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Assessment of the effect of sachet formulation of almond (*Amygdalus dulcis* L.) on diarrhea prominent irritable bowel syndrome (IBS-D) symptoms: A clinical trial

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ABSTRACT

Objectives: Almond has been listed in the low FODMAPs (Fermentable Oligo-, Di-, Mono saccharides And Polyols) and is recommended for infant diarrhea and gastrointestinal problem in Iranian folk medicine. In this work, sachet of almond has been designed, formulated and is studied on the clinical symptoms of diarrhea prominent irritable bowel syndrome (IBS-D). Design: almond was standardized on the basis of total protein and carbohydrate content. A sachet of almond and wheat flour (placebo) was formulated and their physicochemical characteristics were investigated. Intervention: In a double blind randomized trial, fifty IBS-D patients were randomly enrolled into the almond and placebo groups, ranked in respect to the severity of symptoms to mild-moderate and severe disease. The patients received almond or placebo sachet (40 g/day,20 days) respectively. Main outcome measure: Patients were assessed for bowel habit, pain severity and frequency and bloating and data was recorded in a data collecting form. Results: The results showed that none of the primary outcomes of the disease is improved in the patients treated with almond. The bowel movement and severity of the pain was significantly increased in the almond treated patients compared to the placebo and baseline ($p < 0.05$).
Conclusion: Almond contains high content of oligo-fructan which in high intake might result in a large amount of fermentable carbohydrates that can exacerbate the symptoms of the disease. So, despite the almond inclusion in the low-FODMAPs, the amount of almond intake is a determining factor and here we have controversial results for almond intake in patients with IBS. Mental health and physical activity of patients are also involved in the disease.

1. Introduction

Irritable bowel syndrome (IBS) is a common functional disorder of gastrointestinal system with 5–11% prevalence in USA and Europe. The patients who suffer from IBS, experience abdominal pain, sense of abdominal distention, alteration in bowel habit and overgrowth of bacteria.[1] Even though this syndrome is not life threatening, the life quality is impressed and in spite of the avaiability of a wide ranges of medications; its management remains a challenge for physicians and patients. Today, much attention has been devoted to the use of prebiotics to manipulate the intestinal microbiota in order to promote health. Prebiotics can suppress the growth of pathogenic bacteria and may protect gastrointestinal epithelium.[2] To date, no effective treatment has been established for IBS and despite the worldwide popularity of medicinal plants, extensive clinical studies have not been carried out on these natural sources.

Almond (*Amygdalus dulcis* L.) belongs to Rosaceae family and is native to Mediterranean region. The plant has been extensively

Abbreviations: IBS, irritable bowel syndrome; IBS-D, irritable bowel syndrome diarrhea prominent; BSA, bovine serum albumin; FODMAP, fermentable oligo-, di-, mono saccharides and polyols

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distributed throughout Iran especially in different regions of Kerman province [3–5] and contains protein, mono and poly unsaturated fatty acid, catechin, flavonoid, phenolic acid, [6] different types of minerals, vitamins, micronutrients and a great amount of non- digestable carbohydrates. [7] Almond cell wall contains arabinose-rich pectin substances which can be hydrolyzed by the intestinal micro biota.[8] Antioxidant, anti-inflammatory, immune-stimulant and laxative effects have been reported for almond.[9] In Iranian folk medicine, a processed form of almond named "Harire" has been recommended for alleviation of diarrhea in infants in which the fruits are boiled in water for 2 min., peeled off and are re-heated with continuous stirring along some sugar to reach the desired consistency. In this work, considering the experience of folk medicine about the usefulness of almond in the digestive tract, a sachet formulation of the almond powder has been prepared and assessed through a pilot study to find if is effective on improving of clinical symptoms in the diarrhea prominent irritable bowel syndrome (IBS-D) subjects.

2. Materials and methods

2.1. Plant materials

Almond was prepared from Kerman province at June 2017. A voucher specimen of the plant was deposited in the Herbarium Center of Department of Pharmacognosy (KF1234). The kernels were dried in shade. Wheat flour (placebo) was purchased from market.

