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EVALUATION OF TOTAL BLACK SEED (NIGELLA SATIVA) EXTRACT EFFICACY IN PATIENTS WITH IRRITABLE BOWEL SYNDROME - PILOT STUDY

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Abstract

Introduction: Until now, numerous effective compounds of black seed (Nigella sativa) therapeutic benefits and its active ingredient thymoquinone are known; However, effect of this drug to treat and improve clinical symptoms of patients with functional bowel disorders, including IBS, has not been investigated. Present study was conducted with the aim of investigating effectiveness of black seed extract in patients with IBS treatment.

Material and Method: This randomized controlled clinical trial study was conducted on 62 patients with IBS in 1400. The patients were randomly divided into two groups receiving capsules containing 100 mg of black seed extract (test group; 32 people) or similar capsules containing placebo (control group; 30 people) for a period of 8 weeks. The symptoms severity was evaluated based on IBS severity questionnaire (including abdominal pain severity, abdominal distension severity, degree of satisfaction with bowel habits, impact of IBS on quality of life). The data were analyzed with the help of statistical software.

Results: There was no significant difference between two groups in IBS severity before intervention, second week, and fourth week of treatment ($P<0.05$). However, in sixth week ($P=0.004$) and eighth week of treatment ($P=0.018$), the severity of disease in black seed group was significantly lower than placebo group. From sixth week onwards, the average scores of abdominal distention, satisfaction with defecation habits, the impact of IBS on daily life and overall score of IBS severity questionnaire from fourth week onwards in black seed group were significantly lower than placebo ($P<0.05$).

Conclusion: black seed extract supplement is effective and safe in improving all symptoms and severity of IBS.

Keywords: Irritable bowel syndrome, Nigella sativa, extract, Thymoquinone, Black seed

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Introduction

One of the most common digestive system diseases is irritable bowel syndrome (IBS). This disease effects on patient's life quality. IBS is associated with symptoms such as chronic abdominal pain, bloating and abdominal distension, changes in bowel movements along with diarrhea, constipation (1). Epidemiological findings have shown that IBS is observed in 5-20% of general population worldwide (2). Several factors, including changes in intestinal microbiota and intestinal immune system function, inflammation, stress, chronic infections, some specific food items, and genetic factors play a role in IBS pathogenesis (3, 4). Considering that despite many efforts that have been made to improve or treat IBS symptoms, there is still no definitive treatment for it (5 and 6). So far, no definitive treatment has been provided for this disease, and effectiveness of drugs used has not been 100% (7, 8). Most of available treatments have improved IBS symptoms in only 25-30% of patients, so reducing symptoms has been prioritized (8,9).

Nigella sativa or black seed is one of the herbal medicines with high antioxidant properties, which has a special place in Iranian traditional medicine (10). The seeds of this plant contain fixed and volatile oils, fatty acids, amino acids, proteins, carbohydrates, fiber, saponin and alkaloids (11). Thymoquinone is the most abundant substance in black seed essential oil and is the main factor in many beneficial effects of the seed (12, 13). Aqueous and oily extracts of these seeds have antioxidant, antihistaminic, anti-inflammatory, anticancer, analgesic and antimicrobial activities and immune system regulating effects (12). The therapeutic efficacy of black seed has been proven to reduce headache, cough, abdominal pain, diarrhea, asthma, rheumatism and other diseases (13). In relation to safety and security of medicines obtained from black seeds, findings have shown complications absence in digestive system or other organs (12, 13). By affecting digestive system, this plant has positive effects on stomach function and reducing flatulence (12, 14). Research has shown that black seed with its antispasmodic properties has an effect on intestine smooth muscles, and its effects on digestion can be due to this pharmacological property (15, 16). The therapeutic effects of this plant on intestinal motility have been shown in experimental studies (17).

Considering the beneficial effects of this plant on health and its therapeutic roles confirmation on different parts of digestive system, and on the other hand, the effect of this drug to treat and improve clinical symptoms of patients with functional bowel disorders, including IBS, has not been investigated. Therefore, knowing more about this plant can be useful. This study was also conducted with the aim of evaluating the effect of this safe plant as a complementary treatment in irritable bowel syndrome.

Materials and Methods

Study design

This study is a double-blind randomized controlled clinical trial that was conducted as a pilot on 62 patients with IBS who referred to gastroenterology clinic of Imam Khomeini Hospital in Ahvaz in 1400. Eligible patients were included in study after checking the inclusion and exclusion criteria and willingness to participate in study.

