Natural Product Sciences
12(1) : 24-28 (2006)

Analgesic and Antipyretic Activity of *Stachys schimperi* Vatke

Mosa M. Qasheesh and Adnan J. Al-Rehaily*

Department of Pharmacognosy, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia

Abstract – The analgesic and the antipyretic activity of the methanol, chloroform, hexane and acetonitrile extracts of the aerial parts of *Stachys schimperi* Vatke were investigated in mice. The nociceptive response was tested using acetic acid-induced writhing and tail flick method; while hypothermia affect was examined via yeast-induced fever test. The chloroform extract at 500 mg and hexane and acetonitrile extracts at 250 mg produced significant analgesic and antipyretic activity.
Keywords – *Stachys schimperi*, analgesia, hypothermia

Introduction

The genus *Stachys* L. is one of the largest genera of the family Labiatae (Lamiaceae). It is comprised of more than 270 species, which are distributed in temperate and tropical regions of the world, with the exception of Australasia (Mabberley, 1997). These species are mainly herbs and shrubs. In Saudi Arabia, this genus is represented by five species including *Stachys schimperi* Vatke (Collenette, 1999). Several phytochemical investigations of *Stachys* species have been reported and resulted in the isolation of flavonoids (El-Ansari *et al.*, 1995), diterpenes (Paternostro *et al.*, 2000; Fazio *et al.*, 1994), phenyl ethanoid glycosides (Nishimura *et al.*, 1991; Miyase *et al.*, 1996) and saponins (Yamamoto *et al.*, 1994). In addition, few studies about their essential oils (Skaltsa *et al.*, 1999, 2001, 2003; Khanavi *et al.*, 2003, 2004) or solvent extracts (Takeda *et al.*, 1997; Mantle *et al.*, 2000; Maleki *et al.*, 2001) were reported.

In folk medicine, *Stachys palustris* L. and *Stachys sylvatica* L. are used as disinfectant, anti-spasmodic and for the treatment of wounds (Gruenwald *et al.*, 2000). In Iran, the aerial parts of the *Stachys inflata* Benth. are used for infection, asthma, rheumatic and other inflammatory disorders (Maleki *et al.*, 2001). Beside that, various pharmacological studies on the extracts or components of some *Stachys* plants were recorded. These include anti-inflammatory and antinephritic effects (Hayashi *et al.*, 1994; Skaltsa *et al.*, 2000; Maleki *et al.*, 2001; Khanavi *et al.*, 2005), effect on hyaluronidase activity (Takeda *et al.*, 1985) and hypotensive activity (Takeda *et al.*, 1997).

According to our knowledge, no studies have been performed on the analgesic and antipyretic activity of *Stachys schimperi* Vatke and therefore, the aim of the present investigation is to study the analgesic and antipyretic activity of this plant.

Experimental

Plant material – Aerial parts of *Stachys schimperi* Vatke were collected by Dr. Adnan Al-Rehaily from Al-Hada in Taif area of Saudi Arabia in Jan. 2005. The plant was identified by Dr. M. Atiqur Rahman, Medicinal, Aromatic and Poisonous Plants Research Center (MAPPRC), College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. A voucher specimen (# 14510) was deposited at the herbarium of the College of Pharmacy, KSU.

Animals – Male Swiss albino mice weighing 25 - 30 g were used in all experiments. They are obtained from the Animal Care Center, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. They were housed under conventional laboratory conditions. The mice were fed a standard animal pellet diet and allowed free access to water.

Preparation of plant Extracts – The dried powdered aerial parts (1 kg) of *Stachys schimperi* Vatke were extracted at room temperature with petroleum ether (9 g), followed by chloroform to yield after evaporation 12 g of residue and further extracted with 20% aqueous methanol to afford 98 g of residue. The petroleum ether extract was washed with hot methanol to get a precipitate (6.6 g mainly wax materials) and the supernatant (2.4 g) were

*Author for correspondence
Fax: +00966-1-467-7245; E-mail: ajalreha@ksu.edu.sa

partitioned between hexane and acetonitrile pre-saturated with each other to yield pre-saturated hexane fraction (1 g) and pre-saturated acetonitrile fraction (0.4 g).

