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Achillea millefolium is beneficial as an add-on therapy in patients with multiple sclerosis: A randomized placebo-controlled clinical trial

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ABSTRACT

Background: Multiple sclerosis (MS) is a neurological disease for which to date there is no cure and the existing disease-modifying drugs just slow down the disease progression.
Purpose: In this clinical trial we evaluated the efficacy of *Achillea millefolium* (*A. millefolium*) aqueous extract in MS patients.
Methods: A triple-blind randomized placebo-controlled parallel group trial was conducted on 75 MS patients. The patients were randomized into three groups including placebo and two groups receiving *A. millefolium* with two different doses, *i.e.* 250 mg/day and 500 mg/day, for 1 year. The primary outcome was the annualized relapse rate. Also, number and volume of lesions were obtained from magnetic resonance imaging (MRI) scans. Furthermore, we performed a comprehensive neurological and cognitive tests as follows: changes in the expanded disability status scale (EDSS), the multiple sclerosis functional composite (MSFC), fatigue severity scale (FSS), Ashworth spasticity assessment, Beck depression test, State-trait anxiety inventory (STAI), mini-mental status examination (MMSE), Wisconsin card sorting test (WCST), tower of London test (TOL), word-pair learning, paced auditory serial addition task (PASAT) and standard laboratory tests.
Results: This study showed one year administration of *A. millefolium* (both doses) decreased the annual relapse rate in MS patients. The mean volume change of lesions significantly decreased in the 500 mg *A. millefolium* group. The add-on therapy also increased time to first relapse and the MSFC z-score; it decreased the EDSS score and improved performance in word-pair learning, *PASAT*, and WCST.
Conclusion: We found beneficial effects of *A. millefolium* aqueous extract as an add-on therapy in MS patients.

Introduction

Multiple sclerosis (MS) is a neurological disease characterized by chronic progressive demyelinating lesions in the brain and the spinal cord (Rudick et al., 2002b). Its complications include motor and cognitive problems such as muscle weakness and deficits in attention and long-term memory (Polman et al., 2011). These deficits detrimentally affect many aspects of daily life, such as participating fully in the

Abbreviations: MS, multiple sclerosis; A millefolium, *Achillea millefolium*; EAE, experimental allergic encephalitis; RR-MS, relapsing-remitting MS; EDSS, expanded disability status scale; MRI, magnetic resonance imaging; FLAIR, fluid-attenuated inversion recovery; LPA, lesion prediction algorithm; FSS, fatigue severity scale; STAI, Spielberger state-trait: anxiety inventory; MMSE, mini-mental status examination; WCST, Wisconsin card sorting test; TOL, tower of London test; PASAT, paced auditory serial addition task; MSFC, multiple sclerosis functional composite

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society and maintaining employment factors (Chiaravalloti and DeLuca, 2008). Unfortunately, to date, there is no cure for MS and the existing disease-modifying drugs just slow down the disease progression. However, recent research has shown possible usefulness of complementary and alternative medicine for management of MS and its complications (Schmidt, 2017).

Achillea millefolium, is known as a powerful medicinal plant worldwide (Wendy and Daniel, 2011). Since ancient times, it has been used for wounds, digestive problems, respiratory infections, and skin conditions (Wendy and Daniel, 2011). *A. millefolium* flavonoids (apigenin and luteolin) have been identified as the main pharmacologically active ingredients (Wendy and Daniel, 2011). Studies have shown that luteolin protects rats against cognitive dysfunction (Liu et al., 2014), and also against learning deficits in Alzheimer's disease (Schmidt, 2012). It has been shown that apigenin is effective in various neurologic disorders, such as insomnia, Parkinson's disease, and neuralgia (Patil et al., 2014). Moreover, it has been reported that apigenin reduces disease severity and progression in an animal model of MS (experimental allergic encephalitis, EAE) (Ginwala et al., 2016). We had evaluated the effects of *A. millefolium* aqueous extract (the most consumed form of the plant) in the EAE before. Our results demonstrated that this extract could relieve demyelination and inflammatory reaction in the CNS in animal models (Vazirinejad et al., 2014).

In the present study, a randomized placebo-controlled clinical trial was performed to assess the effect of *A. millefolium* aqueous extract on relapsing-remitting MS (RR-MS) patients with a one-year follow-up.

Experimental procedures

Patients

From April 2014 to May 2015, 100 RR-MS patients were recruited from neurology department of the university hospital in Rafsanjan, Iran. The inclusion criteria were the age of 18 to 55 years old, diagnosis of RR-MS according to the McDonald criteria (Polman et al., 2011) with one or more documented relapses within last 2 years, the expanded disability status scale (EDSS) of 0–4 and no evidence of relapses during the last month. The main exclusion criteria were evidence of relapse for at least one month before the beginning of the study and/or during the screening and baseline phases, hypersensitive skin, and pregnancy.

The protocol was approved by the institutional review board and the local Ethics Committee (no. 31/9/3040). All subjects provided written informed consent. The trial was also registered at Iranian Registry of Clinical Trials (IRCT2013121915863N1).

Study design and randomization

The study included a 12-month triple-blind randomized placebo-controlled trial with five time-points (Fig. 1). During this study the patients, investigators, radiologists and analyzers were blind to the treatment assignments. Patients were randomly assigned in a 1:1:1 ratio using PROC PLAN in SAS software version 9.1 (SAS Institute Inc., Cary, NC, USA) to receive either *A. millefolium* aqueous extract (250 mg/day or 500 mg/day) or a matching placebo consisting of starch powder in similar capsules, for 12 months.

