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Original Research

Effect of Squill Oxymel on Knee Osteoarthritis: A Triple-Blind, Randomized, Controlled Clinical Trial

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Abstract

Osteoarthritis (OA) of the knee is a major health problem in the society. Iranian Traditional Medicine (ITM) or Persian Medicine (PM) as a branch of complementary medicine has been practiced in Iran for many centuries. An herbal medication known as squill oxymel has been used by PM physicians for OA. Our aim is to investigate the effect of squill oxymel on OA of the knee joint. Eighty eight patients were assigned to receive a placebo or squill oxymel syrup (10 ml each morning on empty stomach) for 8 consecutive weeks. Acetaminophen tablets were considered as the rescue medicine. Ultimately, 43 patients in the placebo group and 40 patients in the treatment group completed the trial and were included in the statistical analysis. Patients were followed for 4 weeks after cessation of treatment. The Knee injury and Osteoarthritis Outcome Score (KOOS) questionnaire and Visual Analog Scale (VAS) were considered as the main outcome measures. Laboratory tests including AST, ALT, BUN, Cr plus inflammatory tests including WBC, ESR, and CRP with specific tests i.e. interleukin 6 (IL6) and superoxide dismutase (SOD) at the beginning and the end of intervention were measured. The results showed the positive effect of treatment on the outcome of knee pain (p=0.04) and daily activity (p=0.01) of KOOS after Cessation of treatment. On the other hand, VAS decreased in both treatment and placebo groups; while it showed significance intra-group and showed no significance between the two groups. After 4 weeks of cessation of treatment, the positive effect of the squill oxymel on the treatment group continued in some of the subscales of KOOS, including symptoms, knee pain and daily activities, but stopped in the placebo group. In general, both clinically and statistically significant improvement was observed after cessation of treatment. Squill oxymel syrup showed promising results in management of knee OA but future researches with larger sample size and longer duration are necessary.

Keywords: Traditional medicine; Knee osteoarthritis; Squill oxymel; Persian medicine; *Drimia maritima*

Introduction

Osteoarthritis (OA) is the most common degenerative joint disease that causes painful swelling and permanent damage to the joints in the body. The molecular mechanisms of OA are currently unknown. Osteoarthritis (OA) is the most common degenerative pro-

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gressive disease of the joints manifested by painful swelling and permanent damage to the joints in the body. It usually happens in the middle-aged and elder people commonly affecting the knee joint. In terms of health cost, OA is a heavy burden for the patients and hip and knee OA were ranked as the eleventh highest contributor to global disability. Based on the results of several articles, the prevalence of knee OA in Asian Population is in the range of 13.8% to 71.1% which is much higher than its global prevalence [1]. The treatment of OA usually includes change of life style; reduction of pain and inflammation with non-steroidal anti-inflammatory drugs (NSAIDs), analgesics, and joint replacement surgery as the last step [2].

Today, Persian Medicine (PM) is considered as a medical school accepted by the scientific communities and people [3-7]. Due to low cost and lesser side effects, PM has been extensively used among Iranian community, but there is a need for evidence-based studies to clarify its efficacy and safety [3-7]. Squill oxymel is one of the PM remedies which has been traditionally prescribed for OA [4,8,9].

Squill (*Drimia maritima* (L.) Stearn, Asparagaceae) is an herb with oval bulb that grows in the plains and waterways, which is why it is also called the plain onion [4,7]. It is native to southern Europe, western Asia, and northern Africa [10]. Squill has been mentioned in Dioscorides *De Materia Medica* for its activities against jaundice, gripping pain of stomach and bowel, chronic chough, and asthma [11]. Persian scholars including Rhazes and Avicenna have also mentioned squill as antitussive, anti-asthma, and pain reliever of sciatica, joint, and nerves [12,13]. Using squill for medicinal purposes has been continued during 19th century specially for respiratory ailments. For example, squill and its preparations like squill oxymel have been advised several times in London's Pharmacopoeia of the Royal Hospital for Diseases of the Chest (published in 1908) [14]. The British Pharmacopoeia still has a monograph dedicated to squill oxymel [15]. The main bioactive ingredients of squill, found in all parts of the plant but concentrated in the bulbs, are bufadienolides. They are responsible for expectorant activity of the herb. They also have positive inotropic and negative chronotropic effects on the cardiac muscle. Based on traditional history of squill oxymel, resent investigations have focused on the pulmonary activities of this medicine. Nejatbakhsh et al. have recently indicated in a pilot, triple-blind, randomized clinical trial that squill oxymel has positive effects as an add-on treatment in patients with moderate to severe persistent asthma [9]. They have also worked on the effects of squill oxymel in patients with chronic obstructive pulmonary disease (COPD). This research had also positive results and the drug was well-tolerated without any sever adverse effects [16].

The beneficial effects of the squill are also related to the anti-inflammatory and anticholinergic properties bufadienolides [9,17]. These components act against inflammation by suppressing the activation of peripheral T cells [9,17,18]; and are much stronger than steroids and immunosuppressive drugs. In detail, bufadienolides are 16,384 times stronger than cortisol and 256 times more potent than cyclosporine A or tacrolimus [18]. Specifically, proscillaridin A, a bufadienolide in squill, acts like an immunologic biomolecule [9,18-20].

Despite the presence of many studies on the chemical components of the squill [4,17,20-31] and several studies on its therapeutic effects [4,7,9,19,27,32,33], no clinical trial has been conducted to evaluate the efficacy of oral intake of this plant in joints disorders. Thus, the aim of this study was to conduct a triple-blind, randomized controlled clinical trial to evaluate the clinical effect of squill oxymel syrup on chronic knee OA.