Phytochemical screening – A phytochemical analysis of the aerial parts of *Stachys schimperi* Vatke was conducted for the detection of alkaloids, cardiac glycoisdes, flavonoids, coumarins, tannins, anthracens, saponions and sterols and/or triterpenes. 1.0 g of powdered plant was used for each test. All the tests were performed similar to the reported methods (Hildebert, 1983).

Analgesic activity

Acetic acid-induced writhing in mice – The test was carried out using the technique of Siegmund *et al.*, as modified by Koster *et al.* The aqueous methanol, chloroform, hexane and acetonitrile extracts (250 and/or 500 mg/kg body weight) were administered orally, to 16 h fasted mice, and divided into groups of six animals each. One hour after treatment, the mice were injected intraperitoneally with 0.2 ml of 3% acetic acid solution to induce the characteristic writhings. The number of writhings occurring between 5 and 15 min after acetic acid injection in control and treated animals was recorded. The responses of extracts-treated groups were compared with those of animals receiving indomethacin (as standard drug), 4 mg/kg, as well as with control group.

Tail flick test in mice – Acute nociception was induced using a tail flick apparatus (Tail Flick model DS 20 Sorrel Apelex, France) following the method of D'Amour and Smith (1941). Each mouse was placed in a restrainer (six animals in each group); 2 min before treatment, and baseline reaction time was measured by focusing on intensity controlled beam of light on the distal one-third portion of the animal's tail. The extracts were orally administered immediately after this step and 15, 30, 60, 90 and 120 min later; the post drug reaction time was measured. A 10 seconds cut off time was used in order to prevent tissue damage.

Antipyretic activity

Yeast-induced hyperpyrexia in mice – Hyperpyrexia was induced in mice by s.c. injection of (20% aqueous suspension of brewer's yeast) of 20 ml/kg body weight (6 animals in each group) in the back below the nape of the neck (Loux *et al.*, 1972). The animals were then fasted for the duration of experiment (approximately 26 h); water was made available *ad lib.* Control temperatures taken 24 h after the yeast injection to determine the pyretic response to yeast. Rectal temperatures were taken 1 h prior to drug administration in fevered animals served as a pre-drug control. The extracts (250 and /or 500 mg/kg) was given orally 24 h after the yeast injection and temperatures were recorded at 30, 60, 90 and 120 min after their administration.

Statistical analysis – All results were expressed as mean ± S.D. The significance between means was determined using Student's t-test and results were regarded as significant when $P < 0.05$.

Results and Discussion

In this study, the analgesic and antipyretic activities of the different extracts of *Stachys schimperi* Vatke were established. The results as shown in Table 1, indicate that only the chloroform and the acetonitrile extracts at two doses of 500 and 250 mg/kg, respectively, exhibited significant analgesic activity in mice, by inhibiting the acetic acid-induced writhing; which is a model of visceral

Table 1. Effect of various extracts of *Stachys schimperi* on acetic acid-induced writhings in mice

treatment	dose mg/kg orally	number of writhings/10 min	% inhibition
contorl (Acetic acid)	-	33.75 ± 4.57	-
vehicle (CMC)	-	no writhing	-
cmc + acetic acid	-	32.25 ± 3.86	-
aq. methanol ext.	250	26.5 ± 3.69	21.48
aq. methanol ext.	500	22.75 ± 4.78	32.59
chloroform ext.	250	22.75 ± 5.12	32.59
chloroform ext.	500	18.25 ± 2.75[a]	45.92
hexane ext.	250	20.5 ± 3.41[a]	37.40
acetonitrile ext.	250	13.75 ± 2.98[b]	58.00
indomethacin	4	8.75 ± 1.50[b]	73.20

Values are mean ± S.E. (n = 6).
Statistically significance was determined by student t-test.
The significance level showed as [a]$P < 0.05$; [b]$P < 0.01$.
CMC: carboxy methyl cellulose.

