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Influence of the Chirality of (*R*)-(−)- and (*S*)-(+)-carvone in the Central Nervous System: A Comparative Study

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ABSTRACT Many terpenes are used therapeutically, and as flavor and fragrance materials. (*R*)-(−)-Carvone, the main constituent of spearmint oil, and (*S*)-(+)-carvone, found as major component of caraway and dill seed oils, have several applications and are used in cosmetic, food, and pharmaceutical preparations. In this study, the effect of enantiomers of carvone on the central nervous system (CNS) was evaluated in mice. The LD_{50} value was 484.2 mg/kg (358.9–653.2) for (*S*)-(+)-carvone, and 426.6 (389.0–478.6) mg/kg for (*R*)-(−)-carvone. Both enantiomers caused depressant effects, such as decrease in the response to the touch and ambulation, increase in sedation, palpebral ptosis, and antinociceptive effects. (*S*)-(+)- and (*R*)-(−)-carvone caused a significant decrease in ambulation. (*R*)-(−)-Carvone appeared to be more effective than its corresponding enantiomer at 0.5 and 2.0 h after administration. However, (*S*)-(+)-carvone was slightly more potent at 1 h. In potentiating pentobarbital sleeping time, (*R*)-(−)-carvone was more effective than (*S*)-(+)-carvone at 100 mg/kg, but was less potent at 200 mg/kg compared to the (+)-enantiomer, indicating a sedative action. (*S*)-(+)-Carvone at the dose of 200 mg/kg increased significantly the latency of convulsions induced by PTZ and PIC, but (*R*)-(−)-carvone was not effective against these convulsions. These results suggest that (*S*)-(+)-carvone and (*R*)-(−)-carvone have depressant effect in the CNS. (*S*)-(+)-Carvone appears to have anticonvulsant-like activity. *Chirality 19:264–268, 2007.*

KEY WORDS: anticonvulsant activity; sedative effect; spearmint; caraway; *p*-menthane; essential oils; monoterpene; structure–activity relationship

INTRODUCTION

Carvone (*p*-mentha-6,8-dien-2-one) is a monoterpene ketone representative of the terpenes. It is the main active component of the oil of spearmint (*Mentha spicata*), a relative of common mint, and is distilled from the leaves of this plant.[1] Among terpene chirogens, the carvones [(+)- and (−)-forms] are probably the most versatile. The most well-known source of (*R*)-(−)-carvone is spearmint oil, whereas its enantiomer is a constituent of dill and caraway oils. The racemate occurs in gingergrass oil. The cost of (*R*)-(−)-carvone is much less than that of the (*S*)-(+)-carvone.[2–4]

Caraway seeds are used as a flavoring in bread, cheese, sauerkraut, candies, meat products, sauces, and alcoholic liqueurs, and as a source of carvone for cosmetics, toothpaste, chewing gum, and pharmaceutical preparations. The main constituents of the seed are (*S*)-(+)-carvone (50–70%) and (+)-limonene (25–30%). Dill seeds have been used to flavor cakes and pastries, soups, salads, potatoes, meats, sauerkraut, and pickles. Dill seed has an oil, of which 40–60% is (*S*)-(+)-carvone. Spearmint is mostly used in cuisine. Spearmint oil has a minimum of 51% (*R*)-(−)-carvone.[2,5]

In folk medicine as well as in phytotherapy, the essential oils have been used as therapeutic agents, for example as sedatives, relaxants, or anticonvulsants.[6,7] Many essential oils possess a great variety of pharmacological activities, such as anxiolytic,[8] anticonvulsant,[7] and antinociceptive[9] actions. Evidence for the effects of their components on behavior has been supplied. Compounds such as linalool,[10] limonene,[11] and citronellol[12] have anticonvulsant activity, while menthol[13] and myrcene[14] have analgesic

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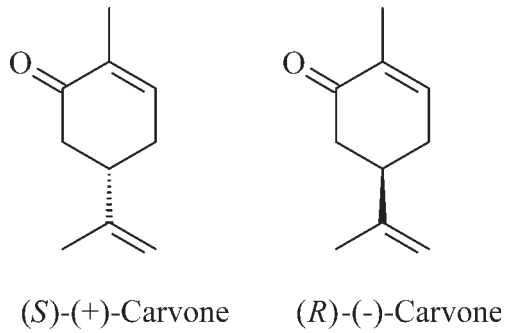

Fig. 1. Chemical structures of carvone.

activity. The monoterpene derivatives also have been shown to have several effects on the central nervous system (CNS), including antinociceptive,[15] sedative,[16] and antidepressant[17] activity.

