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RESEARCH ARTICLE

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The effects of fenugreek seed extract supplementation in patients with Alzheimer's disease: A randomized, double-blind, placebo-controlled trial

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Abstract

The aim of the current randomized control trial (RCT) study was to investigate the effects of fenugreek seed extract on memory, depression, quality of life, blood pressure, and serum malondialdehyde (MDA) and total antioxidant capacity (TAC) levels in adult AD patients. This randomized clinical trial was conducted in geriatric homes in Iran. The study participants included 82 AD patients with mild-to-moderate memory deficit. Patients in the intervention group received 5 cc of fenugreek seed extract for 4 months and subjects in the control group received a placebo. Memory, depression, quality of life, and BP levels, as well as serum MDA and TAC, were assessed before and after the intervention. There was a significant increase in serum levels of TAC ($p < 0.001$) and a reduction in serum MDA status ($p < 0.001$) after 4 months of fenugreek seed extract supplementation. In addition, increasing levels of memory ($p < 0.001$) and quality of life ($p < 0.001$), as well as reduction of depression ($p = 0.002$), systolic BP ($p < 0.001$), and diastolic BP ($p < 0.001$) levels were detected in the intervention group compared with baseline. Fenugreek seed extract supplementation in AD patients shows promising positive effects on memory, quality of life, BP, and selective oxidative indices levels.

KEYWORDS
Alzheimer's disease, fenugreek seeds, memory, oxidative stress, randomized controlled study

1 | INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that commonly occurs in both developed and developing countries (Brookmeyer, Johnson, Ziegler-Graham, & Arrighi, 2007). AD has the ability to destroy memory, cognition, and thinking skills and occurs most often in older people over the age of 65 years, but recently, it can occur earlier due to the mutation of certain genes (Koedam

et al., 2010). The most diffuse type of dementia occurs through AD (Gao, Burney, Callahan, Purnell, & Hendrie, 2019). Worldwide, increasing life expectancy and life conditions are contributed to a higher prevalence of dementia (Catindig, Venketasubramanian, Ikram, & Chen, 2012). A study among 14 World Health Organization (WHO) regions has reported that the prevalence of dementia was 50 million people in 2018 with an actual annual incidence of 4.6 million cases (Ferri et al., 2005; van Praag, 2018). It is estimated that in 2040, 81.1 million people will live with dementia (Mayeux & Stern, 2012). Recent studies were recognized the financial burden imposed, upon the socioeconomic system of countries by treatment and care of those afflicted by AD (Dodel et al., 2015; Schwarzkopf et al., 2011). Further, the results of a simulation study investigated that prolonging of staying a person in every stage of this disease will increase the cost of care for all the AD patients by 2080 (Cimler, Maresova, Kuhnova, & Kuca, 2019). A history of chronic disease such as diabetes, obesity, hyperlipidemia, and hypertension as well as depression may increase AD incidence (Lloret, Monllor, Esteve, Cervera-Ferri, & Lloret, 2019; Madmoli et al., 2019; Xue et al., 2017). It has been suggested that exposure to excessive oxidative stress, lower total antioxidant capacity (TAC), and related cell death in patients with chronic disorders could be partially responsible for AD progression (Volicer & Crino, 1990). On the other hand, increasing cerebral beta amyloid and tau accumulation in the brains of depressive patients has shown a correlation between depression and Alzheimer's pathology (Babulal et al., 2016; Donovan et al., 2018; Gatchel et al., 2017).

In general, the main underlying mechanisms that cause cellular and molecular changes in AD patients have not been clearly identified (Sanabria-Castro, Alvarado-Echeverría, & Monge-Bonilla, 2017). Lifestyle and environmental factors, including diet, physical activity, and weight control, may affect the progression and symptoms of AD (Rahman et al., 2020; Foroumandi et al., 2018). In recent years, use of antioxidant agents was prevalent to resist worsening AD symptoms and there is increasing attention to the use of natural or herbal alternatives that may have fewer side effects (Feng & Wang, 2012; Mancuso et al., 2007).

Fenugreek (also known as Greek hay) is a plant from the Leguminosae family with numerous health benefits due to its flavonoid, alkaloid, steroid, and saponin compounds (Dixit, Ghaskadbi, Mohan, & Devasagayam, 2005). Its seeds are high in vitamins, minerals, fiber, and protein (Altuntaş, Özgöz, & Taşer, 2005). The hypoglycemic and lipid-lowering effects of this medicinal plant have resulted in its wide use in diabetes and hyperlipidemia (Smith, 2003). It has antioxidant properties that may prevent memory deficits through the synergistic action of its active components (Bafadam et al., 2019; Prema, Justin Thenmozhi, Manivasagam, Mohamed Essa, & Guillemin, 2017). Moreover, a neuroprotective effect has reported against Parkinson's disease (Gaur, Bodhankar, Mohan, & Thakurdesai, 2013). To the best of our knowledge, the efficacy of fenugreek seed in AD-induced memory deficits and symptoms has not been studied to date. Therefore, the aim of the current randomized control trial (RCT) study was to investigate fenugreek seed extract effects on memory, depression, quality of life, blood pressure (BP), and on serum MDA and TAC levels as indicators of oxidative stress in adult AD patients.

