PHARMACOLOGICAL SCREENING OF OCIMUM GRATISSIMUM LEAVES FOR ITS ANALGESIC, ANTI-PYRETIC AND ANTI-INFLAMMATORY ACTIVITY

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ABSTRACT
The present work was taken to give the scientific justification of folklore medicinal use of Ocimum gratissimum. The analgesic activity of methanolic and aqueous extract of leaves of Ocimum gratissimum was evaluated using tail immersion method in rats. The anti-pyretic activity of methanolic and aqueous extract of leaves of Ocimum gratissimum was evaluated using rats suffering from yeast induced pyrexia. The rats were assessed for the pain threshold at different interval of time up to 180 minutes. The methanolic extract shows significant activity at 180 min (5.81 ± 0.013) while aqueous extract shows moderate activity at 180 min (3.97 ± 0.006). The results support the traditional use of this plant in some painful and inflammatory conditions. The methanolic extract shows moderate significant anti-pyretic activity at 180 min (36.23 ± 0.08) while aqueous extract shows moderate activity at 180 min (36.82 ± 0.51). The methanolic extract shows significant anti-inflammatory activity at 4 h by inhibiting 61.25 % carrageenan induced paw edema while aqueous extract no activity at 4 h (36.25 %).

KEYWORDS: Ocimum gratissimum, Anti-pyretic activity, analgesic, inflammation.

INTRODUCTION
O. gratissimum (Lamiaceae) is a perennial, woody shrub that is an herbal medicine which has been practiced worldwide and distributed throughout India. Traditionally, it is used in the treatment of diarrhea, as a febrifuge and integral component of anti-malaria remedies, mosquito/insect repellent, stomachic and general tonic, antiseptic in wound dressing, skin infections, conjunctivitis and bronchitis. ‘Ocimum tea ’, is dispensed as a remedy for fever and diaphoretic, roots are used as sedative for children. Ocimum gratissimum is commonly known as fever leaf in general but it is has different native names in different part of the country. There are about 60 or more species of Ocimum and numerous varieties, belonging to the Family Labiatae. This different types of species are represented by the five most important representatives of the more that 60 Ocimum species and these include (i)Ocimum gratissimum, (ii) Ocimum basilicum, (iii) Ocimum americanum, (iv) Ocimum sanctum and (v)Ocimum americanum.
Pain can be simply defined as undesirable physical or emotional experiences. Treatment of chronic pain is a major problem due to the use of available medications and their undesirable side-effect profiles. The side effects of currently used pain medications vary based on the class of agent used however, most medical personnel are concerned with addiction, tolerance, gastrointestinal effects, and abuse. Most recent clinical studies suggest that proper use of pain treatment has low risk of producing addicts and because of this prescribing effort seem to be changing. Regardless, we can separate physical pain into at least four stimuli groups: mechanical, thermal, chemical, or electrical. The stimulation of nociceptive nerve endings of C-fibers or activation of A-fibers carries the painful stimuli.

Puerperal pyrexia is defined as the presence of a fever in a mother greater than or equal to 38°C in the first fourteen days after giving birth. There are many causes of such a fever, but in the days prior to antibiotics, it was a sign which was very much dreaded as it had a very poor prognosis. Pyrexia or fever is caused as a secondary impact of infection, tissue damage, inflammation, graft rejection, malignancy or other diseased states. Prolonged uses of both steroidal and non-steroidal anti-inflammatory drugs are well known to be associated with peptic ulcer formation. Hence, search for new anti-inflammatory factors that hold the therapeutic efficacy and even so are destitute of these untoward effects is warranted. There is much hope of finding active anti-rheumatic compounds from indigenous plants as these are still used in therapeutics. Herbal drugs are being proved as effective as synthetic drugs with lesser side effects.

This research was aimed at investigating the possible analgesic, anti-pyretic and anti-inflammatory activities of leaves extract of the plant in order to support or refute the claims by traditional folklore.

