





Beneficial Effects of *Trachyspermum ammi* (L.) Sprague on Rat Irritable Bowel Syndrome

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Abstract

Background and objective: *Trachyspermum ammi* (*T. ammi*) has been used for the treatment of various digestive disorders with considerable therapeutic effects such as anticholinergic and antioxidant activities. This study aimed to evaluate the efficacy of the hydro-alcoholic extract of the fruits of *T. ammi* in an experimental model of irritable bowel syndrome (IBS). **Methods:** The rats were classified into seven groups, including sham (no stress), control (saline recipients), loperamide and fluoxetine (10 mg/kg/day) (positive controls), and the plant groups at the doses of 150, 250 and 500 mg/kg/day for 5 days under restrictive stress, 2 days before receiving the treatment. All medicines were given as gavage. The effect of the plant extract on gastric emptying and the transit of the small intestine was evaluated. The levels of the inflammatory and oxidative related biomarkers, tumor necrosis factor alpha (TNF- α) and lipid peroxidation (LPO), also the myeloperoxidase (MPO) activity were measured. **Results:** The gastric emptying and the transit of the small intestine were significantly reduced in all *T. ammi* treated groups, and no significant difference was observed at the dose of 500 mg/kg/day compared with the loperamide group. The levels of TNF- α and MPO activities decreased in the treatment groups compared with the control, and the LPO level was decreased at the concentrations of 250 and 500 mg/kg/day compared to the control. The antioxidant levels significantly increased in the rats treated with *T. ammi* at the doses of 250 and 500 mg/kg/day. **Conclusions:** The severity of stress-induced IBS was reduced in a dose-dependent manner by the hydro-alcoholic extract of the fruits of *T. ammi*, confirming the effectiveness of this plant in the management of IBS.

Keywords: irritable bowel syndrome (IBS); lipid peroxidation; myeloperoxidase; *Trachyspermum ammi*; tumor necrosis factor alpha

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Introduction

Irritable bowel syndrome (IBS) is a disease characterized by a series of symptoms and involves about 20 percent of the population. It is a biopsychosocial disorder causing remarkable economic pressure on health-care organization [1]. The exact etiology of IBS is unclear, yet, proper treatment is still challenging [2]. Visceral hypersensitivity is implicated to various disorders such as development of pain, gut dysmotility, stress and psychological disturbances, inflammation, small intestinal bacterial overgrowth, immune activation, and oxidative stress [2,3]. Many therapeutic agents have been introduced to control the IBS symptoms including antidiarrheal, laxatives and bulking agents [2], antispasmodics [4], tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs) [5,6], 5-HT₃ receptor antagonists, 5-HT₄ receptor agonists [7,8], and antibiotics/probiotics [9,10].

Recently, the efficacy of medicinal plants has been proven concerning the treatment of IBS [11]. New findings have shown that inflammatory mediators such as interleukin (IL)-1 β , IL-6, TNF- α and MPO, play effective roles in the pathogenesis of IBS [3,12]. It has been proven that stress can induce colon inflammation by facilitating the entrance of the substances that have been induced in colon by CD₄ T cells to the bowel, which will trigger an inflammatory response [13]. Thus, it seems that natural resources with potential effects on the antioxidant and inflammatory modulators could be considered as therapeutic agents [14].

Trachyspermum ammi (L.) Sprague commonly known as Ajwain is a plant from Apiaceae [15]. The plant grows in Egypt, Iran, Pakistan, Afghanistan and India as well as in the European regions [16]. Traditionally, its fruits have been used for the treatment of various digestive disorders including dyspepsia, colic and diarrhea. Several other therapeutic properties have also reported for the extract and the essential oil of *T. ammi* such as anticholinergic, anesthetic, anti-asthma, anti-cough and anti-oxidant activities [16], as well as gastroprotective properties [17]. Moreover, phenolic and flavonoid components of the fruits extract could affect the gastrointestinal ulcerative disorders [18] through the adjustment of the 5-lipoxygenase pathways of arachidonic acid [19]. The purpose of the present study was to investigate the beneficial effects of the hydro-

alcoholic extract of *T. ammi* fruits on stress-induced IBS rats.

