The present study was designed to investigate analgesic and antipyretic activity of aqueous extract of *Grewia tiliaefolia* Vahl leaves using various techniques viz. hot plate, tail flick, tail immersion, formalin test and acetic acid induced writhing test in albino mice. Antipyretic activity of the extract was studied using yeast induced hyperpyrexia in Wistar rats. Morphine and paracetamol at the dose of 10 and 150mg/kg, i.p. was used as standard drugs for the analgesic and antipyretic activity respectively. Oral administration of *G. tiliaefolia* extract significantly raised the pain threshold at different time interval in comparison with control at doses of 250 and 500mg/kg in hot plate, tail flick, tail immersion tests. Formalin model of nociception was used to discriminate pain in its central and peripheral components. Extract showed the significant nociception in the second phase at both the dose levels, confirming its central analgesic activity, while morphine acts in both, first and second phases. Acetic acid induced writhings; a test for peripheral acting analgesics was significantly inhibited by the extract. The subcutaneous injection of yeast suspension markedly increased the rectal temperature after 19 hour of its administration and was significantly decreased by the extract in a dose dependent manner. The effect observed was comparable to that of paracetamol. In conclusion, aqueous extract of *G. tiliaefolia* leaves possess central and peripheral analgesic activity along with antipyretic activity in a dose dependent manner.

**Keywords:** *Grewia tiliaefolia*, analgesic, morphine, antipyretic, paracetamol.

INTRODUCTION

*Grewia tiliaefolia* Vahl (Family: Tiliaceae) commonly found in the sub Himalayan region from Juma to Nepal upto 4000 ft, and central district of Chennai, Bihar, Orissa, Burma. It is commonly known as Dhamani, Dhaman. It is a well known herb in Ayurvedic system of medicine and has been used in vitiated conditions of pitta and kapha, burning sensation, hyperdipsia, rhinopathy, pharyngopathy, cough, skin diseases, pruritus, wounds, ulcers, diarrhoea, haematemesis, and general debility [1].

The bark of the *G. tiliaefolia* showed the presence of three tri-terpenoids, viz. Betulin, Friedelin and Lupeol. Roots showed the presence of Friedelin and Lupeol [2]. Tri-terpenoids isolated from *G. tiliaefolia* bark at higher concentrations exhibited cytotoxic activity against LEUK-L1210 cells [3]. Stem bark of *G. tiliaefolia* showed the semen coagulant and cardiovascular effects [4].

Although *G. tiliaefolia* is widely used in ethnomedicine, its analgesic and antipyretic properties have not yet been pharmacologically evaluated. Hence present study was undertaken to evaluate analgesic and antipyretic activity of *G. tiliaefolia* vahl leaves in rodents.

**MATERIALS AND METHODS**

**Plant material**

The fresh leaves of *G. tiliaefolia* were collected from the hilly areas of Kasara, District Thane, Maharashtra. The leaves were then authenticated by Dr. Ganesh Iyer, Botanist at Ramnarayan Ruia College, Mumbai. A voucher specimen (2005/11/01A) has been kept in our laboratory for future reference.

**Preparation of plant extract**

The dried powdered leaves of *G. tiliaefolia* were defatted using petroleum ether (60-80°C) and successively extracted with distilled water. Extract was filtered through vacuum filter and the filtrate was concentrated in vacuum evaporator. Dried extract was used for the further study. The yield of the extract was found to be 13.33% w/w.

**Experimental animals**

Wistar albino rats (180–200g) and Swiss albino mice (20-22g) of either sex were procured from Glenmark Pharmaceuticals, Mhape, Navi Mumbai and Haffkin Biopharmaceuticals Parel, Mumbai. Animals were placed in polypropylene cages in a controlled room temperature 22±1°C and relative humidity of 60–70% in registered animal house (87/1999/CPCSEA). They were maintained with standard pellet diet (Amrut brand, Sangli, India) and water *ad libitum*. Animals were acclimatized to laboratory condition for seven days before commencement of experiment. Ethical clearance was obtained from Institutional Animal Ethical Committee.
Chemicals and reagents
Morphine and acetic acid were procured from Sigma Aldrich (St. Louis, MO, USA). All the other chemicals and reagents were of pure analytical grade and obtained from local supplier.

Assessment of analgesic activity
Hot plate method
The hot plate test was performed to measure response latencies according to previously described method with minor modifications [5]. Albino mice were divided into different groups of six animals each. Test groups were treated orally with aqueous extract of *G. tiliaefolia* at dose level of 250 and 500mg/kg respectively, while the control group received 0.1% Na CMC. The hot plate was maintained at 55.0±0.5 °C and the animals were placed into the perspex cylinder on the heated surface and time to discomfort reaction (licking paws or jumping) was recorded as response latency by a stop-watch. The latency was recorded after 30, 60 and 120 min after the administration of test and standard drug. The standard drug used was morphine at the dose of 10 mg/kg, i.p.

