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Efficacy and short-term safety of topical Dwarf Elder (*Sambucus ebulus* L.) versus diclofenac for knee osteoarthritis: A randomized, double-blind, active-controlled trial

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ABSTRACT

Ethnopharmacological relevance: *Sambucus ebulus* L. (S. ebulus) has had long-standing application in Traditional Persian Medicine for joint pain and for a variety of bone and joint disorders. According to traditional use of S. ebulus and its relevant pharmacologic properties, this study was designed to evaluate the efficacy and short-term safety of topical use of S. ebulus in patients with knee osteoarthritis (OA).

Methods and materials: Seventy nine patients with knee OA were randomly enrolled in 2 parallel arms of a pilot randomized, double-blind, active-controlled clinical trial. The patients were treated by topical S. ebulus gel or 1% diclofenac gel, three times a day, as much as a fingertip unit for 4 weeks. Patients were assessed prior to enrollment and, then, 2 and 4 weeks subsequent to the intervention, in terms of scores of visual analogue scale (VAS) for self-grading of their knee joint pain, and according to 3 different domains of Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) questionnaire. Any observed adverse effects were also scrutinized.

Results: The mean values of WOMAC pain score, total WOMAC score and VAS score for pain of the S. ebulus group were significantly lower compared with the diclofenac group ($P=0.004$, $P=0.04$, and $P<0.001$, respectively). In addition, no serious adverse effect was reported.

Conclusion: This pilot study showed that topical treatment with S. ebulus gel can be recommended for alleviating symptoms of patients with knee OA. However, longer trials involving larger samples size, are needed for achieving a comprehensive understanding about the efficacy and safety of S. ebulus in knee OA.

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1. Introduction

Osteoarthritis (OA) can be described as a syndrome mainly characterized by joint pain and loss of function (Altman, 1997). It has been ranked as the most prevalent disease in the whole array of different musculoskeletal disorders (Pereira et al., 2011; Picavet and Hazes, 2003). The WHO Scientific Group on Rheumatic Diseases has reported that OA "affects 9.6% of men and 18% of women aged >60 years" (Woolf and Pfleger, 2003).

According to several reports, knee OA is usually more prevalent than hip OA (Lawrence et al., 1998; Picavet and Hazes, 2003). There are several therapeutic approaches to knee OA, such as lifestyle modification, pharmacologic treatments, physical therapies and surgical interventions (in the last resort) (Gamble et al., 2000; Ibrahim, 2010; Parmet et al., 2003). Pharmacologic treatments (including NSAIDs and analgesics) are the most applied options to treat knee OA (Sarzi-Puttini et al., 2005). There are, however, several challenges in clinical practice when it comes to prescribing medication as for a variety of OA semiology. In point of fact, medicaments' adverse effects (Harirforoosh et al., 2013; Soubrier et al., 2013) and patients' probable comorbidities brought about a

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number of difficulties put together, especially for patients of older cohorts (Dogne et al., 2006; Sale et al., 2006).

Previous investigations had shown an increasing interest for complementary and alternative medicine (CAM) among OA patients (Herman et al., 2004; Rao et al., 1999). Furthermore, our pieces of research prior to the present one, proved CAM to be a popular choice for patients with chronic diseases in Iran (Hashempur et al., 2015; Roozbeh et al., 2013).

One of the plants of possibly the oldest application, whose documentation dates back to "Natural History" of Pliny the Elder (23–79 CE) is *Sambucus ebulus* L. (Adoxacea), or dwarf elder: it was used as the quality prescription to remedy several diseases [including rheumatic pain and joint diseases] (Schwaiger et al., 2011). *Sambucus ebulus* (S. ebulus) is known as *Khamaan* in Traditional Persian Medicine (TPM), recommended as an analgesic remedy for different painful conditions including joint pain and various bone/joint disorders such as fracture and dislocation in TPM literature; sources like *Aghili Shirazi's Storehouse of Medicaments* and *Tohfat ol Moemenin* by *Mohammad Tonkaboni* are among the frequently quoted books where mention has been made of the aforesaid plant. Moreover, in folklore medicine of some nations in the world, use is made of S. ebulus for several therapeutic purposes like treating arthritis, sore throat, bee bites and, also, as a diuretic (Ognyanov et al., 1979; Zargari, 1995).

