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Original Research

Effect of *Mentha aquatica* L. Extract on Bloating Symptom of Irritable Bowel Syndrome Disease: A Double-Blind Randomized, Placebo-Controlled Study

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Abstract

Irritable bowel syndrome (IBS) is a common functional gastrointestinal disorder that causes abdominal pain, distension, change of bowel habit, bloating, constipation, diarrhea, and mucus discharge along with stools. Although the disease causes long-term and agonizing pain, no ideal cure has been found for it so far. This study aimed to investigate the efficacy and compliance of *Mentha aquatica* L. extract for the treatment of bloating caused by IBS. This double-blind, randomized, placebo-controlled trial study was conducted in Tooba gastroenterology clinic in Sari, Iran, during 2019 (from January to May). A total of 104 patients with IBS in the age range of 20-80 years were randomly divided into two parallel groups of herbal medicine (MAC-330) and placebo. Study period consisted of a 4 weeks of administration, and 2 weeks of follow-up. IBS-associated symptoms including severity of bloating (as primary outcome) and frequency of defecation and abdominal pain were evaluated using a questionnaire before treatment, 1, 2 and 4 weeks after beginning treatment and 2 weeks after stopping treatment. There were no significant differences between the two groups in terms of their baseline characteristics ($p > 0.05$). The severity of bloating was significantly reduced in the both groups at fourth week ($p < 0.030$) and 2 weeks after stopping intervention (sixth

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week) ($p < 0.026$). The frequency of defecation has increased with the onset of the intervention until the fourth week of the treatment which was not significant. The abdominal pain reduced during the 4 weeks' intervention, but it was not significant. Based on the obtained results, MAC-330 could not be used as an effective treatment for patients with IBS in short time; however, it reduces the abdominal bloating and pain in these patients, especially in the long-term use.

Keywords: Irritable bowel syndrome; Bloating; *Mentha aquatica*; Herbal; Extract

Introduction

Irritable bowel syndrome (IBS) is a common functional gastrointestinal disorder that causes abdominal pain, distension, change of bowel habit, bloating, constipation and/or diarrhea, and mucus discharge along with stools [1,2]. It should be noted that IBS cannot be diagnosed by a specific test. In lieu of such a specific test, the diagnosis of IBS can be made through an accurate examination of the patient's symptoms, profound history of the patient (e.g. diet, medication, medical, surgical, and psychological history), the performance of a guided physical examination for the presence of warning signs and symptoms (e.g. anemia, hematochezia, unintentional weight loss, and a family history of colorectal cancer or inflammatory bowel disease), and the adoption of Rome's criteria [3,4]. Since bloating IBS is a disorder that is not confirmed by a specific test, the diagnosis is normally based on specific symptoms termed Rome's criteria [5].

However, it should be noted that a definitive diagnosis of IBS is made after a further investigation and rejection of the probability of related diseases [6,7]. Less than 1% of the patients who meet symptom-based criteria for IBS have other diseases, such as colorectal cancer, inflammatory bowel disease, and infectious diarrhea [8,9].

Various drug treatments, such as tricyclic antidepressants (TCAs) [10], antispasmodics (spasmolytic) [11,12], antagonists of 5-hydroxytryptamine-3 receptor (5HT3) [13-15], agonists of 5HT4 [15], selective serotonin reuptake inhibitors [16], antibiotics [17], prebiotics, symbiotic [18] and melatonin [19] are currently recommended. The tendency for finding a way to treat patients with medicinal plants is growing due to the complexity of IBS treatment and the long-term complications of this disease.