Table 2. Effect of various extracts of *Stachys schimperi* on tail flick test in mice

treatment	dose mg/kg orally	pre- drug	reaction time in Sec.			
			30 min	60 min	90 min	120 min
vehicle (CMC)	-	4.57 ± 0.49	4.70 ± 0.46	4.50 ± 0.40	4.80 ± 0.22	4.50 ± 0.20
aq. methanol ext.	250	4.80 ± 0.49	4.90 ± 0.24	5.27 ± 0.35	5.37 ± 0.40	5.52 ± 0.49
aq. methanol ext.	500	5.40 ± 0.71	5.87 ± 0.97	5.97 ± 0.69	6.60 ± 0.40	6.15 ± 0.31
chloroform ext.	250	5.37 ± 0.68	6.87 ± 0.43	7.10 ± 0.31	7.40 ± 0.26^a	6.92 ± 0.35
chloroform ext.	500	4.52 ± 0.42	6.55 ± 0.40^b	6.87 ± 0.9^b	6.92 ± 0.35^b	6.50 ± 0.21^b
hexane ext.	250	4.52 ± 0.49	5.35 ± 0.47	5.72 ± 0.35	6.35 ± 0.31^a	5.35 ± 0.36
acetonitrile ext.	250	4.12 ± 0.44	4.60 ± 0.69	5.95 ± 0.31^a	7.12 ± 0.28^c	6.82 ± 0.47^b
indomethacin	4	4.27 ± 0.35	6.12 ± 0.33^b	7.80 ± 0.31^c	7.97 ± 0.72^b	7.02 ± 0.89^a

Values are mean ± S.E. (n = 6).
Statistically significance was determined by student t-test.
The significance level showed as $^aP < 0.05$; $^bP < 0.01$ and $^cP < 0.001$.
CMC: carboxy methyl cellulose.

Table 3. Effect of various extracts of *Stachys schimperi* on yeast-induced hyperpyrexia in mice

treatment	dose mg/kg orally	rectal temperature (°C)					
		pre- drug	post drug				
			0 min	30 min	60 min	90 min	120 min
vehicle (CMC)	-	35.4 ± 0.60	38.72 ± 0.28	38.85 ± 0.20	38.60 ± 0.45	38.92 ± 0.54	38.75 ± 0.31
aq. methanol ext.	250	34.67 ± 0.28	38.95 ± 0.26^c	37.82 ± 1.26	37.62 ± 0.90	37.47 ± 0.61	37.35 ± 0.46^a
aq. methanol ext.	500	34.97 ± 0.71	38.50 ± 0.66^c	38.22 ± 1.29	37.90 ± 1.02	37.35 ± 0.85	36.92 ± 0.60
chloroform ext.	250	34.80 ± 0.42	38.87 ± 0.33^c	37.27 ± 0.61	36.92 ± 0.41^b	36.40 ± 0.18^c	36.50 ± 0.43^b
chloroform ext.	500	34.60 ± 0.37	38.67 ± 0.35^c	37.02 ± 0.37^a	36.72 ± 0.26^b	36.05 ± 0.17^c	36.12 ± 0.35^b
hexane ext.	250	34.40 ± 0.24	38.57 ± 0.57^c	36.95 ± 0.83	36.27 ± 0.40^a	35.40 ± 0.31^b	36.15 ± 0.26^b
acetonitrile ext.	250	34.25 ± 0.45	38.67 ± 0.36^c	37.02 ± 0.42^a	36.72 ± 0.35^b	36.15 ± 0.28^c	36.80 ± 0.48^a
indomethacin	4	34.27 ± 0.28	38.85 ± 0.12^c	36.87 ± 0.84	36.07 ± 0.50^b	35.02 ± 0.20^c	36.07 ± 0.37^c

Values are mean ± S.E. (n = 6).
Statistically significance was determined by student t-test.
The significance level showed as $^aP < 0.05$; $^bP < 0.01$ and $^cP < 0.001$.
CMC: carboxy methyl cellulose.

pain (Vyklicky, 1979). Acetic acid-induced writhings is a very sensitive and useful test for analgesic drug development, but not a selective pain test. It gives false positives with sedatives, muscle relaxants and other pharmacological activities (Elisabetsky *et al.*, 1995). Both extracts were also found to be very active on tail flick test (Table 2). This test is widely used to investigate the centrally acting analgesic activity. These results suggest that the analgesic effect of the chloroform and the acetonitrile extracts may be due to inhibition of biosynthesis and/or release of prostaglandins and direct stimulation of pain sensation (Adzu *et al.*, 2001).

