EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 3
Issue: 9
30-34
Year: 2016

# IMMUNOMODULATORY EFFECT OF METHANOL LEAF EXTRACT OF *LUPINUS ARBOREUS* IN RATS.

**Sylvester C. Ohadoma***

Department of Pharmacology, College of Medicine, Imo State University, PMB 2000, Owerri, Nigeria.

***Corresponding Author: Dr. Sylvester C. Ohadoma**
Department of Pharmacology, College of Medicine, Imo State University, Owerri, Nigeria.

**Article Received on 29/06/2016** **Article Revised on 20/07/2016** **Article Accepted on 11/08/2016**

## ABSTRACT
**Objective:** To investigate the immunomodulatory effect of methanol extract of *Lupinus arboreus* leaves in experimental rats. **Methods:** Four groups (I-IV) comprising five rats each were used. Group I animals were infected with *Clostrodium sporogenes* (NCIB 532) and Group II animals were infected with *Clostrodium sporogenes* and treated with 250 mg/kg of extract. Groups III and IV received extract (250 mg/kg) only and normal saline which served as control respectively. White Blood Cell count was done using Tork's solution and haematoxytometer. The pack cell volume analysis was carried out using haematocrit centrifuge before reading through the microhaematocrit reader. The differential count was done using a Leishman's stain and view under the microscope. The urinalysis was carried out using Combi 9 urine Test strip for macroscopic analysis and microscope for microscopic analysis. Histopathological evaluation was carried out using light microscopy. **Results:** The results showed an increase in total white blood cell count in the infected rats but the value reduced after treatment with the extract. Conversely, the pack cell volume reduced in the untreated rats. Neutrophil values reduced during infection but the values became normal after treatment. There was reduction in their weights during active infection in those that were dosed with the microorganisms due to infection while those that were dosed with the microorganism and treated with the extract as well as those given the extract only, showed an increase in their body weights respectively. **Conclusion:** The leaf extract of *Lupinus arboreus* possess immunomodulatory effect.

**KEYWORDS:** *Lupinus arboreus*, Chikadoma, immunomodulatory, leaf extract.

## 1. INTRODUCTION
Most people in developing countries are precluded from access to modern therapy, mainly for economic and cultural reasons. Fortunately, these countries are endowed with vast resources of aromatic and medicinal plants.

Plants are considered as a source for identification of bio-active agents that can be employed in preparation of synthetic drugs.[1] The utility of medicinal plants has reached where it is considered more for its capacity to generate other medicines than for its own sake. In developed world where the phytomedicine industry is thriving, extracts from medicinal plants are dispatched for public consumption in purified form or as supplements for the prevention and treatment of various kinds of diseases.[2] This tendency has gained acceptability making phytomedicine an inevitable global discourse, hence, encouraging research into pharmacologic activities of secondary metabolites of plants.[3,2] The English name of *Lupinus arboreus* which is planted widely as ornamental plant, is Yellow bush.[4] In Igbo, South-eastern Nigeria, *L. arboreus* is referred to as "Chikadoma"[5], named after a lead researcher Dr. Chika Ohadoma, who pioneered extensive work on the novelty study of this plant.[6] It is recognised easily as a bushy shrub up to 1.8 m tall with bright yellow, sweet-smelling flowers blended with purple white colours.[7] In Northern California coastal dunes, *L. arboreus* occurs as an invasive species.[4,7] In folkloric medicine, the macerated boiled leaves of *L. arboreus* are applied for toothache and chest pain to reduce swelling and pain. The ancients employed lupines without scientifically proven documentation against scabies, ulcers, deformities of the skin and other cutaneous distemper.[8] The leaf extract and fractions of *L. arboreus* exert antinociceptive and anti-inflammatory effects[5], antimicrobial effect[9]; and have a plethora of phytochemicals[10] including flavonoids, terpenoids, glycosides, alkaloids, steroids and tannins. This present study investigated the immunomodulatory effect of *L. arboreus* leaves in rats.

## 2. MATERIALS AND METHODS

### 2.1. Plant material

Leaves of *L. arboreus* were collected from Owerri, Imo State, Nigeria; and authenticated at the Department of Pharmacognosy, Madonna University, Elele, Nigeria, where a voucher specimen (No. M/PC/193/10) has been deposited in the Herbarium. The leaves were air-dried at room temperature for 28 days and pulverized into fine powder. The powdered leaves (2 kg) were extracted with absolute methanol (Sigma Aldrich, Germany) by cold maceration for 48 h. The mixture was filtered to obtain the crude methanol extract (CME) which was concentrated using a rotary evaporator (RV 05 Basic IB, IKA, Staufen, Germany) and further oven dried and stored in a refrigerator (4 $^{o}$C).