Inclusion and exclusion criteria

Inclusion criteria included patients over 18 years of age with irritable bowel syndrome diagnosis, willingness to participate in study,

absence of malignancy (carcinoid and colorectal tumors), celiac disease, diverticulosis, gastrointestinal infection, hyper and hypothyroidism, lactose intolerance, IBD and ischemic colitis, no previous use of narcotic drugs, calcium channel blockers and antidepressants, and no history of allergy to herbal compounds of black seed family. The exclusion criteria were: occurrence of drug side effects (presence of any side effects caused by drug such as: nausea, heartache, diarrhea, etc.), unwillingness to continue participating in study, not taking drug for a certain period of time, pregnancy.

Statement of Ethics and Ethics Code

Written informed consent was obtained from all patients before treatment start, and for this purpose, the study target was fully explained to them. This study was conducted after obtaining permission from Research Council and approval of Ethics Committee of Ahvaz University of Medical Sciences (ethical code: IR.AJUMS.HGOLESTAN.REC.1400.154) and also registration in country's clinical trial system (IRCT code: IRCT20160709028845N1). Also, in all stages of this research, the provisions of ethics statement in Helsinki research and confidentiality principles of patient information were observed.

Study description and grouping

Considering the pilot nature of this study and the fact that there was no similar article, this study was conducted in phase 2 of a clinical trial, so there was no need for a formula to calculate sample size, and number of at least 30 people in each group was sufficient to use sampling method based on target was selected. Patients were included in study based on ROMA IV diagnostic criteria. The diagnosis was made based on abdominal pain at least one day per week during past three months and with at least two cases of recovery after defecation, change in frequency of defecation or change in consistency (hardness) of stool (8, 18). IBS was diagnosed by a gastroenterologist. In case of age over 50 years, screening colonoscopy was performed to ensure the absence of other diseases and malignancy.

Eligible patients were randomly divided into 2 groups receiving complementary therapy and placebo therapy. Randomization was done using RAS software and by a person who had no involvement in study process. Patient, researcher and doctor who examined results had no knowledge of which group people were placed in. During study period, patients received routine treatment for IBS, and one group was prescribed 100 mg capsules containing black seed extract (intervention group) and other group was prescribed placebo capsules containing starch (control group). The duration of treatment was 2 months and method of taking medicine was 2 times a day. The patients of both groups were given necessary training on how to take drugs, and patients were asked not to change their diet and physical activity level during study.

At the beginning of study, basic characteristics of patients including demographic information, like age, gender, race, occupation, education, height, weight, BMI, medical records and presence of underlying diseases and other digestive disorders, duration of IBS and type of IBS collected and recorded.

Drug preparation

First, extraction of black seed plant was done by maceration method. For this purpose, 70% ethanol was poured on black seed powder and after 48 hours, suspension was smoothed and shaken on a shaker for 2 hours. After that, the extract was concentrated by rotary and dried by freeze dryer, and then black seed extract was standardized by thymoquinone. Each capsule contained 100 mg of black seed extract as an active ingredient. Because the study was a pilot study, dose response of prepared capsule was calculated based on studies conducted in gastrointestinal diseases. Starch was used to prepare placebo. In order to be uniform, same shape capsules and color were used and coded by instructor. Extraction and preparation of capsules containing black seed extract and placebo were carried out under supervision of a referee at Jundishapur Faculty of Medical Sciences, Ahvaz.

Evaluation of consequences

Patients were visited by a gastroenterologist once every two weeks, and their symptoms

were checked every two weeks, and if necessary, liver and kidney tests were requested monthly. IBS Severity, patients' gastrointestinal symptoms, duration of symptoms were recorded at beginning of study (before starting treatment) and then every two weeks. According to type of IBS, based on stool pattern, patients are divided into four groups: IBS-C (IBS with constipation), IBS-D (IBS with diarrhea), IBS-M (IBS with mixed symptoms of diarrhea and constipation) and IBS-U (IBS not classified). were placed (5).

IBS Symptom Severity Scale (IBS-SSS) was used to evaluate severity of IBS. This questionnaire was completed by patient before intervention and at the end of 2nd, 4th, 6th and 8th weeks of treatment. The IBS-SSS questionnaire consists of five main questions, in which patient answers severity of his symptoms in each question with a score of 0 (no pain and discomfort) to 100 (the highest discomfort experienced by the person). The five questions are: 1- Pain intensity, 2- Abdominal pain intensity, 3- Abdominal distention intensity, 4- Level of satisfaction with defecation habit, 5- Impact of IBS on daily life. Finally, according to scores obtained from questionnaire, the severity of disease is classified into three groups: scores from 74 to 174 indicate mild IBS, scores from 175 to 299 indicate moderate IBS, and scores greater than or equal to 300 indicate severe IBS (19).