**MATERIAL AND METHODS**

The fresh leaves of *Ocimum gratissimum* were collected from Vidyabharti trust campus, Bardoli, Surat, Gujarat. All these plants were identified and authenticated by botanist Dr. B. R. Patel, Department of Botany, Patidar Gin Science College, Bardoli, Surat. The fresh leaves were properly washed and cleaned after collection and dried under the shade and were subjected to size reduction to get coarse powder. Around 200 g of powder was subjected to successive hot continuous extraction (soxhlet) with methanol. Finally the drug was macerated with chloroform water. After the effective extraction, the solvents were distilled off, the extract was then
concentrated on the water bath and the extract obtained with each solvent will be preserved in air tight bottles in the refrigerator until use.

**Experimental animals**

Albino rats of either sex weighing 150 to 200 g were selected for the experiment. They were employed for assessing the analgesic, anti-pyretic and anti-inflammatory activity. Rats were divided into four groups, each group having six animals for each activity after washout period. The bedding material of the cages was changed every day. Before conducting the experiment, ethical clearance was obtained from Institutional Animal Ethics Committee, Vidyabharti Trust College of Pharmacy, Umrakh (CPCSEA / VBT / IAEC / 14/01/60)

**Analgesic activity**

**Extract used:** Methanolic and aqueous extracts of *Ocimum gratissimum* were used. Pentazocain (Fortwin, Ranbaxy) used as standard drug.

**Dose selection:** Methanol extract of *Ocimum gratissimum* (100 mg/kg b.wt), Aqueous extract of *Ocimum gratissimum* (100 mg/kg b.wt) c) Control: 5 ml/kg of 5% gum acacia. (p.o.), Standard: Pentazocin 5 mg/kg body weight (i.p.)

**Method:** The analgesic responses of the given samples of extracts were evaluated using the Tail immersion method using analgesiometer. In this method the rats were divided into four groups (each group containing six animals). The first group was served as control and received 5% acacia solution only (5ml/kg bdwt, orally.), second group of animals was served as standard and administered standard drug Pentazocin (5 mg/kg bd wt., i.p.). The animals of remaining groups were treated with different extracts. The analgesic responses of the extracts were evaluated using the tail immersion method. In this method, the albino rats were weighed and marked and then placed into individual restraining cages leaving the tail hanging out freely. The animals are allowed to adapt to the cages for 30 min before testing. The lowest 5 cm portion of the tail is marked. This part of the tail is immersed in a cup of freshly filled water of exactly 55 ºC. Within a few seconds the rat reacts by withdrawing the tail. The standard, test and control doses were injected to the animals and the reaction time was noted at 0, 30, 60, 90, 120 and 180 minutes.

The results are shown in table 1 and figure 1.

**Anti-pyretic activity**

**Extract used:** Methanolic and aqueous extracts of *Ocimum gratissimum* were used. Paracetamol (Dolo, Microlabs) used as standard drug.
**Dose selection:** Methanol extract of *Ocimum gratissimum* (100 mg/kg b.wt), Aqueous extract of *Ocimum gratissimum* (100 mg/kg b.wt) c) Control: 1 ml/kg of normal saline solution. (p.o.), Standard: Pentazocin 200 mg/kg body weight (i.p.)

**Method:** In the beginning of experiment normal rectal temperature was noted by digital thermometer. Pyrexia was induced by sub-cutaneous injection of 2 ml/kg of 15% brewer’s yeast suspension in normal saline. The animals were then fasted for the duration of experiment (approximately 24hrs). After 18 hrs. of yeast injection, the extracts were given orally to all groups except control, which was given 1ml/kg body weight of normal saline (p.o.). A standard group of animals were received Paracetamol 200 mg/kg body weight (i.p.). The rectal temperatures of all the animals were noted at 30 min interval till 3 hrs. After inducing the test drug orally, the rectal temperature was measured by inserting 2 cm of digital thermometer, lubricated with glycerine into the rectum for 2 min. All the temperatures noted were tabulated and difference in the rise of temperature from that of normal rat temperature was computed. The results are shown in table 2 and figure 2.

**Anti-inflammatory activity**

**Extract used:** Methanolic and aqueous extracts of *Ocimum gratissimum* were used. Diclofenac sodium used as standard drug.

