African Journal of
Sciences and Traditional Medicine

ISSN : 0000-0000

Index: Harvard, Boston, Sydney University, Dimensions, Lens, ResearchGet Scilit, Semantic, Google Scholar, Base etc

https://doi.org/10.58578/AJSTM.v1i1.3492

Haematological Studies of the Effects of *Nauclea Latifolia* Ethanolic Root Extracts in Rats

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Article Info:

Submitted:	Revised:	Accepted:	Published:
Jul 1, 2024	Jul 23, 2024	Jul 26, 2024	Jul 29, 2024

Abstract

Nauclea latifolia (Smith) (Family: *Rubiaceae*) also known as 'Pin Cushion tree' or 'African Peach' is a struggling shrub, native in tropical Africa and Asia where the use of folk medicine is prevalent and the search for herbal cure is but common practise. Extraction of the root of *Nauclea latifolia* with ethanol yielded the ethanol extract. The effects of the ethanolic extract of *N. latifolia* on haematological parameters showed a significant elevation ($p < 0.05$) in packed cell volume (PCV), white blood cell count, haemoglobin concentration, red blood cell count and the white blood cell differentials compared to the control group which could be protective mechanisms against infections.

Keywords: Haematology, *Nauclea latifolia,* Haemoglobin, Ethanolic Extract and Toxicity, White Blood Cells, Packed Cell Volume (PCV)

INTRODUCTION

Historically, plants have provided a good source of antiinfective agents. Phytomedicines derived from plants, have shown great promise in the treatment of intractable, infectious diseases, including opportunistic infections with HIV/AIDS – Syphilis, Gonorrhoea and other STDs (Iwu *et al,* 1999; Tian *et al*., 2015; Tchuifon *et al*., 2017). It is believed that nearly 80% of the world population relies primarily on herbal remedies for the treatment of human and animal diseases (Yakubu *et al.,* 2017). Plants containing protoberberines and related alkaloids, picralima-type indole alkaloids and garcinia biflavonones, used in traditional African system of medicine, have been found to be active against a wide variety of microorganisms (Tian *et al*., 2014). More recently, the search for specific plant components that convey health benefits has widened to encompass the vast range of 'non-nutritive' compounds present in plant foods, and their potential to improve health. Evidence is growing that such plant constituents, belonging to the group termed **"**Bioactive compounds**"**, may help to promote optimal health and to reduce the risk of chronic diseases such as cancer, coronary heart disease, stroke and perhaps Alzheimer's disease (Varsha *et al*., 2013)

The plant *Nauclea latifolia* (Family: Rubiaceae) under study, commonly known as 'Pin Cushion tree' or 'African Peach', is a struggling shrub or small tree native to tropical Africa and Asia (Giddado *et al,* 2004) where the use of folk medicine is prevalent and the search for herbal cure is but a common practice. It is a common plant that grows in most parts of Nigeria (Akpanbiatu *et al*, 2005), in the forest and fringe tropical forests (Udobi and Umoh, 2017). Usages as antimalarial, antipyretic and aphrodisiac, for wound-healing activities and as a vermifuge, have all been reported (Udobre *et al*., 2013) as well as for diabetes mellitus, gastroenteritis disorders, sleeping sickness (Giddado *et al*, 2004), hypertension and as a laxative (Akpanbiatu *et al*, 2005). It is also used in combination for the treatment of sexually transmitted diseases (STDs) (Okoli and Iroegbu, 2004), as a tonic, fever medicine, chewing stick, for treating toothaches, dental caries, septic mouth, malaria, diarrhea, dysentery, etc (Owolabi *et al*., 2010). By local nomenclature, *Nauclea latifolia* is called '*Uvuru*' or '*Oto*' in some parts of Enugu state; '*Mbom-mbog*' in Akwaibom and Cross River states and '*Tabasiya*' in Northern Nigeria. However, scanty literature is available on the use of this plant (Akpanbiatu *et al,* 2005).

METHODS

Animal material

The experimental animals used for the study were male and female Wister albino rats (weights 103-230g). The rats were obtained from the animal house of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka and were kept for seven days to acclimatize and given feed and water *ad libitum*.

Plant material

Nauclea latifolia roots were obtained from a forest in Okpome-Agbada Nenwe, Aninri Local Government Area of Enugu State, Nigeria. The roots were authenticated by Mr. Njokuocha in the Department of Botany, University of Nigeria, Nsukka. The roots were dried at room temperature in the absence of sunlight for one week, cut into bits and were ground to coarse powder using a hammer mill.