S. ebulus possesses several active ingredients, for instance: flavonoids, steroids, tannins, glycosides, cardiac glycosides, caffeic acid derivatives, ebulitins, and volatile substances (Ahmadiani et al., 1998; Ebrahimzadeh et al., 2014). It may also exert anti-inflammatory (Ebrahimzadeh et al., 2006), anti-nociceptive (Ahmadiani et al., 1998) and anti-oxidant effects (Ebrahimzadeh et al., 2009a). The said effects are directed against the main pathophysiologic events in knee OA and, therefore, may relieve pain and/or decrease stiffness to improve the physical function of patients with OA.

In spite of traditional uses of S. ebulus for different types of joint pain over and above previous animal evidence for its anti-inflammatory, anti-nociceptive, and antioxidant effects, there is scarcely any human evidence for evaluating its traditional usage in OA patients. Therefore, this study aimed at evaluating the efficacy and safety of the topical use of S. ebulus in patients with knee OA.

2. Materials and methods

2.1. Study design

The study had two parallel interventional arms (i.e., S. ebulus and diclofenac gel groups) with a randomized, active-controlled, double-blind design. Additionally, the protocol of the study has not been modified after the trial's commencement.

2.2. Ethical considerations

The study design was in compliance with the guidelines of Declaration of Helsinki (1989 revision). Also, the study protocol was reviewed, approved and monitored by the Local Ethics Committee of Yazd Shahid Sadoughi University of Medical Sciences (registration number: 17/1/65403/p) to be, then, recorded in Iranian Registry of Clinical Trials (registration ID: IRCT 2014080318494N1). A signed, dated, written informed consent was obtained from all of the patients before enrollment.

2.3. Plant material

S. ebulus leaves were collected in Fouman (Gilan province) to the North of Iran, from an uncultivated site in summer 2014. Taxonomic identification was confirmed by Mr. M. Kamalinejad. A voucher specimen (No. 8101) is stored at the herbarium of Shahid Beheshti University of Medical Sciences, Tehran, Iran.

2.4. Preparation of the materials

One hundred grams of powdered leaves of S. ebulus was placed in a beaker to be mixed with one liter of boiling water; the mixture was left for around 6 h. Later, the contents of the beaker were filtered and condensed on bain-marie. Finally, 10 g of dry extract was obtained. Then, the base gel was made of carbomer with USP method: a gel containing 10% of the extract was made and filled into aluminum tubes. One-percent diclofenac gel of BEHVAZAN co. was filled in the same container as the drug for active control arm of the study.

Besides, both of the drugs were made similar in color (light green) by applying standard natural coloring agent of Merck co. while similarity of odor was created by adding 0.001% rose oil to them.

2.5. Standardization of S. ebulus extract

The total phenolic content of aqueous extract of *S. ebulus* was determined by the Folin-ciocalteu method. One mg/ml of crude extract was made up to 3 ml with distilled water, mixed with 0.5 ml of Folin-ciocalteu reagent for 10 min, and then 4 ml sodium carbonate was added. The final mixture was allowed to stand for a further 30 min in dark. Absorbance was measured to be 765 nm. The total phenolic content was calculated from the calibration curve: the result thereof was expressed as total phenol percentage of quercetin per gr dry weight (Kaur and Kapoor, 2002).

2.6. Inclusion criteria

Male and female patients [from the Imam Khomeini Outpatient Clinic, an academic centre, affiliated to Tehran University of Medical Sciences] with knee OA aged 30–60 and knee joint pain of at least 3 months prior to our visit, were enrolled in the study if they had mild to moderate OA according to Kellgren-Lawrence grading scale. They were required to sign informed consent form.

2.7. Exclusion criteria

Patients who had neuropathy or radiculopathy which could mimic OA symptoms, or secondary arthritis (e.g., gout, septic, metabolic, traumatic or rheumatoid arthritis) were all excluded from the study. Evaluated patients were excluded in case they also showed symptoms of allergy to S. ebulus or diclofenac. Exclusion criteria further covered any skin lesion on the affected knee, pregnancy and/or breastfeeding, plus addiction to alcohol or opiates. Moreover, they were not allowed to participate if they had undergone knee joint replacement surgery or were injected corticosteroids in their knee joint 3 months prior to enrollment. The use of topical or oral corticosteroids in a period of 14 days prior to our evaluation, and acetaminophen demand more than 2 g/day were the other exclusion criteria.

