in basal and vagal stimulation. Also, according to our results this inhibitory effect of the extract could be exerted via gastric vagal parasympathetic nerve.

Keywords: Gastric acid, Rat, Vagotomy, Vagus nerve stimulation, *Ziziphora clinopodioides* Lam.

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Introduction

Gastrointestinal disorders due to abnormal metabolic or physical processes such as gastric and duodenal ulcers, gastritis, dyspepsia, hyperchlorhydria or functional gastrointestinal disorders such as irritable bowel syndrome (IBS) are highly prevalent in the world (1). Several metabolic and physical disorders are related to gastric acid and/or gastric motility disturbances. For over 2000 years, medicinal plants have been used for therapeutic purposes and an increasing attention has been raised to herbal products because of their effectiveness and their low cost in recent years. *Ziziphora clinopodioides*, which is widely used in Iranian traditional medicine for treatment of common cold, gastrointestinal disorders and inflammations, is a member of Labiatae family (2, 3). Several effects such as antibacterial (4, 5), antifungal (6), antioxidant (5, 7) and anti-inflammatory (8) have been reported as the effects of this plant. It has chemical components including pulegone, sis-isopulegone, cineol, thymol, α and β pinen, piperitenone, terpenoides and flavonoids (4, 5, 9, 10). There are no reports on the effects of this plant on gastric acid secretion; therefore, we sought to find any possible effects of aqueous-ethanol extract of *Z. clinopodioides* on gastric acid secretion. In order to further clarify the interaction of the extract with gastric vagal parasympathetic system, we have compared the effects of the extract on gastric acid output in three conditions; basal, vagotomized (VX) and vagal stimulated.

Material and Methods

Plant and extraction

The whole plant of *Z. clinopodioides*, collected in Nishaboor, Khorasan Province, Iran, in March 2006. It was authenticated by Herbarium of Ferdowsi University (voucher No. 157-2016-4). Arial part of the plant (300 g) was soaked in ethanol (50%) for 24 hr and paper filter was used to filter the solute after mixing. The solution was then dried, using a 40 °C oven for 36 hr.

Animals

The experiment was conducted, using 24 male Wistar rats weighed 200-250g. The animals were kept in a 25±2 °C temperature with a 12 hr light /dark cycle and fed with standard diet and tap drinking water. Animals were randomly divided into 2 groups; control and test. The experimental protocol was approved by Ethical Committee at Mashhad University of Medical Sciences (MUMS).

Experimental procedures

The surgical procedure was performed for all groups but the extract was only applied to the test group according to the protocol. All rats were not fed for 24 hr but were free to access to drinking water before operation. To avoid the effect of circadian rhythm, the experiments were started at 8:00 am every day. Anesthesia was induced by sodium thiopental (50 mg/kg; i.p.) and cervical esophagus was ligated after tracheostomy (11). A midline incision was performed on abdomen and a silicone tube (2.5 mm in external diameter) was introduced to the stomach via duodenum (12). For emptying the probable remaining gastric contents, it was washed with 1 ml of 37 °C normal saline (pH= 7) several times (13). In vagal stimulated condition, vagus nerve in the cervical region was dissected and stimulated (15 V, 4 Hz, width 0.05 ms, 30 min) by a stimulator (Harvard, England) (14). Gastric acid output was measured 15 min after the extract was introduced to the stomach (15) and the acid output was calculated and reported as milimole/liter. The gastric content was titrated by NaOH (0.01 N). After the gastric acid output measurement in each condition the stomach was washed with 1 ml of 37 °C normal saline and the interval time between each condition was at least 45 min. To measure gastric acid secretion in the test group, 1ml of 0.5, 1 and 2 mg/kg of the aqueous–ethanol extract was administered intragastrically (i.g.) to each rat for 15 min.

Statistical analysis

The data were shown as mean±SEM and the unpaired t-test and Mann-Whitney were applied to compare the results for control and test groups; $P< 0.05$ was considered as significant.

Results

A significant decrease in basal acid output in the test group was shown by doses 0.5 and 1 mg/kg of the *Z. clinopodioides* extract in compare to control group (37% and 64.5% respectively) (Figure 1).

In VX condition a significant increase in acid output was shown in the test group by 2 mg/kg doses of the extract compare to the control group (84%), but the two other dosages resulted in no significant difference compare to the control group (Figure 2).

In vagal stimulation condition, the test group exhibited a significant decrease in acid output by 1mg/kg dose of the extract compare to the control group (36%) (Figure 3).

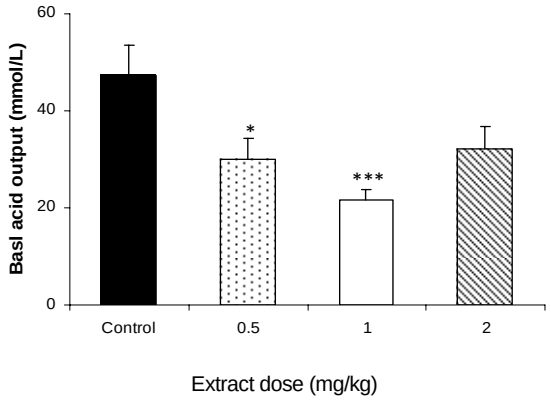

Figure 1. Gastric acid output in the test and control groups in basal condition. Data are shown as Mean±SEM. The *Z. clinopodioides* extract inhibited gastric acid output significantly by 0.5 and 1 mg/kg doses in compare with the control group. (**P*<0.05; *** *P*<0.001; n=12).

