Analgesic Effect of Aqueous Extract of Achillea Millefolium L. on Rat's Formalin Test

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Summary

Achillea millefolium L. (Asteraceae), popularly known as yarrow, has been used in traditional medicine to treat complaints such as gastrointestinal disturbances, hemorrhages, wounds, inflammation and pain. The aim of the present study was to assess the analgesic effects of aqueous extract of Achillea millefolium L. (AE) in the rat's formalin test.

Oral administration of different doses of AE (80, 160 and 320 mg/kg) induced a dose-dependent antinociception, both in the first and second phases of the formalin test.

The results of the present study support the proposal that Achillea millefolium L. has analgesic effects. These findings justify the traditional use of the plant for treating pain and suggest that its activity may be resulted from its central action.

Keywords: Achillea millefolium; Yarrow; Medicinal plants; analgesia; Formalin Test

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Introduction

Achillea millefolium L. (Asteraceae), popularly known as "yarrow", is a widely distributed medicinal plant that has been used for over 3000 years (1). Popular indications of this species include treatment of wounds, hemorrhages headaches, inflammation, pain, spasmodic diseases, flatulence and dyspepsia (2, 3, 4, 5). Some of these reputed traditional effects have been determined showing the potential medicinal use of the plant. The medicinal properties of Achillea millefolium are worldwide recognized and the plant is included in the national Pharmacopoeias of countries such as Germany, Czech Republic, France and Switzerland (1, 4, 6, and 7). In Brazil, Achillea millefolium is included in the list of the 16 medicinal plants of the "Verde Saude" (Green Health), a public health phytotherapy Agency, supported by the Municipal Health Secretary of Curitiba which includes the anti-inflammatory, analgesic, anti-spasmodic and antiseptic properties of the plant. Preparations of Achillea millefolium have been shown to have anti-inflammatory (8, 9), antitumor (10), antioxidant, antimicrobial (11), liver protective (12, 13), anti-secretory and gastro protective activities (14, 15).

Phytochemical studies have identified achilleine (16), azulene, chamazulene (17-19), achimillic acids (A, B and C) (10), 1,8-cineole (18), and flavonoids such as apigenin and luteolin (20,21) in the aerial parts of Achillea milefolium, but only a few studies correlated these substances with pharmacological activities or toxicity. Health hazards associated with long-term exposure to Achillea millefolium's extracts are not well established. Despite the fact that, Food and Drug Administration has classified the plant as non-poisonous and has approved its utilization in alcoholic drinks (22), some toxic effects had been reported after its use by humans and in animal experiments. Toxic effects in human include contact dermatitis (23), headaches and dizziness (7, 24). In animals, Achillea millefolium's preparations reduced fetal weight and increased placental weight when given to pregnant rats (25) and had anti-spermatogenic effects in mice (26) but recent studies did not identify any relevant toxicity on important reproductive biomarkers after 90- day treatments of female Wistar rats (27). Here in this study, the efficacy of the analgesia effect of the aqueous extract (AE) of the aerial parts of plant was investigated in the rat's formalin test.

Materials and Methods

Animals

80 Male Sprague Dawley rats (250–300 g) were used and randomly distributed into 8 groups (n=10). All animals were procured from Iranian Pasture Institute. The rats were maintained under standard laboratory conditions at 25±2 °C, relative humidity 50±15% and normal photo period [12 h dark/12 h light] were used for the experiment. The animals had free access to food and water, except during the time of experiments. Each animal was used only once and was killed immediately after the experiment. The experimental protocol was approved by the Research and Ethics Committee of Kashan University of Medical Sciences, School of medicine.

Collection of plant material

Achillea millefolium L. was provided by the farmers of kashan. The sample flowers used in our study were collected and then allowed to be dried at room temperature. The plant was identified by Hossine Batooli, Ph.D of biology (plant systematic and ecology), Kashan, Iran

Preparation of the aqueous extract

The aqueous extract (AE) was prepared by infusion of the flowers of the plant (3 × 30 min) in water at 70 ◦C (1:10, w/v). The infusion was filtered and concentrated under vacuum (at 56 ◦C) to 1/12 of the original volume and stored at -20 ◦C. The concentrated extract (yield 36%) was diluted in distilled water immediately before use.

Drug treatment:

The concentrated extract and Acetylsalicylic acid (ASA) was dissolved in distilled water. The animals were treated as follows: group 1 (control) received distilled water, group 2-7 received respectively different doses of AE (5,27,40,80,160 and 320 mg/kg, p.o.) and group 8 received Acetylsalicylic acid (ASA)(300 mg/kg, p.o.) in a volume of 1ml/kg, 30 min before formalin injection.