2.8. Intervention

The prescribed medications (i.e., S. ebulus and diclofenac gel) were advised to be taken 3 times per day for a period of 4 weeks, as a fingertip unit. The patients were, of course, trained to apply their medications on their affected knees in such a manner as to fully cover the affected knee by the gels. In order to avoid additional intervention, the patients were instructed not to massage their knees.

Still, all of the participants were allowed to alleviate their symptoms by using standard analgesic (tablet acetaminophen 500 mg) as they needed during the study period. They were advised to document the number of taken analgesics in a diary form.

2.9. Efficacy assessment

The enrolled patients were evaluated by using visual analogue scale (VAS) for self-grading of their knee joint pain at baseline, and at 2 and 4 weeks after the intervention. In addition, Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) questionnaire—which is a popular and valid instrument in OA-related studies (Bellamy et al., 1988)—was chosen as another outcome measure. WOMAC questionnaire assesses the patients in three dimensions of pain [score range from 0 (no pain) to 4 (the worst pain)], physical function [score range from 0 (no difficulty) to 4 (extreme difficulty)], and stiffness [score range from 0 (no stiffness) to 4 (the most severe stiffness)]. Scores of each dimension were summed and averaged. The enrolled patients were evaluated by using WOMAC questionnaire upon enrollment and 2 and 4 weeks after the intervention.

2.10. Safety measures

All of the patients in both groups were asked about possible allergic or adverse reactions at follow-up visits. A check-list questionnaire consisting of several questions on different systems (e.g., gastrointestinal, dermatologic, neurological, and respiratory complains) was applied. Open-ended questions were used for evaluating any adverse reaction.

2.11. Randomization, blinding and concealment

The participants were randomized, using a block-randomization list in the S. ebulus or diclofenac gel groups. The randomization list had blocks of the same length which were non-stratified, actually generated by Microsoft Excel® software. During data collection, neither the researchers nor the physicians had access to the randomization list and were kept unable to predict the allocations: all by the instrumentality of the central dispensary. Not only this, but as described previously, smell and color of both S. ebulus and diclofenac were similar and containers of them were the same aluminum tubes. Therefore, allocation concealment and double-blind design of the study were supported.

2.12. Statistical analysis

The sample size was estimated by considering one-sided significance level of 0.05 and 0.80 power. Finally, by considering a probable 20% drop-out rate, the sample size was calculated to be about 40 patients in each arm of the study.

Descriptive statistics are represented by mean ± standard deviation (SD) or number (percentage) where appropriate. For statistical analysis of the data, Chi-square, independent and paired *t*-test, and repeated measurement analysis were conducted. All of the statistical analyses were performed using the Statistical Package for the Social Sciences, version 18.0 (SPSS Inc., Chicago, IL, USA) at the significance level of 0.05.

