Evaluation of the Central Activity of Hydroxydihydrocarvone

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The central effects of hydroxydihydrocarvone (HC), an analogue of several monoterpenes, were evaluated in animal models. General behavior, locomotor activity, pentobarbital-induced sleeping time, pentylenetetrazol (PTZ)-induced convulsions, and acetic acid-induced writhing were evaluated in mice. The compound caused palpebral ptosis, decreased the response to touch, and increased sedation (general behavioral profile). HC (50—200 mg/kg) caused a significant dose-dependent decrease in the spontaneous motor activity of mice. This compound (100, 200 mg/kg) potentiated the pentobarbital sleeping time, indicating a depressant action. HC also protected the mice against PTZ-induced convulsions at 400 mg/kg. In the acetic acid-induced writhing, the antinociceptive activity of HC was demonstrated with a significant dose-dependent response at a dose range of 25—400 mg/kg. The present results provide evidence that HC has significant psychopharmacologic activity with depressant effects.

Key words hydroxydihydrocarvone; essential oil; monoterpene; antinociceptive activity; anticonvulsant activity; carvone

Essential oils are natural products with different applications, especially in the area of medicine and cosmetics. They contribute to the perception of natural flavors and fragrances.[1,2] In addition, many of them are found to exhibit a variety of biologic properties, such as analgesic,[3] anticonvulsant[4] and anxiolytic[5,6] activity. These effects are probably due to the great structural diversity of essential oil constituents. This notion is supported by previous studies that showed that some monoterpenes present in many essential oils possess sedative, antinociceptive, and anticonvulsant activity in animal experiments, such as linalool.[7,8] Hydroxydihydrocarvone (HC) is a synthetic intermediate prepared by hydration of the monoterpene *R*-(−)-carvone. This compound has functional groups and structural similarities with some monoterpenes with pharmacologic activity.[9,10] In the light of these observations, it was of interest to us to evaluate the psychopharmacologic profile of HC in mice.

MATERIALS AND METHODS

Chemical Compound HC (Fig. 1) was prepared in our laboratory as previously described,[11] and dissolved in 5% Tween 80 as an emulsion.

Animals Male Swiss mice (28—34 g) were obtained from our research animal facility. The animals were maintained at constant room temperature (26±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 13:00 and 18:00.

Statistical Analysis The statistical analysis was performed using analysis of variance followed by Dunnet's test or Kruskal–Wallis analysis of variance on ranks followed by Dunn's test. A probability level of 0.05 was regarded as significant.

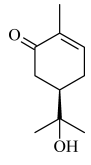

Fig. 1. Chemical Structure of Hydroxydihydrocarvone

Acute Toxicity and Behavioral Effects The toxicity study was performed with different doses of HC to groups of mice ($n=10$) administered intraperitoneal, and mortality was recorded for 48 h for the determination of LD_{50}.[12] The behavioral screening[13] of the mice was performed at 0.5, 1, and 2 h after injection of HC (200 mg/kg i.p.).

Locomotor Activity Mice were divided into five groups of 10 each. Vehicle (control) and HC (25, 50, 100, 200 mg/kg i.p.) were injected. The spontaneous motor activity of the animals was assessed in cages measuring 30×48×48 cm lined with a floor demarcated in squares measuring 12×12 cm. Thirty minutes after treatment, the number of squares traversed was recorded cumulatively every 5 min.[14]

Pentobarbital-Induced Sleeping Time Sodium pentobarbital at a hypnotic dose of 40 mg/kg i.p. was injected in four groups ($n=8$) of mice 30 min after pretreatment with 0.9% saline (control) and HC (50, 100, 200 mg/kg) i.p., respectively. The duration of sleep time (loss and recovery of the righting reflex) was recorded.[7]

Pentylenetetrazol-Induced Convulsions Mice were divided into three groups ($n=8$). The first group served as the control and received saline 0.9%, while the second group was treated with diazepam (5 mg/kg). The remaining group received an injection of HC 400 mg/kg. After 30 min, the mice were treated with pentylenetetrazol (PTZ) at a dose of 60 mg/kg i.p. and observed for at least 15 min to detect the occurrence of the first episode of forelimb clonus.[15]