2 | MATERIALS AND METHODS

2.1 | Participants

The current randomized, double-blind, placebo-controlled trial study was conducted to assess the efficacy of fenugreek seed extract on memory, depression, quality of life, BP, and antioxidant capacity of patients with AD. The inclusion criteria were adult patients with a diagnosis of mild-to-moderate AD according to the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria (McKhann et al., 1984), who were categorized in the clinical dementia rating (CDR) domain of 2 or lower than 2, and consented to participate in the study (the patient or the patient's caregiver). The exclusion criteria were allergic to fenugreek, soy, peas, or their compounds, history of fenugreek consumption, intake of monoamine oxidase inhibitors (MAOI), substance or alcohol abuse, diagnosis of a significant neurological disease other than AD, diabetes, cardiovascular disorders, severe depression, or hepatic, kidney or thyroid disease.

2.2 | Study design

Written informed consent was obtained from both patients and caregivers. The subjects were invited to participate in the study using convenience sampling. Overall, 82 participants were recruited to participate in the study from patients who were living in two geriatrics homes in Sabzevar, Iran, from February to July 2020 ($n = 41$ in each group).

The sample size of the study was calculated using the sample size formula for RCT [$n = 2Sp^2(Z_{1-\alpha/2} + Z_{1-\beta})^2/(\mu^1 + \mu^2)^2$] (Zhong, 2009), considering $\alpha = 0.05$, $\beta = 0.2$ (for a power of 80%), and serum MDA level as an outcome variable. In total, the sample size was calculated at 37 persons for each group. Considering 10% dropout, the total number of subjects needed was calculated at 41.

Participants were randomly assigned in a 1:1 ratio through a central computerized process to the fenugreek supplementation or placebo supplementation. An independent biostatistician generated the random allocation sequence. All patients, physicians, and researchers were blind to randomized allocation during the entire study and nurses were responsible for prescribing supplements to the patients.

2.3 | Intervention

Patients in the intervention group received 5 cc orally of fenugreek seed extract (equivalent to 500 mg of dry extract) for 4 months. Subjects in the control group received a placebo containing simulated

plain water with a taste and color similar to that of fenugreek seed extract. All the patients in both groups were asked to make no changes to their physical activity during the study and to follow their usual diet. They were also asked not to take any dietary supplements other than those prescribed during the study. Further, all the participants in both groups followed a similar routine treatment including Donepezil (5 mg twice a day) and Sertraline (50 mg once a day).

Fenugreek seeds were germinated and extracted at the Sabzevar Pearl Institute. The purchased seeds were cleaned, washed, and dried in a draught oven. The surface of seeds were sterilized by alcohol and then soaked in water. After removing the water, the seeds were spread on a dark screen to germination beginning. The germinated seeds were dried to get rid of excess water. The extract preparation was done by grinding 10 g germinated seeds in 100 ml water and then centrifuged (10 min, 4,000 rpm). The obtained extract was stored at 4°C. In order to accommodate the study's length, these extracts were prepared several times so that no other substances were added to increase the extract's shelf life.

The contents of bioactive compounds in the obtained extract were measured by the validated procedures. As, the total phenolic content was measured by Singleton-Rossi method and was expressed as mg of gallic acid equivalent phenol (GAE) (Slinkard & Singleton, 1977). Further, the flavonoids status was measured by Dowd method, using a UV–Vis spectrophotometer (Beckman Coulter, DU 730 Life Sciences) and the results were calculated as equivalents of rutin (mg RE/g extract) (Berk, Tepe, & Arslan, 2011). According to the extract analysis, total phenolic and flavonoid contents were 96.47 ± 4.38 mg GAE/g extract and 34.95 ± 1.52 mg RE/g extract, respectively.

At the beginning of the study, depression and memory status were assessed and recorded using two validated instruments: a 15-item geriatric depression scale (GDS) for the Iranian population (Malakouti, Fatollahi, Mirabzadeh, Salavati, & Zandi, 2006) and a 75-item Persian clinical dementia rating (CDR) questionnaire (Lotfi, Tagharrobi, Sharifi, & Abolhasani, 2015). Quality of life was assessed using the short form health survey (SF-36) questionnaire (Montazeri, Goshtasebi, Vahdaninia, & Gandek, 2005). Follow-up was done over four sessions, so that patients were visited monthly by traditional medicine and psychiatric specialists and patients' quality of life, depression, and memory status were assessed in each session.

