

The Effects of *Ziziphora Clinopodioides Lam.* Extract on Rat's Gastric Motility at Basal and Vagal Stimulated Conditions

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Summary

Ziziphora clinopodioides Lam. is a plant widely used in traditional medicine especially in Iran. The aim of this study was to investigate the effects of aqueous-ethanol extract of *Z. clinopodioides* on rat's gastric motility in basal and vagal stimulated conditions. Twenty four Wistar rats were randomly divided into two groups: test and control. The extract was prepared by maceration and three doses (0.5, 1 and 2 mg/kg) were used in test group. The same volume of saline was used in the control group. Gastric motility was measured by inserted a small balloon in the stomach through a cut in duodenum. The balloon was connected to a pressure transducer by a 10 cm silicon tube. The data were recorded for 25 minutes duration after each doses and these data were analyzed for 3 intermittent five minute intervals (0-5, 10-15 and 20-25 min). At basal condition the extract increased significantly intragastric pressure (IGP) in the first 5 minutes of recording, but an inhibitory effect was revealed at vagal stimulated condition. The extract showed an inhibitory effect on gastric contraction amplitude in both basal and vagal stimulated conditions but had no effect on gastric contraction frequency in these conditions. The extract exerted an inhibitory effect on gastric motility mainly in vagal stimulated condition. This inhibitory effect could be exerted via gastric vagal parasympathetic nerve.

Key Words: *Ziziphora clinopodioides Lam.*, gastric motility, vagous nerve

Introduction

Gastrointestinal disorders due to abnormal metabolic or physical processes such as gastric and duodenal ulcers, gastritis, dyspepsia, hyperchlorhydria or functional gastrointestinal disorders (e.g. irritable bowel syndrome) are highly prevalent worldwide.(1) The many of metabolic and physical induced disorders are related to gastric acid and/or gastric motility disturbances. Synthetic and herbal medicines have been developed to treat gastrointestinal disorders. Medicinal plants have been used for over 2000 years and an increasing attention has been paid to herbal medicine products because of their effectiveness and lower cost in recent years. *Ziziphora clinopodioides*, which is widely used in Iranian traditional medicine especially for gastrointestinal disorders, common cold and inflammation, is a member of Labiatae (Lamiaceae) family.(2,3) Several effects such as antibacterial (4,5), antifungal (6), antioxidant (5,7) and anti-inflammatory (8) have been reported for this plant. *Z. clinopodioides* has chemical components including pulegone, sis-isopulegone, cineol, thymol, α and β pinen, piperitenone, terpenoides and flavonoids.(5,9,10) To the best of our knowledge no reports on the effects of this plant on gastric motility are available at the moment; therefore, the aim of this study was to investigate possible effects of aqueous-ethanol extract of *Z. clinopodioides* on gastric motility in rats and for more clarification of the extract interaction with gastric vagal parasympathetic system we considered two conditions: basal and vagal stimulated.

Materials & Methods

The experiment protocol was approved by the ethics committee of Mashhad University of Medical Sciences. The experiment was conducted on 24 wistar rats weighed 200-250^g. The animals were kept in a 25±2^oc temperature with a 12 hr light /dark cycle and fed with standard diet and tap drinking water *ad libitum*. *Z. clinopodioides* plant was identified by botanists in the herbarium of Ferdowsi University. Aerial part of the plant (300g) was soaked in ethanol (50%) for 24 hrs and paper filter was used to filter the solute after mixing. The resulting solution was dried using a 40°C ban marry for 36 hr. The average w/w yield was 10.5%. Three doses of 0.5, 1 and 2 mg/kg were prepared. The rats, which were divided into two groups of case and control, were deprived of food but not water for 24 hours before the operation. In order to prevent the effect of the circadian rhythm, the experiments were started at 8 am every day. The animals were anesthetized with sodium thiopental (50mg/kg, i.p). Each rat underwent laparotomy and gastroduodenostomy. A small balloon connected to a pressure transducer by a 10 cm silicon tube, was inserted into the stomach through duodenum. To prevent the gastric distention effect, 0.5 ml of normal saline per 100g BW was introduced into the balloon.(11) The extract (0.5 ml) was introduced into their stomach through an esophageal tube. Before the introducing the extract, the basal intra gastric pressure was measured for 15 minutes, then 3 doses of the extract were introduced at 45 minute intervals, during which the intragastric pressure (IGP) was continuously recorded for 25 minutes and the remaining time was used to withdraw the gastric content and wash the stomach by saline. After the measurement of IGP in basal condition, bilateral cervical vagotomy was carried out in each rat and the vagus nerve was stimulated using a stimulator (15V, 4 Hz, width 0.05 ms, 30 min).(12) To evaluate the gastric motility, we considered 3 parameters including contraction frequency, contraction amplitude and IGP in the basal and vagal stimulation conditions. In order to see whether the effect of the extract is time dependent, pressure measurements at 3 intermittent five minute intervals (0-5, 10-15 and 20-25 min) were chosen.

