**Original Article**

# Effect of Satureja Hortensis Powder Supplementation on Pain and TNF-α in Patients with Knee Osteoarthritis: A Double-blind Randomized Clinical Trial

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**Abstract**

**Introduction:** The most prevalent type of arthritis is osteoarthritis which is known with the degenerative knee disease. Limited pieces of evidence have indicated that satureja with special phenol compounds is effective in inflammation reduction and pain alleviation. The aim of this study was to investigate the effect of Satureja Hortensis powder supplement on pain improvement and serum TNF-α in patients with knee osteoarthritis (OA).

**Materials & Methods:** The present study was conducted as a placebo-controlled double-blind randomized clinical trial. 39 patients with OA with medium pain were randomly divided into two groups which received Satureja Hortensis powder (SG) and placebo (PG) These groups consumed three 200 mg capsules with the same size and form for two months which contained Satureja Hortensis powder and starch, respectively. Pain intensity was determined using visual analogue scale 5(VAS) at the beginning and end of the trial. Response to the treatment was defined as pain reduction by more than 1.5 scores. The concentration of TNF-α were measured with ELISA kit.

**Results:** To distribute pain intensity before the intervention, there was no significant difference between the two groups. Before the intervention, all people had a pain score between 4 and 7. After the intervention, 95% of the SG and 84% of the PG were in this scope. These changes were not statistically different (p=0.12). There was no significant difference between the two groups in concentration of TNF-α before and after the intervention.

**Conclusion:** Results of this study showed that Satureja Hortensis powder supplement with dose of 600 mg per day for 60 days was not effective on pain alleviation and concentration of TNF-α in patients with knee osteoarthritis

**Keywords:** Satureja Hortensis, Osteoarthritis, Pain, TNF-α

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## Introduction

The most prevalent type of arthritis is osteoarthritis which is known with degenerative knee disease. Osteoarthritis is regarded as the fifth debilitating factor of life [(1)]. Prevalence of knee osteoarthritis is 3.8% in the world, which is reported higher among women than in men [(2)]. In Iran, prevalence of knee osteoarthritis at the ages of above 15 years old is 15.34%[(3)] and its symptoms involve painful of joints, mobility reduction, and warmth of joints.

This disease is probably due to daily erosion, joint loss and accumulation of micro trauma in the cartilage, which can lead to degenerative diseases. Its major anatomical feature is degenerative joint disease. Although the most important factor of osteoarthritis is osteolytic age, other factors also play a role in the incidence of this disease including genetic, orthopedic, and anatomical basic disorders, endocrine diseases, obesity, blood circulation disorders, septic arthritis, crystal sedimentation, record of rheumatoid arthritis or articular trauma, repetitive muscular use, and weakness of joints. Regarding the main factors in the progress of this disease, metabolic and mechanical changes can be stated which occur in obesity and are accompanied by pro-inflammatory factors produced with adipose tissue in overweight people [(1)].Specifically, hypertrophy and hyperplasia are important inflammatory effectors. Cytokines such as TNF-α stimulates synovial cells, activates other inflammatory mediators and enhances matrix metalloproteinases (MMPS) gene expression. Enhanced production of MMPS is related to articular tissue damage in osteo arthritis[(4)].

Symptoms of knee osteoarthritis include pain, warmth, stiffness and limited mobility[(1)], which can be treated via pharmacotherapy, surgery, and complementary treatments. There is some concern that some of these treatments cannot be completely effective. Some studies have shown that use of nonsteroidal anti-inflammatory drugs (NSAIDs) could intensify cardiovascular diseases or older types of these medicines could cause digestive system diseases. In addition, a study showed that NSAIDs are usually prescribed, which prevent making cartilage in humans and thus, increases cartilage degeneration in osteoarthritis [(5)]. Therefore, many researchers are searching for drugs such as Satureja Hortensis having less side effects[(6)].

Satureja Hortensis from Lamiaceae group is one of 12 types of Iranian Satureja cultivated in several areas of the country. All parts of the dried plant, particularly areal areas, are added to food as spice that are regarded as carminative, appetizing, and antidiarrheal drugs. In some regions of Iran, its useful effects on pain and inflammatory diseases have been found [(7)].Satureja, having such active compounds as Carvacrol and Cymene, is able to reduce inflammatory factors [(8-11)]. Regarding the lack of clinical trials conducted on the effect of Satureja on the pain, the present study intended to investigate the effect of daily consumption of 600



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mg Satureja Hortensis powder on the pain of patients suffering from knee osteoarthritis.