Material and Method

Ethical considerations

The animals were kept in accordance with the instructions provided by the Tehran University of Medical Sciences Review Board. The procedures implemented throughout the study were approved by the Ethics Committee of Tehran University of Medical Sciences in accordance with the Standards for the Care and Use of Laboratory Animals with code number 90-11-26-6018.

Materials

Rat MPO, lipid peroxidation (LPO) Elisa kits and total antioxidant capacity (TAC) kit were from Zellbio (Germany). The tumor necrosis factor alpha (TNF- α) Elisa kit was purchased from Diaclone (France). Phenol red, trichloroacetic acid (TCA), methyl cellulose and potassium phosphate were obtained from Sigma-Aldrich (GmbH Munich, Germany). Fluoxetine and loperamide were provided by Dr. Abidi Pharmaceutical Co. Iran.

Plant material and extraction

Dried fruits of *T. ammi* were obtained from herbal market (September 2017, Tehran, Iran), identified and authenticated by Dr. GH. Amin and a voucher specimen was deposited at the Herbarium of Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran (PMP-657).

The hydro-alcoholic extract was obtained by maceration method with ethanol 70% (10 litre (L), 72 h), (3 L, 48 h) and (2.11 L, 24 h). The collected extract was concentrated by rotary evaporator at 45 °C [20].

Animals

Male Wistar albino rats weighting 200-230 g taken from animal house of Faculty of the Pharmacy at Tehran University of Medical Sciences were kept under standard requirements of temperature (23 \pm 1 °C), relative humidity (55% \pm 10%) and 12/12 h light/dark cycle with full access to the standard pellet diet and tap water.

Experimental Design

The animals were classified into seven groups

with 12 rats in each group, including sham, control, loperamide, fluoxetine and three groups of *T. ammi*. IBS was induced in all groups except the sham group. Both the sham and control received saline for treatment, and the other groups were set as; the loperamide group (10 mg/kg/day) [21], the fluoxetine group (10 mg/kg/day) [22], and the *T. ammi* groups at the doses of 150, 250, 500 mg/kg/day. All medicines are given as gavage for 7 days, 2 days in form of pre-treatment, followed by 5 days after the induction of IBS [23].

Induction of IBS

The restraint stress method was used to induce IBS. Following respiration of ether, a light (semi-anesthetic) anesthesia was induced in animals. The limitation was caused by limiting plastic tubes. To induce IBS, the rats were placed in tubes with length of 25 and 6 cm in diameter, although the limitation for movement of the rats was considered. This restriction was created by closing two sides of the tubes, thus it was not possible for the animals to leave pipes. Of course, in order to ventilate the air in these pipes, several holes were embedded. Such limitation for 5 consecutive days results in 6 h stress daily, thereby, IBS was induced in animals [24]. As mentioned earlier, medications were ingested before and during the induction of IBS.

Sample preparation

On the 5th day of limitation, each group was divided into three subgroups including four rats. In two subgroups, phenol red was gavaged during the night. One subgroup that received phenol red was put to sleep with sodium pentobarbital (50 mg/kg) after 60 min and the other group after 120 min. Accordingly, the abdomen, small intestine and the colon were removed with laparotomy and placed in a cold saline bath. Thereafter, all rats were subjected to ether overdose. The colon was kept for biochemical and immunological assessments, opened in an ice bath, slowly washed out by cold saline, weighed and homogenized with 10 volumes of cold ice potassium phosphate buffer (50 mmol/L, pH7.4). In the next step, 100 μ L of the homogenate was maintained at -80 °C for evaluation of biochemical factors including TNF- α , MPO, LPO, and TAC assays. Other specimens were sonicated and centrifuged for 30 min at 3500 g, then transferred to the micro tube and stored at -80 °C

until the end of the evaluation. The small intestine was segmented horizontally into three equal parts, and the abdomen and the three parts of the intestine were used for the evaluation of the stomach and intestinal passage.