The data were prepared as mean ± SEM and proper tests including ANOVA, unpaired t- test and Mann-Whitney were applied to compare the results; P<0.05 was considered significant.

Results

The results showed that the *Z. clinopodioides* extract had no significant effects on gastric contraction frequency in basal and vagal stimulation conditions compared with the control group (1A and B).

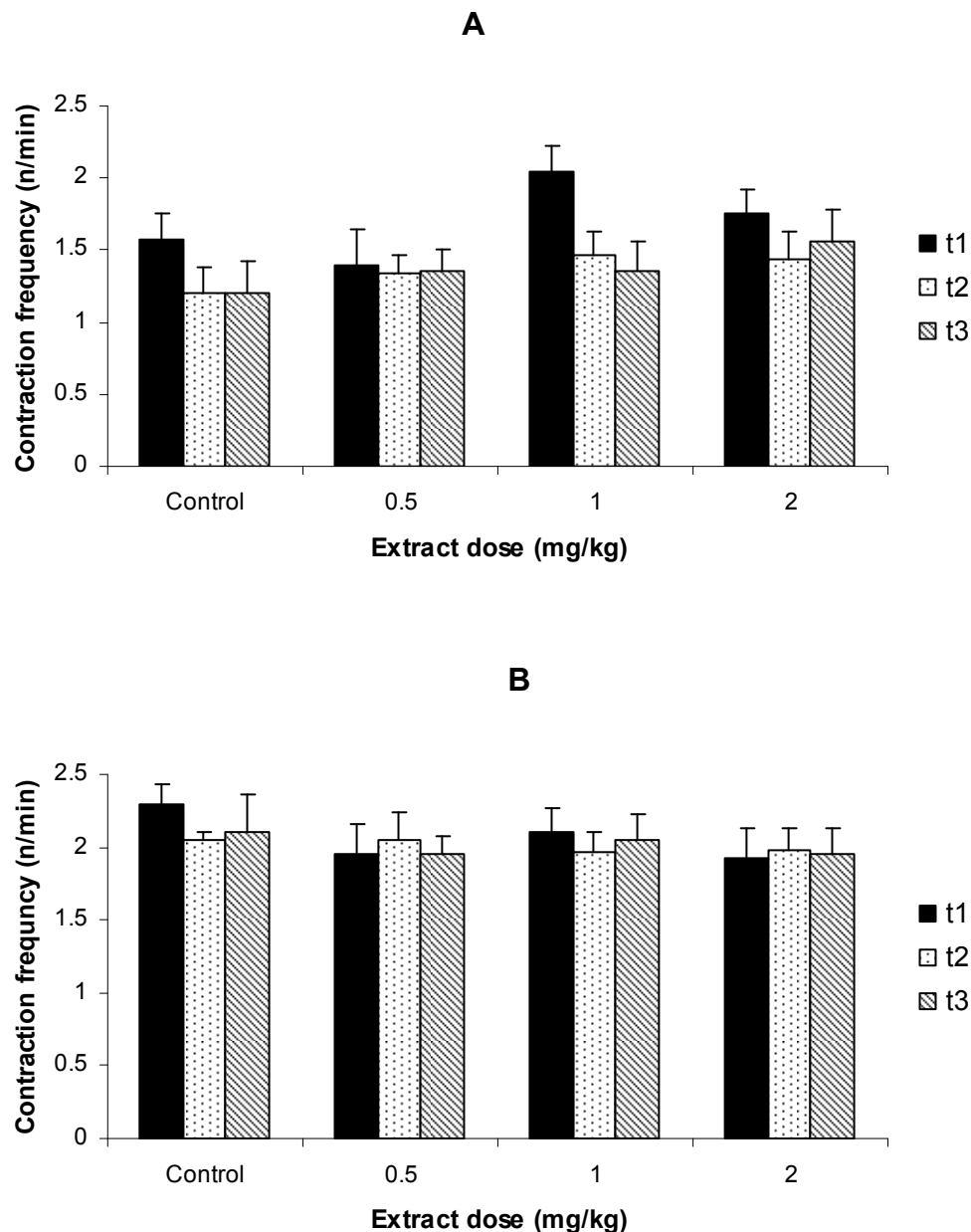


Fig 1: the effect of *Z. clinopodioides* extract on gastric contraction frequency compare with control group at basal (A) and vagal stimulated (B) conditions at 3 intermittent five minute intervals ($t_1=0-5$, $t_2=10-15$ and $t_3=20-25$ min). (n=12)

The extract was able to increase the IGP significantly by using 1 and 2 mg/kg doses in the first 5 minutes in basal condition (2A). These two doses reduced the IGP considerably in the first 5 minutes following vagus nerve stimulation (2B).

