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Anti-inflammatory Activities of Methanolic Extracts of *Stachys persica* and *Stachys setifera* on Rats and Mice

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Abstract: The aim of this study was to evaluate the anti-inflammatory activities of total methanolic extracts isolated from aerial parts of *Stachys persica* and *Stachys setifera*. The anti-inflammatory properties of the extracts were assessed by using two well-characterized inflammatory models, carrageenan-induced paw edema and formalin test in rats and mice. Intraperitoneal injections of the extracts 60 min before carrageenan injection caused significant inhibition on carrageenan-induced paw edema over the dose range 50-200 mg kg^{-1}. The extract of *Stachys persica* showed more inhibitory effect especially with higher doses (100 and 200 mg kg^{-1}). In the formalin test, intraperitoneal injections of different doses (50, 100 and 200 mg kg^{-1}) of either *Stachys persica* or *Stachys setifera* 30 min before formalin injection did not show any inhibitory effects in the first phase (0-10 min) of the formalin-induced pain, but all three doses of the extracts revealed a significant ($p<0.01$) blockade of the second phase (20-30 min) nociception. The anti-inflammatory properties of both extracts were comparable with indomethacin as a potent non-steroidal anti-inflammatory drug. In conclusion, the present findings provide further evidences for inhibitory effects of *Stachys persica* and *Stachys setifera* in inflammatory processes via possible interactions with cyclooxygenase products.

Key words: *Stachys*, inflammation, cyclooxygenase, formalin, carrageenan

INTRODUCTION

The genus *Stachys* L. comprises about 300 species in the world[1] and is considered as one of the largest genera of the Lamiaceae. Iran is an area particularly rich in taxa with more than 34 species including *Stachys setifera* C.A. May and *Stachys persica* G Mel.[2].

The main active biological components of the genus *Stachys* are phenyl ethanoid glycosides[3,4], flavonoids[5], diterpenoids[6,7] and saponines[8]. Also there are other studies on essential oils[9-13] or solvent extracts[14,15] of *Stachys* species.

Several plants of this genus have been used in folk medicine of which *S. palustris* and *S. sylvatica* (wound wort) are approved for healing wounds, treating abdominal pains and as disinfectant, anti spasmodic and anti fever[16].

In Iran, non-flowering stems of *S. inflata* are used for infection, asthma, rheumatic and other inflammatory disorders[17]. It was found that acetoside a phenyl ethanoid glycoside of *Stachys seiboldi* has anti-inflammatory activities in nephritic glumeruli[18]. In addition many other activities have been reported in genus *Stachys* such as anti-inflammatory effect[17,19], effect on hyaluronidase function[20] and hypotensive activity[15]. This study presents antinociceptive and anti-inflammatory effects of methanolic extracts of aerial parts of *Stachys setifera* and *Stachys persica* for the first time.

MATERIALS AND METHODS

Plant material: Aerial parts of *S. setifera* and *S. persica* were collected from Khalkhal, province of Azarbayjan-Sharghi, Iran at an altitude of 1600 and 1100 m, in June 2002 during the flowering stage.

Voucher specimens have been deposited in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran (No. 6529 THE and No. 6528 THE).

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Plant extraction: Dried and finely powdered aerial parts (1000 g) were extracted with methanol (3×5 L) at room temperature for two weeks. After removal of the solvent *in vacuuo* at 50°C, the residue (39 g, 3.9% w/w of *S. setifera* and 34 g, 3.4% w/w of *S. persica*, respectively) were stored at 4°C in sealed vials until required.

Animals: Male albino mice weighing 20-25 g and male Wistar rats weighing (180-230 g) were used for formalin test and carrageenan-induced edema, respectively. The animals were housed in groups of five in conditions of constant temperature (21±2°C) and light-controlled room (12/12 h). Animal studies were conducted in accordance with the internationally accepted principles for laboratory animal use and care in the European community guidelines (EEC Directive of 1986; 86/609/EEC).

