ANTI-INFLAMMATORY, ANTIPYRETIC AND ANALGESIC ACTIVITIES OF EUCALYPTUS GLOBULUS ESSENTIAL OIL IN ALBINO RATS

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Abstract: Medicinal plants are effective natural anti-inflammatory, anti-pyretic, and anti-analgesic agents. Inflammation is a complicated biological defense reaction of vascular tissues to potentially damaging stimuli such as bacteria, damaged cells, or irritants. The current study aimed to establish the anti-inflammatory, antipyretic, and analgesic properties of E. globulus essential oil. In this research, 36 albino rats weighing 140-170g on average were employed. 36 rats were divided into three groups (control, standard, and experimental group). Control and standard group consisted of 6 rats in each group whereas experimental group consisted of 24 rats. All rats in three groups were treated with carrageenan for induction of oedema for anti-inflammatory, brewer’s yeast to induce pyrexia to check anti-pyretic activity and acetic acid to induce pain for analgesic activity. Control group rats were treated with normal saline for all 100µL three activities. Standard group rats were treated with diclofenac for anti-inflammatory and analgesic activities and paracetamol for antipyretic activity. The essential oils were injected into abdominal muscles and intraperitoneal tissues. The maximum percentage of inhibition of leaves oil in anti-inflammatory activity was 54.01%. The maximum percentage of suppression of fever by essential leaves oil was 75.44% in antipyretic efficacy. The highest percentage inhibition against pain in analgesic action was 25%. Significant anti-inflammatory, antipyretic, and analgesic effects were demonstrated at 50µL, 100µL, and 12.5µL/L doses, respectively, when compared to control group. From overall result, it was concluded that injection of E. globulus leaves oil in abdominal and intraperitoneal muscle may possess significant anti-inflammatory, antipyretic and analgesic activities. Our study scientifically supports traditional use of E. globulus as a medicine.

Keywords: Anti-inflammatory, antipyretic, analgesic, Eucalyptus globulus, pain, fever, leaf oil, diclofenac

Introduction

Eucalyptus globulus Labill is in the Myrtaceae family (Abbasi et al., 2020). The Tasmanian blue gum, or E. globulus Labill, is a native of Tasmania, the Bass Strait Islands, and southeast Australia. One of the most well-known and widely distributed fast-growing eucalypt species at the moment, it is thought to cover about 2.3 million acres worldwide (Silva et al., 2021). A tree called E. globulus can naturally reach heights of 60 to 80 metres. Brown or yellowish-brown coloured plants have mostly smooth bark that sheds in long strips, leaving a white or greyish surface that resists rot and can easily detach from the stem. The age of the tree has an impact on its leaf colour as well (Abbasi et al., 2020). Numerous monoterpenes and sesquiterpenes, as well as aromatic phenols, oxides, ethers, alcohols, esters, aldehydes, and ketones, such as 1,8-cineole (eucalyptol), citronellal, citronellol, citronellyl acetate, p-cymene, and eucamalol, are present in the complex composition of eucalyptus (Butnariu, 2021). Asthma, diabetes, and pulmonary tuberculosis are all treated with the essential oil extracted from E. globulus leaf leaves. Additionally, oils from its leaves, fruits, buds, and bark have been shown to have antibacterial, antioxidant, anti-inflammatory, antihelminthic, and anticancer properties (Mehta and Sharma, 2022). Eucalyptus oil can be used as an immunoregulatory agent against infectious diseases,
according to the WHO, because it enhances innate cell-mediated immunity. A bioactive substance known as eucalyptus oil was extracted from Eucalyptus polybractea and is capable of acting as an antiviral agent against the influenza virus as well as inhibiting the avian influenza virus H11N9 in vapour and aerosol form. According to a report, eucalyptus oil exhibits in vitro antiviral activity, which enables it to inhibit enveloped viruses like the mumps and herpes (Asif et al., 2020; Alyas et al., 2020). When used as an anti-inflammatory agent and to treat upper and lower respiratory illnesses in patients with severe asthma, eucalyptol has been shown to have anti-inflammatory properties (Alyas et al., 2020; Vecchio et al., 2016). Patients with severe, ongoing pain depend on a variety of complementary therapies while new medications with minimal or nonexistent side effects become available. Numerous studies have shown that EEO has analgesic properties (Gbenou et al., 2013; Lee et al., 2019; Mondal et al., 2021; Mworia et al., 2020). In general, E. globulus leaf extract showed in vivo antipyretic activities in rats, as shown by a decrease in rectal temperature in response to yeast-induced fever (Mworia et al., 2019). The goal of the present study was to assess the medicinal value of plants for the treatment of inflammation, fever, and pain using an animal model (albino rats).

