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Effects of the Aqueous Root Extract of *Vernonia amygdalina* on the Haematological Profile of *Rattus norvegicus*

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Abstract

The effects of *Vernonia amygdalina* root extract on the haematological profile of 20 male albino rats (Wistar strain) were investigated for a period of 21 days. The rats were divided into three treatment groups A, B and C and a control group D. The experimental groups A, B and C were treated with 50mg.Kg^{-1}, 150mg.Kg^{-1} and 300mg.Kg^{-1} body weight, respectively while the control (group D) received equal volume of normal saline. The extracts were given to the animals orally for 21 days. Blood was collected through the orbital-plexus of rats to assay the effect of the extract on packed cell volume (PCV), white blood cell count (WBC), red blood cell count (RBC) and haemoglobin (Hb) levels. The mean WBC, RBC, PVC and haemoglobin levels ranged from 1560 ± 120 to 3200 ± 1200, 150.00 ± 40.00 to 276.00 ± 44.50, 38.00 ± 1.00 to 41.00 ± 3.00, and 16.00 ± 2.00 to 12.50 ± 0.50, respectively. There was no significant difference ($p>0.05$) in the haematological profile of the treated groups when compared with the control. However, there was significant difference ($p<0.05$) within each group from week one to week three except WBC in group D and PVC in all the groups. This suggested that the effect of the aqueous extract of *V. amygdalina* on the haematological profile of *Rattus norvegicus* was dependent on duration of consumption.

Keywords: *Vernonia amygdalina,* Aqueous root extract, Haematological profile, *Rattus norvegicus*

1. Introduction

The widespread use of *Vernonia amygdalina* both as food and medicine in the rural communities call for urgent attention in order to effectively exploit the maximum benefits of the plant and to avoid/ameliorate the adverse side effects that may emanate from such indiscriminate use of the plant. *V. amygdalina* is a shrub or small tree of 2-5m with petiolate leaf of about 6mm in diameter and elliptic shape (Areghereore *et al.* 1998). The leaves are green with a characteristic odour and bitter taste (Singha 1966). *V. amygdalina* has a variety of names in various languages. In English, it is referred to as bitter leaf (Okokon & Onah 2004), in Yoruba, it is known as "Ewuro"*;* it is called "Etidot"*,* in Efik, Ijaw and Ibibio. The Igbos call it "Onugbo" or "Olubu", it is referred to as "Ityna" in Tiv, "Oriwo" in Edo and "Chusa-doki" in Hausa (Egedigwe 2010). The plant grows throughout tropical Africa. It is drought-resistant and thrives in humid environments (Ijeh & Ejike 2011). It is grown commonly in Benin, Nigeria, Cameroun, Gabon and DR Congo, and to a lesser extent in their neighboring countries.

It is a unique plant, so unique that every part of it has an economic importance. Its leaves are macerated and used in cooking soup, while the extracts are used as tonic for prevention of certain illness. *Vernonia amygdalina* have been shown to be valuable nutritionally. It contains significant quantities of lipids (Ejoh *et al.* 2007), proteins with essential amino acids (Igile *et al.* 1994). It also contains carbohydrates (Eleyinmi *et al.* 2008) and carotenoids, though not in large quantities (Udensi *et al.* 2002). Also contained in this plant are essential elements such as calcium, iron, protein, potassium, phosphorus, manganese, copper and cobalt (Bonsi *et al.* 1995).

V. amygdalina also finds applications in the treatment of various ailments. It is a medicinal herb used popularly by traditional practitioners especially in villages. The plant has been shown to be anti-helminths, blood purifier, anti-laxative and anti-malarial. It is also used by scientists in curing joint pains associated with AIDS, diabetes, persistent headache, fever reduction and a host of others. The roots are used for treatment of gastro-intestinal problems, malaria, toothache and fertility problems (Momoh *et al.* 2010). It is also used as digestive tonic, appetizer and febrifuge, and for topical treatment of wounds as a substitute for iodine (Iwu 1986). Dalziel (1937) was the first to report that the root and twig of the plant are used for the treatment of gastro-intestinal problems by the Hausas of northern Nigeria, while the decoction from leaves are used for treating malaria fever in Guinea and cough in Ghana. Leaf decoctions are also used to treat diarrhea, dysentery and hepatitis. *V. amygdalina* can

also be used as a control agent against diseases in plants. The aquoues leaf extract in combination with *Azadirachta indica* leaf extract is the best cure for type 2 diabetes in Nigeria (Eyong *et al.* 2011). The ash from burnt branches can be used to control seed-borne fungi (Misari 1992). Its stem is used as chewing stick. A lot of researches have been done on its leaf extract but the reverse is the case with the roots. The study therefore was designed to find out the effects of aqueous root extract of *V. amygdalina* on the haematological profile of *Rattus norvegicus.*