Magnetic resonance imaging

MRI experiments were performed using 1.5 Tesla Siemens scanner (Essenza, Germany). The imaging protocol consisted of standard pre-contrast axial T2-weighted, axial T1-weighted, sagittal fluid-attenuated inversion recovery (FLAIR), and post-contrast T1-weighted images in three orthogonal planes using 0.1 mmol/kg intravenous administration of Gadolinium. MR images were obtained at baseline, month 6 and 12 (Fig. 1).

Lesions were segmented by the lesion prediction algorithm (Schmidt, 2017) as implemented in the LST toolbox version 2.0.15 (Schmidt, 2012) for SPM. FLAIR images were used as input of the lesion prediction algorithm (LPA) to calculate the lesion probability maps of different time-points. Then, segmented lesion maps of different time-points were compared using the longitudinal pipeline implemented in the LST toolbox. In this pipeline first lesion maps and FLAIR images are co-registered to the images of the first time-point. Then, relative differences of FLAIR intensities are calculated along all voxels that were segmented as lesions in at least one time point. Finally, significant increase and decrease of lesion voxels are identified if their differences exceed or fall below a certain threshold that has been obtained by analyzing healthy white matter. As a final result, lesion change labels are produced for all consecutive time-points.

From MR images, frequency of patients free from gadolinium-enhancing lesions, number of new lesions on T2-weighted scans and frequency of patients free from new lesions on T2-weighted scans as well as volumes of lesions on T2-weighted scans were extracted.

Clinical and neuropsychological tests

Clinical assessments including EDSS, standard laboratory tests and neuropsychological tests including (fatigue severity scale (FSS) (Krupp et al., 1988), modified Ashworth spasticity assessment (Bohannon and Smith, 1987), beck depression test (Ghassemzadeh et al., 2005), Spielberger state-trait anxiety inventory (STAI) (Schmidt, 2017), mini-mental status examination (MMSE) (Schmidt, 2012), Wisconsin card sorting test (WCST) (Puente, 1985), tower of London test (TOL) (Krikorian et al., 1994), word-pair learning (S., 1963), paced auditory serial addition task (PASAT) (Rudick et al., 2002a)) and multiple sclerosis functional composite (MSFC) (Rudick et al., 2002a) were performed at baseline, and month 3, 6, 9, and 12 (Fig. 1). Except MSFC and magnetic resonance imaging (MRI) which was assessed at month 6 and 12, the rest were examined at each time-point.

Primary end point

The primary end point was the annualized relapse rate defined as the number of confirmed relapses per year. The examining neurologist verified relapses within 7 days after the onset of symptoms. The relapse was confirmed by one of the following criteria (Krikorian et al., 1994): at least a half point increase in the EDSS score; one point increase in each of the two EDSS functional system scores; two point increase in one EDSS functional-system score (not including scores for the bowel–bladder or cerebral functional systems).

Secondary end points

The key secondary end point was the total number of gadolinium-enhancing T1 lesions observed on brain MR images at month 6 and 12 *vs.* placebo. Other secondary end points included the time to the first relapse, EDSS and MSFC z-scores, MMSE, WCST, TOL, word-pair learning, PASAT, FSS, spasticity, depression, STAI and Laboratory tests. Possible adverse events were assessed and reported at each visit by the treating neurologist.

Plant material

The fresh plant was purchased in March 2013 from Isfahan Botany Herbarium (specimen no. 9757). Flowering branches of the plant were removed and ground after washing and drying. 2 g of ground powder was percolated in 200 ml of distilled water for 24 h. The extract was filtered through filter paper (8–10 μm) and then evaporated yielding dried extract. The suggested dose for human use is 2–4 g of dry flowers and stalk (Barnes et al., 2007) that corresponds to 250–500 mg dried aqueous extract which we used in this study. The dried extracts were