Acetic Acid-Induced Writhing The mice were divided into eight groups ($n=8$). The first group was pretreated with saline 0.9% (control). HC (12.5, 25, 50, 100, 200, 400 mg/kg i.p.) and morphine (3 mg/kg i.p.) were administered. After 30 min an acetic acid solution (0.8%; 0.1 ml/10 g i.p.) was injected. After a further 10 min, the number of constrictions was recorded for 10 min.[10]

RESULTS AND DISCUSSION

In this study, the effects of HC were evaluated for central pharmacologic activity. The LD_{50} value of HC after administration to mice was 800.2 mg/kg, with 95% confidence limits

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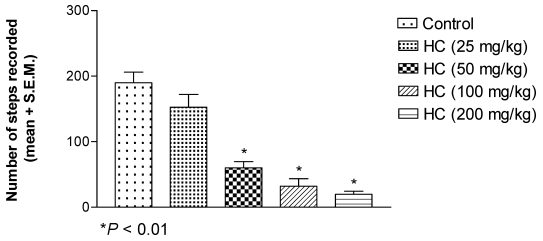

Fig. 2. Effects of on Spontaneous Motor Activity

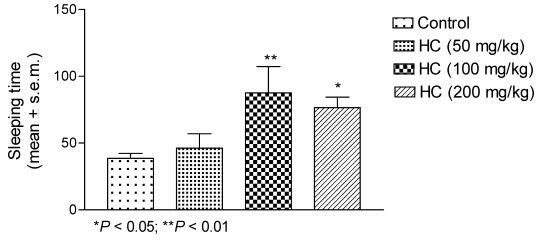

Fig. 3. Effects of HC on Pentobarbital (40 mg/kg) Induced Sleeping Time (min) in Mice

Table 1. Evaluation of HC Effects on PTZ-Induced Convulsive Seizures in Mice

Treatment	Dose (mg/kg)	Latency (s) (mean±S.E.M).
Saline (control)	—	143.1±19.4
Diazepam	5	900.0±0.0**
HC	200	210.9±64.4
	400	365.1±107.5*

*$p<0.05$; **$p<0.01$; $n=8$.

of 724.6—883.6 mg/kg. In the behavioral screening, HC (200 mg/kg) showed depressant action based on the decrease in the response to the touch, increase in sedation, and presence of palpebral ptosis at 0.5 h and 1 h after administration. It also had antinociceptive effects at 2 h, but without palpebral ptosis. Treatment of mice with HC (50, 100, 200 mg/kg) caused a significant dose-dependent decrease in spontaneous motor activity (Fig. 2), indicating a central depressant effect. This central effect of HC (100, 200 mg/kg) was confirmed by an increase in the pentobarbital-induced sleeping time (Fig. 3), indicating sedative activity. However, the effect was not dose dependent. HC was screened for anticonvulsant and antinociceptive activity. The effects on PTZ-induced convulsions are shown in Table 1. HC was effective in protecting against PTZ-induced convulsions at 400 mg/kg, using diazepam (5 mg/kg) as a positive control. In the evaluation of the antinociceptive profile, HC (25, 50, 100, 200, 400 mg/kg) decreased the incidence of acetic acid-induced writhing (Table 2). It induced a significant dose dependent antinociceptive response. Similar to morphine (3 mg/kg), HC 200 mg/kg produced near-maximal inhibition of the writhing response. At the dose of 400 mg/kg, HC showed maximal inhibition.

Table 2. Effects of HC and Morphine on Acetic Acid-Induced Writhing

Treatment	Dose (mg/kg)	Number of writhings
Saline (control)	—	14.2±1.3
Morphine	3	1.1±0.3*
HC	12.5	11.0±2.9
	25	6.7±1.4*
	50	6.0±1.7*
	100	4.5±1.5*
	200	1.6±0.8*
	400	0.0±0.0*

*$p<0.01$; $n=8$.

The data reported in this paper demonstrate the psychopharmacologic activity of HC. The present study showed that HC has a central nervous system depressant effect in mice similar to some monoterpenes. This effect of HC was not different from that observed for the monoterpenes.[9,10]

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