A double-blind, randomized, controlled trial showed that *Aloe vera* (L.) Burm.f. gel was effective in IBS constipation; while it had no effect on abdominal pain [20]. Two separate studies conducted on the artichoke plant revealed that the severity of symptoms has been reduced significantly [21]. It should be noted that this plant significantly improved the total quality-of-life (QOL) in patients with alternating constipation/diarrhea symptoms [22]. No difference was observed between the extracts and the placebo in other researches that have been conducted on aerial parts of *Fumaria officinalis* L. and *Hypericum perforatum* L. and *Curcuma longa* L. rhizome [23-25]. However, significant improvement has been reported in

treatment by *Mentha* × *piperita* L. essential oil [26-28], *Plantago indica* L. (synonym: Plantago psyllium L.) seed [29]. and Carmint drop, including essential oils of *M. piperita, Melissa officinalis* L. (leaf), and *Coriandrum sativum* L. (fruit) [30], suggesting the potential effect of plants, especially the Lamiaceae family to reduce IBS symptoms.

The Lamiaceae family consists of about 220 genera and 3300 species, of which the genus Mentha is an important member [31,32].

Mentha aquatica L. is one of the most important medicinal plants that are used in pharmaceutics as a flavor corrective for some drugs and as herbal medicine because of their analgesic, antigenotoxic, spasmolytic, antibacterial, astringent properties, as a stimulant, antimicrobial, Anti-inflammatory, antioxidant, larvicidal and neuroprotective Effects. It is used against colds, respiratory problems and protection against evil spirits as a treatment for promoting the flow of milk in nursing mothers, sedative, and soothing for intestinal parasites, constipation, diarrhea, stomach troubles, biliousness, liver diseases, impotence, low or high blood pressure, expelling intestinal worms in children, enhancing longevity, and can be mixed with *Senecio asperulus* DC. for joint pain; However, in search of various sources, we did not find any study on the direct effect of this species on bloating [33]. Considering the potential of some members of Lamiaceae family in the treatment of IBS symptoms, this study investigated the therapeutic effects of *M. aquatica* extract on bloating caused by IBS, as well as its effect on the quality of life score.

Materials and Methods

The patients aged between 20-80 years with a major bloating problem were recruited for inclusion in the study. All subjects had IBS according to the Rome IV criteria, and were evaluated by a gastroenterologist [34,35]. In order to find patients willing to enter the study, a number of questionnaires were provided by the researcher to be completed by patients with confirmed irritable bowel syndrome.

Trial registration: This study has been registered on the Iranian Registry of Clinical Trials under the registration number IRCT20190425043371N1.

Ethics committee Number: The written informed consent was obtained from all patients before the enrollment. This study was approved by the Mazandaran University of Medical Sciences, Iran (IR.MAZUMS.IMAMHOSPITAL.REC.1397.089)

Study design

This double-blind, randomized, placebo-controlled clinical trial was performed on patients with known IBS (based on the diagnosis of gastroenterologist and Rome IV criterion for IBS), with bloating signs in Tooba gastroenterology clinic in Sari, Iran, during 2019 (from January to May). Patients were divided into two parallel groups (medicine and placebo). This study was reported according to CONSORT guidelines for reporting parallel group randomized trials [36]. The patients who agreed to participate were evaluated at a screening visit, and inclusion and exclusion criteria were checked (Table 1).

Table 1. Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Signed written informed consent	The history of systematic disorders (e.g., diabetes mellitus, cardiac insufficiency, liver failure, renal insufficiency, asthma, chronic obstructive pulmonary disease (COPD), neoplasm or severe mental illness
Age ≥ 20 and ≤ 80	History of *Helicobacter pylori* in the last three months who received eradication therapy
IBS according to the Rome IV criteria	With the history of gastrointestinal surgery
Willingness to comply to the study procedures	Patients addicted to alcohol or drugs
	Patients with lactose intolerance
	Pregnant and lactating females
	Use of steroids, cardiac, antihypertensive, and anxiolytic drugs
	Use of Supermint® or Colpermin® or other medication consisting *Mentha* spp.

Outcomes

The severity of bloating was a primary outcome by the Visual Analogue Scale (VAS) and was scored from 0 to 10. Scores 0 and 10 represented the lack of bloating and the most severe and annoying type of bloating, respectively.

Sample size

Based on the results of similar studies, including the study conducted by Ahmad Khonche, et al. [37] and using the following formula, the sample size in each group was determined at 50, considering a confidence level of 95% and a power of at least 80% (Equation 1).

