



Journal of
**Pharmacology and
Toxicology**

ISSN 1816-496X



Academic
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**Immunostimulatory Activity of Ethanolic Leaf Extract from
Ocimum gratissimum in Albino Rat Orogastrically Dosed with
Escherichia coli (NCIB 86)**

M.K. Oladunmoye

Department of Microbiology, School of Sciences, Federal University of
Technology, P.M.B. 704, Akure, Nigeria

Abstract: Immunostimulatory activity of ethanolic leaf extract of *Ocimum gratissimum* was investigated in albino rats using immunologic/haematologic indices. The rats were dosed orally with standard inoculum of *Escherichia coli* (NCIB 86) of 1×10^7 cfu mL⁻¹. The extent of infection was carried out by checking the haematologic indices before, during and after treating the infection with ethanolic extract of *Ocimum gratissimum*. The animals were divided into four groups. The first group was dosed with 8ml of the standard inoculum for two days. The second group was dosed with the standard inoculum and treated with 250 mg mL⁻¹ of *Ocimum gratissimum* ethanolic leaf extract. The third group was dosed with the extract alone while the fourth group was given normal saline and this serve as the control. The infected rat that was not given the extract showed a White Blood Count (WBC) of 4,800 mm³ before infection and increased to 13,800 mm³ during infection and later decreased to 2,400 mm³ after oral administration of the extract. The Packed Cell Volume (PCV) was 57% before infection, 47% during infection and 35% after treatment. The neutrophil and lymphocyte percentage in the differential count were 48 and 51% before infection, 62 and 37% during infection and 74 and 26% after treatment of infection respectively. For the rats treated with extract, it showed a White Blood Count (WBC) of 5,000 mm³ before infection, which decreased to 3,000 mm³ during infection and 1,700 mm³ after infections. It has a Packed Cell Volume (PCV), neutrophil and lymphocyte value of 55, 47 and 52% before infection, 50, 42 and 58% during infection and 33, 44, 56% after infection. The rats given the extract of *Ocimum gratissimum* alone showed a value of 4,400 mm³, 48, 41 and 58% for the WBC, PCV, neutrophil and lymphocyte before infection, a value of 3,200 mm³, 63, 43 and 57% during infection and a value of 2,100 mm³, 25, 42 and 56%, respectively after infection. The control showed only a significant increase in WBC with a value of 4,000 mm³ before infection, to 6,100 mm³ after infection and back to 4,400 mm³ after infection. The urinalysis showed a pH value of 5, negative for glucose, Ascorbic acid, Ketone, Nitrite, Protein, Bilirubin, normal for urobilinogen and negative blood value for all the groups before infection. The infected rat without administration of extract showed a pH of 7 and became positive for ketone, nitrite, protein and bilirubin urobilinogen and blood value of Ca. 250 during infection while others remain the same. After infection, the pH turned to 6, became negative for other parameters except protein and bilirubin while the treated rats remain negative. The ethanolic leaf extract of *Ocimum gratissimum* was found effective in inhibiting/preventing the disease condition after infection and capable of reducing excessive breakdown of red blood cells and neutralizing toxin produced by the organism.

Key words: Immunostimulatory activity, haematologic indices, *Ocimum gratissimum*, ethanolic extract

Introduction

Plants are invaluable sources of new drugs. There is an ever-growing interest in investigating different species of plants to identify their potential therapeutic applications. This increasing interest is due to a tremendous historical legacy in folk medicine use of plants as medicine (Rego, 1995) and their easy availability, cost-effectiveness and presumed safety. In the recent past, scientific studies on plants used in ethanol medicine have led to the discovery of many valuable drugs such as pilocarpine and vincristine among others.

Investigations into the chemical and biological activities of plants during the past two centuries have yielded compounds for the development of modern drugs. Despite little information on the composition and biological activity of many plants substances, there has been little effort devoted to the development of chemotherapeutic and prophylactic agents from these plants.

Vertebrates (including humans) are continually exposed to microorganisms and their metabolic products that can cause disease. The immune system is composed of widely distributed cells, tissues and organs that recognize foreign substances and microorganisms and act to neutralize or destroy them.