Materials and Methods

Design

This study was designed to evaluate the clinical effects of squill oxymel syrup on the treatment of patients with mild to moderate chronic knee OA. The study protocol has been reviewed and approved by the ethics research committee of Shahid Beheshti University of Medical Sciences in Tehran (IR.SBMU.RAM.REC.1394.285). The study registered in IRCT (IRCT2015101124474N1). All patients were informed before consenting to the plan by written forms and informed consent.

Sample size calculation

In order to calculate the sample size, visual analog scale (VAS) and five sub-scale Knee injury and Osteoarthritis Outcome Score (KOOS) questionnaires consisting of Pain, Other Symptoms, Activities in Daily Living (ADL), Function in Sport and Recreation (Sport/Rec) and knee-related Quality of Life (QOL) were calculated based on the data obtained from the pilot study in which the maximum sample size was related to the subscale of ADL. In this study, the expected effect size and Standard deviation was 30% and 11.4 units, respectively. The type I and II error were respectively 0.05 and 0.2. Finally, 34 people were assigned to each group and considering a 20% attrition rate, the ultimate sample size was calculated to be 45 people for each group.

Patients

Patients with mild to moderate knee OA according to

the ACR (American College of Rheumatology) criteria who visited the orthopedic clinic of "Baqiyatallah" hospital between December 2015 and May 2017 were included in this study based on inclusion criteria and orthopedic specialist confirmation.
The diagnosis criteria for mild to moderate knee OA were based on the ACR criteria and OA grade I to III according to Kellgren-Lawrence Grading Scale.
Inclusion criteria were as follows: patients aged 40–80 years, affected by mild to moderate knee OA who had ACR clinical and radiological criteria and after a period of drug wash out (7 days for oral NSAIDS and 90 days for injectable NSAIDS), having VAS>40 mm. ACR clinical and radiological criteria consisting of: knee pain along with osteophyte in radiography and at least one of the following three criteria: Age over 50 years or Morning stiffness less than 30 minutes or Crepitus on active joint motion.
Exclusion criteria were as follows: The history of rheumatoid arthritis, gout and crystalopathies, history of surgery on the affected knee, severe advanced cardiovascular disease including heart failure (grade 3 and 4), sick sinus syndrome (SSS), atrioventricular block grade 3, Wolf Parkinson's White syndrome (WPW), ventricular tachycardia (VT), hypertrophic cardiomyopathy, thoracic aortic aneurysm, proven tumor, history of stomach bleeding, presence of liver disease (esophageal varices and bleeding, encephalopathy, ascites), symptomatic biliary stones, severe renal disease (creatinine greater than 3 mg/dL), intra-articular corticosteroid receiving in the past three months, oral corticosteroid receiving in the past four weeks ago, getting oral or injectable NSAIDs in less than a week before intervention, receiving chondroitin or glucosamine, warfarin, clopidogrel, digitalis, capsaicin, quinidine, laxatives, or any other drug that according to the pharmacologist prevents the patient from using the drug of study, pregnancy and breastfeeding.
Discontinuing the drug by the patient for any reason, allergy or experiencing side effects of drug and any conditions that requires clinical attention to change a patient's treatment regimen were conditions for departure of patients from the study.

Preparation of syrup and placebo

Squill oxymel syrup was obtained from Barij Essence Pharmaceutical Company (Kashan, Iran). Based on the company's documents, the syrup was manufactured by boiling squill vinegar extract (33.3%) and honey (66.7%) in a stainless steel double jacket steam syrup mixing tank. The syrup was finally pasteurized and filled in 120 mL syrup bottles and analyzed via titration of acetic acid content based on the method prescribed in British pharmacopoeia under the squill oxymel monograph [15]. As a placebo, brown sugar syrup (50% w/w) was prepared, pasteurized and filled in 120 mL syrup bottles similar to the drugs.

Protocol

The present study is a triple-blind, randomized, controlled clinical trial. Qualified individuals were randomly divided into two groups, the control group (placebo) and treatment group (squill oxymel). Subjects were allocated to A and B groups by block randomization. All patients, researchers and analyzers were blinded to special intervention.
After filling out the information form and questionnaires, radiological X-Ray of the involved knee (in the absence of a photo for 6 months ago) and inflammatory tests of white blood cells (WBC), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and specialized tests of interleukin 6 (IL6) and superoxide dismutase (SOD) were given as a baseline data for comparing with data at the end of the treatment. General liver and kidney laboratory tests such as aspartate transaminase (AST), alanine transaminase (ALT), bod urea nitrogen (BUN), and creatinine (Cr) to determine patients with severe liver and kidney problems were requested.
Duration of study was 12 weeks; 8 weeks of intervention period and 4 weeks after cessation of treatment (follow-up). During this period, patients could use up to 4 g/day of acetaminophen to relieve the pain. Both treatment and placebo groups were consumed each morning, fasting two tablespoons (10 mL) of syrup with a glass of water for 8 weeks.
Patients were visited at weeks 0, 8, and 12. At the end of the eighth week (after the syrup consumption), specialized tests, IL6 and SOD and inflammatory tests WBC, ESR, CRP were performed and were compared with data at the beginning of study. 4 weeks after cessation of treatment, in the 12th week, symptoms and signs were evaluated based on questionnaires and forms for follow-up. The Pharmacist who produced the medication was the only person informed of the type of syrups labeled as A and B (squill oxymel or placebo). During each visit, patients were evaluated for adjuvant medication tolerance and any possible side effects.

