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Impact of *Newbouldia laevis* Root Extract on Hematological Parameters in Rats: A Comprehensive Study on Dosage-Dependent Effects and Long-Term Dynamics

[1,2]Eluu, S.C., [2,3]Oko, A.O., [4]Eluu, K., [5,6]Okoye, C.S., [1]Onyekwere, U.U.[1]and [7]Omoniyi, O.A.

[1]Department of Pharmaceutical Microbiology and Biotechnology,
Nnamdi Azikiwe University, 420110, Agulu, Anambra State, Nigeria
[2]Department of Biotechnology, Ebonyi State University, Abakaliki, Nigeria
[3]Department of Biology and Biotechnology,
David Umahi Federal University of Health Sciences, Uburu, Nigeria
[4]Department of Genetics and Biotechnology, University of Calabar, Calabar, Cross River State
[5]Department of Pharmaceutics and Pharmaceutical Technology,
Nnamdi Azikiwe University, Agulu, Anambra State
[6]Department of Homeopathic Materia Medica, Nigeria Institute of Homeopathy, Enugu, Nigeria.
[7]Department of Material Science, African University of Science and Technology, Galadimawa, Abuja
*Corresponding author's email: eluustanleychijioke@gmail.com

Abstract
Haematological parameters serve as crucial indicators for evaluating essential data related to the body's response to illnesses and medications. This study aimed to investigate the impact of *Newbouldia laevis* root extract on the haematological parameters of rats. A total of sixteen male Wistar rats were randomly divided into four groups, each comprising four rats. The control group, designated as the first group, received only feed and water, while groups two, three, and four were administered doses of 200, 400, and 600 mg/kg of *N. laevis* root extracts, respectively. Upon assessing the percentage packed cell volume fourteen days post-extract administration, no significant variations ($p > 0.05$) were observed among the groups. However, after administering the extract for 14 days, the control group and the 600 mg/kg-treated group exhibited significantly lower red blood cell counts and haemoglobin values ($p < 0.05$) compared to the groups treated with 200 mg of the extract. Notably, at 28 days post-administration, there were no significant differences ($p > 0.05$) in the percentage packed cell volume, red blood cell counts, or haemoglobin values among the groups, despite the observed disparities on the 14-day. Furthermore, a significant increase in percentage packed cell volume, red blood cell counts, and haemoglobin values was noted when the values were compared at 14 and 28 days. The study contributes significantly to our understanding of the plant extract's effects on the physiological components of the haematological system.
Keywords: Newbouldia laevis, red blood cell, haemoglobin, packed cell volume, ethanol extracts, and Wistar rats

Introduction

Often called the African Border Tree or Boundary Tree, *N.* laevis is a flowering plant indigenous to tropical West Africa, which includes countries such as Ghana, Nigeria, Ivory Coast, and Sierra Leone (Okagu *et al*., 2022). Also, the cultivation of *N. laevis* extends beyond its native regions, having been introduced to various tropical areas (Iwu, 2013). This deciduous tree, which is part of the Bignoniaceae family, is valued for its ecological, medicinal, and cultural importance. The bark, leaves, and roots of the plant are abundant in compounds that are believed to confer therapeutic benefits. This has led to its historical use in herbal medicine, wherein the tree's extracts are used to treat a variety of illnesses, including skin disorders, respiratory infections, and malaria (Okagu *et al*., 2022; Hassan *et al*., 2010; Udeozo *et al*., 2014). It has been reported also that compounds and extracts from this plant have a variety of biological activities, including oxytocic, central nervous system modulatory, anti-inflammatory, anti-tubercular, anti-ulcer, hepato-renal protection, antitrypanosomal, anticancer, and antidiabetic effects (Agbodeka *et al*., 2016; Mbagwu *et al*., 2020; Ogbe *et al*., 2020). Moreover, *N. laevis* leaves are used by Tiv traditional healers in central Nigeria to cure illnesses that cause sweet urine and frequent urination (Bosha *et al*., 2016). Various documented medicinal applications include the use of this plant in traditional remedies for