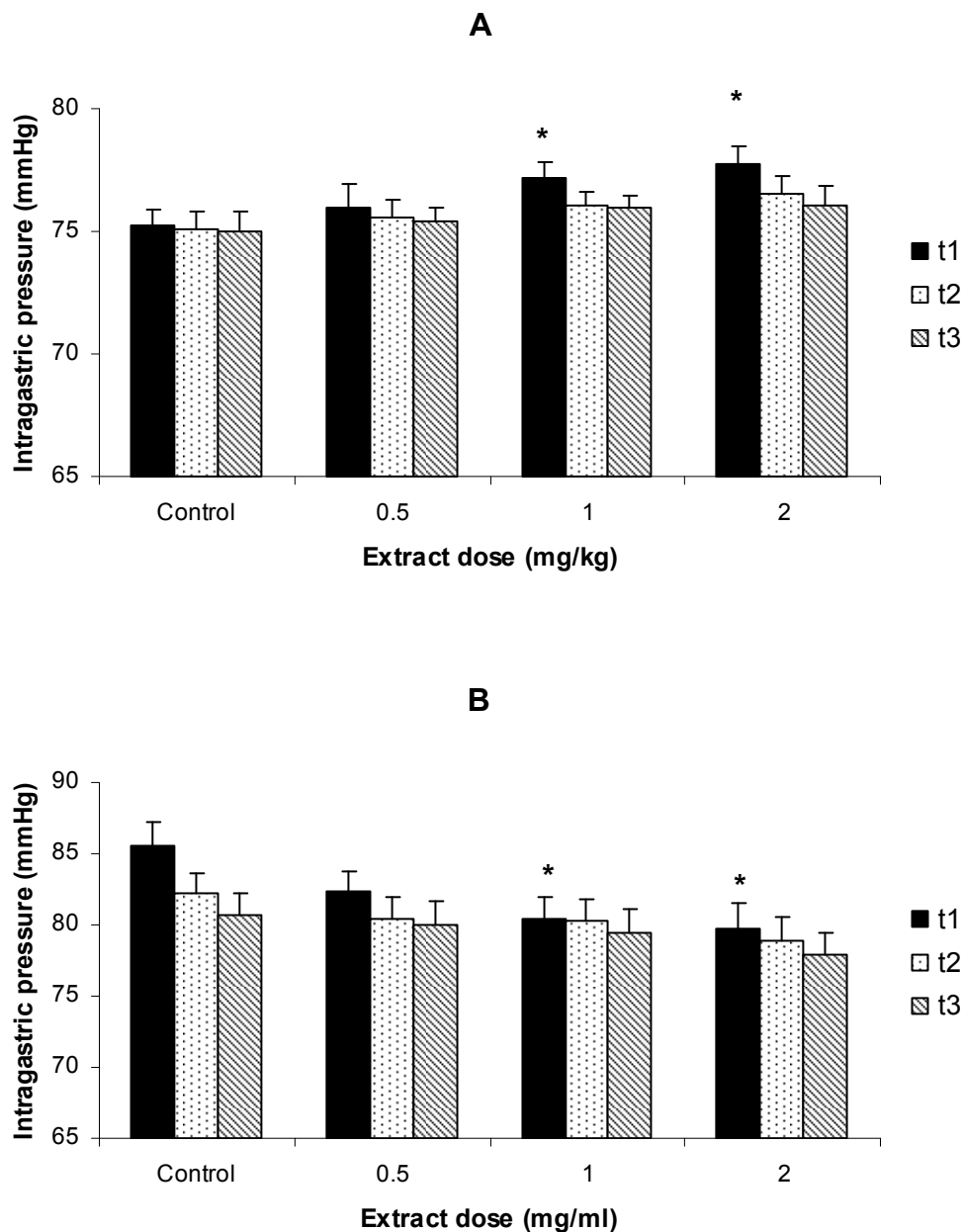


Fig 2: the effect of *Z. clinopodioides* extract on intra gastric pressure compare with control group at basal (A) and vagal stimulated (B) conditions at 3 intermittent five minute intervals ($t_1=0-5$, $t_2=10-15$ and $t_3=20-25$ min). (n=12, unpaired t-test) *P<0.05

The contraction amplitude was meaningfully decreased in the second and last five minutes by using all three doses of the extract (3A). In vagal stimulation, the contraction amplitude was reduced after the administration of the extract in three doses during the 3 intermittent five minutes intervals (3B).

