Analgesic activity of extracts of *Woodfordia fruticosa* stems bark in animal models.

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**Abstract**- *Woodfordia fruticosa* is locally known as Dhawai, belonging to the Lythraceae family. It is commonly used in the treatment of various diseases like diarrhoea, dysentery, fever, headache, haemorrhoids, herpes, internal haemorrhage, leucorrhoea, liver disorders, menorrhagia, ulcer and wounds etc. The present study was designed to evaluate the analgesic activity of *Woodfordia fruticosa* stem bark in albino rats by using thermal and chemical models of nociception hot plate method and acetic acid induced writhing test respectively. The petroleum ether, chloroform, ethanol and aqueous extracts are administered orally to the rats in their respective groups at a dose of 200mg/kg according to their body weight. Analgin is a standard drug which is administered on the standard group. The results of aqueous extract and alcoholic extract exhibited statistically significant (***P‹0.01&*P‹0.05) analgesic activity in albino rats. *Woodfordia fruticosa* extracts produce inhibition effect in thermal and chemical pain models through a mechanism partially linked to either Lipooxygenase or cyclooxygenase via the arachidonic acid cascade or opioid receptors.

**Key words:** Analgesic activity, Dhawai, Medicinal plants, Pain, *Woodfordia fruticosa*.

**Introduction**

*Woodfordia fruticosa* is a wild plant growing in the forest of Jashpur district of Chhattisgarh, India. It is an evergreen shrub up to 5 m tall, with diffuse, irregular branching. It is usually found flowering throughout the year, but a distinct peak in abundance can be observed in March & April. The nectar-rich flowers are regularly visited by insects. The flowers are stimulant and an infusion of the flowers and leaves is used as an herbal tea. Powdered dried flowers in curdled milk are used in the treatment of dysentery, diarrhoea and internal haemorrhages and, with honey are given for leucorrhoea and menorrhagia. Externally, the powder is sprinkled over foul ulcers and wounds to diminish discharge and promote granulation, and used in lotions for the same purpose. Dried flowers are useful in disorders of the mucous membranes, haemorrhoids and disorders of the liver. Flower and root used in the treatment of rheumatism, dysentery, foot and mouth disease, lumbar and rib fracture [1-4].

Previous works reported that flowers contain phytoconstituents they are hydrolysable tannins oenothein A and B, woodfordins A. It also contains contain pelargonidin-3, 5, diglucoside, cyanidin-3, 5, diglucoside and the leaves contain numerous quercetin and myricetin glycosides. Flowers contained chemical constituents are Woodfordin A, B, C, D, E, F, G, H, I. Phytosterols and hydrocarbons octacosanol and β sitosterol [5-8].

The aim of this study was to assess the analgesic activity of different extracts obtained from *Woodfordia fruticosa* stem barks, using thermal and chemical models of pain in albino rats.

**Material and Methods:**

**Plant material:** The stem barks of *Woodfordia fruticosa* were collected during October-November 2008 from Jashpur District of Chhattisgarh, India. The plant material was identified by Dr. Chandrama Prakash Upadhyay, Assistant professor, Department of Botany, Guru Ghasidas Vishwavidyalaya, Bilaspur, C.G. The Voucher specimen (SLT/Med Plant/01/2009) was maintained in research laboratory for further reference in SLT Institute of Pharmaceutical Sciences. The plant material was shade dried and then powdered with mechanical grinder, passing through sieve no 40, and stored in an air tight container. The sieved powder was further taken up for the preparation of the extract.

**Preparation of extract:**

100 gm stems bark powdered material was subjected to successive solvent extraction in Soxhlet extractor using petroleum ether, chloroform, alcohol and aqueous as solvent. All the extracts were filtered and were concentrated and vacuum dried. The all extracts were weighed petroleum ether extract (1.21 gm), chloroform extract (0.45gm), alcohol extract (6.13gm) and aqueous extract (9.53gm) respectively. These extracts were used for the study.
Experimental Animals:
Albino Wister rats (100-150 gm) were obtained from the animal centre of SLT institute of Pharmaceutical Sciences, Bilaspur, C.G. Animals were housed in groups of five at the room temperature of 25±1°C with free access to food and water. The study protocol was approved by Institutional Animal Ethics Committee; Registration no is 994/a/Go/06/CPCSEA. For screening of analgesic activity albino rats were divided into six different groups. The first group served as a control group. The second group was used as standard. And the last four groups received extracts of petroleum ether, chloroform, alcoholic and aqueous of Woodfordia fruticosa stems bark respectively at the dose of 200 mg/kg orally.

