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Analgesic and Anti-inflammatory effect of Different Extracts of *Flemingia wightiania*.

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ABSTRACT

Current study was undertaken to investigate the analgesic and anti-inflammatory effects of the different extracts of *Flemingia wightiania* in mice and rats. For evaluating of analgesic and anti-inflammatory activity, we used the acute and chronic inflammatory activities were studied in rats by formalin induced paw edema models, acetic acid-induced writhing, hot plate method and tail flick. Whereas n-Hexane, ethyl acetate, methanol and aqueous 300 mg/kg extracts also inhibited acetic acid-induced abdominal writhes in a dose-dependent manner. A dose of 300 mg/kg methanol and aqueous extracts exhibited significant (P<0.001) in hot plate method. The extracted compounds exhibited analgesic activity against chemically and thermal noxious stimuli on both early and late phases of pain by the methanol and aqueous extracts (300 mg/kg). Presently diclofenac sodium showed significant 87.14 % inhibition of inflammation at 5\(^{th}\) hour (0.18 ± 0.01) when compared with control (1.40 ± 0.05) respectively. The test compounds showed maximum percentage of inhibition of oedema at 5\(^{th}\) hour significantly in respective dose level i.e., at 300 mg/kg the test compounds HEFW, EAEFW, MEFW and AEFW showed (77.14%, 73.57 %, 85.00% and 83.57%) in acute formalin induced paw edema model. Formalin induced paw oedema is one of the most suitable test procedure to screen chronic anti-inflammatory agents. The mean response of diclofenac sodium 100 mg/kg was 82.40% inhibition of increase in paw thickness after 6 days respectively. In this model at 300 mg/kg dose level of HEFW, EAEFW, MEFW and AEFW extracts showed 27.03%, 20.60%, 65.66%, and 59.22% inhibition of increase in paw thickness after 6 days The data obtained also suggest that the anti-inflammatory and analgesic effects of the extracts may be mediated via both peripheral and central mechanisms.

**Keywords**: Analgesic; Anti-inflammatory; Formalin test; Tail flick; *Flemingia wightiania*.

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INTRODUCTION

Inflammation is considered as a primary physiologic defense mechanism that helps body to protect itself against infection, burn, toxic chemicals, allergens or other noxious stimuli. An uncontrolled and persistent inflammation may act as an etiologic factor for many of these chronic illnesses. [1] Although it is a defense mechanism, the complex events and mediators involved the inflammatory reaction can induce, maintain or aggravate many diseases. [2] Pain has been defined by International Association for the Study of Pain (IASP) as an unpleasant sensory and emotional experience associated with actual or potential tissue damage [3].

Drugs that are currently used for the management of pain are opioids or non-opioids and that for inflammatory conditions are non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids. All these drugs carry potential toxic effects. One study suggests that risk of gastrointestinal bleeding was significantly associated with acute use of non-steroidal anti-inflammatory drugs (NSAIDs) like regular-dose aspirin, diclofenac, ketorolac, naproxen or nimesulide. Piroxicam increased the risk of bleeding in both acute and chronic therapy [4]. Opioids are the commonly used drugs for the management of acute postoperative pain [5].

On the contrary many medicines of plant origin had been used since ages without any adverse effects. It is therefore essential that efforts should be made to introduce new medicinal plants to develop more effective and cheaper drugs. Plants represent a large natural source of useful compounds that might serve as lead for the development of novel drugs.

_Flemingia wightiana_ (Fam. Fabaceae) commonly known as kandaran regu, dandola a crop plant of Andhra Pradesh, Karnataka, Orissa, Tamil Nadu, Nilgiris, Koncan, Canara. [6] _Flemingia wightiana_ is a low erect perennial shrub, with tomentose young shoots. Petiole 1.3 - 2.5 cm., spikes dense, oblong, 2.5-5cm long, often fascicled, bracts under 1.3 cm long, erect – patent, subpersistent. Calyx 1 cm, teeth plumose. Pods oblong, 1 cm long, finely pubescent and often covered with red viscous glands. This plant is used externally for skin disease, inflammation and internally as a purgative and specific for cold [7].

