IRSTI 76.29.29

[1]H. Teimouri, [1]M. Rezaei, [1]S. Abbaszadeh, [1*]M. Azadpour

[1]Lorestan University of Medical Sciences,
Khorramabad, Iran, *e-mail: Mojganazadpour@yahoo.com

The effects of aqueous extracts of *Cuminum cyminum* L. and *Pimpinella anisum* L. seeds on the improvement of irritable bowel syndrome compared with Loperamide in rats

Abstract: Irritable bowel syndrome (IBS) is a common disorder of the digestive system. In this study, the effects of aqueous extracts of *Cuminum* (*C.*) *cyminum* L. and *Pimpinella* (*P.*) *anisum* L. seeds in improving IBS in rats were studied in comparison with Loperamide, selective drug frequently used for the treatment of IBS in Iran. For induction of IBS, rats were anesthetized 24 hours after fasting, after which 1 ml of acetic acid was injected intrarectally at a point 8 cm from the proximal rectum, the intestine was stimulated and 1 ml of phosphate buffer was injected into the same point to dilute acetic acid. Healing process was studied in five groups: control, false control, positive control, aqueous *C. cyminum* extract-treated and aqueous *P. anisum* extract-treated. *C. cyminum* extract at 300 mg/kg reduced the movements of the gastrointestinal tract significantly, when compared to the control group. To analyze the mean differences between the groups, statistical analysis was performed by the Statistical package for the social sciences (SPSS) using continuous multivariate method. P values of less than 0.05 were regarded as statistically significant. According to the results, the most substantial improvement was observed in aqueous *C. cyminum* extract-treated group, which allows us to propose its advantage compared to other treatments. Probably one of the reasons for the beneficial effect of these plants on IBS is the presence of antioxidant substances in these plants.
Key words: irritable bowel syndrome, plant seeds, *Cuminum cyminum* L., *Pimpinella anisum* L., Loperamide.

Introduction

Gastrointestinal disease is one of the most important and common chronic noncommunicable diseases. Gastrointestinal disease imposes a heavy burden on society and healthcare system in developed and developing countries [1; 2]. Among the types of gastrointestinal disorders, recurrent abdominal pain and irritable bowel syndrome (IBS) have been more frequently addressed [1]. Small intestine is the site of the final digestion of food, absorption of nutrients and secretion of hormone. This hollow tube is composed of four layers: mucosa, submucosa, muscularis and serosa [2-4]. The bowel may develop a number of disorders, including IBS. Although the causes of IBS are not definitely known, diet, intestinal infections and psychiatric disorders are risk factors for this disorder. The frequency of depression, anxiety and other major psychiatric disorders is high in people with IBS [5]. Diagnosis of this disease is made based on symptoms such as chronic pain and changes in bowel habits in the absence of any organic cause [6]. IBS accounts for 12% of referrals to gastroenterologists in Europe [7]. The pathophysiology of this disease remains unknown. Genetic and environmental factors are likely to contribute to developing the disease [8]. Some studies have reported abnormal movements of the gastrointestinal tract, visceral irritability, psychological impairment, and emotional stress to be involved in IBS development [7; 8]. Stress, anger, intravenous injection of cholecystokinin and colonic perfusion with deoxycolic acid have also been reported to increase bowel movements in patients with IBS. Increased sensitivity of the bowel due to psychological factors, causing pain and emergency in defecation after balloon expansion in the rectum [9], and increased brain cortical activity after rectal dilation [10] are also observed in IBS. These findings reveal the relationship between the psychological factors and the sensitivity of the visceral neurotransmitters. The brain mediators that have been used include bradykinin, tachykinin, serotonin,

calcitonin gene-related peptides, and neurotrophins [11]. Most patients with IBS suffer from anxiety and depression, and patients with IBS show more severe psychosis than healthy people [12].

The prevalence of IBS in the United States is 12%, and its prevalence is between 3% and 20% in Europe and in the rest of the world with comparable prevalence rates in other parts of the world, but the actual prevalence rate is estimated to be comparatively higher, as this prevalence is reported for clients referring to health centers, while in fact, many patients do not refer to the centers [13; 14]. It has been shown that negative emotions and emotional states such as anger are associated with a decrease in intestinal motor activity in IBS patients. These patients report more stressful events in their daily lives and also throughout their lives than healthy people and may be more sensitive to stress-induced gastrointestinal function changes [15]. The approach to using plant compounds for the production of new drugs that are more effective, stronger and more compatible with the body of living beings is on the agenda of researchers in the pharmaceutical, medical and veterinary sciences, which, given the public interest in herbal drugs and the potentials of plants, such research seems essential. One of the methods that have recently been considered for treatment of IBS is the use of herbal drugs and medicine. Study showed turmeric consumption significantly improved IBS symptoms, especially abdominal pain, and improved quality of life [13].