Outcome measures

Initial value in this study were changes in knee pain that were assessed by VAS. VAS is one of the pain rating scales and is a validated, subjective measure for acute and chronic pain. Scores are recorded by making a handwritten mark on a 10-cm line that represents a continuum between “no pain” and “worst pain.”
We also used the KOOS questionnaire– Persian version for evaluation of treatment [34]. KOOS has 42 items in five separate sub-scales: Pain, Other Symptoms, ADL, Function in Sport and Recreation (Sport/Rec), and knee-related QOL. KOOS is a true, respon-

sive and reliable tool that can be used to track OA in short-term and long-term [2,34]. Likert scale was used for all items with five possible options to respond to scores from 0 (no problems) to 4 (severe problems). Scores were changed to a scale of 0-100. Zero indicates severe knee problems and 100 indicates no knee problems. The total score will not be estimated and should be analyzed in five separate scores. The total amount of acetaminophen consumed was calculated and recorded [2,34].

Inflammatory tests WBC, ESR, and CRP with specific tests IL6 and SOD at the beginning of treatment (first visit) and at the end of treatment (second visit) were laboratory outcomes. Evaluation of side effects of intervention was another outcome of study.

Statistical analysis

Statistical analysis was limited to participants with complete information for all evaluations of actions from the beginning to the end of the study (88 people). The data was analyzed by using the Statistical Packages for Social Sciences (SPSS), version 22.0 (SPSS Inc., Chicago, IL, USA). The normality of the data was evaluated via the Kolmogorov-Smirnov test. The basic characteristics of the data were described by descriptive statistics including frequency, percentage, mean and standard deviations. Differences between groups were analyzed using parametric citation statistics such as independent t-test, chi-square and paired t-test. The main analysis was done by covariance analysis (ANCOVA). Adjusted variables considered in ANCOVA were VAS score at the baseline and use of the number of acetaminophen during intervention when dependent variable of interest was VAS score. In addition, adjusted variable for subscales of KOOS was values of baseline sub-scales. It should be noted that in order to investigate the effect of treatment during four weeks after cessation of treatment (from the end of intervention till the end of study), the paired t-test was used. The continuation of treatment effects has been observed in some of the items described in the following tables in more detail.

Results

Participants

From total of 220 cases, ninety eligible patients did not have inclusion criteria, 23 patients did not participate in study, and 7 patients left the study. Finally, 100 patients were randomly assigned to the study and divided into two groups of treatment and placebo (with block randomization). Of these, 88 patients completed the study and spent eight week taking medication and initial and final laboratory tests. Of the 88 patients, 70 were female (80%) and 18 were male (20%).

There were 12 people who had dropped out, 11 were women and one was a man, 7 (60 %) of the treatment group and 5 (40%) of the placebo group. Causes of drop-out of patients in the treatment group included: nausea, dry stool and constipation, unsatisfactory taste of syrup, gastrointestinal discomfort, anxiety and headache (7 people). In the placebo group, the causes of drop-out included: loss of appetite, allergies and itching, nausea and digestive problems (5 people). Five of 88 patients did not report after cessation of treatment and the third visit (four weeks after the end of the intervention) due to certain problems, such as being away for work and other reasons. A detailed description of the registration, randomization and patient outcomes is shown in the CONSORT chart (Figure 1).

Demographic data

Eighteen men (10 in the treatment group and 8 in the placebo group) and 70 women (33 in the treatment group and 37 in the placebo group) participated in this study. The mean age ± SD was 54.13 ± 7.53 in treatment group and 55.08 ± 7.12 in placebo group with a P value of 0.54.

Based on severity of knee involvement according to ACR criteria, the number of patients with OA grade I and II was 31 (47.6%) in the treatment group and 34 (52.4%) in the placebo group. Twelve patients with OA grade III (52.2%) were assigned to the treatment group and 11 patients (47.8%) in the placebo group.

In this fashion, both groups had the same distribution in terms of severity of knee involvement (P>0.05). The other demographic data and clinical features of the participants such as body mass index (BMI), job, involved knee and menopause are presented in table 1. There was no significant difference between the two groups in terms of age, gender and other demographic characteristics. Blocked randomization was done for age, gender and BMI. By random sampling, the confounding factors were minimized and the study groups were consistent.

Outcomes in intervention duration

As shown in table 2, after intervention, the effect of squill oxymel syrup on the improvement of symptoms of KOOS questionnaire in the treatment group was significantly better than placebo group. For instance, the score of symptoms in the treatment and placebo group before intervention was 62.37 ± 20.52 and 59.76 ± 20.13, respectively; whereas after intervention was 70.84 ± 19.23 and 62.06 ± 20.78, respectively (p=0.04). Other variables in the two groups were not significant. Within group comparisons are presented in the table.

Daily activity and sport item improved in both treatment and placebo groups, but no significant difference was observed between the two groups.