Tail flick method
The activity was screened according to previously reported method with minor modifications [6], Prescreened animals (reaction time: 3–4 sec) were divided into different groups of six animals each. Test extract was administered orally at the dose level 250 and 500mg/kg. The standard drug used was morphine, given intraperitoneally at the dose of 10mg/kg. The tail flick latency was assessed by the analgesiometer (Sci. Eng. Corporation, Delhi, India). The strength of the current passing through the naked nicrome wire was kept constant at 6 Amps. The distance between the heat source and the skin was 1.5 cm. The site of the radiant heat in the tail was maintained at 2.5 cm, measured from the root of the tail. The basal reaction time was recorded at 30, 60 and 120 min after the administration of test drug.

Tail immersion method
The test was performed according to the method of Vogel G H et al and Ghosh MN with minor modifications [7, 8]. Animals were distributed into four groups of six animals each. The test extract was administered orally at 250 and 500mg/kg; whereas standard drug, Morphine, (10 mg/kg) was given intraperitoneally to the respective groups. The lower 5 cm portion of the tail was marked. This part of the tail was immersed in a freshly filled water bath of temperature 55.0±0.5 °C. The reaction time of the typical tail-withdrawal reflex in mice was recorded 30, 60 and 120 min after the administration of test drug.

Formalin test
The test was carried out as described by Hunskaar et al [9]. Animals were injected with 20µL formalin in dorsal hind paw. Animals were treated with test extract (250 and 500mg/kg, p.o.), morphine (10 mg/kg, i.p.) 30 min before the formalin injection. Immediately after the formalin injection the time of pain reactions were registered that the animals remained licking or biting the paw during the first phase (0-5 min) and the second phase (0-20 min) of the test. Percent inhibitions of writhings were calculated by the formula: (Control-Test) X100/Control

Acetic acid induced writhing test [10]
All animals received intraperitoneal injections of 0.6% acetic acid (0.1 ml/10g), for induction of abdominal muscle contractions and/or lengthening of the legs. The control group received only 0.1% Na CMC. One hour before the acetic acid injection, test groups received the plant extract at the doses of 250 and 500mg/kg, p.o. Another group received morphine, 10 mg/kg i.p., 30 min before the acetic acid injection. After 10 min of the acetic acid injection, the numbers of writhings were registered for 20 min for each session. Percent inhibitions of writhings were calculated by the formula mentioned above.

Assessment of antipyretic activity
Antipyretic activity was carried out according to previously reported methods [11]. The initial temperature of animals was recorded. The pyrexia was induced in the albino rats by subcutaneous injection of 10nl/kg of 15% (w/v) Brewer’s yeast suspended in 0.5% (w/v) Na-CMC solution. After 19 hr of yeast injection, the animals with significant rise in the rectal temperature were divided into four groups of six animals each. Group first considered as control and received 0.5% Na CMC, while the group II served as positive control and treated with paracetamol at a dose of 150mg/kg i.p. Group III and IV were treated orally with test extract at the dose of 250 and 500mg/kg respectively. The rectal temperature was recorded upto 3 hours after the drug treatment and compared with the control group.

Statistical analysis
The results are expressed as the mean±SEM for each group. Statistical differences were evaluated using a one-way analysis of variance (ANOVA) followed by Dunnet’s t-test for analgesic activity and Bonferroni test for antipyretic activity. Results were considered to be statistically significant at p<0.05.

RESULTS AND DISCUSSION
Effect of aqueous extract of *G. tiliaefolia* in hot plate method is shown in Figure 1. It is one of the most common test for evaluating the analgesic efficacy of drugs/compound. The paws of mice and rats are very sensitive to heat at temperatures which are not damaging the skin. The responses are jumping, withdrawal of the paws and licking of the paws. The time until these responses occur is prolonged after administration of centrally acting analgesics, whereas peripheral analgesics of the acetylsalicylic acid or phenyl-acetic acid type do not generally affect these responses [8]. *G. tiliaefolia* extract at the dose of 250 and 500mg/kg showed the significant (p<0.05) increase in latency time as compared to control. Positive control; morphine showed significant (p<0.01) analgesic activity at the dose of 10mg/kg, i.p.