Gastric emptying and small bowel transit measurement

The phenol red recovery method was applied to evaluate gastric emptying and passage of the small intestine [25]. Animals received 1 mL of 1.5% methylcellulose solution containing 0.5 mg of phenol via gavages. The abdomen and three parts of the intestine were homogenized in 100 mL NaOH 0.1 mol/L for 30 s. The suspension was kept at room temperature for 60 min. Later, 5 mL of the supernatant was added to 5 mL of 20% W/V TCA and centrifuged at 3000 g for 20 min. The supernatant was added to 4 mL NaOH 0.5 mol/L. Finally, the absorbance of the specimens were read by the ultra violet (UV) visible spectrophotometer at 560 nm [25].

Biochemical assays

TNF- α assay

A rat specific ELISA kit, Diaclone (France), was utilized to quantify the TNF- α in colon tissues. The amount of this cytokine was evaluated at the final step by gauging the absorbance of the sample on a spectrophotometer using 450 nm as the primary wavelength and optionally 620 nm as the reference wave length by the brochure. Data were displayed as pg/mg of protein of the tissue [23].

MPO activity assay

A rat specific ELISA kit, Zellbio (Germany), was utilized to determine the MPO activity, following the instruction of the manufacturer. The absorbance of the sample was evaluated at 450 nm by a UV spectrophotometer. Data were displayed as u/mg protein of the tissues [26].

LPO Assay

Zellbio (Germany) rat specific ELISA kit, was utilized to measure the LPO at 450 nm by a UV spectrophotometer at the final step, following the kit guideline. Data were displayed as mmol/g protein of the tissue.

Total antioxidant capacity assay

A TAC assay kit, Zellbio (Germany), was used to quantitatively measure the TAC on the basis of

the oxidation reduction colorimetric assay. The TAC amount was determined colorimetrically at 490 nm. The TAC concentration (mM) of the sample was calculated based on the standard curve using the standard absorbance against the standard concentration [27].

Total protein of colon homogenate measurement

Total protein was gauged by Bradford method using BSA as the standard. The results were expressed as mg/mL of the homogenized tissue [28].

Statistical Analysis

Data were analyzed using one-way ANOVA followed by Newman Keul's posthoc test for multiple comparisons. P values ≤ 0.05 were assumed significant. The results were reported as mean \pm standard error of the mean (SEM).

Results and Discussion

Chronic stress induced a significant increase in gastric emptying in the control group compared with the normal group ($p < 0.001$). In the fluoxetine and loperamide groups, gastric emptying reduced significantly in comparison with that of the control group ($p < 0.001$). The percentage of gastric emptying was alleviated by the plant extract A groups (125, 250, 500 mg/kg/day) in a dose dependent manner comparing to the control ($p < 0.001$) (figure 1).

Chronic stress significantly augmented the secretion of the small intestine in the control group compared with the normal group ($p < 0.001$). Transfusion from the small intestine did not decrease meaningfully in the fluoxetine group, while a significant reduction was observed in the loperamide group compared with the control group ($p < 0.001$). Transmissions from the small intestine in the extract treated A groups (150, 250, 500 mg/kg/day) were lower than the control group ($p < 0.001$), while there was no significant difference between A-500 and the loperamide group. Transit in the extract treated A groups (150, 250, 500 mg/kg/day) were lower compared with the fluoxetine group ($p < 0.001$) (figure 2).

The level of TNF- α significantly increased by restrained stress in the control group in comparison with the normal group ($p < 0.001$). Treatment with fluoxetine caused a significant decrease in TNF- α level comparing with the control animals ($p < 0.001$). Compared with the

fluoxetine group, there was a further reduction in the A-500 group ($p < 0.001$) (figure 3).

The FRAP value considerably reduced in the control group compared with the normal group ($p < 0.01$), while in the fluoxetine group, the amount of FRAP was significantly elevated ($P < 0.001$). Furthermore, there was a significant promotion in the extract treated A-250 and A-500 groups in comparison with the control group ($p < 0.001$). There was no difference between the A-500 group and the fluoxetine group (figure 4).

The MPO activity in the control group increased dramatically in comparison with the normal group ($p < 0.001$). Treatment with fluoxetine reduced the MPO activity compared with the control group ($P < 0.001$). The MPO level reduced in the all treatment A groups comparing with the control group ($p < 0.001$). The difference between the A-500 and fluoxetine groups was not considerable (figure 5).

