ELSEVIER

Journal of Ethnopharmacology 68 (1999) 251–259

Journal of ETHNO-PHARMACOLOGY

www.elsevier.com/locate/jethpharm

# Antinociceptive and anti-inflammatory effects of *Tanacetum parthenium* L. extract in mice and rats

Naveen K. Jain, Shrinivas K. Kulkarni *

*Pharmacology Division, University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh-160 014, India*

Received 11 May 1999; received in revised form 18 June 1999; accepted 25 June 1999

**Abstract**

Oral administration of the feverfew (*Tanacetum parthenium*) extract led to significant antinociceptive and anti-inflammatory effects against acetic acid-induced writhing in mice and carrageenan-induced paw edema in rats, respectively. These responses were dose-dependent (10, 20, 40 mg/kg, p.o.). Parthenolide (1, 2 mg/kg i.p.), the active constituent of the extract also produced antinociceptive and anti-inflammatory effects. Naloxone (1 mg/kg i.p.), an opiate antagonist, failed to reverse feverfew extract and parthenolide-induced antinociception. Feverfew extract in higher doses (40, 60 mg/kg p.o.) neither altered the locomotor activity nor potentiated the pentobarbitone-induced sleep time in mice. It also did not change the rectal temperature in rats. Feverfew extract exerted antinociceptive and anti-inflammatory effects without altering the normal behaviour of the animals. 

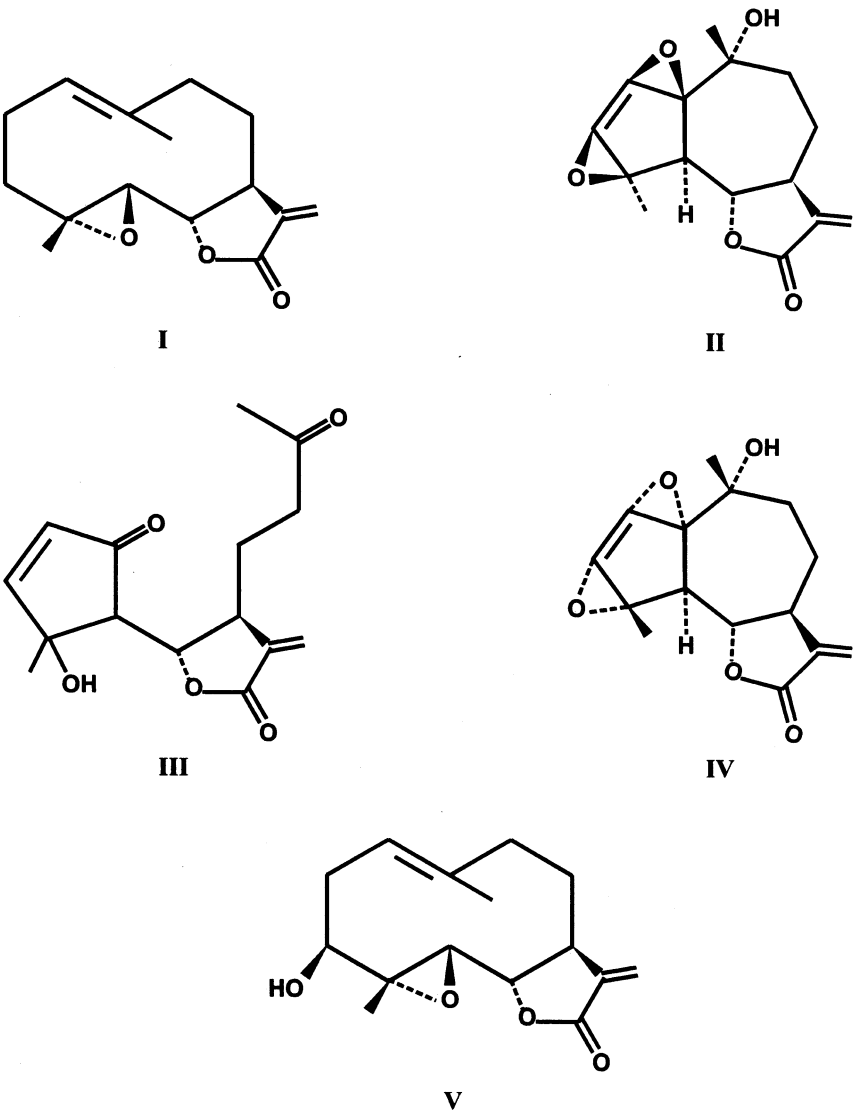
*Keywords:* Feverfew extract; Parthenolide; Antinociception; Anti-inflammatory