## Materials and Methods

Type of study and participants: This placebo-controlled double-blind randomized clinical trial started since October 2013 and ended in December in Yazd, Iran. Inclusion criteria included age of 40-70 years, patients' knee osteoarthritis based on the diagnostic criterion of American College of Rheumatology[5], confirmation of knee osteoarthritis by rheumatologist using radiography, medium knee pain in the past 24h between 4 and 7 cm based on the visual analogue scale **(**VAS), affliction of patients with inflammatory, metabolic diabetes, cancer or severe diseases, liver or renal failure, treatment with edible corticosteroids in the past 4 weeks, injection of corticosteroids in the past 6 weeks, fever, lack of regular consumption of Satureja, lack of allergy to Satureja, and no simultaneous participation in other projects. Moreover, exclusion criteria entailed consumption of less than 80% of capsules, lack of tendency to continue participation in the study, intake of nutritional supplements or tranquilizers during the study, and failure to observe the therapeutic protocol.

Participants of this study were people suffering from knee osteoarthritis who referred to Yazd Khatamol-Anbia Polyclinic and private clinics. The sample size was obtained as 25 people considering the error of 0.05 and test power of 80%. Ultimately, 60 people were entered into the study including 20% of loss. Subjects were randomly assigned to the treatment group (30 people) and placebo group (30 people) using random number table method.

Satureja capsules contained 200 mg Satureja Hortensis powder and placebo capsules contained the same amount of starch in order to be equal in terms of appearance and package. Capsules were prepared by Faculty of Pharmacy in Shahid Sadoughi University of Medical Sciences. Each patient was required to consume 3 capsules every day for 2 months. To make the study a double-blind randomized one, the capsules were put in identical packages before beginning the study by another person than the researcher, which were then coded as A and B. At the beginning of the study, each patient received the ration for one month including 90 capsules. After this period was elapsed, the patients were called and invited to receive the second ration. Within the second session, the number of unconsumed capsules was counted. It should be noted that in case people did not act according to the therapeutic protocol, they were excluded from the study. At the end of the second session, ration of the next month was given to each person. The third session was held after the completion of the project and the number of complements unconsumed by each person was counted. The patients were followed-up by contacting them every week to study the potential side effects and adherence to the study protocol. In case of intake of more than 80% of capsules, adherence of the person was defined perfect; this issue was found by counting the number of capsules remaining at the end of the second month.



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The researcher and patients were not aware of the type of capsules up to the end of the study.

Measurements**:** Pain intensity was assessed at the beginning and end of the study using VAS. This criterion was a 10-cm ruler and the patients were asked to show pain intensity from 0 to 10, who were supposed to mark pain intensity on this ruler before intake of the medicine at the end of the second month. Distance between 0-and3 showed mild pain, between 4 and -7 demonstrated medium pain, and between 8 and -10 indicated intensive pain. Response to the treatment was considered as the pain reduction of more than 1.5 cm on VAS scale [(5)].

Plasma TNF-αwas measured using a sandwich enzyme-linked immunosorbent assay (ELISA). A 10 ml venous blood sample was taken from each patient at baseline and 2 months after treatment via an EDTA-fortified blood collection tube using a 21-gauge needle. The blood was blended with the anticoagulant and then was put on some ice. The samples were centrifuged at 3000 rpm for 5 min twice at room temperature. Then serum was separated and stored for 5 months (-80° C) before analysis. The cytokine assay for TNF-α was carried out using the ELISA kit (ebiosciences, USA).The absorbance was read at 450 nm, and Plasma TNF-α concentration was computed with standard curve generated from recombinant cytokines. At the beginning of the study, a general information questionnaire including such as age, gender, marital status, education, and employment was completed. The patients' weight of was measured using Seca digital balance made in Germany with the accuracy of 0.1 and the patients' height was measured by a tape with the accuracy of 0.5 cm with minimum cover and body mass index (BMI) was calculated by dividing weight (kg) by square root (m) of height. All the interview stages, questionnaire completion, and anthropometric measurements were performed by experienced nutritionists who were not cognizant of the group of patients.

