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Original article

The Effects of *Achillea wilhelmsii* Extract on Rat's Gastric Motility at Basal and Vagal Stimulated Conditions

*[1]Saeed Niazmand, [2] Esmat Khoshnood

Abstract

Objective(s)
Achillea genius is widely used in traditional medicine for gastrointestinal disorders. The aim of this study was to investigate the effects of aqueous-ethanol extract of *Achillea wilhelmsii* on rat's gastric motility in basal and vagal stimulated conditions.

Materials and Methods
Twenty four Wistar rats were randomly divided into two groups: control and test. The extract was prepared by maceration which was used to prepare three 0.5 ml samples of three doses (0.5, 1 and 2 mg/kg) in the test group. The same volume of saline was used in the control group. Gastric motility was measured by inserting a small balloon in the stomach which was connected to a pressure transducer. The data were recorded for 25 min duration after each dose and these data were analyzed for 3 intermittent five min intervals (t_1= 0-5, t_2= 10-15 and t_3= 20-25 min).

Results
The extract at basal condition decreased intragastric pressure (IGP) by 1 mg/kg dose in the t_3 and 2 mg/kg in the t_2 and t_3 intervals. The extract at vagal stimulated condition decreased IGP by 1 and 2 mg/kg doses in the t_2 and t_3 intervals. The extract reduced contraction amplitude at basal condition by 2 mg/kg dose in the t_2 and t_3 intervals. At vagal stimulated condition contraction amplitude was reduced by 1 mg/kg dose in the t_2 and t_3 by 2 mg/kg in all three intervals. The extract showed no effect on frequency of gastric contraction in either basal or vagal stimulated conditions.

Conclusion
The extract showed an inhibitory effect on gastric motility in both basal and vagal stimulated condition. This inhibitory effect may be exerted by an antagonistic effect on acetylcholine dependent calcium influx or release of calcium from intracellular storage in gastric smooth muscle.

Keywords: *Achillea wilhelmsii*, Gastrointestinal Motility, Vagus Nerves

1-Department of Physiology and Cardiovascular Research Center, Medical School, Mashhad University of Medical Sciences, Mashhad, Iran
*Corresponding author: Tel: +98-511-8828565, Fax: +98-511-8828564; email: niazmands@mums.ac.ir
2- Department of Biology, Sciences School, Azad University of Mashhad, Mashhad, Iran

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 worldwide (1). Several metabolic and physical disorders related to gastric acid and gastric motility disturbances have been treated by using synthetic and herbal medicines. 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 (2). *Achillea*, is one of the most important genera of the compositae family and comprises more than 115 species (3). Several effects such as anti-inflammatory (4), antibacterial (5, 6), antihypertensive and anti-hyperlipidemia (7), and antitumoral (8, 9) have been reported for *Achillea*. It is widely used in traditional medicine for gastrointestinal disorders (10) and there are some reports of its effects on gastrointestinal tract such as antispasmotic (11-13), choleretic (14), antiulcer (15), antibacterial (*Helicobacter pylori*) (16) and hepatoprotective (12). *Achillea wilhelmsii* is the major species which is grown in Iran and widely used in Iranian traditional medicine for gastrointestinal disorders. It has chemical components including flavonoids, alkaloids (achilleine), cineol, borneol, α and β pinen, camphor, caryophllene, thujene, rutin, sesquiterpenoids and monoterpenoids (17-20). No reports on the effects of *A. wilhelmsii* on gastric motility are available at the moment. Thus the aim of this study was to investigate possible effects of aqueous-ethanol extract of *A. wilhelmsii* 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 and Methods

Animals

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 20±2 ˚C temperature with a 12 hr light /dark cycle and fed with standard diet and tap drinking water *ad libitum*.

Plant and extraction

A. wilhelmsii was collected from South Khorasan Province, Iran and identified by botanists in the Herbarium of Ferdowsi University (voucher No. 142-2012-4). Dried aerial parts of the plant (300 g) were soaked in ethanol (50%) for 24 hr and paper filter was used to filter the solute after mixing. The resulting solution was dried using a 40 ˚C oven for 24 hr. Three doses of 0.5, 1 and 2 mg/kg were prepared by dissolving the dried extract in distilled water.