### 2.2. Test micro-organism

Standard micro-organism used was *Clostrodium sporogenes* (NCIB 532) obtained from Medical Laboratory Unit of Madonna University Teaching Hospital, Elele, Nigeria.

### 2.3. Animals

Twenty (20) adult Wister rats (160-250 g) of both sexes kept in the Laboratory Animal Facility of Department of Pharmacology and Toxicology, Madonna University, Elele, Nigeria, were used in this study. Prior to experimental uses, the rats were transferred to work area and allowed for two weeks of acclimatization. However, the rats were maintained under standard laboratory conditions and had free access to standard pellets (Vital Feeds Plc, Nigeria) and clean water.

### 2.4. Experimental design

The animals were randomly selected into four groups (I-IV) comprising five rats each.
Group I: rats were infected with *C. sporogenes*
Group II: rats were infected with *C. sporogenes* and treated with extract (250 mg/kg).
Group III: rats were administered with extract (250 mg/kg) only.
Group IV: rats were given normal saline and served as control.

All administration was done orogastrically; and before, during and after infection, the body weight, haematological test and urinalysis were carried out. Histopathological test was lastly carried out to check for damage of internal organs.

### 2.5. Haematological test

Blood samples were collected from the tails of rats and used for white blood cell count (WBC), packed cell volume (PVC) and differential counts of white blood cells. WBC count was done using Tork's solution and haematocytometer. The PCV was carried out using haematocrit centrifuge before reading through a microhaematocrit reader, while the differential count was carried out using a Leishman's stain and viewed under the microscope.

### 2.6. Urinalysis

The urine macroscopy was carried out using a Combi 9 urine Test strip, which measured the value of pH, glucose, ketone, ascorbic acid, nitrate, protein, bilirubin, urobilinogen and blood in urine. The urine microscopy was carried out by collecting the urine into a centrifuge tube and spinned at 12,000 revolution per minute for 5 minutes. The supernatant was decanted and the sediment was dropped on the microscopic slide and covered with cover slide, which was viewed under the microscope.[11]]]

### 2.7. Histopathological evaluation

The formalin-fixed liver and kidney tissues were processed, paraffin wax embedded and microtome sections (5-6 $\mu$m) made from them. These sections were stained with Haematoxylin and Eosin for light microscopy.

## 3. RESULTS

The outcome of the total and differential WBC count and PCV on the infected animals, the treated ones and those given only extract before infection, during infection and after infection are shown in Table I: There was rise in total WBC during the active infection stage and decreased in its values after treatment with extract suggesting an in-vivo antimicrobial activity against the pathogen. There was reduction of PVC in rats infected with *C. sporogenes.* PCV values became elevated after the infection was treated with the extract. The number of neutrophil reduced significantly during infection. The pH of the urine macroscopy remained neutral in the infected rats but the rats given extract only had an acidic pH (Table II). The number of protein at termination in infected rats was 100 while the protein of infected rats treated with extract was negative during active infection due to infection and immunomodulatory effects of the extract. In urine microscopy, the infected rats showed 6-8 HPE of pus cells due to active infection (Table III). The infected rats treated with extract showed 2-4 HPE due to suppression of infection by the extract. The infected rats showed 2-4 HPE of cast in urine when compared to 0-1 HPE in infected rats treated with the extract. The infected rats showed 2-4 HPF of crystals when compared to none HPF of crystal seen in the urine of infected rats treated with the extract. The infected rats showed an average body weight of 220 g before infection and 210 g after infection (Table IV). The treated rats showed an average body weight of 145 g and 160 g after infection. The Group given extract only showed body weight of 172 g at the onset of the infection, 175 g during infection and 180 g after infection; while the control group showed increased body weight for 170, 190 and 199 g before, during and at termination (after) respectively. The histopathological assessment of the vital organs showed no structural changes.