TBARS was significantly increased in the control group in comparison with the normal group ($p < 0.001$). Treatment with fluoxetine caused a decrease in the TBARS level comparing with the control group ($p < 0.001$). TBARS reduced in A-250 and A-500 groups when compared with that of the control ($p < 0.001$). TBARS were similar in the fluoxetine and A-500 groups (figure 6).

The results of this study confirmed the positive effects of the hydro-alcoholic extract of *T. ammi* fruits in reducing the symptoms of IBS. *Trachyspermum ammi* at concentration of 500 mg/kg reduced the TBARS and MPO values, decreased the TNF- α level and the gastric emptying and small bowel transit percentages.

It is well known that IBS is a common chronic disease, covering a wide range of severity. In moderate severity of the disease, the patients education and diet are of great importance, however, in case of severe symptoms, in addition to the identification and management of the exacerbating factors, the application of psychotherapy and behavioral techniques as well as chemical medications are also required [29]. To improve the symptoms of the disease, in addition to the psychotherapy, fluoxetine and loperamide are prescribed by physicians.

Nowadays, various drugs are introduced to improve the conditions of the patients; each with a profile of complications. According to our results, *T. ammi* might be a novel approach to improve and/or treat patients' conditions. This plant has been very much considered in traditional medicine literatures.

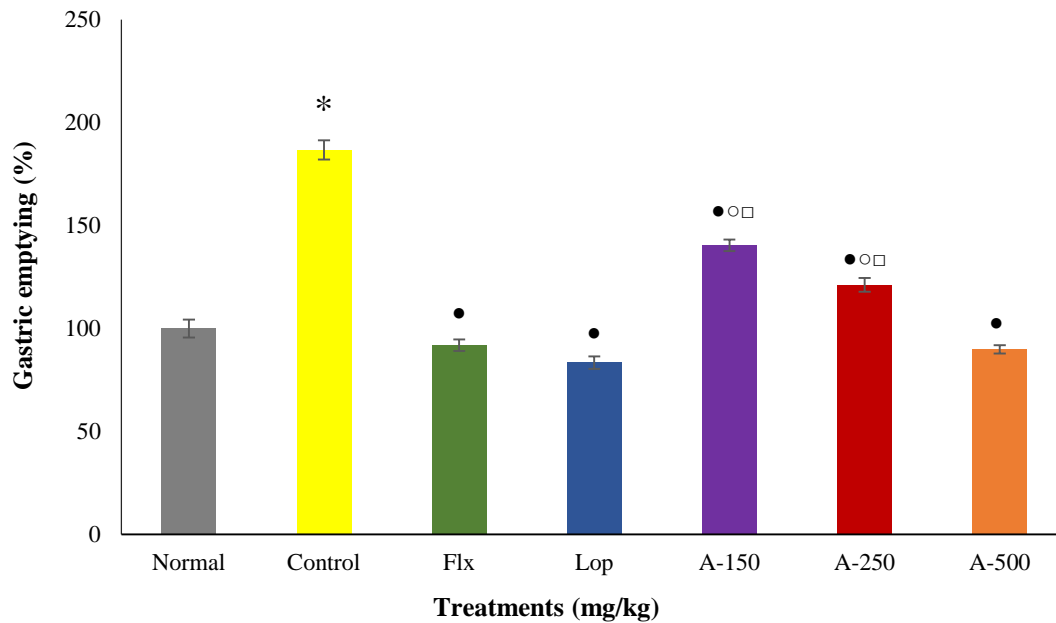


Figure 1. Gastric emptying percentage after 60 minutes; * $p \leq 0.001$, compared with the normal group; ● $p \leq 0.001$, compared with the control group; ○ $p \leq 0.001$, compared with the fluoxetine group; □ $p \leq 0.001$, compared with the loperamide group; Flx: fluoxetine; lop: loperamide; A-150, A-250, A-500: *Trachyspermum ammi* at the doses of 150, 250 and 500 mg/kg respectively