2.2. Protein content determination

Total protein content of the almond was determined using Bradford method due to its convenience and sensitivity. In this colorimetric method, the Coomassie Brilliant Blue G-250 dye forms a strong complex with the carboxyl group of proteins and has absorbance at 595 nm. Bovine serum albumin (BSA) was used as standard.[10]

2.3. Carbohydrates content of almond

Due to the presence of a variety of carbohydrates in almond, it's major carbohydrates were detected using paper chromatography. For determination of water soluble sugars, about 5 g of almond was dried in shade, milled and after sieving was extracted with ethanol 80% (5 ml, 75 °C) and centrifuged for 10 min (3000 rpm). The supernatant was separated and 5 ml concentrated H2SO4 and 1 ml phenol 5% (w/v) were added to 2 ml almond extract for detaching the additional adulterants, mixed thoroughly and let to stand in the room temperature for 30 min. Absorbance was measured at 485 nm and calibration curve of glucose, maltose, sucrose and fructose was provided and total water soluble sugar of the plant was calculated based on these sugars [11].

2.4. Physicochemical characteristics of sachet

Sachet of almond powder was formulated in the Pharmaceutics Department of Faculty of Pharmacy (PDFP), Kerman University of Medical Science. The almond sachet contained 40 g almond powder and 20 g of wheat flour and rice flour. Placebo sachet contained 40 g wheat and 20 g of wheat flour and rice flour with the same size and shape of the almond sachet.To examine the distribution of particle size, a definite weight of powders were passed through 10 different sized meshes (12, 16, 20, 30, 40, 50, 60, 80, 100 and 200) which were stacked one upon the other and were inserted on a sieve shaker (10 rpm) for 20 min. The weight of the remaining powders was determined on each sieve.

For angle of repose, a stainless steel funnel (with hole size of 10 mm and a height of 111 mm) was used. The height (h) and the radius (r) of the formed powder cone were measured (symmetrical cone should be formed), and the angle of repose (θ) was calculated using the following equation:

$$\theta = \tan^{-1}(h/r)$$

Tapped and bulk density were determined as following: a certain amount of powder (approximately 2 g) was shaken to remove any agglomeration and introduced into a graduated cylinder and initial volume and density (D1) of the powder was determined. The powder was tapped until no additional volume changes were noted after tapping. The final volume and density (D2) was measured and the percentage of compressibility was calculated according to the Car's formula: compressibility (%) = (D2-D1)/D2*100. Hausner's ratio (D2/D1) was calculated too [12].

Rheological behavior of the sample was studied at 25 °C with a Brookfield LV rheometer device with spindle speed of 10 rpm. The rheogram was drawn and the rheological specification was recorded.

2.5. Clinical trial design

This double blind randomized, placebo controlled study was conducted in the Gastroenterology and Hepatology Research Center, Afzalipour Hospital of Kerman University of Medical Sciences, Kerman, Iran from September 2016 to April 2017. Approval of the ethic committee of the University was obtained prior to the study and the trial was registered in the Iranian Registry of Clinical Trial (Registry ID: IRCT2017070234861N1). The protocol of the research was in accordance with the Declaration of Helsinki. The IBS-D patients which have been recruited to the trial, met the ROME IV criteria [13] and clinically were confirmed by a specialist. The eligible participants were enrolled to the study and completed consent form before the intervention. Individuals who withdrew the trial or who had the other GI disorders, diabetes mellitus, connective tissue diseases, underlying malignancy, thyroid and any metabolic diseases were excluded from the study. The consort flow diagram of the trial has been shown in Fig. 1. Totally, 50 eligible IBS-D patients were included in the study according to the inclusion and exclusion criteria and were randomly allocated into almond and placebo groups using random allocation rule. Demographic data of the patients was recorded by a technician in a collecting form. Coding of the almond and placebo was taken at the PDFP and the clinical researchers and patients were blinded regarding the medication. The patients received either almond or placebo sachet which was taken with apple juice for 20 consecutive days. The patients were given calculated amount of medication for the whole period of the trial. To control the medication compliance, the patients were urged to return unused drugs and avoid from taking any medications that were

