Effect of leaf extract of ocimum gratissimum on central nervous system in mice and rats

DP Sarraf, GP Rauniar, H Sangraula
Department of Clinical Pharmacology and Therapeutics
B.P. Koirala Institute of Health Sciences, Dharan, Nepal

Abstract

Background: Ocimum gratissimum, known as “Ram Tulsi”, is one of medicinal herbs used in traditional medicinal practice in our country.

Objective: To observe and evaluate various central nervous system activities of cold homogenized leaf extract of Ocimum gratissimum (OGE) in mice and rats. Methods: OGE was given to albino mice (n=240) and Wistar rats (n=30) in three doses 5, 10 and 15 ml/kg for seven days and evaluated for antinociceptive, anticonvulsant, motor coordination, hypnotic and anxiolytic effect using respective experimental procedures. Distilled water was used as vehicle control and various drugs as standard control for comparison. Results: OGE showed significant (p<0.05) analgesic effect as compared to vehicle treatment in hot plate test. Pentobarbital-induced sleeping time was significantly (p<0.05) potentiated by OGE in rats. OGE significantly decreased duration of tonic hind limb extension at doses of 5 and 15 ml/kg in MES test and increased latency to convulsion at dose of 15 ml/kg in PTZ-induced seizure test. OGE at dose of 5 and 10 ml/kg induced significant muscle relaxation. Time spent in central square and number of rearing increased in mice pretreated with OGE at 10 and 15 ml/kg. Conclusion: OGE possess antinociceptive, hypnotic-sedative potentiating, anxiolytic and muscle relaxant properties with probable anticonvulsant properties.

Keywords: Ocimum gratissimum, analgesic, anticonvulsive, sedative, muscle relaxation.

Introduction

Nepal is a repository of wild flora & fauna. It harbors about 2.2% of world’s flowering plants. It is a natural storehouse of medicinal plants. From the vast array of Materia Medica of the indigenous system, many plants have been reported to have activity against CNS disorders and thus act as very useful remedies for the alleviation of human suffering. Ocimum gratissimum (OG) belongs to family Labiate (Lamiaceae). It is a shrubby, perennial 0.2-1.2 meter in height, much branched, woody, below stems and branches sub-quadrangular. It is known to possess many beneficial effects ranging from medicinal to nutritive. Almost every part of the plant (e.g. leaves, seeds and roots) has been used extensively in the ancient system of Indian medicine known as Ayurveda. It is known as Vriddhu tulasi in Sanskrit, Ram tulasi in Hindi and Nepali. Ntezurubanza et al. have reported chemical composition of OG grown in Rwanda. Ueda-Nakamura et al. found antileishmanial activity of eugenol-rich essential oil from OG.

It is known that climatic conditions and water available in the soil can change the plant secondary metabolism and consequently alter the composition of essential oils. Assuming that, OG grown in our country may have different pharmacological properties from that present elsewhere, the present study was aimed to investigate the central nervous system activity of OG obtained from Nepal using different experimental procedures. Since very few experimental studies on this plant were carried out in Nepal, it was considered worthwhile to investigate this plant.

The objective of the study was to observe and evaluate antinociceptive, anticonvulsant, motor in-coordination, hypnotic and anxiolytic effects of cold homogenized
leaf extract of *Ocimum gratissimum* in mice and rats.

**Methods**

**Extract preparation:** OG was collected from thick Tarhara forests (Sunsari district) during month of August to December 2010 and planted in BPKIHS garden. The samples were confirmed and authenticated by Prof. S. N. Jha (Head of the Botany Department, Mahendra Morang College, P. G. Institute, Biratnagar, Nepal). Fresh leaves were collected and washed in tap water and left for shade dry. 100 g of the leaves was ground well in a mixer intermittently (to avoid heating the specimen) with 100 ml of distilled water and filtered using Whatman filter paper number 1. The filtrate (OGE) was used for the experiment. Only the freshly prepared extract was used for the study.