Equation 1. $$n_1, n_2 = \frac{\left(z_{1-\frac{\alpha}{2}} + z_{1-\beta}\right)^2 [P_1(1-P_1) + P_2(1-P_2)]}{(P_1 - P_2)^2}$$

As mentioned above, this study was performed on 100 patients who were divided into two groups of the intervention (water mint group, n = 50) and the control (placebo group, n = 50).

Allocation concealment and Randomization

Permuted-block randomization [38] was used to assign participants to each group for receiving either M. aquatica extract capsules 330 mg (MAC-330) or placebo, using blocks of two. A biostatistician assigned a unique random code to each drug and then arranged the blocks composed of the two drugs (MAC-330 and placebo).

Information regarding randomized block assignments was concealed in opaque and sealed white envelopes. Therefore, random codes were not released until patients were ready to do the research. Once the participants entered the trial, the gastroenterologist recorded their information. Subsequently, an independent research assistant assigned each participant to one of two intervention and control groups after opening the sealed envelopes.

Blinding

Although RCTs are considered the most presti

gious type of epidemiological studies to investigate the best treatment for diseases, the most important factors affecting the credibility of the results of these studies include personal characteristics, impact on physician work, external factors, and appearance of drug [39].

The blinding procedure was done very carefully. For this purpose, all capsules were identical in color, appearance, smell, and taste and were labeled with different codes. Both the researchers and the care provider were blinded to the treatment allocation. The randomized codes were disclosed to the researchers after the completion of statistical analyses.

Material preparation

Initially, the M. aquatica was collected from around the city of Sari, Iran, in locations with a height of 1,450 m above sea level during August 2018. The plant leaves were isolated and dried after they were identified and confirmed by a botanist in the herbarium of Mazandaran University of Medical Sciences (voucher number: E1-36-4114). The dried leaves were then powdered using a milling machine (Pars Khazar GR-123P, Iran) in the pharmacy laboratory of Tehran University of Medical Sciences. Subsequently, the extraction was being carried out for 72 h using a percolator apparatus and a mixture of ethanol-water solvent (70:30). The residual solvent was evaporated using a rotary evaporator (Heidolph Laborota 4001, Germany). The extract obtained with this method was semi-solid due to its non-polar composition, which used a dryer freezer for further drying. Finally, a total of 16 g of the extract was obtained out of every 100 g of dried leaves. To prepare the capsule, considering that the efficacy of the extract was 16%, so consuming 321 mg of the extract per day is approximately equivalent to 7 g of dried M. aquatica. according to Persian medicine references the appropriate dosage of dried M. aquatica is 7 g per day [37]. Accordingly, patients should daily consume four capsules that totally containing 330 mg of dried extract.

The obtained extract should be converted into granules. Drug excipients such as Avicel, Erosil and Lactose were used to prepare the granules. Avicel was used as an emulsifier, filler and anti-mold, Erosil as a binder and lactose as a filler. The mixture of the above three components was mixed with the extract to granulate the extract. For this purpose, 600 g of Avicel, 680 g of Erosil and 600 g of lactose were added to 1848 mg of extract and then the mixture was completely dried in the oven. The resulting mixture was passed through a sieve with a standard mesh for a uniform distribution of particles. Afterward, the powdered extract was poured into the gelatin capsules (as a drug) using a capsule filling device. The placebo capsules were completely similar to drug capsules in shape and size, and had all components of the drug except the extract. In addition to the weight of the extract, starch was used.