There are few scientific studies that show the effects of carvone in the CNS. Recently the effect of (*S*)-(+)- and (*R*)-(−)-carvone on locomotion activity in mice was reported after inhalation.[18] In our earlier studies, we investigated the structure–activity relationship of the analogues of rotundifolone, a monoterpene isolated from the essential oil of the leaves of *Mentha* × *villosa*. In this investigation, the monoterpene (*R*)-(−)-carvone was slightly more antinociceptive than its enantiomer (*S*)-(+)-carvone. Therefore, in continuation in the present paper, we studied the properties of (*S*)-(+)- and (*R*)-(−)-carvone on the CNS, exploring differences between the enantiomers with respect to their pharmacological effects.

MATERIALS AND METHODS

Chemical

(*S*)-(+)-Carvone and (*R*)-(−)-carvone were purchased from Aldrich (USA) (Fig. 1), and dissolved in 5% Tween 80 as an emulsion. Pentobarbital, pentylenetetrazole (PTZ), picrotoxin (PIC), diazepam, and polyoxyethylene-sorbitan monolate (Tween 80) were purchased from Sigma (USA).

Animals

Male Swiss mice (28–34 g) were obtained from our research animal facility. The animals were maintained at constant room temperature (21°C ± 1°C) and on a 12/12-h light-dark cycle (light from 06:00 to 18:00), with free access to food and water. All behavioral observations were conducted between 08:00 and 17:00 h and carried out in accordance with ethical committee approvals.

Statistical Analysis

The statistical analysis was performed using analysis of variance, followed by the Newman–Keuls post-hoc test. A probability level of 0.05 was regarded as significant.

Acute Toxicity and Behavioral Effects

Different doses of (*S*)-(+)-carvone and (*R*)-(−)-carvone were administered intraperitoneally (ip) to groups of mice ($n = 10$), and mortality was recorded for 48 h for the determination of LD_{50}.[19] The behavioral screening of the mice was performed at 0.5, 1, and 2 h after injection of (*S*)-(+)-carvone and (*R*)-(−)-carvone (200 mg/kg, ip).[20]

Pentobarbital-Induced Sleeping Time

Sodium pentobarbital at a hypnotic dose of 40 mg/kg (ip) was injected to three groups ($n = 8$) of mice 30 min after pretreatment with 0.9% saline (control), (*S*)-(+)-carvone, or (*R*)-(−)-carvone at doses of 50, 100, and 200 mg/kg, ip, respectively. The duration of sleep time (loss and recovery of the righting reflex) was recorded.[10]

Locomotor Activity

Mice were divided into three groups of eight each. Vehicle (control), (*S*)-(+)-carvone, and (*R*)-(−)-carvone (200 mg/kg, ip) were injected. The spontaneous motor activity of the animals was assessed in an activity cage (controller model 7441 and Grid-Floor Detecting Arrangement Cage model 7432; Ugo Basile, Italy) at 0.5, 1, and 2 h after administration.

PTZ-Induced Convulsions

Mice were divided into five groups ($n = 8$). The control and positive control groups received 5% Tween 80 or diazepam (4 mg/kg), respectively. The remaining groups received an injection of (*S*)-(+)-carvone or (*R*)-(−)-carvone at doses of 50, 100, or 200 mg/kg. Thirty minutes after drug administration, the mice were treated with PTZ (ip) at a dose of 60 mg/kg and observed for at least 15 min to detect the occurrence of the first episode of forelimb clonus.[21]

PIC-Induced Convulsion

Animals were divided into four groups ($n = 8$), the first group served as control and received 5% Tween 80, while the second group was treated with diazepam (4 mg/kg, ip). The remaining groups received an injection of (*S*)-(+)-