Statistic Analysis

SPSS (SPSS Inc., Chicago, IL, U.S.A.) version 22 was used for statistical analysis. In quantitative variables, mean and standard deviation were used to describe data. In qualitative variables, frequency and percentage were used to describe data. The normality of data was checked by Kolmogorov-Smirnov. In order to compare variables between two groups, independent t-test, Mann-Whitney, chi-square was used. Paired t-test was used to compare average changes of variables before and after intervention. Analysis of variance with repeated measurements was used to evaluate average changes in measured variables over time. The significant level in tests was considered 0.05.

Findings

62 patients with an average age of 38.45 ± 9.53 years were examined. The duration of IBS disease in studied subjects was 35.87 ± 36.67 months. There was no significant difference between investigated variables. Patient characteristics are presented in Table 1. No significant difference was observed between the two groups (P>0.05).

Table 1 - Basic characteristics of patients in two groups under investigation

Variable	Group	Placebo (n=30)	Black seed (n=32)	P-value
Age (years), mean ± S. D		39.10±10.29	37.84±8.89	0.60 *
gender, (%) n	Male	(56.7) 17	(43.8) 14	0.44 **
	Female	(43.3) 13	(56.3) 18	
Duration of IBS (months), mean ± S. D		31.37±31.70	39.16±40.96	0.59 ****
Type of IBS (stool pattern), (%) n	IBS-C	(36.7) 11	(34.4) 11	0.92 ***
	IBS-D	(30.0) 9	(25.0) 8	
	IBS-M	(20.0) 6	(21.9) 7	
	IBS-U	(13.3) 4	(18.0) 6	
Presence of digestive disorder, (%) n		(36.7) 11	(68.8) 22	0.02 **

* Independent T-test, **Fisher's exact test, *** Chi-square test, **** Mann-Whitney test. IBS: Irritable bowel syndrome; IBS-C: constipation dominant; IBS-D: diarrhea dominant; IBS-M: mixed pattern; IBS-U: unclassified

The results related to sign duration during study period in two groups are presented in Table 2. The results showed that sign duration in sixth week (P=0.021) and eighth week (P=0.033) in black seed group was lower than placebo group, so in sixth week, 15.6% of black seed group patients Compared to 6.7% of patients in placebo group, and in eighth week, 40.6% of patients in black seed group had no symptoms, compared to 6.7% of placebo group.

Table 2- Sign duration during the study period in two groups

Time	sign duration	Placebo (n=30)	Black seed (n=32)	P-value
Before treatment	Daily	(16.7)5	(25.0) 8	0.71
	Every 2 days	(13.3) 4	(9.4) 3	
	Every 3 days	(33.3) 10	(28.1) 9	
	Every 4-5 days	(16.7) 5	(12.5) 4	
	Every 6-7 days	(20.0) 6	(18.8) 6	
	Every 10 days or more	0	(6.3) 2	
Second week of treatment	Daily	(36.7) 11	(28.1) 9	0.60
	Every 2 days	(20.0) 6	(12.5) 4	
	Every 3 days	(20.0) 6	(37.5) 12	
	Every 4-5 days	(16.7) 5	(18.8) 6	
	Every 6-7 days	(3.3)1	0	
	Every 10 days or more	(3.3)1	(3.1) 3	
Fourth week of treatment	does not have	(6.7)2	(0) 0	0.17
	Daily	(36.7)11	(31.3) 10	
	Every 2 days	(13.3) 4	(18.8) 6	
	Every 3 days	(33.3)10	(15.6) 5	
	Every 4-5 days	(10.0) 3	(28.1) 9	
	Every 6-7 days	0	(3.1) 1	
	Every 10 days or more	0	(3.1) 1	
Sixth week of treatment	does not have	(6.7) 2	(15.6) 5	0.02
	Daily	(33.3)10	(25.0) 8	
	Every 2 days	(6.7) 2	(28.1) 9	
	Every 3 days	(20.0) 6	(18.8) 6	
	Every 4-5 days	(33.3)10	(6.3) 2	
	Every 6-7 days	0	(6.3) 2	
Eighth week of treatment	does not have	(6.7) 2	(40.6)13	0.03
	Daily	(43.3) 13	(18.8) 6	
	Every 2 days	(16.7) 5	(15.6) 5	
	Every 3 days	(23.3) 7	(15.6) 5	
	Every 4-5 days	(10.0) 3	(6.3) 2	
	Every 6-7 days	0	(3.1) 1	

* Chi-square test

The scores of IBS symptom severity questionnaire in two control (placebo) and test (black seed) groups at different times are presented in Table 3. The average scores of pain intensity in eighth week, abdominal pain intensity from sixth week onwards, and average scores of abdominal distension intensity, satisfaction with defecation habit, the impact of IBS on daily life, as well as overall score of IBS severity questionnaire from fourth week onwards in group Black seed has improved significantly (P<0.05).