Extraction procedure

Preparation of ethanolic extract

About 500g of the powdered root of *Nauclea latifolia* was soaked in 3.4 liters of 70% ethanol. The mixture was left to stand for twenty-four hours with occasional stirring. The mixture was later extracted using a soxhelet extractor to obtain the ethanol extract. The extract was concentrated over a water bath at a temperature of 25 to 30°C to obtain 46g (9.2%) of the ethanol extract.

Determination of concentration of extract

A crucible was weighed and a known weight of the extract was poured into the crucible and their combined weight determined before heating. The crucible was heated until all its content was charred. After heating, the crucible was re-weighed with its content and the weight recorded. The concentration of the solid extract was then determined from the two weights.

Experimental design

A total of sixty Wister albino rats were used in this study. The rats were randomly divided into four (4) groups of 15 rats each.

Group 1: the control group was administered normal saline

Group 2: was administered 10 mg/kg of the ethanolic extract

Group 3: was administered 50 mg/kg of the ethanolic extract

Group 4: was administered 250 mg/kg of the ethanolic extra

The extracts were administered by dissolving accurately measured doses in water and feeding the rats orally on a daily basis for 42 days. At the end, the rats were sacrificed and some internal organs removed, fixed and later used for histopathological studies.

Haematological Assay Methods

Determination of Packed Cell Volume (PCV)

Packed cell volume (PCV) was determined by centrifuging a sample of well-mixed anticoagulated blood, contained in a parallel-sided glass tube for a period of time and then measuring the height of the red cell column and expressing this as a ratio of the height of the total blood column.

$$\text{PCV (\%)} = \frac{\text{Height of Red Cell column}}{\text{Height of Total Blood column}} \times 100$$

Determination of Red Blood Cells (RBCs)

Using the Thoma method, whole blood was diluted with an isotonic diluent which lysed the WBCs, leaving the red cells to be counted microscopically using the 10 x objective.

Calculation

RBCs = Number of cells counted in five squares x 10,000

Determination of Hemoglobin (Hb) concentration

Well mixed sample of EDTA blood (0.02ml) was pipetted into 5ml of cyanmethaemoglobin reagent and mixed. The mixture was allowed to stand for 3 minutes and read against the blank at 540 nm using a spectrophotometer.

Calculation

$$\text{Hb in g/dL} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Concentration of standard (in mg/dL)}$$

Determination of White Blood Cells (WBCs)

Approximately 0.02ml of well mixed EDTA anticoagulated venous blood was added to 0.38ml of diluted fluid dispensed into a small container. One of the grids of the counting chamber was filled with re-mix of the diluted blood sample using a Pasteur pipette, taking care not to overfill the area. The filled chamber was left undisturbed for two minutes to allow time for the white cells to settle, after which the underside of the chamber was dried and placed on the microscope stage. Using the 10x objective with the condenser iris closed sufficiently to give good contrast, the ruling of the chamber and white cells were focused until the cells appeared as small black dots. The cells in the four large squares of the chamber were then counted.

Calculation

The number of white cells per liter of blood was calculated as follows:

- The total number of cells counted was divided by 2
- The number obtained was divided by 10
- The result was then multiplied by 10^9 to give the white cell count.

Determination of differential white cell count

A drop of immersion oil was placed on the lower third of the blood film and covered with a clean cover glass. The film was microscopically examined by focusing the cells using the 10x objective with the condenser iris closed sufficiently to see the cells clearly. The staining and distribution of cells were then checked. A part of the film where the red cells just began to overlap was moved and the 40x objective brought into place. The iris diaphragm was then opened more. The blood film was systematically examined and the different cells seen in each field were counted and recorded in a chart form. Ten cells were recorded in each time. When 10 cells were recorded in line, a total of 100 white cells were then counted. The absolute number of each white cell type was calculated by multiplying the number of each cell counted by the total WBC count.