MATERIALS AND METHODS

Plant Material:

The whole plant _Flemingia wightiana_. is widely found in the South India. The plant was collected from the Chittoor district (Andhra Pradesh), identified and authenticated by Dr. K. Madhava Chetty, Asst. Professor, Dept. of Botany. Sri Venkateswara University, Tirupathi.

Preparation of Plant Extract:

The whole plant was shade dried at room temperature and was chopped into small pieces. Dried plant were powdered and packed in air tight container. The coarse material was subjected to successive soxhlet extraction by using different solvents. Solvents are used based
on their increasing order of polarity i.e., n-Hexane (0.1), Ethyl acetate (4.4), Methanol (5.1) and Water (10.2). The extraction is carried with Methanol, n-Hexane and Ethyl acetate. The aqueous extraction was carried out by cold maceration process. The extracts were concentrated under reduced pressure and stored in desiccators.

**Determination of Acute Toxicity (LD<sub>50</sub>):**

The procedure was divided into two phases. Phase I (observation made on day one) and Phase II (observed the animals for next 14 days of drug administration). Two sets of healthy female rats (each set of 3 rats) were used for this experiment. First set of animals were divided into three groups, each of one in a group. Animals were fasted overnight with water ad libitum. Animals received a single dose of 2000 mg/kg, p.o. was selected for the test, as the test item was a source from herb. After administration of extract, food was withheld for 3-4 hrs. [8]

**Experimental Animals:**

Albino wistar rats weighing 150-200g and Albino mice 20-30 g was procured from Biogen, Bangalore. They were maintained in the animal house of Gautham College of Pharmacy, for experimental purpose. Animals were maintained under controlled condition of temperature at 27° ± 2° C and 12 hr light-dark cycles for one week. They were housed in polypropylene cages and containing paddy husk as bedding. They had a free access to standard pellets and water ad libitum. All the studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) of Gautham College of Pharmacy, Bangalore (REF-IAEC/012/12/2010) according to prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India.

**Evaluation of Analgesic Activity:**

**Acetic acid Induced Writhing in Mice:**

Albino mice weighing 20-30 mg/kg were divided into six groups of six in each group. One hour after the administration of the test drug and diclofenac sodium (10 mg/kg i.p), the mice were given intraperitoneal injection of 0.7%v/v acetic acid solution (volume of injection 0.1ml 10g), the mice were placed individually into glass beakers and 5 minutes, were allowed to elapse. The number of writhes produced in these animals was counted for 15 minutes. For scoring purposes, a writh is indicated by stretching of the abdomen with simultaneous stretching of at least one hind limb. The formula for computing present inhibition was; [9]

- Group-I: Distilled water will be supplied and served as control.
- Group-II: Animals received a dose of 10 mg/kg of Diclofenac sodium i.p. and served as standard
- Group-III: Animals received a dose of 300 mg/kg of HEFW p.o.
- Group-IV: Animals received a dose of 300 mg/kg of EAEFW p.o.
- Group-V: Animals received a dose of 300 mg/kg of MEFW p.o.
Group-VI: Animals received a dose of 300 mg/kg of AEFW p.o.

Tail Flick Method:

Albino wistar rats weighing 150-230 mg/kg were divided into six groups of six in each group. The tail flick latency was assessed by analgesiometer. A light beam is focused (exerting radiant heat) to the proximal third of the tail. The rat tries to pull the tail away and rotates the head; this reaction is known as escape reaction. The reaction time is recorded ½, 1, 2, 3, 4, 5 and 6 hours following intra peritoneal administration of the standard and oral administration of the test compounds.

The strength of the current passing through the naked nichrome wire was kept constant at 6 amperes. The distance between the heat source and tail skin was 1.5 cm. The site of application of the radiant heat in the tail was maintained at 2.5 cm measured from the root of the tail. The cutoff reaction time was fixed at 10 seconds to avoid tissue damage.[10]

Group-I: Distilled water will be supplied and served as control.
Group-II: Animals received a dose of 10 mg/kg of Diclofenac sodium i.p. and served as standard.
Group-III: Animals received a dose of 300 mg/kg of HEFW p.o.
Group-IV: Animals received a dose of 300 mg/kg of EAEFW p.o.
Group-V: Animals received a dose of 300 mg/kg of MEFW p.o.
Group-VI: Animals received a dose of 300 mg/kg of AEFW p.o.

