IN VIVO EVALUATION OF THE INHIBITORY EFFECT OF Ocimum gratissimum ON Salmonella typhi

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ABSTRACTS:
The use of Ocimum gratissimum in treating Salmonella typhi infection was investigated using Wistar rats. Thirty Wistar rats in six groups were used for the study. Weights of the animals were determined during the study while blood and faeces were also collected for analysis. Before infection, the weights of all the rats increased appreciably. Conversely, weights of the rats given prophylactic dose of 200mg/kg before infection decreased from 132 kg to 120 kg within five days after infection. Faecal shedding of S. typhi by the rats treated with the extract decreased with increase in the days of treatment. Among the groups given prophylactic treatment with O. gratissimum, faecal shedding of S. typhi significantly reduced from 2.5 X 10^2 to 1.9 X 10^2 and from 6.0 X 10^3 to 4.0 X 10^2 within four days of treatment. Furthermore, treatment with O. gratissimum caused significant (p<0.05) increase in haemoglobin (Hb), neutrophils and platelets. The observed reduction in the feecal shedding of S. typhi with increase in the days of treatment and increase in the haematological parameters of the Wistar rats may justifiy the use of Ocimum gratissimum in treating Salmonella typhi infection.

Keywords: In vivo, Inhibitory effect, Ocimum gratissimum, Salmonella typhi, Wistar rats

INTRODUCTION

Typhoid fever is a global health problem with approximately 17 million cases and 600,000 deaths occurring annually. It is caused by Salmonella typhi, a pathogenic bacterium which is transmitted by ingestion of faecally-contaminated food and water [28]. After an incubation period of 10-14 days, Salmonella typhi causes low-grade fever, malaise, headache, constipation, bradycardia, and myalgia in an infected individual. The fever rises to a high plateau, the spleen and liver become enlarged, and rose spots (usually seen on the skin of the abdomen or chest) are seen in rare cases [6]. Usually, the clinical picture of typhoid is confused with those of many other febrile infections and the rate of transmission is high especially in individuals between 3 and 19 years of age and in regions such as Central Asia, Latin America, Vietnam, Indonesia, and Sub-Saharan Africa where overcrowding, poor sanitary conditions, and untreated water supplies prevails [22, 28]. Three percent of survivors of typhoid become permanent carriers, harboring the organisms in the gall bladder, biliary tract, or rarely, the intestine or urinary tract. Thus, such carriers and those with unsuspected subclinical disease shed the organisms in their faeces and are very important so urce of contamination [28]. Fluoroquinolone (e.g. ciprofloxacin, ofloxacin) is the drug of choice for the treatment of typhoid fever as it possesses favourable intracellular pharmacokinetics [23]. Nowadays, the major impediment to the effective chemotherapy of typhoid is the ever increasing number of resistant strain of S. typhi. The problem of antibiotics resistance creates an important public health problem that may be related to therapeutic failure [7] since bacteria sensitive to antibiotics becomes resistant in order to survive [13].

Ocimum gratissimum is an herbaceous plant widely distributed in the tropics. In Nigeria, it has various local names which differ from one tribe to another – “Nchuanwu” (Igbo), “Effirin nla” (Yoruba), “Ufuo-yibo” (Urohobo), “Ihiriezia” (Bini), “Dai doyatagida” (Hausa), “Aramagbo” (Edo), and “Ntion” (Efik) [11, 17, 19]. The greatest variety of O. gratissimum is commonly found around the village huts and gardens [19] while it is cultivated in a commercial scale in places like Vietnam [16]. Ocimum gratissimum contains alkaloids, tannins, phytales, flavonoids, oligosaccharides, and cyanogenic glycosides which are secondary metabolites with antimicrobial properties and are stored in varying concentrations in the plant cells [4, 10, 14, 17]. The use of O. gratissimum in the treatment of bacterial infection has to do with the presence of these phytochemicals in the plant. In the South-Western Nigeria, decoction from the whole herb of O. gratissimum is used for the treatment of diarrhoea caused by certain bacteria including S. typhi [1, 2]. Although many herbs have been tested against S. typhi by previous researchers [1, 3, 5, 24], only few works have been done to investigate the antimicrobial activity of O. gratissimum against S. typhi using animal model. In view of this, this study was designed to evaluate the inhibitory effect of Ocimum gratissimum on Salmonella typhi in Wistar rats.
MATERIALS AND METHODS

Plant collection and extraction:
Leaves of *Ocimum gratissimum* were obtained from the Federal School of Agriculture, Akure, Nigeria and were authenticated by Dr J.O. Bifarin of the School of Agriculture. The leaves were shade-dried for two weeks, pulverized using Marlex Excella mixer grinder and kept in an air-tight container. One hundred and fifty gram (150g) of the powder was soaked in 1500ml of 70% ethanol for 72 hours. The mixture was filtered and the filtrate was concentrated using a rotary evaporator before it was freeze-dried.