According to Table 3, pain intensity, abdominal pain intensity, abdominal distension, satisfaction with defecation habits, the impact of IBS on daily life, and overall score of IBS severity between control and test groups showed significant differences at different times (P<0.05).

Table 3- Average scores of IBS severity questionnaire in two groups at different times

IBS severity	Time	Placebo (n=30)	Black seed (n=32)	P-value
Intensity of pain	Before treatment	52.33±28.00	62.34±20.33	0.19
	second week	52.33±22.07	55.94±20.61	0.59
	forth week	49.00±25.64	47.19±21.58	0.61
	sixth week	50.74±26.30	40.48±20.67	0.06
	eighth week	49.63±23.93	38.71±19.27	0.04
Abdominal pain intensity	Before treatment	48.33±31.74	58.59±24.40	0.25
	second week	44.67±30.02	52.19±21.95	0.51
	forth week	46.00±29.66	43.44±20.41	0.31
	sixth week	46.30±28.16	38.55±20.74	0.04
	eighth week	46.30±27.47	36.45±19.92	0.003
Severity of abdominal distension	Before treatment	60.33±27.47	58.59±22.47	0.37
	second week	57.67±25.00	53.78±21.21	0.31
	forth week	58.33±23.35	43.75±19.63	0.004
	sixth week	61.11±23.91	40.16±20.59	<0.001
	eighth week	57.41±23.79	38.71±23.34	0.003
Satisfaction with bowel habits	Before treatment	71.50±12.53	72.81±15.91	0.31
	second week	65.00±10.42	61.41±16.02	0.65
	forth week	62.00±12.42	52.81±17.45	0.02
	sixth week	65.19±10.51	47.26±18.87	<0.0001
	eighth week	64.07±14.48	43.55±18.89	<0.0001
Impact of IBS on daily life	Before treatment	73.17±11.18	72.81±13.73	0.64
	second week	68.33±10.85	61.88±14.68	0.09
	forth week	67.00±12.63	54.69±16.06	0.001
	sixth week	70.37±9.39	47.26±16.06	<0.0001
	eighth week	69.26±13.56	43.55±18.53	<0.0001
The overall score of the IBS severity questionnaire	Before treatment	305.67±78.55	325.16±75.89	0.303
	second week	288.00±76.62	285.16±75.89	0.88
	forth week	282.33±76.60	241.88±79.64	0.04
	sixth week	293.70±70.00	213.71±82.68	<0.0001
	eighth week	286.67±74.78	200.97±90.60	<0.0000

* Independent t test

Discussion

It is important to know the drug that is effective in improving irritable bowel syndrome with the aim of reducing disease complications. Plants that affect digestive system, such as black seed, can be one of the important options in improving IBS due to their healthiness (19). Present study was conducted for the first time to investigate black seed extract effect as a supplement in patients' treatment with different types of IBS. The results showed that during treatment,

gastrointestinal symptoms of patients frequency including nausea, vomiting, constipation and diarrhea decreased in both groups, but this decrease was more in black seed group; So that at the beginning of study, in black seed group, nausea occurred in 56.26%, vomiting in 9.38%, constipation in 65.63%, and diarrhea in 56.25% of patients, and in placebo group, these complications occurred in 23.33% respectively. %, 0%, 56.67% and 50% of patients were reported. But at the end of eighth week, in black seed group, nausea was observed in 6.26%, constipation in 25% and diarrhea in 21.88% of patients; in placebo group, these values were observed in 6.67%, 40% and 30%, respectively. In addition, the results of present study showed that sign duration during treatment and in sixth and eighth weeks in black seed group was lower than placebo group. These results showed black seed effect consumption in improving patients' symptoms. Previous findings have shown that black seed extract has been effective in improving gastrointestinal symptoms, astric mucosal damage and gastrointestinal abnormalities (12, 21, 22).