RESULTS

Effect of NLE on Haematological Parameters

Effect of NLE on Packed Cell Volume

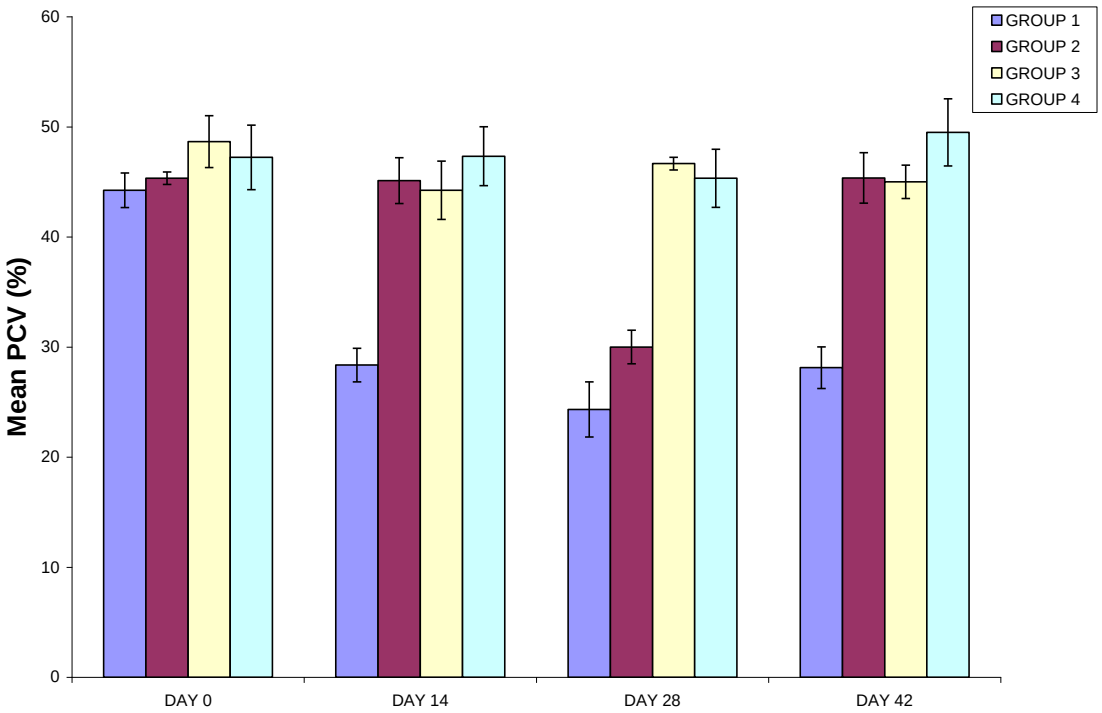

Figure 9: Effect of extract on Packed cell volume

There was no significant difference ($p > 0.05$) in the packed cell volume on day 0 compared to the control. But as administration continued, there was a significant decrease ($p < 0.05$) in the packed cell volume of groups 1 and 2 on day 28, compared to other groups.

Effect of NLE on Haemoglobin concentration

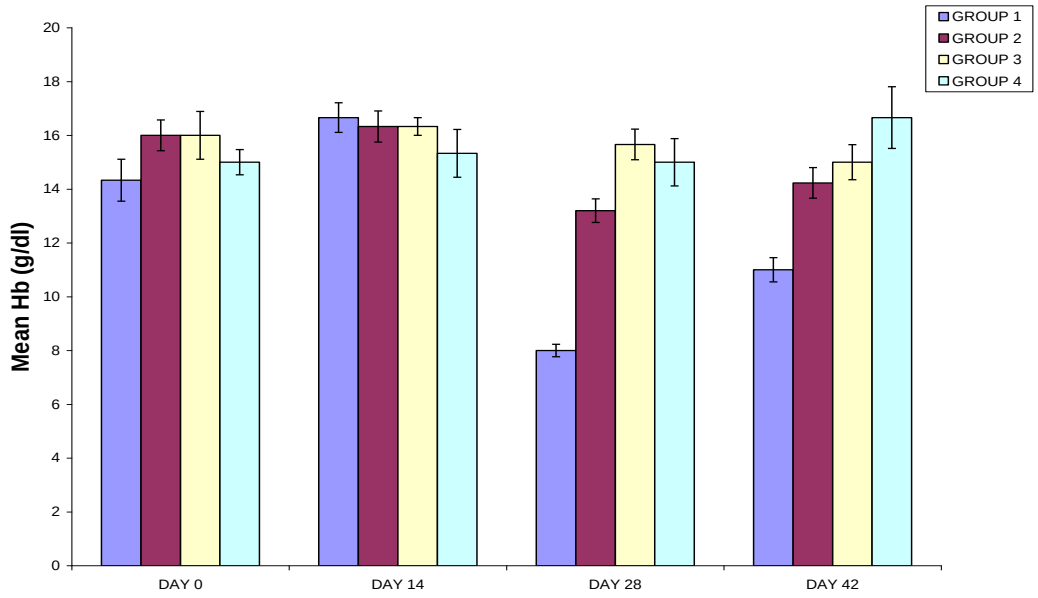

Figure 10: Effect of extract on Haemoglobin concentration

The result indicates that there was no significant difference ($p > 0.05$) in the Hb concentration on days 0 and 14 compared to the control. But as administration continued on days 28 and 42, there were significant ($p < 0.05$) increases of Hb concentration in groups 2, 3 and 4 compared to the control.