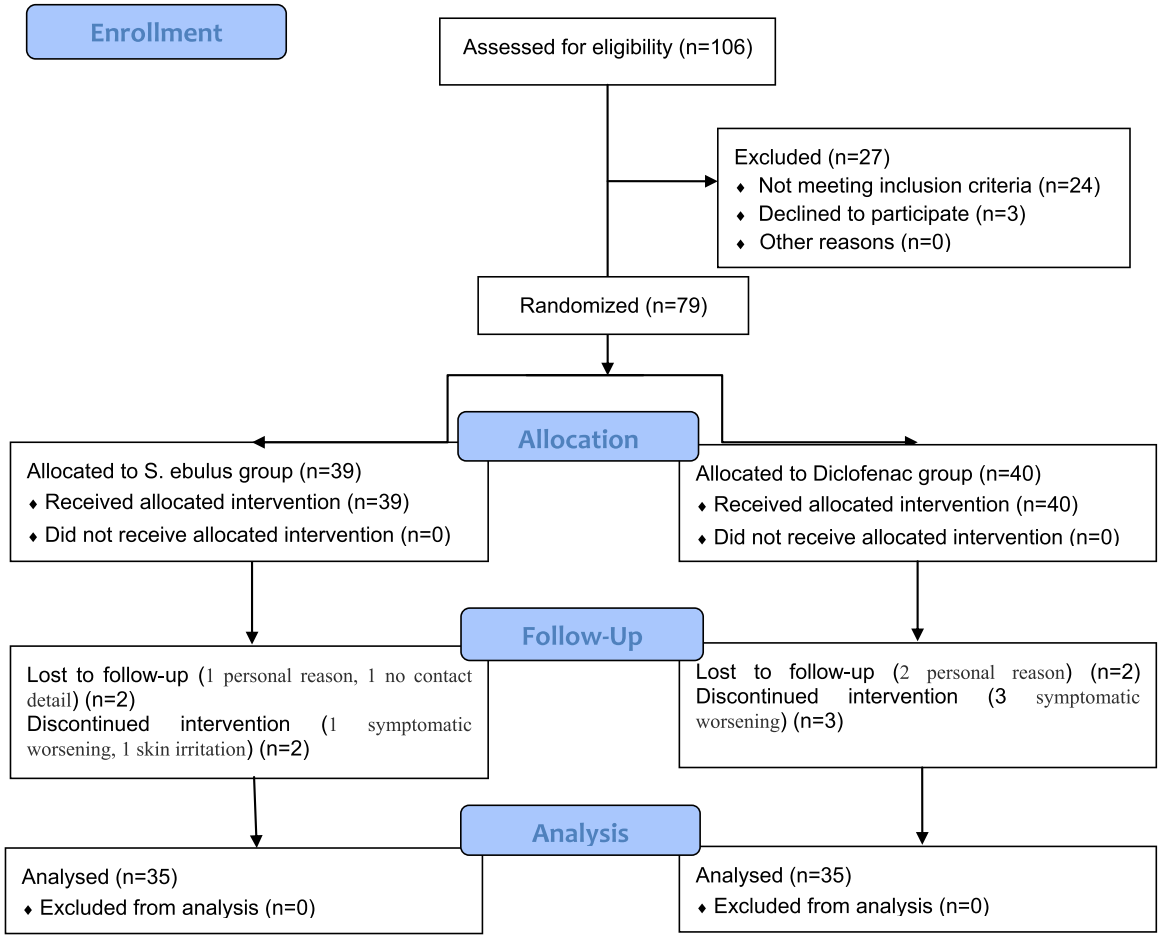

Fig. 1. The CONSORT flowchart of trial.

Table 1
Baseline characteristics of participants in the two groups of S. ebulus and diclofenac gel.

		Diclofenac (n=35)	S. ebulus (n=35)	P-value
Age (years), Mean (±SD)		46.97 (9.39)	47.34 (8.19)	0.86
BMI (kg/m^2), Mean(±SD)		27.02 (4.12)	27.78 (3.75)	0.42
Duration of symptoms (months), Mean (±SD)		4.85 (4.09)	5.28 (4.80)	0.69
Sex	Male, n (%)	7 (20)	11 (31.4)	0.27
	Female, n (%)	28 (80)	24 (68.6)	
Education	Under diploma, n (%)	9 (25.70)	8 (22.90)	0.55
	Diploma, n (%)	14 (40)	12 (34.30)	
	Academic, n (%)	12 (34.30)	15 (42.90)	

SD: standard deviation, BMI: body mass index.

3. Results

3.1. Standardization of the S. ebulus gel

The total phenolic content in 1 g of S. ebulus gel was equivalent to 0.22 mg according to quercetin. The concentration was 1.5 mg/ml and the total phenol/quercetin was 50.36 μg/ml. It is to be mentioned that total phenol was 3.3%.

3.2. Study flow and baseline subjects' characteristics

The process of assessment and recruitment of the patients was initiated from February 2015 to last to August 2015: a total of 79 eligible patients were randomly assigned to the two groups. In the end, 35 patients in each group completed their follow-ups and underwent analysis. Detailed flow of the patients' assessment, enrollment, drop-out and outcomes are presented in Fig. 1.

The mean age of the studied patients was 47.16 ± 8.75, ranging from 30 to 60 years. As many as 52 (74.3%) patients were female and 18 (25.7%) male. Mean duration of symptoms was 5.06 ± 4.43 years. As presented in Table 1, there were no significant differences with regard to age, body mass index (BMI), sex, duration of symptoms and level of education between the two groups.