2.4 | Blood sampling

Biochemical factors of the study were measured twice (at the beginning of the study and after the 4 months of intervention) by taking 5 ml of venous blood from the antecubital vein following 10–12 h fasting. The serums were frozen at −80°C until they were analyzed.

2.5 | Biochemical analysis

To determine serum TAC and MDA concentrations, TAC and MDA human ELISA kits (ZellBio GmbH, Ulm, Germany) were used. According to the order of the manufacturer, serial dilutions of the standard solution were prepared and used to draw the standard curve. Using these curves and their equations, serum TAC and MDA levels were read at 490 and 540 nm, respectively.

2.6 | Blood pressure measurement

Blood pressure of the participants was measured twice (before and after the intervention) in the resting state using an Omron digital blood pressure monitor (Omron Healthcare, Inc, Lake Forest, Illinois).

The informed consent was obtained from all the study participants. The anonymity and confidentiality of participants were assured and their decision to participate voluntarily in this study was respected. The protocol of the study was approved by Ethics Committees of Sabzevar University of Medical Sciences (No.:IR.MEDSAB.REC.1398.040), and also was registered on the Iranian Registry of Clinical Trials website (http://www.irct.ir, identifier: IRCT20160211026511N2).

2.7 | Statistical analysis

Statistical analyses were performed using SPSS version 20 and the intention-to-treat (ITT) method. The normality of data was checked with the Kolmogorov–Smirnov test. Descriptive and frequency statistics were used to present quantitative and qualitative data as mean ± standard deviation (SD) and number (percent), respectively. To compare study variables between intervention and control groups, independent sample *t*-test and Chi-square were used for quantitative and qualitative variables, respectively. Investigation of changes in the studied variables from baseline to the follow-up sessions was performed using general linear model analysis. A p-value of < 0.05 was considered statistically significant.

3 | RESULTS

3.1 | Subject characteristics

A total of 82 AD patients were enrolled in the study and underwent randomization (Figure 1), where 41 patients were assigned equally to the intervention and placebo groups. In the follow-up process, three patients in the intervention group discontinued participation due to their intolerance of the extract's taste. In the placebo group, one patient was withdrawn for personal reasons. In all, 38 and 40 patients in the intervention and placebo groups, respectively, completed the trial. However, all enrolled subjects (41 patients in each group) were included in the study analysis due to the ITT approach. Supplementation with fenugreek seed extract did not show any serious side effects among participants.

As shown in Table 1, 65.9% of the participants were female. The mean ± SD age of the participants was 72.05 ± 2.59 years.