Ethical considerations: A draft of this study was approved by Research Ethics Committee of Shahid Sadoughi University of Medical Sciences, Yazd, Iran, and was registered in the clinical trial website of Deputy of Research and Technology, Ministry of Health (www.irct.ir). Moreover, informed consent was signed by the patients, no cost was paid by the patients and their information was kept confidential. The subjects were voluntarily included in the study and could exclude from the study if they desired to do so.

Statistical analysis: The study data obtained by the patients who underwent the intervention were analyzed by the SPSS software (ver. 16) applying Kolmogorov Smirnov test in order to identify the data distribution. Moreover, Mann–Whitney and Wilcoxon signed-rank tests were utilized in order to compare the means. Fisher's exact test was also used to compare the frequency distribution of pain condition,

## Results

The study participants consisted of 60 people, among which 10 people group stopped cooperation due to travel, imperfect intake of capsules, and other unknown reasons. Out of the remaining



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people, one subject was excluded from the intervention group at the time of analysis due to the imperfect intake of capsules. Overall, 39 people (19 people in SG and 20 in PG) were statistically analyzed (Figure 1). The characteristics of them are shown in Table 1 in terms of the different groups, respectively. Most participants of the study were females, though, frequency of women and men did not demonstrate any significant differences between the two groups ($p$=0.4). Most study participants were obese and overweight in the both groups, since their BMI was reported above 25 $kg/m^2$. Frequency of the people with obesity or overweight was not statistically significant in the both groups ($p$=0.1). Furthermore, no significant difference was observed between the two groups in terms of age, weight, height, and employment.

Side effects observed in the SG were not statistically significant ($p$=0.25), and no significant difference was observed between the two groups in regard with distribution of pain intensity before and after the intervention. Before the intervention, all participants in both groups had pain score of 4-7. After the intervention, 95% of the participants in SG and 84% of the participants in PG lied within this same scope , that these changes were not proved to be statistically significant ($p$=0.12).

The mean score of pain as well as concentration of TNF-α (pg/ml) before and after the intervention are shown in Table 2. As the Table indicates, the mean of pain intensity was not revealed to be significantly different between the two groups before and after the intervention. In addition, there was no significant difference between the two groups in concentration of TNF-α before and after the intervention.

