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Original Article

Evaluation of the effects of *Astragalus fasciculifolius* hydroalcoholic extract on rat ileum contractions: The role of adrenergic, opioid, and nitric oxide receptors

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Abstract

Background and aims: This study aimed to determine the effects of *Astragalus fasciculifolius* hydroalcoholic extract on rat ileum contractions: The role of adrenergic, opioid, and nitric oxide (NO) receptors.

Methods: *A. fasciculifolius* extract was prepared using 70% alcohol by maceration extraction. 2 cm pieces from the end of the rat ileum were placed in a tissue bath containing Tyrode's solution. The tissue bath solution is constantly oxygenated. Potassium chloride (KCl), naloxone, N^{ω}-nitro-L-arginine methyl ester (L-NAME), propranolol, and different concentrations of calcium chloride were used to stimulate the ileum. Ileum contractions were recorded isotonically under 1 g of initial tension. The results were calculated as the amount of shortening (in millimeters).

Results: This study showed that *A. fasciculifolius* extract caused a significant increase in ileum contraction compared to the control group ($P<0.001$). This increase in contraction was amplified in combination with opioid or NO receptor antagonists ($P<0.001$). Also, this effect was not significantly different in association with beta-adrenergic receptor antagonists.

Conclusion: The results of this study showed that *A. fasciculifolius* extract enhances the contractile function of the ileum in rats. This function is reinforced by opioid receptor antagonists and NO.

Keywords: Constipation, *Astragalus fasciculifolius*, Tissue bath

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Introduction

Constipation is still considered a common and essential problem because it harms the patient's life quality and costs the family economy and the health system (1). The problem with constipation is the difference in definition between patients and medical staff (2). There needs to be a match between the dimensions that the general public considers constipation, and health staff community therapy considers constipation (3,4). In general, constipation refers to dissatisfaction with defecation and indicates abnormal bowel function (3). Chronic constipation is prevalent and affects about 15% of people in the United States (5). In 2006, the number of referrals to a physician about constipation was 5.7 million, of which 2.7 million were constipated as a primary diagnosis (6). Constipation is usually multifactorial, but these causes can be divided into two main groups: primary constipation and secondary constipation (7). Adrenergic receptors are members of the G protein-binding receptor family, and stimulation of beta-adrenergic receptors relaxes smooth muscle (7). Also, nitric oxide (NO), an essential non-cholinergic non-adrenergic neurotransmitter, regulates smooth muscle contractile activity and induces a relaxation process in many tissues (8-10). On the other hand, endogenous opioid peptides and opioid agonists control many gastrointestinal functions, including movement, secretion, and transfer of electrolytes and fluids, by activating three significant groups of opioid receptors: opioid receptors: delta, kappa, and mu (11,12). The process of diagnosing constipation is based on examining the patient's history of problems with the main complaint; For example, in addition to constipation, the patient also suffers from hemorrhoids, which is the cause of constipation. A patient with constipation may be completely asymptomatic or complain of gas accumulation in the abdomen, pain during defecation, rectal bleeding, false diarrhea, and back pain (7,13).

Treatment of constipation in the first step includes:

- Stopping medications that cause constipation,
- Increasing the amount of fiber in the daily diet, and
- Using osmotic laxatives for a short time.

Constipation-related diseases should also be identified and treated (14). Today, herbs significantly contribute to traditional medicine in many countries and are essential in modern therapeutic approaches (15). *A. fasciculifolius* plant from the Fabaceae family is a valuable medicinal

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species that has compounds such as saponins, flavonoids, and polysaccharides that play an influential role in treating heart diseases, inhibiting the growth of cancer cells and eliminating the effects of chemotherapy (16). The studies conducted on *A. fasciculifolius* manna have shown that this plant has an abortifacient effect by reducing the progesterone level (17). Also, it can prevent pain and inflammation due to a high amount of saponin (18).

People previously believed that *A. fasciculifolius* could help relieve constipation. *A. fasciculifolius* has properties similar to tragacanth and creates a colloidal and viscous solution due to water absorption (19,20). In the conducted studies, there has been no study on the effects of this plant extract on the contractile activity of the intestine.

In previous studies, for example, the effect of flavonoids extract of parsley leaves on the contraction of the rat ileum caused by KCl was investigated, and the results showed that this extract relaxes the ileum and this action is done through the NO system in higher concentrations (21).

