Anti-Inflammatory Activity of Methanolic Extract of *Ocimum Gratissimum* (Labiatae) on Experimental Animals

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Abstract
The Methanolic leaves extract of *Ocimum gratissimum* was investigated for anti-inflammatory effects in rats. The model used to study the effect on anti-inflammatory is Formalin Induced rat paw edema. The extract demonstrated a significant (P< 0.001) anti-inflammatory activity at all the doses (50, 100 and 200 mg/kg body weight i.p.) tested group and standard group (aspirin 20mg/kg) compared to control normal saline. The significant (P< 0.001) anti-inflammatory activity exhibited by the extract at all the doses used (50, 100, and 200mg/kg body weight extract i.p.) against edema induced by formalin in rats compared to the control group was an indication that, the plant might serve as a useful source of anti-inflammatory agent. The intra-peritoneal LD50 value of the extract was 1285.5mg/kg body weight in mice. Preliminary phytochemical screening revealed the presence of alkaloids, saponins, tannins and flavonoids. The results suggest the extract contained pharmacologically active principles, and supports the local application of the plant in painful conditions. Further studies may reveal the exact mechanisms of action responsible for the anti-inflammatory activity of *O. gratissimum* leaves extract.

Key words: *Ocimum gratissimum*, *Labiatae*, Anti-inflammatory, Asprin, Formalin, and Rat paw edema.

Introduction:
*Ocimum gratissimum* is commonly known as fever leaf in general but is has different native names in different part of the country. In Yoruba language, it is known as Ewirin ajase, Nchu-nwu in Ibo, Bunsuru daji in Hausa, Ireru in Ebira, Ebaubokho in Benin, ufuuo-yibo in Urhobo and ntion in Efik [1]. There are about 60 or more species of *Ocimum* and numerous varieties, belonging to the Family *Labiatae*. These different types of species are represented by the five most important representatives of the more that 60 *Ocimum* species and these include (i)

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Ocimum gratissimum, (ii) Ocimum basilicum, (iii) Ocimum americanum, (iv) Ocimum sanctum and (v) Ocimum americanum\textsuperscript{[2, 3]}. 

Ocimum gratissimum Linn. (Labiaceae) is an herbaceous plant commonly found in tropical Asia especially India. It is used in the treatment of epilepsy in the coastal area of Nigeria\textsuperscript{[4]}, High fever\textsuperscript{[5]}, and Diarrhea\textsuperscript{[5, 6]}. The plant is also used to treat typhoid fever and diabetes\textsuperscript{[7-9]}. Today, basil is used mainly as a culinary herb. It medicinal value is not as widely appreciated in Western World. In France it is used in perfumes and cosmetics\textsuperscript{[10]}.

Inflammation is a physiologic series of responses generated by the host in response to infection or other insults. Inflammation can have rapid onset and last a short period of time (acute inflammation), or it can persist due to a continuous stimulus or injury (chronic inflammation). The initial events of inflammation are derived from vascular reactions at the site of injury. Vascular changes are important for the induction of the response and are characterized by redness, heat, and swelling, usually accompanied by pain and loss of function, and collectively represent the "cardinal signs" of inflammation. These signs of inflammation are the result of vasodilatation and increased vascular permeability, leading to exudation of fluid and plasma proteins and recruitment of leukocytes to the site of injury.\textsuperscript{[11]}

This research was aimed at investigating the possible anti-inflammatory activity of methanolic leaves extract of the plant in order to support or refute the claims by traditional herbalists.

**Material and Methods:**

**Preparation of Extract:**

The fresh leaves of *Ocimum gratissimum* was chopped, cleaned and air dried for minimum one week. After that the size was reduced with a mortar and pestle into a fine powder. 100 g of the powder was extracted with 90% methanol (2.5 litres) using Soxhlet apparatus process for 50-55 h. The liquid extract was then concentrated on a water bath to give a brownish solid extract with a mean yield of 10% w/w.

Methanolic extract obtained was washed with hexane to obtain purified methanolic extract. Methanolic fraction was successively extracted with ethanol (90 % w/w) in Soxhlet apparatus for 50-55 h\textsuperscript{[12]}.