Effect of NLE on Red Blood Cell Count

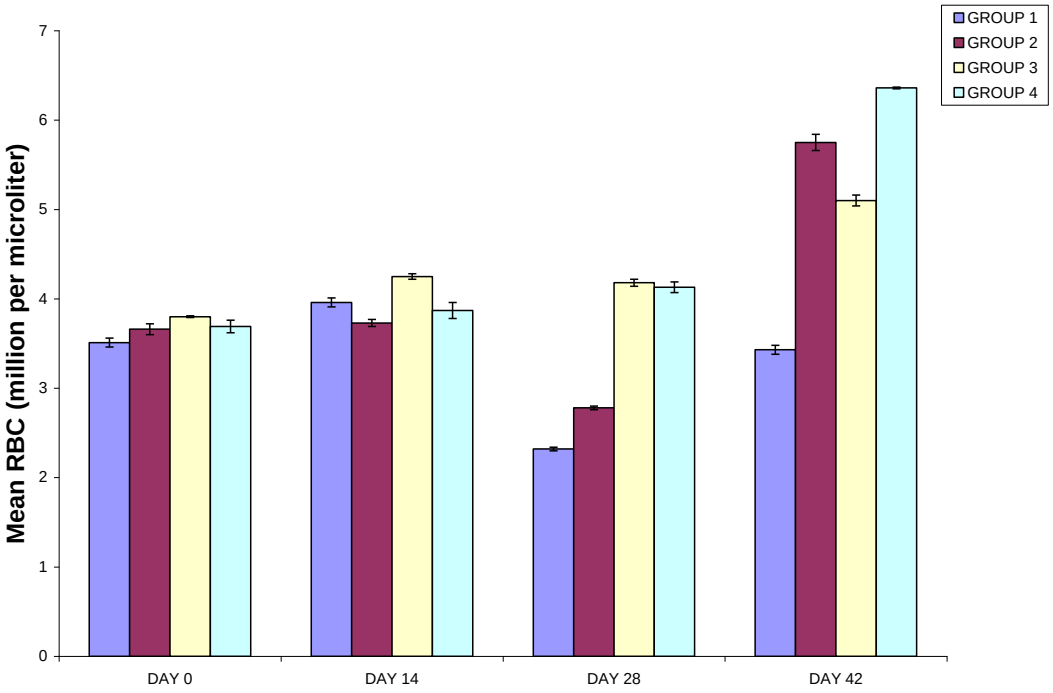

Figure 11: Effect of extract on Red Blood Count

On days 0 and 14, there was no significant difference ($p > 0.05$) in the red blood cell count compared to the control group while on days 28 and 42, there was a significant increase ($p < 0.05$) in the red blood cell count when compared to the control group. The red blood cell count was highest on day 42 with groups 2, 3 and 4 showing higher concentrations compared to the control.