## 1. Introduction

*Tanacetum parthenium* (L.) Schultz-Bip, commonly known as feverfew (Family: Asteraceae), is a common perennial herb, 14–45 cm in height, having a strong smell and greenish yellow feather-like leaves. It is widely distributed in hedges and waste places throughout much of Europe and has long been cultivated, both as an ornamental and medicinal plant. The leaves of this plant are eaten or used as infusions in conditions like arthritis, migraine and asthma. It has also being claimed to be useful for treating conditions like tinnitus, vertigo, fever, menstrual disorders, difficulty in labour, stomach-ache, toothache and insect bites [1,2]. Although the phytochemistry of the plant has not been freely studied in detail, the active principles are mainly sesquiterpene lactones, parthenolide, 3β-hydroxy parthenolide, canin and artecanin, having α-methylene butyrolactone moiety [3] (Fig. 1). Parthenolide is considered as the most important biologically active principal of feverfew [2,4]. It is mainly found in the leaves and flower heads (0.20–0.50%) but not in the stems. On the basis of the traditional uses of this herb, it was decided to study the effects of feverfew extract in nociceptive response in mice and on carrageenan-induced paw edema in rats, respectively.

* Corresponding author. Fax: +91-172-541142.
*E-mail address:* skk@panjabuniv.chd.nic.in (S.K. Kulkarni)

0378-8741/99/$ - see front matter 
PII: S0378-8741(99)00115-4




| | |
|---|---|
| I | Parthenolide |
| II | Canin |
| III | Seco - Tanapartholide |
| IV | Artecanin |
| V | 3β-Hydroxy parthenolide |

Fig. 1. Active compounds isolated from feverfew.

## 2. Materials and methods

### 2.1. Animals

Albino mice (Laka strain 20–30 g) and Albino rats (Wister strain 150–200 g) of either sex (bred in Central Animal House, Panjab University, Chandigarh, India) weighing 20–30 g, were used. The animals were kept under standard 12/12 h light/dark cycle and had food and water ad libitum. All experiments were carried out between 10:00 and 17:00 h.

### 2.2. Materials

The following drugs were used. Feverfew extract (Dabur Research Foundation, New Delhi, Ethanolic extract yield 14–16%, Lot No. DRF/RMD/97), Parthenolide 97% (Aldrich Chemical), Naloxone (Sigma, St. Louis), Pentobarbitone sodium (National Chemicals, Mumbai), Carrageenan type (IV) (Sigma), Acetic acid (S.D. Fine Chemicals), Diazepam (Ranbaxy Labs., New Delhi), Nimesulide (Panacea Biotec, New Delhi).

Feverfew extract was given (10, 20, 40 mg/kg) orally, 30 min before acetic acid/carrageenan challenge. Parthenolide (1, 2 mg/kg), naloxone (1 mg/kg) nimesulide (2 mg/kg) were administered intraperitoneally 30 min before testing. All the drugs were dissolved in distilled water except feverfew extract suspended in distilled water and parthenolide suspended in CMC, respectively.

Parthenolide was determined by HPLC (Water's) method: column ($250 \times 4$ mm, 5 μ, $C_{18}$), mobile phase (acetonitrile:water 55:45), flow rate 2 ml/min, injection volume 20 μl and detection by u.v. at 210 nm against standard parthenolide.