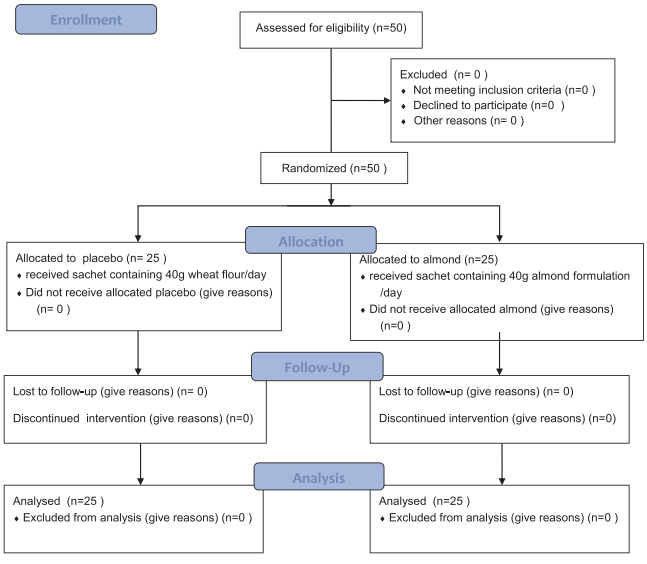

Fig. 1. Consort flow diagram of the trial.

not prescribed by the physician through the study. The primary outcomes were the mean of changes in the bowel movement/day, pain intensity, pain frequency and bloating in comparison to baseline and placebo with a 3-point scale rating. Sleep disturbance, location of abdominal pain, need for analgesics and need to physician visit were assessed as secondary outcomes. A medical student was responsible for follow up the patients via call or visit to sure about their drug regiment adherence as well as the occurrence of any side effects.

For sample size, two sided significance of 5%, power of 80% and standardized effect size 0.8 were considered for comparing the mean difference between two independent groups. Therefore, 25 patients per group were entered to the study. The severity of intestinal symptoms in both groups was recorded in the data collecting form including 7 questions with two to three scales (mild, moderate and severe conditions). To ease the analysis, mild and moderate scales are considered as one level. The concept and meaning of scaling have precisely been explained for the patients.

2.6. Statistical analysis

Due to the normal distribution of symptom score and demographic characteristics, the results were presented as mean ± SD. Data was analyzed using SPSS 19. For description of quantitative data, mean and standard deviation and for qualitative ones, relative frequency and percentage have been used. Independent T test, chi squre, Fisher's Exact Test and McNemar were used for the comparisons. Differences with p value equal or less than 0.05 were considered as significant level.

3. Results

3.1. Total protein content

Almond total protein content was calculated using BSA calibration curve (Fig. 2) (3.37 ± 0.12 mg BSA equivalent /g dried almond) which was almost twice as large as the one reported by De Angelis and et al.,[14].

3.2. Carbohydrate content

As shown, on the basis of calibration curve of sucrose, fructose, glucose and maltose, sugar content of almond was determined as 0.091 ± 0.002 g, 0.086 ± 0.002 g 0.0085 ± 0.002 and 0.078 ± 0.002 g sucrose, fructose, maltose and glucose / g dried almond respectively (Fig. 3a–d).

3.3. Physicochemical characteristic

Characteristics of particle size distribution of almond powder, wheat flour and formulation were determined. The particle size distribution for the formulation was determined. About 78% of particles were more than 1680 microns and about 3% were in the smallest size of 400-400 microns.

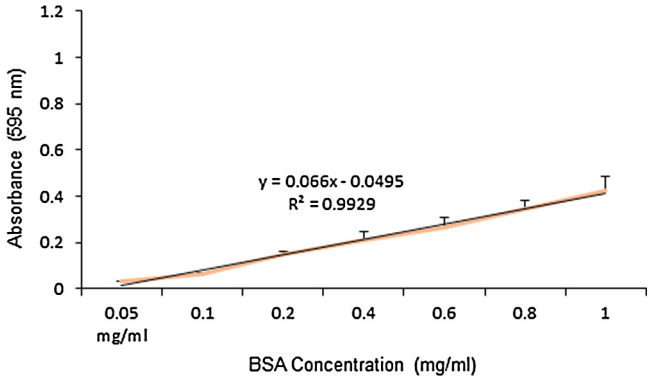

Fig. 2. Calibration curve of different concentration of buvine serum abumin (BSA) in phosphate buffer determined at 595 nm. Each experiment was done in triplicate and the results were reported as mean ± SD.

