Anti-inflammatory and antinociceptive activity of hydroethanolic extract of Woodfordia fruticosa Kurz flowers

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ABSTRACT

In the present study, the analgesic and anti-inflammatory activity of 95% ethanolic extract of Woodfordia fruticosa (WFE) flowers in acute, subacute and chronic models of inflammation was assessed in rats and mice. Oral administration of WFE (250 and 500 mg/kg) exhibited significant anti-inflammatory activity in acute (carrageenin and autacoids induced hind paw edema), subacute (formaldehyde-induced hind paw edema) and chronic (cotton pellet granuloma) models of inflammation. WFE at a dose level of 250 and 500 mg/kg exhibited a significant (P < 0.05) reduction in writhes induced by intraperitoneal administration of acetic acid in mice. The hot plate reaction time was increased at a dose of 250 and 500 mg/kg significantly (P < 0.05).

Key words: Analgesic, Anti-inflammatory, Carrageenin, Woodfordia fruticosa.

INTRODUCTION

Despite progress within medical research during the past decades, the treatment of many serious diseases remains problematic. Chronic inflammatory diseases remain one of the world’s major health problems and may threaten the well-being of the host [1]. Currently, both steroidal and non-steroidal anti-inflammatory drugs (NSAIDs), are used in the relief of inflammation. Steroids have an obvious role in the treatment of inflammatory diseases, but due to their toxicity, can only be used over short periods except in very serious cases where the risks are acceptable. Prolonged use of NSAIDs is also associated with severe side effects, notably gastrointestinal haemorrhage [2]. The newer cyclooxygenase-2 (COX-2) selective drugs do not seem to be free of risk [3]. Consequently, there is a need to develop new anti-inflammatory agents with minimum side effects.

Woodfordia fruticosa Kurz belongs to family Lythraceae popularly known as Dhatki, Dawi, Dhai, Dhavdi etc. The plant is about 3.5 m high occurring abundantly throughout India [4]. They are used as tonic in disorders of mucous membranes, hemorrhoids and in derangement of the liver [5,6]. Flowers are also credited with immunomodulatory [7], antitumor activity [8], useful in diarrhoea [9], small pox [5], urinary disorders, burning sensation, wounds, bleeding injuries and headache [10]. The drug is useful in bowel complaints, cough [11] and shows antipyretic and anti-inflammatory activity. The leaves show antibiotic activity in-vitro against Micrococcus pyogenes var. aurens. The bark is pungent, acrid, cooling, toxic alextic, uterine sedative and anthelmintic. It is used in leprosy, and diseases of the blood [6]. Chemically, oenothein B and woofordin A, B, and C [12], isoschimacoalin-A and five
oligomers-woofordin E, F, G, H, I were reported from dried flowers [13]. Tewari et al has demonstrated the anti-leucorrhoic property of an ayurvedic preparation containing *W. fruticosa* [14]. Based on its diversified ethnopharmacological uses, the objective of the present study was to evaluate effect of 95% ethanolic extract of *Woodfordia fruticosa* flowers on acute, subacute and chronic models of inflammation in rats.

**MATERIALS AND METHODS**

**Plant material**

Fresh flowers of *W. fruticosa* were obtained as gift from M/s Aroma Chemicals, Saharanpur, UP, India. The flowers were identified and authenticated at source by Dr. A.K.S. Rawat (Pharmacognosy and Ethnopharmacology Division, National Botanical Research Institute (CSIR), Lucknow. A voucher specimen No. (NBRI/CIF/174/2010) has been deposited in the herbarium of the institute for future reference.

**Chemicals**

Carrageenin was purchased from CDH, New Delhi. Prostaglandin E₂, 5-hydroxytryptamine and bradykinin were supplied by Sigma Aldrich. Histamine was obtained from Acros Chemicals, USA. Acetic acid was purchased from Sd Fine-Chemicals Ltd., Mumbai. Acetyl salicylic acid, indomethacin, phenylbutazone and pentazocine were procured from Sigma Aldrich Chemicals Pvt Ltd., Bangalore. All chemicals used in the study were of analytical grade.