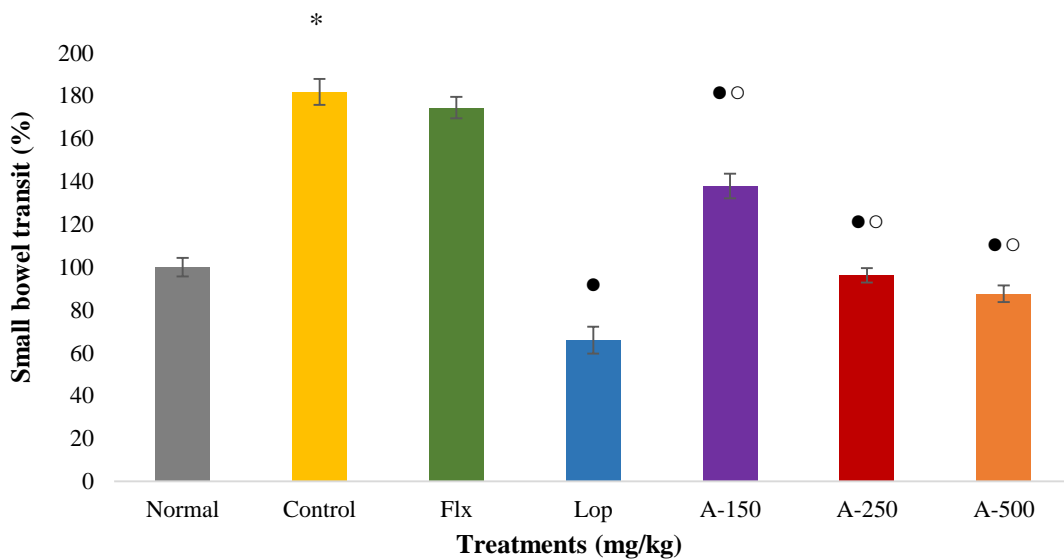


Figure 2. Small bowel transit percentages after 120 min. * $p \leq 0.001$, compared with the normal group; ● $p \leq 0.001$, compared with the control group; ○ $p \leq 0.001$, compared with the fluoxetine group; Flx: fluoxetine; lop: loperamide; A-150, A-250, A-500: *Trachyspermum ammi* at the doses of 150, 250 and 500 mg/kg respectively

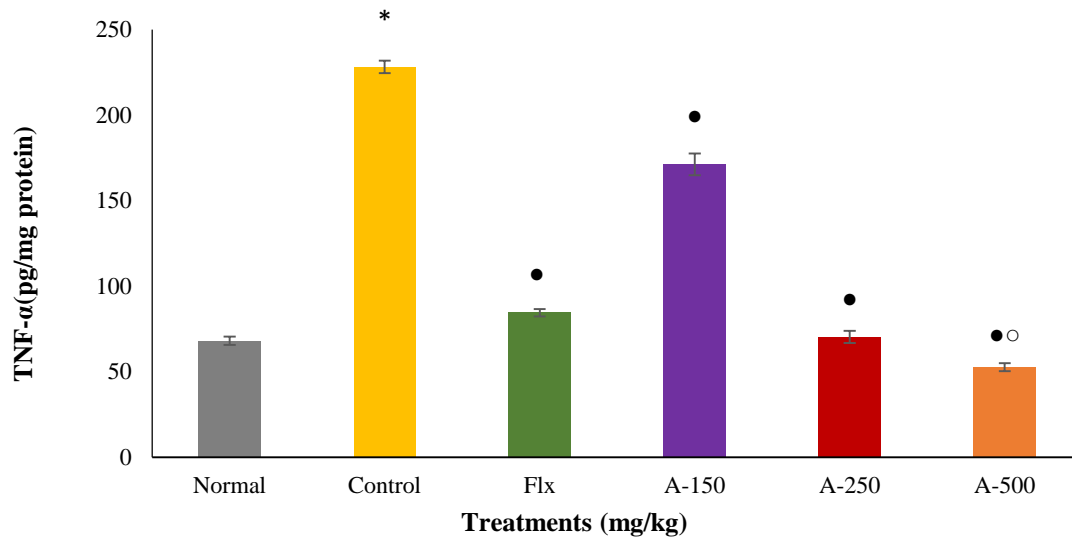


Figure 3. TNF- α level in the colon; * $p \leq 0.001$, compared with the normal group; ● $p \leq 0.001$, compared with the control group; ○ $p \leq 0.001$, compared with the fluoxetine group; Flx: fluoxetine; lop: loperamide; A-150, A-250, A-500: *Trachyspermum ammi* at the doses of 150, 250 and 500 mg/kg respectively