ailments such as fevers (including yellow fever), stomach discomfort, cough, sexually transmitted infections, skin infections, toothaches, constipation, and pain (including pelvic and chest pain, earaches) (Burkill, 1997; Iwu, 2000; Okiei, *et al*., 2009; Romeiras *et al*., 2012). Additionally, it has been employed in treating gonococcal orchitis, elephantiasis, sore feet, ulcers, epilepsy, convulsions, migraines, sickle cell anaemia, acting as a febrifuge and vermifuge (Osigwe *et al*., 2017). It is also utilized in female reproductive healthcare for issues like fibroids, infertility, and haemorrhage, as well as in addressing eye problems, snake bites, wound healing, diabetes, arthritis, rheumatism, and various inflammatory conditions (Dangbo *et al*., 2019; Osigwe *et al*., 2017). The chemical composition of *N. laevis* (Dermane *et al*., 2020; Habu & Ibeh, 2015; Ujah *et al*., 2022; Orakwue & Obiogbolu, 2021; Usman & Osuji, 2007) may be responsible for the medicinal properties of the plant, as many pharmaceutically produced drugs have active ingredients that are made of compounds obtained from phytochemicals found in plants. Furthermore, since this plant is widely used in herbal therapy, it is necessary to understand how various herbal remedies from this plant affect haematological markers. Thus, the goal of this study was to determine how long-term exposure to this natural product affects blood-related components including packed cell volume, red blood cells, and haemoglobin, concentrations (Etim, 2014). Haematological parameters are important markers of an animal's physiological state, and changes in these parameters are crucial for determining how the animal responds to different physiological conditions (Aikpitanyi & Egweh, 2020).

Materials and Methods

Materials

Collection and identification of plant materials

Fresh roots of *N. laevis* were gathered from Izzi in the Abakaliki local government of Ebonyi state, and Mr Emmanuel Nwankwo, a plant taxonomist from the Applied Biology Department at Ebonyi State University, Abakaliki, was responsible for identifying the samples.

Experimental animal

Twenty male Wistar rats, weighing between 160 and 180 g, were procured from the animal house of the Department of Veterinary Medicine at the University of Nigeria, Nsukka. These rats were acclimatized for seven days and were provided with unrestricted access to food and clean water.

Methods

Extraction of plant materials

The plant's root samples were initially washed with distilled water, then air-dried at room temperature away from sunlight, and subsequently pulverized using a grinding machine. The extraction process involved cold maceration at room temperature, where 200 g of the powdered plant material was soaked in 2 L of ethanol (Swem *et al*., 2020). The mixture underwent intermittent vigorous shaking and was later filtered after 48 hours using Whiteman filter paper size 1. The obtained filtrate was concentrated under vacuum conditions using a rotary evaporator.

Experimental design

Sixteen male Wistar rats were randomly divided into four groups, each consisting of four rats. The first group, designated as the control, received only feed and water. Meanwhile, groups two, three, and four were administered the plant extract at doses of 200, 400, and 600 mg/kg, respectively.

Determination of Hematological Assay

Following the initial 14 days, venous punctures were used to obtain blood samples, which were then placed in a sterile specimen bottle for analysis. For the final analysis, the same procedure was carried out after twenty-eight (28) days. The collected blood samples were subsequently used for the quantitative determination of haematological effects.

Red blood cell (RBC) counts

RBC was carried out by the method of Ochei and Kolhatkar (Ochei & Kolhatkar, 2008).

Determination of the Packed Cell Volume (PCV)

PCV was estimated as described by Ochei and Kolhatkar (Ochei & Kolhatkar, 2008).

Determination of the haemoglobin (HB) concentration

Hemoglobin concentration was determined using the technique outlined by Ochei and Kolhatkar (Ochei & Kolhatkar, 2008).