**Preparation of extract**

Flowers were dried in shade and ground to coarse powder and stored in an airtight container. The dried flowers were extracted (250 g) with ethyl alcohol (95%, v/v) in a Soxhlet extractor for 18–20 h. The extract was concentrated to dryness under reduced pressure and controlled temperature (40–50°C). The extract thus obtained was coded as WFE and was preserved in desiccators to prevent degradation by moisture. For the pharmacological studies, WFE was suspended in double distilled water containing gum acacia (2%, w/v).

**Experimental animals**

Studies were carried out using Wistar albino rats (180–220 g) and Swiss mice (25-30 g). The animals were purchased from the animal house of CDRI, Lucknow and were kept in departmental animal house in a cross ventilated room. The animals were housed in polycrystalline cages with not more than six animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2°C) with dark and light cycle (12/12 h). They were allowed free access to standard dry pellet diet and water *ad libitum*. All the animals were acclimatized to laboratory environment for 5 days before experiment. The animals were fasted overnight prior to experiment but were allowed free access to drinking water. All the studies were performed in accordance with the guidelines for the care and use of laboratory animals, as adopted and promulgated by the Institutional Animal Care Committee, CPCSEA, India (Reg. No.222/2000/CPCSEA).

**Anti-inflammatory activity**

**Carrageenin-induced hind paw edema in rats:**

The rats were divided into four different groups comprising of six animals each. The acute hind paw edema was produced by injecting 0.1 ml of carrageenin (prepared as 1% suspension in sterile normal saline) locally into the plantar aponeurosis of the right hind paw of rats [15]. Group 1 and group 2 served as negative and positive controls and received vehicle (gum acacia; 2% w/v) and standard drug, acetylsalicylic acid (ASA, 300 mg/kg, P.O.), respectively. WFE (250 and 500 mg/kg, P.O.) was administered to group 3 and group 4 respectively. WFE and ASA were administered 1 h prior to the injection of carrageenin. The rat pedal volume up to the ankle joint was measured using plethysmometer at 0 (just before) and 3 h after the injection of carrageenin. Increase in the paw edema volume was considered as the difference between 0 and 3 h.

**Autocoid-induced hind paw edema in rats:**

The effect of WFE (250 and 500 mg/kg, P.O.) was tested individually against autacoids, viz., histamine (1 mg/ml), 5-hydroxytryptamine (1 mg/ml), prostaglandin E₂ (1 µg/ml) and bradykinin (20 µg/ml) [16]. Right hind paw edema was induced by the sub plantar injection of 0.1 ml of respective autacoid. WFE (250 and 500 mg/kg, P.O.) and phenylbutazone (PBZ) (100 mg/kg, P.O.) were administered 1 h prior to the inflammatory insult. The pedal volume was measured just before 0 h and 3 h after the inflammatory challenge.
Formaldehyde-induced hind paw volume:
The test was performed according to the technique developed by Brownlee [17]. Pedal inflammation was induced by injecting 0.1 ml of 4% formaldehyde solution below the plantar aponeurosis of the hind paw of the rats. The paw volume was recorded immediately prior to compound administration (0 h) and then at 1.5, 24 and 48 h after formaldehyde injection. Group 1 and group 2 served as negative and positive controls and received vehicle (gum acacia; 2% w/v) and standard drug, acetylsalicylic acid (ASA, 300 mg/kg, P.O.), respectively. WFE (250 and 500 mg/kg, P.O.) was administered to group 3 and group 4 respectively. WFE and ASA were administered 1 h prior to formaldehyde injection.