Results show that after intervention, the VAS in the

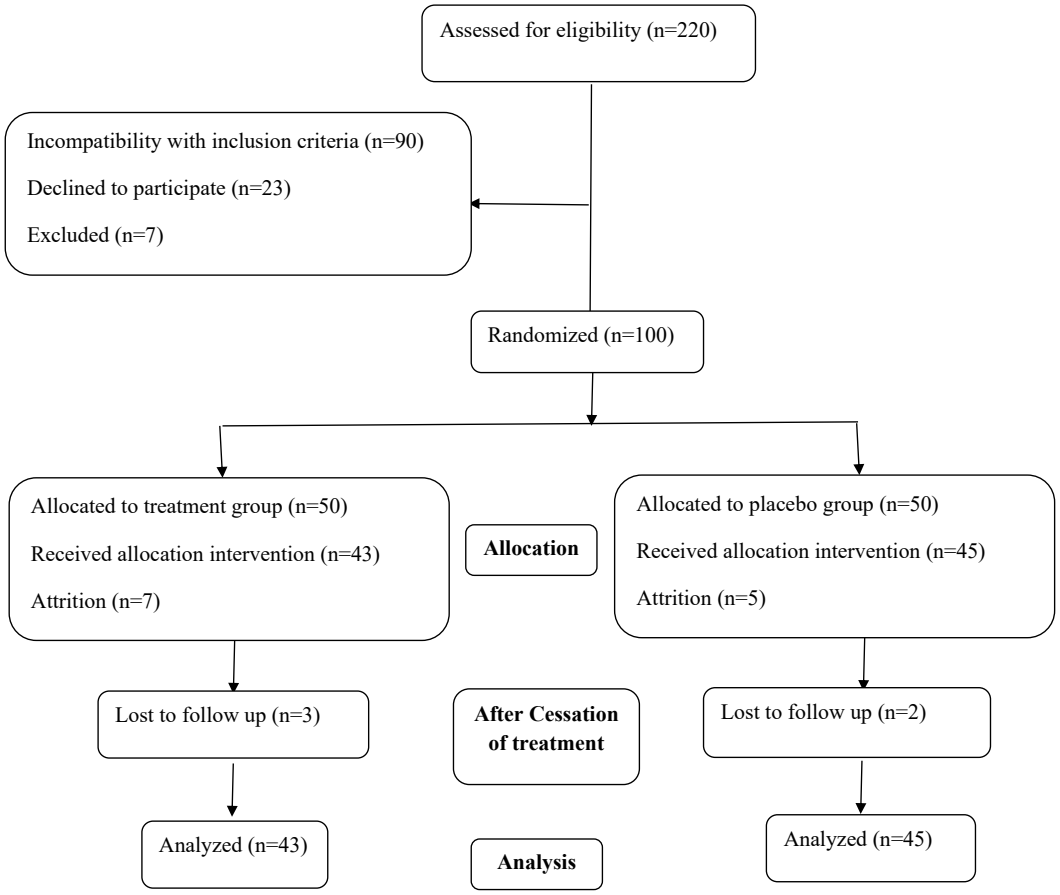

Figure 1. The CONSORT oriented flow diagram

Table 1. Demographic characteristics and clinical features of participants in the study.

Variables	Treatment group n= 43	Placebo group n= 45	P value
Mean ± SD			
Age, y	54.13 ± 7.53	55.08 ± 7.12	0.54
BMI	28.98 ± 4.32	29.35 ± 4.03	0.68
n (%)			
Gender			
Male	10 (55.6%)	8 (44.4%)	0.52
Female	33 (47.1%)	37 (52.9%)	
Job			
Housekeeper	22 (39.3%)	34 (60.7%)	0.01
Employed	21 (65.6%)	11 (34.4%)	
Menopause			
Yes	15 (60%)	10 (40%)	0.1
No	18 (40%)	27 (60%)	
Involved knee			
Just one knee	20 (54.1%)	17 (45.9%)	0.41
Both knees	23 (45.1%)	28 (54.9%)	
Severity of knee involvement			
Grade I & II	31 (47.6%)	34 (52.4%)	0.71
Grade III	12 (52.2%)	11 (47.8%)	

Table 2. Comparison of the mean dimensions of visual analogue scale (VAS), KOOS and laboratory variables before and after the intervention based on treatment and placebo group

Variable	Treatment group Mean ± SD	Placebo group Mean ± SD	P value
VAS			
Before	6.46 ± 1.69	6.66 ± 1.78	0.58
After	4.63 ± 2.50	4.74 ± 2.38	0.84
P value	P <0.001	P <0.001	
KOOS Symptoms			
Before	62.37 ± 20.52	59.76 ± 20.13	0.54
After	70.84 ± 19.23	62.06 ± 20.78	0.04
P value	<0.001	0.30	
KOOS Pain			
Before	50.19 ± 18.30	46.35 ± 17.75	0.32
After	58.72 ± 21.88	51.60 ± 22.19	0.13
P value	<0.001	0.02	
KOOS ADL			
Before	52.42 ± 15.28	51.11 ± 17.14	0.70
After	62.20 ± 22.06	57.90 ± 22.20	0.36
P value	<0.001	0.004	
KOOS Sport/Rec			
Before	26.39 ± 19.89	21.66 ± 20.99	0.28
After	36.97 ± 27.08	28.33 ± 25.09	0.12
P value	0.002	0.03	
KOOS QoL			
Before	35.61 ± 12.61	35.41 ± 11.14	0.93
After	42.29 ± 16.58	39.02 ± 12.86	0.30
P value	0.006	0.05	
WBC			
Before	7.14 ± 2.08	6.94 ± 1.76	0.19
After	6.63 ± 1.47	6.46 ± 1.51	0.59
P value	0.11	0.07	
ESR			
Before	10.74 ± 7.29	12.28 ± 8.17	0.35
After	9.18 ± 8.19	8.26 ± 6.47	0.56
P value	0.17	0.002	
CRP			
Before	6.34 ± 5.22	7.51 ± 9.42	0.47
After	6.57 ± 8.90	6.29 ± 11.09	0.89
P value	0.85	0.22	
IL6			
Before	10.10 ± 7.62	9.58 ± 8.36	0.76
After	10.55 ± 10.65	7.91 ± 9.85	0.23
P value	0.78	0.09	
SOD			
Before	25.91 ± 18.53	25.90 ± 18.97	0.99
After	26.46 ± 22.34	21.96 ± 20.61	0.32
P value	0.88	0.12	

ADL: Activities of Daily Living; Sport/Rec: Sport and Recreation Function; QoL: knee-related Quality of Life; KOOS: Knee injury and Osteoarthritis Outcome Score; CRP: C-reactive protein; SOD: superoxide dismutase; IL6: Interleukin 6; ESR: erythrocyte sedimentation rate; WBC: white blood cells; VAS: visual analogue scale.

treatment group was slightly less than the placebo group, but was not statistically significant so that the score of VAS in the treatment and placebo group before intervention was 6.46 ± 1.69 and 6.66 ± 1.78, respectively; whereas after intervention was 4.63 ± 2.50 and 4.74 ± 2.38, respectively (p = 0.84).

