

Effect of Aqueous and Ethanolic Extracts of *Ocimum gratissimum* on the Histology of the Spleen, Haematological Indices of Wistar Rats and its Antimicrobial Properties

Abdullahi Shehu, U.E. Umana, J.A. Timbuak, Q.W. Hamman, S.A. Musa and D.T. Ikyembe
Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria

Abstract: The effects of the aqueous and ethanolic extracts of *Ocimum gratissimum* (Linn) on the haematological indices and histology of the spleen of adult Wistar rats, as well as its antimicrobial properties were investigated in this study. *Ocimum gratissimum* (Linn) is widely distributed in tropical and warm temperate regions and is commonly called “fever plant”. Twenty five adult wistar rats with weight range of 180-220 g were used. They were randomised into 5 groups. Group 1 was given normal saline 2 mL/kg Group 2 and 3; 500 mg/kg of aqueous and ethanolic extracts respectively while groups 4 and 5 1000 mg/kg of aqueous and ethanolic extracts respectively. Administration was orally for 14 days. Pure strains of *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi* were used for the antibacterial test with concentrations at 2 and 4% for each extract. The plates were then incubated at 37°C for 24 h. The study showed that the extracts of *Ocimum gratissimum* had no antimicrobial effect at these concentrations; however, there was increased white pulp in the spleen more at concentrations of 1000 mg/kg for both extracts. Also, at 1000 mg/kg there was a significant increase in the RBC and lymphocyte count and a decrease in neutrophil count at ($p < 0.05$).

Key words: Haematological indices, histology, spleen, wistar rats, antimicrobial studies, aqueous and ethanolic extracts and *ocimum gratissimum*

INTRODUCTION

Medicinal plants have contributed immensely to health care in Nigeria. This is due in part to the recognition of the value of traditional medical systems, particularly of Asian origin and the identification of medicinal plants from indigenous pharmacopoeias which have significant healing power. In the light of the recent emergence of bacteria which are resistant to multiple antimicrobial drugs, posing a challenge for the treatment of infections, the need to discover new antimicrobial substances for use in combating such microorganisms has become pertinent. *Ocimum gratissimum* L (Lamiaceae) is widely used in the treatment of diseases, for example upper respiratory tract infections, diarrhoea, headache, fever, ophthalmic, skin disease, wound healing and pneumonia (Correa, 1932; Onajobi, 1986; Ilori *et al.*, 1996; Osuagwu *et al.*, 2004). This is due to the numerous phytochemical constituents in the leaves. The volatile aromatic oil from the leaves consists mainly of thymol (32-65%) and Engenol (Pino *et al.*, 1996). It also contains Xanthones, Terpenes and Lactone (Ijaduola *et al.*, 1980). It is the thymol oil that is believed to be the most active constituent and it is regarded as highly antiseptic. In Northern Brazil, it is called “Alfavaca-cravo” (Rabelo *et al.*, 2003) while in India it is called “Vana tulsi” (Ralph and Sam, 2003). In Nigeria, the plant is called “effirinla” by the Yoruba speaking tribe, “Ahuji” by the Igbos,

while in the Northern part of Nigeria, the Hausas call it “Daidoya” (Effraim *et al.*, 2003).

Staphylococcus aureus, *Salmonella typhi* and *Escherichia coli* are common bacteria and all cause harmful infections in the human populace. While *Staphylococcus* are common cause of skin infections and food poisoning, *Salmonella typhi* causes typhoid fever and *Escherichia coli* is implicated in most gastroenteritis or urinary tract infections (Prescott *et al.*, 1996). The spleen being the largest lymphoid organ in the body is essentially concerned with immune responses, cytopoiesis and erythrocyte storage but it is not absolutely essential, since many of its functions can be assumed by the liver and by other lymphoid tissues if the spleen is removed (Williams and Dyson, 1989).

Although much has been documented on the antimicrobial properties of this plant, this study however is designed to evaluate its antimicrobial properties on some selected microorganism and haematological indices correlating it with histological changes in the spleen of wistar rats using different extracts of the plant.