The results of present study showed that there was no significant difference between two groups in terms of disease severity at first, but with time passage in sixth and eighth weeks of treatment, disease severity in black seed group decreased significantly compared to placebo group. Also, in eighth week, in black seed group, 2 cases (6.3%) improved symptoms of disease. Also, average scores of pain intensity in eighth week, abdominal pain intensity from sixth week onwards, and abdominal distention intensity scores, satisfaction with defecation habits, IBS impact on daily life and overall score of IBS severity questionnaire from fourth week onwards in black group, seed was significantly less than placebo group. These results show consuming black seed extract effectiveness for a period of 2 months compared to placebo in reducing IBS severity. The beneficial effects of black seeds for digestive disorders and abnormalities have been reported in experimental and clinical studies (12, 21, 22). In a parallel study, it was reported that thymoquinone extract, one of the main components of black seed, reduces intestinal movements through its effect on muscarinic receptors and calcium receptor block (17). The antispasmodic effects of this plant seeds on intestine smooth muscles are caused by blocking calcium channels. Therefore, due to relaxation nature of adrenergic bread and cholinergic bread, the use of this substance is suitable for functional disorders of digestion treatment, including diarrhea (15). A peer-reviewed review found that black seed extract was effective in improving diarrhea symptoms caused by food allergies. Consumption of black seed extract decreased the number of mast cells in intestine and plasma and decreased pro-inflammatory cytokines (interleukins and interferon-gamma) (23).

Black seed has antioxidant, anti-inflammatory and other therapeutic benefits. These substances protect body against cell damage and chronic diseases. In a systematic review, it has been determined that black seed with Thymoquinone and effective antioxidant compounds affects metabolism and immune system and improves digestive system function (25, 24). Liang et al. showed during their investigations on intestinal microbiome analysis that black seed extract can have immune regulating effects by improving structure and increasing diversity of intestinal flora, regulating metabolic pathways including lipid metabolism and polysaccharide synthesis (26). Also, the results of a clinical study showed that consuming black seeds for a period of 4 weeks can regulate mood, reduce anxiety and improve concentration, without causing specific side effects (27). The beneficial effects of black seed extract in protecting gastrointestinal tract have been investigated in animal models and anti-ulcer effects of black seed have been observed due to prostoglandins regulation nature, as well as its antioxidant and anti-secretory nature (28). Nigella sativa and thymoquinone, as one of active compounds and main cause of therapeutic nature of this plant, can inhibit stomach acid and maintain mucous membrane permeability, which can be used to treat digestive disorders (21). These results support therapeutic and prophylactic effects of black seed extract and thymoquinone (its active substance) in inflammatory, oxidative, and toxic damages caused by toxins, microbes, and various allergens (29, 30).

In a study, the effect of Nigella sativa extract on intestinal pathogen Dientamoeba fragilis,

which is known as one of IBS causes and causes chronic diarrhea, constipation, or changes in both, along with abdominal pain, was investigated. The results showed that different doses of black seed extract performed better in eliminating pathogen compared to standard treatment (Metronidazole). Also, the highest effect was related to highest dose of black seed extract (500 mg), which completely eliminated infection (31).

Therefore, black seed and especially thymoquinone as its active ingredient can help in reducing IBS patients' clinical symptoms. As observed in present study, consumption of black seed extract for 2 months improved irritable bowel syndrome symptoms. Studies have shown that anti-inflammatory properties of black seed oil help to reduce symptoms such as indigestion, nausea, bloating, vomiting, diarrhea or constipation (32). Nerves play an important role in irritable bowel, and among promising medicinal plants, black seed is useful for managing depression and many other neurological disorders, and its pharmacological effects on central nervous system (CNS) have been proven (33).

In general, present study showed that consumption of capsules containing black seed extract is effective in treating and improving IBS patients' symptoms, it does not cause dangerous complications, and it can be used as a cheap and safe drug in the management of different types of IBS.

One of the strengths of this research was IBS severity examination and sign duration during 8 weeks. This study also faced some limitations, such as the fact that only short-term effects of this treatment were investigated in this study and effectiveness of this drug was not investigated in long term. Other limitations of the study include the lack of monitoring and monitoring of the patients' diet and physical activity during the study, and the lack of assessment of the patients' anxiety level before and after treatment using valid questionnaires, including the Beck Anxiety Questionnaire. Also, in the current study, the effectiveness of this drug was compared to placebo, and comparison with standard treatments requires more controlled clinical studies.

Conclusion

Black seed extract was effective in improving irritable bowel syndrome symptoms. Administering a supplement containing black seed extract for a period of 8 weeks has beneficial effects on reducing IBS symptoms severity, including reducing abdominal pain, reducing abdominal distention, increasing satisfaction with bowel habits, and reducing the impact of IBS on daily life. Also, frequency of nausea gastrointestinal symptoms, vomiting, constipation and diarrhea and sign duration decreased more during the study in black seed consuming group. Therefore, black seed can be used as a safe and effective drug supplement in different types of IBS patients to reduce patients' symptoms.

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