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Influence of Some *Stachys* Taxa on Carrageenan-Induced Paw Edema in Rats

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Abstract

This work investigates the anti-inflammatory activity of methanol extracts of four endemic *Stachys* (Labiatae) taxa from the Balkans: *S. beckeana* Dörfler & Hayek, *S. anisochila* Vis. et Pančić, *S. plumosa* Griseb., and *S. alpina* L. subsp. *dinarica* Murb. As a model of acute inflammation, carrageenan-induced paw edema in rats was used. Extracts, applied at doses of 50, 100, and 200 mg/kg p.o., exhibited dose-dependent activity. *S. beckeana* and *S. anisochila* extracts were the most active ones (ED_{50} 154.52 and 162.24 mg/kg, respectively), with the activity comparable with indomethacin at doses of 2 and 4 mg/kg. *S. plumosa* extract has shown less-pronounced anti-inflammatory effect (ED_{50} 220.81 mg/kg). Extract of *S. alpina* subsp. *dinarica* had the lowest efficiency, attenuating inflammation less than 50%.

Keywords: Anti-inflammatory activity, *dinarica*, *S. alpina* subsp., *S. beckeana*, *S. plumosa*, *Stachys anisochila*.

Introduction

The genus *Stachys* (Labiatae) comprises about 300 species distributed in Eurasia and America, some of which have a long traditional use as nervine, tonic, and wound healing agents, and as astringent and antidiarrheal drugs (Naghibi et al., 2005; Kukić et al., 2006a). Hence, several pharmacological activities such as antioxidant (Couladis et al., 2003; Kukić et al., 2006a), antimicrobial (Skaltsa et al., 1999, 2003; Petrović et al., 2006), anxiolytic (Rabbani et al., 2003, 2005), antinephritic (Hayashi et al., 1996), and anti-inflammatory (Maleki et al., 2001; Khanavi et al., 2005) have been attributed to genus.

This work describes the anti-inflammatory activity of four endemic *Stachys* taxa from the Balkans: *S. beckeana* Dörfler & Hayek, *S. anisochila* Vis. et Pančić, *S. plumosa* Griseb., and *S. alpina* L. subsp. *dinarica* Murb. *S. beckeana*, growing on mountains in northern Albania, Montenegro, Herzegovina and southern Bosnia, and *S. anisochila* inhabits mountain regions in western Serbia, Bosnia, Herzegovina, and Albania. Both species belong to a very polymorphic *S. recta* L. complex. *S. plumosa* grows on dry pastures and mountain rocks in southeastern Serbia, Macedonia, western Bulgaria, and northern and central Greece (Ball, 1976). *S. alpina* subsp. *dinarica* Murb. is Balkan endemic (Croatia, Bosnia and Herzegovina, Montenegro, Serbia, and southwestern Bulgaria) (Diklić, 1974; Ball, 1976; Koeva, 1989). Our ethno-medicinal surveys showed that some of these (e.g., *S. alpina* subsp. *dinarica*) are used in traditional medicine for bladder inflammation, stomach complaints, and wound healing.

In the above-mentioned taxa, various secondary metabolites have been identified: glycosides of isoscutellareine, hypolaetine, chrysoeriol, apigenin, and luteolin, along with phenylethanoid glycosides, iridoids, phenolic acids, triterpenes, and essential oils (Marin et al., 2004; Meremeti et al., 2004; Kukić et al., 2006b). Previously, essential oil of *S. plumosa* was assessed for its antimicrobial activity (Petrović et al., 2006). The antioxidant activity of methanol extracts of investigated species was estimated, too (Kukić et al., 2006a).

As a model of acute inflammation in this experiment, carrageenan-induced paw edema in rats was used.