### 2.3. Antinociceptive activity and anti-inflammatory activity

#### 2.3.1. Writhing test (acetic acid writhing assay)

Antinociceptive response was assessed by counting the number of writhes (constriction of abdomen, turning of trunk (twist) and extension of hind legs) induced by 1% acetic acid solution (1 ml/100 g) in mice, as described by [5]. Number of writhes per animal was counted during a 20 min $(5 + 15)$ test period, beginning 3 min after the injection of acetic acid.

#### 2.3.2. Tail-flick test

Antinociceptive response was assessed by measuring tail-flick latency of rats to radiant heat, as described as by D'Armour and Smith [6] and modified by Kulkarni [7]. Baseline latencies to tail withdrawal from the radiant heat (7–8 s) were established. A cut-off time of 15 s was kept to prevent any injury to the tail. Four readings were recorded for each animal in order to calculate mean basal reaction time.

#### 2.3.3. Carrageenan-induced paw edema

Acute edema was induced in the hind right paw of rats by injecting 0.1 ml of freshly prepared 1% solution of carrageenan (type IV). The left paw served as control (non-inflammed paw) for comparison (0.9%, 0.1 ml saline injected). The carrageenan was injected under the planter region of the right hind paw and paw volume was measured using plethysmometer (mercury displacement) at 15, 30, 60, 90, 120, 150, 180 and 210 min after carrageenan challenge. Percent change in paw volume was calculated and expressed as the amount of inflammation [8].

### 2.4. Locomotor activity in mice

Locomotor activity (ambulatory score) was measured using computerised animal activity meter (Opto varimax mino; Columbus Instruments, OH). Animals were individually placed in a transparent plastic cage ($30 \times 23 \times 23$ cm) and the activity was recorded for 5 min after allowing the mouse to adapt to the new environment for a few minutes. An array of 15 infrared emitter/detector pairs (spaced at 2.65 cm intervals; beam wavelength = 875 nm; distance between the sensors = 50 cm) measured the animal activity along a single axis of motion, the digital data being displayed on the front panel meters as ambulatory activity. The ambulatory option in the instrument automatically differentiates between actual ambulatory movements and stereotypic movements such as grooming and scratching. The locomotion was expressed in terms of total photobeam counts per

5 min per animal. Feverfew extract (40, 60 mg/kg, p.o.) was administered 30 min before the assessment of locomotor activity, while diazepam (2 mg/kg, i.p. 30 min before) served as a standard reference drug.

### 2.5. Rectal temperature of rats

The rectal temperature was recorded using a tele thermometer (Yellow Springs Instruments) by inserting the thermister probe to a depth of 5 cm into the rectum of the rat. The rectal temperature of each animal was recorded just before drug administration and thereafter at 15, 30, 60, 120 and 180 min of drug administration. The rectal temperature of an untreated group served as control. The ambient temperature was $20 \pm 2$°C. Feverfew extract was administered at different doses (20, 40, 60 mg/kg, p.o.) and rectal temperature was noted at intervals as mentioned above.

### 2.6. Potentiation of pentobarbitone-induced hypnosis in mice

Laka mice were treated with the feverfew extract of (40, 60 mg/kg, p.o.) and 30 min later pentobarbitone sodium was injected (45 mg/kg, i.p.). Onset of sleeping (loss of righting reflex) and duration of sleep (regain of righting reflex) of each animals was noted.

### 2.7. Statistical analysis

Results were statistically analysed by the ANOVA followed by Student's *t*-test.