Carrageenan-induced edema: Rats were allowed to acclimatize for 60 min before any injection. Hundred microliters of carrageenan (1%) (Sigma-Germany) in saline was injected subplantarly in the right hind paw of the rats. The paw thickness was measured from the ventral to the dorsal surfaces immediately prior to carrageenan injection and then at hourly intervals from 1 to 5 afterwards. The difference of paw thickness between treated animals and control group were expressed at different time points after carrageenan injection. In this part of the study, rats were randomly placed in groups of (a) control (saline), (b) hydro alcoholic extracts of either *S. persica* (50, 100, 200 mg kg^{-1}) or (c) *S. setifera* (50, 100, 200 mg kg^{-1}) and (d) indomethacin (5 mg kg^{-1}). Saline, extracts or indomethacin were administered intraperitoneally 60 min before carrageenan injection.

Antinociception record: Mice were allowed to acclimatize for 30 min before any injection. Twenty five microliters of formalin (0.5%) was injected subcutaneously into the dorsal surface of the right hind paw of the mouse by using a microsyringe with a 26 gauge needle. In formalin test, mice were randomly placed in groups of control (saline), extracts and indomethacin like carrageenan experiment. Immediately after formalin injection, animals were placed individually in glass cylinders (20 cm wide, 25 cm length) on a flat glass floor and a mirror was placed at 45° angle under the cylinder to allow clear observation of the paws of the animals. Saline, extracts or indomethacin were administered intraperitoneally 30 min before formalin injection. The total time (seconds) spent licking in response to the injected paw during periods of 0-10 min and 20-30 min were measured as an indicator of pain.

Statistical analysis: Comparisons between groups were made by analysis of variance (ANOVA) and by the Newman-Keuls post hoc test. Differences with $p<0.05$ between experimental groups and control animals were considered statistically significant.

RESULTS

Effects of methanolic extract of *S. persica* on carrageenan-induced paw edema: Figure 1 shows that injection of carrageenan in control group induced acute inflammation with a prominent increase in paw thickness, began 1 h after intraplantar injection and reached a peak of inflammation after 4 h. Intraperitoneal injection of animals with the different doses of methanolic extract of aerial parts of *S. persica* (50, 100 and 200 mg kg^{-1}) caused potent inhibition on carrageenan-induced inflammation. The extract with dose of 50 mg kg^{-1} showed a significant ($p<0.05$; 0.01) anti-inflammatory activity 4 and 5 h after carrageenan injection. The higher doses of the extract (100 and 200 mg kg^{-1}) decreased inflammation induced by carrageenan 2 h after carrageenan injection and reached to maximum after time points of 4 and 5 h ($p<0.001$). The anti-inflammatory effects of the higher doses of the extract (100 and 200 mg kg^{-1}) were comparable with high dose of indomethacin (5 mg kg^{-1}) as a non-steroidal anti-inflammatory drug (Fig. 1).

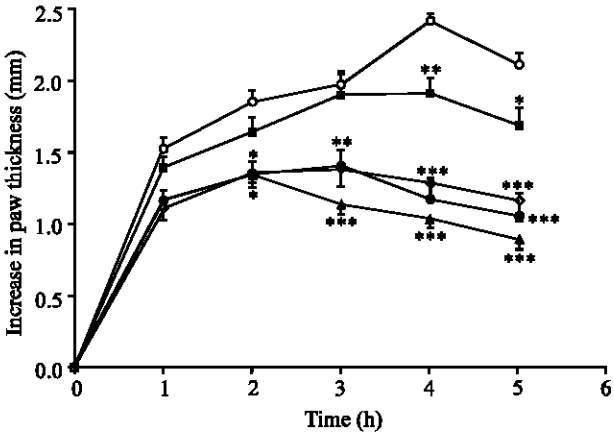

Fig. 1: Effects of different doses of methanolic extract from aerial parts of *Stachys persica* [50 (■), 100 (●), 200 (♦) mg kg^{-1}] and indomethacin (▲, 5 mg kg^{-1}) on carrageenan-induced paw edema in rats. Saline ( ), extracts and indomethacin were administered 60 min before Carrageenan injection. Each point is the mean ± SEM of at least seven animals. * $p<0.05$, ** $p<0.01$ and ***$p<0.001$ compared to the same points in the control (○, saline) group