Angle of repose, bulk density, tapped density, Carr's index and Hausner's ratio for powders have been checked and reported in Table 1 and the rheogram of the formulation has been shown in Fig. 4. Formulation was free from any microbial contamination.

3.4. Clinical trials

A total of 50 participants were enrolled in the study. Regarding demographic characteristics, no significant difference was considered between the almond and placebo subjects in respect to sex and age. Among subjects, 16(64%) and 13(52%) were female in almond and placebo group respectively. Mean ± SD of age was 32.30 ± 8.40 and 27.96 ± 7.15 in almond and placebo groups. In terms of marital status, 17(68%) and 13(52%) of almond and placebo patients were married. Majority of the participants in almond (80%) and placebo (59%) had educational level higher diploma. Mean duration of IBS- D symptoms were 21.56 ± 9.48 and 20.88 ± 8.44 months for almond and placebo groups respectively (p = 0.79) (Table 2).

3.4.1. Primary outcomes

At the end of 20th day of trial, concerning the bowel movement, before the treatment, 13(52%) and 12(48%) and after the trial, 19(76%) and 6(24%) of the patients in the placebo group were inserted in the mild- moderate and severe category which were significantly different (p = 0.02). In the almond group, before the trial, 22(88%) and 3(12%) and after the trial, 3(12%) and 22(88%) of the patients were placed in the mild-moderate and severe level which were significantly different (p = 0.001). The almond recieving patients significantly were different from placebo too(p = 0.001). Concerning the pain intensity, in the placebo group, before the treatment, 19(76%) and 6(24%) and after the treatment, 23(92%) and 2(8%) of the patients complained from mild-moderate and severe pain respectively which were not significantly different (p = 0.09). In the almond group, before the trial, 23(92%) and 2(8%), and after the treatment, 15(60%) and 10(40%) of the patients stated the mild- moderate and severe pain which exhibited significant difference (p = 0.02). The differences between the almond and placebo groups were significant too (p = 0.02). In terms of pain frequency, before the treatment, 25(100%) and 0(0%) and after the treatment, 24 (96%) and 1(4%) of the patients in the placebo group were inserted in the mild- moderate and severe level which were not significantly different (p = 0.3) while in the almond group, before the trial, 24 (96%) and 1 (4%) and after the trial, 13(52%) and 12(48%) of the patients were inserted in the mild-moderate and severe level which were significantly different from each other (p = 0.01) and from the placebo (p = 0.001). In terms of bloating duration, before the treatment, 23(92%) and 2(8%) and after the treatment 25(100%) and 0(0%) of the patients in the placebo were placed in the mild-moderate and severe categories which were not significantly different (p = 0.06). In the almond group, before the treatment, 18(72%) and 7(28%) and after the treatment, 16(64%) and 9(36%) of the patients were in the mild-moderate and severe categories which show significant difference compared to each other (p = 0.002) and placebo (p = 0.001) (Table 3).

3.4.2. Secondary outcomes

Sleep disturbance, and analgesic demand in the almond group exhibited significant difference with the placebo (p = 0.001 and p = 0.004) (Table 4).

4. Discussion and conclusion

Study of the physicochemical properties of a formulation (simple or in combination with excipient and or the other drugs) is crucial and can