## 3. Results

### 3.1. Effect of feverfew extract on acetic acid-induced writhing in mice

Feverfew extract (10, 20, 40 mg/kg p.o.) significantly ($P < 0.05$) increased the pain threshold as the number of writhe responses decreased during 20 min as compared to the control group (Fig. 2). The active constituents of feverfew extract parthenolide (1, 2 mg/kg i.p.) also decreased the number of writhes induced by acetic acid in mice (Fig. 3). The effect was comparable to nimesulide (2 mg/kg). Feverfew extract and parthenolide both produced antinociceptive effect in a dose-dependent manner ($P < 0.01$). Control animals showed $44.15 \pm 2.01$ writhes, which were de-

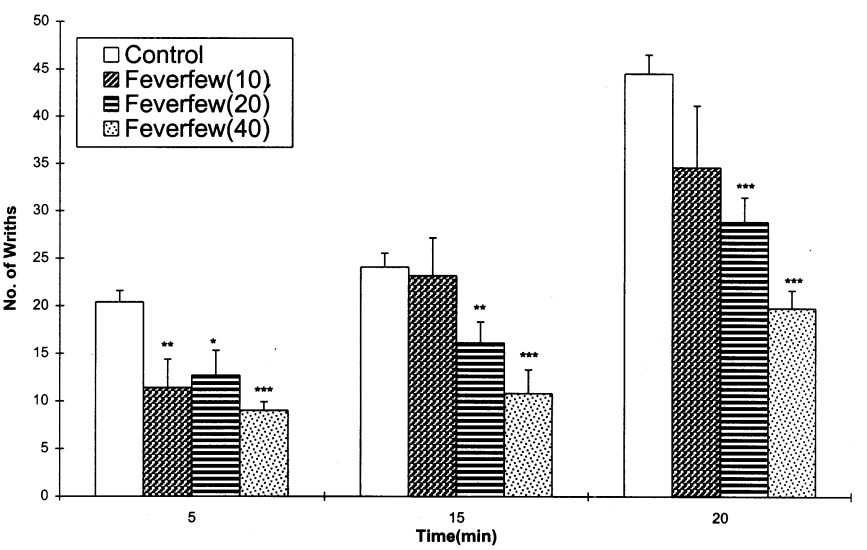



Fig. 2. Effect of feverfew extract on acetic acid-induced writhing in mice. The doses are expressed as mg/kg p.o. Vertical lines show $\pm$ S.E.M. * $P < 0.05$; ** $P < 0.001$; *** $P < 0.001$ as compared with control.

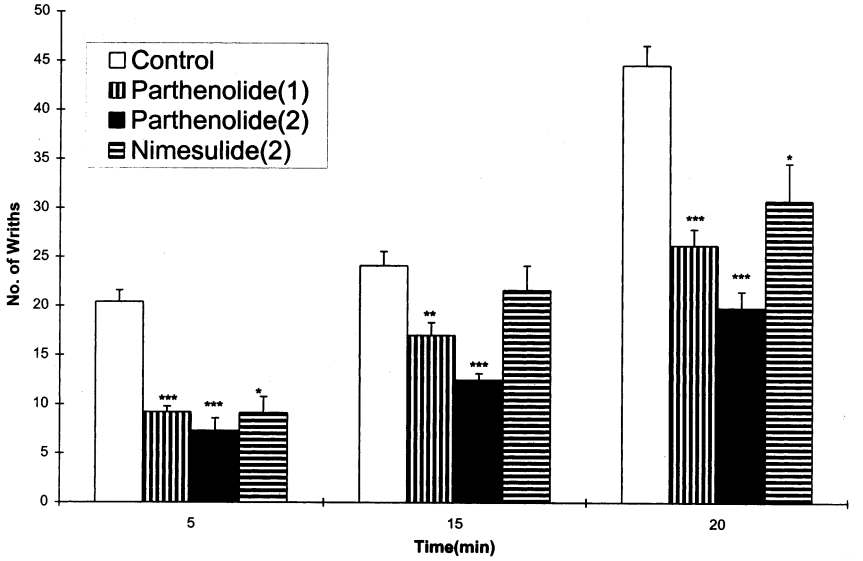



Fig. 3. Effect of parthenolide on acetic acid-induced writhing in mice. The doses are expressed as mg/kg i.p. Vertical lines show ± S.E.M. * $P < 0.05$; ** $P < 0.001$; *** $P < 0.001$ as compared with control.