Test bacteria:
Stock culture of *S. typhi* was obtained from the State Specialist Hospital, Akure, Nigeria on Tryptone Soy Agar (TSA) slant. Discrete colonies of the bacterium were isolated on Salmonella-Shigella agar (SSA) incubated at 37°C for 18 hours and then identified using biochemical tests described by Monica [15].

Laboratory animals:
Sixty albino rats were obtained from the Animal House of the Multi-disciplinary Laboratory, Obafemi Awolowo University, Ile-Ife, Nigeria for this study. The animals were acclimatized for seven days before the commencement of the study and fed with standard rat chow and water *ad libitum*.

Determination of infective dose 50 (ID50) for *Salmonella typhi*:
According to Takumi *et al* [26], *Salmonella typhi* from the stock culture was reactivated in Tryptone Soy Broth (TSB) and then cultured on TSA at 37°C for 18 hours. One hundred millimeter (100ml) TSB was then inoculated with two to three discrete colonies of *S. typhi* from TSA and incubated at 37°C for 18 hours. The broth culture was centrifuged at 2000 rpm for 10 minutes and the supernatant was discarded to obtain whitish pellet which was re-centrifuged in 100ml physiological saline. The pellet was suspended in 4ml physiological saline and re-centrifuged before being serially diluted from 10^1 to 10^6 in physiological saline. Thirty Wistar rats in six groups were then inoculated with bacterial dilutions mixed with equal volume of 6% NaHCO_3 to determine the ID50 of *S. typhi*. Also, corresponding colony-forming units per millimeter (cfu/mL) of the bacterial dilutions was determined using plate count method on Salmonella-Shigella agar (SSA).

*In vivo* evaluation of the inhibitory effect of *Ocimum gratissimum* on *Salmonella typhi*:
Thirty Wistar rats were divided into six groups with Group 1 (G1) being infected with infective dose of *S. typhi* (calculated to be 2.20 X 10^5 cfu/ml) and then treated with the extract of *O. gratissimum* after the infection had set in. Groups 2A and 2B (G2A and G2B) were given prophylactic dose of 200mg/kg and 400mg/kg extract of *O. gratissimum* respectively for two weeks before being infected with *S. typhi* and later treated with the extract of *O. gratissimum*; Group 4 (G4) was infected but not treated; Group 5 (G5) was infected and treated with ciprofloxacin; while Group 6 (G6) was fed with basal meal and water only. All the experimental rats in Groups 1, 2A, 2B, and 5 were observed for signs of infection before being treated.

Faecal shedding of *S. typhi* by the infected rats:
The effect of the leaf extract of *O. gratissimum* on the load of *S. typhi* being shed in the faeces of the infected Wistar rats was investigated as described by Adebolu *et al* [3]. Faecal samples were collected before and after infection. One gram (1g) of the sample was serially diluted in physiological saline, plated on duplicate SSA, and then incubated at 37°C for 24 hours. Typical colonies of *S. typhi* were counted on plates that contained between 30 and 300 colonies.

Collection and examination of blood from the albino rats:
Blood was collected from the experimental rats for haematological assay. The rats were sacrificed and their blood, collected by cardiac puncture into labeled EDTA bottles, was analysed according to Monica [15].

Determination of the rats’ body weight:
Each rat was weighed using a sensitive weighing balance before and during the period of infection and treatment.

Statistical analysis:
The data obtained were analyzed using one-way analysis of variance (ANOVA) and presented as mean ± standard deviation (SD). The level of significance was considered at P < 0.05.
RESULTS

Weights of the albino rats:
Figures 1 and 2 show the average body weights of the rats. Before infection, the weights of all the rats increased appreciably. After infection, weights of the rats given prophylactic dose of 200mg/kg before infection decreased from 132 kg to 120 kg within the period of five days. Conversely, weights of the rats that were pre-treated with 400mg/kg before infection increased from 138 kg to 142 kg during the experiment. The average body weight of the untreated infected rats reduced from 133 kg to 128 kg during the experiment while weights of the rats treated with ciprofloxacin increased from 145 to 150 kg.