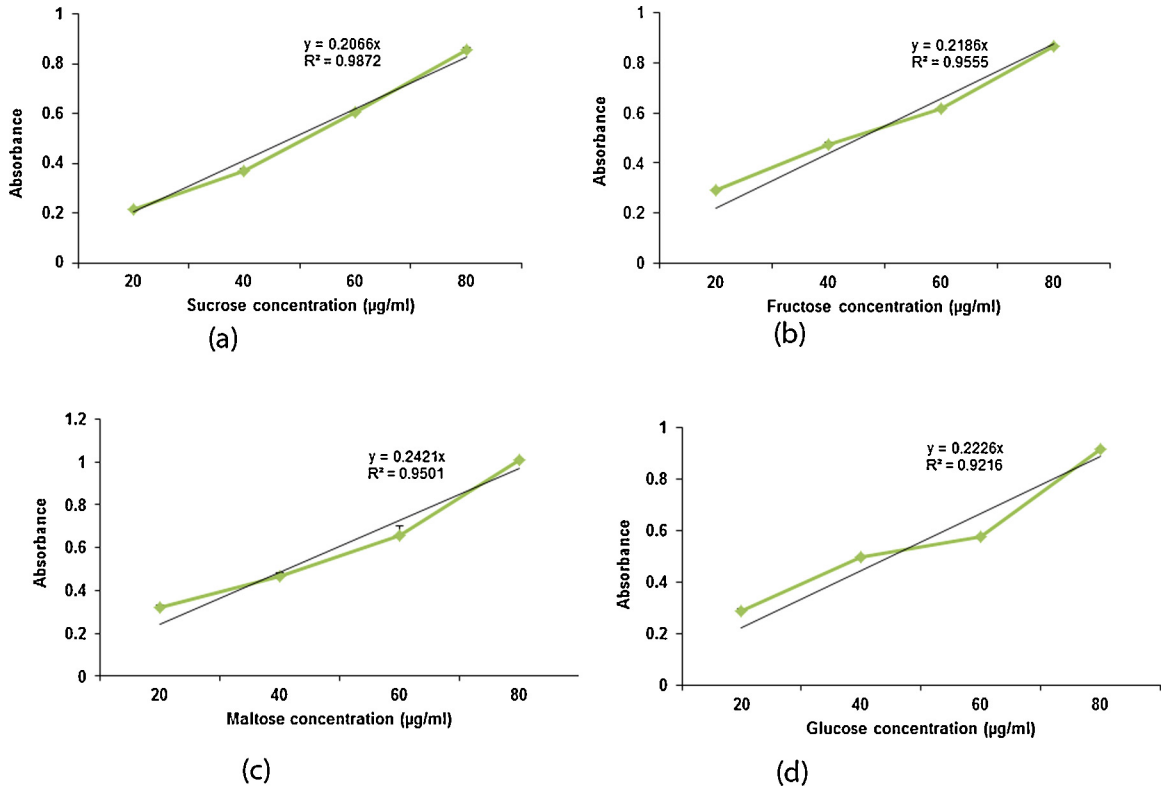

Fig. 3. a–d: Carbohydate determination of almond on the basis of sucrose (a), fructose (b), maltose (c) and glucose (d) calibration curves.

Table 1
Physicochemical characteristics of almond, wheat flour and formulation powders.

Criteria	Sample		
	Almond powder	Wheat flour powder	Formulation powder
Angle of repose	42.61	49.64	41.63
Bulk density	0.48	0.53	0.46
Tapped density	0.51	0.64	0.50
Compressibility (%) = (D2-D1)/D2*100	5.88	17.18	8.00
Hausner's Ratio (D2/D1)	1.06	1.21	1.09

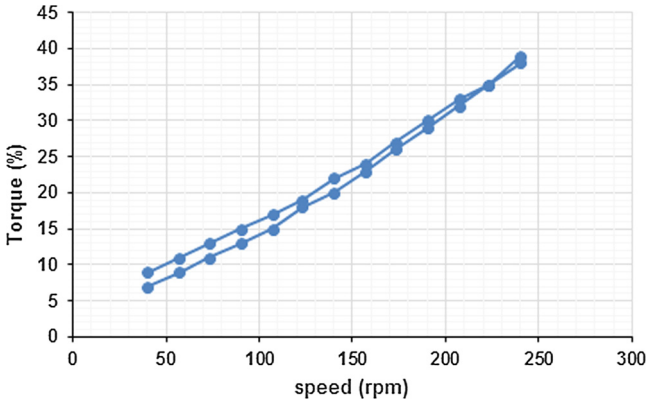

Fig. 4. Rheological behavior of almond formulation at 20–240 rpm.

guarantee the product quality. Angle of repose is a main subject for powder formulations due to the rough surface and rapid change in the capacity of powders. The angle of repose for almond, wheat flour and formulation were determined to be 42.61°, 49.64° and 41.63° respectively which was acceptable and appropriate for the prepared formulation according to the stagnant angle (41-45°). Although the particle size distribution is an important factor on the flow ability of the powders, but their composition especially fat content also strongly influences powder flow ability [15]. The angle of repose is a main factor on their solubility too. Current almond formulation is designed for digestive system and the angle of repose less than 90° would be appropriate for this purpose[16]. Rheological behavior of formulation was studied too using a multi-speed rheometer. This device is available in both single speed and multi-speed models. Single-speed ones are only capable for examining the rheological behavior of Newtonian materials. For the current formulation, the use of a force of 240 rpm led to a strain up to 40%. The rheogram was linear and a Newtonian behavior was observed for almond formulation which indicates that the suspension formed does not resist against the applied force and can easily be leached in

Table 2
Demographic characteristics of the participants involved in evaluation of the almond sachet effectiveness in alleviation of irritable bowel syndrome (IBS) symptoms.