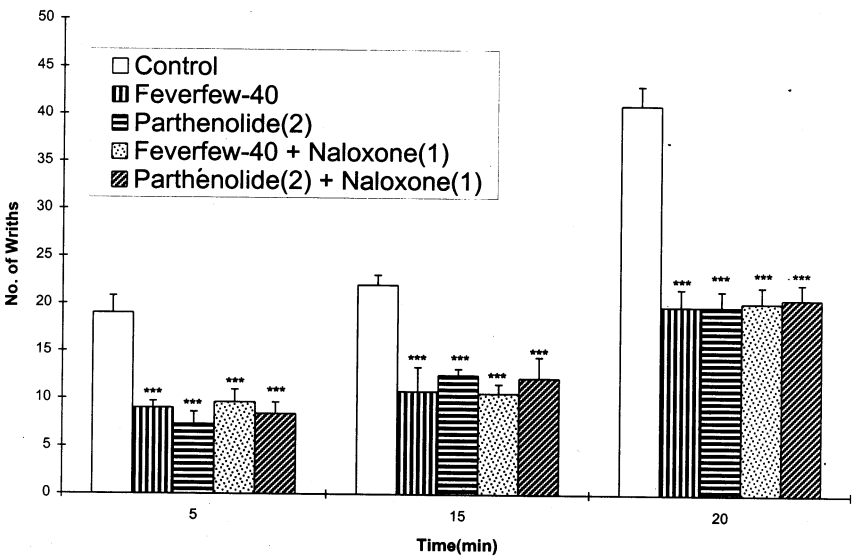



Fig. 4. Effect of naloxone on feverfew extract and parthenolide-induced antinociception in acetic acid-induced writhing in mice. The doses are expressed as mg/kg p.o. or i.p. Vertical lines show ± S.E.M. *** $P < 0.001$ as compared with control.

creased to 19.8 ± 1.85 during a 20-min test upon treatment with feverfew extract (40 mg/kg, p.o.). Naloxone (1 mg/kg) failed to block the parthenolide or feverfew extract-induced antinociception (Fig. 4).

### *3.2. Effect of feverfew extract on tail-flick latency in rats*

Feverfew extract (20, 40, 60 mg/kg) dose dependently increased the tail-flick latency as compared

Table 1
Effect of feverfew extract on tail flick reaction time in rats

| Treatment | Dose (mg/kg) | N | Basal reaction time | Reaction time (seconds ± S.E.M.) after: | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | 15 min | 30 min | 60 min | 120 min | 180 min |
| Feverfew extract | 20 | 5 | 7.4 ± 0.452 | 8.6 ± 0.510 | 7.8 ± 0.860 | 8.1 ± 0.678 | 9.0 ± 1.09 | 8.5 ± 0.452 |
| Feverfew extract | 40 | 5 | 7.4 ± 0.452 | 7.2 ± 0.583 | 8.2 ± 0.339 | 12.0 ± 0.837* | 9.2 ± 0.374 | 8.6 ± 0.245 |
| Feverfew extract | 60 | 5 | 7.4 ± 0.452 | 8.2 ± 0.583 | 11.6 ± 0.510* | 13.0 ± 0.632* | 13.3 ± 0.300* | 9.8 ± 0.374 |

* $P < 0.05$ compared with controls (basal reaction time).

to basal latency (Table 1). Feverfew (60 mg/kg) produced the maximum antinociceptive effect after 120 min (7.4 ± 0.45 to 13.3 ± 0.30 s) ($P < 0.05$).