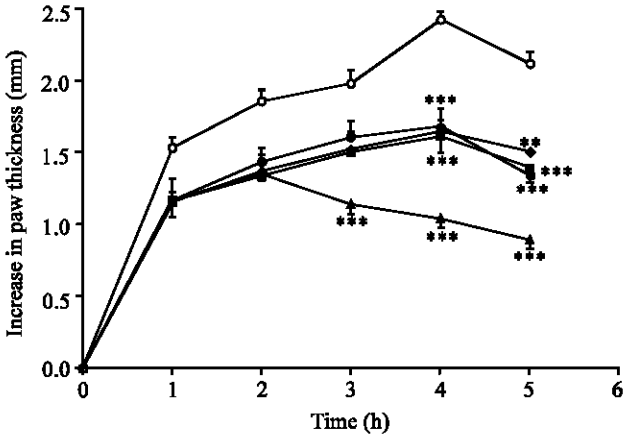

Fig. 2: Effects of different doses of methanolic extract from aerial parts of *Stachys setifera* [50 (■), 100 (●), 200 (♦) mg kg^{-1}] and indomethacin (▲, 5 mg kg^{-1}) on carrageenan-induced paw edema in rats. Saline (○), extracts and indomethacin were administered 60 min before Carrageenan injection. Each point is the mean ± SEM of at least seven animals. ** $p<0.01$ and *** $p<0.001$ compared to the same points in the control (○ , saline) group

Effects of methanolic extract of *S. setifera* on carrageenan-induced paw edema: Intraperitoneal injections of methanolic extract of aerial part of *S. setifera* (50, 100 and 200 mg kg^{-1}) decreased carrageenan-induced inflammation significantly and in a dose-independent manner. The anti-inflammatory effects of all three doses of the extract were started 4 h after carrageenan injection. In comparison with indomethacin (5 mg kg^{-1}) as a potent non-steroidal inflammatory drug, the anti-inflammatory effects of all three doses of extract were less than indomethacin especially at time points of 4 and 5 h after carrageenan injection (Fig. 2).

Effects of methanolic extracts of *S. persica* and *S. setifera* on formalin-induced licking response: Intraplantar injection of formalin (25 µL, 0.5%) induced biphasic licking response. Pretreatment of animals with different doses (50, 100, 200 mg kg^{-1}, 30 min) of methanolic extracts of aerial parts of either *S. persica* or *S. setifera* did not show any significant difference in the duration of licking activity in the early phase (Fig. 3A and 4A), whereas all three doses of the both extracts reduced the licking in the late phase significantly (Fig. 3B and 4B). The maximum inhibitory responses obtained with 50 mg kg^{-1} and 200 mg kg^{-1} of the *S. setifera* and *S. persica*, respectively which reduced licking duration similar to high dose of indomethacin (5 mg kg^{-1}).

