ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY OF METHANOLIC EXTRACTS OF AERIAL PARTS OF *STACHYS SCHTSCHEGLEEVII* SOSN. AND *STACHYS BALANSAE* BOISS. AND KOTSCHY EX BOISS IN RATS.

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ABSTRACT

Extracts of the flowering aerial parts of *Stachys schtschegleevii* Sosn. and *S. balansae* Boiss. and Kotschy ex Boiss have been used in Iranian folk medicine as remedy for rheumatic and other inflammatory disorders and anti-inflammatory and analgesic effects of some species of *Stachys* e.g. *Stachys inflata* have been reported. In this study, the anti-inflammatory and antinociceptive properties of total methanolic extracts of the flowering aerial parts of two *Stachys* species in rat were investigated by carrageenan-induced paw edema and formalin test. Intraperitoneal injection of the extracts, 60 min before induction of inflammation, resulted in inhibition of carrageenan-induced rat paw edema in dose dependant manner (doses 50, 100 and 200 mg/kg). In the formalin test, the extract (50, 100 and 200 mg/kg) had low effect in the first phase (0–5 min) of the formalin-induced pain, but all three doses showed analgesic and anti nociception effects significantly. In conclusion the methanolic extracts of *Stachys schtschegleevii* and *Stachys balansae* have analgesic and anti-inflammatory effects in formalin test and carrageenan-induced paw edema.

Keywords: Anti-inflammatory; Analgesic; *Stachys schtschegleevii; Stachys balansae*

INTRODUCTION

The use of natural products is growing in the world especially in developing countries such as China, India and Arabic countries. For several centuries some *Stachys* species have been used traditionally for their health benefits (1). About three hundred Stachys species have been reported (2); 34 of them are found in Iran; of which 13 are endemic (3, 4). The plants of *Stachys* are widely distributed in tropical and subtropical countries. *Stachys schtschegleevii* and *Stachys balansae* are native plants of Iran (4). In Iranian traditional medicine the extracts of the aerial parts of *Stachys schtschegleevii* (traditionally named Poulk) have been used in infectious, rheumatic and respiratory inflamamatory diseases. Phytochemical investigations on *Stachys* species have shown the presence of phenylethanoid glycosides (5, 6), terpenoids and steroids (7, 8) diterpenes (9) and flavonoids (10, 11). Also the compositions of the essential oils of some species have been reported (12, 13). Pharmacological studies have shown that extracts of some *Stachys* species have anti-inflammatory, anti-toxic (14, 15), anti-nephritic (16, 17), antihepatitis (18) and anti-anoxia (19) properties. Since there are no reports on the pharmacology and phytochemistry of *Stachys schtschegleevii* and *Stachys balansae* Boiss, in this study, anti-inflammatory and analgesic properties of methanolic extracts of these species were evaluated by carrageenan-induced paw edema and formalin tests in rat (20, 21).

MATERIALS AND METHODS

Plant material

Aerial flowering parts of these two *Stachys* species were collected during flowering period (June and July 2001), identified in Central Herbarium of Iran (Research Institute of Forest and Rangelands, Tehran) and a voucher specimen of both species (*S. balansae* and *S. schtschegleevii*) (83579 TARI and 83580 TARI respectively) were deposited in herbarium. Plants were dried in shadow before extraction.

Chemicals

Carrageenan was prepared from Sigma- Aldrich Company (Germany); indometacin was obtained from Hakim Pharmaceutical Company (Iran); all other solvents were prepared from Panreac Company (Spain).

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Extract preparation

200 g of crushed aerial parts of plant was subjected to Soxhlet extraction with methanol (99.9%) for 3 hours. Methanolic extracts were rotary evaporated to volume of 200 ml at 50ºC, and after addition of 50 ml of water, the solution was extracted with 3 x 100 ml ether (40-60ºC) (3x100ml). The extracts were then filtered and filtrates were evaporated at maximum 50 ºC to an amorphous gum under reduced pressure.