Ocimum gratissimum belongs to the family lamiaceae. It is traditionally called 'Efirin'. It has been reported to have medicinal properties. The leaf extracts are popularly used for the treatment of diarrhea while the cold-leaf infusions are used for the relief of stomach upset and haemorrhoids (Akinyemi *et al.*, 2005). The leaf is rich in thymol and has been reported to have antimicrobial properties.

The plant contains tannins, alkaloid and saponin. Also, it contains a trace amount of anthraquinone. Eugenol which is the component obtained as essential oil from *Ocimum gratissimum* is about 93.9% (Sartoratto *et al.*, 2004).

The aim of the present study is to investigate the Immunostimulatory activity of ethanolic leaf extracts of *Ocimum gratissimum* on the blood of Swiss albino rats with *E. coli* orogastrically dosed with *E. coli* to check for the effect on the internal organs and ability to modulate and stimulate the production of certain immunological parameters involved in body defense against infectious agents.

Materials and Methods

Plant Sample Collection, Preparation and Extraction

The *Ocimum gratissimum* plant was collected from the vicinity of Federal University of Technology, Akure. It was identified by Mr. Aduloju of Crop, Soil and Pest Management Department, Federal University of Technology, Akure Nigeria.

The leaves of *Ocimum gratissimum* were dried and then blended with blender into powdery form. About 500 g of the powdered leaves was weighed into 60% Ethanol (extraction solvent) until it was super-saturated. The mixture was sieved with muslin cloth after 72 h and concentrated in vacuo using rotary evaporator. The extract was purified using the method of Silva and Parente (2001). Typed pure isolate of *Escherichia coli* (NCIB 86) was obtained from Microbiology Department, Obafemi Awolowo University, Ile-Ife. The isolate was maintained in pure culture prior to use.

Laboratory Animals Used

Swiss albino rats were obtained from Pharmacy Department, Obafemi Awolowo University, Ile-Ife. The rats have average body weight between 100 and 220 g. They were given water and feed *ad libitum*.

Standard Inoculum Preparation

The organism was transferred from slant to plate of nutrient agar and a pure colony was picked before inoculating into a nutrient broth. The broth was incubated at 37°C for 24 h.

Serial dilution was made from the stock solution of broth and diluted serially up to 10^{-5} . One milliliter from 10^{-5} test tube was dispensed into Petri-dish and already prepared molten agar was poured on it. It was allowed to set before incubating at 37°C for 24 h. After incubation, plate count was done.

Evaluation of the Effects of Ethanolic Extract of Ocimum gratissimum on Some Immunologic Indices

Seventy two albino rats were used to assess the effect of the plant extract on the immune system. The rats were divided into four groups. The first group was given normal saline (placebo). Two groups were given the standard inoculum. Out of the two groups, one was given the standard inoculum and booster shot of the standard inoculum after three days. Also, one of the groups was treated with 250 mg mL^{-1} of the ethanolic extract of *Ocimum gratissimum*. One group was given the extract only.

On infection and after infection, the weight, haematologic test and urinalysis were carried to assess the progression of the infection and damage done to the internal organs.

Blood samples were obtained from each of the animals, White Blood Count (WBC) total and differential, Packed Cell Volume (PCV), haemoglobin levels (Hb concentration) determined. White blood count was estimated using the haemocytometer method. Packed Cell Volume was measured by the microhaematocrit technique using a Hawksley microhaematocrit centrifuge and spinning for 5 min at 12,000 rev/sec before reading with the haematocrit reader. Haemoglobin levels were measured colorimetrically by the oxyhaemoglobins methods using Reichert's haemoglobinometer (Ogwumike, 2002). The differential was done by using Leishman's stain before viewing under the microscope.

Urinalysis

For the urinalysis, test strip for the rapid determination of blood, urobilinogen, bilirubin, protein, nitrite, ketones, ascorbic acid, glucose and pH value in urine produced by Macherey-nagel was used.