Variable	Amond group	Placebo group	P value
Age (Year)(µ ± SD)	32.30 ± 8.40	27.97 ± 7.15	0.289
Sex N (%)			
Male	9(36%)	12(48%)	0.39
Female	16(64%)	13(52%)	
Marriage N (%)			
Single	8(32%)	12(48%)	0.248
Married	17(68%)	13(52%)	
Disease duration (month) (µ ± SD)	21.56 ± 9.48	20.88 ± 8.44	0.790
Education N(%)			
Under diploma	2(8%)	3(12%)	0.09
Diploma	3(12%)	5(20%)	
B.S.	18(72%)	9(36%)	
M.S.	0(0%)	3(12%)	
Ph.D	2(8%)	5(20%)	

Table 3
Comparison of primary outcomes after 20 days treatment with almond in comparison to placebo and baseline.

Symptom	Placebo group			Almond group			P Between group comparison Before / After
Primary outcomes	Baseline N (%)	Treatment N (%)	P	Baseline N (%)	Treatment N (%)	P	
Bowel movement							
Moderate (1-2/day)	13(52)	19(76)	0.02	22(88)	3(12)	0.001	0.005/ 0.001
Severe (3-4/day)	12(48)	6(24)		3(12)	22(88)		
Pain intensity							
Mild-moderate	19(76)	23(92)	0.09	23(92)	15(60)	0.02	0.51/0.02
Severe (Needs to drug)	6(24)	2(8)		2(8)	10(40)		
Pain frequency							
Mild-moderate	25(100)	24(96)	0.3	24(96)	13(52)	0.01	0.6/0.001
Severe (Once daily)	0(0)	1(4)		1(4)	12(48)		
Bloating duration							
Mild-moderate	23(92)	25(100)	0.06	18(72)	16(64)	0.002	0.05/0.001
Severe (Continues more than 12 hours)	2(8)	0(0)		7(28)	9(36)		

Newtonian fluids.

It is imagined that IBS is a multifactorial disorder in which different factors have been involved for example alteration in the bowel movement, overgrowth of the small bacteria, microscopic inflammation and visceral over sensitization [17]. Almond has been used in Iranian folk medicine for diarrhea in the form of “Harire”, a baked form of skinned kernel. In this work, the effectiveness of almond was studied in alleviation of the clinical symptoms of IBS-D. The IBS-D patients were ranked into mild- moderate and severe in respect to severity and frequency of the symptoms. The results showed that none of the primary and secondary outcomes of the disease is improved in the patients treated with almond and the bowel movement and severity of the pain was significantly increased compared to the placebo and baseline ($p < 0.05$) (Table 3).

In recent decade, a multidisciplinary team from Monash University has developed FODMAP (Fermentable Oligo-, Di-, Mon saccharides And Polyols) model of diet which seems to be superior to the other advised food styles of intake. FODMAPs contain a group of poorly- absorbed carbohydrates including sugar alcohols and short chain carbohydrates which could not be absorbed or digested, increase the osmotic pressure and pull water into the intestine. Consequently lead to the fluid secretion into the intestine, distend it and induce abdominal discomfort. The high-FODMAP diets are prone to fermentation by the microbiota of colon and raise the gas production and as a result contribute to trigger or mimic IBS symptoms such as bloating and diarrhea. So adhere to a low-FODMAP diet might lessen the symptoms of disease and there are some controlled studies that assessed the role of low-FODMAP diets in the patients with IBS [18–20]., The evidence and clinical trials to date show that limitation of FODMAPs has an important role for alleviation of the IBS symptoms, however further studies are needed to evaluate the safety and effectiveness of this diet in the long term use. Although, in most of the references, almond has been listed as lowFODMAP, [20,21] there are still some baffling about almond inclusion. The researchers in Monash University have suggested that while the taking about 10 unit almonds is categorized in low-FODMAPs, the use of 20 almonds does not fall into this group due to the high content of almond oligo-fructan. This amount of almond intake is also associated with the size of almond. So that Monash app notes that 20 almonds (24 g) contain high amounts of galactan oligosaccharides (GOS), but a smaller serving of 10 almonds (12 g) can be listed in low-FODMAPs. Furthermore, the apple juice used in this study has considerable fructose content and can worsen the intestinal complaints especially in fructose- intolerant patients. Fructose has osmotic effect and causes motility increasing, abdominal symptoms, bloating and dose dependently abdominal pain [22].