Carrageenan induced paw edema

The anti-inflammatory activities of the extracts were determined by the carrageenan-induced edema test in the hind paws of rats by the reported method (22). Male albino Wistar rats (150-200g) were fasted for 24 hours before the experiment with free access to water and then 150 µl of 1% suspension of carrageenan in saline, which was prepared 1 hour before each experiment, was injected into the plantar side of both hind paws of the rats. The extracts were dissolved in saline and passed through a weighed filter paper and the filtrate was used for intraperitoneal injection. Following filtration the filterate was dried and weighed again, in order to obtain the real concentration of the extract. Rats were allocated randomly to groups of six; a) for controls; b) for extracts (50, 100 and 200 mg/kg) and c) for indometacin as the reference drug (5mg/kg). Indometacin solution was prepared in saline using tween 80 as dispersing agent. Saline and the extracts or indometacin in 0.5 ml of saline were injected intraperitoneally 60 minutes before induction of inflammation. The paw thickness was measured from the ventral to the dorsal surfaces using a dial caliper prior to carrageenan injection and then at 1 hour intervals for 5 hours. Data are expressed as a percentage increase in thickness compared with pre-injection values.

Formalin-test

Male Albino Wistar rats (n=6), weighing 180-220 g, were kept in Plexiglas cages with free access to food and water. Test was conducted in the middle of the light period of a 12-h light: 12-h dark cycle. Each animal was tested once only. Plant extracts (50, 100 and 200 mg/kg) were dissolved in 0.9% saline and indometacin (5 mg/kg) was suspended in 0.9% (w/v) saline containing tween 80 and administered intraperitoneally in a volume of 1 ml. Control group received only vehicles (1 ml). The analgesic activities of the extracts were determined by the reported method for formalin test (23). One hour before testing, animals were placed in a standard cage (30×12×13 cm), that served as an observation chamber and then 60 µl of 5.0% formalin were injected to the dorsal surface of the left hind paw. The rats were observed for 60 min after injection of formalin, and the amount of time that animals spent licking the injected hind paw was recorded. The first 5 min post formalin injection was assigned as the early phase and the period between 15 and 60 min as the late phase. Pain rate was calculated according to formula:

$$Pain\ rating = \frac{1T_1 + 2T_2 + 3T_3}{Timebloke(\text{second})}$$

T_1, T_2 and T_3 are every 15 seconds during 5 minutes, in which animals had 1, 2 or 3 type habit. The samples were administered 15 min before injection of formalin.

Statistical analysis

ANOVA followed Student-Newman-Keuls test was used to determine significant differences between groups and $P < 0.05$ were considered significant.

RESULTS

Anti-inflammatory effects of Stachys schtschegleevii

Induction of acute inflammation in control rats resulted in a prominent increase in paw thickness which began 1 hour after intraplantar injection of carrageenan and reached to a peak after 4 hours (fig. 1). Intraperitoneal injection of the extract of aerial flowering parts of *Stachys schtschegleevii* resulted in inhibition of carrageenan-induced paw edema dose dependently. All doses (50, 100 and 200 mg/kg) of extracts induced a significant ($P<0.01$) anti-inflammatory effects 5 hours after carrageenan injection. The doses of 100 and 200 mg/kg showed more potent effects ($P<0.001$) at all time points. Indometacin (5 mg/kg, i.p) showed less activity than the extracts. The extracts (100 and 200 mg/kg) showed the maximum inhibitory effects at fourth hour.

Anti-inflammatory effects of Stachys balansae

Doses of the aerial flowering parts of *Stachys balansae* showed different anti-inflammatory effects in comparison with *Stachys schtschegleevii*. Dosage of 50mg/kg of extract showed no significant differences with controls during first 3 hours (fig. 2) but represented significant anti-inflammatory effect 4 hours after carrageenan injection ($P<0.05$). The dose of 100 mg/kg at first 2 hour showed inhibitory effect in carrageenan induced edema ($P<0.01$). The dose of 200 mg/kg showed significant antiinflammatory effect during all 5 hours ($P<0.01$) and reached to a maximum 4 hours after injection.