Hot plate Method:

Albino mice weighing 20-30 mg/kg were divided into six groups of six in each group. The temperature is controlled for 55 ± 1°C. The animals were placed into the Perspex cylinder on the heated surface and the time (sec) to discomfort reaction (licking paws or jumping) was recorded as response latency, period to and 30, 60, 90, 120 and 180 minutes following intra peritoneal administration of the standard and oral administration of the test compounds. A latency period of 15 seconds was identified as complete analgesia and the measurement was terminated if it exceeded the latency period in order to avoid injury. [11]

Group-I: Distilled water will be supplied and served as control.
Group-II: Animals received a dose of 10 mg/kg of Pentazocine lactate i.p. and served as standard.
Group-III: Animals received a dose of 300 mg/kg of HEFW p.o.
Group-IV: Animals received a dose of 300 mg/kg of EAEFW p.o.
Group-V: Animals received a dose of 300 mg/kg of MEFW p.o.
Group-VI: Animals received a dose of 300 mg/kg of AEFW p.o.
Evaluation of Anti-Inflammatory Activity

Acute Anti-inflammatory Activity

Formalin-induced Paw Oedema in Rats:[12]

Acute inflammation was induce by injecting formalin (0.1 ml of 1% suspension in 0.9% saline) in sub-plantar region and paw volume was measured 0, 1, 2, 3, 4 and 5 hours with the help of Plethysmometer. All the treatment compounds compound were administered 30 minutes prior to formalin. Acute inflammation was induced in right hind paw. A mark was put on the leg second at the leg at the mallaleous region to facilitate the dipping of the leg to the same level at the second and subsequent times.

The initial reading was taken at 0th hour, i.e., immediately after injecting formalin and the procedure was repeated at 1, 2, 3, 4 and 5 hours after formalin injection. The difference between 0 hour reading and one of the subsequent reading provides the actual edema volume at the time. The mean paw volume at different times was calculated and compared with the control and the percentage inhibition was then calculated by using the formula;

Group-I: Distilled water will be supplied and served as control.
Group-II: Animals received a dose of 10 mg/kg of Diclofenac sodium i.p. and served as standard
Group-III: Animals received a dose of 300 mg/kg of HEFW p.o.
Group-IV: Animals received a dose of 300 mg/kg of EAEFW p.o.
Group-V: Animals received a dose of 300 mg/kg of MEFW p.o.
Group-VI: Animals received a dose of 300 mg/kg of AEFW p.o.

Chronic Anti-inflammatory Activity

Formalin Induced Paw Oedema: [13]

Albino wistar rats weighing 170-230 g were divided into six groups of six in each group. All these animals were fasted for 18 hours before the beginning of the experiment and water was given ad libitum. In animals of all the groups chronic inflammation was produced by sub plantar injection of 20µl of freshly prepared 2% suspension of formalin in normal saline in right hind paw of rat was used as the oedematogenic agent. Animals were treated with drugs for 6 consecutive days.

The paw volume was measured using a plethysmometer before and 6 days after formalin challenge in each group. The increase in paw volume and percent of inhibition was calculated.

Group-I: Distilled water will be supplied and served as control.
Group-II: Animals received a dose of 100 mg/kg of Diclofenac sodium i.p. and served as standard
Group-III: Animals received a dose of 300 mg/kg of HEFW p.o.
Group-IV: Animals received a dose of 300 mg/kg of EAEFW p.o.
Group-V: Animals received a dose of 300 mg/kg of MEFW p.o.
Group-VI: Animals received a dose of 300 mg/kg of AEFW p.o.

Statistical Analysis:

The values are expressed as Mean ± SEM. The data was analysed by using one way ANOVA followed by Dunnett’s test using Graph pad prism software. Statistical significance was set at P ≤ 0.05.

RESULTS AND DISCUSSION

Analgesic Activity

Effect of Flemingia wightiania Plant Extracts on Acetic acid Induced Writhing in Mice

Control and various treated groups were tested for analgesic activity against acetic acid induced writhing, which is nothing but the painful reaction. Thirty minutes after the treatment each mouse was 0.1 ml 0.7% v/v aqueous solution of acetic acid injected i.p. The number of abdominal constrictions was cumulatively counted from 0 - 10 minutes. The % reduction of writhing in standard Diclofenac sodium 10 mg/kg treated group was found to be 55.20% against control.

