# LOCAL ANAESTHETIC ACTIVITY OF MONOTERPENES AND PHENYLPROPANES OF ESSENTIAL OILS

# Local Anaesthetic Activity of Monoterpenes and Phenylpropanes of Essential Oils

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***Abstract:*** **The local anaesthetic activity of a number of compounds with different structures, contained in essential oils, was studied. Anaesthetic activity was evaluated *in vivo* in the rabbit conjunctival reflex test and *in vitro* in a rat phrenic nerve-hemidiaphragm technique. Among the substances tested terpineol and *trans*-anethole ($10^{-3}$ – 1 μg/ml) were able to drastically reduce the electrically evoked contractions of rat phrenic nerve-hemidiaphragm in a concentration-dependent manner, but not eugenol, (–)- and (+)-citronellal, (–)- and (+)-carvone, *trans* cinnamaldehyde and α-terpinene. In the rabbit conjunctival reflex test, the treatment with a solution of terpineol and *trans*-anethole (10 – 100 μg/ml) effected a concentration-dependent increase in the number of stimuli required to evoke the reflex, thus confirming *in vivo* the local anaesthetic activity observed *in vitro*. Eugenol, (–)- and (+)-citronellal, *trans*-cinnamaldehyde, (–)- and (+)-carvone and α-terpinene were as ineffective in the *in vivo* test as they were in the *in vitro* results.**

Essential oils obtained from several plants, including *Rosmarinus officinalis* L., *Hyssopus officinalis* L. var. *decumbens*, *Origanum compactum* Benth. and *Mentha piperita* L., have been shown to possess myorelaxing activity which justifies the traditional use of these medicinal plants in gastrointestinal disorders (1), (2), (3). The myorelaxing activity of the essential oils has been related to some of their chemical components such as bornel, linalool, and menthol (1), (2).

Studies performed *in vitro* on the antispasmodic action of essential oils showed that they inhibit the contractions induced by several spasmogens that have various pharmacological mechanisms to cause contraction, thus pointing to an unspecific antagonism (2), (3). It has been suggested that, due to their high lipid solubility, the essential oils may interact with the lipid bilayer of the plasma membrane, inhibiting $Ca^{++}$ influx or preventing the increase in $Na^{+}$ permeability, and thereby blocking the neurotransmission (3).

Moreover, our recent studies have shown that the essential oil of *Lavandula angustifolia* Mill. and some monoterpenes of essential oils, such as linalool and menthol, possess local anaesthetic activity (4), (5), which could be responsible, at least in part, for their muscle-relaxing properties. In this work we have tested a number of the monoterpenes and phenylpro-

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panes contained in essential oils, in order to evaluate their potential local anaesthetic activity.

Terpineol and *trans*-anethole, in a concentration range of 0.001 – 1 μg/ml, were able to reduce, in a concentration-dependent manner, the electrically evoked contractions of the isolated rat phrenic nerve hemidiaphragm, up to complete abolishment of the contractions (Table **1**). In the same experimental conditions, the classic local anaesthetic procaine (Table **1**) exhibited a profile similar to that shown for terpineol and *trans*-anethole. The other substances tested, up to a concentration of 1 μg/ml, did not inhibit the electrically evoked contractions (Table **1**). Terpineol and *trans*-anethole did not modify the contractions induced through direct stimulation of the diaphragm muscle (data not shown).

The local anaesthetic activity of terpineol and *trans*-anethole was confirmed *in vivo* in the conjunctival reflex test in rabbit. Treatment with a solution of terpineol and *trans*-anethole (10 – 100 μg/ml), administered in the conjunctival sac, induced a concentration-dependent increase in the number of stimuli required to evoke the reflex (Table **2**). The application of a solution of eugenol, (–)- and (+)-citronellal, (–)- and (+)-carvone, *trans*-cinnamaldehyde and α-terpinene, in a range of concentrations of 3 – 1000 μg/ml, did not exhibit any local anaesthetic effect (Table **2**). The vehicle DMSO, at the maximal concentration used (1 : 4 in $H_2O$), was devoid of any effect when administered in the conjunctival sac alone (Table **2**). The local anaesthetic activity of terpineol and *trans*-anethole was observed starting 5 min after administration, then it quickly diminished and disappeared within 15 min. Both compounds injected subcutaneously at a concentration of 300 μg/ml, also inhibited the cutaneous muscle reflex in guinea-pig dorsal skin (data not shown).