Due to the unavailability of the results of the effects of this plant on the smooth muscle of the ileum and, on the other hand, its traditional use in digestive disorders, this research aims to determine the effect of the hydroalcoholic extract on the contractions of the rat ileum and investigate the role of adrenergic, opioid and NO receptors was done.

Materials and Methods

Preparation of A. fasciculifolius hydroalcoholic extracts

Propranolol hydrochloride, naloxone hydrochloride dehydrate, and N-Nitro-L-arginine methyl ester hydrochloride (L-NAME) were purchased from Sigma Aldrich USA. *A. fasciculifolius* manna was purchased from a local Naturopathic store in Shahrekord. After the approval of a botanist, it was registered in Shahrekord Medicinal Plants Research Center with herbarium no. 721. After powdering, 70% was soaked in ethanol for maceration extraction. After filtering the extract, a rotary vacuum distillation device removed the solvent (22). All chemicals used had a standard analytical grade.

Animals and treatment

Sixty female Wistar rats weighing 180-200 g were purchased from the Institute Pasteur Research and Production Complex laboratory animal sciences department in Tehran. Standard water and food were freely available to the rats. Rats were kept in standard conditions and 12 hours of darkness and 12 hours of light and plastic cages of animals in the animal nest of Shahrekord University of Medical Sciences. The study was conducted according to the instructions of the University Ethics Committee.

Experimental design

Rats were deprived of food 24 hours before the experiment but had free access to water and were randomly selected and divided into six groups of 10:

1. Control group: ileum in a tissue bath containing potassium chloride
2. Extract: cumulative concentrations of the extract between 0.0625 and 0.5 mg/mL
3. Propranolol + Extract: Propranolol 1 µM + cumulative concentrations of the extract between 0.0625 and 0.5 mg/mL
4. Naloxone + Extract: Naloxone 1 µM + cumulative concentrations of the extract between 0.0625 and 0.5 mg/mL
5. L-NAME + Extract: L-NAME 100 µM + cumulative concentrations of the extract between 0.0625 and 0.5 mg/mL
6. CaCl2 + Extract: high potassium chloride (60 mM) + cumulative concentrations of calcium chloride (0.5 to 8 mM)

On the day of the experiment, each rat was anesthetized with chloroform. Then, by cutting the abdomen from the midline, four pieces of 1.5 to 2 cm length were cut from the end of the ileum (except the last 2 cm). The separated pieces were immediately placed in glass containers containing oxygenated Tyrode's solution at laboratory temperature (23). The specimens were then transferred to a tissue bath (containing 50 cc of oxygenated Tyrode's solution at 37 °C) and placed vertically between two stainless steel hooks. The upper hook was attached to the isotonic transducer and, from there, to the physiography. A one-gram weight was hung on the opposite side of the transducer lever to provide initial traction to the tissue. The ileum tissue was kept in a tissue bath for one hour before the experiment to adapt to the new conditions. It was oxygenated and fed with fresh Tyrode's solution with a constant flow of air bubbles into the bath every 15 minutes. The composition of Tyrode's solution in mill moles was as follows:

(136) NaC1, (5) KCl, (2) CaCl2, (9/11) NaHCO3, (98/0) MgCl, (36/0) NaH2PO4, (55/5) glucose.

After compatibility, potassium chloride (60 mM) was added to the tissue bath to shrink the ileum (15). Then the cumulative extract concentrations (0.0625 to 0.5 mg/mL) were added to the bath. In order to investigate the involvement of beta-adrenergic and opioid receptors, new ileum fragments were immobilized for 30 minutes with a beta-adrenergic antagonist (propranolol 1 µM) or an opioid receptor antagonist (naloxone 1µM). Tissue shrinkage and anti-contractile performance of the extract were recorded. Also, in Tyrode's solution without calcium but with high potassium chloride (60 mM), the ileum was contracted by adding cumulative concentrations of calcium chloride (0.5 to 8 mM). The same steps were repeated after incubating the tissue (3 minutes) with a specific extract concentration. Each tissue was affected by only one stimulant and one antagonist (24). Contraction force changes were calculated (including 100% contraction caused by the stimulus) as a percentage of contraction changes in each research stage as mean ± SEM.

Statistical analysis

Statistical analysis of data was performed using GraphPad

Prism 8 software. Then, a one-way ANOVA and Tukey post hoc test were used to determine the significant differences between the treatments. Data were recorded as mean and standard deviation, and $P < 0.05$ was considered statistically significant.