Experimental procedures

The rats, which were divided randomly into two groups of control and test, were deprived of food but not water for 24 hr before the experiment. In order to prevent the effect of the circadian rhythm, the experiments were started at 8 am. The animals were anesthetized with sodium thiopental (50 mg/kg, i.p.). Each rat underwent laparatomy and gastroduodenostomy. A small balloon connected to a pressure transducer (UFI 1050.1) by silicon tube (10 cm length and 2 mm internal diameter), was inserted into the stomach through duodenum. To prevent the gastric distention effect, 0.5 ml of normal saline per 100 g body weight was introduced into the balloon (21). The extract (0.5 ml, 37 ˚C) was introduced into their stomach through an esophageal tube in the test group and the same volume of normal saline in the control group. Before introducing the extract, the basal intra gastric pressure was measured for 25 min, then each of the 3 doses of the extract were introduced to the stomach at 50 min intervals respectively, during which the intragastric pressure (IGP) was continuously recorded for 25 min 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 distal part of vagus nerve was

stimulated using a stimulator (15V, 4 Hz, width 0.05 ms, 30 min) (Harvard, England) (22). To evaluate the gastric motility, we considered 3 parameters including IGP, contraction frequency and contraction amplitude under the basal and vagal stimulation conditions. The IGP was defined by the base line pressure, contraction frequency was defined by the mean of contraction per min and contraction amplitude was defined by the mean of contraction amplitude per min. In order to evaluate whether the effect of the extract is time dependent, pressure measurements at 3 intermittent five min intervals (t_1=0-5, t_2=10-15 and t_3= 20-25 min) were chosen (22).

Statistical analysis

The data were represented as mean±SEM and one way repeated measured ANOVA with Tukey post test was applied to compare the results between groups; $P< 0.05$ was considered as significant.

Results

The extract decreased the IGP significantly by using 1 mg/kg dose in the third intermittent 5 min interval and 2 mg/kg dose in the second and third intermittent 5 min intervals ($P< 0.05$) (Figure 1A). The extract reduced considerably the IGP in the second and third intermittent five min intervals by using 1 mg/kg and also 2 mg/kg dose following vagus nerve stimulation (Figure 1B). The IGP was not changed by extract in a dose dependent fashion.