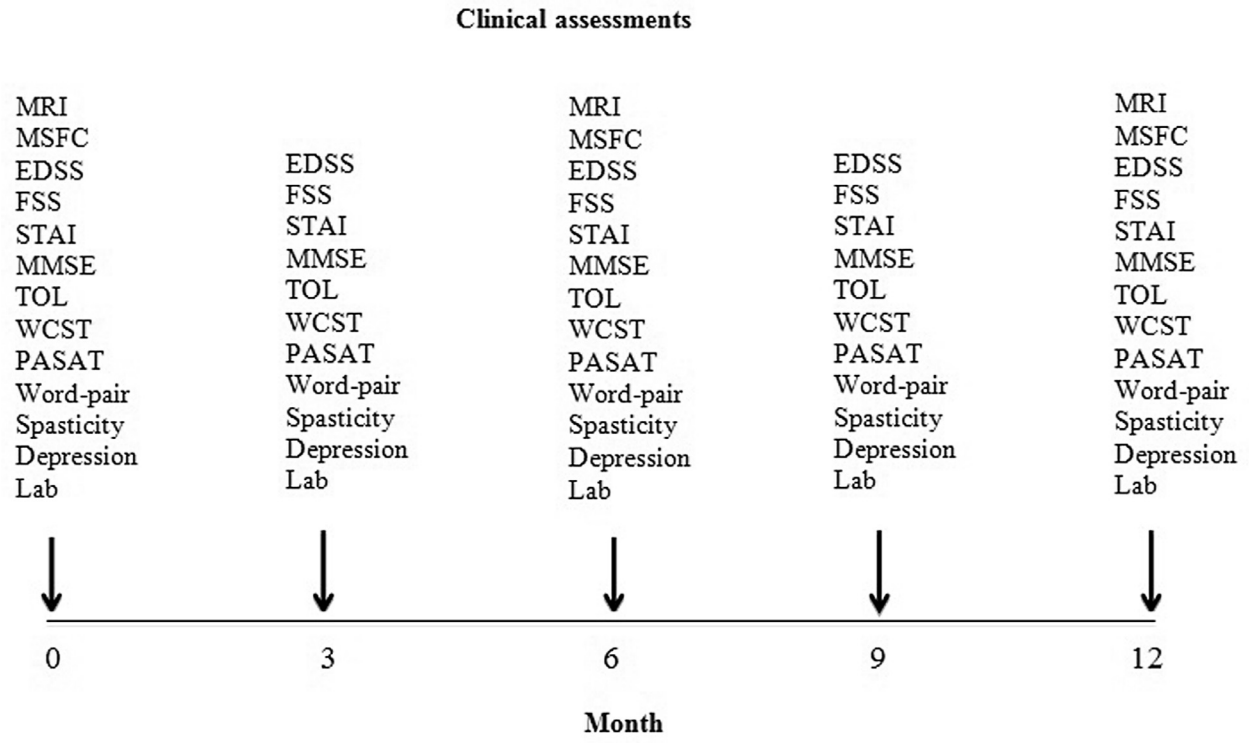


Fig. 1. Diagram of clinical assessments at different time-points. MRI: magnetic resonance imaging; MSFC: multiple sclerosis functional composite; EDSS: expanded disability status scale; FSS: fatigue severity scale; STAI: state-trait anxiety inventory; MMSE: mini-mental status examination; TOL: tower of London test; WCST: Wisconsin card sorting test; PASAT: paced auditory serial addition task; and Lab: laboratory tests.