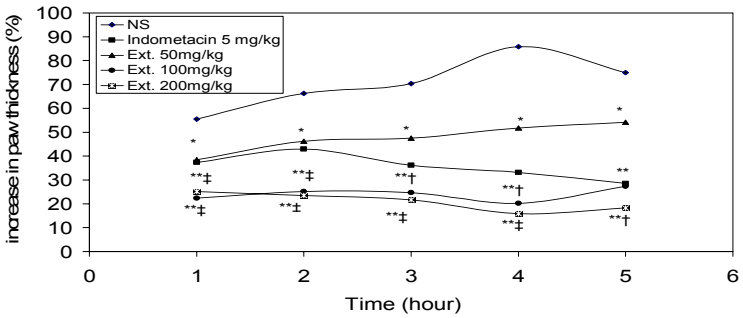

Figure 1. Anti-inflammatory effects of different doses of methanolic extract prepared from aerial parts of *Stachys schtschegleevii* in carrageenan induced paw edema in rat compared to the same points in control group (saline) [* P<0.01, ** P<0.001] and Indometacin 5 mg/kg [† P<0.05, ‡ P<0.01].

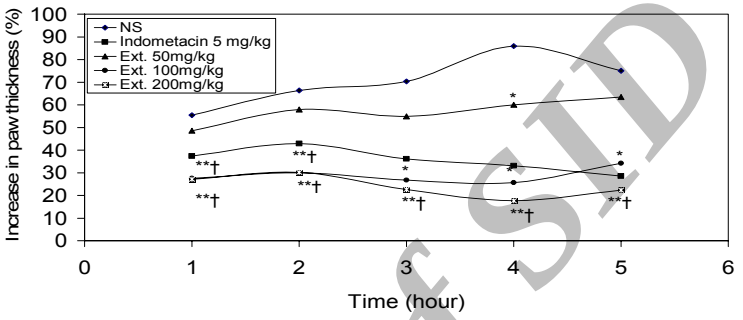

Figure 2. Anti-inflammatory effects of different doses of methanolic extract prepared from aerial parts of *Stachys balansae* in carrageenan induced paw edema in rat compared to the same points in control group (saline) [* P<0.05, ** P<0.01] and indometacin 5 mg/kg [† P<0.05]

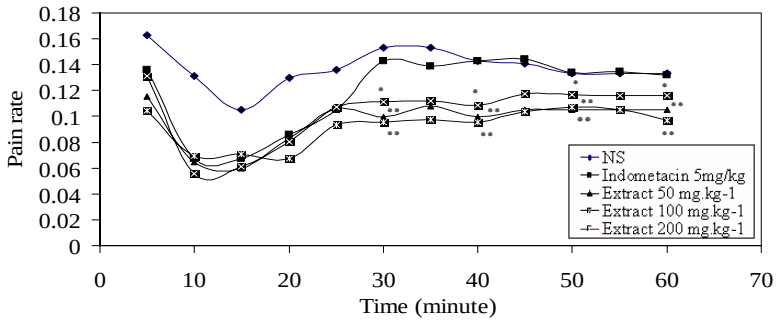

Figure 3. Analgesic effects of different doses of methanolic extract prepared from aerial parts of *Stachys schtschegleevii* and indometacin (5 mg/kg, ip) in formalin-induced paw licking in rat compared to the same points in control group (saline), [* P<0.01, ** P<0.001].

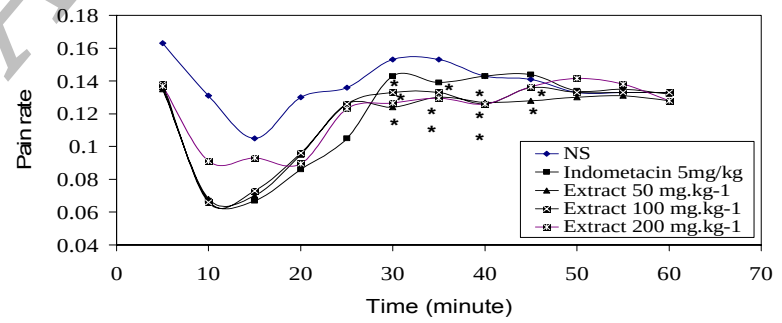

Figure 4. Analgesic effects of different doses of methanolic extract prepared from aerial parts of *Stachys balansae* and Indometacin (5 mg/kg ,ip) in formalin-induced paw licking in rat compared to the same points in control group (saline) (*P<0.05).

