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Inhibition of Carrageenan-Induced Edema by Tripleurospermum Disciforme Extract in Rats

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Abstract: In this research anti-inflammatory effect of Tripleurospermum disciforme extract was studied in rats. The effect of the extract against acute inflammation was studied by hind paw edema test. Intraperitoneal injection of different doses (15, 30, 45, 60, 90 and 120 mg kg^{-1}) of Tripleurospermum disciforme which was followed by hind paw carageenan injection 1 h later were investigated. The resultant edema was quantified by measuring changes of diameter of hind foot. After 2, 3, 4 and 6 h we measured the anti-inflammatory effect of Tripleurospermum disciforme extract. Tripleurospermum disciforme extract with doses of 45, 60, 90 and 120 mg kg^{-1} and indomethacin 5 mg kg^{-1} showed significant effect. It is concluded that Tripleurospermum disciforme extract has anti-inflammatory effect against acute inflammation.

Key words: Tripleurospermum disciforme, acute inflammation, hind paw edema test, rat

INTRODUCTION

Tripleurospermum disciforme, a kind of *Matricaria chamomilla*, commonly referred to as the chamomile plant, is a member of the Asteraceae (Aster family) and is native to Europe and Western Asia (Renuka, 1992). Chamomile has been used as a medicinal plant for centuries. Today, chamomile is the most widely used herb for relaxation in the Western world. Throughout history, chamomile has been (and still is) used in a variety of ways: healing baths, teas, air fresheners, hair rinse, cosmetics, insect repellents, wine flavoring, dyes, companion planting, potpourris and landscaping (Ibid). From a medicinal point of view, Chamomile is useful for the following: soothing, calming, sedative, relaxation, anti-inflammatory, tenseness, aching muscles, indigestion, acidity, hay fever, asthma, morning sickness, eczema, mouthwash and exhaustion (Yarosh *et al*., 2006; Fidler *et al*., 1996; Budzinski *et al*., 2000; Clement *et al*., 2005; Maday, 1999). Chamomile is known for its calming effect on smooth muscle tissue and is still a popular remedy for nervous stomach, menstrual cramps and other common problems related to stress (Avallone *et al*., 2000). Used externally, it is also useful in treating skin inflammation and hemorrhoids. Used as a mouthwash, Chamomile can relieve the pain of a toothache. *Matricaria chamomilla* is among several medicinal herbs that are popular in Hispanic folk medicine (Appelt, 1985). *Matricaria chamomilla* is a very common plant used for its medical attributes. It can be used to treat very unique disorders as well as the common cold and other ailments that people come across on a daily basis. There are many species of chamomilla one of which is Tripleurospermum disciforme. The purpose of the present study is to investigate the anti-inflammaroty effects of Tripleurospermum disciforme given that no such investigation has been reported.

MATERIALS AND METHODS

Animals: Male Sprague-Dawley rats weighing 200-250 g were used. They were maintained on normal diet in the animal house of Tehran University of Medical Sciences. They were housed in Wire-Mesh cages in a room with a 12/12 h light/dark cycle at 21±2°C.

Plant material: The plant was collected 20 km north of Tehran, Iran, in June 2006 and dried in shadow followed by grinding. Tehran university properly identified the Tripleurospermum disciforme and voucher samples were preserved for reference in the herbarium of the Department of Pharmacology, Tehran University, Tehran.

The preparation of extracts: Powder of the plant was extracted using aqueous infusion and maceration with water. In the infusion method 100 g of the plants was added to 1 L of hot water for 15 min and then filtered with cloths.

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Drugs: Indomethacin and Carrageenan was purchased from Sigma (U.S.A.). Indomethacin was used with dose of 5 mg kg^{-1} in this study. Carrageenan was injected with dose of 0.1 mL of 1% solution in the plantar surface of the right hind paw of rats 1 h before intraperitoneal Tripleurospermum disciforme extract injection. Tripleurospermum disciforme was used with doses of 15, 30, 45, 60 and 90 mg kg^{-1}.