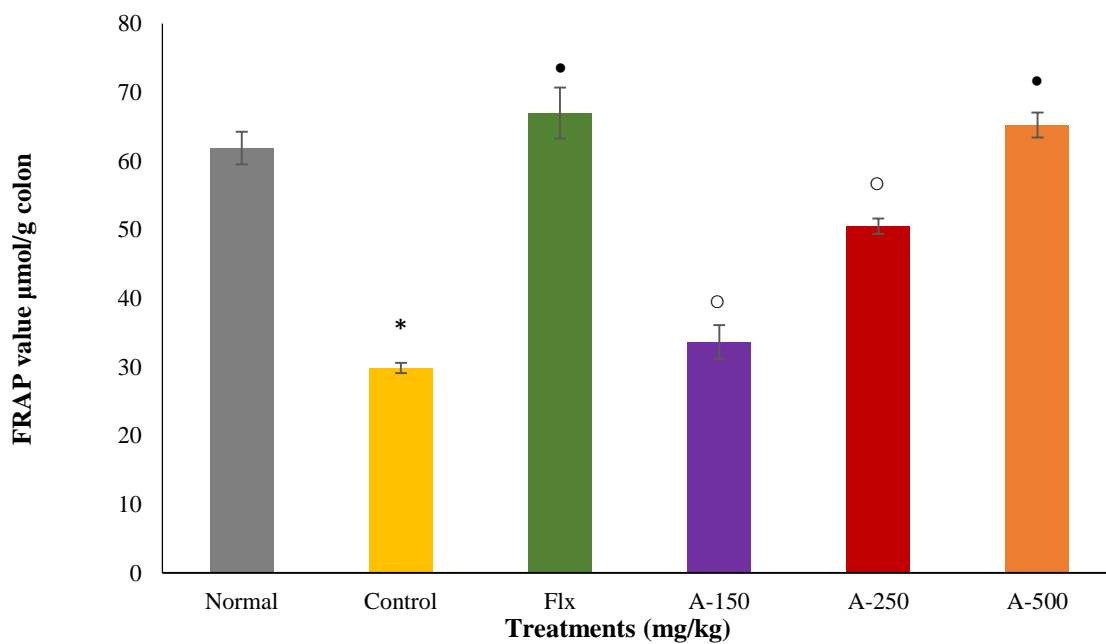


Figure 4. Ferric-reducing anti-oxidant power (FRAP) of the colon. * $p \leq 0.001$, compared with the normal group; ● $p \leq 0.001$, compared with the control group; ○ $p \leq 0.001$, compared with the fluoxetine group; Flx: fluoxetine; lop: loperamide; A-150, A-250, A-500: *Trachyspermum ammi* at the doses of 150, 250 and 500 mg/kg respectively