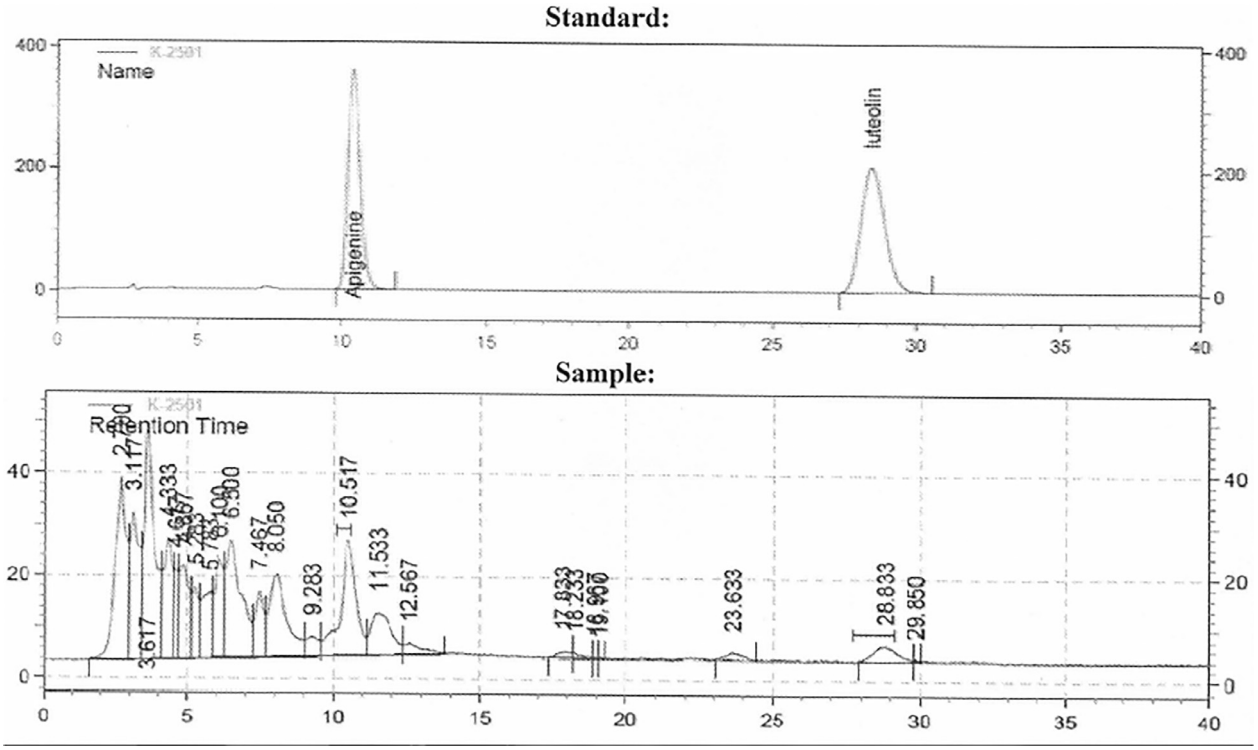


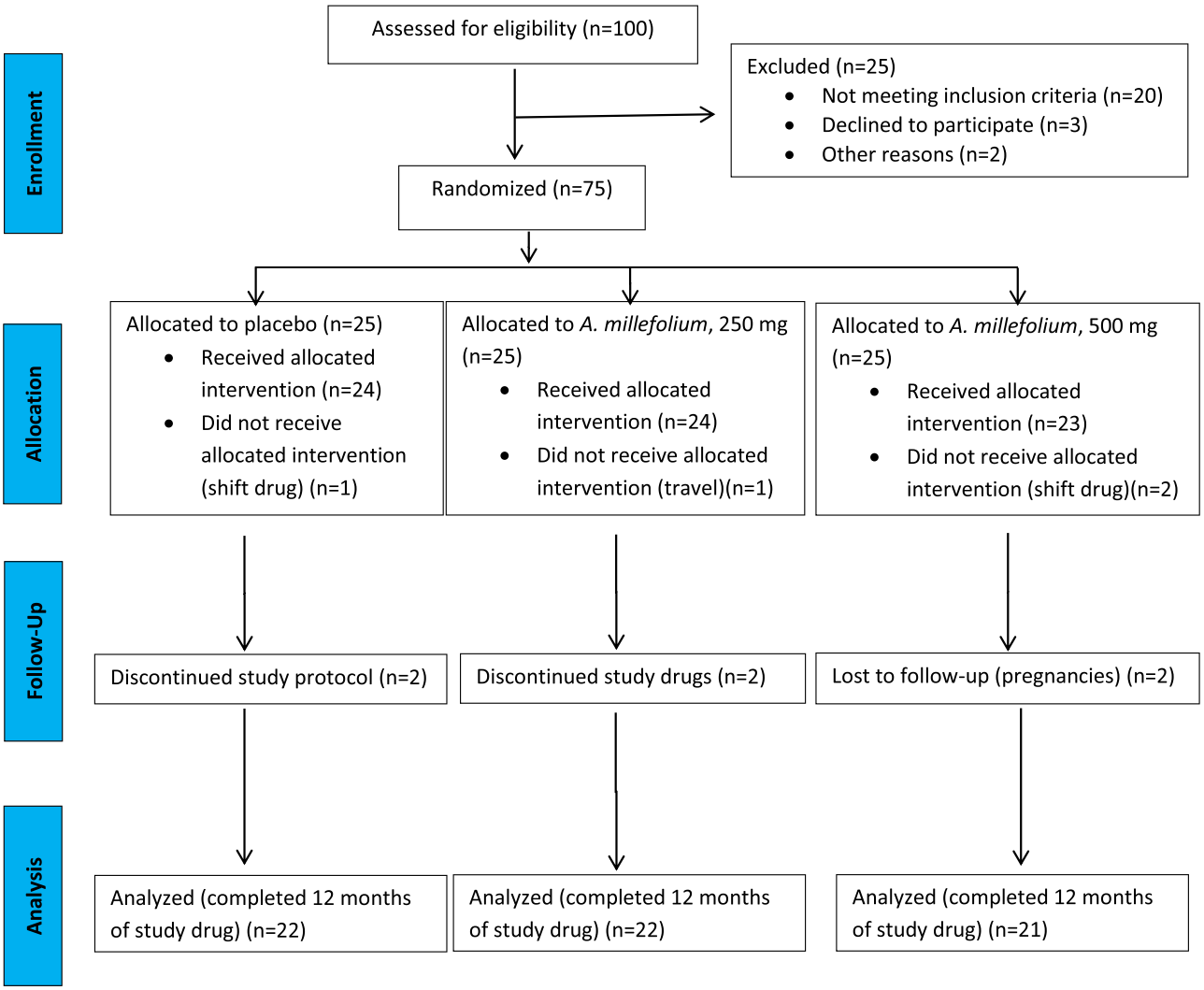
Fig. 2. HPLC analysis of aqueous extract of *Achillea millefolium*.

packed in 250 and 500 mg capsules. The results for quantification of the flavonoids in our sample were 0.28 mg/g of luteolin and 1.58 mg/g of apigenin (Fig. 2).

Statistical analysis

The sample size was determined assuming the mean and standard deviation of EDSS in MS patients in previous similar study (Masoud Etemadifar et al., 2007). In order to detect a statistically significant

Table 1
CONSORT flow diagram of patient disposition. Numbers of patients who underwent randomization and who completed treatment. The most common reasons for premature discontinuation were discontinued according to the study protocol. In addition to the patients who prematurely discontinued study medication, 2 discontinued due to pregnancies.



difference $p < 0.05$) between the two groups with a 90% power, the required sample size was calculated as 25 subjects in each study arm. At month 12, missing data were handled using last observation carried forward if there was a premature withdrawal visit prior to month 12, otherwise missing data were not replaced.

Baseline characteristics of the three groups were compared using one-way ANOVA (analysis of variance) for numeric variables, and *chi*-square or *Fisher's* exact test for categorical variables.

Variable of primary efficacy was represented by the annualized relapse rate after 12 months of treatment. Proportions of patients who were free of relapse at month 12 were estimated using the Kaplan–Meier method and between-group comparisons were made with the log-rank test. Two-way repeated measures ANOVA was performed to compare secondary outcomes, MRI-related outcomes, cognitive tests and clinical outcomes across all groups through the times. The interaction between study group and time period was also introduced into the model.

Changes from baseline month 6 and 12 for secondary outcomes were compared using one-way ANOVA across all groups. The analysis was performed on randomized coded subjects. SPSS version 15.0 (SPSS Inc., Chicago, IL) was used. All p-values were 2-tailed, with statistical significance defined as $p \leq 0.05$.

Results

Study population

Patients were recruited from April 2014 to May 2015. Seventy-five patients fulfilled the inclusion criteria and were randomized into three groups (25 patients/group) including placebo, 250 mg, and 500 mg *A. millefolium* for 12 months. In the 12-month follow-up study, the assessment for the last patient was completed in April 2016. 65 patients completed the study (CONSORT flow diagram in Table 1). Baseline demographic and disease characteristics were balanced between the trial groups (Table 2).

Primary outcome

As reported in Table 3, the mean annualized relapse rate was lower in treatment groups compared with placebo ($p = 0.003$ for 250 mg and $p = 0.013$ for 500 mg *A. millefolium*).

Secondary outcomes

In the *A. millefolium* groups compared with the placebo group, the

Table 2
Baseline characteristics of the patients.