Faecal shedding of S. typhi by the infected albino rats:
Before infection, no S. typhi was isolated in the faeces of all the experimental rats. Generally, faecal shedding of S. typhi by the rats treated with the extract decreased with increase in the days of treatment. The infected-ununtreated rats continually shed S. typhi during the period of observation while fecal-shedding of S. typhi by the rats in group 2A decreased from 2.5 X 10^5 to 1.9 X 10^3 c.f.u/ml and 6.0 X 10^3 to 4.0 X 10^2 c.f.u/ml in group 2B by the fourth day of treatment with the extract (Table 1).

Collection and examination of blood from the albino rats:
The lowest mean value of haemoglobin (Hb), 12.7g/dl, was observed in the group given prophylactic treatment with 400mg extract/kg body weight while rats in group one (G1) had the highest Hb value. Platelets counts increased in the experimental rats treated with the extract of O. gratissimum. In relation to the control group, there was increase in neutrophils and decrease in lymphocytes among the experimental groups of wistar rat (Table 2).

DISCUSSION

The weight loss observed in the untreated group might be due to the debilitating effect of S. typhi on the host. Also, the increase observed in the average weight of the rats treated with 400mg/kg extract and the decrease in average body weight observed in those treated with 200mg/kg extract might be attributed to the dose-dependent nature of the antimicrobial activity of extract of O. gratissimum [25]. The infected-ununtreated group shed more S. typhi in their faeces than any of the groups treated with the extract of O. gratissimum. The reduction in faecal-shedding of S. typhi by the rats treated with ciprofloxacin when compared with the rats treated with extract of O. gratissimum might not be unconnected with a known mechanism of action that is common with conventional antibiotics [9, 27].

In relation to the group treated with ciprofloxacin, mean value of haemoglobin (Hb) was higher in the group treated with the extract after infection since ciprofloxacin could cause decrease in haemoglobin (Hb) due to high permeability of erythrocyte membranes to the quinolone [8]. On the other hand, leaf extract of O. gratissimum may contain active agents which promote the action of erythropoietin and consequently increase production of erythrocytes [18, 20]. This finding is in agreement with the report of Sheu et al [25]. The value of Hb obtained for the infected-ununtreated group was lower than the values obtained for the groups infected and treated with the extract since most pathogenic microorganisms cause acute inflammation which leads to haemolysis and decrease in Hb. However, anti-inflammatory potentials inherent in the plant extract prevent inflammation and haemolysis [21]. Total WBC was higher in the group treated prophylactically with 400mg extract/kg than those given 200mg extract/kg. This might be due to variation in the dose administered. There was increase in neutrophils and decrease in lymphocytes across the experimental groups in relative to the control group since neutrophils are the primary white blood cells that respond to bacterial infection [12]. There was no significant difference between the differential WBC values obtained for the infected-treated rats when compared with the rats given prophylactic treatment. Thus, the use of O. gratissimum leaf extract for prophylactic treatment is not encouraged. Contrary to the findings of Okon et al [20], platelets count was higher in the experimental groups than the control group. The highest platelet count was observed in the infected-ununtreated group followed by the group treated with ciprofloxacin. This might be attributed to the response of the host’s endogenous defense system to the adverse effects of the pathogen and the ability of fluoroquinolone to cause increase in platelets production [8]. In conclusion, the reduction in the faecal shedding of S. typhi among the rats treated with ethanolic leaf extract of O. gratissimum and the effect of the extract on haematopoiesis justify the use of Ocimum gratissimum in the treatment of Salmonella typhi infection.
REFERENCES


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Table 1: Faecal shedding of *S. typhi* by the infected albino rats (cfu/mL)