**Drugs:** Following drugs and chemicals were used: indomethacin (Indocap, Jagsonpal Pharmaceuticals, India), phenytoin (M-Toin, Medopharm, India), sodium valporate (Encoreate, Sun Pharmaceuticals, India), diazepam (Valium, Piramal Healthcare, India), pentylenetetrazole (Sigma Chemicals, USA), pentobarbitone sodium (LobaChemie, India), morphine (Martindale Pharmaceuticals, UK) and acetic acid (Qualigens fine chemical, India).

**Animals:** The experiments were performed on adult albino mice (n = 240) of either sex weighing 20-30gm and Wistar rats (n = 30) of either sex weighing 100-200gm. The animals were bred in the animal house of the Department of Clinical Pharmacology and Therapeutics. They were maintained under controlled room temperature (25±2°C), and light and dark (12:12 hour) conditions. The animals were given food pellets and water *ad libitum* but fasted overnight before the experiment.

Before conducting the experiment, ethical clearance was obtained from the Local Ethical Committee on Animal Research, BPKIHS. The ethical guidelines for investigations were followed in accordance with Indian National Science Academy (INSA).

**Experimental design**

All animals were randomly divided into five groups consisting of six animals. The dose of the test drug was chosen according to the study done Archana et al. and Volume Guidelines for Compound Administration. All standard drugs were administered to the animals through oral route 45 minutes before the experiments. The experiment was conducted on 7th day, 45 minutes after the last test drug administration. Indomethacin, morphine, pentylenetetrazole, phenytoin, sodium valporate, diazepam and pentobarbitone sodium were dissolved in distilled water.

The different groups received drugs and vehicles as follows:

- **Group 1** (vehicle control 10 ml/kg b. w.);
- **Group 2** (standard control);
- **Group 3** (OGE 5 ml/kg b. w.);
- **Group 4** (OGE 10 ml/kg b. w.) and
- **Group 5** (OGE 15 ml/kg b. w)

**Experimental models**

1. **Antinociceptive effect**
   - **(i) Hot plate test:** The test was carried out using a hot plate apparatus (UGO Basile, Italy) to evaluate central analgesic activity as described by Woolfe and MacDonald. The thermal noxious stimulus was induced to mice by placing them individually in the hot-plate maintained at 53±1°C, 10 minutes prior to the experiment. The mice that showed initial nociceptive response within 30 seconds were selected for the experiment. The latency of nociceptive response such as licking, flicking of a hind limb or jumping was noted. The experiment was terminated 30 second after their placement on the hot plate to avoid damage to the paws.
   - **(ii) Tail flick test:** For the tail-flick method, pain was induced by giving infrared heat on the tail of the mice (Tail-Flick Unit, UGO Basile, Italy) 1-2 cm away from the tip of the tail. The tail withdrawal from the heat (flicking response) was taken as the end point. The animals which showed a flicking response within 3-5 seconds were selected for the study. Tail-flick latency was noted. A maximum radiation exposure period of 15 seconds was taken as the cut off time to avoid damage to the tail.
   - **(iii) Acetic acid induced writhing test:** The method described by Koster *et al.* was employed to assess the peripheral analgesic activity. After 45 minutes post dosing with the drugs, the number of abdominal constrictions (writhings) in mice for a period of 15 minutes was counted following intraperitoneal injection of 0.6% acetic acid in a dose of 10 ml/kg b.w. For scoring purposes, a writhe is indicated by stretching of the abdomen with simultaneous stretching of at least one hind limb. Any significant reduction in the number
of abdominal constrictions when compared with vehicle-treated animal was considered as antinociceptive response. Antinociception was expressed as the percent reduction in writhing numbers compared between vehicle treated control and animals pretreated with OGE or indomethacin.