	Placebo (n = 25)	*A. millefolium*, 250 mg (n = 25)	*A. millefolium*, 500 mg (n = 25)	p-value
Characteristic				
Intention-to-treat population				
Age — yr				
Mean ± SD	34.68 ± 7.828	31.36 ± 9.859	34.00 ± 8.515	p = 0.422
Median (interquartile range)	34.50 (28.50–41.25)	28.50 (24.00–40.75)	34.00 (27.50–42.50)	
Age — no. (%)				p= 0.418
≤30yr	9 (36)	12 (48)	10 (40)	
31–40yr	10 (40)	8 (32)	9 (36)	
>40yr	6 (24)	5 (20)	6 (24)	
Male— no. (%)	3 (12)	3 (12)	2 (8)	
Education-no. (%)				p= 0.191
Elementary	4 (16)	3 (12)	4 (16)	
Guidance school	6 (24)	7 (28)	4 (16)	
High school	9 (36)	10 (40)	13 (52)	
College	6 (24)	5 (20)	4 (16)	
Marital status				p = 0.997
Single	6 (24)	4 (16)	7 (28)	
Married	19 (76)	20 (80)	18 (72)	
Divorced	0	1 (4)	0	
Interval since first symptoms — yr.				
Mean ± SD	3.14 ± 3.256	2.82 ± 3.724	3.52 ± 4.332	p= 0.830
Median (interquartile range)	2.00 (1.00–4.25)	1.00 (1.00–3.25)	1.00 (1.00–5.00)	
Course of disease — no. (%)				
Relapsing–remitting	24 (96)	24 (96)	24 (96)	
Secondary progressive	1 (4)	1 (4)	1 (4)	
No. of relapses in the previous year				
Mean ± SD	1.00 ± 0.926	1.09 ± 0.610	1.48 ± 0.750	p= 0.111
Median (interquartile range)	1.00 (0.00–1.25)	1.00 (1.00–1.00)	1.00 (1.00–2.00)	
Drug-no (%)				p= 0.635
interferon beta	22 (88)	24 (96)	23 (92)	
Glatiramer acetate	3 (12)	1 (4)	2 (8)	
EDSS score*				
Mean ± SD	1.383 ± 1.10	1.182 ± 1.38	1.952 ± 1.39	p= 0.142
Median (interquartile range)	1.50 (0.00–2.00)	0.50 (0.00–2.50)	2.00 (0.50–3.50)	
Primary MRI analysis population				
No. of T1-weighted, gadolinium-enhanced lesions at baseline				
Mean ± SD	2.14 ± 4.580	3.45 ± 4.183	2.24	p = 0.566
Median (interquartile range)	0.00 (0.00–2.75)	0.50 (0.00–7.50)	0.00 (0.00–2.00)	
Patients with T1-weighted, gadolinium-enhanced lesions at baseline — no. (%)	8 (36)	11 (50)	8 (38)	p = 0.608
Volume of T1-weighted gadolinium-enhanced lesions				
Mean ± SD	856.72 ± 1758.45	770.28 ± 1218.99	653.85 ± 1180.95	p= 0.899
Median (interquartile range)	0.00 (0.00–1095.56)	120.92 (0.00–1077.72)	0.00 (0.00–1144.37)	
Volume of T2-weighted lesions				
Mean ± SD	3705.76 ± 4237.97	3971.08 ± 4123.28	3635.80 ± 5549.56	p= 0.970
Median (interquartile range)	2168.70 (661.47–49,381.16)	2933.25 (346.67- 6230.07)	1682.20 (412.42–5582.20)	
MSFC z-score				
Mean ± SD	−0.13 ± 0.79	0.08 ± 0.81	0.05 ± 0.68	p= 0.575
Median (interquartile range)	−0.11 (−0.72–0.56)	0.18 (−0.27–0.69)	0.12 (−0.47–0.52)	
PASAT test				
Mean ± SD	25.55 ± 14.46	30.32 ± 13.43	27.38 ± 12.35	p= 0.499
Median (interquartile range)	27.00 (15.00–35.00)	29.00 (22.50–36.25)	25.00 (19.50–36.00)	

EDSS = expanded disability status scale; MSFC = multiple sclerosis functional composite; PASAT = paced auditory serial addition test.
* Scores range from 0 to 10; higher scores indicate a greater degree of disability.

time to a first relapse was longer in the treatment group (p = 0.013 for 250 mg, and p = 0.039 for 500 mg dose of *A. millefolium*) (Fig. 3). At month 12, the proportion of patients who were free of relapse rate was greater in both *A. millefolium* groups than in the placebo group. Moreover, the risk of relapse was reduced, and proportionately more patients remained free of relapse during the 12-month period (Table 3). Comparison of the EDSS score, at month 12, revealed that it increased in the placebo group while in the *A. millefolium* groups it decreased. This improvement in the EDSS score was significantly higher in the treatment group taking 500 mg dosage compared to the other two groups (p = 0.030 versus 250 mg dose and p = 0.001 versus placebo, Fig. 4). Likewise, we found that, at month 12, the MSFC z-score significantly increased in the group with 500 mg dose of *A. millefolium* compared to placebo (p = 0.003, Table 3).

Analysis of the MR imaging data showed that, at month 12, mean volume change of lesions on T2-weighted scans significantly decreased in the 500 mg *A. millefolium* group compared to the placebo group (p = 0.004, Table 3). This trend in decreasing the mean volume change was already seen in 250 mg group compared to the placebo (p = 057).