The mean response of control and standard was 41.50 ± 1.25 and 18.59 ± 092 respectively. The respective test compounds HEFW, EAEFW, MEFW and AEFW in its 300mg/kg dose, showed mean writhing responses as 25.59 ± 1.43, 28.33 ± 1.66, 20.67 ± 1.11 and 23.83 ± 1.30. In terms of percentage inhibition of writhing by Diclofenac sodium was 55.20% while with the test compound it was 38.33%, 31.73%, 50.19% and 42.57% respectively. The values are tabulated in the Table 1.

Acetic acid-acid-induced writhing model represents pain sensation by triggering localized inflammatory response. Such pain stimulus leads to the release of free arachidonic acid from tissue phospholipids [14]. The acetic acid induced writhing response is a sensitive procedure to evaluate peripherally acting analgesics. The response is thought to be mediated by peritoneal mast cells acid sensing ion channels and the prostaglandin pathway [15-17].
**Table 1: Effect of Flemingia wightiana Plant Extracts on Acetic acid Induced Writhing in Mice**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Mean no of writhing ±SEM</th>
<th>% Inhibition of writhes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I</td>
<td>Saline</td>
<td>41.50 ± 1.25</td>
<td>-</td>
</tr>
<tr>
<td>Group-II</td>
<td>Diclofenac sodium (10mg/kg)</td>
<td>18.59 ± 092</td>
<td>55.20%</td>
</tr>
<tr>
<td>Group-III</td>
<td>HEFW (300mg/kg)</td>
<td>25.59 ± 1.43</td>
<td>38.33%</td>
</tr>
<tr>
<td>Group-IV</td>
<td>EAEFW (300mg/kg)</td>
<td>28.33 ± 1.66</td>
<td>31.73%</td>
</tr>
<tr>
<td>Group-V</td>
<td>MEEW (300mg/kg)</td>
<td>20.67 ± 1.11</td>
<td>50.19%</td>
</tr>
<tr>
<td>Group-VI</td>
<td>AEFW (300mg/kg)</td>
<td>23.83 ± 1.30</td>
<td>42.57%</td>
</tr>
</tbody>
</table>

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett’s test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant.

HEFW - n-Hexane extract of Flemingia wightiana, EAEFW - Ethyl acetate extract of Flemingia wightiana, MEEW - Methanolic extract of Flemingia wightiana, AEFW - Aqueous extract of Flemingia wightiana