Cotton pellet granuloma in rats:
The effect of WFE on chronic or proliferative phase of inflammation was assessed by cotton pellet granuloma rat model [18]. Autoclaved cotton pellets (35 ± 1 mg) were implanted subcutaneously along the axilla or flank region of the rats anesthetized with ether. Group 1 and group 2 served as negative and positive controls and received vehicle (gum acacia; 2% w/v) and standard drug, acetylsalicylic acid (ASA, 300 mg/kg, P.O.), respectively. WFE (250 and 500 mg/kg, P.O.) was administered to group 3 and group 4 respectively once daily for seven consecutive days from the day of cotton pellet insertion. On the eighth day, all the rats were sacrificed and the cotton pellets covered by the granulomatous tissue were excised and dried in hot air oven at 60°C till a constant weight was achieved. Granuloma weight was obtained by subtracting the weight of cotton pellet on 0 day from the weight of the cotton pellet on eighth day.

Analgesic activity
Acetic acid -induced writhing:
A total of 24 mice were divided into four groups of 6 in each group. Mice of group 1 (control) received gum acacia (2% w/v) and groups 2 and 3 administered 250 and 500 mg/kg orally WFE and group 4 received indomethacin (10 mg/kg), respectively. Analgesic activity was assessed by abdominal writhing test using acetic acid. In the writhing test, 0.6% acetic acid was injected intraperitoneally and the number of writhes was counted starting 10 min after injection for a period of 20 min [19]. Indomethacin and the WFE were administered 1 h before acetic acid injection.

Hot plate method:
The mice were divided into three groups of 6 in each group. Groups 1 was received 10 mg/kg pentazocine (PZ) intra peritoneal and group 2 and 3 were administered 250 and 500 mg/kg orally WFE respectively. The basal reaction time of all animals towards thermal heat was recorded. The animals which showed fore paw licking or jumping response within 6-8 seconds were selected for the study. After the 60 min administration of WFE and PZ, the animals in all groups were individually exposed to the Eddy’s hot plate maintained at 55 ± 1°C. The time for fore paw licking or jumping on the hot plate was taken as a reaction time [20]. A cut off period of 15 sec was observed to avoid damage to the paws.

Statistical analysis
The values are expressed as mean ± SD of six observations. The results obtained were statistically analyzed by Student’s t-test.

RESULTS AND DISCUSSION

Anti-inflammatory activity
Carrageenan-induced hind paw edema:
The mean increase in paw edema volume was about 0.84 ± 0.15 ml in the vehicle-treated group 1 control rats. WFE (250 and 500 mg/kg, P.O.) exhibited anti-inflammatory activity in a dose-dependent manner with the percent inhibition of paw edema of 15.00 and 51.11 (P < 0.05, P < 0.01), respectively, as compared with the control group. However, the standard drug, ASA (300 mg/kg, P.O.) showed highly significant (P < 0.001) anti-inflammatory activity with the percent inhibition of 75.00 (Table 1).

Autacoid-induced hind paw edema:
The mean increase in paw edema volume produced at 3 h after injection of different autacoids, viz., histamine, 5-HT, PGE2 and bradykinin was 0.24 ± 0.03, 0.47 ± 0.04, 0.26 ± 0.02 and 0.31 ± 0.04 ml respectively. WFE (500 mg/kg, P.O.) significantly (P < 0.01) inhibited hind paw edema induced by histamine, 5-HT, PGE2 and bradykinin.
However, phenylbutazone (100 mg/kg, P.O.) significantly (P < 0.001) inhibited all autacoids including bradykinin-induced hind paw as shown in Figure 1.

Formaldehyde-induced hind paw edema:
WFE (500 mg/kg, P.O.) significantly diminished the mean paw edema volume at 1.5 h (P < 0.01). However no significant effect was observed at 24 and 48 h for both the doses (Table 2). Aspirin showed a significant reduction in paw volume at 1.5 h (P < 0.001, 50.72%) but at 24 and 48 h no significant reduction in paw volume was observed as compared to control.

Cotton pellet granuloma:
The study of WFE on proliferative phase of inflammation indicated that WFE (500 mg/kg, P.O.) significantly (P < 0.01) reduced the granuloma formation with percentage inhibition of 37.55 as compared with control group. However, the standard drug, ASA (300 mg/kg, P.O.) showed highly significant (P < 0.001) anti-inflammatory activity with the percent inhibition of 46.72 (Table 1).