Intervention and follow-up

All patients in both intervention groups (drug and placebo) received 4 capsules, 2 after breakfast and 2 after dinner, for 4 weeks. The severity of bloating, frequency of defecation, and abdominal pain have been investigated in both MAC-330 and placebo groups at the beginning of medica-

tion and one, two, four, and six weeks later. The visual analog scale (VAS) has been used to measure the amount of bloating and the patient indicated the bloating severity by selecting a number from 0 (the least severe) to10 (the most severe). The standard SF-36 quality of life questionnaire was completed by the patients once before the study and once two weeks after stopping the study. The SF-36 questionnaire consists of 36 items, which are used to calculate eight subscales: physical functioning (PF), role physical (RP), bodily pain (BP), general health (GH), vitality (VT), social functioning (SF), role emotional (RE), and mental health (MH). The first four scores can be summed to create the physical composite score (PCS); while the last four can be summed to create the mental composite score (MCS). Scores for the SF-36 scales range between 0 and 100, with higher scores indicating a better Health-related quality of life.

Additionally, the severity of symptoms was recorded three times through the phone call: before and two weeks after the beginning of the medication and four weeks after the end of the treatment (i.e., week 0,2,6). They were followed in 0, 1,2,4 weeks after interventions and 2 weeks after stopping any interventions (6^{th} week).

Safety assessment

The participants were requested to report any unexpected adverse effects including abdominal pain, vomiting, urticarial vasculitis, headache, and heartburn. The assessment of these effects was made every week by calling the patients. The patients reported no specific side effects for *M. aquatica*. However, during the four weeks of treatment, patients were visited daily by primary healthcare workers who directly observed the treatment and short course (DOTS) agents. They were asked to inform the researcher about any complication or physical and mental problem (related and unrelated) in patients, within at most 24 h. The researcher was in turn supposed to notify the executor, sponsor, and Ethics Committee if necessary. In addition, the researcher called the patients on a weekly basis to know about their problems. The participants who met at least one of the exclusion criteria at any stage during the study were supposed to be excluded from the study.

Statistical analysis

The basic parameters of the study are presented as mean ± SD and frequency (percentage). Means were compared between two groups using the students t-test, whereas nominal data were compared by use of the Pearson's Chi-squared test. Data were analyzed using SPSS software (version 18). A p value less than 0.05 was considered statistically significant. Our analysis method is intention to treat (ITT) and per protocol. For this purpose, all patients who entered the study underwent ITT analysis, but for the calculation of per protocol, only patients who took more than 90% of the drug were included in the analysis.

Results

Out of the 104 patients who visited the gastroenterologist for bloating from during 2019 (from January to May), 100 patients with IBS fulfilled the inclusion criteria and were randomized to receive either MAC-330 or placebo. There were 50

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Results

Out of the 104 patients who visited the gastroenterologist for bloating from during 2019 (from January to May), 100 patients with IBS fulfilled the inclusion criteria and were randomized to receive either MAC-330 or placebo. There were 50

people in each group. The population was Iranian and the mean (±SD) age was 36.5 ± 9.9 years There were 6 men and 44 women in the placebo group and 16 men and 34 women in the medicine group. Baseline demographic characteristics, duration of illness, the severity of bloating, the frequency of defecation, the frequency of feeling of abdominal pain and quality of life were examined at the beginning of the study and at 1, 2, 4, 6 weeks after intervention.

The mean ± SD age of patients in MAC-330 and placebo groups was estimated at 35.8 ± 8.4 and 37.4 ± 11.2 years, respectively, which did not have a significant difference ($p < 0.393$). On the other hand, the range of individuals' height in both groups was obtained at 164-165 cm, which was quite close to each other ($p < 0.271$). Based on the Kolmogorov-Smirnoff test the variables had a normal distribution. It is worth mentioning that there were no significant differences between the two groups in terms of their baseline characteristics ($p > 0.05$). However, there is a significant difference between the two samples in terms of marital status ($p < 0.021$). (Table 2 and Figure 1).