Percentage inhibition of writhing was calculated using the formula:

\[ \text{Percent inhibition} = \left( \frac{\text{Mean no. of writhes (control)} - \text{Mean no. of writhes (test)}}{\text{Mean no. of writhes (control)}} \right) \times 100 \]

45 minutes of post dosing with drugs, mice were subjected to MES of 150 mA of alternating current from the convulsiometer (Techno, India) for 0.2 sec through a pair of electrodes attached to each ear.\(^{12}\) Seizures were manifested as tonic hind-limb extension (THLE). The duration of THLE phase and the number of animals protected from convulsions were noted. The ability to prevent this feature or prolong the latency and/or onset of the THLE was considered as an indication of anticonvulsant activity.

(ii) Pentylenetetrazole (PTZ) induced seizures: PTZ was used in a dose of 40 mg/kg i.p. The latency to first convulsion and the no. of mice which exhibited seizure were observed immediately after the PTZ injection for a period of 30 minutes.\(^{13}\)

3. Muscle relaxation (Rota rod) test
The method described by Dunhan and Miya was used.\(^{14}\) A rota rod (Techno, India) consists of a horizontal wooden rod or metal rod coated with rubber with 3cm diameter attached to a motor with the speed adjusted to 15 rotations per minute (15 RPM). The rod is 75 cm in length and is divided into 5 sections by plastic discs, thereby allowing the simultaneous testing of five mice. To avoid a bias due to inability not related to drug treatment, on the previous day, animals were evaluated and those that showed the ability of walking on the bar for at least 1 minute were selected for the experiment. One hour after the administration of drugs, each mouse was placed on the rotating rod for 60 seconds, at intervals of 30 minutes for two hours. The latency (in seconds) to drop off the rod (endurance time) was recorded up to a limit of 60 seconds.

4. Pentobarbitone induced sleeping time
Method described by Williamson \textit{et al} was used.\(^{15}\) 45 minutes after administration of drug or vehicle, all the animals received 40 mg/kg i. p. of pentobarbitone sodium and kept in individual cage for observation. Sleeping time was calculated as the interval lapsing between the loss and the recovery of the righting reflex. The rat was considered as being awake if it could right itself, i.e. return to upright position.

5. Open field test (OFT)
OFT consists of a wooden box (40 cm×40 cm with 30 cm high walls) with painted black floor, subdivided into nine equal fields by white lines. The experimental room is a sound attenuated dark room. OFT illuminated with a 40W bulb, focusing on the field from a height of about 50cm, was placed in the experimental room. The mice were placed individually in a corner square of the OFT and the ambulation (number of squares crossed at periphery), total locomotion activity in the centre (number of central squares crossed) and rearing (number of times the animal stands on the rear paws) were recorded for five minutes. Rearing reflects an exploratory tendency of the animal that can be reduced due to a high level of fear.\(^{16}\) Enhanced peripheral, central and total numbers of squares crossed are taken as increased locomotor activity. In addition, increased rearing, number of inner squares crossed and time spent in them reflect enhanced exploratory activity and reduced fear. All the above parameters are inversely proportional to the level of anxiety. The observation was made on a closed circuit TV.

All data were presented as mean ± Standard Error of Mean (SEM). Statistical differences between the treatment ant control groups were calculated by one way ANOVA followed by LSD (least significant difference) post hoc test for multiple comparisons. A probability (p-value) level less than 0.05 were considered as significant.

Results
\textit{Effect of OGE on nociception:} OGE caused dose dependent increase in hot plate latency. Higher doses of OGE (15 ml/kg b.w.) significantly (p<0.05) prolonged hot plate latency as compared to vehicle treated mice. The analgesic effect of OGE at 15 ml/kg b.w. was comparable to that of morphine. The effect of OGE at 10 and 15 ml/kg b. w. on TFL was statistically significant (p <0.05) as compared to that of vehicle treated mice.
OGE significantly (p<0.05) reduced the number of writhing. OGE pretreatment at 5, 10 and 15 ml/kg produced 59%, 69.27% and 60.45% protection against abdominal writhing respectively. The maximum inhibition was produced by OGE at dose of 10 ml/kg b. w. (Table 1).