Analgesic activity
Acetic acid-induced writhing:
In the acetic acid-induced writhing test, WFE demonstrated dose dependently significant analgesic effect at 500 mg/kg dose, inhibiting pain by 38.14 % compared to control, and 25.4% at a dose of 250 mg/kg. (Figure 2)

Hot plate method:
WFE significantly increased the reaction time and it was 102.6 seconds (P < 0.05) and 126.7 seconds (P < 0.01) at a dose 250 and 500 mg/kg, respectively. Pentazocine significantly increased reaction time (P < 0.001) compared to respective group. (Figure 3)

Table 1  Effect of *Woodfordia fruticosa* extract on carrageenin-induced hind paw edema and cotton pellet granuloma in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg body weight)</th>
<th>Carrageenin induced hind paw edema volume (ml; mean ± SD)</th>
<th>Weight of cotton pellet granuloma (mg; mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Gum acacia (2 % w/v)</td>
<td>0.84 ± 0.3</td>
<td>109.19 ± 4.18</td>
</tr>
<tr>
<td>Group 2</td>
<td>ASA (300)</td>
<td>0.21 ± 0.05</td>
<td>58.17 ± 2.09</td>
</tr>
<tr>
<td>Group 3</td>
<td>WFE (250)</td>
<td>0.71 ± 0.04</td>
<td>101.49 ± 1.88</td>
</tr>
<tr>
<td>Group 4</td>
<td>WFE (500)</td>
<td>0.41 ± 0.03</td>
<td>68.18 ± 3.18</td>
</tr>
</tbody>
</table>

n=6 in each group; * P < 0.05; ** P < 0.01; *** P < 0.001 vs. control group.

Table 2 Effect of *Woodfordia fruticosa* extract on formaldehyde induced hind paw edema in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg)</th>
<th>Formaldehyde induced hind paw edema volume (ml; mean ± SD)</th>
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</thead>
<tbody>
<tr>
<td></td>
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<td>1.5h</td>
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<tr>
<td>Group 1</td>
<td>Gum acacia (2 % w/v)</td>
<td>0.72 ± 0.05</td>
</tr>
<tr>
<td>Group 2</td>
<td>ASA (300)</td>
<td>0.39 ± 0.06</td>
</tr>
<tr>
<td>Group 3</td>
<td>WFE (250)</td>
<td>0.58 ± 0.03</td>
</tr>
<tr>
<td>Group 4</td>
<td>WFE (500)</td>
<td>0.45 ± 0.04</td>
</tr>
</tbody>
</table>

n=6 in each group; * P < 0.05; ** P < 0.01; *** P < 0.001 vs. control group.