Material and methods
Sample collection
Leaves of E. globulus were collected from botanical garden, Quaid-e-Azam campus Punjab University, Lahore. The species were identified by botanists of IMBB department of The University of Lahore, Pakistan. Essential oil was extracted by hydrodistillation in a Clevenger apparatus. 100 g of E. globulus leaves were boiled for two hours in distilled water. The distillate was collected once the temperature had settled. The distillate was mixed with sodium chloride (5 g) to help separate the organic and aqueous phases. The mixture was then poured into a separate funnel, and the distillate was repeatedly cleaned with cyclohexane. After stirring, the organic phase was used to perform rotary evaporation to get the essential oil and get rid of the cyclohexane. After the yield estimate was calculated, the essential oil was kept at 4°C (Belkhodja et al., 2022).

Experimental rats
Albino rats of either female and male sex (140-170 g) were used for all three activities. For experimental study, albino rats were purchased from (UVAS) University of Veterinary and Animal Sciences Lahore. Rats were kept in animal house, University of Lahore in polypropylene cages. Before experimental work, rats were kept in fasting condition. After that, they were given distilled water and balanced feed.

Drugs Used and Chemicals
- Normal saline 100 µl/L
- Diclofenac 50 µl/L
- Eucalyptus globulus leaf essential oil (oil) 100 µl/L
- Carrageenan (inflammation) 100 mg/kg
- Yeast (pyrexia) 100mg/kg
- Paracetamol 100mg/kg
- Acetic acid (writhing) 100mg/kg
- Distilled water 100 µl/L

Anti-inflammatory activity model
Firstly, all rats were treated with carrageenan in which 12.5 mg/kg, 25 mg/kg, 50 mg/kg and 100mg/kg was carrageenan injected into sub-plantar region of paw. Measurement of paw was taken before and after the induction of carrageenan. Group I was considered as control group. In control group rats were treated with normal saline 12.5 µl/L, 25 µl/L, 50 µl/L and 100 µl/L according to their body weights. Normal saline was injected into right hind paw. Volume of paw was measured in millimeter cube. Group II was considered as standard group. Rats of standard group were treated with diclofenac (12.5 µl/L, 25 µl/L, 50 µl/L and 100 µl/L). Group III was considered as experimental group. Rats of experimental group were treated with essential oil of E. globulus leaves 12.5 µl/L, 25 µl/L, 50 µl/L and 100µl/L 3hrs after the carrageenan injection. The volume of paw was measured after the interval of 1hr, 2hr and 3hr of treatment doses of essential oil. Anti-inflammatory activity was calculated by the given formula:

\[
\text{Anti-inflammatory} = (Ct - Co) - (Ct - Co) \times 100 \frac{C}{(Ct - Co)}
\]

Where, Co= Reading of paw before carrageenan, Ct= Volume of the hind paw of after carrageenan (Ct-Co) = Volume of the hind paw of the treated group after carrageenan injection.

Anti-pyritic activity model
Firstly, all rats were treated with brewer’s yeast, which were injected below the nape of neck 12.5, 25, 50 and 100µl/L according to their body weight. After the interval of 0, 1hr 2hrs, 3hrs and 4hrs temperature were measured with help of thermometer (rectum). Group I was considered as control group. In control group rats were treated with normal saline 12.5 µl/L, 25 µl/L, 50 µl/L and 100µl/L according to their body weights. Normal saline was injected into nape of the neck. Group II was considered as standard group. Rats of standard group were treated with paracetamol injection 12.5 mg/kg, 25 mg/kg, 50 mg/kg and 100 mg/kg in nape of the neck. After the interval of 0, 1hr, 2hrs, 3hrs and 4hrs temperature were measured with help of thermometer (rectum). Group III was considered as experimental group. The rats of experimental group were treated with leaves

oil of *E. globulus* (12.5 µL/L, 25 µL/L, 50 µL/L and 100µL/L). After the interval of 0, 1hr, 2hrs, 3hrs and 4hrs temperature were measured with help of thermometer (rectum). Percent production = \( \frac{B - C_b \times 100}{B - A} \)

Where, \( B = \) Temperature after pyrexia induction, \( C_b = \) Temperature before 1, 2 and 3 hours and \( A = \) Normal body temperature.