The mean ± SD duration of illness, as another study variable, was estimated at 45 ± 10.5 and 41.5 ± 11.7 months in placebo and MAC-330 groups, respectively. Based on the analysis, the two groups did not show a significant difference in terms of the duration of the study variable ($p < 0.122$)

The severity of bloating in the MAC-330 and placebo groups was measured through VAS and obtained at 6.4 and 6.2, respectively, before the intervention (p value < 0.418). Simultaneously, the patient's bloating rate decreased by two units in the second week after the intervention in both MAC-330 and placebo groups ($p < 0.438$). However, four weeks after the beginning, the severity of bloating in the case and placebo groups decreased ($p < 0.030$). The severity of bloating increased again in both groups after six weeks (two weeks after stopping the treatment) and was much higher in the placebo group than in the MAC-330 group ($p < 0.026$). A significant difference of 5% was observed at the probability level between the two groups four weeks after the study and 2 at weeks follow up. Table 3

The frequency of defecation per week was recorded from the week before the start of the intervention until the fourth week of the follow-up period. In general, the frequency of defecation has increased with the onset of the intervention until the fourth week of the treatment. Although this increase has been observed in both groups, it has been higher in the MAC-330 than in the placebo group (Table 3).

Based on the trend of abdominal pain reduction in both case and control groups, it can be inferred that both medicine (MAC-330) and placebo were effective in reducing abdominal pain; however, these changes were not significant. It should be noted that, although the reduction in abdominal pain in patients is a little different in the MAC-330 group, these changes are not significant (Table 3).

Table 2. Baseline characteristics of participants

	MAC- 330 n = 50	Placebo n = 50	Total n =100	P-value
Age (mean ± SD, years)	35.8 ± 8.4	37.4 ± 11.2	36.5 ± 9.9	*0.393
Height (mean ± SD, centimeter)	164.0 ± 5.5	165.6 ± 8.3	164.8 ± 7.0	*0.510
Weight (mean ± SD, Kg)	70.9 ± 10.7	72.6 ± 15.0	71.8 ± 13.0	*0.271
BMI (mean ± SD, kg/m2)	26.4 ± 4.0	26.4 ± 4.6	26.4 ± 4.2	*0.980
Duration of illness (mean ±SD, months)	41.5 ± 11.7	45 ± 10.5	43.25 ± 11.1	*0.122
Education status (%) Under diploma Diploma	 46 54	 56 44	 51 49	**0.317
occupational status (%) Unemployed Employed	 38 62	 38 62	 38 62	**1.000
Marital status (%) Single Married	 6 94	 22 78	 14 86	**0.021

* t- test , **chi square- test

Table 3. Comparison of response variables between the groups and across time

	MAC- 330 (mean ±SD)	placebo (mean ±SD)	*p- value
Severity of bloating (Score) *p- value			
The start of medication	6.4 ± 1.4	6.2 ± 1.3	0.418
Week 1	no report	no report	no report
week 2	4.2 ± 1.6	4.4 ± 1.2	0.438
week 4	3.7 ± 1.6	4.3 ± 1.4	**0.030
week 6	4.4 ± 1.5	5.0 ± 1.2	**0.026
Frequency of defecation			
The start of medication	8.2 ± 5.8	7.8 ± 4.5	0.730
Week 1	8.8 ± 4.4	8.7 ± 4.3	0.908
week 2	9.9 ± 3.6	9.0 ± 3.9	0.222
week 4	9.0 ± 3.9	9.0±4.1	**0.026
week 6	10.5 ± 3.1	9.4 ± 4.1	0.106
Abdominal pain			
The start of medication	20.6 ± 6.7	18.8 ± 6.5	0.271

Week 1	17.4 ± 6.0	16.3 ± 6.0	0.528
week 2	14.6 ± 5.7	14. 5± 5.2	0.913
week 4	13.2 ± 5.8	14.0 ± 5.6	0.506
week 6	13.2 ± 5.7	14.3 ± 5.1	0.293