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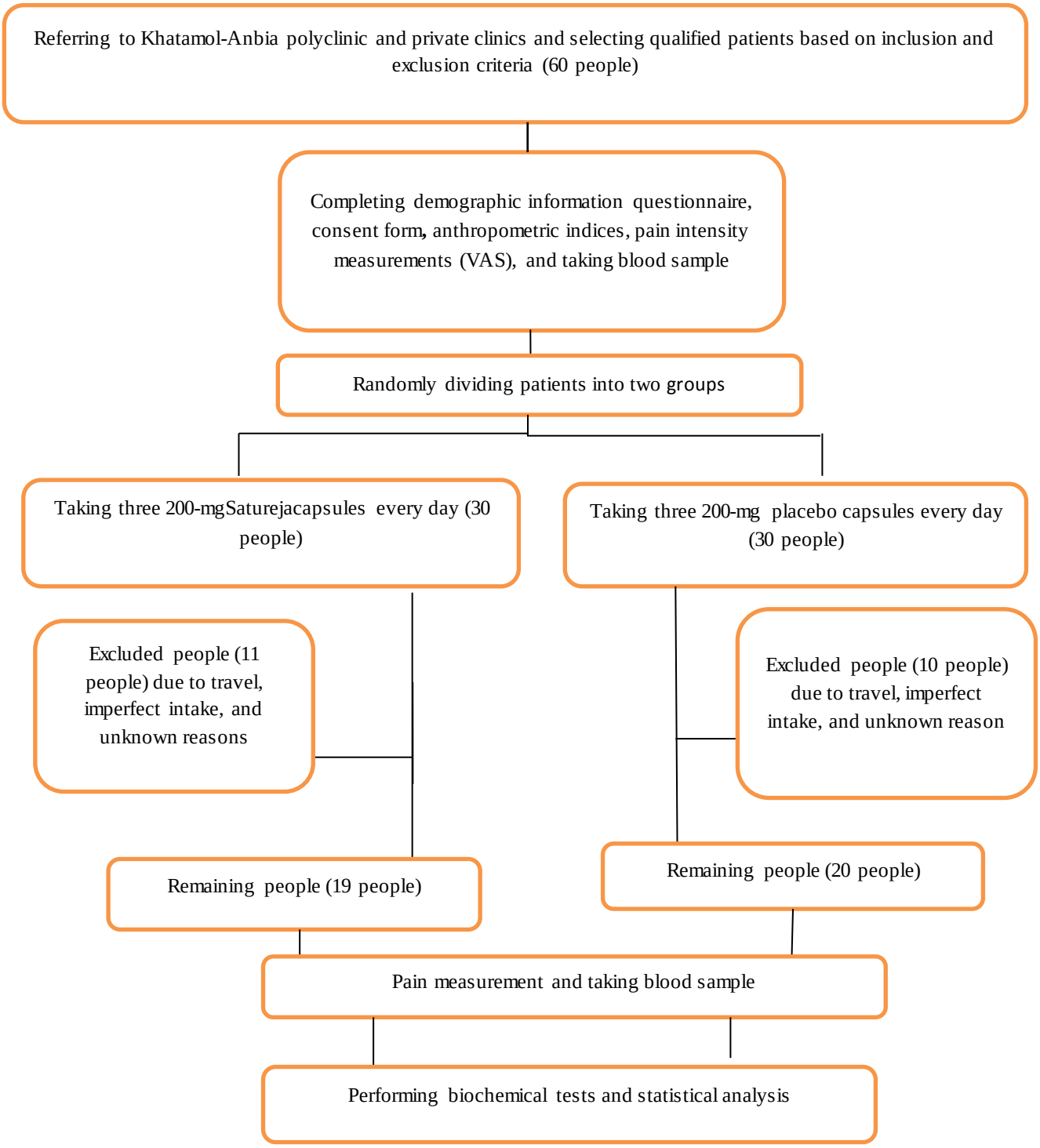


**Figure 1**- Flowchart of the study



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**Table 1**: Characteristics of the patients at the beginning of the study in the studied groups

| Characteristics | Placebo Group | Satureja Hortensis Group | P_value |
|---|---|---|---|
| **Age (year )** | 55.10±9.48 | 52.73±8.18* | 0.41 |
| **Weight (kg)** | 83.65± 10.66 | 76.84±10.66 | 0.12 |
| **Height (cm)** | 157±9.8 | 157±8.7 | 0.96 |
| **BMI (kg/m$^2$)** | 33.59± 4.63 | 31.29± 5.39 | 0.16 |
| **Sex** | N (%) | N (%) | |
| **Male** | 4(20) | 2(10.5) | 0.41 |
| **Female** | 16(80) | 17(89.4) | |
| **Weight status**** | | | |
| **Obese** | 16(80) | 9(47.3) | 0.08 |
| **Overweight** | 3(15) | 8(42.1) | |
| **Normal** | 1(5) | 2(10.5) | |
| **Education** | | | |
| **Illiterate** | 1(5) | 3(15.7) | 0.14 |
| **Primary school** | 9(45) | 11(57.8) | |
| **Junior high school** | 3(15) | 1(5.2) | |
| **High school** | 6(30) | 4(21.05) | |
| **University** | 1(5) | 0 | |

***:**Mean±standard deviation ;

**: According the body mass index or BMI (Obese; BMI> 30 kg/m2, Overweight; BMI 25-30 kg/m2 and Normal; BMI; 18.5-24.99 kg/m2)



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**Table 2-** Comparing mean of pain score and concentration of TNF-α (pg/ml) before and after the intervention in the studied groups

| Groups | Before | After | Difference | P_value* |
|---|---|---|---|---|
| **Pain Score** | | | | |
| **Satureja Hortensis group** | 7±2 | 5±1.50 | -2±3 | 0.009 |
| **Placebo group** | 7±1.75 | 5±3 | -0.5±2 | 0.005 |
| **P-value**** | 0.57 | 0.33 | 0.38 | |
| **TNF-α ( pg/ml)** | | | | |
| **Satureja Hortensis group** | 0.23±0.4 | 0.21±0.59 | 0.51±1.4 | 0.68 |
| **Placebo group** | 0.57±1.36 | 0.44±0.87 | 0.19±0.4 | 0.64 |
| **P-value**** | 0.18 | 0.48 | 0.3 | |

*Wilcoxon Signed Ranks Test, **Mann-Whitney Test

## Discussion

In this study, 250 mg intake of three Satureja Hortensis powder capsules per day for 2 months was not effective in alleviating pain and decreasing concentration of TNF-α (pg/ml) in patients suffering from knee osteoarthritis.

