

Antinociceptive Effects of Carum Copticum Extract in Mice Using Formalin Test

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Abstract: Medicinal plants are an important source of medications. Pain is a universal complaint, which needs further investigations for new pain relieving agents. Carum Copticum L. is a plant in umbelliferae family is used as an antinociceptive medication in Iranian folk medicine, but there are not enough scientific reports to prove its effect on pain using formalin test. So, we conducted to design an experimental trial study to assess and compare the antinociceptive effect of hydroalcoholic extract of Carum Copticum fruit with morphine sulphate by using formalin test. The results showed that Carum Copticum L. extract had antinociceptive effect on both early and late phases, which showed more antinociceptive effect on late phase than early phase. The present study supports the traditional use of it in Iran as an antinociceptive medication. However, further investigations are required to study the efficacy and safety of this herbal medication in man.

Key words: Carum copticum • antinociceptive • formalin test • mice

INTRODUCTION

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. Thus, study of plant species that traditionally have been used as pain killers should still be seen as a strategy in research for new antinociceptive drugs. Carum Copticum L. is a plant in Umbelliferae family and its seeds contain an aromatic volatile essential oil and a crystalline substance called stearoptene. The stearoptene is known as crude thymol [1-4]. It has been mentioned in Iranian traditional literature that Carum Copticum has therapeutic effects on flatulence, indigestion, colic, dyspepsia and diarrhea [4-6]. But there are a few scientific reports about the therapeutic effects mentioned above. So, we decided to study the antinociceptive effect of ethanolic extract of Carum Copticum fruit in compare with different doses of morphine sulphate in mice.

MATERIALS AND METHODS

The experimental protocol used in this study was approved by the ethics committee of the Yazd Shahid Sadoughi University, Yazd, Iran.

Plant material: The Carum Copticum was authenticated by herbal museum of the faculty of agriculture, Azad Meybod University, Meybod, Yazd, Iran. The plant extract was prepared by maceration of 50 g of the chopped and dried fruit of Carum Copticum in a mixture of ethanol and distilled water (200/200 ml) and shaking them for 48 hours and extracting the solution using a filter press. Then, the solvent was removed until the extract was completely dried like gum.

Animal material: Male Balb C mice (Mus Domesticus) weighed 30-35 g were obtained from the Yazd University animal house in Iran. They were kept at standard environmental conditions (12/12h light/dark cycle) and were allowed free access to food (standard pellet diet) and water ad libitum. The mice were randomly divided into four groups of seven as control, sham and test subjects.

Preparation of dose: The dose of 10 mg kg⁻¹ of the extract was used. The dose was selected based on the extract dry weight. Normal saline 0.9 % was used as solvent. Concentrations of 1 and 2 mg kg⁻¹ of morphine sulphate, 10 mg kg⁻¹ of the extract in a volume of 0.5 ml normal saline and 0.5 ml normal saline in control group were administered interperitoneally (IP) 30 min before formalin injection to animals.

Formalin test: Each animal was allowed 15 min to explore the chamber before injection and its behavior was rated according to the scale described below. These data constituted the pain-free baseline. Each mouse received 25 µl formalin 1% subcutaneously into the dorsal surface of the right hind paw using a microsyringe. Each animal was placed individually on a flat glass floor under the chamber and a mirror was arranged at an angle of 45°C under the glass to allow clear observation of the paw of the animal. The mouse was observed continuously by a blinded observer for 60 min. Formalin injection was associated with an early phase (0-15 min) and late nociceptive phase (15-60). The antinociceptive activity of the compound was determined using the method described by Dubuisson and Denis and Abbot *et al.* [7,8].

- The injected paw is elevated and not in contact with any surface. The uninjected paw is placed firmly on the floor.
- The injected paw is licked, bitten or shaken, while the uninjected paw is not.

The mouse was observed for 60 minutes after the injection of formalin and the amount of time (sec) spent in each scale (0, 1, 2 and 3) was recorded.

Ratings are averaged over 3min blocks. Numerical ratings are calculated from the following formula:

$$\text{Pain rating} = \frac{T1+2T2+3T3}{180}$$

Where T1, T2 and T3 are the durations (in sec) spent in categories 1, 2 or 3 respectively during each 3min block.