In addition, the chloroform and the acetonitrile extracts were also found to produce a marked hypothermia in a dose-dependent manner especially after 1.5 h of the administration (Table 3). This again further support that these extracts (chloroform and acetonitrile) are probably working through inhibition of prostaglandin biosynthesis similar to nonsteroidal anti-inflammatory drugs (NSIDs) (Vane, 1987).

Since the results showed that the aqueous methanol extract did not exert any significant analgesic and antipyretic activities and all the effects are due to the chloroform and acetonitrile extracts with some significant antipyretic activity in the hexane fraction, therefore, it can be concluded that the analgesic and antipyretic activities of *Stachys schimperi* are resided mainly in the nonpolar and intermediate polarity compounds. In addition, the

phytochemical analysis of the plant reveals the presence of coumarins, flavonoids, sterols/triterpines, saponins and tannins. This in turns will let us to suggest that the analgesic and the antipyretic activities could be due to coumarins, flavonoids and/or triterpenes. Such compounds are reported to exert these effects (Iwalewa *et al.*, 2003).

Finally, it can be concluded that *Stachys schimperi* Vatke produce analgesic activity, which is both central and peripheral analgesia, and also a very significant antipyretic effect. Further studies are needed to better evaluate these activities, to isolate the active principle, and the potential of the plant.

Acknowledgement

The authors are thankful to Mr. Malik Saud for his assistance.

References

Adzu, B., Amos, S., Wambebe, C., and Gamaniel, K., Antinociceptive activity of *Zizyphus spina-christi* root bark extract. *Fitoterapia* **72**, 344-350 (2001).

Collenette, S., An Illustrated Guide to the Flowers of Saudi Arabia. Scorpion Publishing Limited, London, UK, 1985.

D'Amour, E.F., and Smith, D.L., A method for determinating loss of pain, sensation. *J. Pharmacol. Exp. Ther.* **72**, 74-79 (1941).

El-Ansari, M.A.El., Nawwar, M.A., and Saleh, N.A., Stachysetin, a diapigenine-7-glucoside-*p*-*p*'-dihydroxy-truxinate from *Stachys aegyptiaca*. *Phytochemistry* **40**, 1543-1548 (1995).

Elisabetsky, E., Amador, T.A., Albuquerque, R.R., Numes, D.S., and Carvalho, A.C.T., Analgesic activity of *Psychotria colorata* (Wild ex R.&S.) Muell Arg. alkaloids. *J. Ethnopharmacol.* **48**, 77-83 (1995).

Fazio, C., Passannanti, S., Paternostro, M.P., and Arnold, N.A., Diterpenoids from *Stachys mucronata*. *Planta Med.* **60**, 499 (1994).

Gruenwald, J., Brendler, T., and Jaenicke, C., PDR for herbal medicines, 2nd ed. Medical Economics Company, Montvale, NJ, p. 832 2000.

Hayashi, K., Nagamatsu, T., Ito, M., Hattori, T., and Suzuki, Y., Acotoside, a component of *Stachys siebodii* MIQ, may be a promising antinephritic in rats. *Jap. J. Pharm.* **65**, 143-151 (1940).

Hildebert, W., Sabina, B., and Eva, M.Z., Plant drug analysis. Springer-Verlag, Berlin, Heidelberg, Germany, 1983.

Irwin, S., Pharmacologic Techiques in drug Evaluation. 35, Year Book, Chicago, 1964.

Khanavi, M., Hadjiakhoondi, A., Amin, G., Amanzadeh, Y., Rustaiyan, A., and Shafiee, A., Comparison of the volatile composition of *Stachys persica* Gmel. and *Stachys byzanthina* C. Koch. oils obtained by hydrodistillation and steam distillation. *Z. Naturforsch.* **59**, 463-467 (2004).

Iwalewa, E.O., Iwalewa, O.J., and Adeboye, J.O., Analgesic, antipyretic, anti-inflammatory effects of methanol, chloroform and ether extracts of *Vernonia cinerea* less leaf. *J. Ethnopharmacol.* **86**, 229-234 (2003).