Our experiments show that terpineol and *trans*-anethole have a remarkable local anaesthetic activity, comparable with that of the classic local anaesthetic procaine. These results justify, at least in part, the traditional use of herbal drugs such as the leaves of *Origanum majorana* L. and the fruits of *Pimpinella anisum* L., whose essential oils contain terpineol and anethole respectively (6), (7), as antispasmodics and carminatives in gastrointestinal disorders. The other substances tested resulted almost inactive; among them eugenol, the main constituent of clove oil, reduced the electrically evoked contractions of the rat phrenic nerve-hemidiaphragm, starting from a concentration of 100 μg/ml : this result is in accordance with the data of Brodin and Roed (8). However, it must be underlined that at concentrations higher than 100 μg/ml all substances tested reduced, to a certain extent, the contractions of the

**Table 1** Reduction of electrically-evoked contractions of phrenic nerve-hemidiaphragm induced by monoterpenes and phenylpropanes in comparison with procaine. Values (in percent) are expressed as mean ± S.E. of 4 experiments

| Substance | Concentration (μg/ml) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | $10^{-4}$ | $10^{-3}$ | $10^{-2}$ | $10^{-1}$ | 1 | 10 | $10^2$ | $10^3$ |
| Eugenol | 0 | 0 | 0 | 0 | 0 | 0 | 13.2 ± 5.5 | 38.4 ± 5.1 |
| Terpineol | 0 | 25.8 ± 6.6 | 63.5 ± 7.2 | 92.4 ± 9.3 | 100 | – | – | – |
| (–)-Citronellal | 0 | 0 | 0 | 0 | 0 | 4.3 ± 1.1 | 11.2 ± 3.9 | 12.6 ± 2.5 |
| (+)-Citronellal | 0 | 0 | 0 | 0 | 0 | 0 | 5.6 ± 2.4 | 9.8 ± 2.2 |
| *trans*-Cinnamaldehyde | 0 | 0 | 0 | 0 | 0 | 0 | 9.2 ± 3.6 | 11.0 ± 3.5 |
| (–)-Carvone | 0 | 0 | 0 | 0 | 0 | 6.7 ± 4.0 | 15.9 ± 4.7 | 19.3 ± 7.1 |
| (+)-Carvone | 0 | 0 | 0 | 0 | 0 | 0 | 10.7 ± 5.5 | 21.0 ± 9.5 |
| α-Terpinene | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 22.5 ± 6.2 |
| *trans*-Anethole | 0 | 10.3 ± 9.5 | 43.9 ± 9.8 | 79.7 ± 10.2 | 100 | – | – | – |
| Procaine | 0 | 39.7 ± 6.4 | 67.6 ± 5.1 | 89.3 ± 9.4 | 100 | – | – | – |

– Not tested.

**Table 2** Activity of monoterpenes and phenylpropanes in comparison with procaine, in rabbit conjunctival reflex test evaluated 5 min after administration. Results are expressed as number of stimuli required to evoke the conjunctival reflex. Each value represents the mean ± S.E. of 4 experiments

| Substance | Concentration (μg/ml) | | | | |
|---|---|---|---|---|---|
| | 3 | 10 | 30 | 100 | 1000 |
| Vehicle | 1.6 ± 0.4 | 3.0 ± 1.6 | 2.8 ± 1.1 | 3.1 ± 1.5 | 2.7 ± 1.3 |
| Eugenol | 1.5 ± 0.5 | 3.2 ± 1.4 | 1.7 ± 0.4 | 3.9 ± 1.6 | 7.7 ± 1.5 |
| Terpineol | 1.7 ± 0.3 | 21.5 ± 4.4* | 39.8 ± 6.5* | 66.5 ± 8.2* | 62.1 ± 6.8* |
| (–)-Citronellal | 1.1 ± 0.1 | 2.3 ± 0.5 | 1.6 ± 0.4 | 1.5 ± 1.0 | 4.5 ± 1.6 |
| (+)-Citronellal | 4.8 ± 2.7 | 5.1 ± 1.9 | 2.6 ± 0.6 | 1.5 ± 1.7 | 2.9 ± 1.0 |
| *trans*-Cinnamaldehyde | 3.3 ± 2.0 | 6.3 ± 2.1 | 5.4 ± 2.9 | 5.6 ± 2.2 | 4.5 ± 1.7 |
| (–)-Carvone | 1.0 ± 0.3 | 3.5 ± 1.3 | 3.5 ± 1.2 | 4.7 ± 1.9 | 4.4 ± 1.5 |
| (+)-Carvone | 2.9 ± 0.6 | 3.1 ± 1.2 | 2.7 ± 2.0 | 1.8 ± 1.3 | 2.8 ± 1.6 |
| α-Terpinene | 2.8 ± 0.9 | 4.5 ± 2.6 | 4.6 ± 2.7 | 5.5 ± 1.8 | 3.0 ± 1.4 |
| *trans*-Anethole | 1.5 ± 0.4 | 9.7 ± 3.2* | 31.8 ± 5.1* | 65.2 ± 9.5* | 57.8 ± 5.2* |
| Procaine | 1.6 ± 0.6 | 18.5 ± 2.6* | 45.8 ± 6.0* | 69.4 ± 8.1* | 67.5 ± 4.4* |

* $P<0.01$.

isolated preparation (Table **1**), suggesting that such an effect is unspecific.