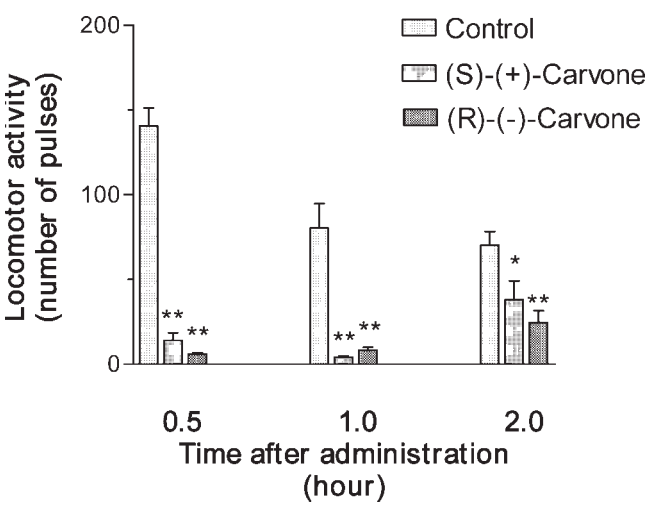

Fig. 2. Effect of (*S*)-(+)-carvone and (*R*)-(−)-carvone on locomotor activity on mice. The parameters evaluated were the total number of pulses measured in activity cage. Values are the mean ± SEM for eight mice; $*P < 0.05$, $**P < 0.01$ when compared with vehicle (control), one-way ANOVA, followed by Newman–Keuls post-hoc test.

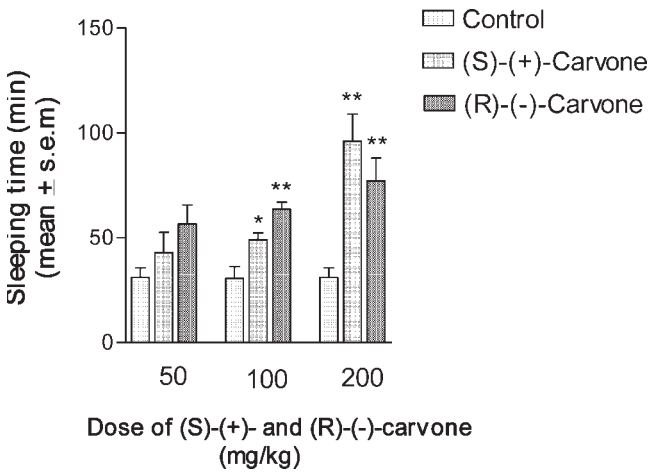

Fig. 3. Effect of (*S*)-(+)-carvone and (*R*)-(−)-carvone on pentobarbital-induced hypnosis in mice. Values are the mean ± SEM for eight mice; $*P < 0.05$, $**P < 0.01$, when compared with vehicle (control), one-way ANOVA, followed by Newman–Keuls post-hoc test.

carvone or (*R*)-(−)-carvone (200 mg/kg, ip) 30 min after of drug administration, and the mice were treated with PIC at a dose of 8 mg/kg (ip). Immediately after the injection of the convulsant agent, mice were placed individually in plastic boxes and observed for the onset of clonic seizures.[22]

RESULTS

Acute Toxicity

The LD_{50} values of (*S*)-(+)-carvone and (*R*)-(−)-carvone after administration to mice were 484.2 mg/kg (95% confidence limits 358.9–653.2 mg/kg) and 426.6 mg/kg (95% confidence limits 389.0–478.6 mg/kg), respectively.

Behavioral Effects

(*S*)-(+)-Carvone (200 mg/kg) showed depressant action, decreased the response to touch and ambulation, increased sedation, and presence of palpebral ptosis at 0.5 and 1 h after administration. It also had an antinociceptive effect at 1 and 2 h, with palpebral ptosis. (*R*)-(−)-Carvone (200 mg/kg) decreased the response to touch and ambulation, increased sedation, antinociceptive effect, and palpebral ptosis and catalepsy at 0.5 and 1 h. At 2 h it showed only palpebral ptosis and catalepsy.