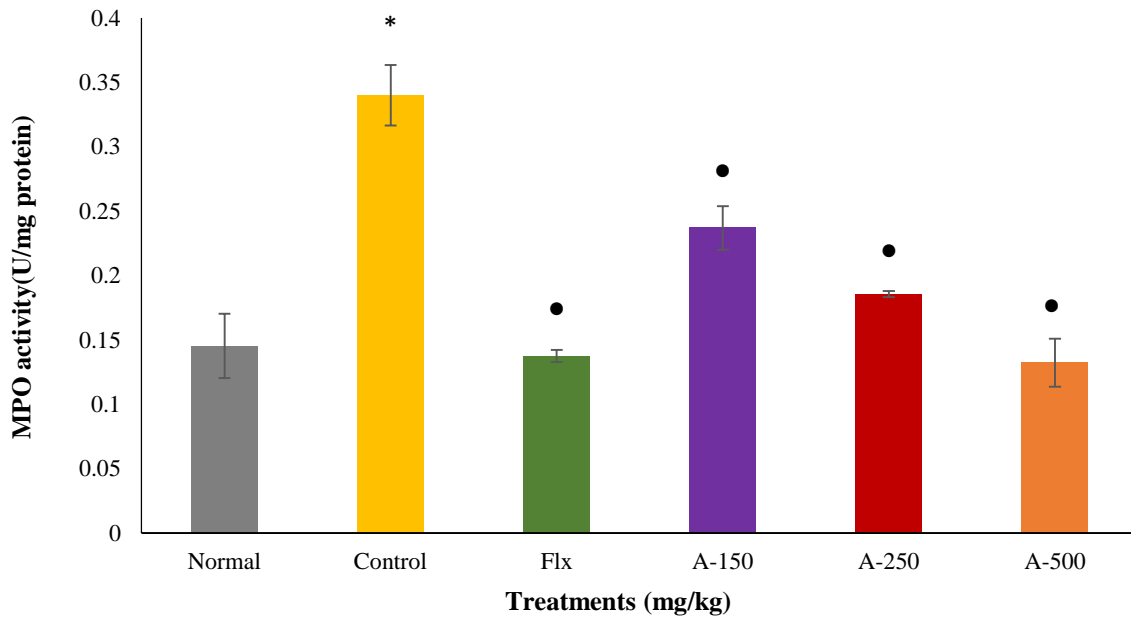


Figure 5. MPO activity of the colon; * $p \leq 0.001$, compared with the normal group; ○ $p \leq 0.001$, compared with the control group; ● $p \leq 0.001$, compared with the control group; Flx: fluoxetine; lop: loperamide; A-150, A-250, A-500: *Trachyspermum ammi* at the doses of 150, 250 and 500 mg/kg respectively

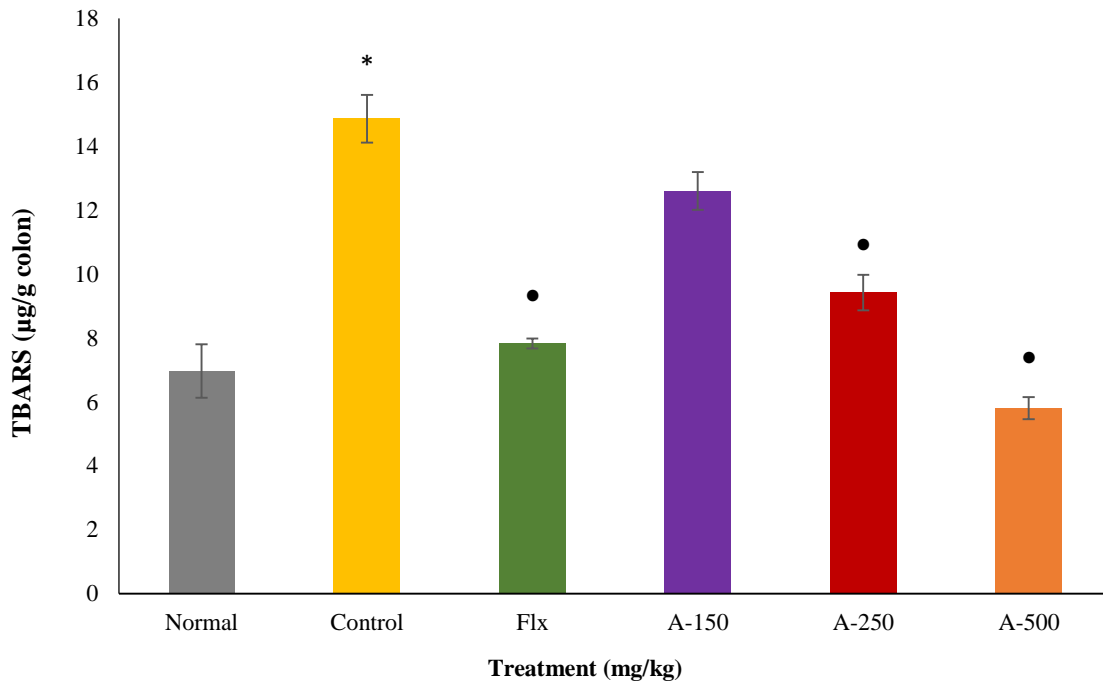


Figure 6. TBARS value of the colon; * $p \leq 0.001$, compared with the normal group ○ $p \leq 0.001$, compared with the control group; Flx: fluoxetine; lop: loperamide; A-150, A-250, A-500: *Trachyspermum ammi* at the doses of 150, 250 and 500 mg/kg respectively