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Materials and Methods

Plant material

The flowering aerial parts of plants were collected from natural populations: *S. beckeana* from Mt. Durmitor (Montenegro), August 2003; *S. anisochila* in the gorge of Beli Rzav (western Serbia), June 2003; *S. plumosa* in Jelašnička klisura gorge (southeastern Serbia), June 2002; and *S. alpina* subsp. *dinarica* on Mt. Jahorina (Bosnia and Herzegovina), July 2004. Voucher specimens were identified by Mr. Sc. Marjan Niketić (custodian) and deposited in the herbarium collection of the Natural History Museum in Belgrade (BEO ko820033/6, ko620041/4, ko320025/6, and ko720049/83, respectively).

Extraction

Plant material (100 g) was air-dried at room temperature and finely ground. Each sample was bimacerated with chloroform (3 and 2 days; plant material/solvent ratio = 1:7). The marc was further extracted in the same way with methanol and the solvent evaporated under reduced pressure. The obtained dry methanol extracts of *S. beckeana*, *S. anisochila*, *S. plumosa*, and *S. alpina* subsp. *dinarica* (11.66, 14.88, 12.57, and 13.94 g, respectively) were used for investigations.

Anti-inflammatory activity

The carrageenan-induced rat paw edema test was used as an experimental model for screening the anti-inflammatory activity as reported earlier (Petrović et al., 2003). The investigated *Stachys* extracts, dissolved in DMSO, were administered orally (p.o.) in doses of 50, 100, and 200 mg/kg. Indomethacin, dissolved in DMSO, was used as a reference drug in doses of 1, 2, 4, and 8 mg/kg p.o. The control animals were given the vehicle (DMSO) in a dose of 1 ml/kg p.o. One hour after the oral administration of the extracts or indomethacin, 0.1 mL carrageenan-saline solution (0.5%) and saline were injected into the plantar surface of the right and left hind paws, respectively. The left paw served as the control (noninflamed paw). The animals were sacrificed 3 h after the carrageenan and saline injection, and paws were cut off for weighing. The difference in weight between right and left paw, active drug-treated versus vehicle-treated (control) rats, served as an indicator of the anti-inflammatory activity.

Animals

Animal studies were conducted in accordance with the internationally accepted principles for laboratory animal use and care in the European Community (EEC Directive of 1986; 86/609/EEC) adopted by the Ethical Committee of Military Medical Academy, Belgrade. Adult male Wistar rats, weighing 200–250 g, were used in the experiments. Experimental groups consisted of 6–10 animals each. The animals were deprived of food for 18–20 h before the beginning of the experiments but had free access to tap water.

Statistical analysis

Statistical analysis was performed with the Mann–Whitney *U*-test and ANOVA. Differences were accepted as statistically significant when $p < 0.05$.

The mean effective anti-inflammatory doses (ED_{50}) of extracts and indomethacin were calculated by the method of Litchfield and Wilcoxon (1949).

Results

All investigated *Stachys* extracts expressed a dose-dependent anti-inflammatory effect (Table 1). The most prominent reduction of carrageenan-induced inflammation was achieved at the highest dose applied. *S. beckeana* and *S. anisochila* extracts were the most active ones. These extracts applied at 200 mg/kg p.o. suppressed inflammation by 53.30% and 54.04%, with ED_{50} of 154.52 and 162.24 mg/kg, respectively. These effects were comparable with those achieved by 2 and 4 mg/kg of indomethacin (50.37% and 58.06%, respectively). *S. plumosa* extract was slightly less active reaching the

Table 1. Anti-inflammatory effect of investigated *Stachys* extracts.

Treatment	Dose (mg/kg, p.o.)	Anti-inflammatory effect (%)	ED_{50} (mg/kg)
Control (DMSO)[a]	1 mL/kg	0.00 ± 20.57	
Indomethacin[b]	1	27.14 ± 11.83	2.53
	2	$50.37 \pm 5.96^{*}$	
	4	$58.06 \pm 13.87^{*}$	
	8	$74.32 \pm 15.70^{**}$	
S. beckeana extract	50	$20.65 \pm 8.97^{*}$	154.52
	100	$45.43 \pm 10.02^{**}$	
	200	$53.30 \pm 5.72^{**}$	
S. anisochila extract	50	$20.33 \pm 12.11^{*}$	162.24
	100	$40.39 \pm 6.06^{**}$	
	200	$54.04 \pm 11.20^{**}$	
S. plumosa extract	50	$25.65 \pm 7.19^{*}$	220.81
	100	$40.65 \pm 14.23^{*}$	
	200	$46.52 \pm 14.70^{*}$	
S. alpina subsp. *dinarica* extract	50	$24.67 \pm 9.67^{*}$	—
	100	$30.43 \pm 5.39^{*}$	
	200	$37.17 \pm 16.63^{*}$	