Preliminary Phytochemical Analysis: Phytochemical screening was carried out the various constituents present in the extracts. The extract was tested for the presence of glycoside, alkaloids, steroids, flavonoids, tannins, mucilages, fixed oils, phenolic compounds, proteins and sterols standard qualitative tests [9, 10].

Experimental design:
Hot plate method: The animals were divided into six groups of six rats in each group of albino rat’s 100-150gm body weights [11-14]. The test drugs of 200mg/kg and standard drug ibuprofen of 30mg/kg were administered orally, 30 min before the experiment started. Albino rats were placed on a hot plate maintained at 55±1°C and the reaction time (latency in seconds) for licking of hind paw or jumping noted. The reaction time was observed at 0, 30, 60 and 90 min after drug administration.

Acetic Acid-Induced Writhing Test:
The writhing test in albino rats was conducted by acetic acid induced writhing method using six groups of albino rats 100-150 gm body weight of either sex selected by random sampling technique. The Standard drug ibuprofen (30 mg/kg) and the extracts (200 mg/kg)) were given orally 30 minutes prior to the administration of the writhing agent of 1%v/v aqueous acetic acid. The number of writhing produced in the animal was observed for 10 minutes. The number of writhing and stretching was recorded and compared the same with the effects of the Control drug. The percent was calculated using the following ratio: % of protection = Control mean- treated mean X 100/Control mean [15-16].

Statistical Analysis:
The results are expressed as mean ± SEM. The Dunnett’s test was used to make a statistical comparison between the groups. The results with P<0.01 and P<0.05 were considered as significant.

Results and discussion:
The present study revealed the positive analgesic activity of extracts of Woodfordia fruticosa stem bark in hot plate model and acetic acid induced writhing model. Pain sensation in acetic acid induced writhing method is elicited by triggering localized inflammatory responses resulting the release of the free arachidonic acid from tissue phospholipid via cyclooxygenase (COX), and prostaglandin biosynthesis [17-18]. A significant increase in the reaction time for hot plate method is indicated the analgesic effect of the involvement of central mechanism in analgesic action. The Analgesic effect mediated through central mechanism indicated the involvement of endogenous opioid peptides and biogenic amines like 5HT. The Analgesics of narcotic central e.g. Morphine, pentazocine etc and non narcotic peripheral type e.g. aspirin, ibuprofen indomethacin etc. can also inhibit the writhing response in rats.

The results of analgesic activity of stems bark extracts of Woodfordia fruticosa are presented in table-1& table-2. In both the hot plate method and acetic acid induced writhing method is normally used to evaluate the central analgesic and peripheral analgesic effect of drugs and chemicals. Analgin produced a significant (p<0.01) in the reaction time at 30, 60 and 90 min as compared with control. Alcoholic and aqueous ext exhibited significant analgesic activity as compared to control group. The extract of ethanol and aqueous significantly inhibited writhing responses as compared to control groups at 200 mg/kg. After 30 min of drug administration reaction time was increased for test samples and standard samples when compared to reaction time of pre-treatment test. The present study exhibited on the base of obtained result mentioned in table1&2 the alcoholic and aqueous extracts showed significant analgesic action which has properly presented reaction time at 0 hrs, 30min, 60min, and 90 min in graph (fig1, 2, 3 & 4). The graphical writhing response has presented in Fig 5. The aqueous extract found to be the most potent in comparison to alcoholic and petroleum ether. Comparison with standard group and test groups aqueous extract group followed by alcoholic and petroleum ether extract exhibited significant analgesic effect in animal models. Phytoconstituents present in bark are flavonoids, steroids, glycosides and tannin. Analgesic activity of Woodfordia fruticosa stem bark may be due to present of flavonoid, steroids or glycosides. In conclusion this study has established that aqueous and alcoholic extracts having the central and peripheral analgesic properties. It can be an importance in drug development, especially in the field of nociception.
References:


Table 1

Effects of various extracts of Woodfordia fruticosa stems bark on hot plate response in albino rats