The reported constituents are fiber, carbohydrate, tannin, glycoside, moisture, protein, fat, saponins, flavones and other components such as calcium, phosphorus, iron, cobalt, copper, iodine, manganese, thiamine, riboflavin and nicotinic acid [30-32]. Based on the gas chromatography and mass spectrometry (GC-MS) analysis, the main components of the plant were reported as iso-thymol (carvacrol), terpinene, terpineol, emersol, ethylester, cymene and phenol4methoxy2,3-6trimethyl [33]. Synergistic effects of carvacrol, p-cymene and γ -terpinene were found to reduce the antimicrobial resistance [34], whereas, synergistic interactions of carvacrol and terpineol were shown to have anti-larvae effect [35].

Ajwain has traditionally been used for a variety of medical and therapeutic applications. Oral administration of the extract of Ajwain seeds has been reported to be useful for paralysis, tremor, palsy and other neurological disorders [36]. Iranian traditional medicine professionals also used the eye and ear drops made from Ajwain to control the infections and to correct hearing loss [37]. In respiratory depression, the extract of *T. ammi* improved coughing, pleurisy and dysfunction [38]. Ajwain has also been reported to improve gastrointestinal disorders, stomach problems, colic, chronic fever and female sexual dysfunction. Additionally, *T. ammi* was found to reduce the unwanted effects associated with the withdrawal of addiction [36-37].

Antipyretic, antifungal, anticoagulant and bronchodilator effects, diuretic and anti-lithiasis activities, antihyperlipidemic properties, detoxification activity, hepatoprotective and antiviral effects, anti-hypertensive and anti-spastic activities are other properties of the species [16]. Digestive stimulating activity and the antioxidant properties of this species have been reviewed by Zarshenas et al., confirming the results obtained in this study [16]. In vivo studies have demonstrated that Ajwain extract has antioxidant properties, accordingly, it was shown that the aqueous fraction of Ajwain reduced the toxicity of hepatic free radical stress [40]. In another study, the aqueous and alcoholic extracts of *T. ammi* exhibited significant anti-inflammatory effects [41].

The aim of the present study was to investigate the effects of *T. ammi* on the stress-based empirical model of IBS by examining the gastric

emptying and transmission of the small intestine and the colon. In this regard, the overall gastrointestinal function and also the effect of this extract on the intestinal inflammatory and oxidative markers were evaluated in comparison with fluoxetine and loperamide, as approved psychological and symptomatic treatments for IBS. The results demonstrated that *T. ammi* was able to reduce the colon secretion of TNF- α , MPO and LPO; in addition, enhanced the ability of mucosal antioxidants to cope with oxidative stress induced by 5 days restraint stress in rats. However, *T. ammi* was able to significantly reduce the stress caused by gastric emptying.

It is concluded that the hydro-alcoholic extract of the fruits of *T. ammi* can be suggested as a natural treatment for IBS. However, further studies are necessary to evaluate the precise mechanism of action of the plant phytochemicals. Moreover, clinical trials are needed to obtain more definitive results regarding the efficacy and safety of this plant in IBS.

Author contributions

Shadi Izadpanah committed to the practical parts and drafted the manuscript; Amir Hossein Abdolghaffari, Mohammad Abdollahi, Mohammad Reza Shams Ardekani and Roja Rahimi designed the study and all works have been done under their supervision; Fatemeh Farjadmand and Mahdiah Eftekhari participated in plant extraction; Maryam Baeri, Saeideh Momtaz and Mahban Rahimifard contributed in biochemical assays and data analysis; Saeideh Momtaz and Amir Hossein Abdolghaffari revised the manuscript.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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Abbreviations

IBS: irritable bowel syndrome; TNF- α : tumor necrosis factor alpha; TCAs: tricyclic antidepressants; LPO: lipid peroxidation; MPO: myeloperoxidase; SSRIs: selective serotonin reuptake inhibitors; IL: interleukin; LPO: lipid peroxidation; TAC: total antioxidant capacity; L: litre; SEM: standard error of the mean; GC-MS: gas chromatography and mass spectrometry; Flx: fluoxetine; lop: loperamide; A-150, A-250, A-500: *Trachyspermum ammi* at the doses of 150, 250 and 500 mg/kg