[a]Vehicle.
[b]Reference drug.
$^{*}p < 0.05$; $^{**}p < 0.001$ vs. control group.

highest activity (46.52%) at maximum dose applied and with ED_{50} at 220.81 mg/kg. The ED_{50} for *S. alpina* subsp. *dinarica* extract could not be determined, because the uppermost dose exhibited an anti-inflammatory effect of 37.17%.

Discussion

The obtained results are consistent with investigations concerning some other *Stachys* species. *S. inflata* (Maleki et al., 2001) and *S. byzantina* (Khanavi et al., 2005) also attenuated carrageenan-induced rat paw edema similarly to indomethacin (5 mg/kg p.o.). Extract of aerial parts of *S. inflata* exhibits a cardioprotective effect, causing a pronounced reduction in myocardial infarct size, and such activity is likely the result of the inhibition of inflammatory components at the site of the reperfused area (Garjani et al., 2004).

As previously reported, secondary metabolites in investigated *Stachys* species include various compounds (Marin et al., 2004; Meremeti et al., 2004). Many of these compounds also demonstrated anti-inflammatory activity. In *S. plumosa*, phenylethanoid glycosides acteoside and forsithoside B were identified (Bankova et al., 1999), which showed selective inhibition toward the COX-2 enzyme (Sahpaz et al., 2002). Acteoside, isolated from *S. sieboldii*, had a suppressive effect on the accumulation of leukocytes in the nephritic glomeruli (Hayashi et al., 1994) and a modulating activity on NO production, a key mediator in carregeenan-induced rat paw edema (Ohno et al., 2002).

All investigated *Stachys* taxa are characterized by the presence of several hypolaetin derivatives. It was previously shown that hypolaetin and its 8-β-D-glucoside are selective inhibitors of lipoxygenase activity *in vitro* (Ferrandiz & Alcaraz, 1991). Few luteolin glycosides were identified in *S. plumosa* (Marin et al., 2004). Flavonoids resembling luteolin in structure (sharing 3′, 4′, 5, 7-tetrahydroxy substitution) resemble its anti-inflammatory activity, obtained by inhibition of lipopolysaccharide-induced TNF-α production (Ueda et al., 2004). In *S. anisochila* and *S. beckeana*, harpagide and 8-acetylharpagide were identified (Lenherr et al., 1984). These iridoids selectively inhibit thromboxane-synthase enzyme, which may be the primary target of their action and one of the mechanisms through which they exert their anti-inflammatory effects (Bermejo Benito et al., 2000).

It is reasonable to presume that presence of the above-mentioned compounds contributes to the demonstrated anti-inflammatory effects of investigated *Stachys* taxa. Our previous experiments revealed the antioxidant and free radical scavenging activities of the investigated extracts (Kukić et al., 2006a). It is well-known that antioxidants directly scavenge reactive oxygen species (ROS), which have been considered to exert their inflammatory effects via a direct toxic action on target cells and also through gene induction and especially through the activation of the redox-sensitive transcription factor NF-κB (Menegazzi et al., 2005). Some antioxidants may also mediate their anti-inflammatory activities by inhibiting different proinflammatory enzymes (COX, LOX, NOS) (Sadik et al., 2003; Sala et al., 2006) and also by preventing induction of the cytokine cascade and upregulation of the expression of adhesion molecules (Menegazzi et al., 2006). It had been shown that the extracts of *S. beckeana* and *S. anisochila*, the most active ones in this study, also exhibited stronger antioxidative activity than extracts of the other two *Stachys* species. Such correlation between antioxidant activity and anti-inflammatory effects obtained in the current study suggests that antioxidant activity of investigated *Stachys* extracts is at least partly involved in their anti-inflammatory effect. However, for determining the precise mechanisms of the anti-inflammatory action and for recognition of active compounds responsible for such activity, further investigations are needed.