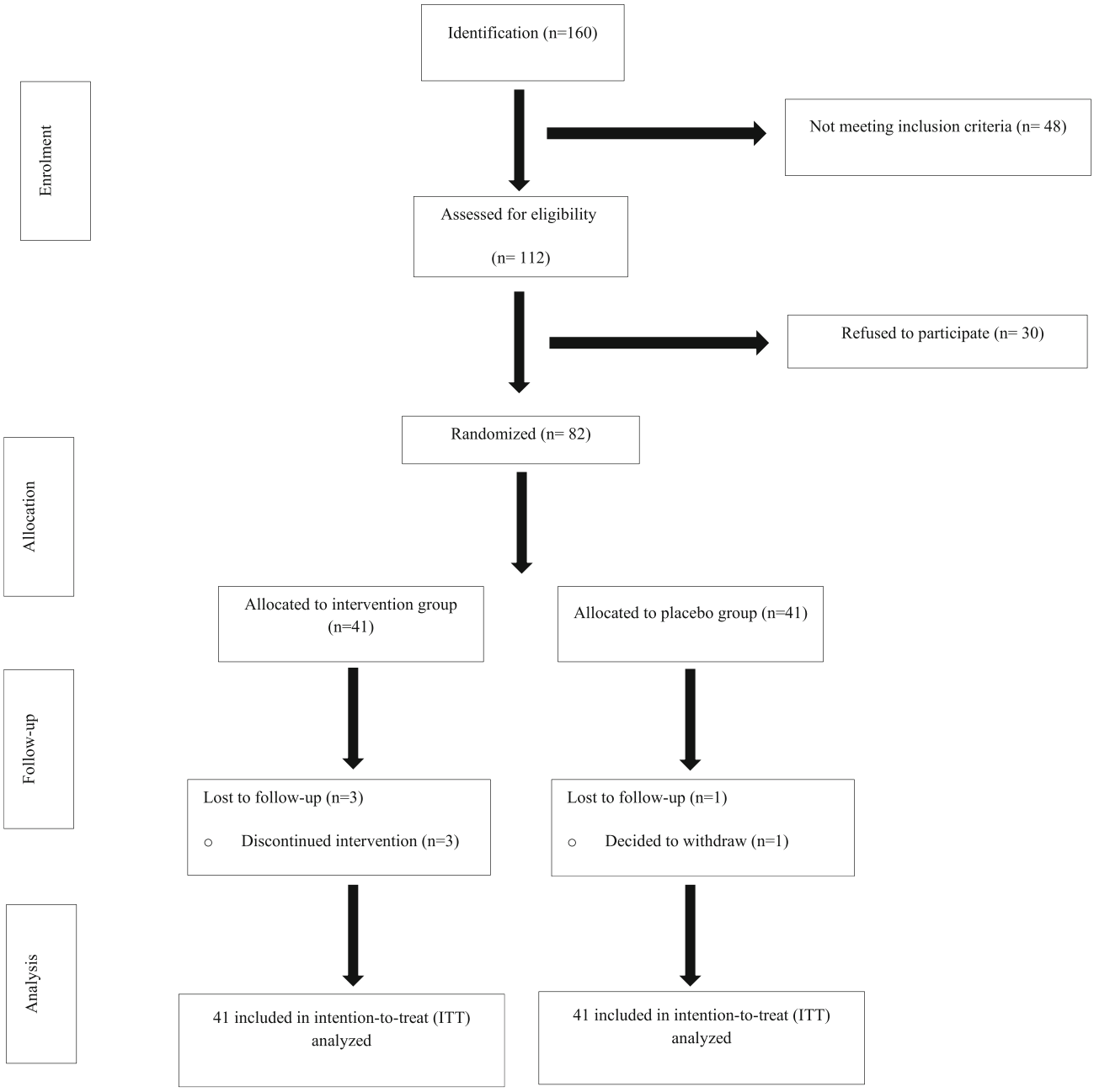

FIGURE 1 Flow diagram of patient recruitment and randomization process

TABLE 1 Demographic characteristics of study participants

	Study groups		
Gender	**Intervention ($n = 41$)**	**Placebo ($n = 41$)**	***p* value**
Male	14 (34.1)	14 (34.1)	1.000[a]
Female	27 (65.9)	27 (65.9)	
Age (years)	72.05 ± 2.59	71.12 ± 1.98	0.854[b]
Systolic blood pressure (SBP) (mmHg)	115.24 ± 6.35	115.85 ± 5.98	0.658[b]
Diastolic blood pressure (DBP) (mmHg)	74.80 ± 1.40	74.63 ± 1.60	0.608[b]

[a]Based on Chi-square.
[b]Based on independent sample *t*-test.

TABLE 2 Depression, memory, and quality of life status of the participants throughout the study

Variables	Follow-up	Study groups						F value[b]	p value[a]
		Intervention ($n = 41$)			Placebo ($n = 41$)				
		1	2	3	1	2	3		
Depression status	Baseline	21 (51.2)	8 (19.5)	12 (29.3)	17 (41.5)	10 (24.4)	14 (34.1)	7.68[d]	0.671[c]
	1	21 (51.2)	8 (19.5)	12 (29.3)	15 (36.6)	15 (36.6)	11 (26.8)	4.12[d]	0.205[c]
	2	20 (48.8)	9 (22.0)	12 (29.3)	14 (34.1)	14 (34.1)	13 (31.7)	2.51[d]	0.335[c]
	3	17 (41.5)	12 (29.3)	12 (29.3)	10 (24.4)	17 (41.5)	14 (34.1)	1.117[d]	0.243[c]
	After intervention	12 (29.3)	17 (41.5)	12 (29.3)	6 (14.6)	21 (51.2)	14 (34.1)	7.41[d]	0.276[c]
	Overall p value[e]	0.002			0.007				
Quality of life	Baseline	49.01 ± 1.27			48.39 ± 1.17			1.12	0.124
	1	48.85 ± 0.73			49.47 ± 1.03			5.40	0.002
	2	48.41 ± 0.88			49.97 ± 1.46			12.24	<0.001
	3	48.88 ± 1.22			51.28 ± 1.02			0.49	<0.001
	After intervention	50.19 ± 1.42			49.86 ± 1.17			1.76	0.256
	Overall p value[e]	<0.001			<0.001				
Memory status	Baseline	10.66 ± 1.98			10.39 ± 1.84			0.11	0.524
	1	10.00 ± 1.78			10.32 ± 1.51			0.43	0.388
	2	8.85 ± 1.52			10.61 ± 1.97			2.58	<0.001
	3	7.68 ± 1.55			10.90 ± 2.21			1.49	<0.001
	After intervention	7.16 ± 1.27			12.29 ± 2.52			6.88	<0.001
	Overall p value[e]	<0.001			<0.001				

Data presented as n(%) where applicable.
[a]Based on independent sample t-test.
[b]Levene's test for equality of variance.
[c]Based on Chi-square test.
[d]Chi-square test.
[e]Based on general linear model.