Table 1: Effect of OGE in antiociception tests

<table>
<thead>
<tr>
<th>Groups of animals</th>
<th>Hot-plate latency (sec)</th>
<th>Tail-flick latency (sec)</th>
<th>Writhing test</th>
<th>No. of writhes</th>
<th>Inhibition of writhing</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>8.37±0.61*</td>
<td>3.47±0.54</td>
<td></td>
<td>98.17±5.16</td>
<td>71.69%</td>
</tr>
<tr>
<td>II</td>
<td>21.75±1.89*</td>
<td>11.70±1.44*</td>
<td></td>
<td>13.17±2.15</td>
<td>86.9%</td>
</tr>
<tr>
<td>III</td>
<td>12.77±0.93*</td>
<td>6.65±1.21</td>
<td></td>
<td>40.33±4.9*</td>
<td>69.27%</td>
</tr>
<tr>
<td>IV</td>
<td>14.4±1.06*</td>
<td>7.28±0.71*</td>
<td></td>
<td>30.17±3.39**</td>
<td>59%</td>
</tr>
<tr>
<td>V</td>
<td>17.5±1.11*</td>
<td>9.63±1.71*</td>
<td></td>
<td>30.83±4.24**</td>
<td>60.45%</td>
</tr>
</tbody>
</table>

*: p < 0.05, compared to control; n=6

Effect of OGE on convulsion: Pretreatment with OGE in three different doses (5, 10 and 15 ml/kg b. w.) did not reduce the number of convulsing animals, suggestive of no role in protecting mice against occurrence of maximal electroshock induced convulsion; however, the duration of tonic hind limb extension in the mice pretreated with OGE at 5 and 15 ml/kg b. w. was significantly (p <0.05) less as compared to that of vehicle treated mice (Table 2).

Table 2: Effect of OGE in MES test

<table>
<thead>
<tr>
<th>Groups of animals</th>
<th>Duration of tonic hind limb extension in MES (sec)</th>
<th>Protected animals (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>15.83±0.60</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>0.67±0.67</td>
<td>83.33</td>
</tr>
<tr>
<td>III</td>
<td>12.83±1.74*</td>
<td>0</td>
</tr>
<tr>
<td>IV</td>
<td>13.83±0.60</td>
<td>0</td>
</tr>
<tr>
<td>V</td>
<td>11±0.97*</td>
<td>0</td>
</tr>
</tbody>
</table>

*: p < 0.05, compared to control; n=6

In PTZ induced seizure test, there was dose dependent increase in the latency to convulsions with the OGE pretreatment in three different doses (5, 10 and 15 ml/kg b. w.); however, the response was only significant (p<0.05) at dose of 15 ml/kg. The OGE pretreatment did not decrease the incidence of convulsions induced by PTZ (Table 3).

Table 3: Effect of OGE in PTZ test

<table>
<thead>
<tr>
<th>Groups of animals</th>
<th>Latency to convulsion in PTZ (sec)</th>
<th>Protected animals (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>3.15±0.27</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>25.83±4.17*</td>
<td>83.33</td>
</tr>
<tr>
<td>III</td>
<td>4.93±0.35</td>
<td>0</td>
</tr>
<tr>
<td>IV</td>
<td>8.62±0.80</td>
<td>0</td>
</tr>
<tr>
<td>V</td>
<td>10.40±1.10*</td>
<td>0</td>
</tr>
</tbody>
</table>

*: p < 0.05, compared to control; n=6

Effect of OGE on motor co-ordination in rota rod test: OGE in three different doses (5, 10 and 15 ml/kg b. w.) caused decrease in endurance time at 1 hour in mouse; however, the responses were statistically not significant (p>0.05) at any doses as compared to that of vehicle. Pretreatment with OGE also caused decrease in endurance time at 1.5 and 2 hour in mouse; however, the responses were statistically significant (p<0.05) at all the tested doses (Table 5).