Four groups, each containing seven mice, were run:

- A control group received formalin and 2ml normal saline (IP).
- A 10mg kg⁻¹ extract test group
- A sham group received 1 mg kg⁻¹ morphine sulphate
- A sham group received 2 mg kg⁻¹ morphine sulphate

Statistical analysis: Comparison between groups were made by one-way analysis of variance (ANOVA) followed by Tukey test. Differences with P<0.005 between experimental groups were considered statistically significant. SPSS was used to analyze the data.

Pain intensity was rated according to the following numerical scale:

- Both forepaws are placed on the floor and weight is evenly distributed.
- The injection paw rests lightly on the floor or on another part of the animal's body and little or no weight is placed upon it.

RESULTS

Figure 1 shows the baseline and pain rating curves for the four groups of mice. Ratings are averaged over 3min blocks. The left hand side of the Fig.1 shows the baseline data. The average rating given to these do not favor one forepaw over the other. The formalin injected

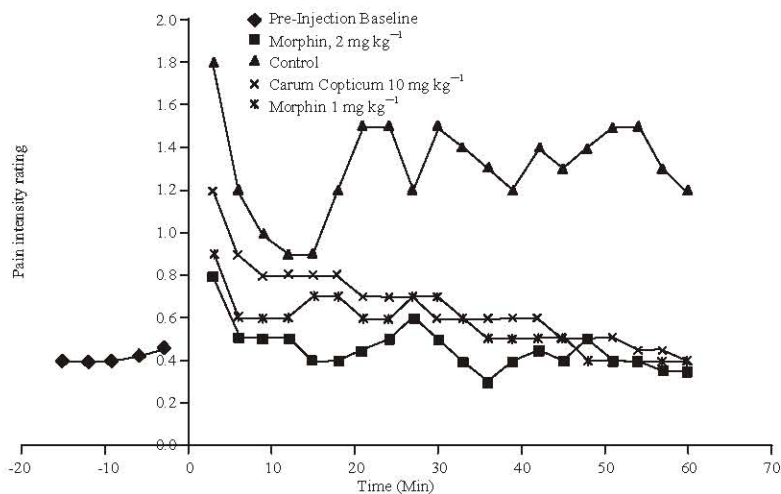


Fig. 1: Quantitative data from the 4 groups of mice used. On the ordinate is pain intensity; lower values signify less pain. On the abscissa is time. The zero time point represents the pain rating in the first 5 seconds following injection. The remaining points represent weighted averages over 3 min blocks

paw in control group mice were shaken, licked or bitten and generally kept elevated.

The results showed that *Carum copticum* L. extract had antinociceptive effect on both early and late phases, which showed more antinociceptive effect on late phase than early phase. The results of Tukey test showed a significant difference ($P < 0.5$) between the test group with control group on both phases. On the late phase, there was no difference between the test group and the group received 1 mg kg^{-1} morphine sulphate.

DISCUSSION

Pain is a universal complaint that needs further studies for new antinociceptive agents. *Carum copticum* L. has been used as a traditional medicine in some part of Iran. It was not found any scientific reports concerning the antinociceptive effects of *Carum copticum* using formalin test. But there are some reports of its antiparasitic effect [9], antigout effect [10], antihelminthic effect [11], antihistaminic and relaxant effects on guinea pig tracheal chains [12, 13] and cholinomimetic effect [14]. Some new reports indicate that it has antitussive effect in guinea pigs [15] and Bronchodilatory effect in airways of asthmatic patients [16]. Antifungal activity of *Carum copticum* is also reported [17]. Also it has been reported to have antidiarrheotic activity [9] and antibacterial activity [18]. Thangam and Dhananjayan [19] reported the anti-inflammatory effect of *Carum copticum* seed extract. In the present study, which used a model of acute analgesiometric test, the antinociceptive action for the alcoholic extract of *Carum copticum* L. was found the antinociceptive effect of the same type of morphine sulphate. As well it might be due to its parasympatometric action. The results of this study parallel the traditional use of it in some parts of Iran. The mechanism of action and its efficacy and safety in man remain to be elucidated by further studies.

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