3.2 | Memory and depression status

Memory and depression status of the participants are shown in Table 2. Compared to baseline values, patients in both groups had significantly lower GDS scores after 4 months of supplementation. There was no difference in GDS score between the intervention and placebo groups.

At baseline, the mean ± SD of the CDR score was 10.66 ± 1.98 and 10.39 ± 1.84 in intervention and placebo groups, respectively, but this difference was not significant ($p > 0.05$). At the endpoint, the CDR score was significantly different between the groups ($p < 0.001$). The score was lower (7.16 ± 1.27) in the intervention group, while it increased to 12.29 ± 2.52 in the placebo group. This difference was apparent since the second follow-up session.

3.3 | Quality of life

After intervention, the participants in both groups experienced higher quality of life scores compared to baseline ($p < 0.001$), but there was no significant difference between the groups ($p > 0.05$).

3.4 | Oxidative Stress-Related parameters

The serum MDA and TAC levels within and between groups are presented in Figures 2 and 3. The intervention group had significantly lower serum MDA levels after the intervention ($p < 0.001$), while the placebo group experienced higher MDA levels after the study ($p < 0.001$). Conversely, fenugreek seed extract supplementation caused a significant increase in serum TAC levels ($p < 0.001$) compared to baseline values in the intervention group and no significant changes were seen in the placebo group. There were significant changes in oxidative stress-related parameters between the two groups after the intervention.

3.5 | Blood pressure

The effect of fenugreek seed extract and placebo supplementation on the systolic blood pressure (SBP) and diastolic blood pressure (DBP) is shown in Figures 4 and 5. In the intervention group, both SBP and DBP were significantly reduced after the intervention ($p < 0.001$). Although a higher SBP level was observed in the placebo group

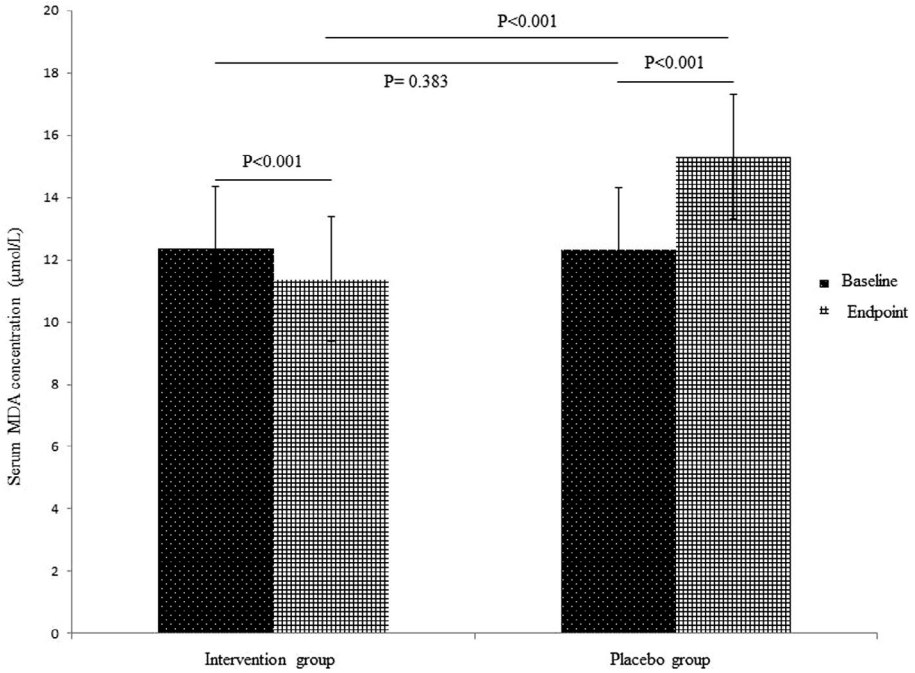

FIGURE 2 Effects of fenugreek seeds extract supplementation on malondialdehyde (MDA) status. Data were presented as mean ± SD