Antinociception recording

One hour before formalin test, the animal was placed in a glass cage (30 × 12 × 30 cm), that served as an observation chamber. Twenty five µl of formalin (2.5%) was injected subcutaneously into the dorsal surface of the right hind paw of the rat using a microsyringe with a 26-gauge needle 30 min after the administration of herbal extracts.

Immediately after formalin injection, the animals were placed individually in a glass cage (30 × 12 × 30 cm), that served as an observation chamber on a flat glass floor and a mirror was arranged in a 45◦ angle under the glass cage to allow clear observation of the paws of the animals. Pain response was scored immediately after formalin injection for a period of 60 min. The nociceptive response was scored at the end of each 15 sec. as follows: 0; the injected paw is not favoured, 1; the injected paw rests lightly on the floor and little or no weight is placed on it, 2; the injected paw is elevated and not in contact with any surface, 3; the injected paw is licked, bitten or shaken. The total scores between 0–5 min (early phase) and 15–60 min. (late phase) after formalin injection was presented.

The formalin test is a valid and reliable model of nociception and is sensitive for various classes of analgesic drugs. Formalin test produced a distinct biphasic response and different analgesics may act differently in the early and late phases of this test (28).

Statistical analysis

One-way ANOVA followed Student–Newman–Keuls test to determine significant differences between groups and $P < 0.05$ was considered significant**.**

Results

Effect of different doses of aqueous extract of Achillea millefolium L. on nociceptive Responses in the formalin test

The s.c. injection of 2.5% formalin into the right hind paw of rat induced a biphasic nociceptive response. The effects of different doses of AE (5, 27,40,80,160 and 320 mg/kg, p.o.) on formalin test have been shown in Table 1. Statistical analysis showed that extract (80,160 and 320 mg/kg, p.o.) were endowed with a significant antinociceptive activity compared to the control group and extract (5, 27, 40 mg/kg, p.o.) on the early and late phases ($P < 0.001$).

On the other hand extract (160 and 320 mg/kg, p.o.) were endowed with a significant antinociceptive activity compared to the extract (80 mg/kg, p.o.) on the early ($P < 0.05$) and late phases ($P< 0.001$). The maximum Antinociceptive occurred at a dose of 160 mg/kg AE, and larger doses did not further effect in the formalin test

Table 1**. Antinociceptive effect of different doses of aqueous extract of Achillea millefolium L. in the formalin test.**

Treatment	Dose(mg/kg)	**pain score** (mean±S.E.M)	
		0–5 min	**15–60 min**
Control(water)	**Water**	20.01±1	63± 4
Extract	5	18.5±1.6	70± 5
	27	19.8±1.5	66± 6
	40	18.5±1.2	60± 7
	80	12.7±1.5**, ††, ##,€€	50± 6**,††,##
	160	9.9±1**, ††, ##,€€,¶	30± 7**,††, ##,¶¶
	320	9.8±1.1**, ††, ##,€€, ¶	27± 7**,††, ##,¶¶

Rats were injected either with vehicle (water, 1 ml/kg, p.o.) or aqueous extract of Achillea millefolium L. (5, 27, 40, 80,160 and 320 mg/kg, p.o.) 30 min before formalin injection. Antinociception during 0–5 min. (first phase) and 15–60 min. (second phase) after formalin injection was recorded. Each data represents the mean ± S.E.M. for 10 animals. Statistical comparison was performed using analysis of variance (ANOVA) followed by Tukey's test.

** P< 0.001 statistically significant compared to control group (water, 1 ml/kg, p.o.)

†† P< 0.001 statistically significant compared to extract group (5mg/kg, p.o.)

P< 0.001 statistically significant compared to extract group (27mg/kg, p.o.)

€€P< 0.001 statistically significant compared to extract group (40mg/kg, p.o.)

¶¶ P< 0.001, ¶ P< 0.05 statistically significant compared to respectively extract group (80mg/kg, p.o.)

Analgesic Effectiveness of aqueous extract of Achillea millefolium L. (160mg/kg, p.o.) Compared to ASA (300 mg/kg, p.o.) in the Rat Formalin Test

The effects of AE (160 mg/kg, p.o.) and ASA (300 mg/kg, p.o.) on formalin test have been shown in Table 2. Statistical analysis showed that extract (160 mg/kg, p.o.) and ASA(300 mg/kg, p.o.) were endowed with a significant antinociceptive activity compared to the control group on the early and late phases ($P < 0.001$). On the other hand extracts (160 mg/kg, p.o.) had a significant antinociceptive activity compared to the ASA (300 mg/kg, p.o.) on the early($P < 0.05$) phase.

This experiment demonstrates that ASA(300 mg/kg, p.o.) when compared to extract (160 mg/kg, p.o.), is less effective in treating pain from direct nerve stimulation and is equal effective in treating pain from inflammatory origin.