**Table I: Haematological effect of leaf extract of *L. arboreus* in rats**

| | Before infection | | | | | During infection | | | | | After infection | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Grou | WBC (mm³) | PCV (%) | E (%) | N (%) | L (%) | WBC (mm³) | PCV (%) | E (%) | N (%) | L (%) | WBC (mm³) | PCV (%) | E (%) | N (%) | L (%) | M (%) |
| I | 2,520 | 51 | ---- | 58 | 49 | 8,800 | 24 | 2 | 48 | 50 | 2,800 | 23 | ---- | 40 | 60 | ---- |
| II | 2,540 | 54 | ---- | 43 | 57 | 2,000 | 30 | 1 | 40 | 51 | 3,100 | 34 | ---- | 68 | 32 | ---- |
| III | 2,740 | 55 | ---- | 43 | 57 | 2,400 | 33 | ---- | 41 | 60 | 26,000 | 35 | ---- | 60 | 36 | 4 |
| IV | 4,000 | 57 | 1 | 48 | 51 | 5,000 | 22 | ---- | 48 | 52 | 4,400 | 30 | 1 | 59 | 40 | ---- |

Groups I: Rats infected with *Colstridium sporogenes*
II: Rats infected with *Clostridium sporogenes* given extract
III: Rats given extract only
IV: Rats given normal saline only, serving as control: --- means no value

**Table II: Urine macroscopic effect of extract of *L. arboreus* in rats**

| | Groups | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Before Treatment | | | During Treatment | | | After Treatment | | | Control |
| Parameters | I | II | III | I | II | III | I | II | III | IV |
| pH | 6 | 6 | 6 | 7 | 7 | 6 | 7 | 6 | 6 | 6 |
| 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 | -ve |
| Nitrite | -ve | -ve | -ve | +ve | -ve | -ve | -ve | -ve | -ve | -ve |
| Protein | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve |
| Bilirubin | -ve | -ve | -ve | ++ | -ve | -ve | ++ | -ve | -ve | -ve |
| Urobilinogen | Norm | Norm | Norm | Norm | Norm | Norm | Norm | Norm | Norm | Norm |
| Blood | -ve | -ve | -ve | Ca 250 | Ca 10 | Ca 50 | Ca 10 | -ve | -ve | -ve |

Groups I: Rats infected with *Colstridium sporogenes*
II: Rats infected with *Clostridium sporogenes* given extract
III: Rats given extract only
IV: Rats given normal saline only, serving as control: -ve = negative, +ve = positive

**Table III: Urine microscopic effect of leaf extract of *L. arboreus* in rats**

| Group | Pus cell / HPF | Cast / HPF | Crystal / HPF | Bacterial cell / HPF |
|---|---|---|---|---|
| I | 6 - 8 | 2 - 4 | 2 -4 | 4 - 6 |
| II | 2 - 8 | 0 - 1 | 0 - 1 | 2 - 4 |
| III | 0 - 1 | 0 - 1 | 0 - 1 | 0 - 1 |
| IV | 0 - 1 | 0 - 1 | 0 - 1 | 0 - 1 |

Groups I: Rats infected with *Colstridium sporogenes*
II: Rats infected with *Clostridium sporogenes* given extract
III: Rats given extract only
IV: Rats given normal saline only, serving as control:

**Table IV: Average body weights during the course of the experiment**

| Group | Weight before treatment (g) | Weight during treatment (g) | Weight after treatment (g) |
|---|---|---|---|
| I | 220 | 200 | 210 |
| II | 165 | 145 | 160 |
| III | 172 | 175 | 180 |
| IV | 170 | 190 | 199 |

Groups I: Rats infected with *Colstridium sporogenes*
II: Rats infected with *Clostridium sporogenes* given extract
III: Rats given extract only
IV: Rats given normal saline only, serving as control:

## 4. DISCUSSION

From the result of this study, the immunomodulatory effect of *L. arboreus* leaves could be seen from many indices. The increase of total WBC during infection may have arisen from proliferation of lymphocytes required for phagocytosis of the foreign agents.[12] PCV reduced in infected rats suggested possible haemolytic activity. Report has it that anemia as a result of haemolysis has been associated with most microbial infections.[13] The elevation of PCV values after the infection was treated with the extract might be that the extract had successfully inhibited the growth of microbes involved in the haemolytic process thereby enhancing the oxyhaemoglobin values of the red blood cells. The significant reduction of neutrophil during infection may be as a result of migration of neutrophil into tissues to destroy the invading pathogen.[14] The effect of the extract on the urine macroscopy supported documented reports that in urinary infection, proteins and nitrites are often found in the urine.[15] Urinary pathogens such as *E. coli*, *Proteus* and *Klebsiella species* are able to reduce the nitrate normally present in urine to nitrite.[12] Proteinuria is found in most bacteria urinary tract infection.[16] The infected rats showed 6-8 HPF, treated with extract showed 2-4 HPE. This was due to suppression of infection by the extract.[17] The infected rats showed 2-4 HPF of crystals in urine when compared to none HPF of crystal seen in the urine of infected rats treated with the extract. This is in agreement because crystals were not expected in urine of normal subject; and the presence of crystals may signify renal abnormalities.[16] Due to no infection in the control rats as well as immunomodulatory effects in those given extract only, the gain in body weight may not be unconnected with the higher value of the PCV permitting great amount of nutrient to be circulated into the body system. The histopathological assessment of the leaf extracts of *L. arboreus* showd no deleterious effect on the vital organs. The capability of the extract to preserve vital organs histoarchitecture could be due to its ability to stabilize cell membranes through free radical scavenging activities in consonance with many plants in Fabaceae family.[18] The antioxidant properties of *L. arboreus* which belongs to the family of Fabacaea has been reported.[5]