The local anaesthetic activity observed in the rabbit conjunctival reflex test with terpineol and *trans*-anethole cannot be attributed to an increase in the pain threshold since even drugs able to induce a strong analgesia such as morphine, diphenydramine, amitriptyline and acetylsalicilic acid, proved to be completely ineffective in this test; the only effect observed using analgesic drugs is a potentiation of the effect induced by local anaesthetics (9).

In this work we have tested several monoterpenes and phenylpropanes, typical components of essential oils, differing in structure and in their functional groups, i.e., $\alpha$-terpinene (hydrocarbon), terpineol (alcohol), (–)- and (+)-carvone (ketons), (–)-citronellal, (+)-citronellal and *trans*-cinnamaldehyde (aldehydes), eugenol (phenol), anethole (phenolic ether). Although it is not possible to outline a structure-activity relationship on the basis of present results, it can nevertheless be pointed out, when considering these data, along with those obtained previously (4), (5) with linalool and menthol, that local anaesthetic activity appears more frequently in terpene alcohols.

## Materials and Methods

Male Wistar rats (150 – 200 g), guinea-pigs (300 – 400 g) and New Zealand albino rabbits (2.5 – 3.0 kg) from the Morini (San Paolo d'Enza, Italy) breeding farm were used. All experiments were carried out according to the guidelines of the European Council on animal care.

Experiments on the rat phrenic nerve-hemidiaphragm were performed according to Bülbring (10), and as modified by Wessler and Kilbinger (11).

The rabbit conjunctival reflex test was carried out according to the method described by Donatelli and Buffoni (12). The external side of the rabbit eye was stimulated with a cat whisker to induce the conjunctival reflex and consequently the closure of the eyelids. Data were analysed by analysis of variance (ANOVA), followed by Fisher's Protected Least Significant Difference (PLSD) procedure for post-hoc comparison, to verify significance between two means.

The following drugs (purity higher than 90%) were used: eugenol, terpineol, (–)-citronellal, *trans*-anethole, $\alpha$-terpinene (Sigma, Milan, Italy); (+)-citronellal, *trans*-cinnamaldehyde, (–)-carvone, (+)-carvone, (Aldrich, Milan, Italy); procaine hydrochloride (RBI, Milan, Italy). Monoterpenes and phenylpropanes were solubilised in a solution of DMSO in $H_2O$ (1 : 4) in order to obtain a concentration of 1000 $\mu$g/ml, then were diluted in $H_2O$; procaine was solubilised in $H_2O$.

## References


[1] Taddei I, Giachetti D, Taddei D, Mantovani P, Bianchi E. Spasmolytic activity of peppermint, sage and rosemary essences and their major constituents. Fitoterapia 1988; 59 (6): 463 – 8

[2] Mazzanti G, Lu M, Salvatore G. Spasmolytic action of the essential oil from *Hyssopus officinalis* L. var. *decumbens* and its major components. Phytotherapy Research 1998; 12 (Supplements): 92 – 94

[3] Van Den Brouke CO, Lemli JA. Antispasmodic activity of *Origanum compactum*. Planta Medica 1980; 38: 317 – 31

[4] Ghelardini C, Galeotti N, Salvatore G, Mazzanti G. Local anaesthetic activity of the essential oil of *Lavandula angustifolia*. Planta Medica 1999; 65: 700 – 3

[5] Galeotti N, Ghelardini C, Mannelli Di Cesare L, Mazzanti G, Braghiroli L, Bartolini A. Local anaesthetic activity of (+)- and (–)-menthol. Planta Medica 2001; 67: 174 – 6

[6] Van Hellemont J. *Origanum majorana*. In: Compendium de Phytotherapie. Association Pharmaceutique Belge Bruxelles: 1986: 272 – 3

[7] Newall CA, Anderson LA, Phillipson JD. Aniseed. In: Herbal Medicines – A guide for health-care professionals. The Pharmaceutical Press London: 1996: 30 – 1

[8] Brodin P, Roed A. Effects of eugenol on rat phrenic nerve and phrenic nerve-diaphragm preparations. Archives of Oral Biology 1984; 29(8): 611 – 5

[9] Ghelardini C, Galeotti N, Fantetti L, Scapecchi F, Bartolini A. Antinociceptive effect of *R*-(+)-hyoscyamine on the conjunctival reflex test in rabbits. Japanese Journal of Pharmacology 1999; 81: 34 – 7

[10] Bülbring E. Observation on the isolated phrenic nerve-diaphragm preparation on the rat. British Journal of Pharmacology 1946; 1: 38 – 61

[11] Wessler I, Kilbinger H. Release of [$^3$H] acetylcholine from a modified rat phrenic nerve-hemidiaphragm preparation. Naunyn-Schmiedeberg's Archives of Pharmacology 1986; 334: 357 – 64

[12] Donatelli L, Buffoni F. Potenziamento dell'anestesia locale operato da mefenidina. Archivio Italiano della Società di Farmacologia 1948; 1: 4 – 20



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