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References

Ball PW (1976): *Stachys* L. In: Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walters SM, Webb DA, eds., *Flora Europaea*, Vol. IV. Cambridge, Cambridge University Press, pp. 151–157.

Bankova V, Koeva-Todorovska J, Stambolijska T, Ignatova-Groceva M-D, Todorova D, Popov S (1999): Polyphenols in *Stachys* and *Betonica* species (Lamiaceae). *Z Naturforsch C 54*: 876–880.

Bermejo Benito P, Diaz Lanza AM, Silvan Sen AM, De Santos Galindez J, Fernandez Matellano L, Sanz Gomez A, Abad Martinez MJ (2000): Effects of some iridoids from plant origin on arachidonic acid metabolism in cellular systems. *Planta Med 66*: 324–328.

Couladis M, Tzakou O, Verykokidou E, Harvala C (2003): Screening of some Greek aromatic plants for antioxidant activity. *Phytother Res 17*: 194–195.

Diklić N. (1974): *Stachys* L. In: Josifović M, ed., *The Flora of FR Serbia*, Vol. VI. Belgrade, Serbian Academy of Sciences and Arts, Department of Natural and Mathematical Sciences, pp. 408–432.

Ferrandiz ML, Alcaraz MJ (1991): Anti-inflammatory activity and inhibition of arachidonic acid metabolism by flavonoids. *Agents Actions 32*: 283–288.

Garjani A, Maleki N, Nazemiyeh H (2004): Effects of hydroalcoholic extract from aerial parts of the sterile stems of *Stachys inflata* on myocardial infarct size in rats. *Iran J Pharm Res 3*: 165– 170.

Hayashi K, Nagamatsu T, Ito M, Hattori T, Suzuki Y (1994): Acteoside, a component of *Stachys sieboldii* MIQ, may be a promising antinephritic agent. (2): Effect of acteoside on leukocyte accumulation in the glomeruli of nephritic rats. *Jpn J Pharmacol 66*: 47–52.

Hayashi K, Nagamatsu T, Ito M, Yagita H, Suzuki Y (1996): Acteoside, a component of *Stachys sieboldii* MIQ, may be a promising antinephritic agent. (3): Effect of acteoside on expression of intercellular adhesion molecule-1 in experimental nephritic glomeruli in rats and cultured endothelial cells. *Jpn J Pharmacol 70*: 157–168.

Khanavi M, Sharifzadeh M, Hadjiakhoondi A, Shafiee A (2005): Phytochemical investigation and anti-inflammatory activity of aerial parts of *Stachys byzanthina* C. Koch. *J Ethnopharmacol 97*: 463–468.

Koeva Ĭ (1989): *Stachys* L. In: Jordanov D, Velčev V, Kuzmanov B, eds., *Flora Republicae Popularis Bulgaricae*, Vol. IX. Sofia, Academia Scientiarum Bulgaricae, pp. 388–410.

Kukić J, Petrović S, Niketić M (2006a): Antioxidant activity of four endemic *Stachys* taxa. *Biol Pharm Bull 29*: 725–729.

Kukić J, Petrović S, Pavlović M, Couladis O, Tzakou O, Niketić M (2006b): Composition of essential oil of *Stachys alpina* L. ssp. *dinarica* Murb. *Flavour Fragr J 21*: 539–542.

Lenherr A, Meier B, Sticher O (1984): Modern HPLC as a tool for chemotxonomical investigations: Iridoid glucosides and acetylated flavonoids in the group of *Stachys recta*. *Planta Med 50*: 403–409.

Litchfield JT, Wilcoxon F (1949): A simplified method of evaluating dose-effect experiments. *J Pharmacol Exp Ther 96*: 99–113.