Rat hind paw edema: Edema or swelling is a cardinal sign of acute inflammation. Edema is a useful parameter to look at when testing for agents which may be active in treating acute inflammation. Originally the rat paw edema model was induced by the injection of 0.1 mL of 1% Carrageenan solution subcutaneously under the plantar surface of the right hind foot. The resultant edema, which is maximal by approximately 6 h, can then be quantified by measuring changes of diameter of hind foot.

The rats were divided into 8 groups with 6 rats per group. At first we measured the hind paw diameter with qullis.

Tripleurospermum disciforme extract was injected intraperitoneally in the first 6 groups of rats with doses of 15, 30, 45, 60, 90 and 120 mg kg^{-1}.

Also Indomethacin with dose of 5 mg kg^{-1} was injected in one group of rats. One hour after Tripleurospermum disciforme extract or indomethacin injection, 0.1 mL of 1% Carageenan solution was injected subcutaneously under the plantar surface of the right hindpaw.

In the 8th group we injected only carageenan subcutaneous in the plantar surface of right hind paw with dose of 0.1 mL of 1% solution and was our control group. Following 2, 3, 4 and 6 h of the injection, the anti-inflammatory effect of Tripleurospermum disciforme was measured on hind paw edema. The rat hind paw edema remains the most commonly used test for anti-inflammatory activity.

Statistical analysis: The data were expressed as mean values±SEM and tested with analysis of variance (ANOVA) followed by the multiple comparison test of Tukey.

RESULTS

In the present study, inhibition of carrageenan-induced hindpaw edema was used as an estimate for anti-inflammatory activity of Tripleurospermum disciforme extract.

Comparison of the anti-inflammatory effect of Tripleurospermum disciforme and indomethacin at 2 h: The intraperitoneal injection of aqueous extract of Tripleurospermum disciforme and indomethacin and saline were compared at 2 h following Carageenan injection.

Intraperitoneal injection of indomethacin with a dose of 5 mg kg^{-1} 1 h before carrageenan hind foot injection, showed a significant reduction in inflammation (Table 1).

Administration of different doses of aqueous extract of Tripleurospermum disciforme didn't produce any significant anti-inflammatory effect at 2 h (Table 1).

Comparison of the anti-inflammatory effect of Tripleurospermum disciforme and indomethacin at 3 h: Indomethacin at dose of 5 mg kg^{-1} shows significant anti-inflammatory effect at 3 h (Table 2). Aqueous extract of Tripleurospermum disciforme at the doses of 45, 60 and 90 mg kg^{-1} showed significant anti-inflammatory effect. Tripleurospermum disciforme at the doses of 15 and 30 mg kg^{-1} didn't produce any significant effect at 3 h (Table 2).

Comparison of the anti-inflammatory effect of Tripleurospermum disciforme and indomethacin at 4 h: Indomethacin at dose of 5 mg kg^{-1} showed significant anti-inflammatory effect at 4 h (Table 3).

At this time all doses of chamomilla showed significant anti-inflammatory effect but the doses of 45, 60 and 90 mg kg^{-1} displayed the best anti-inflammatory effect (Table 3).

Table 1: Effect of Tripleurospermum disciforme extract and indomethacin on rat hind paw edema induced by carrageenan after 2 h

Groups	Hind paw edema (mm/2 h)
Saline	1.95±0.20
Indomethacin	1.12±0.06
Tripleurospermum disciforme 15	1.12±0.13
Tripleurospermum disciforme 30	0.96±0.10
Tripleurospermum disciforme 45	0.92±0.17
Tripleurospermum disciforme 60	1.02±0.56
Tripleurospermum disciforme 90	1.02±0.26
Tripleurospermum disciforme 120	1.45±0.17

Each bar represents the mean±SEM of the 6 rats

Table 2: Effect of Tripleurospermum disciforme extract and indomethacin on rat hind paw edema induced by carrageenan after 3 h

Groups	Hind paw edema (mm/3 h)
Saline	2.80±0.20
Indomethacin	1.20±0.12
Tripleurospermum disciforme 45	0.92±0.28**
Tripleurospermum disciforme 60	1.12±0.32*
Tripleurospermum disciforme 90	1.12±0.32*