**Acute Toxicity Study:**

This was conducted by using the method described by Lorke. In the initial phase, mice were divided into 3 groups of three and treated with the methanolic leaves extract of the plant at doses of 10, 100 and 1000mg extract/ kg body weight intra peritoneal (i.p.) and were then observed for 24 hrs for signs of toxicity including death . In the final phase, mice were divided into 4 groups of one mouse each and treated with the ethanol extract at doses of 600, 1000, 1600 and 2900
mg / kg body weight i.p. The median lethal dose (LD50) was calculated from the second phase [13].

**Anti-inflammatory activity:**

**Formalin induced rat paw edema**

In this method rats were divided in five groups of five each. Thirty minutes before the injection of formalin (50μl of 2.5% v/v) into the sub planter tissue of left hind paw of each rat, the groups were treated i.p. as follows: group 1, normal saline (10ml/kg as a negative control); group 2, 20mg/kg of Asprin; groups 3, 4 and 5 received extract of *O. gratissimum* at the doses of (50, 100 and 200mg/kg) respectively [14]. Paw linear diameter (cm) was measured using vernier caliper at 1, 2, 3 4 and 5 hrs after formalin injection.

\[
\text{Inhibition (\%)} = \frac{\text{Dc} - \text{Dt}}{\text{Dc}} \times 100
\]

Dc = Control mean paw diameter
Dt = Treated mean paw diameter

**Statistical analysis:**

Results were expressed as mean ± Standard Error of Mean (SEM). The data was statistically analyzed using the one-way ANOVA to determine whether results in a particular group were significantly different from those in the corresponding control groups. Results were statistically significant when P values are less than 0.001 (P <0.001) as described by Duncans [15].

**RESULT:**

The freshly prepared extracts were subjected to preliminary phyto-chemical screening test for various constituents. This revealed the presence of alkaloids, tannins, saponins, flavonoids, terpenoids and steroids. The sign of toxicity was first noticed after 4-6 hrs of extract administration. There was decreased loco-motor activity and decreased sensitivity to touch and jerking. Also there was decreased feed intake, and prostration after 10 hrs of extract administration. The median lethal dose (LD50) in mice was calculated to be 1285.5 mg/kg body weight.

The extract demonstrated a significant (P< 0.001) anti-inflammatory activity at all the doses (50, 100, and 200 mg/kg body weight i.p.) tested compared to control normal saline. The activity resides more at the highest dose 200mg/kg body weight i.p. that was found to have the highest percentage of inhibition of the paw edema, induced by Carrageenan in rat paw (Table 1 & figure 1).

**DISCUSSION & CONCLUSION:**

The significant (P< 0.001) anti-inflammatory activity exhibited by the extract at all the doses used (50, 100, and 200mg/kg body weight extract i.p.) against edema induced by formalin in rats compared to the control group was an indication that, the plant might serve as a useful source of anti-inflammatory agent. This anti-
inflammatory effect of the extract observed might be due to the presence of flavonoids in the plant. This was supported by other workers, who found that flavonoids inhibited phosphodiesterases which are involved in cell activation, and their effect depend upon the biosynthesis of protein cytokines that mediate adhesion of circulating leucocytes to the sites of injuries \[^{16}\]. In conclusion, these preliminary investigations and data obtained from this study demonstrated that effect of herbal plant *Ocimum gratissimum* methanolic leaves extract have good anti-inflammatory activity.

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REFERENCES:

Table 1: Effect of aqueous leaves extract of *Ocimum gratissimum* on formalin -induced paw Edema in rats

<table>
<thead>
<tr>
<th>Treatment Groups (n=5)</th>
<th>Dose (mg/kg)</th>
<th>Edema diameter (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 hr</td>
</tr>
<tr>
<td>Normal saline</td>
<td>10ml/kg</td>
<td>0.68±0.72</td>
</tr>
<tr>
<td>Aspirin</td>
<td>20</td>
<td>0.61±0.25</td>
</tr>
<tr>
<td><em>O. gratissimum</em></td>
<td>50</td>
<td>0.55±0.15</td>
</tr>
<tr>
<td><em>O. gratissimum</em></td>
<td>100</td>
<td>0.46±0.66</td>
</tr>
<tr>
<td><em>O. gratissimum</em></td>
<td>200</td>
<td>0.58±0.09</td>
</tr>
</tbody>
</table>

Each value is mean ±SEM of 5 rats. *a* p <0.05; *b* p<0.01; *c* p<0.001 compared to control

Figure 1: Graphical Presentation of effect of aqueous leaves extract of *Ocimum gratissimum* on formalin -induced paw Oedema in rats.