Khanavi, M., Hadjiakhoondi, A., Shafiee, A., Masoudi, Sh., and Rustaiyan, A., Chemical composition of the essential oil of *Stachys byzanthina* C. Koch. from Iran. *J. Essent. Oil Res.* **15**, 77-78 (2003).

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

Koster, R., Anderson, M., and De Beer, E.J., Acetic acid for analgesic screening. *Fed. Proc.* **18**, Abstract P. 412 (1959).

Loux, J.J., DePalma, P.D., and Yankell, S.L., Antipyretic testing of aspirin in rats. *Toxicol. Appl. Pharmacol.* **22**, 672-675 (1972).

Mabberley, D.J., The Plant-Book. Cambridge University Press, Cambridge, New York, Melbourne, 1997.

Maleki, N., Garjani, A., Nazemiyah, H., Nilfouroushan, N., Eftekhar Sa-dat, A.T., Allameh, Z., and Hasannia, N., Potent anti-inflammatory activities of hydroalcoholic extract from aerial parts of *Stachys inflata* on rats. *J. Ethnopharmacol.* **75**, 213-218 (2001).

Mantle, D., Eddeb, F., and Pickering, A.T., Comparison of relative antioxidant activities of British medicinal plant species in vitro. *J. Ethnopharmacol.* **72**, 497-510 (2000).

Miyase, T., Yamamoto, R., and Ueno, A., Phenyl ethanoid glycosides from *Stachys officinalis*. *Phytochemistry* **43**, 475-479 (1996).

Nishimura, H., Sasaki, H., Inagaki, N., Chin, M., and Mitsuhashi, H., Nine phenethyl alcohol glycosides from *Stachys seiboldii*. *Phytochemistry* **30**, 965-969 (1991).

Paternostro, M.P., Maggio, A.M., Piozzi, F., and Servettaz, O., Labdane diterpenes from *Stachys plumosa*. *J. Nat. Prod.* **63**, 1166-1167 (2000).

Siegmund, E., Cadmus, R., and Lu, G.A., Method for evaluating both non-narcotic and narcotic analgesics. *Proc. Soc. Exp. Biol. Med.* **95**, 729-731 (1957).

Skaltsa, H.D., Bermejo, P., Lazari, D.M., Silvan, A.M., Skaltsounis, AL., Sanz, A., and Abed, M.J., Inhibition of prostaglandin E2 and leukotriene C4 in mouse peritoneal macrophages and thromboxan B2 production in human platelets by flavonoids from *Stachys chrysantha* and *Stachys candida*. *Bio. Pharm. Bull.* **23**, 47-53 (2000).

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

Skaltsa, H.D., Lazari, D.M., Chinou, I.B., and Loukis, A.E., Composition and antibacterial activity of the essential oil of *Stachys candida* and *S. chrysantha* from Southern Greece. *Planta Med.* **65**, 255-256 (1999).

Skaltsa, H.D., Mavrommati, A., and Constantividis, T., A chemotaxonomic investigation of volatile constituents in *Stachys* subsect *Swain-sonianeae* (Labiatae). *Phytochemistry* **57**, 235-244 (2001).

Takeda, Y., Fujita, T., Satoh, T., and Kakegawa, H., On the glycosidic constituents of *Stachys sieboldi* MIQ. and their effects on hyaluronidase activity. *Yakugaku Zasshi* **105**, 955-959 (1985).

Takeda, Y., Zhang, H., Masuda, T., Honda, G., Otsuka, H., Sezik, E., Yesieada, E., and Sun, H., Megastigmane glucosides from *Stachys byzanthina*. *Phytochemistry* **44**, 1335-1337 (1997).

Vane, J.R., The evolution of non-steroidal anti-inflammatory drug and their mechanisms of action. *Drugs* (supply) **33**, 18-27 (1987).

Vyklicky, L., Techniques for the study of pain in animals. In: Bonica, J.J., Liebeskind, J.C., Albe-Fessard, D.G. (Eds.), Advances in Pain Research and Therapy. Reven Press, New York, pp. 727-745 (1979).

Yamamoto, R., Miyase, T., and Ueno, A., Stachyssaponins I-VIII, new oleanane-type triterpene saponins from *Stachys riederi* Chamisso, *Chem. Pharm. Bull.* **42**, 1291-1296 (1994).

(Accepted March 1, 2006)