

Nepetalactone: A New Opioid Analgesic from *Nepeta caesarea* Boiss.

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Abstract

The essential oils of *Nepeta* species including *Nepeta phyllocllamys* P. H. Davis, *N. nuda* L. ssp. *nuda*, and *N. caesarea* Boiss. have been screened by use of the tail-flick and tail immersion (52.5°C) methods.

Of the species studied, only *N. caesarea* showed significant analgesic activity, besides marked sedation, which was also blocked by naloxone, indicating involvement of opioid receptors. Moreover, it was only active on mechanical, not thermal, algic response which suggests specificity for specific opioid receptor subtypes, excluding μ -opioid receptors.

Because 4 α ,7 α ,7 α -nepetalactone is the main component of the essential oil of *N. caesarea*, and is present at very high levels (92–95%), it is concluded that 4 α ,7 α ,7 α -nepetalactone is the active principle and has a specific opioid receptor subtype agonistic activity.

The search for analgesic substances dates back to prehistoric times and successfully resulted in the discovery of opium and its use for thousands of years. Plants have served as considerably rich sources of analgesic substances which have become prototypes for other analgesics—morphine for opiates and salicylates for non-steroidal anti-inflammatory agents.

Opioid drugs such as morphine and the endogenous opioid peptides, namely enkephalins, endorphins and dynorphins, exert a wide spectrum of physiological and behavioural effects, including effects on pain perception, mood control and autonomic functions. Opioid receptors are classified as δ , κ and μ , membrane-bound G protein-linked receptors; recently a new nomenclature has been proposed—OP1 for δ , OP2 for κ and OP3 for μ (Dhawan et al 1996). Opioid receptor-mediated actions have been shown to be complex because of the presence of many opioid receptor subtypes and also many limitations and side effects of the opiates have stimulated a search for new drugs. This search is complicated by the identification of new opioid receptor subtypes (Sofuoglu et al 1991) and the difficulty of purification and identification of new endogenous opioid agonists (Zadina et al 1997). At this point pharmacologically active and new com-

pounds are needed which are devoid of side effects and are also specific to opioid receptor subtypes. With the advent of molecular biological techniques, molecular cloning of opioid receptors has stimulated and paved the way for discovery of drugs selective for opioid receptor subtypes.

One method leading to new drug discovery is the screening of plants and their chemical constituents. Turkish folk medicine is one of the richest in the world, possibly because of the large number of plant species in the Turkish flora, a third of which are endemic. The presence of a variety of analgesic plants in the flora has been shown by pharmacological experiments (Çakici et al 1997). In a recent study (Aydin et al 1996) the analgesic activity of the essential oil of kekik (*Origanum onites* L.) was reported by our group; this stimulated us to focus on the analgesic action of essential oils and to continue screening for other essential oil-containing species.

The genus *Nepeta* (Labiatae) is represented in Turkey by 33 species and altogether 38 taxa of which 17 are endemic (Davis 1982). The genus is known to be poor in oil and although the essential oils of some species have been investigated chemically as part of continuing research (Baser et al 1996), to the best of our knowledge, none was subject to pharmacological studies. Although *Nepeta* species are not reported to be used for analgesia in Turkish folk medicine, it is known that

several are ethnomedically used for analgesia (Duke 1985) and very few experimental studies are published on their analgesic activities (Ahn 1981; Abraham et al 1986). In this study we have investigated the analgesic action of a few randomly selected *Nepeta* species which gave interesting positive results.

Materials and Methods

Drugs

Morphine sulphate (TMO, Turkey) and naloxone hydrochloride (Sigma, St Louis, MO) were dissolved in saline.

Preparation of the essential oils

Plant materials were collected from the wild and authenticated by an authority. Voucher specimens were deposited at the Herbarium of the Faculty of Pharmacy of Anadolu University (ESSE) in Eskişehir, Turkey. *Nepeta phyllocllamys* P. H. Davis was collected from Tahtali dag, Antalya, in June 1995, altitude 1080 m; *N. caesarea* Boiss. was collected from Eskişehir, Saricakaya, in August 1992, altitude 1210 m; and *N. nuda* L. ssp. *nuda* from Uludag, Bursa, in July 1992, altitude 1480 m (Table 1).

Steam distillation of the plants was performed in a Clevenger-type glass apparatus for approximately 3 h. The essential oils were diluted ten times in olive oil and administered to animals intraperitoneally at a standard dose (0.03 mL kg⁻¹).

Animals

Adult Swiss albino mice, 28–38 g, of both sexes were used in this study. They were housed in well-ventilated rooms at 18–25°C. All mice were fed with standard diet (Esyem A. S., Eskişehir) and water was freely available.

Behavioural observation of the animals

After intraperitoneal injection of saline, olive oil and test substances (essential oils of *N. caesarea*, *N. nuda* ssp. *nuda*, and *N. phyllocllamys* (0.03 mg kg⁻¹) all the animals were placed separately in transparent containers and observed for 30 min; the animals were then subjected to tail-clip or water immersion tests.