Effect of NLE on White Blood Cell Count

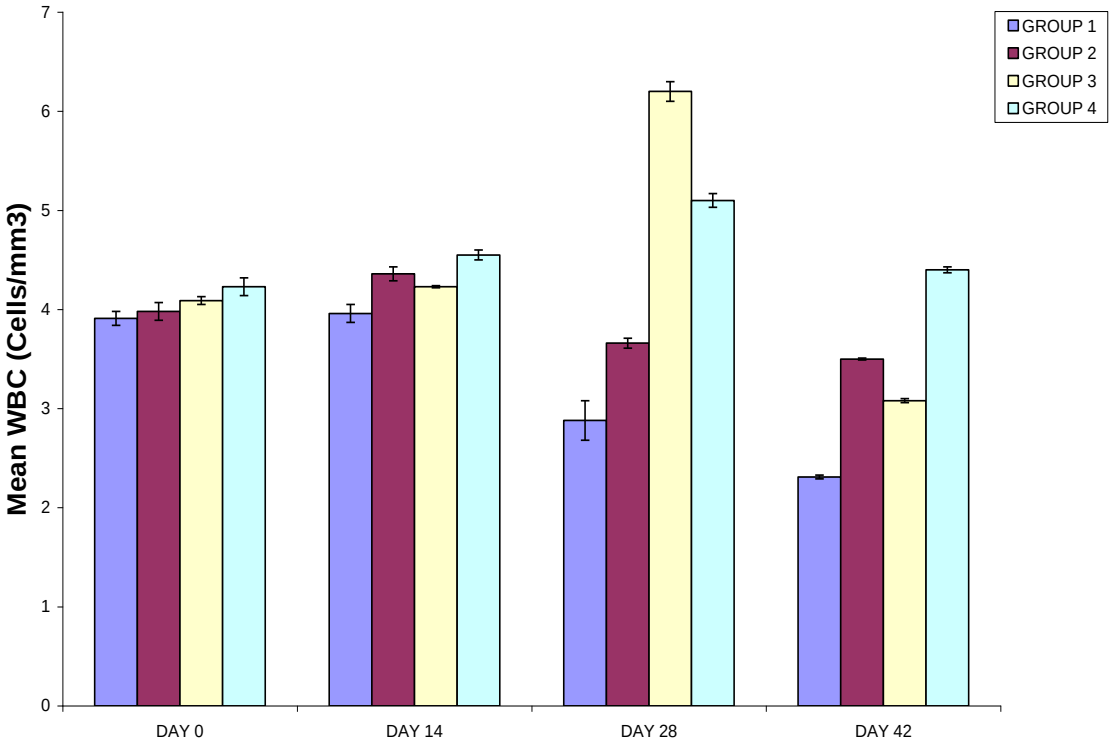

Figure 12: Effect of extract on White Blood cell count

Figure shows that there was no significant difference ($p > 0.05$) in the white blood cell count on days 0 and 14 among the treatment groups when compared to the control but on days 28 and 42, there were significant increases ($p < 0.05$) in white blood cell count within the treatment groups compared to the control, implying that as days of administration of the extract increased, increase in white blood cells occurred.

Effect of NLE on Monocyte Count

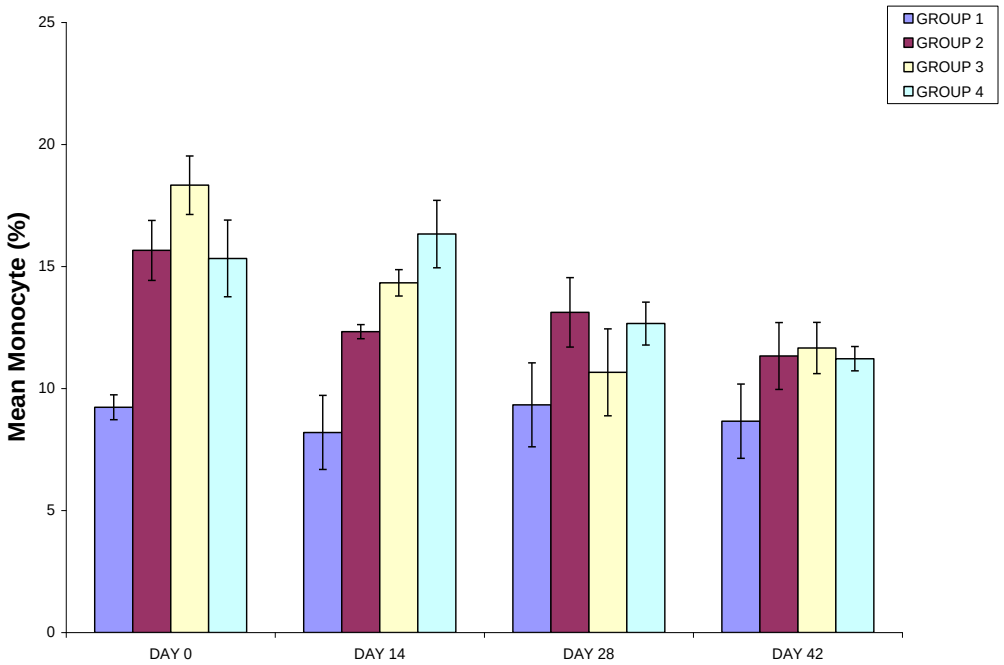

Figure 13: Effect of extract on Monocytes count

There was a significant decrease ($p < 0.05$) in the monocytes count in all the treatment groups when compared to the control group as extract treatments continued from day 1 to 4.