Previous studies have shown that, apart from the diet, other factors such as mental health status and physical activity have been complicated especially in the severe IBS and at least 16–30% of the patients with IBS suffer from depression and or anxiety. In addition, the small sample size of patients was one of the limitations in this trial which enhances the chance of bias.

Conclusion: Recalling the periodic dietary through the study is encouraged because it affords information which help to find if there is any factors have influenced on severity of the symptoms in the clinical trial for example, the amount of carbohydrate, fiber and fat intake with known effects on IBS end points [23]. The use of multiple enrolling sites is strongly encouraged in order to maximize external validity and to minimize the baffling influence of regional variations in diet, exercise habits, and ethnicity [24]. There is still a gap between existing knowledge and understanding the different styles of diet. The controversial issue in the use of almond as a low-FODMAPs in patients with IBS in this study

Table 4
Comparison of secondary outcomes after 20 days treatment with almond in comparison to placebo and baseline.

Symptom	Placebo group			Almond group			P Between group comparison Before / After
Secondary outcomes	Baseline N (%)	Treatment N (%)	P	Baseline N (%)	Treatment N (%)	P	
Sleep disturbance							
Mild-moderate	20(80)	22(88)	0.8	13(52)	11(44)	0.2	0.005/0.001
Daytime fatigue (lethargy)	5(20)	3(12)		12(48)	14(56)		
Pain location							
Lower abdomen	10(40)	14(56)		7(28)	6(24)		
Upper abdomen	13(52)	10(40)	0.32	16(64)	12(48)	0.05	0.8/0.02
Both lower and upper abdomen	2(8)	1(4)		2(8)	7(28)		
Analgesic drug							
Demand Moderate (1-2/week)	10(40)	15(60)	0.06	19(76)	5(20)	0.04	0.01/0.004
Severe (3-4/week)	15(60)	10(40)		6(24)	20(80)		
Need to physician visit							
1-2/month	25(100)	25(100)	0.05	20(80)	20(80)	0.05	1/1
3-4/ month	0(0)	0(0)		5(20)	5(20)		

and the obtained results show that still many unknown factors involved that require further researches. This is the first study about effectiveness of almond and clinical trials to arrive at the accurate and correct conclusion.