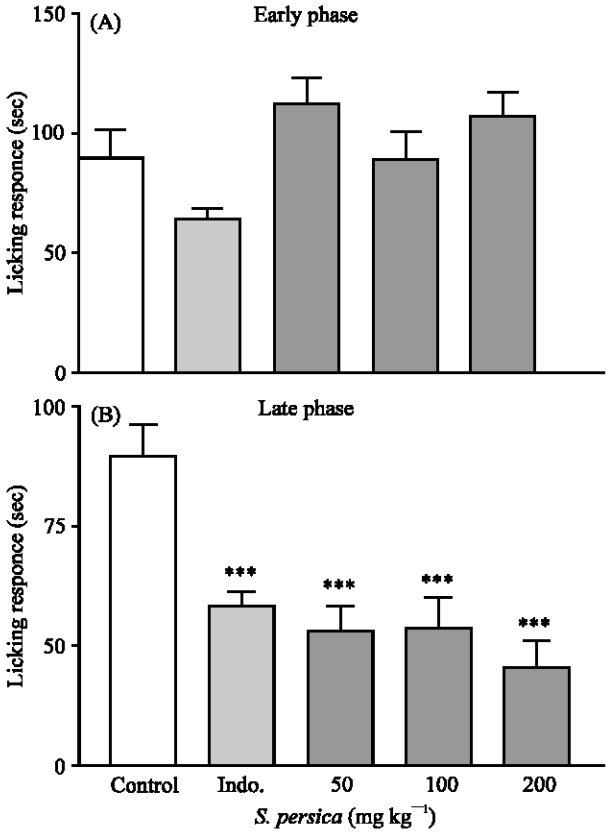

Fig. 3: Effects of methanolic extract of *Stachys persica* on formalin test. Different doses of the extract (50, 100, 200 mg kg^{-1}) were administered to mice. Control group received saline. Methanolic extract of *Stachys persica* was administered 30 min before formalin injection. Antinociception was recorded 0-10 min (A, early phase) and 20-30 min (B, late phase) after formalin injection. Each point is the mean±SEM of at least ten animals. *** $p<0.001$ different from control group

DISCUSSION

The results of the present experiment demonstrate that the methanolic extracts of both *S. persica* and *S. setifera* can play a significant role in the inhibition of pain and inflammatory processes. The results support previous work showing that hydroalcoholic extract of *Stacys inflata* induced antinociception and anti-inflammatory effects in rats[17]. Carrageenan-induced inflammation is a suitable method for evaluation of the anti-inflammatory effects of the agents[21]. The inflammation induced by carrageenan includes early and delayed phases. It seems that early phase is related to the

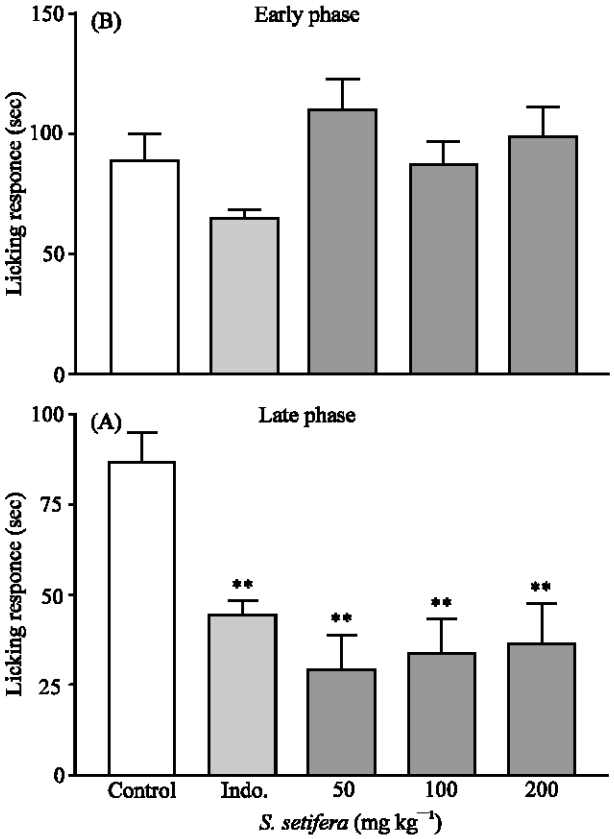

Fig. 4: Effects of methanolic extract of *Stachys setifera* on formalin test. Different doses of methanolic extract (50, 100, 200 mg kg^{-1}) were administered to mice. Control group received saline. Methanolic extract of *Stachys setifera* was administered 30 min before formalin injection. Antinociception was recorded 0-10 min (A, early phase) and 20-30 min (B, late phase) after formalin injection. Each point is the mean±SEM of at least ten animals. **p<0.01 different from control group

production of 5-hydroxytryptamin, histamine, bradykinnin and cyclooxygenase products while delayed phase is related to neutrophil infiltration, as well as to the continuing of the production of arachidonic acid (AA) metabolites[22,23].