### 3.3. Effect of feverfew extract on carrageenan-induced paw edema in rats

Carrageenan (1%) produced a significant increase in paw volume in the control group indicating an inflammatory response. Nimesulide (2 mg/kg) showed significant anti-inflammatory effect as compared to control group ($P < 0.05$). Feverfew extract (10, 20, 40 mg/kg p.o.) dose dependently and significantly decreased the carrageenan-induced increase in paw volume as compared to control rats ($P < 0.05$) (Fig. 5). Parthenolide, the active constituent of feverfew, also produced anti-inflammatory effect (Fig. 6) similar to feverfew extract but the effect of parthenolide was superior to nimesulide.

### 3.4. Effects of feverfew extract on locomotor activity, pentobarbitone-induced sleep in mice and rectal temperature in rats

Feverfew extract (40, 60 mg/kg p.o.) did not show any depressant effect on locomotor activity as compared to diazepam (2 mg/kg), which showed a significant ($P < 0.05$) reduction in ambulatory score. In addition, feverfew extract (40, 60 mg/kg) did not alter the pentobarbitone-induced sleep time in mice (onset as well as duration of sleep). Feverfew extract (20, 40 mg/kg) did not alter the rectal temperature of rats but, at a higher dose (60 mg/kg), it showed insignificant reduction

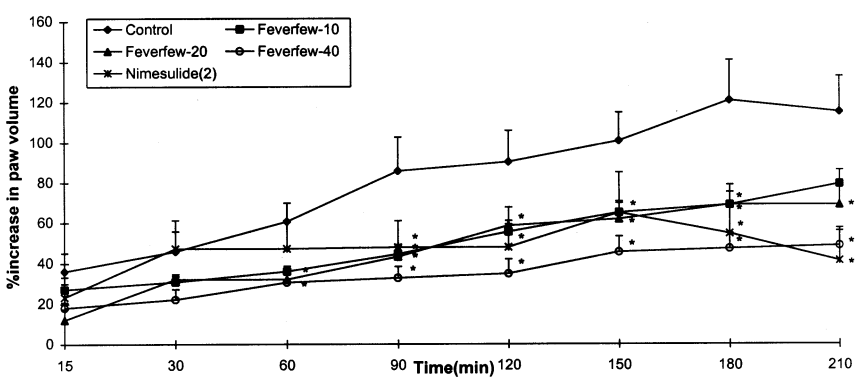



Fig. 5. Effect of various doses of feverfew against carrageenan-induced paw edema in rats. The doses are expressed as mg/kg (p.o. or i.p.). Vertical lines show ± S.E.M. * $P < 0.05$ as compared with control.

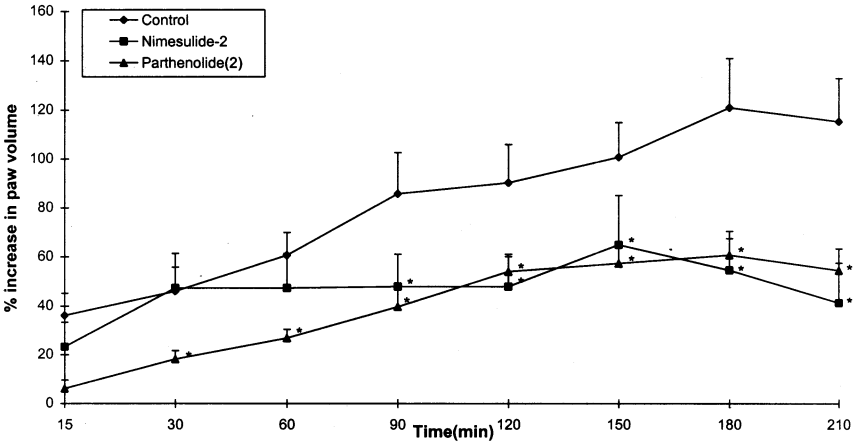



Fig. 6. Effect of parthenolide treatment on carrageenan-induced paw edema in rats. The doses are expressed as mg/kg (p.o. or i.p.). Vertical lines show ± S.E.M. * $P < 0.05$ as compared with control.