Results

The one-way ANOVA analysis results showed a significant difference between the groups in this study ($P < 0.001$). The following results were obtained by pairwise comparison between the studied groups using the Tukey test.

Results of determining the effect of A. fasciculifolius on ileum contractions of the studied groups

There was a statistically significant difference between the control group (ileum in a tissue bath containing potassium chloride) and the group receiving *A. fasciculifolius* extract (cumulative concentrations of the extract between 0.0625 and 0.5 mg/mL) ($P < 0.05$). The rate of contraction increased. This effect was in line with the widespread belief that our contractile effect was *A. fasciculifolius*, acting as a laxative (Figure 1).

The results of determining the effect of A. fasciculifolius on ileum contractions in the presence of beta-adrenergic antagonist (propranolol) in the studied groups

Because stimulation of adrenoceptors reduces the mechanical activity of the ileum, at this stage, it was tried to prevent the occurrence of this possible effect by using the antagonist of these receptors (propranolol). There was no statistically significant difference between the group receiving *A. fasciculifolius* extract (cumulative concentrations of the extract between 0.0625 and 0.5 mg/mL) and the group receiving *A. fasciculifolius* extract with the beta-adrenergic antagonist (propranolol) at a dose of 1 µM ($P > 0.05$).

The results of determining the effect of A. fasciculifolius on ileum contractions in the presence of opioid receptor antagonists (naloxone) in the studied groups

All three groups of opioid receptors are present in the neuronal tissue of the rat intestinal nervous system. In addition, opioids inhibit smooth muscle contractions caused by electrical stimulation. Therefore, at this stage, it was tried to prevent the occurrence of this possible effect by using the antagonist of these receptors (naloxone). There was a significant difference between the group receiving *A. fasciculifolius* extract (cumulative concentrations of the extract between 0.0625 and 0.5 mg/mL) and the group receiving *A. fasciculifolius* extract with opioid receptor antagonist (naloxone) at a dose of 1 µM ($P < 0.05$). Furthermore, the rate of contraction increased. This result may indicate the antagonistic effect of *A. fasciculifolius* on opioid receptors.

The results of determining the effect of A. fasciculifolius on ileum contractions in the presence of L-NAME NO receptor antagonist in the studied groups

Nitric oxide relaxes the ileum in rats, which can directly affect the smooth muscle of the small intestinal wall. Therefore, at this stage, we tried to prevent this possible effect by using the antagonist of these receptors (L-NAME). In comparison between the group receiving *A. fasciculifolius* extract (cumulative concentrations of the extract between 0.0625 to 0.5 mg/mL) with the group receiving *A. fasciculifolius* extract with L-NAME NO receptor antagonist at a dose of 100 µM, there was a statistically significant difference ($P < 0.05$). Therefore, the rate of contraction increased (Table 1). This result could indicate the antagonistic effect of *A. fasciculifolius* on NO receptors (Figure 2).

Results of determining and comparing the effect of A. fasciculifolius on ileum contractions in the presence of different concentrations of calcium ions

There was a statistically significant difference between the group receiving *A. fasciculifolius* extract (cumulative concentrations of the extract between 0.0625 to 0.5 mg/mL) and the group receiving *A. fasciculifolius* extract with different concentrations of calcium ions ($P < 0.0001$). This result could indicate the effectiveness of *A. fasciculifolius* by facilitating the opening of calcium receptors. The above results can also be seen in Figure 3.