2. Materials and Methods

2.1 Plant Material

Fresh roots of *V. amygdalina* used were collected from Enugu-Ezike, Igbo Eze North Local Government of Enugu State. Thereafter, they were transported to the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka where they were authenticated and voucher specimen kept in the herbarium (VAR112111). The roots were taken to the Department of Zoology and Environmental Biology Physiology Laboratory where they were washed to remove impurities and then cut into smaller sizes. The roots were air-dried under room temperature over a period of three weeks. The dried roots were pulverized to get a coarse powder used for the extraction. 540g of the powder was macerated in 2.16 litres of distilled water. The mixture was stirred continuously for 10 minutes and allowed to stand for 48 hours, after which it was filtered using Whatman filter paper (grade 1: 11 µm) and the filtrate dried into powder using a rotary evaporator (Stuart, model RE-300, UK) at a temperature of 40^{o}C. The residue referred to as the crude extract was stored in an air-tight container under refrigeration until used (Momoh *et al.* 2010).

2.2 Management of Experimental Animals

Twenty male albino rats of six weeks old and weighing between 115 - 184g were used for the experiment. The animals were procured from Animal Breeding and Genetics Unit of the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. Approval to use albino rats was obtained from the ethical committee of University of Nigeria, Nsukka, and full protocol for animal experimentation was adhered to during the study. They were kept in stainless wire rat cages equipped with drinkers and faecal collecting tray in a clean fly proof experimental animal house and were fed (Chick Growers Mesh, Guinea Feed, Nigeria) and watered *ad libitum* during the course of acclimatization and experimentation. The faecal droppings in the tray were removed daily.

2.3 Methodology

The albino rats were divided into four groups A, B, C and D of 5 rats per group. Groups A, B and C were the treatment groups while group D served as the control. Group A, B and C were given 50mg/kg, 150mg/kg and 300mg/kg of the root extracts of *V. amygdalina,* respectively on a daily basis for a period of 3 weeks, while group D received equal volume of normal saline. Each extract concentration was resuspended in 1 ml of normal saline and administered orally to the albino rats using 2ml syringe. Blood was drawn weekly from the eyes of the albino rats with a haematocrit by inserting the tube carefully into the cantus to puncture the orbital-plexus. This is to enable the flow of blood into the EDTA treated bottles.

2.4 Haematological Parameters

The haematological parameters such as: red blood cell count (RBC), white blood cells count (WBC) and packed cell volume (PCV) were determined using the method of Sood (2006).

2.5 Haemoglobin Determination

Drabkin's solution was used and this composed of 50mg of Potassium cyanide (KCN), 20 mg Potassium ferrycyanide [$K_3Fe(CN)_6$] and 1000ml of distilled water. Spectrophotometer was used to measure the Optical Density (OD) of the Drabkin's solution. This was compared with the standard reagent blank in spectrophotometer. The reading obtained was then read-off as standard graph of OD and haemoglobin.

Haemoglobin concentration was calculated thus: Hb concentrated (g/100ml) = Optical density of test solution × Hb concentration standard solution / Optical density of standard × b × 1000 (Sood 2006).

2.6 Data Analysis

The data collected were subjected to Analysis of Variance (ANOVA) to test for variations of the different parameters observed in the study. Test of significance was at 0.05% probability.

3. Results

3.1 Effects of Aqueous Root Extract of V. amygdalina on the Haemoglobin Level of Rattus norvegicus

The haemoglobin level of the albino rats ranged from 16.00 ± 2.00 in week one in group D to 12.50 ± 0.50 in group C in week two. There was no significant difference ($p>0.05$) in haemoglobin level with respect to dosage of administration between the treatment groups and the control group during the period of the study. However, within the groups there was a significant decrease in haemoglobin level in group A ($p<0.05$) and a significant increase in haemoglobin level in B and C ($p<0.05$), but in D the decrease in haemoglobin level was not statistically significant ($p>0.05$) (Table 1).

3.2 The effects of Aqueous Root Extract of V. amygdalina on the Total White Blood Cell Count (WBC) of Rattus norvegicus

There was no significant change ($p>0.05$) in the WBC of the rats in treatment groups when compared with those of the control group. Within the groups, there was significant increase ($p<0.05$) of white blood cell count from week one to week three in all the groups except group D (the control) (Table 2).

3.3 Effect of Aqueous Root Extract of V. amygdalina on Red Blood Cell Count (RBC) of Rattus norvegicus

The effect of the extract on RBC was not concentration dependent as no significant change ($p>0.05$) was observed in groups A, B and C when compared with group D. Within each group, there was a significant increase ($p<0.05$) of the red blood cell count of the rats from week one to week three of administration of the extract (Table 3).

3.4 Effects of Aqueous Root Extract of V. amygdalina on Packed Cell Volume (PCV) of Rattus norvegicus

There was increase in PCV of the treatment groups compared to the control however; the increase was not statistically significant ($p>0.05$). There was no significant difference ($p>0.05$) observed within the groups (Table 4).