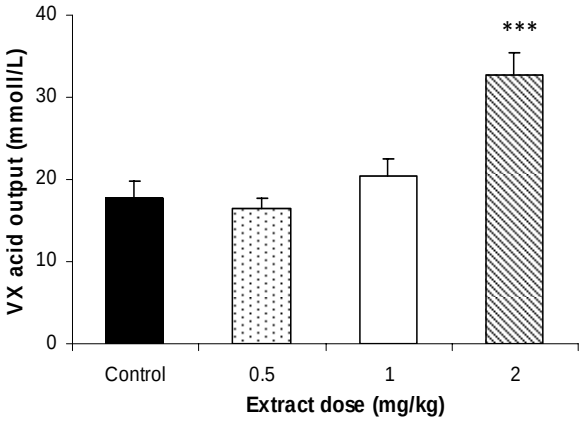

Figure 2. Gastric acid output in the test and control groups in vagotomized condition (VX). Data are shown as Mean±SEM. The *Z. clinopodioides* extract revealed an excitatory effect on the gastric acid output with 2 mg/kg dose in comparison with the control group. (****P*< 0.001; n=12)

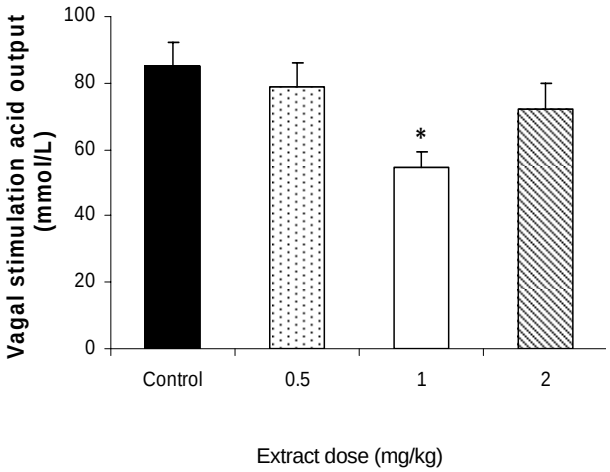

Figure 3. Gastric acid output in the test and control groups in vagal stimulated condition. Data are shown as Mean±SEM. The *Z. clinopodioides* extract with 1 mg/kg dose inhibits the gastric acid output significantly in compare with the control group. (**P*< 0.05; n= 12).

Discussion

The results of this study indicate that the aqueous-ethanol extract of *Z. clinopodioides* at doses 0.5 and 1 mg/kg had inhibitory effects on the gastric acid output in basal condition in comparison with the control group. These inhibitory effects were disappeared at VX condition; thus these effects of the extract in basal condition may be exerted via gastric vagal parasympathetic nerve. The dose of 2 mg/kg had no inhibitory effect on the gastric acid output in basal condition but it showed an excitatory effect on the gastric acid output at VX condition in comparison with the control group. This may indicate the presence of compound/s in the extract with an excitatory effect on the gastric acid output that act at higher concentrations through processes other than gastric parasympathetic system. The inhibitory effect of 2 mg/kg was masked by excitatory effect of the extract component/s in basal condition. The inhibitory effect of the extract on gastric acid secretion was unable to overcome the excitatory effect of vagal stimulation on the gastric acid secretion with dose of 0.5 mg/kg, but the inhibitory effect of the extract was shown at dose of 1 mg/kg in vagal stimulated condition. The dose of 2 mg/kg of the extract had no inhibitory effect on the gastric acid secretion at vagal stimulated condition. These findings confirm that the extract has compound/s with the

excitatory effect on gastric acid secretion that is dominant at higher concentration. On the base of our knowledge, there are no reports to show the effect of *Z. clinopodioides* extract on gastric acid secretion but previous studies have shown that 1,8-Cineole (cineole), which is one of the main components of *Ziziphora* essential oil (4) has gastroprotective, gastric acid secretion and compliance reduction (16, 17) effects. Considering our data, the inhibitory effect of the *Z. clinopodioides* could be partly due to the cineole component of the extract, but for more clarifications, future studies are needed and recommended.

Conclusion

Our findings show that the *Z. clinopodioides* extract has an inhibitory effect on the gastric acid output in basal and vagal stimulated conditions. Comparison of acid output in basal, vagotomized and vagal stimulated conditions shows that the inhibitory effect of the extract was exerted through gastric vagal parasympathetic nerve. Also, these findings indicate that the extract may have compound/s with excitatory effect on the gastric acid secretion which exerts its effects at higher concentrations independent of the gastric vagal parasympathetic nerve.

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