As the tail of the animals are very sensitive to heat or thermal stimuli, tail flick and tail immersion tests were used for the evaluation of analgesic activity of drug. These tests are also available for the study of central analgesic activity [7]. Here, *G. tiliaefolia* extract exhibited significant (p<0.05) analgesic activity at higher dose level, 500mg/kg by increase in latency time. (Figure 1)

The advantage of using the formalin model of nociception is that it can discriminate pain in its central and peripheral components [12]. The test consists of two different phases which can be separated in time: the first one is generated in the periphery through the activation nociceptive neurons by the direct action of formalin and the second phase occurs through the activation of the ventral horn neurons at the spinal chord level. Morphine, a typical narcotic drug, inhibits nociception in both phases [13], but drugs with peripheral action, such as indomethacin and corticosteroids inhibit only in the second phase. Moreover, drug such as aspirin and paracetamol, which inhibit prostaglandin synthesis, block only in the first phase of the formalin test [9,14]. Animals treated with *G. tiliaefolia* extract showed significant (p<0.01) inhibition in formalin induced paw licking time only in second phase. It showed the maximum inhibition 31.90 and 45.39% at the dose of 250 and 500mg/kg; however morphine at the dose of 10mg/kg, i.p. showed 45.22 and 93.38% inhibition in first and second phase respectively. (Table 2)

Pain is induced by injection of irritants into the peritoneal cavity of mice. The animals react with a characteristic stretching behavior which is called writhings. The test is suitable to evaluate peripheral analgesic activity of drug. In this test, acetic acid is injected into the peritoneal cavity of mice. The pain is generated via endog-
Figure 1: Effect of aqueous extract of *G. tiliaefolia* on hot plate method, tail flick method and tail immersion method

Table 2: Effect of aqueous extract of *G. tiliaefolia* on formalin test and acetic acid induced writhing test

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (mg/kg)</th>
<th>Formalin induced paw licking time in sec. Acetic acid induced writhing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0-5 min</td>
</tr>
<tr>
<td><strong>First phase</strong></td>
<td><strong>Second phase</strong></td>
<td>(First phase)</td>
</tr>
<tr>
<td>Control</td>
<td>0.1 % Na CMC, p.o.</td>
<td>83.00±3.63</td>
</tr>
<tr>
<td>Morphine</td>
<td>10, i.p.</td>
<td>45.46±2.76**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(45.22)</td>
</tr>
<tr>
<td><em>G. tiliaefolia</em></td>
<td>250, p.o.</td>
<td>75.02±1.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(09.61)</td>
</tr>
<tr>
<td><em>G. tiliaefolia</em></td>
<td>500, p.o.</td>
<td>80.25±2.93</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(03.31)</td>
</tr>
</tbody>
</table>

Values in the results are expressed as mean±SEM, (n=6). *p<0.05, **p<0.01, ***p<0.001 significantly different in comparison with Control group (ANOVA followed by Dunnet’s t-test), values in the parentheses are % inhibitions

Table 3: Effect of aqueous extract of *G. tiliaefolia* on the brewer’s yeast induced pyrexia in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (mg/kg)</th>
<th>Rectal Temperature in Fahrenheit (°F) at different time intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial Temp.</td>
</tr>
<tr>
<td>Control</td>
<td>0.5 % Na CMC, p.o.</td>
<td>101.8±0.61</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>150, i.p.</td>
<td>101.2±0.90</td>
</tr>
<tr>
<td><em>G. tiliaefolia</em></td>
<td>250, p.o.</td>
<td>101.6±0.68</td>
</tr>
<tr>
<td><em>G. tiliaefolia</em></td>
<td>500, p.o.</td>
<td>102.8±0.84</td>
</tr>
</tbody>
</table>

Values in the results are expressed as mean±SD, (n=6). *p<0.05, **p<0.01, ***p<0.001 significantly different in comparison with Control group, *p<0.05 significantly different as compared to paracetamol (ANOVA followed by Bonferroni test)
REFERENCES

leaves of Ramnarayan Ruia College, Mumbai for authentication of the fresh New Delhi for financial support and Dr. Ganesh Iyer, Botanist, Authors are thankful to University Grant Commission, Govt. of India, ACKNOWLEDGEMENTS

antipyretic agent with a low toxicity and better therapeutic index. quired to find active component of the extract and to confirm the mechanism of action in the development of a potent analgesic and antipyretic effect. Hence the presence of flavonoids may be responsible for the activity of Grewia tiliaeolia leaves extract.

CONCLUSION

In conclusion, present study revealed the analgesic and antipyretic activity of aqueous extract of Grewia tiliaeolia at 250 and 500mg/kg showed significant (p<0.01) decrease in the rectal temperature at different time interval in a dose dependent manner and this effect was comparable to that of paracetamol at the dose of 150 mg/kg, i.p. (Table 3)

Phytochemical evaluation of aqueous extract of G. tiliaeolia revealed the presence of tannins, flavonoids and tri-terpenoids. Flavonoids are known to target prostaglandins which are involved in the late phase of the acute inflammation and pain perception [16]. In many earlier studies, flavonoids have been reported to exhibit analgesic [17] and antipyretic effect [18, 19]. Hence the presence of flavonoids may be responsible for the activity of Grewia tiliaeolia leaves extract.

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