MATERIALS AND METHODS

The plant was collected in Zaria city and authenticated at the herbarium of Department of Biological Sciences Ahmadu Bello University, Zaria, Nigeria. Fresh leaves of the plant were air dried under

shade at room temperature and then pounded into a mash. The extraction was done by maceration method.

Animal: Animals were kept in a controlled environment in the department of Anatomy, Ahmadu Bello University, Zaria, Nigeria of 12 h light/dark cycle, temperature 24±2°C. They were fed with pelletized mash and water was provided ad libitum. The animals were fasted overnight prior to administration of extracts which was done at the same time in the morning daily for a period of 14 days. The procedures were in accordance with the guidelines for the care and use of Ahmadu Bello University Zaria, Nigeria.

Procedure for extract administration: Dosage was determined in relation to the value of LD 50 given by Rabelo *et al.* (2003) as >2.4 g/kg and was put at 500 and 1000 mg/kg for a lower and higher dose of both extracts respectively. Twenty five adult wistar rats weighing between 180-220 g were randomly divided into 5 groups of 5 each. Group 1 received normal saline 2 mL/kg body weight; group 2 and 3 received 500 mg/kg body weight of aqueous and ethanolic extracts respectively while group 4 and 5 received 1000 mg/kg body weight of aqueous and ethanolic extracts, respectively. Administration was by oral intubation.

Antimicrobial assay: The organisms used were clinical isolates obtained from Microbiology laboratory of the Department of Microbiology, Ahmadu University, Zaria, Nigeria. Cultures of these bacteria was done using nutrient agar at 37°C and maintained on slant. Each of the test organisms was transferred into a separate test-tube containing nutrient broth to reactivate them by culturing overnight at 37°C. The different prepared extracts of the leaves of *Ocimum gratissimum* was tested for antimicrobial activity against the test organism using the agar diffusion method of Navarro *et al.* (1966).

The organisms were used to seed different nutrient agar plates; one organism per plate, two wells were made on each plates with a sterile cork borer of 6 mm diameter to contain the different extracts of *Ocimum gratissimum* at 2 and 4%. Each microorganism was cultured on two plates at concentrations 2 and 4%, for both aqueous and

ethanolic extracts making a total of six plates. Thereafter the plates were incubated at 37°C for 24 h. The plates were observed for zones of inhibition at the end of the incubation period.

Tissue processing: After 14 days of administration of the extract, the animals were sacrificed by anaesthesia followed by cardiac puncture; and blood was collected into EDTA bottles and used to determine the haematological parameters viz, Packed Cell Volume (PCV), Haemoglobin (Hb), White Blood Cell counts (WBC), Red Blood Cell counts (RBC), monocytes, neutrophils and lymphocytes using an automated dialyser machine (Cell-Dyn, Abbott, US). (Bishop and Fody, 2005). The spleen was excised and fixed in 10% formalin. The tissue was processes for staining using standard Haematoxylin and Eosin method.

Statistical analysis: Data are reported as Mean±Standard Error of Mean (SEM). One way Analysis of Variance (ANOVA) was to be statistically used to compare the means with p<0.05 was deemed statistically significant. Sigmastat 2.0 (Systat Inc, Point Richmond, CA) was used for the statistical analysis.

RESULTS

All the haematological parameters showed slight increase as the concentration of both extracts increases; the Red blood cell count, lymphocyte count and neutrophil count showed statistical significance difference with p values of 0.004, <0.001 and <0.001 respectively, (Table 1). The extract having the greatest impact on the haematological parameters was the highest dose of the ethanolic extract.

There was no pathological change to the histology of the spleen but a slight increase in the white pulp across the groups from the control up to group 1000 mg/kg group of both extracts was noticed. This was observed as increased purple colouration on the histological slide and the higher density of the cells. But groups 2 and 3 that had 500 mg/kg of both extracts had slight increase in white pulp, while for groups 4 and 5 that had 1000 mg/kg of both extracts showed a increase in white pulp especially in group 5.

