



Reversal effect of *Achillea millefolium* extract on ileum contractions

Mehrnoosh Sedighi¹, Hamid Nasri², Mahmoud Rafeian-kopaei^{1*}, Seifollah Mortazaei¹

¹Medical Plants Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran

²Department of Nephrology, Division of Nephropathology, Isfahan University of Medical Sciences, Isfahan, Iran

ARTICLE INFO

Article Type:
Original Article

Article History:
Received: 11 September 2012
Accepted: 22 September 2012
ePublished: 1 June 2013

Keywords:
Achillea millefolium
Ileum
Ileum contraction
Spasm

ABSTRACT

Introduction: Traditionally *Achillea millefolium* L. has been used to treat gastrointestinal disorders. In this study the hydroalcoholic extract of *A. millefolium* was evaluated on ileum contractions of Wistar rats. **Materials and Methods:** In this experimental study 32 male Wistar rats were designated into 4 equal groups, including: acetyl choline, KCl, extract plus acetyl choline and the group which received extract plus KCl. The isotonic contractions of ileum (induced by 60 mM KCl or 1 μ M acetyl choline) in tyrode solution were recorded, under 1 gr tension. Then, the effects of normal saline or extract (1%) were evaluated. The percentage changes were calculated and compared in different groups using ANOVA and Tukey tests.

Results: The mean of ileum contractions in acetyl choline and KCl groups were 18.83 ± 4.91 and 18.31 ± 11.12 ($p=0.5$). The percentage of contraction relieve in extract plus acetyl choline group was 53.16 ± 12.06 and in extract plus KCl group was 62.96 ± 11.08 ($p=0.4$). The contractions in extract groups were significantly less than acetyl choline or KCl groups ($p<0.05$).

Conclusion: The results indicate that *Achillea millefolium* extract inhibits ileum contractions. Therefore, it might be used in patients to reduce ileum spasms.

Implication for health policy/practice/research/medical education:

Achillea millefolium possesses relaxation effect on ileum contraction activity and might be used in gastrointestinal spasms.

Please cite this paper as: Sedighi M, Nasri H, Rafeian-kopaei M, Mortazaei S. Reversal effect of *Achillea millefolium* extract on ileum contractions. J HerbMed Pharmacol. 2013; 2(1): 5-8.

Introduction

Gastrointestinal disorders the most common problems affecting general population. Synthetic drugs are present, however, with low efficacy and high side effects.

Medicinal plants have been recently been considered as reliable sources for drug preparation. Significant results are also presented from clinical (1,2) and nonclinical (3-5) researches and they have shown positive effects of these plants in different diseases. Meanwhile, medicinal plants show usually few adverse drug reactions from themselves although their possible adverse drug reaction also should be considered (6-8). *Achillea millefolium* L. is one of the plants that are traditionally used to treat gastrointestinal disorders in Chaharmahal and bakhtiyari province. *Achillea* is a genus of about 110-140 species. One of the species is *Achillea millefolium*. *Achillea millefolium* is a plant which is grown in many regions specially Asia and Europe (9). It has straight stems, yellow flowers with pleasantly fragrant

smell and thin leaves which are highly dissected. Used parts are flowering twigs which has bitter flavor and sharp smell and are gathered in flowering time in summer (10,11). The most important compounds available in this part are essential oil, polyphenol compounds and some of flavonoides such as Apigenin, Quercetin, Borneol, fatty acids, Sesquiterpenes, lactone, betaine, Luteolin, Cineol, Linalool and tannins (12). *Achillea millefolium* plant has useful applications in different cases such as hemorrhage control, menstrual disorder, treating nighttime urinary incontinence; ascaris worm excretion, acne and insomnia control (13,14). *Achillea millefolium* has effect on heart and nervous systems and is used in different problems like general fatigue, heart failure, kidney stone and also nervous diseases like neurasthenia, hysteria, epilepsy, hysterogenic colic (15). Tozy *et al.* have proved in their studies the antibacterial, anticancer and anti-inflammatory effects of this plant (16). Aerial parts of *Achillea millefolium* containing high amounts of