3.3. Clinical response

Table 2 lays out the mean ± SD score of VAS, WOMAC questionnaire in addition to its 3 dimensions at baseline, 2 weeks, and 4 weeks after the intervention. There was a significant improvement in WOMAC pain score, total WOMAC score and VAS score for pain of the S. ebulus group as compared with the diclofenac group (P=0.004, P=0.04 and P<0.001, respectively). Nonetheless, comparison of the outcome measures regarding the mean values at each time-spot showed no significant difference between the two groups, except for the baseline VAS (P=0.01).

Fig. 2 demonstrated a visual comparison between the two groups of the study, by collating mean values of the outcome measures at each time in the two groups.

Moreover, improvement of all the outcome measures (i.e., VAS, WOMAC questionnaire and all of its dimensions) over time was significant. In fact, there was a therapeutic trend in both groups for all of the assessed outcomes (P <0.001 for total WOMAC score, pain, physical function and VAS, and P=0.001 for stiffness).

As a final point, in order to investigate the difference between each pair-wise time, paired *t*-test was performed. It is noticeable that the differences between baseline- and after-two-weeks values were significant for all combinations of group and time, except for stiffness scores in the S. ebulus group. It is to be noted that the mean differences between the baseline- and after-4-weeks figures were significant for the totality of the measures in both groups.

The significant improvement of the total WOMAC score, physical function, and pain, compared with mean differences of 2–4 week(s) is remarkable: P=0.001, 0.002, and 0.005 were, respectively, registered as for WOMAC score, physical function, and pain within the diclofenac group. When it comes to the S. ebulus group, the recorded P-value for all the three scores turned out to be below 0.001 [that is to say, P<0.001]. Correspondingly, comparison of the mean differences of 2–4 weeks between the groups implied a significant improvement in VAS of the S. ebulus group (P=0.006), but not for the diclofenac group (P=0.095). Lastly, knee stiffness did not emanate any significant change from 2 to 4 weeks–neither in the diclofenac group nor in the S. ebulus group. Description of the mean differences of the three time-spots of patients' assessment and exact P-values for these comparisons are all shown in Table 3.

As patients were allowed to take and wanted to record their acetaminophen use during the course of the study, the final outcome showed no significant differences between the two groups of the study (Table 4).

3.4. Short-term safety and tolerability

There was no report on any local or systemic adverse effects in the diclofenac group. In addition, S. ebulus gel was well tolerated

Table 2
Baseline measures of and changes in the outcome measures of the study, comparing mean values of two groups at each time-spot; trend of changes within groups (time); and repeated measurements analysis between groups.

		Time			P-value (repeated measurement)		
		Baseline	After 2 weeks	After 4 weeks	Time	Group	Time × Group
		Mean (SD)	Mean (SD)	Mean (SD)			
Pain	Diclofenac	1.55 (0.57)	1.32 (0.69)	1.18 (0.59)	<0.001	0.82	0.004
	S. ebulus	1.81 (0.69)	1.30 (0.57)	1.04 (0.49)			
Stiffness	Diclofenac	1.36 (0.82)	1.06 (0.83)	1.03 (0.80)	0.001	0.58	0.34
	S. ebulus	1.17 (0.81)	1.07 (0.78)	0.91 (0.70)			
Physical function	Diclofenac	1.78 (0.61)	1.51 (0.71)	1.32 (0.67)	<0.001	0.64	0.13
	S. ebulus	1.84 (0.69)	1.38 (0.59)	1.19 (0.52)			
Total WOMAC	Diclofenac	1.66 (0.51)	1.41 (0.64)	1.25 (0.59)	<0.001	0.81	0.04
	S. ebulus	1.78 (0.64)	1.33 (0.53)	1.11 (0.48)			
VAS	Diclofenac	4.81 (1.60)	4.16 (1.74)	3.96 (1.70)	<0.001	0.86	<0.001
	S. ebulus	5.69 (1.07)*	3.9 (1.29)	3.51 (1.20)			

SD: standard deviation, VAS: visual analogue scale, WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index.

* Significant difference according to independent *t*-test at P=0.01.