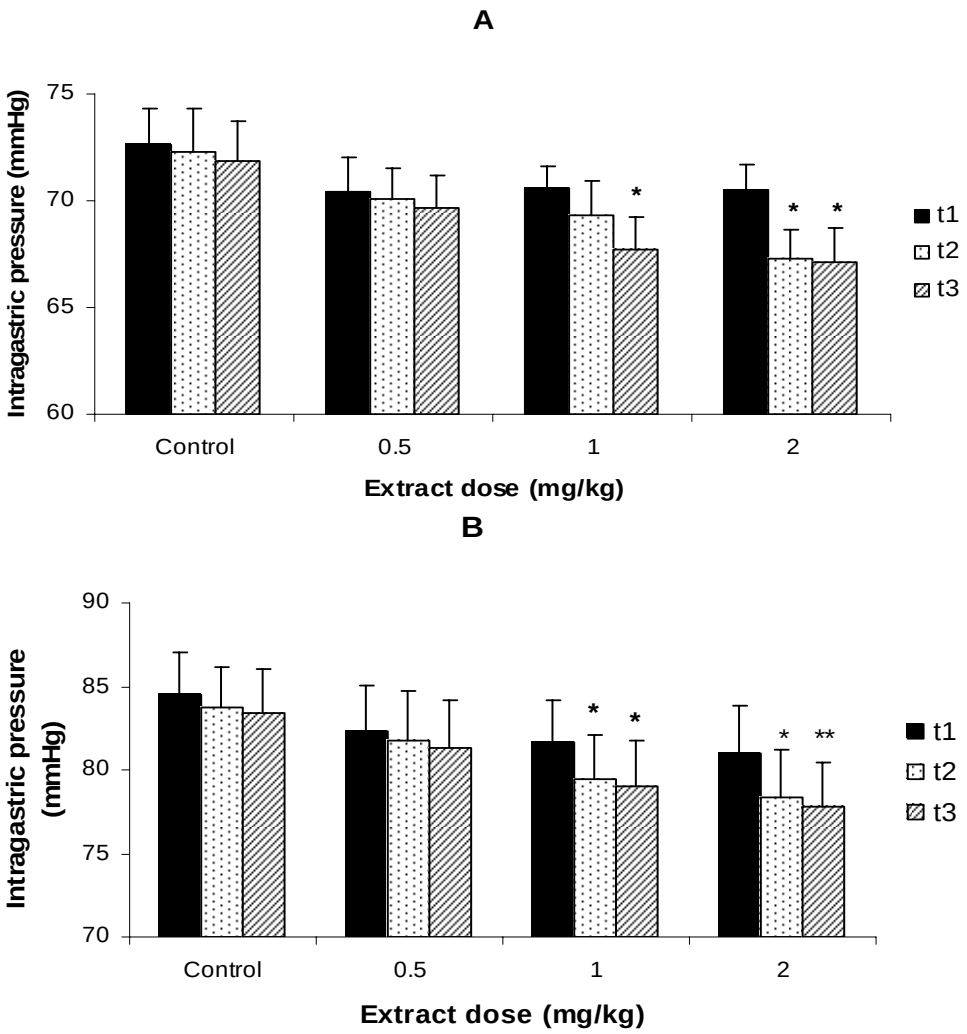

Figure 1. The effect of *A. wilhelmsii* extract on intragastric pressure compared with control group at basal (A) and vagal stimulated (B) conditions at 3 intermittent five min intervals (t_1= 0-5, t_2=10-15 and t_3= 20-25 min). The extract had no dose dependent effect on contraction amplitude. (n= 12) *$P< 0.05$, **$P< 0.01$.

The contraction amplitude was meaningfully decreased in the second and third intermittent five min intervals by using 2 mg/kg dose of the extract in basal condition ($P< 0.05$) (Figure 2A). In vagal stimulation, the contraction amplitude was reduced considerably after the administration 1 mg/kg dose of the extract during the second and third intermittent five min intervals and 2 mg/kg dose during the all three intermittent five min intervals ($P< 0.05$) (Figure 2B).

The results showed that the *A. wilhelmsii* extract had no statistically significant effects on gastric contraction frequency in basal and vagal stimulation conditions compared with the control group (Fig 3A and B).