Table 5: Effect of OGE in Rota rod test:

<table>
<thead>
<tr>
<th>Group of animals</th>
<th>Endurance time in 1 hr (sec)</th>
<th>Endurance time in 1.5 hr (sec)</th>
<th>Endurance time in 2 hr (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>59.50±0.34</td>
<td>57.17±1.82</td>
<td>55.17±3.19</td>
</tr>
<tr>
<td>II</td>
<td>13.67±1.94*</td>
<td>15.83±3.42*</td>
<td>22.83±2.71*</td>
</tr>
<tr>
<td>III</td>
<td>44.5±4.50*</td>
<td>54.83±2.61</td>
<td>46.17±4.50</td>
</tr>
<tr>
<td>IV</td>
<td>44.6±4.41*</td>
<td>46.67±6.22</td>
<td>47±4.8</td>
</tr>
<tr>
<td>V</td>
<td>49.83±4.56</td>
<td>46.02±4.68</td>
<td>51.17±4.55</td>
</tr>
</tbody>
</table>

*: p < 0.05, compared to control; n=6
**Effect of OGE on anxiety in open field test:** Number of squares crossed was increased in all tested doses of OGE as compared to vehicle; however, the response was not statistically significant (p>0.05) at any dose. The OGE pretreatment at all tested doses increased time spent in central square as compared to that of vehicle and the responses were statistically significant (p<0.05). The OGE pretreatment increased no. of rearing as compared to that of vehicle; however, the responses were statistically significant (p<0.05) only at doses of 10 and 15 ml/kg b.w. (Table 6).

**Table 6: Effect of OGE in Open-field test**

<table>
<thead>
<tr>
<th>Group of animals</th>
<th>No. of squares crossed</th>
<th>Time spent in central square (sec)</th>
<th>No. of rearing</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>92.5±8.31</td>
<td>0.83±0.54</td>
<td>23.17±1.72</td>
</tr>
<tr>
<td>II</td>
<td>152.5±11.40*</td>
<td>6.33±1.38*</td>
<td>49±4.49*</td>
</tr>
<tr>
<td>III</td>
<td>111.5±10.16</td>
<td>3.67±0.84*</td>
<td>31.83±2.39</td>
</tr>
<tr>
<td>IV</td>
<td>117.5±7.16</td>
<td>4.33±0.61*</td>
<td>41.17±6.20*</td>
</tr>
<tr>
<td>V</td>
<td>116.5±6.58</td>
<td>3.83±1.14*</td>
<td>39.83±5.33*</td>
</tr>
</tbody>
</table>

*: p<0.05, compared to control; n=6

**Discussion**

Despite the widespread traditional use of OG for treating various disorders, there are very few scientific evaluations of its CNS activities. In our study, OGE was evaluated for its antinociceptive activity in both peripheral and central analgesic models. The antinociceptive tests used in the present work were chosen in order to test different nociceptive stimuli namely thermal (hot plate test), radiant (tail flick test) and chemical visceral nociceptive stimuli (acetic acid). It was essential to employ more than one test to confirm the antinociceptive action, as it has been shown that some ‘false positive’ activity can be observed with agents that are not normally considered as analgesic.17

It was found that OGE in three dose (5, 10 and 15 ml/kg) for seven days were effective in producing analgesic activity in hot plate test; the effects were statistically significant and dose dependent. In tail flick test, significant analgesic activity of OGE extract was also observed. In acetic acid induced writhing, OGE at all doses (5, 10 and 15 ml/kg) significantly produced a dose dependent inhibition of the number of abdominal writhing. This result is in agreement with the previous studies done in Brazil with leaf essential oil of OG and in India with methanol extract of leaf of OG.18,19