**Effect of Flemingia wightiana Plant Extracts on Tail Flick method in Rats**

In the tail flick method, the increase in latency period at different time points significantly differed (P<0.001) compared to baseline values within the same drug treated groups. The extracts and diclofenac sodium caused significant increase (P<0.001) in the percentage reaction time whilst the control and dose of extracts (300 mg/kg). The percentage increase in reaction time was dose dependent. At all the specified time intervals, the percentage of tail flick elongation time differed significantly (P<0.001) between the extracts and diclofenac sodium at the doses of plant extracts, being greater for diclofenac sodium. At the peak of activity, MEEF and AEFW extracts showed (P<0.001) and significantly of tail flick elongation time respectively, whilst diclofenac sodium gave (P<0.001) elongation of tail flicking time. The values are tabulated in the Table 2.
### Table 2: Effect of *Flemingia wightiana* Plant Extracts on Tail Flick Method Test in Rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Reaction Time (Sec)</th>
<th>0 hr</th>
<th>½ hr</th>
<th>1 hr</th>
<th>2 hr</th>
<th>3 hr</th>
<th>4 hr</th>
<th>5 hr</th>
<th>6 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group-I</td>
<td>Saline</td>
<td></td>
<td>4.51±0.29</td>
<td>4.48±0.23</td>
<td>5.05±0.34</td>
<td>5.30±0.28</td>
<td>5.70±0.38</td>
<td>5.76±0.36</td>
<td>4.86±0.57</td>
<td>5.65±0.51</td>
</tr>
<tr>
<td>Group-II</td>
<td>Diclofenac sodium (10mg/kg)</td>
<td>4.71±0.31</td>
<td>8.86±0.39***</td>
<td>10.05±0.45***</td>
<td>11.52±0.98***</td>
<td>12.32±0.77***</td>
<td>13.17±1.19***</td>
<td>14.28±0.90***</td>
<td>13.62±0.63***</td>
<td></td>
</tr>
<tr>
<td>Group-III</td>
<td>HEFW (300mg/kg)</td>
<td>4.50±0.27</td>
<td>7.10±0.62 ns</td>
<td>7.86±0.80*</td>
<td>9.13±0.68**</td>
<td>9.33±0.57**</td>
<td>10.13±0.81**</td>
<td>11.12±0.82**</td>
<td>9.48±0.84**</td>
<td></td>
</tr>
<tr>
<td>Group-IV</td>
<td>EAEFW (300mg/kg)</td>
<td>4.71±0.48</td>
<td>6.95±0.86*</td>
<td>7.71±0.74ns</td>
<td>8.61±1.01*</td>
<td>8.71±0.63*</td>
<td>9.80±0.75**</td>
<td>10.90±0.90***</td>
<td>8.86±0.82*</td>
<td></td>
</tr>
<tr>
<td>Group-V</td>
<td>MEFW (300mg/kg)</td>
<td>4.43±0.44</td>
<td>8.51±0.99**</td>
<td>9.71±0.87***</td>
<td>10.58±0.56***</td>
<td>11.25±0.86***</td>
<td>12.02±0.87***</td>
<td>13.07±0.65***</td>
<td>11.13±0.69***</td>
<td></td>
</tr>
<tr>
<td>Group-VI</td>
<td>AEFW (300mg/kg)</td>
<td>4.70±0.24</td>
<td>7.60±0.83*</td>
<td>8.60±0.97**</td>
<td>9.86±0.96**</td>
<td>10.47±0.66***</td>
<td>11.33±0.63***</td>
<td>12.58±0.74***</td>
<td>9.93±0.73***</td>
<td></td>
</tr>
</tbody>
</table>

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett’s test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. HEFW - n-Hexane extract of *Flemingia wightiana*, EAEFW - Ethyl acetate extract of *Flemingia wightiana*, MEFW - Methanolic extract of *Flemingia wightiana*, AEFW - Aqueous extract of *Flemingia wightiana*

### Effect of *Flemingia wightiana* Plant Extracts on Hot Plate Method in Mice

The standard pentazocine lactate (10 mg/kg) was given i.p., n-Hexane, Ethyl acetate, Methanol and Aqueous extracts given orally, in a dose of 300 mg/kg, elicited a significant analgesic activity in the hot plate method as evidenced by increase in latency time in seconds as compared with vehicle control. The increase in latency time was dose dependant. Latency time was noted 30, 60, 90, 120 and 180 minutes after administration of vehicle, standard and plant extracts. The values are tabulated in the Table 3.

To evaluate the analgesic activity, hot plate method was chosen. In this method pentazocine lactate (10 mg/kg) was used as reference standard. The HEFW, EAEFW, MEFW and AEFW extracts of *Flemingia wightiana* produced antinociception against thermal induced pain stimuli in mice at various time points of post treatment. The hot plate test is considered to be selective for opioid like compounds, which are centrally acting analgesic in several animal species. The hot plate method has been found to be suitable for evaluation of centrally acting analgesic [18, 19]. The HEFW, EAEFW, MEFW and AEFW (300 mg/kg p.o.) increase the reaction time in dose dependant manner to the thermal stimulus.
### Table 3: Effect of *Flemingia wightiania* Plant Extracts on Hot plate Method test in Mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Reaction time (Sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>Group-1</td>
<td>Saline</td>
<td>2.66 ± 0.33</td>
</tr>
<tr>
<td>Group-II</td>
<td>Pentazocine lactate (10mg/kg)</td>
<td>2.50 ± 0.22</td>
</tr>
<tr>
<td>Group-III</td>
<td>HFW (300mg/kg)</td>
<td>2.16 ± 0.16</td>
</tr>
<tr>
<td>Group-IV</td>
<td>EAEFW (300mg/kg)</td>
<td>2.83 ± 0.30</td>
</tr>
<tr>
<td>Group-V</td>
<td>MEEW (300mg/kg)</td>
<td>3.16 ± 0.47</td>
</tr>
<tr>
<td>Group-VI</td>
<td>AEFW (300mg/kg)</td>
<td>3.00 ± 0.36</td>
</tr>
</tbody>
</table>