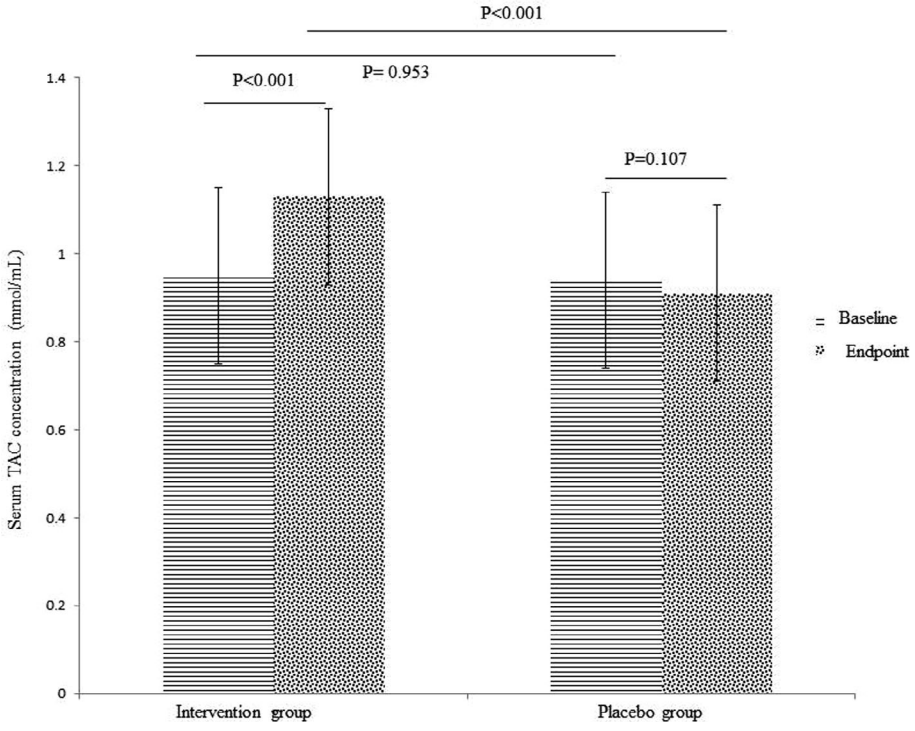

FIGURE 3 Effects of fenugreek seeds extract supplementation on total antioxidant capacity (TAC) status. Data were presented as mean ± SD

($p < 0.001$), there was no significant change in DBP status after the intervention period. The changes of SBP and DBP were significantly different between the groups ($p < 0.001$).

4 | DISCUSSION

To the best of our knowledge, this is the first study of a controlled clinical trial in human patients with AD to examine the effects of fenugreek seed extract supplementation on memory, depression, quality of life, BP, and selective oxidative stress indices. The results of the study demonstrated that 4 months of supplementation with fenugreek seed extract resulted in improvement in memory status and quality of life, as well as reductions in serum MDA levels and SBP and DBP. A significant increase was also seen in serum TAC levels.

The effect of fenugreek seed extract on oxidative stress indices in animal models with AD was seen in a recent experimental study by Prema et al., who reported attenuation of memory deficits, oxidative