Statistical analysis

The means ± standard deviation was used to express the data. Using the Statistical Package for the Social Sciences (SPSS) version 20, comparisons between the control and treated groups were carried out at the same time point using a one-way analysis of variance (ANOVA). The least significant differences were identified at $p < 0.05$.

Results and Discussion

Results

Effect of N. leavis on percentage packed cell volume

The % PCV observed 14 days after administering the ethanol extract were 42±2, 39±3, 39±4, and 40±2% for the control group and the groups that received 200, 400, and 600 mg/kg of the extracts, respectively. Subsequently, at 28 days post-administration, the percentages were 47±3, 45±4, 44±2, and 43±4% for the control group, and the groups administered 200, 400, and 600 mg/kg of the extracts, respectively (Fig.1). There were no significant variations ($p > 0.05$) in the percentage packed cell volume among the groups when compared.

Effect of N. leavis on red blood cell count

RBC 14 days after the plant extracts were given were 5.4±0.9, 4.4±0.5, 4.8±0.2, and 5.3±0.8 $X10^6/\mu L$ for the control group and the groups that received 200, 400, and 600 mg/kg of the extracts, respectively. After the extracts were administered for 28 days, the RBC were 6.54±0.3, 6.28±0.2, 6.32±0.6, and 6.16±0.4 $X10^6/\mu L$ for the control group and the groups that received 200, 400, and 600 mg/kg of the extracts, respectively (Fig. 2). After administering the extract for 14 days, the control group and the group treated with 600 mg/kg of the

extract had significantly higher RBCs ($p<0.05$) compared to the groups treated with 200 mg of the extract, but there was no difference in the RBC of the group administered 400 mg/kg of the extract compared to the other groups. However, when all the groups were compared to one another on the 28th day, there were no significant differences ($p > 0.05$) in the RBC.

Effect of N. leavis on haemoglobin levels

Fourteen days after the administration of the plant, the haemoglobin values were 12.3±0.9, 10.2±0.6, 9.6±0.8, and 11.7±0.8 g/dL for the control group and the groups that received 200, 400, and 600 mg/kg of the extracts, respectively. Subsequently, at 28 days post-administration, the haemoglobin values were 13.4±0.4, 12.5±0.8, 12.3±0.6, and 11.96±0.47 for the control group and the groups that received 200, 400, and 600 mg/kg of the extracts, respectively (Fig.3). Hemoglobin levels in the control and 600 mg/kg groups of extract-treated rats were significantly ($p<0.05$) higher on the 14th-day post-extract administration compared to the 200 and 400 mg/kg groups. On the 28th day, however, when the haemoglobin values of each group were compared with one another, there were no significant differences ($p>0.05$).

Discussion

The crucial indicators for hematological assessment include erythrocytes, packed cell volume (PCV), and hemoglobin. The hematological parameters are important because they are used to assess critical parameters regarding the body's reaction to various illnesses and medications (Delwatta *et al*., 2018). These indicators play a significant role in the diagnosis of anemia (Peters *et al*., 2011). The results obtained in this study imply that the administration of the ethanol root extract of *N. leavis* did not lead to any substantial deviations from the normal packed cell volume levels in rats, as per the referenced range of packed cell volume outlined by Delwatta *et al*. (Delwatta *et al*., 2018). These results contribute to a broader understanding of the effects of the *N. leavis* root extract on hematological parameters and provide a foundation for further exploration of its physiological impact. When the 14-day results were compared, it was shown that the groups treated with 200 mg/kg of the extract had significantly lower red blood cell counts ($p<0.05$) compared to the control group and the group treated with 600 mg/kg of the extract. It's interesting to note that at this time, the red blood cell counts of the group that received 400 mg/kg of the extract did not significantly differ from those of the other groups. This points to a dosage-dependent effect, with the greatest dosage (600 mg/kg) having a more noticeable influence on red blood cell counts. Hemoglobin levels were considerably ($p<0.05$) lower in the control and 600 mg/kg groups of *N. leavis* extract-treated rats compared to the 200 and 400 mg/kg groups, according to the values of the study of day 14. This suggests that hemoglobin levels may be affected by dosage at initial administration. However, after 28 days, PCV, RBC, and Hb values did not differ significantly ($p >0.05$) amongst all the groups, despite the disparities that were seen on day 14 when they were compared to each other. It appears from this that the differences in RBC and Hb values between the groups gradually diminished, which may point to the enhancing effects of the *N. leavis* extracts. Also, a significant increase in PCV, RBC, and Hb values was noted when the values at 14 and 28 days were compared. Red blood cells and hemoglobin actively participate in the transport of carbon dioxide and oxygen throughout the body of an animal and an increase in the number of the blood parameters indicates an increase in the amount of oxygen delivered to the tissues and cells (Shittu *et al*., 2022). This finding corroborates the results of other studies which demonstrated an increase in hematological parameters as the days progressed (Jacob Filho *et al*., 2018). This result is also in line with the result of a different study (Kolawole *et al*., (2013) that found a slight increase in red blood cells and their indices in normal rats given supplements of *N. laevis* leaves. It was also suggested that the increase could be the result of the extract's stimulating effect on the bone marrow's stromal cells and macrophages, which stimulate the production of hemopoietic regulatory elements like erythropoietin and colony-stimulating factors (Osigwe *et al*., 2017).