**Dose selection:** Methanol extract of *Ocimum gratissimum* (100 mg/kg b.wt), Aqueous extract of *Ocimum gratissimum* (100 mg/kg b.wt) c) Control: 5 ml/kg of normal saline solution. (p.o.), Standard: Diclofenac sodium 100 mg/kg body weight (i.p.)

**Method:** Anti-inflammatory activity was assessed by the method described by Winter et al., The rats were divided into four groups of six animals each. First group (control) received 5 ml/kg body wt of normal saline; second group (standard) received 100 mg/kg body wt (i.p) diclofenac sodium, third group received methanolic extract (100 mg/kg body wt, p.o.) and fourth group received aqueous extract (100 mg/kg body wt, p.o.) of *Ocimum gratissimum*, respectively. After 1 h, the rats were challenged with subcutaneous injection of 0.1 ml of 1% w/v solution of carrageenan (Sigma chemical co, St. Louis MO, USA) into the plantar side of the left hind paw. The paw was marked with ink at the level of lateral malleolus and immersed in solution up to the mark. The plethysmograph apparatus used for the measurement of rat paw volume was of UGO Basil company. The paw volume was measured immediately after injection (0 h) and then every hour till 4 h after injection of carrageenan to each group. The difference between the initial and subsequent reading gave the actual edema volume. Percent inhibition of inflammation was calculated using the formula,
% inhibition = 100 (1 - Vt/Vc)

Where ‘Vc’ represents edema volume in control and ‘Vt’ edema volume in group treated with test extracts.

The results are shown in table 3 and figure 3.

**Statistical analysis**

All data were expressed as Mean ± SEM and analyzed statistically by using Dunnett’s t-test. A difference was considered significant at P value less than 0.05.

**RESULTS AND DISCUSSION**

The results of analgesic activity showed that the methanolic extract and aqueous extracts does not possess the analgesic activity as compared with the standard drug pentazocaine at the initial time points. At 90 minutes, the aqueous extract (2.30) and the methanolic extract (2.88) showed moderate analgesic activity as compared to standard drug. At 180 minutes the aqueous extract of *Ocimum gratissimum* showed moderate analgesic activity (5.81) while methanolic extract showed highly significant analgesic activity (3.97) when compared to standard drug pentazocaine (5.72).

The results of analgesic activity showed that at 90 min. interval methanolic extract showed moderate anti-pyretic activity (37.62 °C) as compared to aqueous extract (37.82 °C). After 180 minutes, methanolic extract showed moderately significant anti-pyretic activity (36.23 °C) and aqueous extract of *Ocimum gratissimum* does not show any moderate significant anti-pyretic activity, when compared with the standard drug paracetamol (35.18 °C).

The results of anti-inflammatory activity obtained indicates that the methanolic extract had significant anti-inflammatory activity in rats, while aqueous extract had moderate anti-inflammatory activity. The methanolic and aqueous extracts of *Ocimum gratissimum* reduced the edema induced by carrageenan by 61.25 % and 36.25 % respectively on oral administration of 100 mg/kg body wt, as compared to the untreated control group. Diclofenac sodium at 100 mg/kg body wt. inhibited the edema volume by 75.00%.
Table 1. Analgesic activity of extracts of *Ocimum gratissimum*

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Group</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 min.</td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>1.39 ± 0.012</td>
</tr>
<tr>
<td>2</td>
<td>Standard</td>
<td>1.81 ± 0.030</td>
</tr>
<tr>
<td>3</td>
<td>MEOG</td>
<td>1.18 ± 0.017</td>
</tr>
<tr>
<td>4</td>
<td>AEOG</td>
<td>1.30 ± 0.010</td>
</tr>
</tbody>
</table>

MEOG – Methanolic extract of *Ocimum gratissimum*, AEOG – Aqueous extract of *Ocimum gratissimum*, * = Significant, ** = highly significant. (p<0.05), n = 6, number of animals used in each group