Tail-clip test

Experiments were performed on freely moving Swiss albino mice, 28–38 g. The analgesic (antinociceptive) activity of morphine and the essential oils were measured by application of mechanical tail-clip as described elsewhere (D'Amour & Smith 1941). A control response (2–4 s) was determined by administration of 0.1 mL olive oil and 0.9% physiological saline solution, because essential oils and standard pure chemicals (morphine sulphate and naloxone hydrochloride) were diluted in olive oil and in saline solution, respectively. Test latencies were assessed 30 min after administration of drugs to mice for all test substances and control groups. Naloxone, a specific antagonist for opioid receptors, was administered intraperitoneally 15 min before drugs and test substances (0.4 mg kg⁻¹). Morphine sulphate (1 mg kg⁻¹ i.p.) was used as standard opioid agonist. To avoid

Table 1. ESSE numbers, collection places and dates, endemism and main constituents of *Nepeta caesarea*, *Nepeta phyllocllamys* and *Nepeta nuda* ssp. *nuda*.

Name of plant (Endemism)	Collection Place	ESSE no.	Main constituents (%)*	Reference
<i>Nepeta phyllocllamys</i> (Endemic)	Antalya 1995	11296	β -Pinene (16.26) Terpinen-4-ol (8.37) Caryophyllene oxide (7.78) Linalool (7.64) Sabinene (6.56) 1,8-Cineole (5.91)	Baser et al (1996)
<i>Nepeta nuda</i> ssp. <i>nuda</i> (—)	Bursa 1992	10265	Geijerene (23.31) 4 $\alpha\beta$,7 α ,7 $\alpha\alpha$ -Nepetalactone(13.47) 1,8-Cineole (7.99) 4 $\alpha\alpha$,7 α ,7 $\alpha\alpha$ -Nepetalactone(7.73) Pregeijerene(7.36)	Baser et al (1996)
<i>Nepeta caesarea</i> (Endemic)	Eskişehir 1992	9751	4 $\alpha\alpha$,7 α ,7 $\alpha\alpha$ -Nepetalactone (91.2–95.34) Germacrene D (1.46)	Baser & Özek (1994)

*Only compounds present at levels > 5% are listed (except germacrene D in *N. caesarea* because of the unusual composition of the oil).

damage to the tails of the mice, a maximum latency of 15 s for the tail-clip test was imposed if no response occurred within that time. Analgesia is expressed as a percentage of the maximum possible effect (%MPE) where:

$$\%MPE = \left(\frac{(\text{post-drug latency}) - (\text{pre-drug latency})}{(\text{cut-off time} - \text{pre-drug latency})} \right) \times 100$$

Tail-immersion test

Experiments were performed on Swiss albino mice, 28–38 g. The details of the tail-immersion test procedure used were essentially similar to those published elsewhere (Schmauss & Yaksh 1984). By use of a circulating water heater (Heto, Allerod, Denmark) a constant temperature of $52.5 \pm 0.2^\circ\text{C}$ was maintained in a water bath in which the terminal 3 cm of the animal's tail was immersed. The nociceptive endpoint was characterized by a jerk of the tail. While nociception measurements were being made the animals were briefly immobilized by gentle wrapping. A control response was determined by administration of 0.1 mL olive oil and 0.9% physiological saline solution. Test latencies were assessed 30 min after the administration of drugs to mice for all test substances and control groups. Naloxone, a specific antagonist for opioid receptors, was administered intraperitoneally 15 min before the drugs and test substances (0.4 mg kg^{-1}). Morphine sulphate (1 mg kg^{-1} i.p.) was used as standard opioid agonist. To avoid any damage to the tails of the mice a maximum latency of 60 s was imposed if no response occurred within that time. Analgesia is expressed as percent of maximum possible effect (%MPE) according to the formula given above.

Statistics

Results are presented as means \pm s.e.m. and statistical significance between groups was determined by analysis of variance then Tukey's HSD multiple comparison test. $P < 0.05$ was considered significant.

Results

Behavioural observations

Behavioural differences were noticed during observation of animals, including control groups, subject to analgesic tests. Only the essential oil of *N. caesarea* (0.03 mL kg^{-1}) was found to cause marked inhibition of spontaneous activity of the animals.

Tests for analgesia

Intraperitoneal injection of animals with the essential oil of *N. caesarea* (0.03 mL kg^{-1}) induced marked inhibition of the tail-clip response in mice. This activity was inhibited by pretreatment with naloxone. Other essential oils obtained from *N. nuda* ssp. *nuda* and *N. phyllocllamys* had no analgesic effect (Table 2). Because none of the essential oils was active in the warm water (52.5°C) tail-immersion test (Table 3), naloxone pretreatment was not performed for this test.

Discussion

In addition to known endogenous opioid peptides, new compounds are being synthesized in the search for novel opioid agonists (Portoghesi et al 1992; Chang et al 1993), and there are reports of new plant-based analgesics, some of which are components of essential oils (Aydin et al 1996) and are sensitive to naloxone (Almeida et al 1996).