*Corresponding author: Prof. Mahmoud Rafeian-kopaei, Medical Plants Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran. E-mail: rafeian@skums.ac.ir

flavonoides have antioxidant and anti-inflammatory effect (17). Colberg *et al.* extracted a compound from *Achillea millefolium* which decreased inflammation up to 35% in mice (18). Khori *et al.* showed that methanolic extract of this plant could decrease relative activity of atrioventricular node (19). It was shown in other study that achillea, chamomile and crataegus hawthorn plants could increase the red blood cells resistance to oxidizing substances (20). This plant is used to treat gastrointestinal diseases in Chaharmahal and bakhtiyari province but no research has been done on the effect of *Achillea millefolium* alcohol extract on function of smooth muscles such as gastrointestinal tract. This research aims to determine in vitro effect of plant on locomotor activity of ileum in Wistar rats.

Materials and Methods

Extraction

Achillea millefolium was prepared from Hafshejan located in Chaharmahal and Bakhtiyari province and confirmed in the Herbariorum Unit of Shahrekord University of Medical Sciences. A herbarium sample was provided from it and deposited there (No: 285). Then flowering twigs prepared from plant were maintained in suitable (dark and dry) environment and dried completely. After drying and grinding, they ground to powder. Then 50 gram of the plant powder was soaked in ethanol alcohol for 72 hours and was filtered by Büchner funnel. The obtained solution was put in a rotary evaporator to evaporate its solvent in 35°C. Finally the solution was poured into a watch glass and then was put in incubator. Obtained extract powder was maintained in refrigerator until it was used and 1% fresh solution was produced and used in the experiment day.

Animals

Twenty four male Wistar rats (200–250 gram) were obtained from Shahrekord University of Medical Sciences and maintained under 12-hour dark/12-hour light condition. Rats had free access to water and food.

Ileum preparation

Each rat was anesthetized by chloroform in the experiment day. Then, a 1.5–2 cm part was cut from the end of ileum and immediately put into a glass containers containing oxygenated Tyrode solution in laboratory temperature. Then the sample was transferred into organ bath containing oxygenated Tyrode solution at 37°C and pH 7.4 and was placed vertically between two stainless steel clasps. Upper clasp was attached to isotonic transducer (Harvard, UK) by thread and then from there to a physiograph device (Harvard Universal Oscillograph, UK). 1 gram weight was hung in front of transducer axel to provide first tension in tissue. Before starting the experiment, the Ileum was maintained in organ bath for 1 hour to be adjusted with new condition and the Tyrode solution was changed every 15 minutes. Tyrode solution at mM was as below:

NaCl (136), KCl (5), CaCl₂ (2), NaHCO₃ (11.9), MgCl₂ (0.98), NaH₂PO₄ (0.36) and glucose 5.55. In the end of compatibility period the ileum was contracted by potassium chloride (60 mM) (21) and acetylcholine (1 µg/ml) (21). By reaching the contractions to plateau state, the extract of *Achillea millefolium* (1%) or saline was added to organ bath and finally the related responses were recorded by physiograph device on paper and the percent of changes in the contraction force compared to the plateau state of contraction were measured and recorded as

Mean± SEM. Statistical analysis was done by ANOVA test and p<0.05 was considered as statistically difference.

Results

Effects of hydroalcoholic extract of *Achillea millefolium* on ileum contractions of Wistar rats resulted from potassium chloride and acetylcholine.

In all experiments potassium chloride (60 Mm) and acetylcholine (1µ g/ml) increased ileum contractions. The mean of contractions provided by acetylcholine and potassium chloride was obtained as 18.83±4.91 and 18.31±11.12, respectively (p>0.05). Contractions developed to plateau after a short time. Adding 1% extract to organ bath decreased the ratio of contractions resulted from acetylcholine and potassium chloride. The mean percentages of ileum contraction relieve in extract plus potassium chloride and in extract plus acetylcholine was obtained as 59.96±11.8 and 54.16±12.06, respectively (p<0.05).