Effect of NLE on Lymphocyte Count

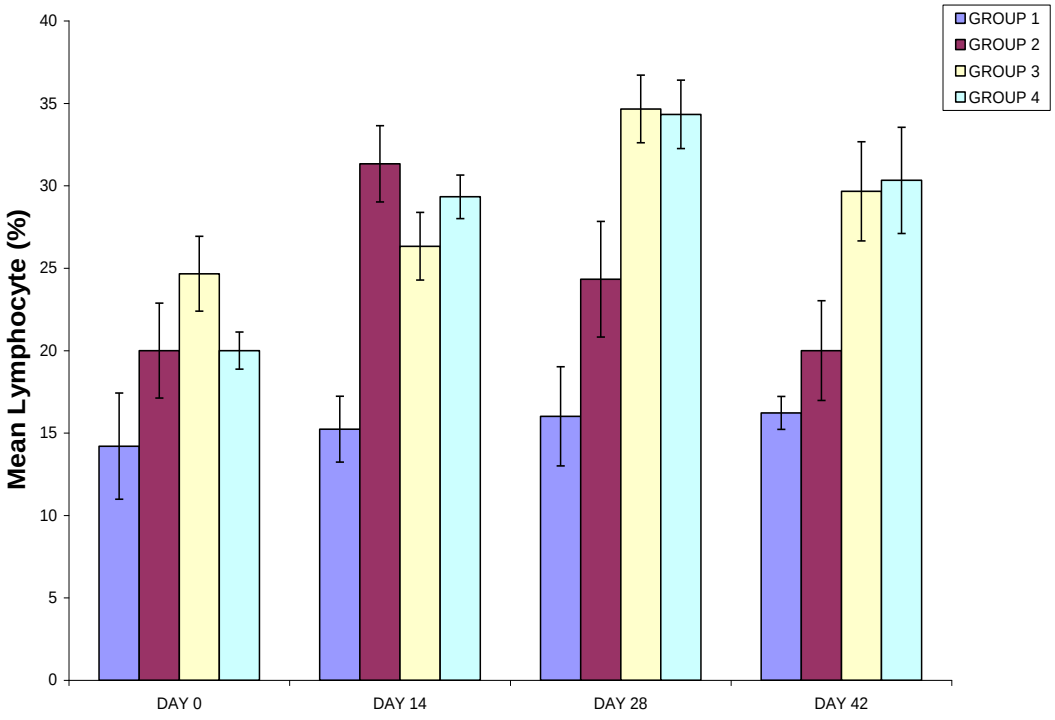

Figure 14: Effect of extract on Lymphocyte count

The result above shows a significant increase ($p < 0.05$) in the lymphocyte counts within the treatment groups when compared to the untreated control group. The increase in lymphocytes was highest on day 28 with groups 3 and 4 having higher responses to the extract compared to the control.