### Analgesic activity model

Acet ic acid induced writhing. Group I was considered as control group. In control group rats were treated with normal saline 12.5mg/kg, 25mg/kg, 50mg/kg and 100 mg/kg before 1 hrs treated with acet ic acid, according to their body weights. Normal saline was injected in the intra peritoneal cavity. Group II was considered as standard group. Rats of standard group were treated with diclofenac (12.5 µg/kg, 25 µg/kg, 50 µg/kg and 100 µg/kg) was given 1-hour before the administration of acet ic acid. Group III was considered as experimental group. Rats of experimental group were treated with leaves oil of *E. globulus* (12.5 µL/L, 25 µL/L, 50 µL/L and 100 µL/L) before 1hr treated with acet ic acid. A writhing was recorded with the help of stopwatch.

### Analgesic activity

\[
\text{Percent production} = \frac{B - C_b \times 100}{B - A} 
\]

Where, \( B = \) Temperature after pyrexia induction, \( C_b = \) Temperature before 1, 2 and 3 hours and \( A = \) Normal body temperature.

### Table 1. Anti-inflammatory of *E. globulus*

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Pre-inflammation</th>
<th>Post-inflammation</th>
<th>1hr</th>
<th>2hrs</th>
<th>3hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I</td>
<td>Control group</td>
<td>3.33±0.17</td>
<td>4.05±0.12</td>
<td>3.74±0.11</td>
<td>3.60±0.09</td>
<td>3.53±0.09</td>
</tr>
<tr>
<td></td>
<td>Standard group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Treat at (12.5 µL/L)</td>
<td>2.94±0.14</td>
<td>5.65±3.1</td>
<td>5.29±0.32</td>
<td>4.97±0.33</td>
<td>4.41±0.21</td>
</tr>
<tr>
<td></td>
<td>Treat at (25 µL/L)</td>
<td>2.76±0.12</td>
<td>6.30±0.29</td>
<td>5.77±0.29</td>
<td>5.03±0.09</td>
<td>4.59±0.17</td>
</tr>
<tr>
<td></td>
<td>Treat at (50 µL/L)</td>
<td>3.15±0.08</td>
<td>5.97±0.1</td>
<td>5.97±0.1</td>
<td>5.48±0.09</td>
<td>5.07±0.16</td>
</tr>
<tr>
<td></td>
<td>Treat at (100 µL/L)</td>
<td>2.41±0.1</td>
<td>6.45±0.12</td>
<td>5.76±0.12</td>
<td>5.35±0.04</td>
<td>5.02±0.08</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

Anti-inflammatory of *E. globulus* leaves oil Values are mean ±S.D; n=4 in each group *P*< 0.0001 compare to control group.

### Figure I Anti-inflammatory activity

[https://doi.org/10.54112/bcsrj.v2023i1.207](https://doi.org/10.54112/bcsrj.v2023i1.207)
Alyas et al., (2023)

Anti-inflammatory, antipyretic and analgesic activities of eucalyptus globulus essential oil in albino rats.

Table 2. Anti-Pyretic Activity of *E. globulus*

<table>
<thead>
<tr>
<th>No. of Groups</th>
<th>Treatment</th>
<th>Initial temperature</th>
<th>0hr</th>
<th>1hr</th>
<th>2hrs</th>
<th>3hrs</th>
<th>4hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I</td>
<td>Control group</td>
<td>98.08±0.3</td>
<td>100.82±0.47</td>
<td>100.62±0.47</td>
<td>100.40±0.45</td>
<td>100.15±0.42</td>
<td>99.98±0.42</td>
</tr>
<tr>
<td></td>
<td>Standard group</td>
<td>97.77±0.16</td>
<td>100.43±0.44</td>
<td>99.03±0.3</td>
<td>98.57±0.22</td>
<td>97.93±0.17</td>
<td>97.78±0.17</td>
</tr>
<tr>
<td>Group-II</td>
<td>Treated at (12.5 µL/L)</td>
<td>99.23±0.32</td>
<td>100.05±0.5</td>
<td>98.85±0.25</td>
<td>98.38±0.14</td>
<td>97.88±0.17</td>
<td>97.12±0.31</td>
</tr>
<tr>
<td></td>
<td>Treated at (25 µL/L)</td>
<td>99.32±0.25</td>
<td>100.90±0.26</td>
<td>99.57±0.27</td>
<td>99.10±0.19</td>
<td>98.87±0.12</td>
<td>98.17±0.18</td>
</tr>
<tr>
<td>Group-III</td>
<td>Treated at (50 µL/L)</td>
<td>98.68±0.49</td>
<td>100.22±0.36</td>
<td>99.32±0.14</td>
<td>99.02±0.22</td>
<td>98.23±0.59</td>
<td>97.05±0.62</td>
</tr>
<tr>
<td></td>
<td>Treated at (100 µL/L)</td>
<td>99.05±0.21</td>
<td>99.78±0.28</td>
<td>98.28±0.29</td>
<td>96.83±0.95</td>
<td>95.90±0.79</td>
<td>95.28±0.76</td>
</tr>
<tr>
<td>p-value</td>
<td>0.004</td>
<td>0.3265</td>
<td>0.0002</td>
<td>0.0004</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
</tbody>
</table>

Antipyretic activity of *E. globulus* of leaves oil
Values are mean ±S.D; n=4 in each group *P*<0.005 compared to control group.