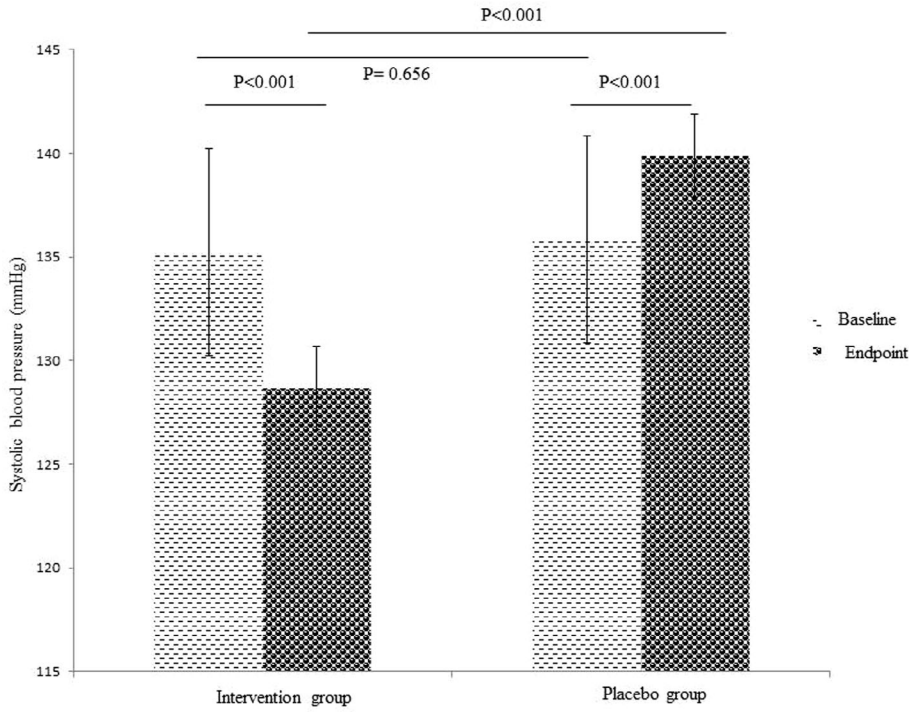

FIGURE 4 Effects of fenugreek seeds extract supplementation on systolic blood pressure (SBP) status. Data were presented as mean ± SD

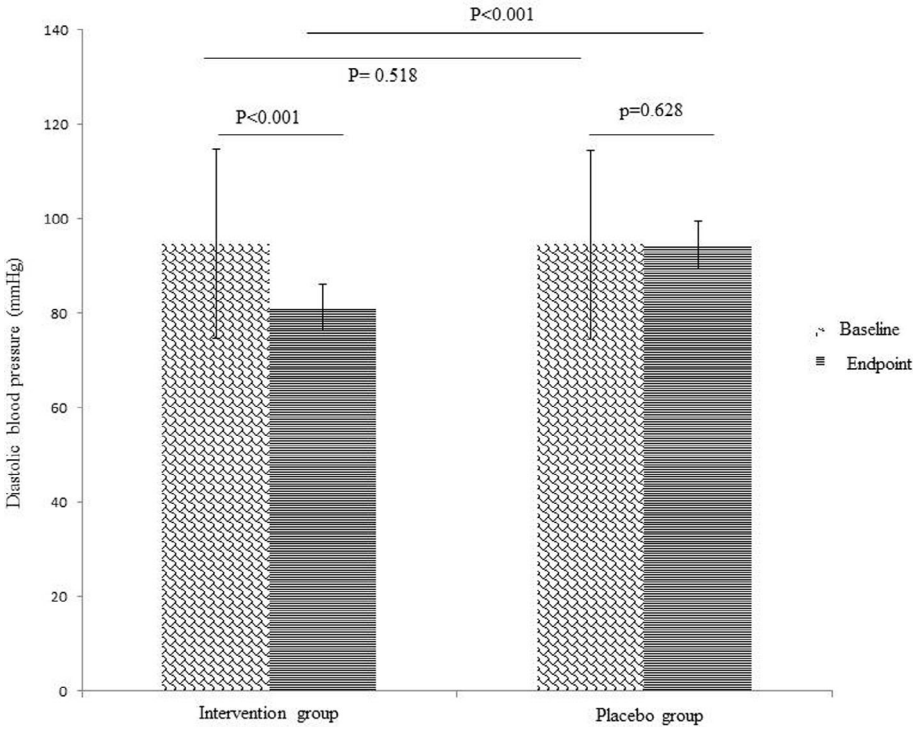

FIGURE 5 Effects of fenugreek seeds extract supplementation on diastolic blood pressure (DBP) status. Data were presented as mean ± SD

stress, and inflammation in AD rats (Prema, Justin Thenmozhi, Manivasagam, Mohamed Essa, & Guillemin, 2017). In Mohammad-Sadeghipour et al.'s study on fructose-fed rats found that administration of 4-week hydro-Alcoholic extract of fenugreek seed contributed to increasing serum TAC levels and MDA reduction (Mohammad-Sadeghipour et al., 2020). Furthermore, fenugreek seed powder was able to enhance IL-6 expression and modulate pro-oxidant-related effects in Wistar rats that exposed to chronic aluminum (Belaïd-Nouira et al., 2013). The antioxidant effects of fenugreek seed extract is due to the therapeutic roles of its phenolic content and active phytochemical components, including trigonelline, gitogenin, tigogenin, and yamogenin diosgenin choline (Choudhary & Yousuf, 2019). These components participate in scavenging hydroxyl radicals, 2,2′-diphenyl-1-picryl hydrazyl hydrate (DPPH), and also 2,2′-azinobis

3-ethylbenzothiazoline-6-sulfonate (ABTS−) radicals (Kaviarasan, Naik, Gangabhagirathi, Anuradha, & Priyadarsini, 2007). Development of AD may be attributed in part to the possible contributive role of oxidative stress markers, as higher oxidative stress is seen in AD compared to healthy rats (Moslemnezhad, Mahjoub, & Moghadasi, 2016). Therefore, complementary therapies, including herbs with antioxidant properties such as fenugreek, can help control the progression of AD and its symptoms.