Table 1: Showing the result of the haematological indices

	Mean±SEM normal saline group 1	Mean±SEM AE (500 mg) group 2	Mean±SEM EE (500 mg) group 3	Mean±SEM AE (1000 mg) group 4	Mean±SEM EE (1000 mg) group 5	F-value	p-value
RBC count (x10 ¹² /L)	4.78±0.15	4.03±0.88	4.65±0.09	5.13±0.06	4.83±0.24	6.45	0.004
PCV (%)	50.50±0.65	51.33±0.88	51.50±1.19	51.75±1.70	52.00±1.96	0.17	0.950
Hb conc. (g/dL)	16.83±0.22	17.11±0.29	17.17±0.40	17.25±0.57	17.34±0.65	0.17	0.950
WBC count (x10 ⁹)	6.88±0.22	7.17±0.22	8.05±0.46	8.20±0.47	8.45±0.79	1.86	0.173
Neutrophils (%)	31.00±1.47	29.33±2.33	30.75±0.75	16.75±1.65	15.00±1.08	31.08	<0.001
Lymphocytes (%)	63.00±1.41	63.33±1.20	63.25±1.18	77.50±1.44	78.75±1.25	38.98	<0.001
Monocytes (%)	6.00±0.41	5.67±0.33	6.00±0.58	6.00±0.58	6.25±0.50	0.15	0.959

p<0.05; AE: Aqueous Extract; EE: Ethanolic Extract

There was no zone of inhibition on any of the plates treated with the experiment plant.

DISCUSSION

There was increase in mean PCV, Haemoglobin concentration, WBC and monocytes count across the groups relative to the control. While for the RBC count, there was an initial decrease in groups 2 and 3, but the slight increase in group 4 and 5 might have been due to a decrease in plasma volume (Kumar *et al.*, 2005) as it has been shown by Effraim *et al.* (2003) that *Ocimum gratissimum* is diuretic. This was confirmed during the study and could have been the reason for the slight increase in RBC count.

There was significant decrease in neutrophil count in groups 4 and 5. In the majority of clinical conditions with neutropaenia diminished production significantly caused by drugs appear to be the most important mechanism (Kumar *et al.*, 2005). There was an increase in lymphocyte count across the groups especially in groups 4 and 5 which was statistically significant. Also according to Agomo *et al.* (1992) this increase is expected as reticulocyte numbers increased in normal mice administered with *Ocimum gratissimum*. Therefore, the increase seen in the lymphocyte count could have only been as a result of this plant.

The increase seen in the white pulp could be correlated from the haematological parameters which showed a significant increase in the lymphocyte count and this is what constitutes the bulk of the white pulp. Since lymphopoiesis in the white pulp contributes to lymphocytes in the circulation (Williams and Dyson, 1989), it is likely that the plant possibly caused increase in lymphocyte by causing lymphopoiesis in the white pulp of the spleen.

Ilori *et al.* (1996) was able to show a weak antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus* using the aqueous extract with a high minimum inhibitory and minimum bactericidal concentration which agrees with this study of why we could not see zones of inhibition at concentrations of 2 and 4% of both extracts. Finally, looking at the whole results it will be noted that the ethanolic extract had more effect at the same concentration and could be attributed to the following reasons; firstly, the nature of biological active components (Saponins, tannins, alkaloids and anthraquinone), which could be enhanced in the presence of ethanol (Tschesche, 1970). Secondly the stronger extraction capacity of ethanol could have produced greater number of active constituents (Kabir *et al.*, 2005). For the haematological parameters, the extracts caused increased RBC count by virtue of its diuretic and laxative properties, it also caused neutropaenia and lymphocytosis

which manifests as increase in the white pulp of the spleen, while it showed no antibacterial properties with the above mode of extraction and concentration.