Table 2. Antinociceptive effect of aqueous extract of Achillea millefolium L. (160mg/kg, p.o.) and ASA (300 mg/kg, p.o.) in the formalin test.

Treatment	Dose(mg/kg)	**pain score** (mean±S.E.M)	
		0–5 min	**15–60 min**
Control(water)	-	20.01±1	63± 4
Extract	160	9.9±1**	30± 7**
ASA	300	13.2 ± 2**,†	33±8**

Rats were injected either with vehicle (water, 1 ml/kg, p.o.), aqueous extract of Achillea millefolium L. (160 mg/kg, p.o.) or ASA (300 mg/kg, p.o.) 30 min before formalin injection. Antinociception during 0–5 min. (first phase) and 15–60 min. (second phase) after formalin injection was recorded. Each Data represents the mean ± S.E.M. for 10 animals. Statistical comparison was performed using analysis of variance (ANOVA) followed by Tukey's test.

** P< 0.001 statistically significant compared to control group.

† P< 0.05 statistically significant compared to extract group (160mg/kg, p.o.)

Discussion

In the present study, antinociceptive effect of AE has been investigated in the rat's formalin test. The present data shows that since higher dose the aqueous extract of the plant was more potent when compared to the lower dose indicating that analgesic effect is dose-dependent in both phases of the formalin test. Such an effect for AE not has been reported previously. Achillea millefolium L is widespread species used in Iranian and Brazilian folk medicine to treat diverse diseases including inflammation and pain. (5, 15). It has been reported that the oil of Achilla aleppica another species of achilla showed a significant anti-inflammatory and antinociceptive activities (29).
The obtained results give further insights into the pharmacological activity of Achillea and confirm the traditional application as antinociceptive drug.

In the formalin test, it is considered that first phase of formalin-induced behavior reflects direct activation of A delta and C afferent fibers while the second phase reflects
both ongoing peripheral sensory input and central sensitization. Therefore, the test can be used to clarify the possible mechanism of antinociceptive effect of a proposed analgesic (30). Centrally acting drugs such as opioids inhibit both phases equally (31) on the contrary, peripherally acting drugs such as aspirin; indomethacin and dexamethasone only inhibit the late phase. The late phase seems to be an inflammatory response with inflammatory pain that can be inhibited by anti-inflammatory drugs (32, 33). The effect of extract on the first and second phases of formalin test suggests that its activity may be resulted from its central or/and peripheral action.

On the other hand, our result showed that ASA at dose of 300 mg/kg (p.o.) reduced significantly nociceptive response both first phase and second phase of the formalin test. These data is in agreement with those of Giovanni Vitale and et al (1998), who showed that ASA (300 mg/kg, I.P) suppressed the pain behavior of the both phases of the formalin test (34) and Bjo rkman in 1995 also studied the antinociceptive activity of ASA through other experimental models, suggesting the involvement of a number of steps in the nociceptive system acting both centrally and peripherally (35,36).
In other series of experiments, comparing to ASA (300 mg/kg, p.o.), the potency of the AE (160 mg/kg, p.o.) appeared to have a greater potent in the early phase and equal in the late one.
On the other hand, this experiment demonstrates that ASA(300 mg/kg, p.o.), when compared to extract (160 mg/kg, p.o.), is less effective in treating pain when direct nerve stimulation is concerned and is equally effective in treating pain when inflammatory origin is considered.
What chemical composition is involved in the analgesic response needs to be elucidated by further experiments.
Furthermore, essential oil and sesquiterpenes phenolic compounds such as flavonoids and phenolcarbonic acids are a major group of plant constituents present in yarrow. Due to their high solubility in water and ethanol, those polar substances are completely extracted into teas and tinctures which are the traditional application forms of yarrow. Recently, the spasmolytic activity of the flavonoids (37) and topical anti-inflammatory activity of the sesquiterpenes have been already shown being caused by inhibition of the arachidonic acid metabolism (38). B Benedek (2007) showed anti-inflammatory action of crude plant extract, flavonoid and dicaffeoylquinic acids fraction of Achillea millefolium (39). The late phase seems to be an inflammatory pain due to the inflammatory response that can be inhibited by topical anti-inflammatory activity of the flavonoids.
Moreover, the safety of the plant after chronic exposure was previously reported (15). Ana Maria Cavalcanti et al (2006) showed that after repeated dose of 90-day oral exposure to AE, rats exhibited no treatment-related toxicological or histopathological abnormalities. The doses of AE tested in rats were higher than that anticipated for human consumption. Thus, it is likely that no long-term toxicological risk would occur with the doses of AE commonly consumed by humans. However, this extrapolation should be made with caution, since the real human risk cannot be assessed on the basis of the present study.

Conclusion

In conclusion, the present study has shown that AE exert significant antinociceptive effects on the first and second phases of formalin test. In addition, its activity may be resulted from its central / peripheral action.

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