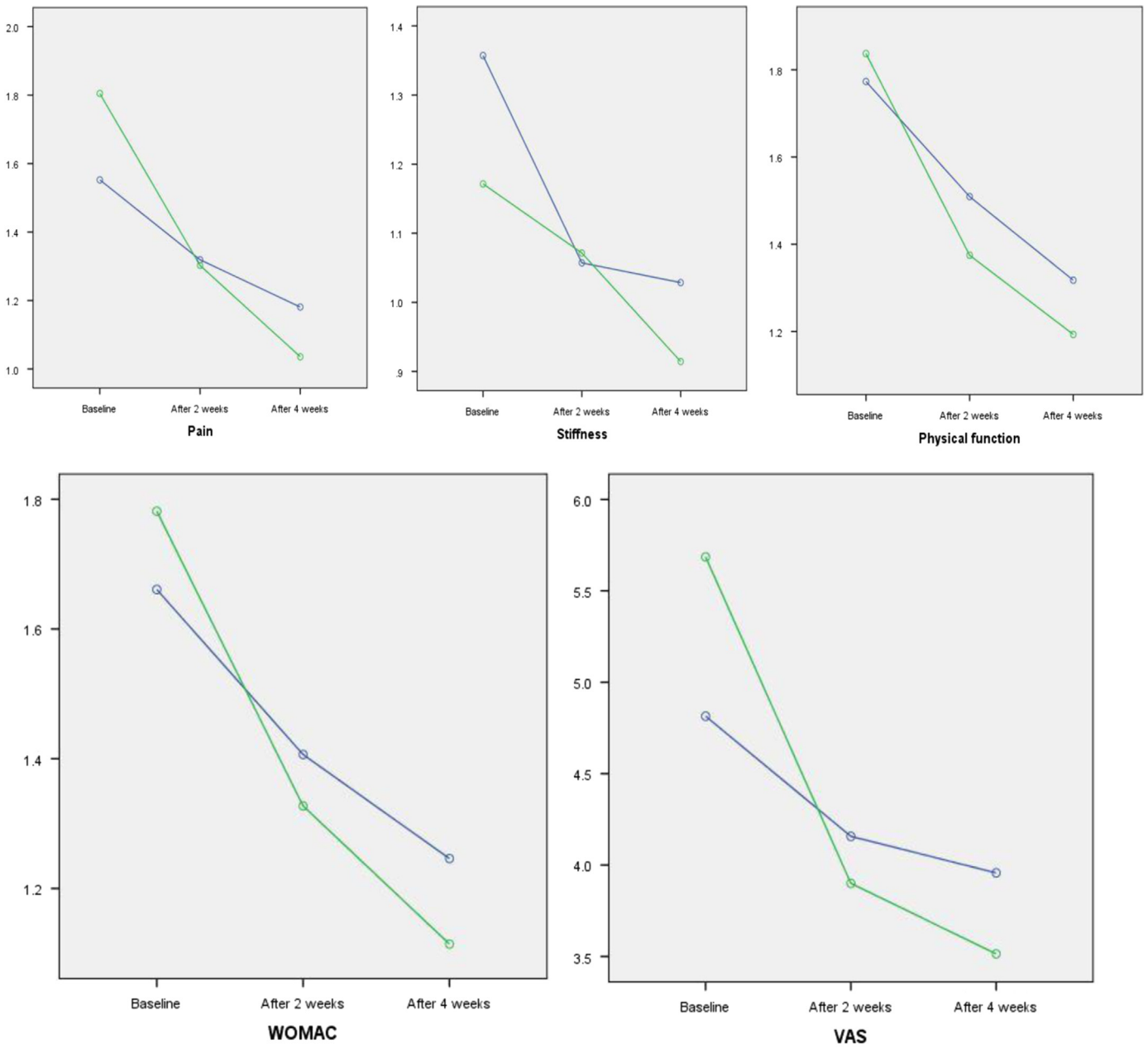

Fig. 2. Trend of mean values of the outcome measures during the intervention period. The S. ebulus group is shown by green color while the diclofenac group is shown by blue. VAS: visual analogue scale, WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
Mean of the difference (± SD) of time pair-wise and within-group analysis of mean values for each time-pair.