The present study demonstrates the anti-inflammatory and analgesic activity of the 95% ethanolic extract of *Woodfordia fruticosa* flowers on different models of inflammation. The paw edema induced by carrageenin has been extensively studied in the assessment of the anti-inflammatory action of steroidal and non-steroidal drugs involving several chemical mediators such as histamine, serotonin, bradykinin and prostaglandins. The edema and inflammation induced by carrageenin is shown to be mediated by histamine and 5-HT during first 1 h, after which increased vascular permeability is maintained by the release of kinins up to 2.30 h and from 2.30 to 6 h, the mediators appear to be prostaglandins, the release of which is closely associated with migration of leucocytes into the inflamed site [21]. In autacoid-induced inflammations, WFE produced significant inhibitory activity against 5-HT induced hind paw edema in rats at 500 mg/kg dose but failed to exhibit activity against histamine, PGE₂ and bradykinin-induced hind paw edema. Inflammation induced by formaldehyde is biphasic, an early neurogenic...
component is mediated by substance P and bradykinin followed by a tissue mediated response where histamine, 5-HT, prostaglandins and bradykinin are known to be involved [22]. In the formaldehyde-induced inflammation, the WFE at a dose of 500 mg/kg demonstrated significant anti-inflammatory activity that lasted up to 1.5 h. The present study demonstrates the anti-inflammatory and analgesic activity of the 95% ethanolic extract of *Woodfordia fruticosa* flowers on different models of inflammation. The paw edema induced by carrageenin has been extensively studied in the assessment of the anti-inflammatory action of steroidal and non-steroidal drugs involving several chemical mediators such as histamine, serotonin, bradykinin and prostaglandins. The edema and inflammation induced by carrageenin is shown to be mediated by histamine and 5-HT during first 1 h, after which increased vascular permeability is maintained by the release of kinins up to 2.30 h and from 2.30 to 6 h, the mediators appear to be prostaglandins, the release of which is closely associated with migration of leukocytes into the inflamed site [21]. In autacoid-induced inflammations, WFE produced significant inhibitory activity against 5-HT induced hind paw edema in rats at 500 mg/kg dose but failed to exhibit activity against histamine, PGE2 and bradykinin-induced hind paw edema. Inflammation induced by formaldehyde is biphasic, an early neurogenic component is mediated by substance P and bradykinin followed by a tissue mediated response where histamine, 5-HT, prostaglandins and bradykinin are known to be involved [22]. In the formaldehyde-induced inflammation, the WFE at a dose of 500 mg/kg demonstrated significant anti-inflammatory activity that lasted up to 1.5 h.

Figure 1. Effect of *Woodfordia fruticosa* extract on autacoid induced hind paw edema in rats. Group 1: normal control; Group 2: rats treated with phenylbutazone 100 mg/kg; Group 3: rats treated with WEF 250 mg/kg; Group 4: rats treated with WEF 500 mg/kg body weight.

*n=6 in each group; *P < 0.05; **P < 0.01; ***P < 0.001 vs. control group.

Figure 2. Effect of *Woodfordia fruticosa* extract on acetic acid induced writhing in mice. Group 1: normal control; Group 2: rats treated with indomethcin 10 mg/kg; Group 3: rats treated with WEF 250 mg/kg; Group 4: rats treated with WEF 500 mg/kg body weight.

*n=6 in each group; *P < 0.05; **P < 0.01; ***P < 0.001 vs. respective group.

Figure 3. Effect of *Woodfordia fruticosa* extract on hot plate method in mice. Group 1: rats treated with pentazocine 10 mg/kg; Group 2: rats treated with WEF 250 mg/kg; Group 3: rats treated with WEF 500 mg/kg body weight.

*n=6 in each group; *P < 0.05; **P < 0.01; ***P < 0.001 vs. respective group.
In order to assess the efficacy of WFE against proliferative phase of inflammation in which tissue degeneration and fibrosis occur, the widely used cotton pellet granuloma test was employed. During the repair process of inflammation, there is proliferation of macrophages, neutrophils, fibroblasts and multiplication of small blood vessels, which are the basic sources of forming a highly vascularised reddish mass, termed granulation tissue [23,24]. Though WFE (500 mg/kg, P.O.) significantly reduced the granuloma formation, the effect was of less intensity when compared with ASA (300 mg/kg, P.O.). The mechanism of anti-inflammatory activity of WFE on proliferative phase of inflammation in a rat model of cotton pellet granuloma is not exactly known and needs further study. Acetic acid causes inflammatory pain by increasing capillary permeability [25]. Writhes induced by noxious chemicals injected intraperitoneally is due to sensitization of nociceptors by prostaglandins. This test is useful for evaluation of mild analgesic nonsteroidal antiinflammatory compounds [26]. In the acetic acid-induced writhing test, the WFE demonstrated a significant analgesic effect at a dose of 500 mg/kg, inhibiting pain by 40.14 % compared to control, while at lower dose i.e. 250 mg/kg, the inhibition was not significant, 31.34 %. In hot plate method WFE showed significant increase in reaction time. Thus, our study shows that the flowers of *Woodfordia fruticosa* affords anti-inflammatory and analgesic activity by preventing the inflammation and inhibiting the pain in different animal models.

REFERENCES