Neuropsychological results

The mean score of Word-pair learning test in *A. millefolium* groups was decreased compared to placebo (p = 0.039 for 250 mg, and p = 0.049 for 500 mg at month 9, and p = 0.037 for 500 mg at month 12) (Table 2 in Supplement). These data may suggest that *A. millefolium* extract could improve the hippocampal function in MS patients.

The PASAT is used to measure the information processing speed as a valuable cognitive task in MS. In our study, lower performance on the PASAT was detected in the placebo group compared to *A. millefolium*

Table 3
MRI and clinical outcomes at month 12 follow up.

Outcome	Placebo (n = 22)	*A. millefolium*, 250 mg (n = 22)	*A. millefolium*, 500 mg (n = 21)	p-value		
Relapse				250 mg *vs.* Placebo	500 mg *vs.* Placebo	250 mg *vs.* 500 mg
Annualized relapse rate-no (%)	11 (50.0)	5 (22.7)	7 (33)	0.060	0.268	0.438
Mean ± SD	1.09 ± 1.30	0.23 ± 0.42	0.33 ± 0.48	0.003	0.013	0.911
Median (interquartile range)	0.50 (0.00–2.00)	0.00 (0.00–0.25)	0.00 (0.00–1.00)			
Time to a first relapse - day						
Mean ± SD	230.64 ± 142.67	323.68 ± 86.02	310.05 ± 91.54	0.013	0.039	0.913
Median (interquartile range)	282.50 (92.00–365.00)	365.00 (341.25–365.00)	365.00 (250.00–365.00)			
Patients with no confirmed relapse - no. (%)[a]	11 (50.0)	17 (77.3)	14 (66.7)	0.060	0.268	0.438
Patients with confirmed relapse - no. (%)				0.007	0.018	0.438
1 relapse	3 (13.6)	5 (22.7)	7 (33.3)			
2 relapse	4 (18.2)	0 (0.0)	0 (0.0)			
≥ 3 relapse	4 (18.1)	0 (0.0)	0 (0.0)			
EDSS score at month 12[b]						
Mean ± SD	1.77 ± 1.43	0.95 ± 1.28	0.97 ± 0.96	0.084	0.100	0.998
Median (interquartile range)	1.75 (0.00–3.12)	0.50 (0.00–1.12)	1.00 (0.00–1.75)			
Change in EDSS score from baseline						
Mean ± SD	0.39 ± 1.09	−0.23 ± 0.78	−0.98 ± 0.91	0.085	0.000	0.030
Median (interquartile range)	0.00 (−0.50–1.13)	0.00 (−0.50–0.00)	−1.00 (−1.50–0.00)			
Change in MSFC z-score from baseline at month 12[c]						
Mean ± SD	0.06 ± 0.521	0.32 ± 0.35	0.50 ± 0.33	0.111	0.003	0.371
Median (interquartile range)	0.09 (−0.14–0.31)	0.35 (0.13–0.55	0.32 (0.29–0.79)			
No. of gadolinium-enhanced lesions at month 12						
Mean ± SD	1.05 ± 1.78	1.77 ± 2.75	1.05 ± 2.95	0.613	1.000	0.621
Median (interquartile range)	0.00 (0.00–1.25)	0.00 (0.00–3.00)	0.00 (0.00–1.00)			
Patients free of gadolinium-enhanced lesions at 12 mo — no. (%)	14 (63.6)	12 (54.5)	15 (71.4)	0.540	0.586	0.404
Volume of gadolinium-enhanced lesions at month 12mm^3						
Mean ± SD	232.74 ± 427.08	222.35 ± 385.47	133.67 ± 425.95	0.996	0.724	0.767
Median (interquartile range)	0.00 (0.00–414.94)	0.00 (0.00–354.28)	0.00 (0.00–33.45)			
Volume of T2 lesions at month 12mm^3						
Mean ± SD	3793.81 ± 4439.83	3535.92 ± 3975.58	3484.92 ± 5558.41	0.982	0.976	0.999
Median (interquartile range)	1803.80 (729.56–6257.39)	2355.95 (322.51–5314.87)	1460.30 (383.28–5268.90)			
Number of new T2 lesions						
Mean ± SD	0.13 ± 2.41	−0.27 ± 1.03	−0.76 ± 1.37	0.432	0.091	0.354
Median (interquartile range)	0.00 (−0.25–1.00)	0.00 (−1.00–0.00)	0.00 (−2.00–0.00)			
Change in volume of T2 lesions from baselinemm^3						
Mean ± SD	97.42 ± 342.23	−65.48 ± 198.24	−150.87 ± 212.23	0.057	0.004	0.307
Median (interquartile range)	0.00 (−25.30–429.30)	−7.91 (−177.50–0.00)	−41.47 (−304.10–0.00)			
PASAT test at month 12						
Mean ± SD	27.32 ± 14.91	36.67 ± 12.20	38.24 ± 12.53	0.025	0.009	0.703
Median (interquartile range)	25.00 (21.75–40.75)	35.00 (27.50–45.50)	45.00 (28.00–49.50)			

EDSS = expanded disability status scale; MSFC = multiple sclerosis functional composite; PASAT = paced auditory serial addition test.
[a] Values are Kaplan–Meier estimates from the analysis of time to first relapse.
[b] Scores range from 0 to 10; higher scores indicate a greater degree of disability.
[c] Scores on the multiple sclerosis functional composite (MSFC) are expressed as z-score, with higher scores indicating improvement in disability.

groups at month 12. The mean PASAT score in the placebo group was lower than both *A. millefolium* groups (p = 0.025 and p = 0.009 for 250 and 500 mg, respectively) (Table 3).