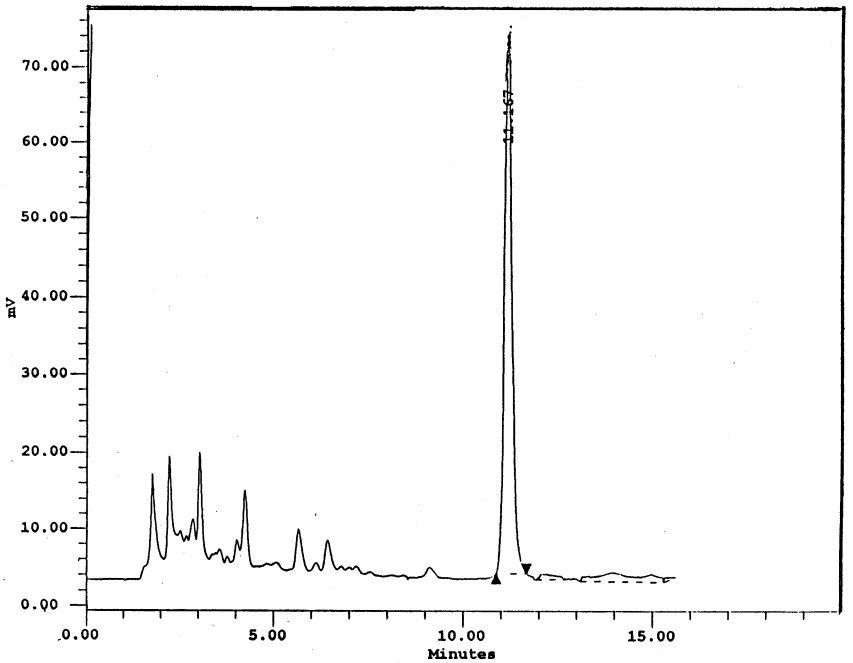
in rectal temperature after 60 and 120 min of its administration as compared to control animals (results not shown).

### 3.5. HPLC analysis

Parthenolide content of feverfew extract was found to be 0.856% (Fig. 7).

## 4. Discussion

Feverfew (*T. parthenium*) is a herb with a traditional reputation of treating a variety of disorders. Studies performed by Johnson et al. [4] have demonstrated feverfew to be an effective remedy for the prophylactic treatment of migraine, as it inhibits platelet aggregation, histamine release from mast cells, [9] and the production of prostaglandins, thromboxanes and leukotriens. In the present study, the analgesic and anti-inflammatory effect of feverfew extract was investigated. Feverfew extract significantly and dose-dependently (10–40 mg/kg) increased the pain threshold in acetic acid-induced chemonociception and radiant heat-induced nociception. Parthenolide is an active constituent (0.856%) in feverfew extract. It is a sequiterpene lactone with α-methylene-γ-lactone moiety. Various studies suggest that sesquiterpene lactone have analgesic and anti-inflammatory effect [10]. It is reported that the anti-inflammatory activity of these lactones is due to α-methylene-γ-lactone and cyclopentenone moieties that undergo Michael-type addition with various biological nucleophiles like L-cysteine, glutathione and a number of sulphahydryl-bearing cell enzymes [11–13]. In the present study, feverfew extract and parthenolide showed an anti-inflammatory effect against carrageenan induced paw-edema and their effect was comparable to that of nimesulide (2 mg/kg), a newer nonsteriodal anti-inflammatory drug. HPLC analysis of feverfew extract showed that parthenolide is the only major active constituent in extract, therefore, it can be speculated that antinociception induced by extract is mainly due to parthenolide itself. Naloxone opiate antagonist failed to block the feverfew extract or parthenolide induced antinociception, therefore, the involvement of opiate system has been ruled out. The antinociceptive effect of parthenolide was superior compared to nimesulide, a drug known to have good safety profile [14]. Feverfew extract neither altered the locomotor activity nor potentiated the pentobarbitone induced sleep time in mice. It also did not change the rectal temperature in rats. It indicates that the feverfew extract exerted analgesic and anti-inflammatory effects without altering the normal behaviour of the animals.