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett’s test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. HFW - n-Hexane extract of *Flemingia wightiania*, EAEFW - Ethyl acetate extract of *Flemingia wightiania*, MEEW - Methanolic extract of *Flemingia wightiania*, AEFW - Aqueous extract of *Flemingia wightiania*

### Anti inflammatory Activity:

#### Acute Anti inflammatory Activity:

**Effect of *Flemingia wightiania* Plant Extracts on Formalin-induced paw Oedema in Rats**

All the test compounds were tested with the diclofenac sodium as a standard drug in the dose of 10 mg/kg for the anti-inflammatory activity. Presently diclofenac sodium showed significant 87.14 % inhibition of inflammation at 5th hour (0.18 ± 0.01) when compared with control (1.40 ± 0.05) respectively. The test compounds showed maximum percentage of inhibition of oedema at 5th hour significantly in respective dose level i.e., at 300 mg/kg the test compounds HFW, EAEFW, MEFW and AEFW showed 77.14%, 73.57 %, 85.00% and 83.57%. The values are tabulated in the Table 4. It is well known that inhibition of formalin-induced pedal oedema in rats is one of the most suitable test procedures to screen anti-arthritic and anti-inflammatory agents as it closely resembles human arthritis [20]. Injection of formalin subcutaneously into hind paw of rats produces localized inflammation and pain. The nociceptive effect of formalin is biphasic, an early neurogenic component followed by a later tissue mediated response [21]. Thus formalin-induced arthritis is a model used for the evaluation of an agent with probable anti-proliferative activity. This experiment is associated with the proliferative phase of inflammation. Results with *Flemingia wightiania* of HFW, EAEFW, MEFW and AEFW are showed quite compatible with those of the standard drug diclofenac sodium. Therefore, the test drug appears to be effective against formalin-induced arthritis.
Table 4: Effect of *Flemingia wightiana* Plant Extracts on Formalin-induced Paw Oedema in Rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>0 hr</th>
<th>1hr</th>
<th>2hr</th>
<th>3hr</th>
<th>4 hr</th>
<th>5 hr</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I</td>
<td>Saline</td>
<td>0.66 ± 0.01</td>
<td>0.78 ± 0.05</td>
<td>0.99 ± 0.05</td>
<td>1.20 ± 0.06</td>
<td>1.25 ± 0.07</td>
<td>1.40 ± 0.05</td>
<td>-</td>
</tr>
<tr>
<td>Group-II</td>
<td>Diclofenac sodium (10mg/kg)</td>
<td>0.15 ± 0.01</td>
<td>0.37 ± 0.02***</td>
<td>0.55 ± 0.04***</td>
<td>0.36 ± 0.2***</td>
<td>0.30 ± 0.03***</td>
<td>0.18 ± 0.01***</td>
<td>87.14%</td>
</tr>
<tr>
<td>Group-III</td>
<td>HEFW (300mg/kg)</td>
<td>0.13 ± 0.01</td>
<td>0.60 ± 0.04*</td>
<td>0.76 ± 0.05**</td>
<td>0.63 ± 0.03***</td>
<td>0.48 ± 0.02***</td>
<td>0.32 ± 0.05***</td>
<td>77.14%</td>
</tr>
<tr>
<td>Group-IV</td>
<td>EAEFW (300mg/kg)</td>
<td>0.14 ± 0.02</td>
<td>0.67 ± 0.06ns</td>
<td>0.79 ± 0.03*</td>
<td>0.61 ± 0.02***</td>
<td>0.51 ± 0.02***</td>
<td>0.37 ± 0.01***</td>
<td>73.57%</td>
</tr>
<tr>
<td>Group-V</td>
<td>MEFW (300mg/kg)</td>
<td>0.15 ± 0.02</td>
<td>0.52 ± 0.03**</td>
<td>0.61 ± 0.03***</td>
<td>0.46 ± 0.02***</td>
<td>0.36 ± 0.02***</td>
<td>0.21 ± 0.02***</td>
<td>85.00%</td>
</tr>
<tr>
<td>Group-VI</td>
<td>AEFW (300mg/kg)</td>
<td>0.13 ± 0.01</td>
<td>0.56 ± 0.03**</td>
<td>0.65 ± 0.03***</td>
<td>0.50 ± 0.02***</td>
<td>0.37 ± 0.02***</td>
<td>0.23 ± 0.03***</td>
<td>83.57%</td>
</tr>
</tbody>
</table>