The results of the patients' laboratory tests showed no significant differences in the two groups.

Outcomes after cessation of treatment

Based on table 3 after cessation of treatment, the efficacy of treatment with squill oxymel syrup continued on knee pain and ADL. There was a significant difference between the two groups after cessation of

treatment; while there was no significant difference between knee pain and ADL during the intervention. For instance, pain score in the treatment and placebo group after intervention was 58.19 ± 22.04 and 50.19 ± 21.66, respectively; whereas after cessation of treatment was 61.31 ± 21.41 and 49.80 ± 18.25 respectively (p=0.01). Also ADL score after intervention was 61.91 ± 22.06 and 56.66 ± 21.86 in the treatment and placebo group, respectively; whereas after cessation of treatment was 63.75 ± 21.11 and 53.42 ± 17.99, respectively (p=0.01). This indicates the delay and continuous effect of squill oxymel syrup on the treatment. Comparing the results of this table with table 2 shows that the recovery trend continued after cessation of treatment especially in pain and daily activity.

After cessation of treatment, VAS did not change significantly in the two groups.

In the next stage, covariance analysis was used which was adjusted based on VAS in the beginning of the study and the number of acetaminophen consumed during the intervention.

As shown in Table 4, two covariance analyses were performed after the intervention and after cessation of treatment in the treatment and placebo groups.

In summary, our results showed the effect of treatment on the KOOS sub-scales (pain and ADL). The crude mean of pain score after cessation of treatment in the treatment and placebo group was 61.31± 21.41 and 49.80 ± 18.25, respectively; whereas after modification, the adjusted mean in the treatment and placebo group was 58.70 ± 14.49 and 52.23 ± 14.48, respectively (p=0.04). In addition, crude mean in the subscale of ADL was more than in the treatment group compared with the placebo group (63.75 ± 21.11 vs. 53.42 ± 17.98), and after adjustment, adjusted mean in the treatment and placebo group was 61.91 ± 12.90 and 55.12 ± 12.89, respectively (p=0.01). These results indicated that the clinical effect of squill oxymel syrup might be started with delay after cessation of treatment and therapeutic effects can be continuous (Table 4). Furthermore, laboratory variables and specific tests were compared in the two groups (Table 5).

Table 3. Comparison of the mean dimensions of the VAS, KOOS and laboratory variables after intervention and after cessation of treatment by treatment and placebo group

Variable	Treatment group Mean ± SD	Placebo group Mean ± SD	P value
VAS			
After treatment	4.62 ± 2.54	4.84 ± 2.39	0.84
After cessation	4.25 ± 2.66	4.87 ± 2.68	0.29
P value	0.11	0.92	
KOOS Symptoms			
After treatment	70.71 ± 18.58	60.71 ± 20.23	0.04
After cessation	66.87 ± 21.18	60.63 ± 17.20	0.14
P value	0.07	0.96	
KOOS Pain			
After treatment	58.19 ± 22.04	50.19 ± 21.66	0.13
After cessation	61.31 ± 21.41	49.80 ± 18.25	0.01
P value	0.20	0.88	
KOOS ADL			
After treatment	61.91 ± 22.06	56.66 ± 21.86	0.36
After cessation	63.75 ± 21.11	53.42 ± 17.99	0.01
P value	0.40	0.17	
KOOS Sport/Rec			
After treatment	36.00 ± 27.36	27.09 ± 24.37	0.12
After cessation	34.75 ± 28.23	24.53 ± 20.49	0.06
P value	0.71	0.39	
KOOS QoL			
After treatment	42.34 ± 15.98	38.22 ± 12.58	0.30
After cessation	42.34 ± 16.89	38.22 ± 13.02	0.21
P value	1.00	1.00	

QoL: knee-related Quality of Life; KOOS: Knee injury and Osteoarthritis Outcome Score; ADL: Activities of Daily Living; VAS: visual analogue scale.

Table 4. Comparison of adjusted VAS and KOOS score in two groups (treatment and placebo) after intervention and after cessation of treatment using covariance analysis