Locomotor Activity

At doses of 200 mg/kg (*S*)-(+)-carvone and (*R*)-(−)-carvone significantly decreased ambulation at 0.5, 1, and 2 h after administration (Fig. 2).

Pentobarbital-Induced Hypnosis

(*S*)-(+)-Carvone and (*R*)-(−)-carvone increased sleeping time at 100 and 200 mg/kg compared with control animals (Fig. 3).

PTZ-Induced Seizure

The latency of convulsions was prolonged significantly for (*S*)-(+)-carvone at dose of 200 mg/kg against PTZ-induced convulsions (Fig. 4).

PIC-Induced Seizure

(*S*)-(+)-Carvone at dose of 200 mg/kg against PIC-induced convulsion produced a significant increase of the latency. The positive control drug diazepam (4 mg/kg) also caused an increase of the latency (Fig. 5).

DISCUSSION

Because the carvone enantiomers are common in many plant species, and are used in cosmetic and pharmaceutical preparations, as well as in the food industry, it is important to know the effects and the enantioselectivity of the CNS receptors of these monoterpenes. Chiral recognition by receptors and enzymes is well demonstrated in biochemical, pharmaceutical, and chemosensory research. We report in this comparative study the findings from the central effects of (*S*)-(+)- and (*R*)-(−)-carvone on toxicological and behavioral parameters in mice.

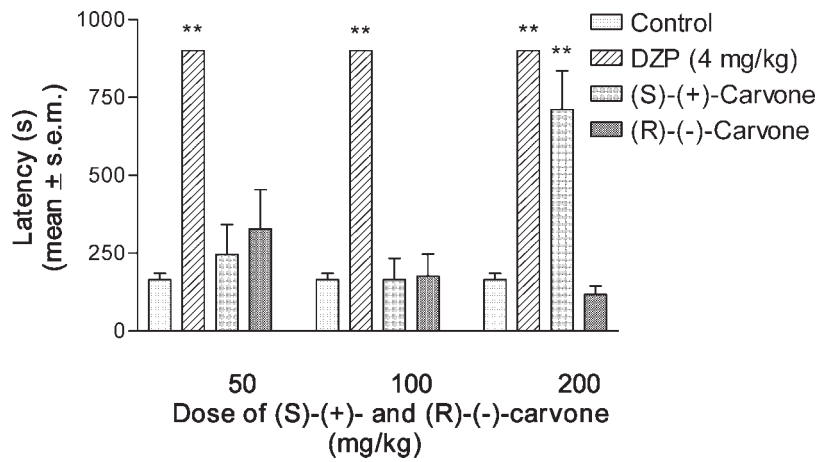

Fig. 4. Effects of (*S*)-(+)-carvone and (*R*)-(−)-carvone on PTZ-induced seizure in mice. Values are the latency of convulsions. Values are presented as mean ± SEM for eight mice; $**P < 0.01$, when compared with vehicle (control), one-way ANOVA, followed by Newman–Keuls post-hoc test.

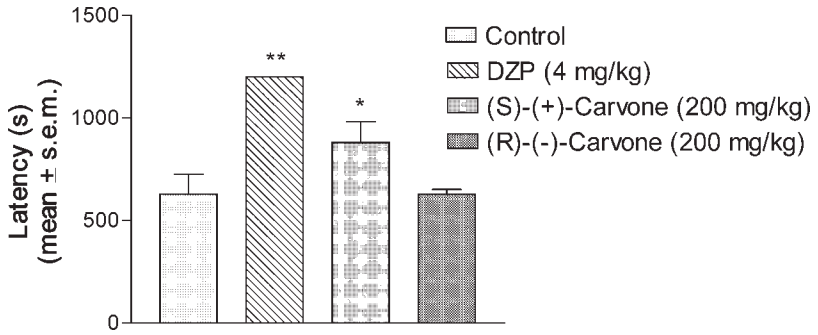

Fig. 5. Effects of (*S*)-(+)-carvone and (*R*)-(−)-carvone on PIC-induced seizure in mice. Values are the latency of convulsions. Values are presented as mean ± SEM for eight mice; $*P < 0.05$, $**P < 0.01$, when compared with vehicle (control), one-way ANOVA, followed by Newman–Keuls post-hoc test.

