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The analgesic activity of *Mentha piperita* (MP) leaves extract

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Summary

In the present work, the antinociceptive action was assayed in several experiment models in mice, Hot plate, writhing and formalin test. The alcoholic extract of MP leaves at a dose of 150mg/kg B.W and 300 mg/kgB.W showed antinociceptive effects in different methods, where the dose of 300 mg/kg B.W showed significant reduction of the nociception by acetic acid. In the formalin test, the extract (300mg/kg B.W) also significantly reduced painful stimulus in both phases of the test. Treatment with extract (300mg/kg B.W) when given orally produced significant increase of the reaction time in hot plate test. These results showed that the leaves extract of MP contain active analgesic principles acting both centrally and peripherally.

Key words:analgesic, menthe piperita,leaveextract.

الفعالية التسكينية لخلاصة أوراق النعناع

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الخلاصة

استعمل في هذه الدراسة ثلاثة اختبارات لإحداث الألم في الفئران وهي اختبار الصفيحة الساخنة واختبار حامض الخليك واختبار الفورمالين لمعرفة التأثير المسكن لمستخلص أوراق النعناع . أظهر المستخلص الكحولي لأوراق النعناع وبجرعة 150 ملغم/ كغم من وزن الجسم و 300 ملغم/ كغم من وزن الجسم تأثيرا" مسكنا" للألم في الاختبارات المختلفة ، إذ أظهرت جرعة المستخلص 300 ملغم/كغم من وزن الجسم انخفاضا" معنويا" للإحساس بالألم في اختبار حامض الخليك مقارنة مع حيوانات السيطرة ، كما اظهر المستخلص في اختبار الفورمالين وبجرعة 300 ملغم/كغم من وزن الجسم انخفاضا" معنويا" بالمقارنة مع مجموعة السيطرة في الاستجابة للحافز المسبب للألم في كلا الطورين المبكر والمتأخر . أدى العلاج بالمستخلص بجرعة 300 ملغم/ كغم من وزن الجسم أيضا" إلى زيادة معنوية في زمن الاستجابة للألم في اختبار الصفيحة الساخنة . تُظهِر هذه النتائج إن مستخلَص أوراق نبات النعناع له فعل مسكّن ويعمل بصورة مركزية ومحيطية .

Introduction

Plants are the oldest friend of man kind; they not only provide food and shelter but also serve humanity by preventing and curing ailment. Peppermint (English name) and Latin name is Mentha piperita (MP)is one of these plants which is widely used in food, cosmetic and medicine. The plant M.piperita is a perennial, 50-60cm high, the square stems are usually reddish-Purple and smooth, the leaves are short, oblong-ovate and serrated(1).Peppermin leaves contains about 0.5-4% volatile oil that is composed of 50-78% free menthol, monoterpene, menthofurane and trace of jasmine (0.15%) to improve the oil quality (2),also the plant contain flavonoids, tannins, monoterpenes and caffeic acid(3). Peppermint is a medicinally important plant which

have broad spectrum of activity, a combination of peppermint oil and ethanol lead to significant analgesic effect with reduction in sensitivity to headache(4),also external application of peppermint extract raised the pain threshold in human(5),Davies(6) noted a case studied of 76-years old woman whose pain had been resistant to standard therapies, the application of peppermint oil on her skin resulting in significant decrease in her pain(6), also peppermint have antibacterial action (7), it may also increase the flow of bile from the gall bladder (8).Aqueous extract of peppermint leaves have antiviral action against influenza A, newcastle disease virus in egg and cell culture system(9). Peppermint have antihelmenthic activity which at a concentration of 20 mg/ml caused death of the worms (10). Peppermint also exhibited dose dependent inhibition on mycelial growth of *Fusarium oxysporum* (11).In the present study we established the analgesic activity of Peppermint in laboratory animal.

Materials and methods

Preparation of extract: Apparently healthy plants were collected from local area and identified by national herbarium at Abu-Ghraib, at first washed thoroughly in tap water,then leaves were dried at room temperature for 15 days and powdered, then extracted by 70% ethanol using magnetic stirrer for 72hrs at 50°C then the extract was filtered and evaporated to dry it by rotary evaporator 45°C under reduced pressure(12). **Experimental animals:** Swiss albino mice 20-25gm were used. The animals kept in suitable cages and provided with food and water adlibitum, whereas the animals acclimatized for a period of 7 days prior performing the experiments. **Nociceptive assay: Hot plate (thermal) method:** The hot plate test done as described by LeBars (13).Twenty four mice were used in this test and divided into four groups(6 for each), the first one treated orally with distilled water only and served as control , the second and third groups treated orally with MP extract(2.5%) at a dose of 150mg/kg.BW(0.12-0.15ml/mouse) and 300mg / kg. BW(0.24-0.3ml/mouse) respectively, while the last group were treated with morphine i.p.(5mg /kg B.W) which used as reference drug. After 30min from treatment the animals were placed on a hot plate maintained at 55±1°C and the time taken by the animals to lick the fore or hind paw or jump out of the place was taken as the reaction time and each test continued for 60 second.**Acetic acid-induced writhing test :** This test was done by using the method described by (14).Twenty four mice were used in this test and divided into four groups (6 for each).The animals were treated with distilled water orally for first group and MP extract (2.5%) orally at a dose of 150mg/kg.BW (0.12-0.15ml/mouse) and 300 mg/kg.BW(0.24-0.3ml/mouse)for second and third group respectively and indometacin(10mg/kg.BW) orally for last group.30 min. after treatment the animals were injected i.p with 70% solution of acetic acid (10ml/kg) and immediately after administration of acetic acid animals were placed in cages and the number of stretching per animal was recorded during the following 15 min. **Formalin-induced pain test:** The method described by (15) was used. Twenty four mice were used in this test and divided into four groups (6 for each). Animals were injected s/c with 20 µl of 2.5%formalin in the dorsal right hind paw of mice. Distilled water and MP extract (2.5%) at a dose of 150mg/kg.BW (0.12-0.15ml/mouse) and 300mg/kg.BW (0.24-0.3ml/mouse) and indometacin (10mg/kg.BW) were orally administrated to different four groups of mice, where these treatments were given 30 min. prior to formalin injection. The time (seconds) that spent licking or biting the injected paw indicative of pain was monitored. The first period (earlier or neurogenic phase) was recorded 0-5 min. after formalin injection and the second period (later or