Variable	Group	Crude* Mean ± SD	Adjusted** Mean ± SD	F	P value***
VAS					
After treatment†	Treatment	4.63 ± 2.50	4.52 ± 2.39	0.31	0.57
	Placebo	4.74 ± 2.38	4.78 ± 1.97		
After cessation††	Treatment	4.25 ± 2.66	4.44 ± 2.08	0.56	0.45
	Placebo	4.87 ± 2.68	4.75 ± 1.63		
KOOS Symptoms					
After treatment	Treatment	70.84 ± 19.23	69.88 ± 13.70	5.53	0.02
	Placebo	62.06 ± 20.78	62.98 ± 13.68		
After cessation	Treatment	66.87 ± 21.18	62.82 ± 12.02	0.34	0.55
	Placebo	60.63 ± 17.20	64.39 ± 12.01		
KOOS Pain					
After treatment	Treatment	58.72 ± 21.88	56.92 ± 14.76	1.30	0.25
	Placebo	51.60 ± 22.19	53.31 ± 14.75		
After cessation	Treatment	61.31± 21.41	58.70 ± 14.49	4.05	0.04
	Placebo	49.80 ± 18.25	52.23 ± 14.48		
KOOS ADL					
After treatment	Treatment	62.20 ± 22.06	61.54 ± 15.33	0.84	0.36
	Placebo	57.90 ± 22.20	58.54 ± 15.34		
After cessation	Treatment	63.75 ± 21.11	61.91 ± 12.90	5.70	0.01
	Placebo	53.42 ± 17.98	55.12 ± 12.89		
KOOS Sport/Rec					
After treatment	Treatment	36.97 ± 27.08	35.08 ± 20.79	1.23	0.26
	Placebo	28.33 ± 25.09	30.14 ± 20.78		
After cessation	Treatment	34.75 ± 28.23	31.82 ± 18.49	1.24	0.26
	Placebo	24.53 ± 20.49	27.25 ± 18.49		
KOOS QoL					
After treatment	Treatment	42.29 ± 16.58	42.23 ± 12.99	1.29	0.25
	Placebo	39.02 ± 12.86	39.08 ± 13.00		
After cessation	Treatment	42.34 ± 16.89	40.94 ± 11.86	0.58	0.58
	Placebo	38.22 ± 13.02	39.52 ± 11.86		

* Crude mean without any adjustment

** The mean based on VAS in the beginning of the study and the number of acetaminophen consumed during the intervention were modified.

*** Significant value for comparison between two groups based on covariance analysis.

† The adjusted mean values were calculated after modification of values of variable in the baseline (phase I).

†† The adjusted mean values were calculated after modification of values of variable in the end of treatment duration (phase II).

QoL: knee-related Quality of Life; KOOS: Knee injury and Osteoarthritis Outcome Score; ADL: Activities of Daily Living; VAS: visual analogue scale.

The results shown that there was not any statistically significant difference between two groups.

Adverse events

In the squill oxymel group three female patients experienced nausea, two patients suffered from constipation, one patient suffered from exacerbation of knee stiffness and one patient had headache and dizziness. In the placebo group, a woman who had a history of allergy to cold water, experienced redness, itching and inflammation of the palms. Two female patients left the study due to nausea and oth-

er gastrointestinal problems after taking the syrup and one woman had loss of appetite after taking the syrup. With useful recommendations, complications were improved and controlled in some patients. No serious adverse effect occurred during the study.

Table 5. Comparison of crude and adjusted mean of laboratory tests in two groups (treatment and placebo) after intervention using covariance analysis

Variable	Group	Crude* Mean ± SD	Adjusted** Mean ± SD	F	P.Value***
WBC	Treatment	6.63 ± 1.47	6.60 ± 1.39	0.14	0.70
	Placebo	6.46 ± 1.51	6.49 ± 1.38		
ESR	Treatment	9.18 ± 8.19	9.53 ± 6.55	1.31	0.25
	Placebo	8.26 ± 6.47	7.93 ± 6.56		
CRP	Treatment	6.57 ± 8.90	7.10 ± 7.46	0.69	0.40
	Placebo	6.29 ± 11.09	5.78 ± 7.46		
IL6	Treatment	10.55 ± 10.65	10.37 ± 8.69	1.52	0.22
	Placebo	7.91 ± 9.85	8.08 ± 8.69		
SOD	Treatment	26.46 ± 22.34	26.46 ± 18.84	1.24	0.26
	Placebo	21.96 ± 20.61	21.97 ± 18.85		

* Crude mean without any adjustment
** The mean based on VAS in the beginning of the study and the number of acetaminophen consumed during the intervention were modified.
*** Significant value for comparison between two groups based on covariance analysis.
CRP: C-reactive protein; SOD: superoxide dismutase; IL6: Interleukin 6; ESR: erythrocyte sedimentation rate; WBC: white blood cells.

Discussion

To the best of our knowledge, this is the first clinical trial to examine the effectiveness of squill oxymel in patients with knee OA. Despite the fact that there are several studies on the composition and therapeutic effects of squill, there is little information about its role in OA. Previously and based on local experiences, a clinical study was conducted on musculoskeletal pains by prescription of topical taxifolin, proscillaridin-A, and scilliroside, the active components of squill bulb. This study showed that these natural chemicals significantly reduce knee, joint, calf, hip, shoulder, upper back, low back (lumbago), tailbone and fibromyalgia paints of the participants. Topical application of taxifolin, proscillaridin-A, and scilliroside also significantly reduced higher values of Antistreptolysin O (ASO), C-reactive protein (CRP) and Rheumatoid Factor (RF) [35]. Our results are in line with the above-mentioned research. We showed that oral intake of squill oxymel syrup can reduce knee pain, improve symptoms of OA, and enhance daily activity in knee OA. Comparing treatment and cessation phases indicated that, this improvement continues after the cessation of treatment which suggests the long-term effect of squill oxymel syrup on pain and symptoms of knee osteoarthritis.

Previous in vitro and in vivo studies have shown that the natural antioxidants of the white squill bulb have protective effects against free radicals and have a wide range of antimicrobial, anti-allergic, anti-inflammatory and anticancer activity [9,11,20,28,30,32].