Jung et al. designed a clinical trial in order to study the effect of SKI 306X (a plant anti-arthritic factor) on the pain of patients suffering from knee osteoarthritis. This anti-arthritic factor was a mixture of 3 plant extracts, one of which was obtained from a plant from Lamiaceae group with scientific name of Prunella Vulgaris. Ninety six patients with knee osteoarthritis were included in a double-blind randomized clinical trial in 4 groups, which included placebo and doses of 200, 400, and 600 mg of SKI306X extract as tablet for three times a day. After the intervention, lasting for four weeks, the patients' pain intensity was measured using VAS, and a significant difference was detected between the intervention and placebo groups in regard with the pain mean score[12].

Different animal studies have been conducted on Satureja plant from Lamiaceae group. Haj Hashemi et al. designed a study on analgesic and anti-inflammatory of hydroalcoholic extract (2000 mg/kg), polyphenol (1000 mg/kg) and extract (200 mg/kg) of aerial bodies of Satureja plant. Acute and chronic pains as well as anti-inflammatory effects were studied using light tail-flick, formalin, and Carrageenan-induced paw edema tests among rats. The results of this study demonstrated that effect of Satureja extract was not significant on the acute pain, whereas significant results were detected concerning formalin test and chronic pain measurement [7].



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In another study which was conducted by Haj Hashemi et al., Satureja Hortensis extract (200 mg/kg) was utilized to study anti-inflammatory and analgesic effects among rats. In this study, acute pain test results were not significant; but chronic results of pain test were significant [13].

Bonjardim et al. designed another study aimed to evaluate analgesic and anti-inflammatory characteristics of one of two effective materials inSatureja, Cymene, in rats. Analgesic activity was evaluated by acetic acid-induced writhing response tests. Cymene in intra-peritoneal doses of 50 and 100 mg inhibited the inflammatory activity inspired by acetic acid and formalin [7]. In another study, Satureja Hortensis proved to be effective in decreasing concentration of pro-inflammatory cytokines such as TNF-α in the mice[14].

Herbal medicines have been studied for their anti-inflammatory inhibition of MMPS and expression of COX-2[1]. Landa et al. indicated inhibitory activity of carvacrol on production of PGE2 catalyzed by Cyclooxygenase 2(COX-2) in vitro. The study findings revealed that the production of PGE2 was significantly decreased[10].

In the animal and in vitro studies which have used Satureja extract, analgesic and anti-inflammatory effects have been observed, whereas the present study in which Satureja Hortensis powder was applied, no significant difference was demonstrated in the pain score of patients. Different studies have demonstrated that Satureja has analgesic and anti-inflammatory effects, which is induced by inhibiting pro -inflammatory cytokines and cyclooxygenase route[9-11, 13, 15].

Satureja plant consists of 29% thymol**,** 26.5% Carvacrol, 22.6% Terpinen, and 9.3% Cymene [16]. The present study has been the only work investigating the effect of Satureja Hortensis powder supplement on pain alleviation of patients with knee osteoarthritis. Hence, mechanism of satureja action on anti-inflammatory effects needs further investigations. The placebo-controlled double-blind randomized clinical trial design of the present study can be mentioned as the study strength. This study suffers from some limitations as small sample size, low therapeutic dose, loss of samples during intervention, and exclusion of some subjects due to failure to follow the study's protocol. Since this study was the first clinical trial on patients with knee osteoarthritis, it is recommended to conduct other studies with larger sample size and higher drug dose. Pain intensity in the osteoarthritis of other joints can be estimated as well.

## Conclusion

Results of this study demonstrated that Satureja Hortensis powder supplement with dose of 600 mg per day for 60 days was not effective in the pain alleviation and concentration of TNF-α in patients suffering from knee osteoarthritis; however, more studies should be conducted.



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