We measured frontal lobe function with the WCST test. *A. millefolium* could improve performance over the placebo group in some sectors including NPE, CLR, and the number of failures. No significant difference was observed in other indices between the study groups (Table 3 in Supplement). The treatment groups with *A. millefolium* had improved performance on mental preplanning in the TOL task; however, this was not statistically significant (Table 4 in Supplement). The interaction term between groups and the time period was not statistically significant between the *A. millefolium* and the placebo groups on the MMSE task (Table 2 in Supplement).

Other clinical outcomes

Our findings also showed a significant decrease in depression at month 6 (p = 0.033) and month 12 (p = 0.046) for 250 mg *A. millefolium* versus placebo. Comparison of *A. millefolium* treated groups with placebo on STAI, fatigue, and Ashworth tests revealed no significances (Table 2 in Supplement).

Monitoring the side effects

In order to monitor the possible side effects of *A. millefolium,* comprehensive laboratory tests including CBC, FBS, TG, Cholesterol, SGOT, SGPT, BUN, Cr, and TSH were performed at baseline, month 3, 6, 9, and 12. Provided that skin rashes and allergic skin reactions has been reported as adverse effect of the *A. millefolium* (Wrangsjö et al., 1990)., the patients were asked to report any allergic or hypersensitive reactions. Since *A. millefolium* had been used in complementary medicine for abortion, we did not include pregnant patients in the study, and also we excluded one patient during the study due to her temptation for pregnancy. The results showed no prominent abnormality in laboratory tests, indicating no significant side effects or adverse events throughout the course of the study (Table 5 in Supplement). In addition, *A.*

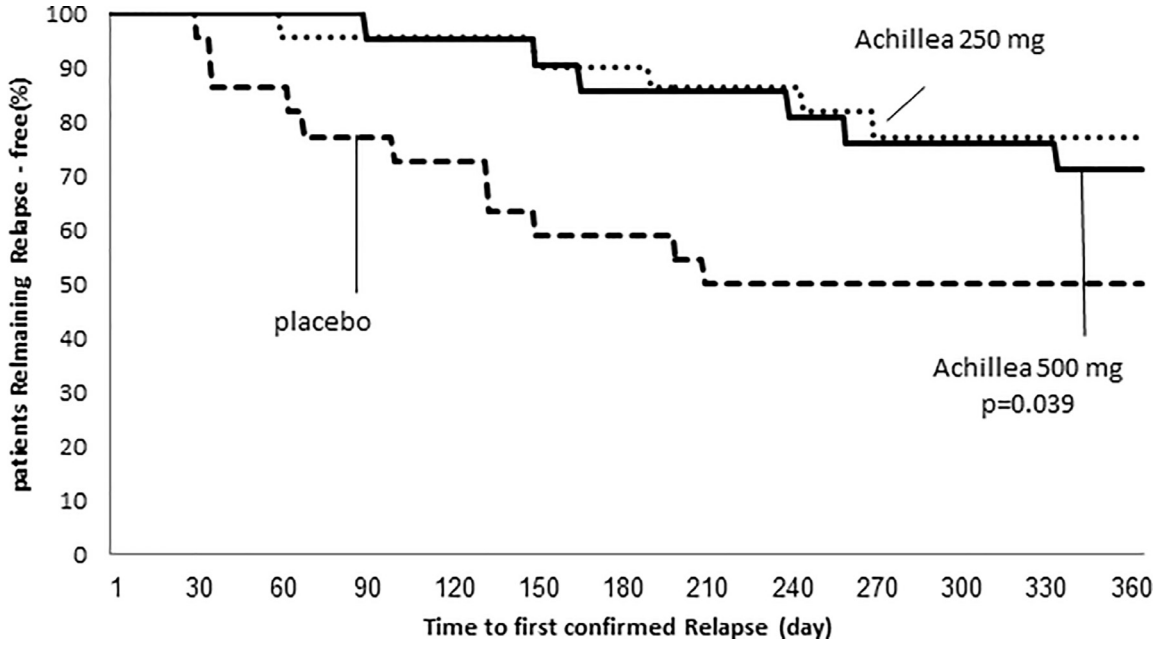


Fig. 3. Kaplan–Meier survival curves. The estimated time to a first confirmed relapse in all three groups. *P*-values are reported for each A. millefolium dose as compared to placebo.