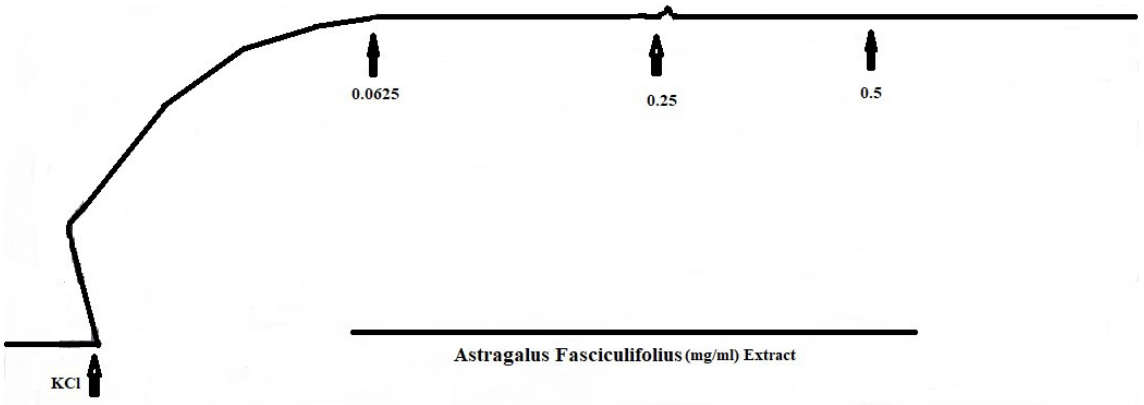

Figure 1. An example of the actual recording of the effect of different concentrations of *A. fasciculifolius* on ileum contractions of rats in response to adding potassium chloride: The arrow on the left shows the time of adding potassium chloride, and the arrow on the right shows the time of adding extract

Table 1. Comparison of the contraction values of the ileum in the groups receiving saline, extract, naloxone, and propranolol

	Groups					
	Control	Extract	Naloxone + Extract	Propranolol + Extract	L-NAME + Extract	CaCl2
% Contraction	0	1.63	2.155	1.6	2.698	2.76
% Contraction	0	1.0652	3.122	2.78	2.1033	2
% Contraction	0.523	1.342	3.78	1.853	3.459	3.01
% Contraction	0.432	2	2.3	1.78	2.831	3.15

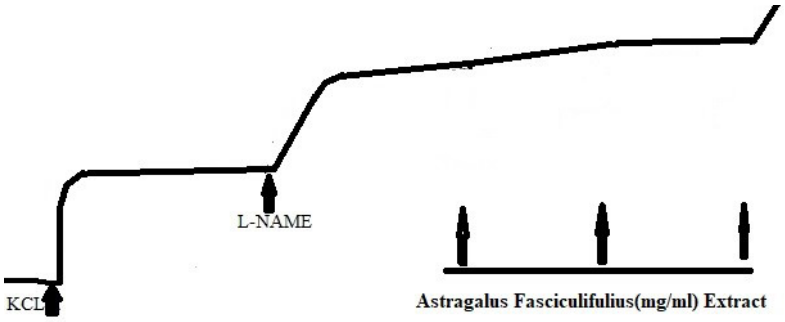

Figure 2. An example of the actual recording of the effect of different concentrations of *A. fasciculifolius* on ileum contractions of rats in response to adding potassium chloride and the presence of nitric oxide (NO) receptor antagonist. The arrow on the left shows the time of adding L- NAME, and the arrow on the right shows the time of adding extract

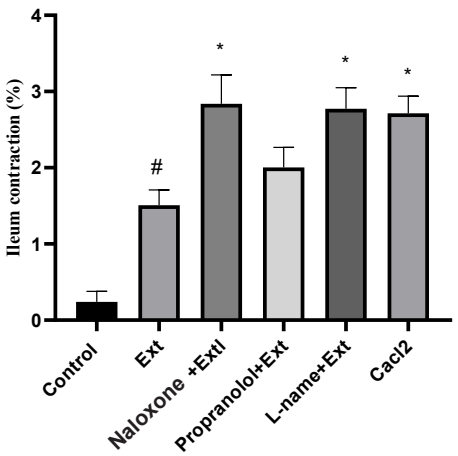

Figure 3. Results of intergroup comparison of the contractile effect of *A. fasciculifolius* on the ileum of the studied groups. Ext: Extract and CaCl2: Calcium chloride. # $P<0.01$ compared to the control group; * $P<0.001$ compared to the extract group

Discussion

This study is the first to investigate the effect of *A. fasciculifolius* extract on rat ileum contractions. The present study results align with the view of folk medicine, which introduces *A. fasciculifolius* as a laxative and enhancer of bowel movements (19). The present study results also show that this plant enhances intestinal contraction through several mechanisms other than adrenergic, opioid, and NO pathways. Many studies have been conducted on the aqueous and alcoholic extracts of various plants such as *Allium cepa* (25), *Teucrium polium* (26), and *Apium graveolens* (27) on the contractile activity of the ileum and have shown that their extracts inhibit the contractions of the ileum.

This study showed that the contractile function of *A. fasciculifolius* might be due to the facilitative effect on the opening of calcium receptors. Potassium chloride is one of the most well-known causes of gastrointestinal smooth muscle contraction, which causes voltage-dependent calcium channels to open by depolarizing cells and activates Ca^{2+}-dependent myosin light chain (MLC) kinase, and an increase in MLC phosphorylation (28,29). The Enhancing Effect of *A. fasciculifolius* extract on potassium chloride contraction can confirm the presence of substances in the extract that enhance the opening of voltage-dependent calcium channels or may increase the sensitivity of the contractile system to Ca^{2+} (30).