Maleki N, Garjani A, Nazemiyeh H, Nilfouroushan N, Eftekhar Sadat AT, Allameh Z, Hasannia N (2001): Potent anti-inflammatory activities of hydroalcoholic extract from aerial parts of *Stachys inflata* on rats. *J Ethnopharmacol 75*: 213–218.

Marin PD, Grayer RJ, Grujić-Jovanović S, Kite GC, Veitch NC (2004): Glycosides of tricetin methyl ethers as chemosystematic markers in *Stachys* subgenus *Betonica*. *Phytochemistry 65*: 1247–1253.

Menegazzi M, Di Paola R, Mazzon E, Muià C, Genovese T, Crisafulli C, Suzuki H, Cuzzocrea S (2006): *Hypericum perforatum* attenuates the development of carrageenan-induced lung injury in mice. *Free Radic Biol Med 40*: 740–753.

Meremeti A, Karioti A, Skaltsa H, Heilmann J, Sticher O (2004): Secondary metabolites from *Stachys ionica*. *Biochem Syst Ecol 32*: 139–151.

Naghibi F, Mosaddegh M, Motamed SM, Ghorbani A (2005): Labiatae family in folk medicine in Iran: From ethnobotany to pharmacology. *Iran J Pharm Res 2*: 63–79.

Ohno T, Inoue M, Ogihara Y, Saracoglu I (2002): Antimetastatic activity of acteoside, a phenylethanoid glycoside. *Biol Pharm Bull 25*: 666–668.

Petrović SD, Dobrić S, Bokonjić D, Niketić M, Garcia-Piñeres A, Merfort I (2003): Evaluation of *Tanacetum larvatum* for an anti-inflammatory activity and for the protection against indomethacin-induced ulcerogenesis in rats. *J Ethnopharmacol 87*: 109–113.

Petrović S, Ristić M, Kukić J, Milenković M, Antić-Stanković J, Niketić M (2006): Composition and antimicrobial activity of essential oil of *Stachys plumosa* Griseb. *Flavour Fragr J 21*: 250–252.

Rabbani M, Sajjadi SE, Jalali A (2005): Hydroalcohol extract and fractions of *Stachys lavandulifolia* Vahl: Effects on spontaneous motor activity and elevated plus-maze behaviour. *Phytother Res 19*: 854–858.

Rabbani M, Sajjadi SE, Zarei HR (2003): Anxiolytyic effects of *Stachys lavandulifolia* Vahl on the elevated plus-maze test of anxiety in mice. *J Ethnopharmacol 89*: 271–276.

Sadik CD, Sies H, Schewe T (2003): Inhibition of 15-lipoxygenases by flavonoids: Structure-activity relations and mode of action. *Biochem Pharmacol 65*: 773–781.

Sahpaz S, Garbacki N, Tits M, Bailleul F (2002): Isolation and pharmacological activity of phenylpropanoid esters from *Marrubium vulgare*. *J Ethnopharmacol 79*: 389–392.

Sala A, Recio MC, Schinella GR, Máñez S, Giner RM, Cerdá-Nicolás M, Ríos JL (2003): Assessment of the anti-inflammatory activity and free radical scavenger activity of tiliroside. *Eur J Pharmacol 461*: 53–61.

Skaltsa HD, Demetzos C, Lazari D, Soković M (2003): Essential oil analysis and antimicrobial activity of eight *Stachys* species from Greece. *Phytochemistry 64*: 743–752.

Skaltsa HD, Lazari DM, Chinou IB, Loukis AE (1999): Composition and antibacterial activity of the essential oils of *Stachys candida* and *S. chrysantha* from southern Greece. *Planta Med 65*: 255–256.

Ueda H, Yamazaki C, Yamazaki M (2004): A hydroxyl group of flavonoids affects oral anti-inflammatory activity and inhibition of systemic tumor necrosis factor-α production. *Biosci Biotechnol Biochem 68*: 119–125.