The urine microscopy was carried out by collecting the urine into a centrifuge tube and centrifuging for 5 min at 12,000 rev/sec. After this, the supernatant is decanted and the sediment is placed on slide before covering with cover slip and view under the X40 objective.

Results and Discussion

Table 1 showed that White Blood Count (WBC), Packed Cell Volume (PCV), Haemoglobin concentration (Hb) and the differential count of the white blood cells in percentage of the animals infected with *E. coli*, the ones treated and the those given extract alone was shown before infection during infection and after dosing with extract.

From the results obtained, it was evident that *Ocimum gratissimum* is highly effective in treating *Escherichia coli* infection. This can be seen in the haematologic tests for the untreated infected rats The white blood count increased from 5,400 to 13,800 mm^3 showing 60.8% increase in the white blood count while the treated rats showed a decrease in the white blood count showing the capability of normalizing or decreasing the level of infection and suppressing the effect of the *Escherichia coli* infection (Ashida and Okimasu, 2005). The increase in total WBC may be due to production of T-lymphocytes at elevated level.

The rats treated with extract also showed a decrease in packed cell volume and haemoglobin value This suggest a possible haemolytic activity of the extract. From the value obtained at infection while in comparison with the untreated rats, it showed an increase in the packed cell volume and the haemoglobin concentration showing infection has been successfully reduced.

There is also higher value of neutrophil and lower value of lymphocytes in the untreated compared with the treated rats because neutrophil are usually higher during active infection while the

Table 1: Effect of ethanolic extract of *Ocimum gratissimum* on haematologic indices of albino rat orally dosed with *Escherichia coli*

Group	WBC (mm ³)	PCV (%)	Hb (g dL ⁻¹)	Differential			
				N	L	E	M
Before infection							
A	4,800	57	19.0	48	51	1	-
B	5,000	55	18.3	47	52	1	-
C	4,400	48	16.0	41	58	1	-
D	4,000	25	8.3	48	51	1	-
During infection							
Group	WBC (mm ³)	PCV (%)	Hb (g dL ⁻¹)	Differential			
				N	L	E	M
A	13,800	47	15.7	62	37	1	-
B	3,000	50	16.7	42	58	-	-
C	3,200	63	22.0	43	57	-	-
D	6,100	22	7.3	48	52	-	-
After infection							
Group	WBC (mm ³)	PCV (%)	Hb (g dL ⁻¹)	Differential			
				N	L	E	M
A	2,400	35	11.7	74	26	-	-
B	1,700	33	11.0	44	56	-	-
C	2,100	25	8.3	42	56	-	2
D	4,400	30	10.0	47	51	1	-

A: Rats infected with *Escherichia coli* (1×10^7 cfu mL⁻¹)

B: Rats infected with *Escherichia coli* and treated with ethanolic extract of *Ocimum gratissimum*

C: Rats given ethanolic extract of *Ocimum gratissimum* alone

D: Control

WBC-White Blood Count N-Neutrophil L-Lymphocyte

PCV-Packed Cell Volume M-Monocyte E-Eosinophil Hb-Haemoglobin

lymphocytes are lower. This shows that the extract has been able to reduce or suppress the infection, therefore making a lower neutrophil circulating in the blood in the case of the treated rat. This is similar to the report of Sheng *et al.* (2000), using the extract *Uncaria tomentosa*.

In the rats given the extract alone, it showed that is able to normalize the immune system making it suitable as nutrient supplement. In the traditional settings, where is it used as vegetable soup, it has the capacity to serve as immune boosters when it is taken continuously. This support the work of Ezekwesili *et al.* (2005).

From the urine macroscopy, the positive result of ketone and nitrite in urine may be indicative of urinary tract infection or bacterial infection of *Escherichia coli* because it was absent in *Ocimum gratissimum* treated rats (Table 2).

Positive protein and bilirubin value in untreated rats may be indicative of excessive breakdown of red blood cells while in the treated rats, it shows a negative result. This is in agreement with report of a similar investigation by Ogwumike (2002).