The main compounds of the *Astragalus* include glycosides, saponins, flavonoids, and polysaccharides (16). In previous studies, it has been found that the flavonoid obtained from the Astragalus has a spasmolytic effect on the smooth muscles of the gastrointestinal tract (31). So this contraction effect is not related to flavonoids.

Because stimulation of adrenoceptors in the ileum inhibits contraction (7), therefore, it may be hypothesized that the present extract contains inhibitory compounds of these receptors, but propranolol (beta-adrenergic antagonist) did not significant change the number of contractions caused by the extract.

Our results showed that the presence of NO and opioid receptor antagonists enhances the contraction effect of the extract. Nitric oxide relaxes the ileum in rats, which can directly affect the smooth muscle of the small intestinal wall (8-10). Using L-NAME (NO synthesis inhibitor) increased the contraction caused by the extract. The studies conducted on the role of NO in the relaxation effect of three plants, *Anethum graveolens* (32) *and Rosmarinus officinalis* (33), resulted in no involvement of NO. However, in the *Ruta chalepensis* leaf (34) extract, the role of NO in the relaxing effect of the extract was evaluated positively.

All three groups of opioid receptors are present in the neuronal tissue of the rat intestinal nervous system. In addition, opioids inhibit smooth muscle contractions induced by electrical stimulation (11,12). Using naloxone (an opioid receptor blocker) increased the contraction rate of the extract. Considering that NO and opioid receptors cause relaxation of smooth muscles, the extract probably caused ileum contraction by reducing NO and inhibiting opioid receptors. On the other hand, the results showed that the use of antagonist naloxone and L-NAME amplified the contractile response of the extract. It seems that the extract performed this contraction action without the involvement of NO and opioid receptors.

In the last part of the study, it was observed that the contraction effect of the extract increased by adding calcium chloride. This result could indicate the effectiveness of A. fasciculifolius by facilitating the opening of calcium receptors.

In this study, the role of acetylcholine was not investigated, but it is considered one of the essential neurotransmitters in muscle contraction (26). Acetylcholine also increases intracellular calcium concentration after binding to M2 and M3 muscarinic receptors in two ways. First, activating these receptors and, with the intervention of inositol triphosphate (IP3), causes calcium release from intracellular sources. Second, acetylcholine facilitates extracellular calcium entry through the receptor-operated calcium channel. *Astragalus* hydroalcoholic extract may have increased ileum contractions by affecting the amount of acetylcholine (26).

The results of the present study showed the effect of *A. fasciculifolius* extract on rat ileum contractions. The effectiveness of this extract was to increase the rate of contraction in this tissue, and the presence of opioid receptor antagonists and NO enhanced this increase in contraction. These results align with widespread belief in the past that *A. fasciculifolius* can help relieve constipation. To evaluate the effectiveness of receptors involved in this process, further studies with the simultaneous use of antagonists and receptor agonists that are effective in bowel movements will be helpful. Still, our study could have been more extensive in this regard. The effect of this extract on other body systems, including the cardiovascular system, renal filtration, liver markers, and brain function, requires further studies, and in general, there is a long way for researchers to use this substance in the clinic as a laxative.

Conclusion

This study showed that *A. fasciculifolius* extract enhances the contractile function of the ileum in rats, which is enhanced in the presence of opioid receptor antagonists and NO.

Authors' Contribution

Conceptualization: Najmeh Asgharzadeh.
Data curation: Parisa Zamani, Elham Bijad.
Formal analysis: Mehrdad Shahrani-Korani.
Funding acquisition: Mehrdad Shahrani-Korani.
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Project administration: Parisa Zamani, Elham Bijad.
Resources: Parisa Zamani.
Software: Najmeh Asgharzadeh.
Supervision: Fatemeh Azizi.
Validation: Hossein Amini-Khoei.
Visualization: Najmeh Asgharzadeh.
Writing–original draft: Mehrdad Shahrani-Korani.
Writing–review & editing: Fatemeh Azizi.

Competing Interests

None.

Ethical Approval

All procedures were carried out per the regulations of the University and the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (Ethics code: IR.SKUMS.REC.1397.113) and Guide for the Care and Use of Laboratory Animals (8th edition, National Academies Press). Full efforts were made to reduce the use of animals and to advance their welfare.

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