		Baseline–After 2 weeks		Baseline–After 4 weeks		After 2 weeks–After 4 weeks	
		Mean (SD) of difference	P-value	Mean (SD) of difference	P-value	Mean (SD) of difference	P-value
Pain	Diclofenac	0.23 (0.44)	0.004	0.37 (0.42)	< 0.001	0.14 (0.27)	0.005
	S. ebulus	0.50 (0.62)	< 0.001	0.77 (0.58)	< 0.001	0.27 (0.30)	< 0.001
Stiffness	Diclofenac	0.3 (0.72)	0.019	0.33 (0.80)	0.021	0.03 (0.34)	0.62
	S. ebulus	0.10 (0.48)	0.23	0.26 (0.57)	0.012	0.16 (0.47)	0.054
Physical function	Diclofenac	0.26 (0.78)	0.002	0.46 (0.52)	< 0.001	0.19 (0.33)	0.002
	S. ebulus	0.46 (0.48)	< 0.001	0.64 (0.53)	< 0.001	0.18 (0.25)	< 0.001
Total WOMAC	Diclofenac	0.25 (0.43)	0.001	0.41 (0.44)	< 0.001	0.16 (0.25)	0.001
	S. ebulus	0.45 (0.46)	< 0.001	0.67 (0.49)	< 0.001	0.21 (0.21)	< 0.001
VAS	Diclofenac	0.66 (0.96)	< 0.001	0.86 (0.97)	< 0.001	0.20 (0.69)	0.095
	S. ebulus	1.79 (1.13)	< 0.001	2.17 (0.89)	< 0.001	0.39 (0.79)	0.006

SD: standard deviation, VAS: visual analogue scale, WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index.

Table 4
Mean number ± standard deviation of used acetaminophen tablets by each group.

	Week 2	Week 4
Diclofenac	0.66 ± 1.66	0.31 ± 1.10
S. ebulus	0.66 ± 1.67	0.17 ± 0.56
P-value	1	0.499

by the enrolled patients: only one case of local skin irritation by one person was reported. Nevertheless, no systemic adverse effects were reported. No abnormal physical examination was detected by researchers on the follow-up contacts, either.

4. Discussion

Topical application of S. ebulus gel—3 times a day for a period of 4 weeks—appears to be effective for clinical improvement of knee-joint pain, physical function and stiffness arising from knee OA. To the best of our knowledge, this is the first study evaluating the efficacy and safety of S. ebulus gel in patients with knee OA. Of course, S. ebulus has been used in a variety of dosage forms and for several diseases (Nabati et al., 2012).

It is notable that diclofenac gel is considered as an effective treatment for knee OA in several studies. It was superior to placebo and comparable to oral NSAIDs, such as diclofenac and ibuprofen (Derry et al., 2012; Mason et al., 2004; McVeigh, 2013; Stanos and Galluzzi, 2013). Consequently, in England, as a prominent example, the National Institute for Health and Clinical Excellence has recommended topical therapies as the first choice for patients with OA in 2008 (National Collaborating Centre for Chronic Conditions (Grande Bretagne), 2008). All these having been said, as indicated in Table 2, there were significant differences between the efficacy of S. ebulus gel and diclofenac gel, regarding WOMAC pain score, total WOMAC score and VAS score of pain. This spells that the authoritative [topical use] recommendation on the part of the National Institute for Health and Clinical Excellence is maintained while, at the same time, the level of efficaciousness is also improved by the usage of S. ebulus gel.

The exact, definite mechanism of this effect of S. ebulus is not known. However, there are several plausible mechanisms of action which could potentially be attributed to different active ingredients such as quercetin 3-O-glucoside, Ebulitin, ebulin 1, flavonoid, anthocyanin (Shokrzadeh and Saeedi Saravi, 2010; Süntar et al., 2010).

Firstly, S. ebulus has been approved for its significant anti-nociceptive activity in animal models. It is notable that this effect is not related to opioid system and not only by inhibiting the prostaglandin synthesis pathway. Its analgesic activity seems to be hinged to a variety of mechanisms such as endogenous release of glucocorticoids or exogenous effect of steroids, interfering with tachykinin pathway, alpha-2 adrenoceptor or serotonergic system (Ahmadiani et al., 1998).