Haemoglobin in urine (haemoglobinuria) indicates breakdown of red blood cells or poisoning. The infected-untreated rats showed a high level of haemoglobin in urine while the treated does not. The infection causes breakdown of red blood cells and destruction of the blood cells by the enterotoxin produced by the *E. coli*. The extract could be seen to reduce it below detectable or harmful level (Bang *et al.*, 2003).

From the urine microscopy, the untreated rats showed a high level of pus cells, casts, crystals and bacterial cells compared with the treated rats, rats given extract only and the control (Table 3).

Presence of the pus cells, cast crystals, bacteria cells indicate active infection which was reduced by the extract (Taylor *et al.*, 1992).

Table 2: Effect of ethanolic extract of *Ocimum gratissimum* on urine biochemical indices

Parameters	Before infection			During infection			After infection			
	A	B	C	A	B	C	A	B	C	D
pH	5	5	5	7	7	7	6	5	6	5
Glucose	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Ascorbic acid	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Ketone	-ve	-ve	-ve	++	-ve	-ve	-ve	-ve	-ve	-ve
Nitrite	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve
Protein	-ve	-ve	-ve	30	-ve	-ve	100	-ve	-ve	-ve
Bilirubin	-ve	-ve	-ve	++	-ve	-ve	+++	-ve	-ve	-ve
Urobilinogen	norm	norm	norm	2	norm	norm	norm	norm	norm	norm
Blood	-ve	-ve	-ve	Ca 250	Ca 5-10	-ve	Ca 5-10	-ve	-ve	-ve

A: Rats infected with *Escherichia coli*
 B: Rats given *E. coli* and treated with ethanolic extracted of *Ocimum gratissimum*
 C: Rats given ethanolic extracted of *Ocimum gratissimum*
 D: Control

Table 3: Effect of ethanolic extract of *Ocimum gratissimum* in urine microscopy of rat during and after infection

Group	Pus cell/HPF	Cast/HPF	Crystal/HPF	Bacterial cell/HPF
A	6-8	2-4	2-4	Numerous
B	2-3	0-1	0-1	2-4
C	0-1	0-1	0-1	0-1
D	0-1	0-1	0-1	0-1

A: Rats infected with *Escherichia coli*
 B: Infected rats treated with ethanolic extract of *Ocimum gratissimum*
 C: Rats given ethanolic extract of *Ocimum gratissimum* alone
 D: Control

Table 4: Body weights of the rats in relation to stages of infection

Group	Before infection Weight (g)	During infection Weight (g)	After infection Weight (g)	Treatment with extract
A	200	195	198	
B	200	205	210	
C	170	175	180	
D	170	180	198	

A: Rats infected with *Escherichia coli*
 B: Rats infected with *Escherichia coli* and treated with ethanolic extract of *Ocimum gratissimum*
 C: Rats given *Ocimum gratissimum* alone.
 D: Control

It could be seen that the extract of *Ocimum gratissimum* reduces / stop the infection of *E. coli* by modulating the immunological indices. It can also serve as immune boosters as seen in the rats given extract only. It can also be seen to reduce bacterial infection, prevent excessive breakdown of red blood cells and destruction or lysis of the blood cells by enterotoxin produced by the organism. This result also showed that low concentration or regular intake of the plant extract is able to cure *Escherichia coli* infection (Table 4).

The result further shows from the urinalysis (both macroscopy and microscopy) and from the dissection, that the organism did not induce damage to any of the internal organ nor the *Ocimum gratissimum* extract only.

Recommendation and Conclusions

This research project shows that *Ocimum gratissimum* is able to inhibit/cure *E. coli* infection in rats and it is also capable of modulating or normalizing the immune system during infection. It can also prevent excessive breakdown of red blood cells and destruction of the cells by enterotoxin produced by the organism.

Based on these facts, *Ocimum gratissimum* extracts have good antimicrobial activity especially against the enteric organisms and can boost the immune system.

The result justified traditional usage of *Ocimum gratissimum* against enteric organisms and haemorrhoids. Further tests i.e., further *in vivo* studies are necessary and should be carried out to determine toxicity of the active constituents, their side effect and diffusion in different body sites. The antimicrobial activities could be enhanced if the active components are purified and adequate dosage determined for proper administration.

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