Discussion

The aim of this research was to evaluate the alcoholic extract of *Achillea millefolium* on locomotor activity of ileum. As it was observed in results section, alcohol extract of *Achillea millefolium* decreased ileum contractions resulted from acetylcholine and potassium chloride. Smooth muscle membrane contained more voltage-dependent calcium channels than skeletal muscle but contained less voltage-dependent sodium channels. Generally, flow of calcium ions via slow calcium-sodium channels into fiber is influenced by potassium chloride which is cause of contraction phenomenon (23). So, substances which control the contractions resulted from potassium chloride in smooth muscle are defined as blockers of voltage-dependent calcium channels (24). Acetylcholine decrease the M4 by effect on muscarinic receptors and increase the potassium by effect on cAMP receptors and activation of calcium channels, M3 muscarinic and increase in internal calcium and also effect on nicotinic receptors, opening of ion channels and providing depolarization cause contraction phenomenon (25). Observed control effect of *Achillea millefolium* extract in stimulated tissue (by acetylcholine and potassium chloride) was not completely reversible by washing tissue and changing bath solution but was decreased. Relative decrease in extract effects after washing tissue is possibly resulted from removing extract from surface of receptors which were reversibly bandaged with extract (26). Also results related to long term contraction of ileum by *Achillea millefolium* and acetylcholine without using extract determined that ileum could remain in contraction state as long term without any decrease in contraction force and fatigue. So, contraction force decrease in this research is caused by extract performance not muscle fatigue. Increase of calcium and potassium inside cell is controller factor of smooth muscle tension in gastrointestinal tract (27,28). It could be found that extract has prevented normal effects of calcium and potassium. Likely, effective substances of the present extract used the same method to control contraction. Apigenin is one of the plant flavonoides and its antispasmodic effect has been reported (29). In a research conducted by Gharib Naseri *et al.* on normal celery, inhibitory effect of ileum was attributed to flavonoides of plant apigenin (30). Effect of apigenin available in celery caused aortic-endothelium dependent relaxation (31). So, it is possible that observed anti-contraction effects in this research be resulted from apigenin flavonoides available in *Achillea millefolium*

extract. Also, Quercetin is another flavonoid compound of the plant which possesses medicinal properties and help to decrease blood pressure in affected patients (32). Anti-contraction effects of this compound on small intestine movements and also its calcium antagonist property have been proved (33). The flavonoid Quercetin in aorta causes endothelial-dependant relaxation. Also anti-contraction effects of flavonoides in smooth muscles in vessel wall (34) and on ileum contraction in Guinea pig have been reported (35). Flavonoides available in *Althaea officinalis* which is available in other plants provide endothelial-dependant relaxation or independent in human arteries (36). Luteolin by preventing increase in calcium release from Sarcoplasmic reticulum, decreasing intercellular calcium and activation of potassium channels relax aorta smooth muscle (37). Also, antispasmodic property of carvacrol, another plant compound, on tracheal of guinea pigs (38) is another reason that proves *Achillea millefolium* compounds, especially flavonoides compounds, have effect on smooth muscle relaxation.

Conclusion

Hydroalcoholic extract of *Achillea millefolium* has relaxation effect on ileum contraction activity, likely related effect could be attributed to flavonoids properties of plant specially quercetin and apigenin.

Authors' contributions

All contributed doing the study, MS, SM prepared the draft, HN, MRK edited the final version of the manuscript.

Conflict of interests

The author declared no competing interests.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the author.

Funding/Support

This study was financially supported by Research deputy of Shahrekord University of Medical Sciences, Shahrekord, Iran. The article was derived from the MSc thesis of the first author.

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