## CONCLUSION

The leaf extract of *L. arboreus* showed immunomodulatory effect as seen in the humoral and cell-mediated response of the treated experimental rats.

## CONFLICT OF INTEREST

No conflict of interest declared.

**SOURCE OF SUPPORT:** NIL.

## ACKNOWLEDGEMENT

The author is grateful to Dr. I.N. Nnatuanya of the Department of Medical Laboratory Sciences, Madonna University, Elele, Nigeria, for his technical assistance.

## REFERENCES

1. Wink M. Biochemistry of Secondary product metabolism. 2nd ed, Boza Raton: CRC Press., 1999; 5-16.
2. Ohadoma SC. Comperative analysis of hepatoprotective effect of ethanol extracts of Gongronema latifolium and Alternanthera dentate leaves in rats. World Journal of Pharmacy and Pharmaceutical Sciences 2016; 5(1): 156-163.
3. Sofowara EA. Medicinal plants and traditional medicines in Africa. Nigeria: John Wiley and Sons Ltd., 1982; 64-79.
4. Wear KS. Hybridization between native and introduced lupines in Humboldt county. Thesis, Humboldt State University Arcata, 1998; 8: 20-22.
5. Ohadoma SC. Akah PA, Enye JC, Pharmacological assessment of the leaf extract and fractions of Lupinus arboreus for antinociceptive and anti-inflammatory effects. Asian Journal of Science and Technology., 2015; 6(8): 1663-1668.
6. Ohadoma SC. Stigmast steroids of the leaf extract of Lupinus arboreus Sims. Literati Journal of

Pharmaceutical Drug Delivery Technologies., 2016; 2(1): 01-02.
7. Pickart AJ, Miller LD. Yellow bush lupine invasion in Northern California coastal dunes. Ecological impacts and manual restoration techniques. Restoration Ecology., 1998; 6: 59-68.
8. Pliny IO. The family of lupines. A collection of Miller Institute for Basic Research, Miller Publishers, USA, 2009.
9. Ohadoma SC, Nnatuanya IN, Amazu LU, Okolo CE. Antimicrobial activity of the leaf extract and fractions of Lupinus arboreus. Journal of Medicinal Plants Research. 2014; 8(8): 386-391.
10. Ohadoma SC, Akah PA, Nkemnele CA, Ikeduba EN, Nwokoma EI. Determination of the acute toxicity and phytochemical constituents of the methanol leaf extract of Lupinus arboreus. Journal of Scinece, Engineering and Technology., 2010; 17(3): 9738-9743.
11. Ogwunike OO. Haemotopoietic effect of aqueous extract of the leaf sheath of Sorghum bicolor in albino rats. African Journal of Biomedical Research 2002; 5(1): 69-71.
12. Jawetz E, Melnick J, Adelberg EA. Medical microbiology, 3rd ed. New York: McGraw-Hill Company 2004; 764.
13. Tortora GI, Funke BR, Case CL. Microbiology: An introduction 8th ed., New Zealand: Pearson Education Inc. 2002; 777.
14. Oladunmoye MK. Immunostimulatory activity of ethanolic extract from Ocimum gratissimum in albino rats orogastrically dosed with Esherichia coli (NCIB 86). Journal of Pharmacology and Toxicology 2006; 1(4): 389-394.
15. Brock TD, Madigan TM. Biology of microorganisms. 9th ed., London: Prentice-Hall Int. 2000; 864
16. Monica C. District laboratory practice in tropical countries. Part 2 Low Price ed. UK: Cambridge University Press. 2000; 108-326.
17. Prescott LM, Harley JP, Klein DA. Microbiology. 4th ed. N.Y: McGraw-Hill Inc. 2000; 978.
18. Okwuosa CN, Achukwu PU, Azubike NC. Biochemical and toxicological studies on the crude leaf extract of Abrus precatorius in rats. Journal of Applied Sciences. 2010; 13(2): 9165-9175.