Figure II Antipyretic activity

Table III. Analgesic activity of *E. globulus*

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Analgesic Time</th>
<th>Writhing (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>18.83±0.4</td>
<td>0</td>
</tr>
<tr>
<td>Standard group</td>
<td>12.17±0.91</td>
<td>35.36</td>
</tr>
<tr>
<td>Treated at (12.5 µL/L)</td>
<td>14.00±0.26</td>
<td>25.65</td>
</tr>
<tr>
<td>Treated at (25 µL/L)</td>
<td>18.83±0.87</td>
<td>0</td>
</tr>
<tr>
<td>Treated at (50 µL/L)</td>
<td>18.50±0.76</td>
<td>1.75</td>
</tr>
<tr>
<td>Treated at (100 µL/L)</td>
<td>18.67±0.56</td>
<td>0.85</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt; 0.0001</td>
<td></td>
</tr>
</tbody>
</table>

Analgesic effect of *E. globulus* leaves oil
Values are mean ±S.D; n=4 in each group *P*<0.0001 compare to control group.
Discussion
In our study, *E. globulus* leaf oil was used to evaluate anti-inflammatory, antipyretic and analgesic activities on albino rats. Due to the drug’s lag in entering rats’ bodies, time lag arises in analgesic action (Parra et al., 2019). Carrageenan’s anti-inflammatory activity causes edema in rat paws whereas brewer's yeast's antipyretic activity causes pyrexia in rats, and acetic acid's analgesic activity is used to assess the writhing test's analgesic effect (Alyas et al., 2022; Subedi et al., 2016). In inflammation process main symptoms are fever and pain (Deka et al., 2018). In our results, highest paw oedema inhibition of leaves oil of *E. globulus* was at dose of 50µL/L. Anti-inflammatory activity was dose dependent. The essential oil declined the paw edema induced by carrageenan injection when compared with the control group. The presence of chemical substances which are inflammatory process mediators result in increase in the vascular permeability at the site of inflammation, thus promoting accumulation of fluid in tissues and this resulted in oedema (Germolec et al., 2018).

Brewer’s yeast causes pyrexia in the body by boosting prostaglandin production when it is injected into the albino rats’ nape of the neck. Brewer’s yeast is used to create pathogenic fever, which is characterised by the generation of prostaglandins as its etiological cause. Synthesis of prostaglandins can be used to produce an antipyretic effect. Paracetamol and prostaglandins both work to suppress the cyclooxygenase enzyme’s activity. To lower the fever, intraperitoneal injection of *E. globulus* leaf oil is used (Muhammad et al., 2021). According to results the group that received leaf oil of *E. globulus* at a dose level of 100µL/L recorded the highest antipyretic effects, which was 85.43%, 78% 72% and 75% at 1hr, 2hrs, 3hrs and 4hrs respectively. Acetic acid induction induces abdominal and visceral pain in rats, which generates prostaglandin pain from peritoneal fluid (Bairagi et al., 2017). Acetic acid is used as a painkiller to block endogenous substances that cause pain and release from nerve endings (Subedi et al., 2016). In contrast to the control group, our study found that intra-peritoneal treatment of the leaf oils of *E. globulus* dramatically reduced the number of acetic acid-induced writhes in rats. The maximum writhing inhibition and the highest analgesic effect were caused by the leaves oil of *E. globulus* (12.5 l/L).

Conclusion
*E. globulus* leaves oil may possess significant anti-inflammatory, antipyretic and analgesic activities due to its phytocomponents. Our study scientifically supports traditional use of *E. globulus* as a medicine. In future prospective, secondary metabolites present in plant will help us to understand identification and isolation of compound that can be clinically used. Natural compounds of plant are responsible for above activities. Due to the anti-inflammatory, antipyretic, and pain-relieving properties of its bioactive compounds, it was suggested that this essential oil be used in scientific research and industry as a natural alternative and drug.

Conflict of interest
The authors declared absence of conflict of interest.

References


Figure III Analgesic activity

![Analgesic activity graph](image-url)