Conclusion

The study contributes significantly to our understanding of the plant extract's effects on the physiological components of the haematological system by offering a comprehensive understanding of the short- and long-term impacts of the ethanol roots extract *N. laevis* on haematological parameters.

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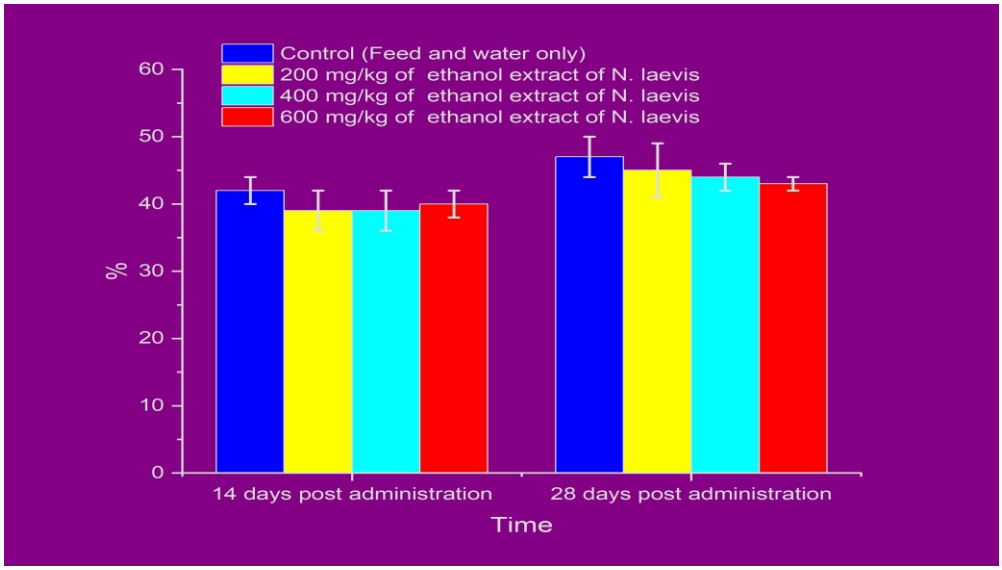

Figure 1: ***Effects of* N. leavis *on percentage packed cell volume. The values are presented as mean ± standard deviation, with a sample size (n) of 4. The asterisk (*) denotes significant differences at p < 0.05.***