<table>
<thead>
<tr>
<th>S. no</th>
<th>Group Description</th>
<th>Dose</th>
<th>Reaction time in second (Mean ± SEM)</th>
<th>Pre treatment</th>
<th>30 min</th>
<th>60 min</th>
<th>90min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control( distilled water)</td>
<td>2.8±0.04</td>
<td>3.25±0.040</td>
<td></td>
<td>3.42±0.210</td>
<td>4.28±0.460</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Standard (Analgin) 30mg</td>
<td>3.08±0.010</td>
<td>6.24±0.026**</td>
<td>6.54±0.310**</td>
<td>7.32±0.420**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Petroleum ether extract of barks 200mg</td>
<td>2.21±0.016</td>
<td>2.5±0.050**</td>
<td>4.12±0.600**</td>
<td>4.46±0.620**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Chloroform extract of barks 200mg</td>
<td>3.28±0.020</td>
<td>3.62±0.032**</td>
<td>2.62±0.260**</td>
<td>3.65±0.530**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Alcoholic extract of barks 200mg</td>
<td>3.20±0.041</td>
<td>4.02±0.30**</td>
<td>5.41±0.380**</td>
<td>6.36±0.61*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Aqueous extract of barks 200mg</td>
<td>2.32±0.018</td>
<td>4.82±0.040**</td>
<td>5.83±0.460**</td>
<td>7.02±0.021**</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Compare control vs all group, Mean ±SEM, (n=6) followed by Dunnett’s test, Significant at **P<0.01,*P<0.05, ns P>0.05, ns-Not significant.
**Table 2**  
Effects of different extracts of *Woodfordia fruticosa* barks on acetic acid induced writhing response in albino rats

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Mean writhing %</th>
<th>% Inhibition of writhing reflex</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (Acetic acid 1%v/v)</td>
<td></td>
<td>48.21</td>
<td>......</td>
</tr>
<tr>
<td>2</td>
<td>Acetic acid + Standard (Analgin)</td>
<td>30mg</td>
<td>10.43</td>
<td>78.36</td>
</tr>
<tr>
<td>3</td>
<td>Acetic acid + Petroleum ether extract of barks</td>
<td>200mg</td>
<td>43.26</td>
<td>10.26</td>
</tr>
<tr>
<td>4</td>
<td>Acetic acid + Chloroform extract of barks</td>
<td>200mg</td>
<td>40.64</td>
<td>15.70</td>
</tr>
<tr>
<td>5</td>
<td>Acetic acid + Alcoholic extract of barks</td>
<td>200mg</td>
<td>30.52</td>
<td>36.69</td>
</tr>
<tr>
<td>6</td>
<td>Acetic acid + Aqueous extract of barks</td>
<td>200mg</td>
<td>26.04</td>
<td>45.98</td>
</tr>
</tbody>
</table>

**Fig1** - Graphical representation of reaction time in second on hot plate method in albino rats before drug administration. (Data are presented as Mean±SEM of 6 albino rats).

Before the administration of drugs, the reaction time observed in the control group, standard group and four extract groups these are pet. ether group, chloroform group, alcohol group and aqueous group. In the above column diagram shows that the reaction time indicated by the pet. ether extract group is the minimum (2.21±0.016sec.) while the reaction time indicated by the chloroform extract group is maximum (3.28±0.02 sec.).
In the above graphical representation indicates that the reaction time taken by the pet. ether group is minimum (2.5±0.05 sec.) while the reaction time taken by standard group is maximum (6.24±0.026 sec.) hence its effect is quite significant, similarly the aqueous extract (4.82 ±0.46 sec.) also indicate a significant analgesic effect.

The given column diagram indicates that the standard extract (6.54±0.31sec), aqueous extract (5.83±0.46sec) and alcohol extract (5.41±0.38sec) show the significant analgesic effect as compare to the control group.
Fig 4 - Graphical representation of reaction time in seconds of albino rats after 90min of drug administration. (Data are presented as Mean ± SEM of 6 albino rats).

The above diagram representing the reaction time of drugs on albino rats after the administration of 90 min. It indicates that the analgesic reaction of the standard group (7.32±0.42 sec.), aqueous extract (7.02±0.021 sec.) and alcoholic extract (6.36±0.61 sec.) is very significant in comparison to the control group.

Fig 5 - Graphical representation of % inhibition of writhing of acetic acid induced in Albino rats after drug administration

The above graphical representation indicates the inhibiting effects of writing in albino rats after the administration of the drugs here it is quite clear that the inhibiting effect of the standard extract (78.36%) is maximum and similarly the inhibiting effect of pet.ether (10.26%) is negligible. Even the inhibiting effect of aqueous extract (45.98%) and alcoholic extract (36.69%) are also quite noticeable.