Along with anti-nociceptive activity, S. ebulus is a known potent anti-inflammatory, anti-arthritic agent (Ebrahimzadeh et al., 2006; Yesilada, 1997). S. ebulus leaves' extract demonstrated a significant decrease of the concentration of IL-1 alpha, IL-1 beta, and TNF alpha. Likewise, it had a significant inhibitory action against carrageenan- and serotonin-induced hind paw edema in addition to adjuvant-induced arthritis models in mice. Following an activity-guided isolation approach, a recent study on S. ebulus suggested ursolic acid to be assumingly playing an anti-inflammatory part (Schwaiger et al., 2011). Ursolic acid can play its anti-inflammatory role via interfering with COX-2 pathway (Subbaramaiah et al., 2000), inhibitory effect on IL-8 production (Thuong et al., 2005), release promotion of macrophage migration inhibitory factor (Ikeda et al., 2005), and also infiltration decreasing of agranulocytes across basal lamina (Cha et al., 1996).

As well as anti-inflammatory effect of S. ebulus, previous studies showed that it had valuable total antioxidant capacity (TAC), total polyphenol content (TPC), and total anthocyanin content whilst its TAC and TPC were proven to be the highest when compared with other anthocyanin containing plants (Ebrahimzadeh et al., 2009a; Ivanova et al., 2014). Oxidative stress in the pathophysiology and progression of OA has a substantial role. Destruction of different organic molecules of the joints by free radicals (Yudoh et al., 2005), impaired homeostasis of the cartilage matrix, induction of apoptosis in chondrocytes, and excess production of pain mediators by reactive oxygen forms (Abramson, 2008; Im et al., 2008), can describe the role of oxidative stress in OA. On the other hand, S. ebulus exhibited a significant scavenging activity and chelating properties (Ebrahimzadeh et al., 2009b) which can explain its potent anti-oxidant activity. The abovesaid anti-nociceptive, anti-inflammatory, and antioxidant activities of S. ebulus can elucidate, though partially, the functional and symptomatic improvement of patients with knee OA.

The safety of S. ebulus, as we observed during the patients' follow up, may be yet another reason for its prescription for knee OA. According to Ebrahimzadeh et al., no toxicity was observed ensuing from intra-peritoneal use of S. ebulus in mice (up to 2 g/kg body weight), except for its ethyl acetate extract (Ebrahimzadeh et al., 2007). Conjointly, in another study LD_{50} of the methanol extract of S. ebulus was estimated to be 600 mg/kg body weight (Schwaiger et al., 2011).

4.1. Study limitations

In spite of the clear results about the efficacy of S. ebulus, there are some limitations which ought to be taken into account for interpretation and generalization of our results. The small size of the study population, resulting from its type as a pilot study, is one of the most significant limitations of this present trial. In reality, we had to choose the minimum sample size, yet large enough, to demonstrate a significant effect size of S. ebulus gel and its efficacy as contrasted with a standard treatment for knee OA (i.e., diclofenac gel).

Lack of objective measures for assessing patients' functional status, despite its reliable and valid assessment by WOMAC questionnaire, signifies itself as another important limitation. Short duration of the follow-up was another limitation, too. OA is a disease of chronic nature: longer duration of the study leads to even more realistic judgment on efficacy, tolerability and safety of the tested drugs. Longer duration of the study may also show some adverse events, either locally or systematic, arising from S. ebulus regular use.

Another constraint was clinical and demographical characteristics of our patients–mainly: age (i.e., 30–60 years old) and degree of knee OA (i.e., mild to moderate). Despite our attempts to make only necessary restrictions on the patients' enrollment, some inclusion limitations were inevitable due to required homogeneity on the study population of an interventional investigation.

5. Conclusion

Considering the results of this pilot randomized double-blinded controlled clinical trial, it seems that the topical use of S. ebulus gel can be recommended as an effective alternative for patients with mild-to-moderate knee OA. Longer trials involving larger samples size, however, are necessitated for achieving comprehensive understanding about the efficacy and safety of S. ebulus in knee OA.

The positive results of this study of ours may prove motivating for other students to conduct trials for assessing efficacy of other dosage forms of S. ebulus and even on other joints pains.

Conflict of interest

The authors have declared that there is no conflict of interest.

Authors' contributions

MJ, MK, SZER and ME participated in the design of the study. MJ and SZER participated in the patients enrollment and evaluation. MK prepared the formulation and contributed in the standardization of the herbal preparation. HRS performed the statistical analysis. HRS and MHH contributed to interpretation of the results. MHH wrote the first draft of the manuscript. All authors contributed to the final manuscript. All authors read and approved the final manuscript.

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