Figure 1. Analgesic effect of various extracts of *Ocimum gratissimum* in rats
Table 2. Anti-pyretic effect of different extracts of Ocimum gratissimum on yeast induced pyrexia

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Group</th>
<th>°C (Mean ± SEM)</th>
<th>Without Pyrexia</th>
<th>0 min.</th>
<th>30 min.</th>
<th>60 min.</th>
<th>90 min.</th>
<th>120 min.</th>
<th>180 min.</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>35.62 ± 0.23</td>
<td>37.03 ± 0.13</td>
<td>38.19 ± 0.10</td>
<td>38.95 ± 0.15</td>
<td>39.07 ± 0.11</td>
<td>39.99 ± 0.10</td>
<td>39.72 ± 0.10</td>
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<td>2</td>
<td>Standard</td>
<td>35.52 ± 0.24</td>
<td>37.58 ± 0.22</td>
<td>37.75 ± 0.28</td>
<td>37.00 ± 0.12</td>
<td>36.12 ± 0.16</td>
<td>35.82 ± 0.16</td>
<td>35.18 ± 0.21</td>
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</tr>
<tr>
<td>3</td>
<td>MEOG</td>
<td>36.32 ± 0.32</td>
<td>38.07 ± 0.22</td>
<td>38.53 ± 0.18</td>
<td>38.24 ± 0.12</td>
<td>37.62 ± 0.16</td>
<td>37.09 ± 0.16</td>
<td>36.23 ± 0.19</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>AEOG</td>
<td>36.68 ± 0.28</td>
<td>37.45 ± 0.18</td>
<td>38.8 ± 0.18</td>
<td>38.11 ± 0.12</td>
<td>37.82 ± 0.09</td>
<td>37.31 ± 0.10</td>
<td>36.82 ± 0.51</td>
<td></td>
</tr>
</tbody>
</table>

MEOG – Methanolic extract of *Ocimum gratissimum*, AEOG – Aqueous extract of *Ocimum gratissimum*, * = Significant, ** = highly significant (p<0.05), n = 6, number of animals used in each group

Figure 2. Anti-pyretic effect of different extracts of Ocimum gratissimum on yeast induced pyrexia in rats
Table 3. Anti-inflammatory effect of different extracts of *Ocimum gratissimum* on carrageenan induced rat paw edema

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Group</th>
<th>Mean paw edema volume in ml ± SEM</th>
<th>Difference</th>
<th>% inhibition in paw edema after 4 h</th>
</tr>
</thead>
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<td></td>
<td></td>
<td>0 h.</td>
<td>1 h.</td>
<td>2 h.</td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>1.01</td>
<td>1.10</td>
<td>1.26</td>
</tr>
<tr>
<td>2</td>
<td>Standard</td>
<td>1.03</td>
<td>1.31</td>
<td>1.20</td>
</tr>
<tr>
<td>3</td>
<td>AEOG</td>
<td>1.89</td>
<td>1.95</td>
<td>1.82</td>
</tr>
<tr>
<td>4</td>
<td>MEOG</td>
<td>1.84</td>
<td>2.00</td>
<td>1.98</td>
</tr>
</tbody>
</table>

MEOG – Methanolic extract of *Ocimum gratissimum*, AEOG – Aqueous extract of *Ocimum gratissimum*, * = Significant, ** = highly significant. (p<0.05), n = 6, number of animals used in each group

Figure 3. Anti-inflammatory effect of different extract of *Ocimum gratissimum* on carrageenan induced rat paw edema
CONCLUSION

From the results and discussion it can be finally concluded that the methanolic and aqueous extract of leaves of *Ocimum gratissimum* does possess highly significant to moderate analgesic, anti-pyretic and anti-inflammatory effects in laboratory animals at the dose investigated. It can also be concluded that if the dose increases the activity may also increases. The results support the traditional use of this plant in some aching and inflammatory conditions and propose the presence of biologically active components which may worth further investigation and elucidation. Further studies may disclose the exact mechanisms of action responsible for the analgesic anti-pyretic and anti-inflammatory activities of *O. gratissimum* leaves extract.

REFERENCES

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