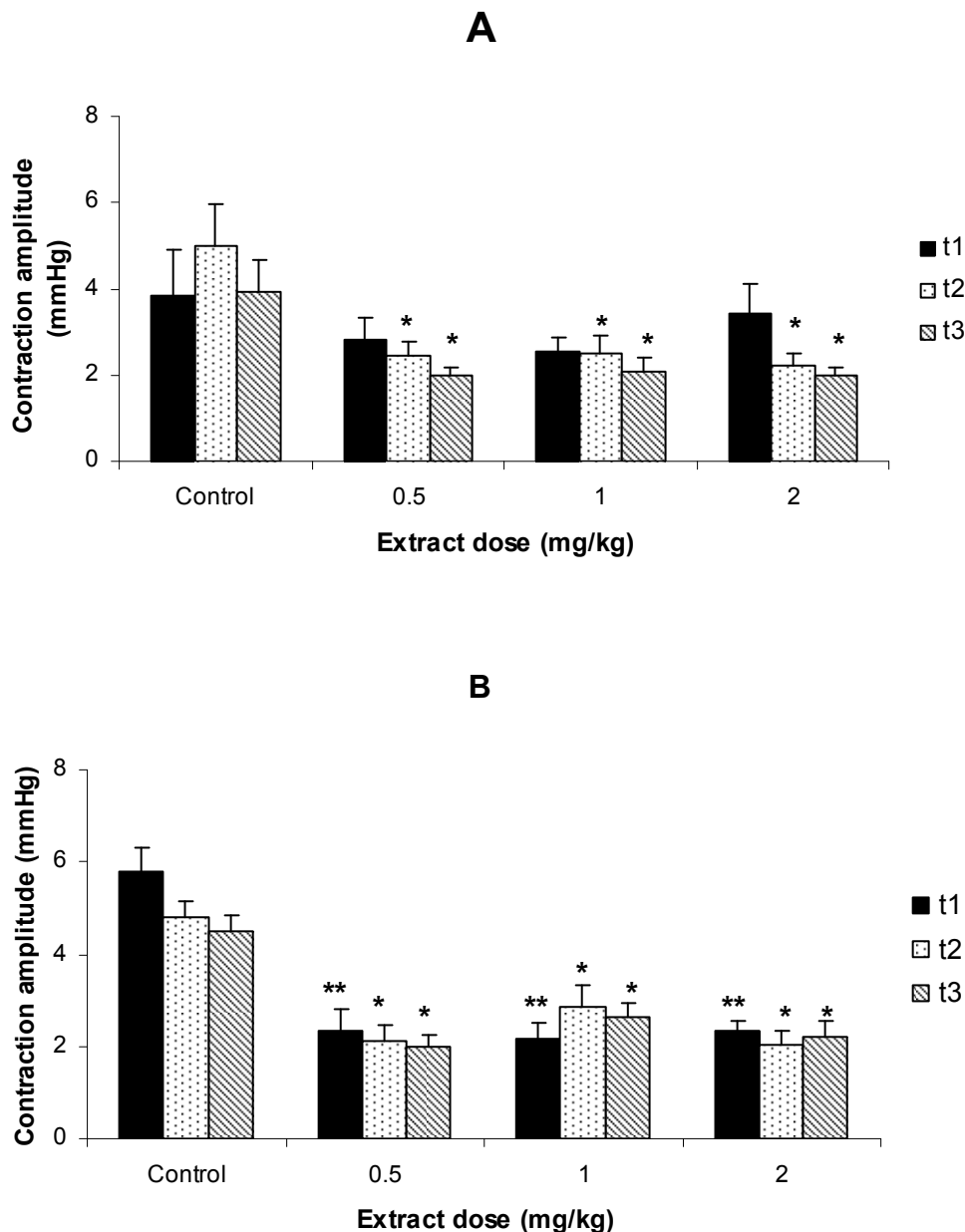


Fig 3: the effect of *Z. clinopodioides* extract on contraction amplitude compare with control group at basal (A) and vagal stimulated (B) conditions at 3 intermittent five minute intervals ($t_1=0-5$, $t_2=10-15$ and $t_3=20-25$ min). (n=12, unpaired t-test) *P<0.05, **P<0.01

Discussion

As mentioned before there are some reports that show the medical effects of medicinal plants on gastrointestinal disorders which are highly prevalent worldwide. Among these plants, *Ziziphora clinopodioides* is used traditionally for treating gastrointestinal disorders. In our study the results showed that the *Ziziphora clinopodioides* extract increases the IGP in the first five minutes in basal condition which leads to the conclusion that it has a fast but transient stimulatory effect on the intra gastric pressure. This stimulatory effect maybe is due to the presence of compound/s in the extract which has stimulatory effect on gastric smooth muscles.

Considering the inhibitory IGP effect of the extract in vagal stimulation during the first five minutes, it could be concluded that this fast and short lived effect is the result of an inhibitory interaction with the gastric parasympathetic nerves. The extract showed an inhibitory effect on contraction amplitude in both basal and vagal stimulation conditions. However, this inhibitory effect was appeared to be sooner and lasted longer in the vagal stimulation condition. The inhibitory effect of the extract on contraction amplitude in vagal stimulation condition is more clearly compared to basal condition, so partly of