Moreover, previous clinical studies have also shown that squill has a protective effect on oxidative DNA damage and therefore, increase the antioxidant level [9,30,32]. Oxidative stress plays an important role in the pathophysiology of OA. Free radicals may destroy various types of molecules in the joint. In fact, the cartilage matrix is damaged due to excessive production of inflammatory mediators by activated oxygen forms and chondrocyte apoptosis [36,37]. Therefore, antioxidant and radical scavenging activity may be one of the mechanisms by which squill oxymel acts against OA.

Moreover, honey also demonstrates considerable effects on pain and inflammation of OA. In an experimental study, the effect of honey and glucosamine/chondroitin sulphate compared with each other in controlling knee edema, tactile allodynia, some serum bi-

omarkers like tumor necrosis factor (TNF)-α, and histopathological changes on rat OA model induced by intra-articular injection of monosodium iodoacetate. The results showed that both honey and glucosamine/chondroitin sulphate comparably reduce knee edema, tactile allodynia, and TNF-α but have no effect on histopathological features [38]. Anti-inflammatory, chondroprotective effect, and regulation of articular homeostasis of honey are related to its natural compounds e.g. chrysin, luteolin, quercetin, baicalin, and bulein [39]. Therefore, positive activity of squill oxymel on OA is partly attributed to honey.

The main limitation of our study was the short duration of intervention. On the other hand, according to PM, due to temperament, race, season, sex, age, occupation, etc., there are differences in bioavailability of the product in patients, which causes differences in the effect of the drug. Analgesic use by patients during the study was another limitation of the study.

Conclusion

As a conclusion, our results indicate the positive effect of squill oxymel syrup on knee OA even after cessation of treatment which suggests the long-term effect of the syrup. Larger sample size and longer period of study would be fruitful to consolidate our results.

Conflict of Interests

None.

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References

[1] Zamri NAA, Harith S, Yusoff NAM, Hassan NM, Qian Ong Y. Prevalence, risk factors and primary prevention of osteoarthritis in Asia: a scoping review. Elderly Health J 2019;5:19-31.

[2] Taheri M, Kazem Emami meybodi M, Mokaberinejad R, Dehghan S. The role of nutrition in management of joint disorders according to Iranian traditional medicine: a narrative review. Indo American J Pharm Sci 2017;4:4455-4459.

[3] Shoara R, Hashempur MH, Ashraf A, Salehi A, Dehshahri S, et al. Efficacy and safety of topical Matricaria chamomilla L. (chamomile) oil for knee osteoarthritis: a randomized controlled clinical trial. Complement Ther Clinic Pract 2015;21:181-187.

[4] Bozorgi M, Amin G, Shekarchi M, Rahimi R. Traditional medical uses of Drimia species in terms of phytochemistry, pharmacology and toxicology. J Tradit Chinese Med 2017;37:124-139.

[5] Naseri M, Mojab F, Khodadoost M, Kamalinejad M, Davati A, et al. The study of anti-inflammatory activity of oil-based dill (Anethum graveolens L.) extract used topically in formalin-induced inflammation male rat paw. Iran J Pharm Res 2012;11:1169-1174.

[6] Alijaniha F, Naseri M, Afsharypuor S, Fallahi F, Noorbala A, et al. Heart palpitation relief with Melissa officinalis leaf extract: double blind, randomized, placebo controlled trial of efficacy and safety. J Ethnopharmacol 2015;164:378-384.

[7] Abdolhosseini S, Mokaberinejad R, Alian Nezhadi V, Bozorgi M, Nazem E. Evaluation of treatment with squill vinegar in 10 patients with head pediculosis. Asian J Clin Case Rep Tradit Altern Med 2017;1:57-64.

[8] Ziaei A, Sahranavard S, Faizi M. Topical herbal remedies for treatment of joint pain according to Iranian Traditional Medicine. Res J Pharmacognosy 2016;3:63-72.

[9] Nejatbakhsh F, Karegar-Borzi H, Amin G, Eslaminejad A, Hosseini M, et al. Squill oxymel, a traditional formulation from Drimia Maritima (L.) stearn, as an add-on treatment in patients with moderate to severe persistent asthma: A pilot, triple-blind, randomized clinical trial. J Ethnopharmacol 2017;196:186-192.

[10] Mozaffarian V. Identification of medicinal and aromatic plants of Iran. Farhang-e Moaser. Tehran 2012; pp 636-637.

[11] Dioscorides. De Materia Medica. Robert P. A. Wood, Tess Anne Osbaldeston (Editors). IBIDIS Press Johannesburg 2000; pp 336-339.

[12] Rhazes. Alhawi fit-Tibb. Vol. 20. Dar ul-Ihya At-Torath al-Arabi. Beirut 2001; p 42 [Arabic].

[13] Avicenna. Al-Ghanoon fit-Tibb (Canon of Medicine). Vol. 1. Dar ul-Ihya At-Torath al-Arabi. Beirut 2005; p 323 [Arabic].

[14] London Hospital for Diseases of the Chest. Pharmacopoeia of the City of London Hospital for Diseases of the Chest. Victoria Park, London 1908; p 35.

[15] British Pharmacopoeia Commission. British Pharmacopoeia 2022. London; IV-486.

[16] Mohammadi-Araghi M, Eslaminejad A, Karegar-Borzi H, Mazloomzadeh S, Nejatbakhsh F. An add-on treatment for moderate COPD with squill-oxymel (a traditional formulation from Drimia maritima (L.) Stearn): a pilot randomized triple-blinded placebo-controlled clinical trial. Evid Based Complement Altern Med 2022;2022:5024792.