Effect of NLE on Neutrophil Count

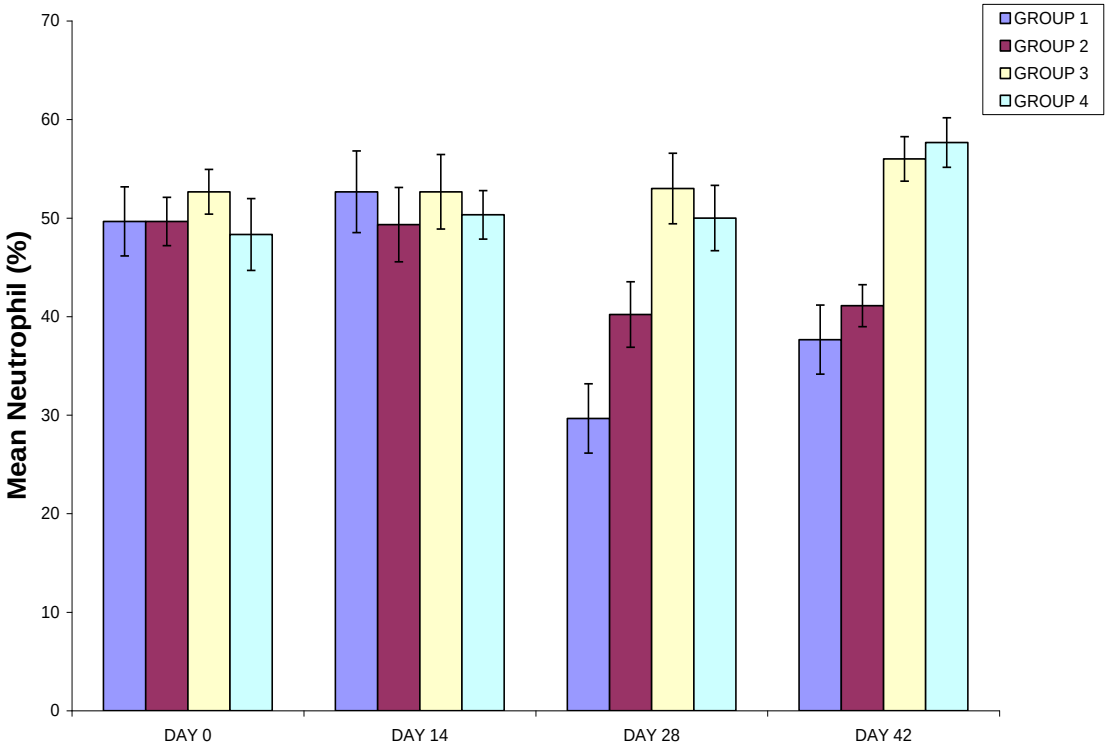

Figure 15: Effect of extract on Neutrophil count

On days 0 and 14, there was no significant difference ($p > 0.05$) in neutrophil counts among the treatment groups compared to the control but as administration continued, there was a significant ($p < 0.05$) increase in the neutrophil count within the treatment groups compared to the untreated control group. On day 42, the neutrophil count was highest compared to the other three groups.

Effect of NLE on Basophils Count

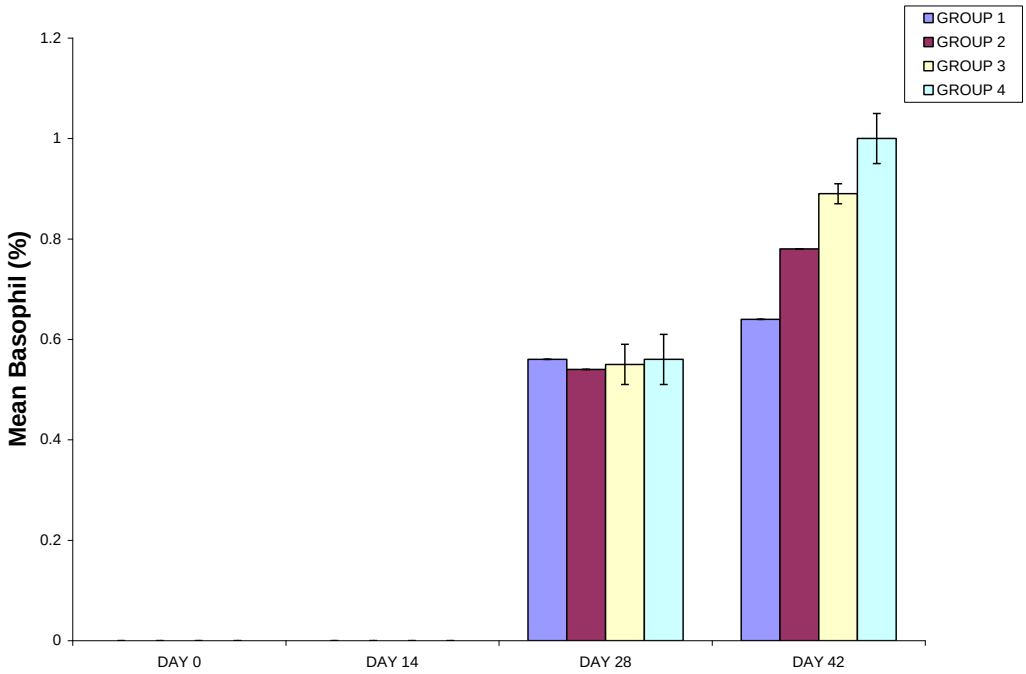

Figure 16: Effect of extract on Basophil count

From the result above, there were no basophils seen on days 0 and 14. On day 28 there was no significant ($p > 0.05$) change in basophils count **among** the treatment groups compared to the control. But on day 42, there was a significant ($p < 0.05$) increase in basophils within the treatment groups 3 and 4 compared to control group 1.