The use of medicinal plants is common in traditional Iranian medicine. Traditionally, *Cuminum (C.) cyminum* L. and *Pimpinella (P.) anisum* L. are used to treat gastrointestinal disorders. *P. anisum* is a perennial and high plant from the *Umbeliferae* family that is very fragrant and has pink-shaped green, and small seeds. The upper part of the plant is sharp with five striking lines easily observed on it. The most important ingredient of the plant is its essential oil, which accounts for 1.5-5% of the plant. The most important compound in the essential oil is the trans-anethole (80-90% of the essential oil). Coumarins are other important compounds of *P. anisum* [19]. *P. anisum* seeds are used as an analgesic in migraine, and are traditionally used as a fragrant, disinfectant and diuretic agent, and the extract obtained from its distillation also serves these purposes. It has also antibacterial, antispasmodic, anti-inflammatory, digestive, hepatotonic, laxative, sedative, stimulant and gastrotonic properties. The pharmacological effects of *P. anisum* are more closely related to the anethole in its essential oil, whose formulation is similar to those of catecholamines (including adrenaline, adrenaline and dopamine) [20]. Green cumin, *C. cyminum* L., belongs to the *Umbeliferae* family, and is an aromatic plant. Its major ingredients include sabinene, flavonoids, polysaccharides, coumarin, cuminaldehydes, pinene and terpinene, vitamins B_1, B_2, B_3, B_9, C, E, and K, as well as various minerals such as calcium, iron, magnesium, phosphorus, potassium and sodium. Green cumin has a very warm and dry nature. In the treatment of gastrointestinal diseases, *C. cyminum* acts as an anti-inflammatory agent and facilitates digestion, and is also used in pulmonary diseases for the treatment of cough [21]. Cumin, of any kind, serves as a tonic and energizing agent for the body. These properties are enhanced when cumin is mixed with honey [22]. *C. cyminum* is effective for the treatment of gastrointestinal disorders, gastrointestinal tract weakness, bloating, and heartburn. In fact, consuming *C. cyminum* will strengthen the stomach. It is the strongest herbal carminative agent and reduces the spasm of the intestinal smooth muscles [22].

Cumin plant contains such compounds as sabine, flavonoids, polysaccharides, coumarin, cumin aldehyde, pinen and terpinene, which have antioxidant properties. *P. anisum* has flavonoids and phenols that have antioxidant properties [19-21]. Therefore, this study was conducted to investigate effects of *C. cyminum* and *P. anisum* seed aqueous extracts on the improvement of IBS in rat model.

Materials and methods

Loperamide (Eksir-danesh Co., Iran) is a type of opioid receptor agonist, used to treat symptomatic acute diarrhea (especially gastroenteritis, inflammatory bowel disease, and irritable bowel syndrome). It affects the onset of the middle cerebral nervous system of the intestine, decreasing the smooth muscle length of the intestine, thereby slowing down the intestinal activity and thus decreasing the amount of water and salt in the body.

Extraction. Plant material for study was obtained from the Agricultural Research Center of Lorestan province, Khorramabad city, southwest of Iran. The aqueous extract was prepared by maceration from plant material dried at 23±2°C. For this purpose, 100 g of seeds were weighed, put in 100 ml of water (so that the seeds were completely under the water surface), poured into a large Erlenmeyer flask and placed on a heater to boil. Then, they were boiled for 15 minutes, cleaned with a clean cloth, filtered with

filter paper, and poured into a crystallizer and dried on a Bain Marie. Due to the long drying time of the extract, exceeding 72 hours, it was stored in the refrigerator during the drying intervals. Drying continued until the weight of the extract became constant. After preparation and drying, the lid of the extract container was covered with aluminum foil and the container placed in the refrigerator until subsequent tests.

Studied animals and experiment procedure. In this study, 40 male Wistar rats weighing 220-250 g were used. They were collected from the Animal House of Razi Herbal Medicines Research Center of Lorestan University of Medical Sciences, Khorramabad city, Lorestan province, Iran. Rats were housed in the animal room under controlled conditions with respect to temperature and light, with free access to water and food as follows: 12/12 hours of light and darkness, standard food and water, 40±10 % r.h., 22±2 °C.