<table>
<thead>
<tr>
<th>DAYS</th>
<th>G1</th>
<th>G2A</th>
<th>G2B</th>
<th>G4</th>
<th>G5</th>
<th>G6</th>
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<tr>
<td></td>
<td>0.0</td>
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<tr>
<td>1</td>
<td>2.0 X 10^5</td>
<td>2.5 X 10^5</td>
<td>6.0 X 10^5</td>
<td>4.0 X 10^6</td>
<td>10.2 X 10^3</td>
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<tr>
<td>2</td>
<td>8.0 X 10^3</td>
<td>8.0 X 10^4</td>
<td>5.0 X 10^3</td>
<td>7.0 X 10^6</td>
<td>3.0 X 10^3</td>
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</tr>
<tr>
<td>3</td>
<td>1.3 X 10^3</td>
<td>1.0 X 10^4</td>
<td>8.0 X 10^3</td>
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<td>2.5 X 10^2</td>
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</tr>
<tr>
<td>4</td>
<td>8.7 X 10^2</td>
<td>1.9 X 10^3</td>
<td>4.0 X 10^2</td>
<td>2.0 X 10^6</td>
<td>1.0 X 10^2</td>
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</tr>
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<td>5</td>
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<tr>
<td>6</td>
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<td>0.0</td>
<td>0.0</td>
<td>4.0 X 10^5</td>
<td>0.0</td>
<td>0.0</td>
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<tr>
<td>7</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>2.0 X 10^3</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

**Keys:**
- G1 = Rats infected with *S. typhi* and treated;
- G2A = Rats pre-treated with 200mg extract/kg body weight before infection;
- G2B = Rats pre-treated with 400mg extract/kg body weight before infection;
- G4 = Rats infected but not treated with the extract;
- G5 = Rats infected and treated with ciprofloxacin;
- G6 = Rats fed with normal diets and water only (control)

Table 2: Effects of *Ocimum gratissimum* on haematological parameters of the infected Wistar rats

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>G1</th>
<th>G2A</th>
<th>G2B</th>
<th>G4</th>
<th>G5</th>
<th>G6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (g/dL)</td>
<td>13.6 ± 0.59</td>
<td>13.4 ± 0.82</td>
<td>12.7 ± 1.19</td>
<td>12.1 ± 0.5</td>
<td>12.3 ± 1.28</td>
<td>13.4 ± 0.47</td>
</tr>
<tr>
<td>Total WBC (X 10^3) mm^3</td>
<td>6.7 ± 4.54</td>
<td>5.8 ± 0.86</td>
<td>7.0 ± 2.11</td>
<td>6.9 ± 1.18</td>
<td>10.5 ± 3.47</td>
<td>7.6 ± 4.45</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>73.0 ± 10.70</td>
<td>78.0 ± 9.20</td>
<td>80.7 ± 10.99</td>
<td>72.3 ± 18.3</td>
<td>70.5 ± 14.98</td>
<td>87.0 ± 5.57</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>27.0 ± 10.74</td>
<td>22.0 ± 9.20</td>
<td>19.2 ± 10.99</td>
<td>27.6 ± 18.3</td>
<td>29.5 ± 14.98</td>
<td>13.0 ± 5.57</td>
</tr>
<tr>
<td>Platelets</td>
<td>44.7 ± 6.72</td>
<td>146.3 ± 7.09</td>
<td>116.6 ± 6.68</td>
<td>285.9 ± 9.3</td>
<td>252.9 ± 7.54</td>
<td>56.2 ± 7.74</td>
</tr>
</tbody>
</table>
**Keys:**

G1 = Rats infected with *S. typhi* and treated; G2A = Rats pre-treated with 200mg extract/kg body weight before infection; G2B = Rats pre-treated with 400mg extract/kg body weight before infection; G4 = Rats infected but not treated with the extract; G5 = Rats infected and treated with ciprofloxacin; G6 = Rats fed with normal diets and water only (control)

![Figure 1: Average weights of Rats before infection](image-url)

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G1 = Rats infected with *S. typhi* and treated; G2A = Rats pre-treated with 200mg extract/kg body weight before infection; G2B = Rats pre-treated with 400mg extract/kg body weight before infection; G4 = Rats infected but not treated with the extract; G5 = Rats infected and treated with ciprofloxacin; G6 = Rats fed with normal diets and water only (control)
Figure 2: Average weights of Rats after Infection

**KEY:**
- **G1** = Rats infected with *S. typhi* and treated;  
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- **G2B** = Rats pre-treated with 400mg extract/kg body weight before infection;  
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- **G6** = Rats fed with normal diets and water only (control)