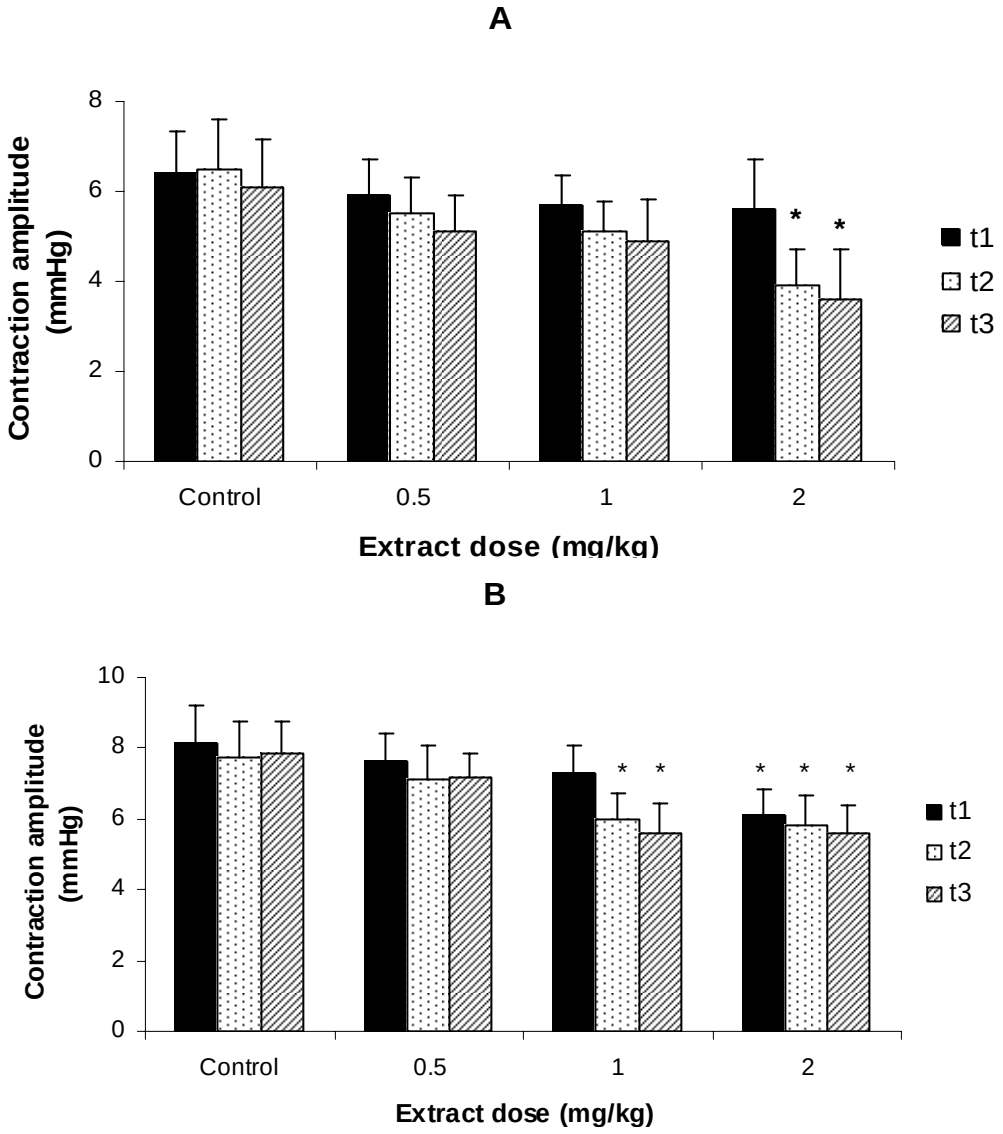

Figure 2. The effect of *A. wilhelmsii* extract on contraction amplitude compared with control group at basal (A) and vagal stimulated (B) conditions at 3 intermittent five min intervals (t_1= 0-5, t_2= 10-15 and t_3= 20-25 min). The extract had no dose dependent effect on contraction amplitude (n= 12) *$P< 0.05$.