Analgesic effects of Stachys schtschegleevii
Intraplantar injection of 5% formalin evoked a characteristic biphasic licking response. All three doses of extract (50, 100 and 200 mg/kg) showed analgesic effects like indometacin (5 mg/kg, intraperitoneal) in the first phase of study (0-5 min) (fig. 3). During the second phase, extracts showed higher analgesic effects (50 mg/kg, $P<0.05$; 100 and 200 mg/kg, $P<0.01$).

Analgesic effects of Stachys balansae
All three doses of extracts (50, 100 and 200 mg/kg) showed analgesic effects similar to that of indometacin (5 mg/kg, intraperitoneally) in the first phase and higher effects after the second phase ($P<0.05$) up to 45 minutes and thereafter no significant differences were observed.

DISCUSSION

Carrageenan injection into the rat paw provokes a local, acute inflammatory reaction that is a suitable criteria for evaluation of anti-inflammatory agents (24). The inflammation consists of two phases, early phase which is related to the production of histamine, 5-hydroxytryptamin, bradykinins and cyclooxygenase products and delayed phase which has been linked to neutrophil infiltration, as well as production of arachidonic acid metabolites (25, 26 and 27). Extract of flowering aerial parts of *Stachys schtschegleevii* and *Stachys balansae* produced anti-inflammatory effects in carrageenan induced inflammation in rats. As shown in Fig.1, *S.schtschegleevii* had anti-inflammatory effects higher than saline at all three doses (50, 100 and 200 mg/kg) ($P<0.01$) and higher than indometacin in doses of 100 and 200 mg/kg. Doses of 100 and 200 mg/kg of this extract showed higher effect ($P<0.001$) than saline and indometacin ($P<0.01$) and there was no significant differences between these two doses. Extract of the *Stachys balansae* showed similar anti-inflammatory trends but lower than extract of *S. schtschegleevii*. In addition, all three doses of the extracts (50, 100 and 200 mg/kg) prepared from both *Stachys schtschegleevii* and *Stachys balansae* significantly inhibited the pain associated with the second phase (inflammatory component) of the formalin test, and the effect of *Stachys schtschegleevii* was more pronounced. Doses of 100 and 200 mg/kg showed similar potency which were high than those of dose of 50 mg/kg. In the formalin test, the initial nociceptive scores normally peaked 5 min (first phase) and then 15–30 min after injection (second phase), which represent the neurogenic and inflammatory pain responses, respectively (28). Phenylethanoid glycosides, triterpenoids and flavonoids have been considered as active components responsible for the biological actions of the *Stachys* genus (10, 11, 8, and 6). However, the anti-inflammatory effects of *Stachy*s, or its components, have not been elucidated completely so far. It has been reported that acteoside, a phenylethanoid glycoside of *Stachys sieboldii*, has a suppressive effect on the accumulation of leukocytes in the nephritic glomeruli through prevention of the upregulation of adhesion molecules (17). The discrepancy between the inhibitory effect upon low doses and the lack of increase in potency phenomena at higher doses of the extracts might be explained by hypotheses that some of the active constituent(s) of these two *Stachys* genus at high concentrations may exhibit pro-inflammatory properties. It is also likely that the extracts may have components with different anti- and pro-inflammatory effects. The co-existence of both anti-nociceptive and anti-inflammatory effects which was observed with this extract is well-defined for various non-steroidal antiinflammatory drugs (NSAIDS) particularly salicylates. It is therefore interesting that the extract behaved liked NSAIDS in this study which correlates well with the traditional application of the plant. The results presented in this study should be taken as a basis for further investigation for determination of the exact mode of action of individual constituents of the extracts.

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