*paired t test, ** statically significant

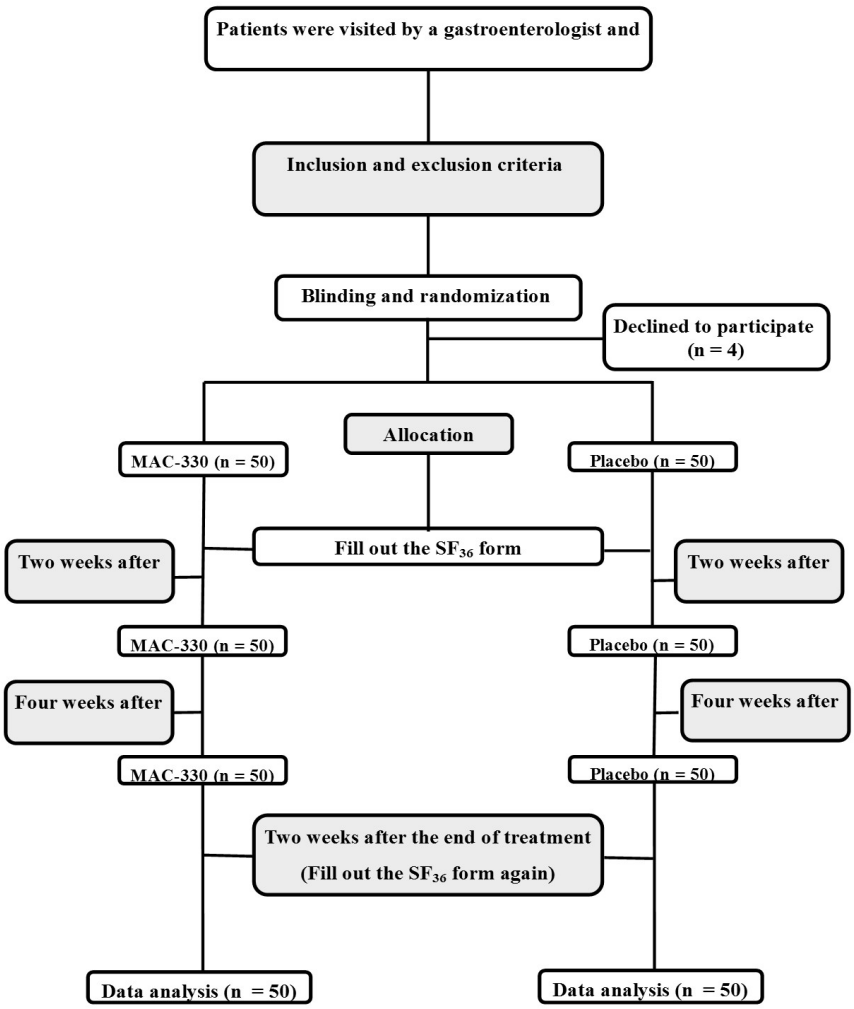

Figure 1. CONSORT Flow diagram

Quality of life

Patients were asked to consider their physical and mental health status within 4 weeks and answer relevant questions. Analysis of each question of questionnaire showed no significant differences between the two groups of MAC-330 and placebo before and after the intervention. Paired t-test also showed that the sum of scores of each question before and after the intervention in both groups were not significantly different.

Outcomes

The comparison of the mean scores of the two groups before and after drug treatment showed

that there was no significant difference between the two groups in terms of the severity of bloating, frequency of defecation, and abdominal pain after two weeks from the start of the intervention. However, 4 weeks after the study and 2 weeks after the end of the study, a significant difference was observed between the two groups in terms of severity of bloating ($p < 0.030$, $p < 0.026$ respectively). The frequency of defecation was increased after 4 weeks of intervention in both groups but this increase was not significant. The abdominal pain decreased after 4 weeks of intervention in both group but this was not significant (Table 3 and Figure 2).