this inhibitory effect in vagal stimulation condition exerted by gastric parasympathetic inhibition. Any effects on the contraction frequency were not found in the present study. It seems that the extract has no effect on the membrane potential of the gastric smooth muscle.

Although on the best of our knowledge there is no study on the effects of *Z. clinopodioides*, in one study the effects of *Zataria multiflora* Boiss, a plant which is another member of the Labiatae family, on the isolated rat ileum showed that the extract had an anticholinergic effect on the smooth muscle by inhibiting the contractions induced by acetylcholine therefore it was concluded that this inhibitory effect is exerted by the reduction of the calcium influx through the calcium channel blockage.(13) This is in agreement with the results of previous study that is the inhibitory effects of *Z. clinopodioides* on the IGP and contraction amplitude in vagal stimulation condition can be attributed to the anticholinergic effects of the extract.(13)

Gharib Naseri et al (2005) showed that *Zataria multiflora* Boiss has an antispasmodic effect on the uterine smooth muscle of rat. It was concluded that this effect was due to a reduction in the intracellular calcium release and so probably it could be concluded that this inhibitory effect of the *Z. clinopodioides* is partly related to the inhibition of the intracellular calcium release. In order to more clarify the mechanism of effects of *Z. clinopodioides* extract on the gastric motility, further studies is recommended.

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