[17] Hamouda AB, Chaieb I, Zouari L, Zarrad K, Laarif A. Toxicological effects of Urginea maritima (L.) against the red flour beetle (Coleoptera: Tenebrionidae). J Entomol Zool 2016;4:17-20

[18] Terness P, Navolan D, Dufter C, Kopp B, Opelz G. The T-cell suppressive effect of bufadienolides: structural requirements for their immunoregulatory activity. Int Immunopharmacol 2001;1:119-134.

[19] Bozorgi M, Amin GR, Ostad SN, Samadi N, Nazem E, et al. Toxicological, chemical and antibacterial evaluation of squill vinegar, a useful product in Persian Traditional Medicine. Res

J Pharmacognosy 2017;4:33-39.
[20] Li R-Z, Fan X-X, Duan F-G, Jiang Z-B, Pan H-D, et al. Proscillaridin A induces apoptosis and suppresses non-small-cell lung cancer tumor growth via calcium-induced DR4 upregulation. Cell Death Dis 2018;9:696.
[21] Geetha HL, Shivakameshwari MN, Nijagunaiah R. Karyotypic variations in different accessions of urginea indica (Roxb) Kunth. and urginea wightii lakshmin. hyacinthaceae. Cytologia 2016;81:329-334.
[22] Sharma H, Devi N. Phytochemical analysis of Drimia species. Int J Appl Sci Res Rev 2017;4:12.
[23] Hammad I, Elbakry A, Mohammed E. DNA polymorphism within different six forms of Urginea maritima (L.) Baker (Liliaceae) from the Mediterranean coast, Egypt. Egypt J Exp Biol (Bot.) 2017;13:353-360.
[24] Mohanarangan J, Ravikumar S, Dhamotharan R. Phytochemistry and HPTLC Fingerprinting Analyses of Scilla indica (Wt.) Baker. J Acad Ind Res 2016;5:85.
[25] Prathima P, Kameshwari MNS, Nijagunaiah R. Morphometric studies in Urginea indica (Roxb) Kunth. Hyacinthaceae. Int J Pure Appl Biosci 2015;3:82-87.
[26] Bozorgi M, Amin G, Kasebzade S, Shekarchi M. Development and validation of a HPLC-UV method for determination of Proscillaridin A in Drimia maritima. Res J Pharmacogn 2016;3:1-7.
[27] Knittel DN, Stintzing FC, Kammerer DR. Metabolic fate of cardiac glycosides and flavonoids upon fermentation of aqueous sea squill (Drimia maritima L.) extracts. J Pharm Biomed Anal 2015;110:100-109.
[28] Jarjes ZA, Mahmood A. Identification of some chemical constituents and antibacterial activity of Uriginea maritima (L.) extracts from Kurdistan region, Bozan area, Alqosh. Zanco J Pure Appl Sci 2016;28:122-131.
[29] Belhaddad O, Noureddine C, Amamra S, Zerargui F, Baghiani A, et al. Chromatographic fractionation, antioxidant and antibacterial activities of Urginea maritima methanolic extract. Pakistan J Pharm Sci 2017;30:127-134.
[30] Namjoyan F, Jahangiri A, Azemi ME, Mousavi H. Inhibitory effects of Urginea maritima (L.) Baker, Zhumeria majdae Rech. F. and Wendelbo and physalis divaricata D. Don ethanolic extracts on mushroom tyrosinase. Pharm Sci 2016;22:81-86.
[31] Alluri N, Shivakameshwari MN, Manohar SH, Majumdar M. Diversity of Drimia indica (Roxb.) Jessop and its relationship to Drimia nagarjunae using phenotypic traits and molecular markers. Indian J Exp Biol 2015;53:412-416.
[32] Gupta A, Singh S, Yadav A. Pharmacological evaluation of antidiabetic activity of Urginea indica in laboratory animals. Intl J Nutr Pharmacol Neurol Dis 2015;5:63-68.
[33] Akhtari E, Mokaberinejad R. Treatment of fallopian tubes obstruction in an infertile woman using Iranian traditional medicine. Asian J Clin Case Rep Tradit Altern Med 2017;1:9-16.
[34] Salavati M, Mazaheri M, Negahban H, Sohani S, Ebrahimian M, et al. Validation of a Persian-version of knee injury and osteoarthritis outcome score (KOOS) in Iranians with knee injuries. Osteoarthr Cartil 2008;16:1178-1182.
[35] Bayazıt V, Konar V. Analgesic efects of scilliroside, proscillaridin-a and taxifolin from squill bulb (Urginea maritima) on pains. Dig J Nanomater Biostructures 2010;5:465-467.
[36] Jabbari M, Hashempur MH, Razavi SZE, Shahraki HR, Kamalinejad M, et al. Efficacy and short-term safety of topical Dwarf Elder (Sambucus ebulus L.) versus diclofenac for knee osteoarthritis: A randomized, double-blind, active-controlled trial. J Ethnopharmacol 2016;188:80-86.
[37] Hashempur MH, Sadrneshin S, Mosavat SH, Ashraf A. Green tea (Camellia sinensis) for patients with knee osteoarthritis: A randomized open-label active-controlled clinical trial. Clin Nutr 2018;37:85-90.
[38] Oluwamayowa H, Jimoh-Abdulghaffaar J, Owoyele BV. Honey reverses disease progression, has anti-nociceptive and anti-inflammatory effects in a rat model of knee osteoarthritis induced by monosodium iodoacetate. Clin Nutr Open Sci 2021;36:14-25.
[39] Martinez-Armenta C, Camacho-Rea MC, Martínez-Nava GA, Espinosa-Velázquez R, Pineda C, et al. Therapeutic potential of bioactive compounds in honey for treating osteoarthritis. Front Pharmacol 2021;12:642836.