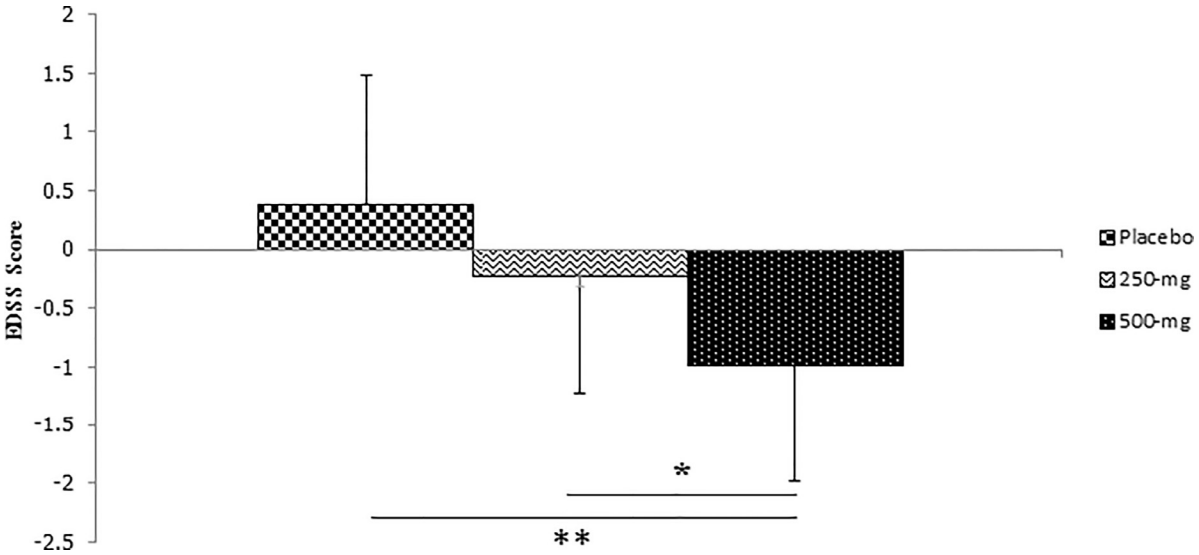


Fig. 4. The EDSS changes from baseline in the three groups. * $p < 0.05$ and ** $p < 0.001$.

millefolium was well tolerated by patients and they did not report any prominent adverse effects.

Discussion

MS is considered to be a disease with high disability rates in young adults worldwide (Miller et al., 2008). Controlling the relapse rate, slowing down the progression, and reducing the incidence of disability is very important for the prognosis of patients (Johnson et al., 1995). Our results demonstrated that 1-year oral administration of *A. millefolium* aqueous extract provides a significant improvement in both MR-related measures and in relapse-related clinical outcomes in RRMS patients. To our knowledge, this is the first time to assess the efficacy of *A. millefolium* as an add-on therapy in MS.

In traditional medicine, *A. millefolium* has been used to decrease inflammation in various diseases (Benedek et al., 2007). It has been reported that *A. millefolium* reduces disability and progression in EAE mice, suggesting that it might be beneficial for the complementary treatment of MS patients (Vazirinejad et al., 2014). In this study, add-on therapy with *A. millefolium* reduced annual relapse rate and increased the time to the first relapse in MS patients. Also, the disease progression was delayed in *A. millefolium* treatment patients, as evidenced by decreased EDSS scores. Our further investigations revealed that *A. millefolium* provides a significant improvement in MRI measures of inflammation by decrease in the volume of lesions and number of new enhancing lesions on T2-weighted and T1-weighted images, respectively. These findings could be partly due to anti-oxidant and anti-inflammatory effects of flavonoids in this herb (mainly luteolin and apigenin). Hendriks and colleagues reported that oral administration of luteolin prevented relapse and reduced inflammation in EAE rats (Hendriks et al., 2004). Also, it has been reported that oral administration of apigenin reduced the progression and relapse in two mouse models of multiple sclerosis (Ginwala et al., 2016).

MS impacts various aspects of cognition which may appear either

early or late in the disease process (Grigsby et al., 1994). The most common cognitive deficits in MS are thought to happen in information processing speed and working memory (Audoin et al., 2005). In the present study, *A. millefolium* could improve some cognitive aspects in MS patients as assessed by PASAT and word-pair learning tests. Some studies have demonstrated that anti-oxidative flavonoid compounds, especially apigenin and luteolin, could inhibit neuronal death and provide beneficial effects on memory and learning (Liu et al., 2014). Also, it has been reported that *A. millefolium* aqueous extract did not impair recognition memory in mice (Ayoobi et al., 2013) and did not exert any adverse effect on the electrophysiological properties of neurons in the rat barrel cortex (Salari et al., 2016). In addition, a recent research indicated that 15%%– 20% of MS patients have impaired executive functions (Drew et al., 2009). Our results showed that the treatment groups had better performance in some parameters of WCST. As the mean disease duration in patients of this study was shorter than 4 years, so it is likely that *A. millefolium* might improve executive and cognitive performance in early stages of the disease process.

Several studies have reported depression prevalence in the range of 37% to 54% in MS (Sadovnick et al., 1996). Major depression has a negative impact on quality of life and treatment adherence in MS patients (Drew et al., 2009). Some findings have indicated that luteolin and apigenin have antidepressant-like effects in mice and attenuate the expression of endoplasmic reticulum stress-related proteins in the hippocampus of corticosterone- treated depression model mice (Ishisaka et al., 2011). In line with these findings, we found that *A. millefolium* reduced depression in MS patients.

Spasticity is a common problem in MS patients causing pain, spasms, loss of function, and difficulties in nursing care (Bohannon and Smith, 1987). Several flavonoids in *A. millefolium* including quercetin, luteolin, and apigenin had potent antispasmodic activities. The concentrations of these flavonoids in *A. millefolium* tea (3.5 g) would be high enough to produce this effect *in vivo*(Benedek et al., 2007). In our study, the results indicated that the spasm decreased in treatment groups, but not significantly versus placebo.

Regarding the optimum dosage of the *A. millefolium*, we did not see a major difference between the effects of high (500 mg) and low (250 mg) dose. Also, we did not observe any remarkable side effects for even the higher dose,*i.e.* 500 mg. Therefore, use of 250 mg dose is considered to be beneficial and safe.

Conclusion

A. millefolium significantly reduced the relapse rate and prevented the progression in MS associated with alleviating inflammation. Therefore, *A. millefolium* could be an effective add-on treatment for RRMS. However, the underlying mechanism of *A. millefolium* needs to be elucidated in future experiments and would be interesting to test it on other sub-types of MS. It should be noted that as the sample size was tailored on the primary outcome, these data might not have sufficient power to assess other aspects of disease activity. Therefore, assessing the efficacy of the treatment on a larger sample size is warranted.

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Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.phymed.2018.06.017.

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