Prostanoids including prostaglandins (PGs) and thromboxanes (Txs) are metabolites of arachidonic acid (AA) that synthesized and released by most cell-types[24-26]. These metabolites participate in various physiological and pathological processes ranging from synaptic transmission to inflammation[26]. Cyclooxygenase (COX) enzymes catalyze the first two committed steps in the biosynthesis of prostanoids[27].

Two Cox isoforms, cyclooxygenase-1 (Cox-1) and cycoloxygenase-2 (Cox-2) have been identified[28-30]. Cox-2 is often referred to as the inducible isoform of Cox, since levels of Cox-2 increase in response to several forms of stimulation in various types of tissue[27]. In contrast, the constitutive form of Cox, Cox-1 appears to be involved in housekeeping cellular function[31,32].

Both the extracts of the aerial parts of *S. persica* and *S. setifera* attenuated delayed phases of carrageenan-induced inflammation, but *S. persica* showed more anti-inflammatory activity. In comparison with high dose of indomethacin as a non-steroidal anti-inflammatory drug, the methanolic extract of *S. persica* inhibited the inflammation similar to high doses of indomethacin.

Considering the production of arachidonic metabolites via Cox-2 enzyme that is the main responsible factor for both early and delayed phases of the carrageenan-induced inflammation, these findings demonstrate that the anti-inflammatory activities of the extracts are probably related to the inhibition of the synthesis or release of Cox-2 products. There is also an evidence that inflammation processes are accompanied with increase of free radicals activity[14]. Previous investigations also showed that extracts of the some species of *Stachys* have appreciable levels of antioxidant activity[14]. On the basis of this function, the extracts may exert their anti-inflammatory effects at least partially through the relative antioxidant activity. However some other activities such as inhibition of neutrophils infiltration and prevention of leukocyte accumulation[18] could be involved in anti-inflammatory effects of the genus of *Stachys*. In formalin test the initial pain (early phase) is explained as a direct stimulation of nociceptors. The late phase is thought to be secondary to the inflammatory reactions[33,34]. Several chemical mediators, such as histamine, kinnin, serotonin and prostaglandins (PGs) are released from damaged cells[35]. These mediators take part in the inflammatory response and are able to stimulate nociceptors and thus induce pain. Late phase pain may be blocked by drugs that are known to reduce the inflammatory response[34,36,37]. In most cases, stimulation of pain endings in the periphery is chemical in origin. Excessive mechanical or thermal stimuli can obviously cause acute pain, but the persistence of such pain after the stimulus has been removed and the pain resulting from inflammatory or ischemic changes in tissues generally reflect a chemical stimulation of the pain afferents[38]. Many studies have confirmed that inflammation take place in the late phase[35,38,39]. In this study all three doses of both extracts of *S. persica* and *S. setifera* (50, 100 and 200 mg kg^{-1}) significantly inhibited

the pain associated with the second phase (inflammatory component) of the formalin test. Compared to indomethacin as a NSAID, both extracts decreased licking response like indomethacin. Since antinociceptive activities of both extracts were observed in late phase similar to NSAIDs, therefore the analgesic effects of the extracts is probably mediated by interactions with inflammatory mediators especially arachidonic acid metabolites. However findings of the present study showed the anti-inflammatory and antinociceptive activities of *Stachys persica and Stachys setifera* but for determination the exact mechanisms of total extracts and their biologically active components more investigations are needed.

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