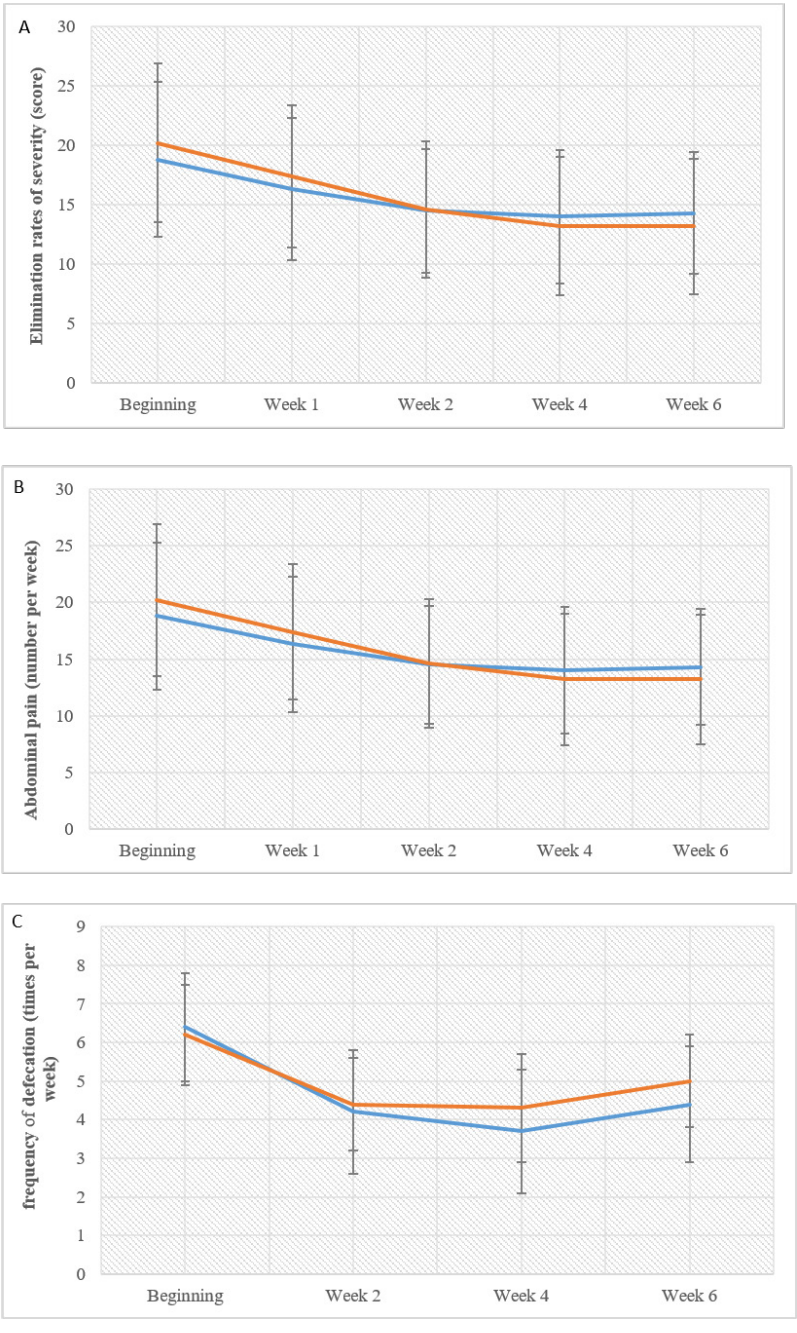

Figure 2. The severity of bloating (A), abdominal pain (B) and frequency of defecation during study

Quality control

A proper framework was considered before the beginning of the trial to ensure the quality of the study. Moreover, weekly monitoring was performed by the executor through telephone, and daily face-to-face monitoring was also performed by the primary health workers during the implementation of DOTS. Any possible problems or complication (related or unrelated to the study) was supposed to be reported to the researcher. Furthermore, the completion and conformity of the forms and clinical trial procedures, such as medication compliance and withdrawal of the participants were also investigated in this study.

Confidentiality

The present study was conducted in accordance with the ethics codes and the collected data were kept confidential, according to the ethical principles stated in the declaration of Helsinki [40].

Data collection and management

All data and documents including informed consent forms, questionnaires, and test results have been collected in accordance with standard operating procedures. The standard SF-36 quality was completed and collected by the researcher. This questionnaire included 36 items and its validity and reliability were confirmed in Iran. The VAS for the amount of bloating was completed five times at two-week intervals. The researcher ensured the participants that the questionnaires and forms would remain anonymous and that all the collected data would be kept confidential.