The hot plate and tail flick tests are considered to be selective for opioid-like compounds, which are centrally acting analgesics in several animal species.20 The increase in the reaction time of the mice on the hot plate following administration of the OGE suggests that the extract possess central analgesic activity. The suppression of the acetic acid-induced writhing suggests, however, that the extracts may act via local peritoneal receptors.21 The i.p. injection of acetic acid produces pain through the activation of chemosensitive nociceptor or irritation of the visceral surface, thereby leading to liberation of bradykinin, histamine, prostaglandin and serotonin. Acetic acid causes inflammatory pain by inducing capillary permeability and liberating endogenous substances that excite pain nerve endings.22 NSAIDs inhibit COX in peripheral tissues and, therefore, interfere with the mechanism of transduction of primary afferent nociceptors.23 OGE has inhibited the pain induced by acetic acid which indicates that it act through peripheral mechanisms. Therefore, it can be inferred that the OGE may have produced analgesic effects via both central and peripheral mechanisms. It is not known whether this action is opioid-like in nature and/or involves acetylcholine or other receptors. The use of selective antagonists (e.g. naloxone or atropine) might delineate this, The mechanism of analgesic effect of OG in acetic acid induced writhing could probably be due to blockade of the effect or the release of endogenous substances that excite pain nerve endings similar to that of indomethacin and other NSAIDs.24,25

To evaluate anticonvulsant properties of OGE, PTZ induced seizure and MES test were used. In this test, OGE on chronic dosing delayed the onset of PTZ-induced seizures and the maximum delay on onset of latency to convulsion was observed at dose of 15 ml/kg; however, the extract pretreatment did not protect any mice from having convulsion. Similarly in MES test, OGE in all doses decreased duration of tonic hind limb extension (THLE) in mice; however, the extract did not protect any mice from having convulsion. The maximum decrease in THLE was observed with OGE at 10 ml/kg. This study shows that OGE have probable anticonvulsant properties. This result is in accordance with the study done in Nigeria in which methanol extract of leaf of OG demonstrated significant anticonvulsant activity in mice.26

For evaluating sedative-hypnotic potentiating effect of OGE, pentobarbital-induced sleeping time test was used. The interval between loss and recovery of righting reflex was used as the index of hypnotic effect. The OGE
significantly prolonged the duration of pentobarbital induced sleeping time at all doses. All doses of OGE were equally effective in prolonging the sleeping time. Potentiation of pentobarbital-induced sleeping time strongly suggests central depressant activity of the OGE.\textsuperscript{27}

For evaluating effect of OGE on muscle coordination, rota rod test was used. Loss of coordinated motor movement is one of the pharmacological effects of anxiolytic drugs. The OGE showed muscle relaxation effect only at 5 and 10 ml/kg which may be due to presence of lower active constituents in the extract. The extract had no observable effects on motor coordination in this experiment at dose of 15 ml/kg.

In open field test, the locomotor activity was increased as indicated by the enhanced total number squares crossed. Increased rearing and time spent in central square reflect enhanced exploratory activity and reduced fear.\textsuperscript{28} Increase in locomotor activity reveals anxiolytic activity of the OGE. In open-field tests when the animals are taken out from their home cage and placed in a novel environment, they express their anxiety and fear by a decrease in ambulation, rearings and other exploratory behaviors. All these behaviors are increased by anxiogenic agents and attenuated by anxiolytics under identical experimental conditions.\textsuperscript{29,30} The findings of the present study indicate that OGE pretreatment induced anxiolytic behavior patterns in mice in the open-field test.

During our study period of seven days, no mortality was observed in the experimental animals pretreated with OGE. In MES test, one case of mortality was observed during the experiment in vehicle treated animals.

**Conclusion**
The study revealed that cold homogenized leaf extract of OG possess antinociceptive, hypnotic-sedative potentiating, anxiolytic and muscle relaxation activity with probable anticonvulsant activity.

Despite showing good pharmacological effects, there is still a need for more precise studies to isolate the active constituents responsible for these activities and to elucidate its mechanisms of action. So, further studies with isolated fractions of OG are needed to determine the exact mechanism(s) of action for antinociceptive, sedative and anxiolytic action of the aqueous extract and to reveal their potential use in relevant diseases.

**References:**