The results of current study have demonstrated that fenugreek seed extract supplementation can significantly improve the memory and quality of life, but not depression status in AD patients. The significant positive effects of fenugreek extract on learning and memory, as well as diminished effects of that on neural cell deficits were seen in rats (Anjaneyulu, Rai, Rajesh, Nagamma, & Bhat, 2018; Khalili, Alavi, Esmaeil-Jamaat, Baluchnejadmojarad, & Roghani, 2018). The behavioral and electrophysiological methods in Sadraie et al. study have revealed that administration of fenugreek in AD rats was a beneficial approach to improve their learning and memory levels and alleviate neuronal loss (Sadraie et al., 2019). Short-term spatial and recognition memory of Aβ-microinjected rats were increased by fenugreek at a dose of 100 mg/kg in another study (Fahanik-Babaei, Baluchnejadmojarad, Nikbakht, & Roghani, 2019). Further, upregulation of brain-derived neurotrophic factor, a key molecule for memory, by fenugreek was also reported in another study (Chowdhury, Gawali, Munshi, & Juvekar, 2018). The high alkaloid and saponin levels of this herbal extract have a potential role in increasing learning and memory among animal models (Chiu et al., 2011; Jiang et al., 2007). Taken all together, it is suggested that therapeutic and healing effects of fenugreek seeds on memory may be due to multiple pathways, including activation of the PI3K/Akt signaling pathway, attenuation of amyloid and tau pathology, preservation of mitochondrial integrity, suppression of oxidative stress, apoptotic pathway, and astrocyte activity. Since from the point of view of the authors this is the first human study to investigate the effects of fenugreek seed extract on AD patients, further clinical trial studies are indicated to determine its effects on memory and quality of life.

In contrast with our results, the effects of fenugreek seed on menopausal depression were investigated by recent studies, which reported a significant beneficial effect on decreasing depression without any adverse effects (Akbari Torkestani, Atarha, Heidari, Amiri Farahani, & Roozbehani, 2013; Khanna et al., 2020). In animal models with Huntington's disease, ethanolic extract of fenugreek also significantly reduced depressive-like behavior thorough monoamine oxidase (MAO) suppression and neurotransmission improvement (Garcia-Miralles et al., 2016). The effects of fenugreek seed on depression levels in AD patients can be investigated through more human studies in this area.

The results of current study revealed that SBP and DBP decreased by supplementation of fenugreek seeds extract. In line with our study, a recent study reported that fenugreek essential oil can significantly suppress angiotensin-converting enzyme (ACE) in rats and resulted in lower BP (Strømgaard & Nakanishi, 2004). Further, human studies have shown reduction in SBP through fenugreek supplementation in diabetic patients and in healthy subjects (Hadi et al., 2020; Prasath, Priya, Devi, & Arivarasu, 2021). It has been suggested that the serotonergic antagonistic property involving the 5-HT2 receptor subtype may play an important role in the antihypertensive effect of fenugreek seeds (Balaraman, Dangwal, & Mohan, 2006; Hamden, Keskes, Belhaj, Mnafgui, & Allouche, 2011).

5 | LIMITATIONS

This study had some limitations that should be considered in future studies. First, our study was conducted on the patients who had mild-to-moderate AD, but not patients in advanced stages. Second, just two oxidative stress indices including MDA and TCA were assessed in the study. Third were the duration of supplementation and small sample size that may affect the effects of fenugreek seed extract on some of the study variables.

6 | CONCLUSION

It is concluded that fenugreek seed extract supplementation on AD patients has shown promising effects on memory, quality of life, BP, serum MDA, and TAC levels over 4 months in a double-blind randomized placebo-controlled study. No adverse effects were detected during the intervention.

AUTHOR CONTRIBUTIONS

AZ and RJ designed this study. FS, FK, and LM participated in data collection. RJ and HF participated in process of intervention. MN and EF analyzed data. EF wrote the first draft of the manuscript and revised it. All authors have read and approved the final manuscript.

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None.

FUNDING INFORMATION

Sabzevar University of Medical Sciences, Sabzevar, Iran.

CONFLICT OF INTEREST

The authors reported no conflict of interest.

DATA AVAILABILITY STATEMENT

The dataset used and analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The informed consent was obtained from all the study participants. The anonymity and confidentiality of participants were assured and their decision to participate voluntarily in this study was respected. The protocol of the study was approved by Ethics Committees of Sabzevar University of Medical Sciences (No.:IR.MEDSAB.REC.1398.040), and also was registered on the Iranian Registry of Clinical Trials website (http://www.irct.ir, identifier: IRCT20160211026511N2).

CONSENT FOR PUBLICATION

Not applicable for the current analysis.

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