Discussion

Participants were randomized and assigned to placebo and MAC-330 groups. In the placebo group, 12% and 88% of the participants were male and female, respectively; while this rate was 32% and 68% in the MAC-330 group. Therefore, the percentage of female patients in both groups was significantly higher than males, and this difference in the prevalence of IBS in females has been observed in other studies as well. In a case study conducted in North America, the ratio of female to male was estimated at 2 to 1. Moreover, in a study conducted by Mansouri et al. in Iran, the incidence of females was twice that of males [21,41,42]. However, this significant difference was not observed in other studies, and cannot be generalized [43,44]. Higher percentage of female patients can be more attributed to the females' lifestyle and less to their activity.

The comparison of the two groups in terms of age, height, weight, and BMI did not show a significant difference before the intervention. All three factors have improved during the treatment in both groups; however, the graph pattern showed that the effects of the drug and placebo seem to have diminished two weeks after the intervention, especially in terms of the frequency of defecation.

Based on the analysis, the two groups did not show a significant difference in terms of the duration of the study variable (p value < 0.122). This variable has been reported in other randomized clinical trials [42-46]. The review of all aforementioned articles indicated that there was no relationship between the duration of the

illness and the severity of symptoms.

The results showed that the patient's bloating rate decreased in the second week and fourth weeks after the intervention in both MAC-330 and placebo groups. The severity of bloating increased again in both groups after six weeks (two weeks after stopping the treatment) and was much higher in the placebo group than in the MAC-330 group. In general, the decrease in bloating in both groups after the start of the intervention indicated that this improvement is at least partly due to the effects of self-inculcation. Therefore, it seems that the MAC-330 is not preferable to treatment by placebo in a short time (two weeks after intervention). However, in the long time (four weeks after intervention), the effect of MAC-330 intervention was significant, compared to placebo.

The frequency of defecation was increased after 4 weeks of the treatment. Although this increase has been observed in both groups, it has been higher in the MAC-330 than in the placebo group.

The results of this study also showed that both drug (MAC-330) and placebo were effective in reducing abdominal pain; however, these changes were not significant.

In a study by May et al., which showed the effectiveness of a 29-day use of a combination of peppermint and cumin essential oil compared with placebo in patients with gastrointestinal disorders [47], the lack of response in the first 2 weeks of intervention was suggested to be due to short time of the intervention. The results in the fourth week after the intervention and the sixth week, despite stopping the intervention, were in favor of MAC-330 group.

In a study of Khonche et al. on peppermint as a plant with digestive effects, it was found that peppermint extract significantly improves the quality of life and reduces digestive problems such as bloating, diarrhea, gastric contraction, indigestion [37]. In the present study, the positive effects of MAC-330 on gastrointestinal symptoms were in line with this study. Perhaps due to the short duration of the study and measuring the quality of life, the effect of MAC-330 on quality of life was not evident. Due to the short duration of the intervention, none of the markers of quality of life was significant in the two groups.

In a study by Agah et al., which compared the effect of dimethicone with Carmint herbal medicine on bloating, the result showed that the therapeutic effects of Carmint on improving bloating complaints, especially in the short term, were greater than the effect of dimethicone [48]. However, in the present study, in the short term (after 2 weeks), no difference was shown between MAC-330 and placebo, but in the long-term use (4 weeks), this difference was significant.

Finally, the small number of participants and single-center design of the study, the lack of similar trials on "*M. aquatica*" (MAC-330) and the large number of capsules that each patient must be used can be mentioned as study limitations.

Conclusion

According to the results of this study, MAC-330 cannot be applied to treat patients with bloating

IBS in short time; however, it reduces the abdominal bloating and pain in these patients especially in the long-term use. Further research is needed to establish the role of *M. aquatica* extract in the treatment of irritable bowel syndrome and to determine the optimal duration of treatment, dosage of M. aquatica extract, and its possible side effects related to long-term use.

Conflict of Interests

The authors declare that there is no conflict of interest regarding the publication of this study.

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