Induction of IBS. For induction of IBS, rats were anesthetized with ether after 24 hours fasting, after which 1 ml of 4% acetic acid was injected intrarectally at a point 8 cm from the proximal rectum, the intestine was stimulated and 1 ml of phosphate buffer was injected into the same point to dilute acetic acid. The protocol of this study fully conformed to the ethical principles regarding the use of laboratory animals in research.

Animals were randomly divided into 5 groups:

– control group: after stress induction, normal saline was intrarectally injected to rats;

– false control group: after stress induction, acetic acid was intrarectally injected to rats and distilled water (10 mg/kg) was orally administered (by gavage) for 26 days (21 days before stress induction until 4 days after);

– positive control group: Loperamide (10 mg/kg) was orally administered (by gavage) for 26 days (21 days before stress induction until 4 days after);

– *P. anisum* extract-treated group: aqueous *P. anisum* seed extract (150 and 300 mg/kg) was orally administered (by gavage) for 26 days (21 days before stress induction until 4 days after);

– *C. cyminum* extract-treated group: aqueous *C. cyminum* seed extract (150 and 300 mg/kg) was orally administered (by gavage) for 26 days (21 days before stress induction until 4 days after).

Measuring bowel movements. The rats were separately exposed to immobility stress (at certain times) and the number of stools for 1 hour was determined to be used as measure of bowel movement. Finally, the comparison was made with the corresponding results in the control group.

Measuring myeloperoxidase (MPO) activity. Myeloperoxidase activity in the intestinal tissue was measured in order to determine intestinal inflammation. 8 cm piece was removed from the distal intestine and homogenized in 1 ml of phosphate buffer (pH 6.0), containing 14 mM of hexadecyl trimethylammonium bromide. Put into freezer. Centrifuged for 2 minutes. Finally, the absorbance of the samples in all groups was read by a spectrophotometer at a wavelength of 480 nm.

Measuring lipid peroxidation. Lipid peroxidation was evaluated by determining the concentration of thiobarbituric acid reactive substances (TBARS). In order to evaluate lipid peroxidation, malondialdehyde levels in the intestinal tissue were measured by spectrophotometer at a wavelength of 532 nm.

Statistical data analysis. To analyze the mean differences between the groups, statistical analysis was performed by the Statistical Package for the Social Sciences (SPSS 16.0; https://www.ibm.com/analytics/data-science/predictive-analytics/spss-statistical-software), using continuous multivariate method. P values of less than 0.05 were regarded as statistically significant.

Results and discussion

Based on our results, regarding the movements of gastrointestinal tract, the mean (±SD) number of stools in the false control group (29±2.34) compared to the control group (8.67±3.74), indicates development of certain symptoms in comparison with the control group in which the number of stools was higher. The mean number of stools in the positive control group was 11. ±5.53, in *C. cyminum* seed extract (300 mg/kg)-treated group 18.88±5.12, and in *P. anisum* seed extract (300 mg/kg)-treated group 24±1.92, with significant changes when compared to the control group (8.67±3.74). According to our results, there was a significant improvement in rats treated with 300 mg/kg of *C. cyminum* seed extract. Comparison of the group treated with 300 mg/kg of *C. cyminum* extract and false control group showed in the group receiving the *C. cyminum* extract, the number of stools significantly decreased (P<0.01). In general, the results showed that treatment with aqueous *C. cyminum* extract (300mg/kg) for 26 days significantly reduced the symptoms of the disease (Table 1).

Table 1 – Summative analysis of effects of cumin and anise plant extracts on gastrointestinal tract

Test	Control	False control	Positive control	Cumin extract	Anise extract	ANOVA P value
Activity and movement of the colon	8.67±3.74	29±2.34	11±5.53	18.88±5.12*	24±1.92**	0.002
Peroxidation of lipids	1.05±0.12	3.36±1.08	0.47±0.04	1.16±0.28*	1.96±0.34	0.076
Measurement of mylioproxidase	15.42±1.33	45.21±1.75	25.18±1.07	19±1.52*	31.76±1.57**	0.008
* $P < 0.05$:; ** $P < 0.01$, compared to false control group						

The number of stools in the false control group at all 1 hour intervals and the total number of stools per hour in comparison with the control group, indicated the development of IBS, while in the group treated with 300 mg/kg of *C. cyminum* seed extract (P<0.01) and positive control group (P<0/05), stool numbers at all intervals were significantly lower than false control group, which can be indicative of the prevention of IBS development.

The rate of lipid peroxidation in the false control group (3.36±1.08) was higher than the control group (1.05±0.12), and in the positive control group (0.47±0.04) and the group receiving 300 mg/kg of *P. anisum* extract (1.96±0.34) was significantly lower than false control group. In the group receiving 300 mg/kg of the *C. cyminum* extract (1.616±0.28), lipid peroxidation was significantly lower than the false control group (3.36±1.08) and approximately equal to the control group (Table 1).