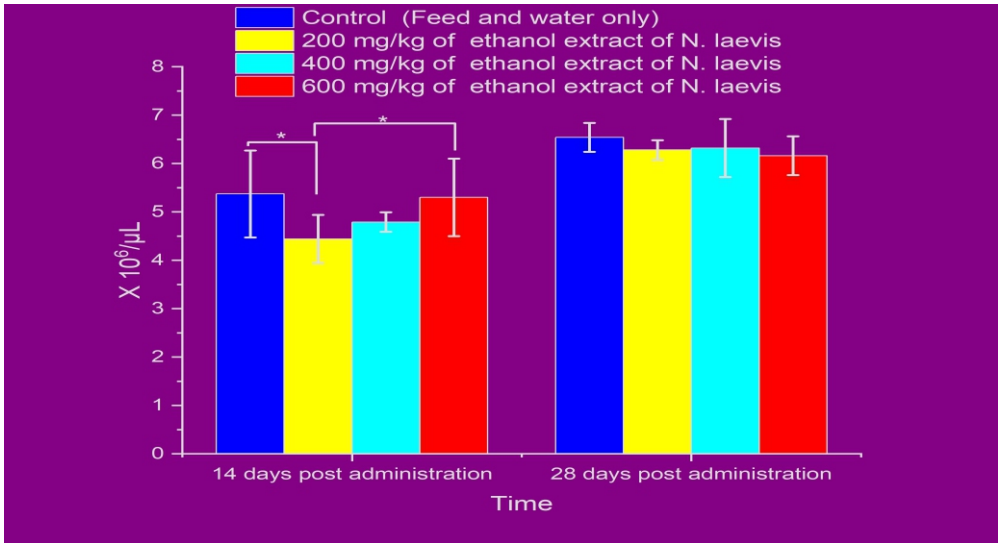

Figure 2: ***Effects of* N. leavis *on red blood cell count. The values are presented as mean ± standard deviation, with a sample size (n) of 4. The asterisk (*) denotes significant differences at p < 0.05.***

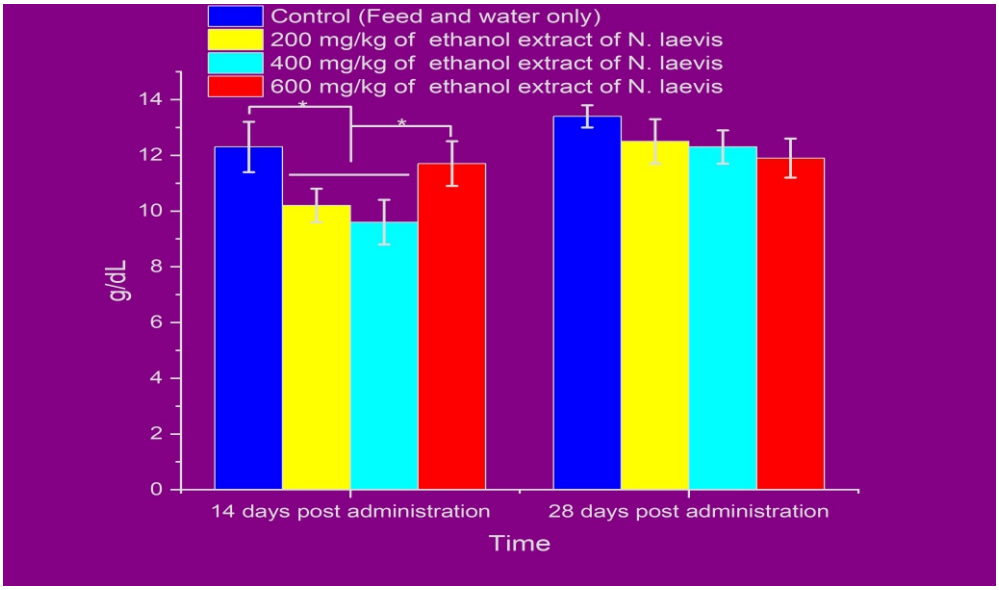

Figure 3: ***Effects of* N. leavis *on hemoglobin levels. The values are presented as mean ± standard deviation, with a sample size (n) of 4. The asterisk (*) denotes significant differences at p < 0.05.***