REFERENCES

- Agomo, P.U., J.C. Idigo and B.M. Afolabi, 1992. "Antimalaria" medicinal plants and their impact on cell populations in various organs of mice. *Afr. J. Med. Sci.*, 21(2): 39-46.
- Bishop, M.L. and E.P. Fody, 2005. *Clinical Chemistry, Principles, Procedures, Correlations*. 5th Edn., Lippincourt, Williams and Williams, Philadelphia, pp: 230-484.
- Correa, M.P., 1932. *Dicionario das plantas uteis do Brasil*. IBDF, Rio de Janeiro.
- Effraim, K.D., T.W. Jacks and O.A. Sodipo, 2003. Histopathological studies on the toxicity of *ocimum gratissimum* leaf extract on some organs of rabbit. *Afr. J. Biomed. Res.*, 6(1): 21-25.
- Ijaduola, G., I. Anyiwo and C. Thomas, 1980. *Ocimum gratissimum* and blood coagulation. *J. Res. Ethinomed.*, 1: 19-21.
- Ilori, M., A.O. Sheteolu, E.A. Omonibgehin and A.A. Adeneye, 1996. Antidiarrhoeal activities of *Ocimum gratissimum* (Lamiaceae). *J. Diarrhoeal. Dis. Res.*, 14(4): 283-285.
- Kabir, O.A., O. Olukayode, E.O. Chidi, C.I. Christopher and A.F. Kehinde, 2005. Screening of crude extracts of six medicinal plants used in southwest Nigerian unorthodox medicine for anti-methicilin resistant *staphylococcus aureus* activity. *BMC Complement. Alternat. Med.*, 5: 6-11.
- Kumar, V., Abbas, A.K. and N. Fausto, 2005. *Robbins and Cotran Pathologic Basis of Disease*. 7th Edn., Elsevier, Philadelphia, pp: 619-665.
- Navarro, V., M.L. Villareal, G. Rojas and X. Lozoya, 1966. Antimicrobial evaluation of some plants used in Mexican traditional medicine for the treatment of infectious diseases. *J. Ethnopharmacol.*, 53: 143-147.
- Onajobi, F.D., 1986. Smooth muscle contracting lipid soluble principles in chromatographic fractions of *Ocimum gratissimum*. *J. Ethnopharmacol.*, 18: 3-11.
- Osugwu, F.C., O.W. Oladejo, I.O. Imosemi, B.A. Adewoyin, A. Aiku, O.E. Ekpo, O.O. Oluwadara, P.C. Ozegbe and E.E. Akang, 2004. Wound healing activities of methanolic extracts *Ocimum gratissimum* leaf in Wistar rats-a preliminary study. *Afr. J. Med. Med. Sci.*, 33(1): 23-26.
- Pino, J.A., A. Rosado and V. Fuestes, 1996. Composition of the essential oils from the leaves and flowers of *Ocimum gratissimum* L. grown in Cuba. *J. Essential. Oil Res.*, 8: 139-141.

- Prescott, L.M., J.P. Harley and D.A. Klein, 1996. Microbiology. 3rd Edn., McGraw-Hill companies Inc. Indiana U.S.A.
- Rabelo, M., E.P. Sonza, P.M.G. Soares, A.V. Miranda, F.J.A. Matos and D.N. Criddle, 2003. Antinociceptive properties of the essential oil *ocimum gratissimum* L. (Labiatae) in mice. *Braz. J. Med. Biol. Res.*, 36(4): 521-524.
- Ralph, M. and M. Sam, 2003. Tulsi, the queen of herbs. The Green Isle Enterprise. Salt spring Island, pp: 250-653.
- Sofowora, L.A., 1993. Medicinal plant and traditional Medicine in Africa, 3 Mn. Spectrum books Ltd, Ibadan. Pp: 55-71.
- Tschesche, R., 1970. Advances in the chemistry of antibiotics substances from higher plants: *Pharmacognosy and phyto-chemistry*. 1971:274-289.
- Williams, P.L. and M. Dyson, 1989. Gray's Anatomy. 37th Edn., Churchill livingstone, Norwich, PP: 827-832.