The MPO activity in the groups receiving *C. cyminum* extract (19±1.52) and *P. anisum* extract (31.76±1.51) significantly decreased in comparison with the false control group (45.21±1.75). The corresponding decrease in *C. cyminum* extract-treated group was also significant when compared to the control group (15.42±1.33). The results shown in the Figure indicate that the *C. cyminum* extract decreases MPO activity significantly compared to the false control group (Table 1).

The results of this study show that the *C. cyminum* extract at 300 mg/kg reduces movements of gastrointestinal tract significantly compared to the control group, which is comparable to the standard drug Loperamide, reducing the movements compared to the control group. However, the P. anisum extract did not show a significant inhibitory effect on the movements of gastrointestinal tract contents compared to the *C. cyminum*. Although the extract of the *C. cyminum* reduces the frequency of diarrhea, its antidiarrheal mechanism remains unknown. Medicinal plants are of great value and importance in providing health care to communities, in terms of both treatment and prevention of diseases. The *P. anisum* is a gastrotonic plant and therefore can be effective in preventing some gastrointestinal problems. Due to its carminative effect, the plant is also used in the formulation of some gastrointestinal antispasmodic antiflatulent drugs. From the perspective of traditional Iranian medicine, *P. anisum* seeds have anti-inflammatory and analgesic properties and can used to prevent and treat many of diseases in which inflammation is a main factor [28]. *C. cyminum* is known as helpful medicinal plant in Iran [29]. From the ancient times in traditional medicine, *C. cyminum* seeds have been used to treat diseases, such as bloating, abdominal colic, pulmonary inflammation and gastritis [31]. In addition, aqueous and oily extracts of the plant have antioxidant properties and are used as natural antioxidants to reduce oxidative stress levels and increase the levels of antioxidant agents [32]. A study has shown that essential oil, containing 26% γ-terpinene (compound of *C. cyminum* essential oil) has antioxidant and antimicrobial properties [33]. γ-terpinene also decreases the linoleic acid peroxidation [34]. Some compounds present in *C. cyminum* essential oil, such as z-ß-ocimene, limonene and carvone, have antibacterial and antifungal effects and are effective in the treatment of infections caused by various pathogenic fungi and bacteria such as *Candida albicans* [35]. In the study of Sabbaghian *et al*. [36], the effect of aqueous *Aloe vera* extract on the intestinal and brain water content and gastric acid secretion was investigated after acetic acid-induced gastric ulcer in male rats. Their results showed that the use of *Aloe vera* produced an inhibitory effect on gastric acid secretion. The inhibitory effect of *Aloe vera* is due mainly to the formulation and gastroprotective activity of the plant. Improved tissue conditions also indicated the healing properties of this plant [36].

Cruz *et al*. [37] and Ismaili *et al*. [38], found that oral intake of silymarin (a flavonoid combination derived from *Silybum marianum*) improves bowel

tissue inflammation and damage. Factors such as increased vascular permeability, long-term infiltration of neutrophils, and increased mediators of inflammation that play a role in the onset of human colitis are also associated with the formation of colitis [39]. The anti-inflammatory effects of sulfasalazine and its metabolite, 5-amino-salicylic acid, are well known [40]. The use of *Hypericum perforatum* extract has been reported as effective in improving the symptoms of IBS in rats [17]. The property of turmeric property in IBS improvement has been demonstrated [18]. Inflammatory bowel disease has been considered an important public health issue due to its impact on young people, recurring and progressive clinical course, and impact on the quality of life and social status of individuals, and the ability to work and education [41-43].

Conclusion

Although irritable bowel syndrome is a benign condition and definitely not life threatening, the duration of this illness is long and the likelihood of its recurrence is high. It is a functional gastrointestinal disease with a high population prevalence. The disorder can be debilitating in some patients, whereas others may have mild or moderate symptoms. Probably one of the reasons for the effect of aqueous extracts from *Cuminum cyminum* L. and *Pimpinella anisum* L. seeds on IBS is the presence of antioxidant substances in these plants. It has been found that these two herbs contain active substances that have antioxidant activity. In the prospective future, there is hope for a better understanding of the disease and therefore its treatment.

Acknowledgment

Research was funded within the framework of the project 33/90 from Deputy for Research and Technology, Lorestan University of Medical Sciences, Khorramabad, Iran. The Ethics Committee of Lorestan University of Medical Sciences (code 9/26/1) approved conducted animal studies (code 33/90).

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