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References

1. Saha L. *Irritable bowel syndrome: Pathogenesis, diagnosis, treatment, and evidence-based medicine.* . Jun 14 [cited 2019 Feb 14];20(22):6759–73. Available from:. World J Gastroenterol [Internet]. Baishideng Publishing Group Inc; 2014http://www.ncbi.nlm.nih.gov/pubmed/24944467.
2. Conlon MA, Bird AR. The impact of diiet and lifestyle on gut microbiota and human health. *Nutrient.* 2019;7(1):17–44.
3. Mohamadi N, Sharififar F, Koohpayeh A, Daneshpajouh M. Traditional and Ethnobotanical uses of medicinal plants by ancient populations in Khabr and Rouchon of Iran. *Int J Appl Pharm Sci Res.* 2015;5(11).
4. Sharififar F, Koohpayeh A, Motaghi MM, Amirkhosravi A, Puormohseni Nasab E, Khodashhenas M. Study of medicinal plants in Sirjan county, Kerman province, Iran. *J Herb Drug.* 2010;1:19–28.
5. Sharififar F, Moharamkhani MR, Moattar F, Babakhanloo P, Khademi M. Ethnobotany study of medicinal plants of Joopar mountains of Kerman province, Iran. *J Kerman Univ Med Sci.* 2014;21:37–51.
6. Milbury PE, Chen CY, Dolnicowski GG, Blumberg JB. Determination of flavonoids and phenolics and their distribution in almond. *J Agric Food Chem.* 2006;54(14):5027–5033.
7. Zhu Y, Wilkinson KL, Wirthensohn MG. *Lipophilic antioxidant content of almonds (Prunus dulcis): A regional and varietal study.* . May 1 [cited 2019 Feb 15];39:120–7. Available from:. J Food Compos Anal [Internet]. Academic Press; 2015https://www.sciencedirect.com/science/article/pii/S0889157515000046.
8. Dourado F, Barros A, Mota M, Coimbra MA, Gama FM. Anatomy and cell wall polysaccharides of almond (Prunus dulcis D. A. Webb) seeds. *J Agric Food Chem.* 2004;52:1364–1370.
9. Barreira JCM, ICFR Ferreira, MBPP Oliveira, Pereira JA. Antioxidant activity and bioactive compounds of ten Portuguese regional and commercial almond cultivars. *Food Chem Toxicol.* 2008;46(6):2230–2235.
10. Noouroozi R, Noroozi M, Ahmadizadeh M. Determination of protein concentration using Bradford microplate protein quantification assay. *Int Electron J Med.* 2018;4(1):11–17.
11. Schlegel H_G. Die verwertung orgngischer souren durch chlorella lincht. *Planta J.* 1956;47:47–51 47-51. Plant J.
12. Saw HY, Davies CE, Paterson AHJ, Jones JR. The influence of particle size distribution and tapping on the bulk density of milled lactose powders. *Chemeca, Brisbane, Aust.* 2013:299–303.
13. Drossman DA, Hasler WL. Rome IV-Functional GI Disorders: Disorders of Gut-Brain Interaction. *Gastroenterology.* 2016;150(6):1257–1261.
14. De Angelis E, Bavaro SL, Forte G, Pilolli R, Monaci L. Heat and pressure treatments on almond protein stability and change in immunoreactivity after simulated human digestion. *Nutr Switzerland.* 2018;10(November 11).
15. EH-J Kim, Chen XD, Pearce D. Effect of surface composition on the flowability of industrial spray-dried dairy powders. *Colloids Surf B Biointerfaces. Netherlands.* 2005;46(December 3):182–187.
16. Bachmann J, Ellies A, Hartge KH. Development and application of a new sessile drop contact angle method to assess soil water repellency. *J Hydrol (Amst).* 2000;231:66–75.
17. Ghoshal UC, Srivastava D. Irritable bowel syndrome and small intestinal bacterial overgrowth: Meaningful association or unnecessary hype. *World J Gastroenterol.* 2014;20(10):2482–2491.
18. Staudacher HM, Irving PM, Lomer MCE, Whelan K. Mechanisms and efficacy of dietary FODMAP restriction in IBS. *Nat Rev Gastroenterol Hepatol. England.* 2014;11(April 4):256–266.
19. Ostgaard H, Hausken T, Gundersen D, El-Salhy M. Diet and effects of diet management on quality of life and symptoms in patients with irritable bowel syndrome. *Mol Med Rep. Greece.* 2012;5(June 6):1382–1390.
20. Shepherd SJ, Gibson PR. Fructose malabsorption and symptoms of irritable bowel syndrome: Guidelines for effective dietary management. *J Am Diet Assoc. US.* 2006;106(October 10):1631–1639.
21. Fedewa A, Rao SSC. Dietary fructose intolerance, fructan intolerance and FODMAPs. *Curr Gastroenterol Rep. US.* 2014;16(January 1):370.
22. Manning AP, Thompson WG, Heaton KWMA. *Towards positive diagnosis of irritable bowel.* 1978; 1978:653–654 (September):2.
23. Austin GL, Dalton CB, Hu Y, et al. A very low-carbohydrate diet improves symptoms and quality of life in diarrhea-predominant irritable bowel syndrome. *Clin Gastroenterol Hepatol.* 2009;7(6):706–708.
24. Miller LE. Study design considerations for irritable bowel syndrome clinical trials. *Ann Gastroenterol.* 2014;27(4):338–345.