*p>0.05, **p>0.01; Each bar represents the Mean±SEM of the 6 rats

Table 3: Effect of Tripleurospermum disciforme extract and indomethacin on rat hind paw edema induced by carrageenan after 4 h

Groups	Hind paw edema (mm/4 h)
Saline	3.50±0.25
Indomethacin	0.80±0.05***
Tripleurospermum disciforme 15	1.60±0.20**
Tripleurospermum disciforme 30	1.88±0.65*
Tripleurospermum disciforme 45	0.88±0.25***
Tripleurospermum disciforme 60	1.00±0.38***
Tripleurospermum disciforme 90	1.00±0.38***
Tripleurospermum disciforme 120	1.43±0.15***

*p>0.05, **p>0.01, ***p>0.001; Each bar represents the Mean±SEM of the 6 rats

Table 4: Effect of Tripleurospermum disciforme extract and indomethacin on rat hind paw edema induced by carrageenan after 6 h

Groups	Hind paw edema (mm/6 h)
Saline	3.70±0.33
Indomethacin	1.65±0.35**
Tripleurospermum disciforme 30	1.88±0.23**
Tripleurospermum disciforme 45	1.90±0.55**
Tripleurospermum disciforme 60	1.72±0.35**
Tripleurospermum disciforme 90	1.73±0.35**

**p>0.01; Each bar represents the Mean±SEM of the 6 rats

Comparison of the anti-inflammatory effect of Tripleurospermum disciforme and indomethacin at 6h: Doses of 30, 45, 60 and 90 mg kg^{-1} showed significant anti-inflammatory effect, whereas the dose of 15 mg kg^{-1} did not have any effect at this time (Table 4).

DISCUSSION

The present results indicate the aqueous extract of Tripleurospermum disciforme has anti-inflammatory activity in rat hind paw edema test. The hind paw edema has been analyzed by a variety of different workers and it has been found that the reaction depends upon the activation of the complement system (Dale *et al*., 1994). Injected kinins mimic inflammation. Measurement of the components of the kinin cascade and the effects of bradykinin antagonists indicates that kinins plays a role in a variety of inflammatory diseases. Plasma kinins increase permeability in the microcirculation. The effect, like that of histamine and serotonin in some species, is exerted on the small venules and involves separation of the junctions between endothelial cells. This, together with an increased hydrostatic pressure gradient, causes edema. Such edema, coupled with stimulation of nerve endings, results in a wheal-and-flare response to intradermal injections in human beings (Goodman and Gilman's, 2001). Thus depletion of the complement titre will cause a striking suppression of the reaction. The edema is produced by a sequential release of pharmacological mediators, including histamine, 5-hydroxytryptamine, kinin and prostaglandins (Dale *et al*., 1994). When using this mode, it is important to assess the effect of the potential anti-inflammatory agent at the appropriate time during the swelling of the hind paw. If the anti-inflammatory agent is administered early, an antihistamine could be found, or if a little later possibly a kinin antagonist. Ideally the foot should be measured at more than one time but certainly at 3-4 h. this allows the participation of all the chemical mediators.

The rat hind paw edema remains the most commonly used test for anti-inflammatory activity. The system has several advantages, when compared to other models, mainly the ease and reproducibility of the system. By using one group of rats a time course of edema can be examined which keeps the cost down. Tripleurospermum disciforme extract with doses of 45, 60, 90 and 120 mg kg^{-1} and Indomethacin at a dose of 5 mg kg^{-1} showed significant effect. The present results indicate that the anti-inflammatory effect of Tripleurospermum disciforme is showing a time dependent phenomenon and as the time goes by the anti-inflammatory effect significantly increases. The isolation of active ingredients may increase the potency of the extract, although in low doses the extract was significantly effective (such as 15 and 30 mg kg^{-1}, Table 3 and 4). It is concluded that Tripleurospermum disciforme extract has anti-inflammatory effect against acute inflammation.

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