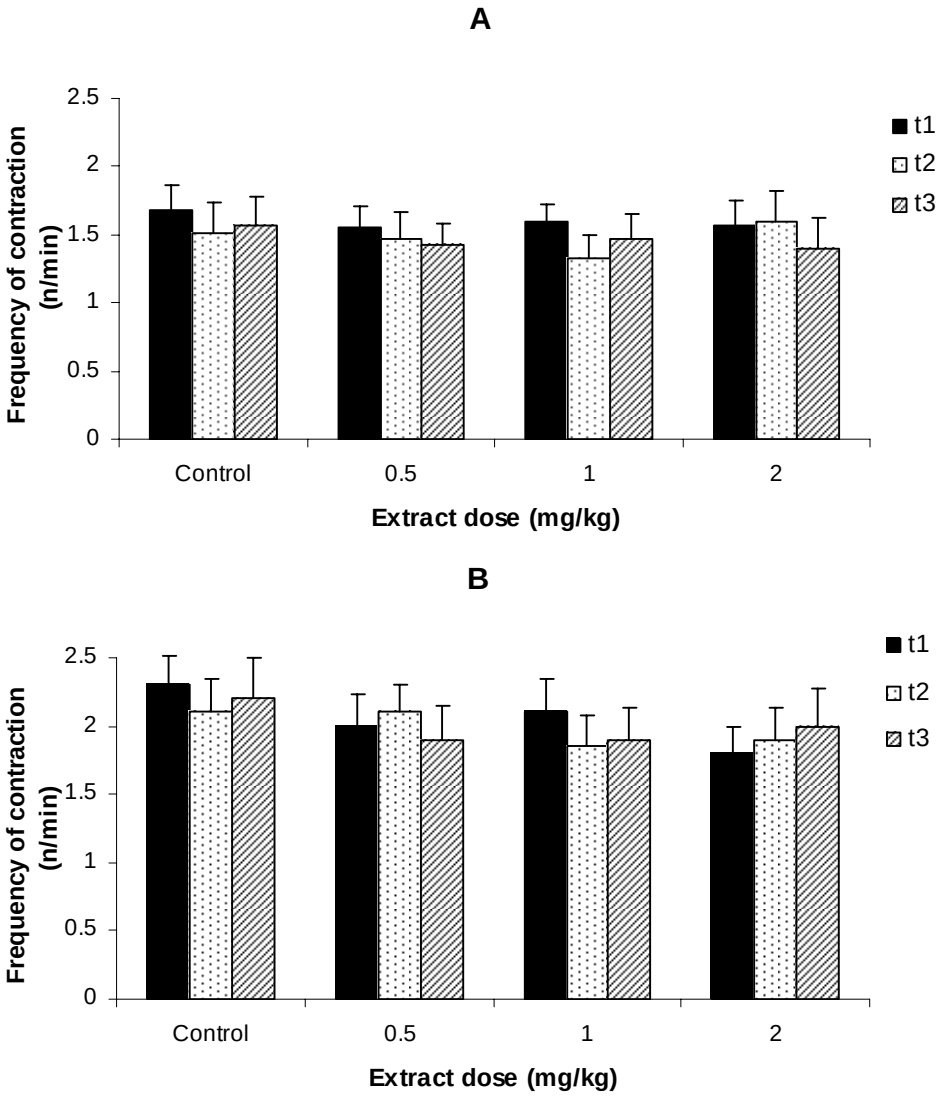

Figure 3. The effect of *A. wilhelmsii* extract on gastric contraction frequency compared with control group at basal (A) and vagal stimulated (B) conditions at 3 intermittent five min intervals (t_1= 0-5, t_2= 10-15 and t_3= 20-25 min) (n= 12).

Discussion

Achillea genus is used traditionally for treating gastrointestinal disorders. The results showed that inhibitory effect of *A. wilhelmsii* extract on IGP was more profound in vagal stimulated condition, thus it could be concluded that the extract exerted its effect in part by inhibition of gastric vagal nerve effect. The extract showed its inhibitory effect in the second and third intermittent five min interval under basal and vagal stimulated conditions which leads to the conclusion that it has a delay inhibitory effect on the IGP. The extract showed an inhibitory effect on contraction amplitude in both basal and vagal stimulated conditions. However, this inhibitory effect was stronger in the vagal stimulated condition compared to basal condition, thus this inhibitory effect in vagal stimulated condition may be exerted by an inhibitory effect on acetylcholine-induced contraction. The gastric motility under basal condition is also controlled by gastric vagal nerve and the extract can intervene with this effect. Although on the base of our knowledge there is no study on the effects of *A. wilhelmsii* on gastric motility, the pervious studies on isolated ileum (11) and jejunum (12) smooth muscle showed antispasmodic effect of *A. millefolium* which is mainly caused by blockade of the calcium influx. The main flavenoids of *A. millefolium* (quercetin, luteolin and apigenin) exhibited antispasmodic

activities by negative modulation of calcium influx *in vitro* (9, 23). Karamenderes et al (2003) (13) showed that the *A. nobilis* extract had an inhibitory effect on acetylcholine-induced contraction in rat's duodenal smooth muscle. Therefore, it is reasonable to suppose that the extract antagonized the effect of gastric vagal nerve effect by an antagonistic effect on acetylcholine dependent calcium influx or release of calcium from intracellular storage in gastric smooth muscle (23, 24) and reduction of the IGP and contraction amplitude.

No effect on the contraction frequency was found in the present study. It seems that the extract has no effect on the membrane potential of the gastric smooth muscle. Thus the extract may exert its effect by acetylcholine dependent release of calcium from intracellular storage in gastric smooth muscle which did not affect membrane potential.

The *A. wilhelmsii* has an inhibitory effect on gastric motility and this inhibitory effect may be partly due to the inhibition of gastric vagal nerve effect probably by antagonizing the acetylcholine effect, more probably by acetylcholine dependent release of calcium from intracellular storage in gastric smooth muscle. In order to clearly clarify the mechanism of effects of *A. wilhelmsii* extract on the gastric motility, further studies is recommended.

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