*Peak Results*

| # | Name | Retention Time (min) | Area (uV*sec) | Amount % | % Area |
|---|---|---|---|---|---|
| 1 | FF | 11.17 | 977112 | 0.856 | 88.95 |

Fig. 7. HPLC pattern of feverfew extract.

## 5. Conclusion

Feverfew extract exerted dose-dependent analgesic and anti-inflammatory effects. The active constituent of it, parthenolide, also produced the same effect. The effect of parthenolide was superior to nimesulide, a new generation of NSAID. The study indicated that the analgesic and anti-inflammatory effects of the extract are attributed to parthenolide, the active constituent.

## Acknowledgements

The authors appreciate M/s Dabur Research Foundation, New Delhi and Panacea Biotec Ltd., New Delhi for their kind assistance in providing the samples of feverfew extract, parthenolide pure and nimesulide, respectively for this work.

## References

[1] Berry, M.I., 1984. Feverfew faces the future. Pharmacy Journal 232, 611–614.
[2] Murphy, H., Mitchell, J.R., Heptinstall, S., 1988. Randomized double-blind placebo controlled trial of feverfew in migraine prevention. Lancet 11, 189–192.
[3] Berry, M., 1994. Feverfew. The Pharmaceutical Journal 253, 806–808.
[4] Johnson, E.S., Kadam, N.P., Hylands, D.M., Hylands, P.J., 1985. Efficacy of feverfew as prophylatic treatment of migraine. British Medical Journal 291, 569–573.
[5] Koster, R., Anderson, M., Debber, E.J., 1959. Acetic acid for analgesic screening. FASEB J. 18, 412.
[6] D'Armour, F.E., Smith, D.C., 1941. A method for determining loss of pain sensation. Journal of Pharmacology and Experimental Therapeutics 72, 74–79.
[7] Kulkarni, S.K., 1980. Heat and other physiological stress induced analgesia: catecholamine mediated and naloxone reversible response. Life Science 27, 185–188.
[8] Kulkarni, S.K., Mehta, A.K., Kunchandy, J., 1986. Anti-inflammatory actions of clonidine, guanfacine and B-HT 920 against various inflammagen-induced acute paw

edema in rats. Archives Internationales de Pharmacodynamic et de Therapie 279 (Suppl 2), 324–334.

[9] Hayes, N.A., Foreman, J.C., 1987. The activity of compounds extracted from feverfew on histamine release from rat mast cells. Journal of Pharmacy and Pharmacology 39, 466–470.

[10] Hall, L.H., Lee, K.H., Stanres, C.O., Sumida, T., Wu, R.Y., Waddell, T.G., Cochran, J.W., Gerhart, K.G., 1979. Anti-inflammatory activity of sesquiterpene lactones and related compounds. Journal of Pharmaceutical Sciences 68 (5), 537–542.

[11] Heptinstall, S., Groenewegen, W.A., Spangenberg, P., Loesche, W., 1987. Extract of feverfew may inhibit platelet behaviour via neutralization of sulphydryl groups. Journal of Pharmacy and Pharmacology 39, 459–465.

[12] Groenewegen, W.A., Knight, D.W., Heptinstall, S., 1986. Compounds extracted from feverfew that have anti-secretary activity contains an α-methylene butyrolactone unit. Journal of Pharmacy and Pharmacology 38, 709–712.

[13] Summer, M.J., Feniuk, W., Mc Cormick, J.D., Humphrey, P.P.A., 1992. Studies on the mechanism of 5-HT1 receptor-induced smooth muscle contraction in dog saphenous vein. British Journal of Pharmacology 105, 603–608.

[14] Rabasseda, X., 1990. Nimesulide: A selective cyclo-oxygenase-2 inhibition anti-inflammatory drug. Drug of Today 32 (Suppl 5), 365–384.