inflammatory phase) was recorded 15-30 min. after the injection. **Statistical analysis:** Data were analysed statistically by using Complete Random Design (C.R.D.) to analysis using SPSS programming (16), and to compare between treatments was used Dunnett's t- test(17).

Results and discussion

Mentha piperita extract at a dose of 150 mg/kg. BW showed no significant difference may be because it sub effective dose while at a dose of 300 mg/ kg. BW showed significant increase in latency time to heat stimulus as compared with control group, also morphine produced analgesia and induced an increase in time latency of pain (table 1). The hot plate induced pain test was performed in order to determine whether the analgesic activity of the extract was caused by central or peripheral mechanisms, where the hot plate test is believed to show the involvement of central mechanisms (14).

Table 1: Shows the effect of Mentha piperita extract and morphine on hot plate induced pain in mice.

Treatment	Dose mg/ kg	Reaction time(s)
Control	0.5 ml	5.16±0.48
Extract	150mg/kg	7.00±0.47
Extract	300mg/kg	15.16±0.48*
Morphine	5mg/kg	36.66±0.88*

Values: M ±SE. *significantly different from control p<0.05

In acetic acid-induced writhing test dose dependent antinociceptive effect was noted with the extract at the tested dose levels. The percentage of inhibition of writhing responses exhibited by the MP extract at 150mg/kg B.W and 300mg/kg. B.W was 6.09% and 37.40% respectively, while indometacin was 72.51% (table 2).Acetic acid-induced abdominal constrictions is believed to show that of peripheral mechanism, so it can be considered a model of prostaglandins synthesis response (18), the enhanced analgesic effect of MP may be due to inhibition of the synthesis of arachidonic acid metabolites via inhibiting COX-2.

Table2: Shows the effect of Mentha piperita extract and indometacin on acetic acid-induced abdominal constrictions in mice.

Treatment	Dose mg/kg	No.of abdominal constriction	Inhibition%
Control	0.5ml	43.66±1.49	--
Extract	150mg/kg	41.00±1.15	6.09
Extract	300mg/kg	27.33±1.11*	37.40
Indometacin	10mg/kg	12.00±0.68*	72.51

Values:M±SE. *Significantly different from control p≤0.05

Using a classical pain model, MP and indometacin were evaluated in the formalin-induced pain in mouse. The effect of MP extract and indometacin on the time spent liking the injected paw during the early phase (0-5 min) and later phase (15-30 min) of the formalin test is shown in table 3, MP at a dose of 300 mg/kg B.W caused significant decrease (as compared with control) in the time spent licking hind paw during early phase and the percentage of inhibition was 25.21% and during the later phase and percentage of inhibition was 35.86% and there is no any significant effect of MP extract at a dose of 150 mg/kg B.W on both phase. Indometacin had no effect on the first phase but it produced a significant reduction in the second phase and the percentage of inhibition was 49.75%. The formalin test it used to investigate both central and peripheral mechanisms (15).In this test the early phase is thought to be produced by direct activation of nociception neuron by formalin , whereas the late phase reflects pain generated in actually injured tissues(19).Centrally acting drugs such as opioids, inhibit both phases of pain by equally(20) involving the effect produced by prostaglandins released at this level in response to inflammation(19) and may be endogenous opioids through their action on the central nervous system(15). The extract of MP was shown to possess antinociceptive activity evident in all the nociceptive models signifying the presence of both centrally and peripherally mediated activities. The action of the mentioned extract as analgesic agent may be related to its major important constituents (menthol, flavonoids and tannins), where the menthol is able to block voltage gated calcium channels in human neuroblastoma cell (21) and the modulation of calcium currents is involved in the regulation of pain threshold, so inhibition of calcium current by administration of voltage-sensitive calcium channels blockers produce antinociception in laboratory animals (22),also menthol stimulate opioid receptors(23), it well known that stimulation of central k-opioid receptors induce increase of the pain threshold(24). Also flavonoids reported to have analgesic activity by reducing availability of prostaglandins (25), or the analgesic action of MP may be due to other important compound (tannins) which demonstrated significant antinociceptive activity against abdominal constrictions and formalin test (26).

Table3: Shows the effect of Mentha pipterita extract and indometacin on formalin-induced licking in mouse.

Treatment	Licking time(s) 0-5min	Inhibition%	Liking time(s) 15-30min	Inhibition%
Control	40.33±0.99	--	132.50± 3.21	--
Extract 150mg/kg	37.50±1.05	7.01	127.5± 0.67	3.40
Extract 300mg/kg	30.16±0.79*	25.21	84.66± 1.33*	35.86
Indometacin10mg /kg	36.83±0.70	8.67	66.33± 1.90*	49.75

Values: M±SE Significantly different from control p<0.05.

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