(*S*)-(+)-Carvone was less toxic than (*R*)-(−)-carvone. The parameters of the behavioral screening were suggestive of a central depressant effect.[20] Both (*S*)-(+)- and (*R*)-(−)-carvone presented characteristic effects of depressant drugs because the animals were found to be dull, calm, and relaxed. Comparing the behavioral changes of the carvones, (*R*)-(−)-carvone showed antinociceptive effect only up to 1 h. This monoterpene also differed from (*S*)-(+)-carvone by the induction catalepsy in the mice. The depressant action on CNS was confirmed by the significant decrease of ambulation. (*R*)-(−)-Carvone appeared to be more effective than its enantiomer at 0.5 and 2.0 h after administration. However, (*S*)-(+)-carvone was slightly more potent at 1 h. The carvones also potentiated pentobarbital-induced sleeping time, which may be attributed to an action on the central mechanisms involved in the regulation of sleep or inhibition of pentobarbital metabolism.[23,24] In this test (*R*)-(−)-carvone was more effective than (*S*)-(+)-carvone at 100 mg/kg, but was less potent at 200 mg/kg when compared with the (+)-enantiomer. The present results showing differences in sedative effect of enantiomers of carvone are in agreement with the obtained results in mice after inhalation of carvones.[18] Administration of PTZ caused clonic convulsions in mice. Pretreatment of the mice with (*S*)-(+)-carvone at the dose of 200 mg/kg increased the latency of convulsions significantly, close to that of diazepam, a standard anticonvulsant drug. (*R*)-(−)-Carvone had no effect on the onset of convulsions. The present result shows that the chiral center at carbon 4 in the carvone molecule is important in the interaction with the receptor. The molecule with isopropenyl group in *S* configuration at carbon 4 is clearly capable to reduce the convulsive effect of PTZ in terms of onset time. PTZ is the prototype agent in the class of systemic convulsants, and is used as a screening test for anticonvulsants in part because the anti-absence drug, ethosuximide, which is effective against PTZ-induced seizures, fails to alter MES thresholds. Therefore, it became common practice to presume that drugs effective against PTZ seizures would be potential anti-absence therapies. The mechanism of action of PTZ is only partially understood. At a synaptic level, PTZ appears to interact with the (GABA receptor–benzodiazepine–chloride ionophore) complex, decreasing the potency of inhibition and leading to seizures.[25] The enhancement of neural inhibition by GABA is a common therapeutic strategy for treating CNS diseases such as sleep disturbances, muscle spasms, and seizure disorders.[26] Generally, compounds with anticonvulsant activity against petit mal epilepsy are effective in PTZ-induced seizure model.[27] So (*S*)-(+)-carvone may be useful in petit mal epilepsy. (*S*)-(+)-Carvone also inhibited the action of PIC, but (*R*)-(−)-carvone had no prolonged the onset of convulsions induced. This is a popular systemic convulsant and is known to be a GABA antagonist exerting its effect by binding to the PIC-binding site which is closely related to the chloride ionophore in the $GABA_A$ receptor complex. Classical anticonvulsants such as carbamazepine, phenytoin, and DZP have a protective effect against PIC-induced seizures.[28]

It has been reported that some ketonic compounds have anticonvulsant properties and act at the PIC receptor to decrease neuronal activity.[29] Recent reports have demonstrated that some fragrance compounds in essential oils, which increase the pentobarbital-induced sleep time in mice and potentiate the $GABA_A$ receptor-mediated response, such as a cyclic ketone the cis-jasmone, might have a tranquillizing effect.[30] The activity of other oxygenated monoterpenes on the $GABA_A$ receptor has been shown, for example, (+)- and (−)-borneol, and α-thujone.[31,32] Therefore, our experiments suggest that possibly the carvones interacted with $GABA_A$ receptors in the brain after crossing the blood–brain barrier.

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