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett’s test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. HEFW - n-Hexane extract of *Flemingia wightiana*, EAEFW - Ethyl acetate extract of *Flemingia wightiana*, MEEW - Methanolic extract of *Flemingia wightiana*, AEFW - Aqueous extract of *Flemingia wightiana*

**Chronic Anti inflammatory Activity**

**Effect of *Flemingia wightiana* Plant Extracts on Formalin-induced Paw Oedema in Rats**

Formalin induced paw oedema is one of the most suitable test procedure to screen chronic anti-inflammatory agents. The results obtained as mean increase in paw volume (ml) and % inhibition are represented in Table 5. The mean response of standard was 82.40% inhibition of increase in paw thickness after 6 days respectively. In this model at 300 mg/kg dose level of HEFW, EAEFW, MEFW and AEFW extracts showed 40.77%, 39.48%, 62.23% and 54.93% inhibition of increase in paw thickness after 6 days, However at MEFW, AEFW extracts showed 62.23% and 54.93% inhibition of increase in paw thickness after 6 days. All the results were compared with solvent control and diclofenac sodium reference drug control.
Table 5: Effect of *Flemingia wightiania* Plant Extracts on Formalin-induced Paw Oedema in Rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Initial Paw Volume</th>
<th>Paw Volume After 6 Days</th>
<th>Increase in Paw Volume</th>
<th>% of Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I</td>
<td>Saline</td>
<td>1.28 ± 0.07</td>
<td>3.61 ± 0.12</td>
<td>2.33 ± 0.06</td>
<td>-</td>
</tr>
<tr>
<td>Group-II</td>
<td>Diclofenac sodium (100 mg/kg)</td>
<td>1.23 ± 0.04</td>
<td>1.65 ± 0.05</td>
<td>0.41 ± 0.07</td>
<td>82.40%</td>
</tr>
<tr>
<td>Group-III</td>
<td>HEFW (300mg/kg)</td>
<td>1.30 ± 0.05</td>
<td>2.85 ± 0.13</td>
<td>1.38 ± 0.22</td>
<td>40.77%</td>
</tr>
<tr>
<td>Group-IV</td>
<td>EAEFW (300mg/kg)</td>
<td>1.30 ± 0.07</td>
<td>2.86 ± 0.14</td>
<td>1.41 ± 0.19</td>
<td>39.48%</td>
</tr>
<tr>
<td>Group-V</td>
<td>MEEW (300mg/kg)</td>
<td>1.23 ± 0.07</td>
<td>2.08 ± 0.14</td>
<td>0.88 ± 0.11</td>
<td>62.23%</td>
</tr>
<tr>
<td>Group-VI</td>
<td>AEFW (300mg/kg)</td>
<td>1.26 ± 0.07</td>
<td>2.26 ± 0.16</td>
<td>1.05 ± 0.14</td>
<td>54.93%</td>
</tr>
</tbody>
</table>

Results are expressed on Mean + SEM from four observations Paw Volume was measured after 6 days.

HEFW - n-Hexane extract of *Flemingia wightiania*, EAEFW - Ethyl acetate extract of *Flemingia wightiania*, MEEW - Methanolic extract of *Flemingia wightiania*, AEFW - Aqueous extract of *Flemingia wightiania*

**CONCLUSION**

In conclusion, this study demonstrated that the n-Hexane, ethyl acetate, methanol and water extracts of *Flemingia wightiania* have a significant analgesic and anti-inflammatory activity. Further studies will be necessary to establish the probable mechanism of action of anti-inflammatory and analgesic activities of different extracts of *Flemingia wightiania*.

**REFERENCES**