Effect of NLE on Eosinophil Count

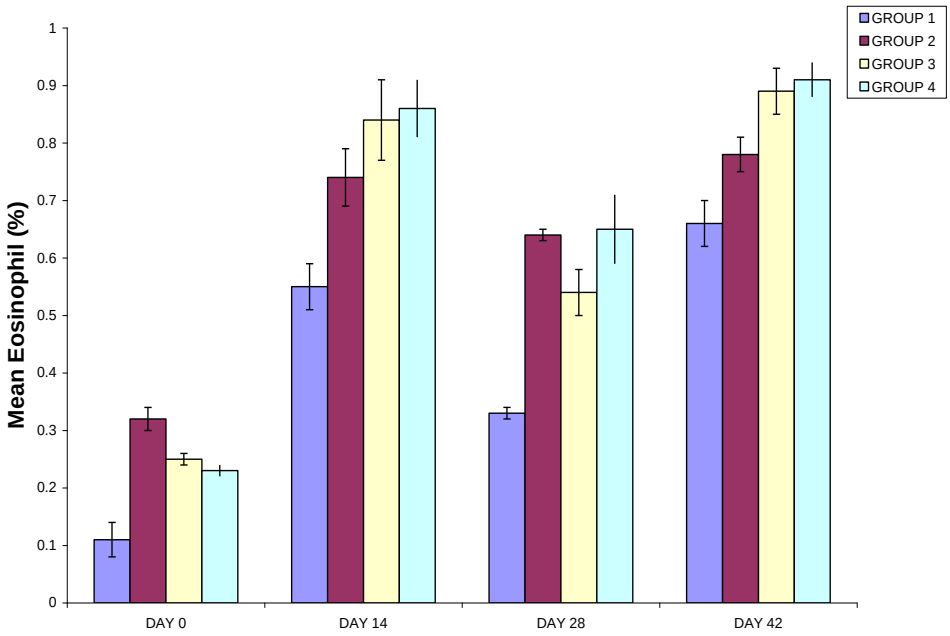

Figure 17: Effect of extract on Eosinophil count

From the result, eosinophil count was lowest on day 0 compared to other days. There was a significant ($p < 0.05$) increase in eosinophil count from days 14 to 42 compared to day 0. From days 14 to 42, there was a significant ($p < 0.05$) eosinophil increase among the treatment groups on each day when compared to the untreated control group 1

DISCUSSION

Evaluation of haematological parameters are not only used to determine the extent of deleterious effect of herbal extracts, but also to explain the functions of plant extracts or their products on the blood of animals (Bin-Jaliah *et al*., 2014). Haematological parameters provide valuable information on the health status of an animal (Yakubu *et al.,* 2017). The hematopoietic systems represent a sensitive target of toxic compounds and an essential index of physiological and pathological status in experimental animals (Mukinda and Syce, 2007).The effects of ethanolic extract of *Nauclea latifolia* on haematological parameters showed a significant elevation ($P<0.05$) in packed cell volume (PCV), white blood cell count, haemoglobin concentration, red blood cell count and the white blood cell differentials compared to the control group which could be a protective mechanism against infection (Awodu *et al*, 2002). The raised haematocrit is an indication of haemo-concentration which may be due to increased RBC mass. The higher values of RBC and associated parameters are suggestive of polycythemia (Oso *et al*., 2019). The administration of the plant extract also caused a slight increase in the level of haemoglobin. In view of this, the plants can also be of benefit in some anaemic conditions characterized by decrease in erythrocyte number. Several medicinal plants such as *Xylopia aethiopica* (Oso *et al*., 2019), *Tectona grandis* (Diallo *et al*., 2008) and extracts of *M. indica*, *A. hybridus* and *T. occidentalis* (Ogbe *et al*., 2010) have also been reported to elevate RBC, hemoglobin and packed cell volume. The blood stimulating effects could be due to the presence of dietary bioactive constituents that stimulate activities of haematopoietic cells and stabilization of blood in circulation (Anthonia *et al*., 2019). White blood cell counts usually increase following foreign invaders (pathogens) resulting in normal body physiological responses that boost the body's defense mechanisms (Stover and Caudill, 2008). The increase in WBC and WBC differentials, suggests that the ethanolic extract of *N. latifolia* root probably contains agents that stimulate production of leucocytes (Yakubu *et al*., 2017). The presence of such agents had been reported in other commonly prescribed medicinal plants (Al-Mamary 2002;

Imoru *et al*., 2005). The crucial role of WBC in defending the body against infection and tissue damage is well known. Thus the results of this study imply that *Nauclea latifolia* root extract is a potent immuno-stimulant and its use to treat immune-related diseases in herbal medicine can be justified. Such immune boosters are usually recommended to strengthen and harmonize degenerative body systems and assist the immune system to fight invading agents such as bacteria and viruses (Bendich, 1993; Al-Mamary, 2002).

CONCLUSION

In conclusion, most people in developing nations depend on herbal medical care (Valdez-Solana *et al*., 2015). These herbs are orally administered or can be applied onto the skin surface as ointment. If the results obtained from this study are applicable to man, ethanolic extracts of *Nauclea latifolia* root can be used as immune boosters and blood tonics in herbal medicine. Unfortunately, it seems that it may contain particles that may exert or have allergenic and inflammatory properties hence, the need to exercise caution on the excessive or prolonged use of the plant traditionally, especially the crude forms of the root extracts.

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