In a continuing screening study we have studied the essential oils of three *Nepeta* species and observed that only one, which is endemic to Turkey (*N. caesarea* Boiss.), has marked analgesic activity

Table 2. Results from tail flick testing of *Nepeta caesarea*, *Nepeta phyllocllamys* and *Nepeta nuda* ssp. *nuda*.

Treatment	n	Latency (s)	Percentage of the maximum possible effect
Control	11	02.18 ± 0.772	—
<i>Nepeta phyllocllamys</i>	5	04.60 ± 2.64	16.0 ± 21.5
<i>Nepeta nuda</i> L. ssp. <i>nuda</i>	5	04.60 ± 1.83	16.4 ± 17.7
<i>Nepeta caesarea</i>	5	12.40 ± 2.60	$80.0 \pm 20.0^*$
Naloxone + <i>Nepeta caesarea</i>	5	05.00 ± 2.61	26.2 ± 18.9
Morphine	5	11.60 ± 1.89	$72.92 \pm 14.4^*$
Naloxone + morphine	5	04.80 ± 1.46	19.7 ± 11.3

Results are means \pm s.e.m. * $P < 0.05$, significantly different from control result.

Table 3. Results from tail immersion (52.5°C) testing of *Nepeta caesarea*, *Nepeta phyllocllamys* and *Nepeta nuda* ssp. *nuda*.

Treatment	n	Latency (s)	Percentage of the maximum possible effect
Control	11	08.45 ± 1.38	–
<i>Nepeta phyllocllamys</i>	5	09.00 ± 1.90	5.51 ± 5.23
<i>Nepeta nuda</i> L. ssp. <i>nuda</i>	5	08.00 ± 2.00	3.24 ± 7.22
<i>Nepeta caesarea</i>	5	07.20 ± 3.12	2.06 ± 16.42
Morphine	5	41.60 ± 10.7	68.3 ± 18.4*
Naloxone + morphine	5	13.00 ± 2.83	13.03 ± 5.99

Results are means ± s.e.m. * $P < 0.05$, significantly different from control.

which is sensitive to naloxone (Tables 2 and 3). Because thermal algnesia was not abolished by *N. caesarea* essential oil, we suggest that a specific subtype of opioid receptor is involved in *N. caesarea* analgesia.

Somatostatin rather than substance P is known to mediate noxious heat-induced signals (Tiseo et al 1990). It is also shown that spinal κ (Schmauss & Yaksh 1984) and δ opioid receptors do not mediate thermal algseic stimulus in the mouse warm-water tail-flick test (Heyman et al 1989; Jiang et al 1990), which suggested the involvement of κ or δ opioid receptors, or both, in the analgesic action of *N. caesarea* essential oil. Thus, our current results might indicate specific activity or a mechanism which discriminates the actions involving substance P and somatostatin. Furthermore, the results strongly indicate subtype-selective action on κ or δ opioid receptors, or both.

Interestingly, it is known that the *N. caesarea* essential oil is almost entirely (92–95%) $4\alpha,7\alpha,7\alpha$ -nepetalactone (Figure 1) (Baser & Özek 1994). Major components of other species are: *N. phyllocllamys*, β -pinene (16%) and *N. nuda* L. ssp. *nuda*, geijerene (23.31%) (Table 1) (Baser et al 1996). Among the essential oils tested, that from *N. nuda* ssp. *nuda* also contains nepetalactones (Table 1). Although the activity was not at the baseline

level, the lack of significant analgesic activity of *N. nuda* ssp. *nuda* might be a result of a different isomeric structure (4β form) and the amount of nepetalactones (Table 1), or the presence of possible algseic compounds which are yet to be discovered.

Moreover, the marked sedation observed also implies action of $4\alpha,7\alpha,7\alpha$ -nepetalactone on the central nervous system. Thus we conclude that the analgesic action is completely attributable to $4\alpha,7\alpha,7\alpha$ -nepetalactone which is a new opioid agonist with a subtype-specific action, which might be selective on κ or δ opioid receptors, or both, but not on μ opioid receptors. Furthermore, the nepetalactone skeleton might serve as a new prototype opioid analgesic for synthetic and medicinal chemists because it has a lipophilic structure that enables it to pass through the blood–brain barrier easily and thus be active in the central nervous system. The opioid agonistic activity of $4\alpha,7\alpha,7\alpha$ -nepetalactone is, to the best of our knowledge, the first report of such activity for a compound with the nepetalactone skeleton.

It is obvious that further studies are required to identify whether δ or κ receptors are involved in the analgesic action of nepetalactones. Dose–response studies should also be performed. These were not conducted in the current study because of insufficient plant material.

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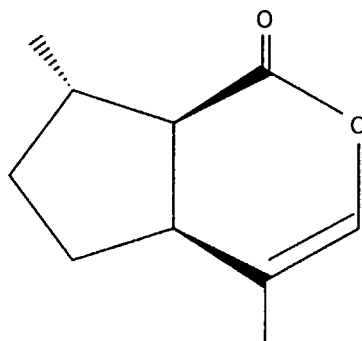


Figure 1. The chemical structure of $4\alpha,7\alpha,7\alpha$ -nepetalactone.

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