4. Discussion

The study revealed that the effect of the root extracts on the haematological profiles of the albino rats were dependent on the duration of consumption rather than the dosage. Considering the response of the haematological parameters to the dose of extract, there was a decrease in the haemoglobin and increase in the RBC for all the treatment groups. Reduction in haemoglobin might have resulted to lower oxygen supply to different tissues, while the increase of the red blood cell count may be as a result of increased formation and reduced destruction of red blood cells (El-Demerdash 2004).

The decreased WBC in treated groups might indicate depressed immune-response. The reduction in the total white blood cell count could also be due to reduced production of white blood cells, re-distribution of white blood cells from peripheral blood into the tissues or rapid destruction of white blood cells (Guton & Hall 1996). This was however increased as more of the extract was consumed. The significant increase in WBC might indicated activation of the immune system, a normal cell-mediated immune response (El-Demerdash 2004).

The PCV of the treated rats remained relatively constant. PCV levels reflect the extent and efficiency of oxygen uptake and transfer to the tissues of the rat (Carpenter 1975; Ots *et al.* 1998). The relatively constant PCV might indicate that the extent and efficiency of oxygen uptake and transfer to tissue of the rats was at a normal rate.

Our study contrast Oboh (2001), who had reported decreased PCV, due to induced haemolysis in normal rats treated with aqueous extracts of *V. amygdalina* leaves.

5. Conclusion

The root extracts of *V. amygdalina* had no dose-dependent significant effect on the haematological profile of *R. norvegicus*. The effect observed was dependent on the time, with considerable boost observed in the WBC and RBC of treated animals. Therefore, the extract was safe for consumption, because of the observed increase in RBC. The increase in the WBC suggested that the root extract of *V. amygladina* boosted immune system.

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Table 1. Effects of aqueous root extract of *V. amygdalina* on haemoglobin level of *Rattus norvegicus*

Groups	Dosage (mg/kg)	Week 1	Week 2	Week 3
A	50	15.75 ± 0.25^{a2}	14.00 ± 1.00^{a1}	14.40 ± 1.10^{a1}
B	150	15.50 ± 0.50^{a2}	13.00 ± 0.00^{a1}	15.5 ± 0.75^{a2}
C	300	15.50 ± 1.50^{a2}	12.50 ± 0.50^{a1}	14.35 ± 0.45^{a2}
D	Normal saline	16.00 ± 2.00^{a1}	15.30 ± 1.30^{a1}	15.50 ± 0.50^{a1}

Data expressed as Mean ± SD. Mean values in a column with different alphabets are significantly different ($p < 0.05$). Mean values in a row with different figures are significantly different ($p < 0.05$).

Table 2: Effects of aqueous root extract of *V. amygdalina* on white blood cell count of *Rattus norvegicus*

Groups	Dosage (mg/kg)	Week 1	Week 2	Week 3
A	50	1900 ± 100^{a2}	1560 ± 120^{a1}	3150 ± 350^{a3}
B	150	3200 ± 1350^{a2}	2820 ± 950^{a1}	3200 ± 1200^{a2}
C	300	1860 ± 40^{a1}	1800 ± 180^{a1}	2300 ± 1000^{a2}
D	Normal saline	3325 ± 1325^{a1}	3300 ± 1300^{a1}	3400 ± 900^{a1}

Data expressed as Mean ± SD. Mean values in a column with different alphabets are significantly different ($p < 0.05$). Mean values in a row with different figures are significantly different ($p < 0.05$)

Table 3. Effect of aqueous root extract of *V. amygdalina* on red blood cell count (RBC) of *Rattus norvegicus*

Groups	Dosage (mg/kg)	Week 1	Week 2	Week 3
A	50	195.00 ± 5.00^{a1}	220.00 ± 20.00^{a2}	267.50 ± 22.50^{a3}
B	150	200.00 ± 10.00^{a1}	210.00 ± 10.00^{a1}	272.50 ± 2.50^{a2}
C	300	184.00 ± 14.00^{a1}	190.00 ± 10.00^{a1}	276.00 ± 44.50^{a2}
D	Normal saline	152.50 ± 37.50^{a1}	150.00 ± 40.00^{a1}	185.00 ± 65.00^{a2}

Data expressed as Mean ± SD. Mean values in a column with different alphabets are significantly different ($p < 0.05$). Mean values in a row with different figures are significantly different ($p < 0.05$)

Table 4. Effects of aqueous root extract of *V. amygdalina* on packed cell volume (PCV) of *Rattus norvegicus*

Groups	Dosage (mg/kg)	Week 1	Week 2	Weeks 3
A	50	38.00 ± 1.00^{a1}	41.00 ± 3.00^{a1}	39.00 ± 1.00^{a1}
B	150	38.00 ± 1.00^{a1}	38.50 ± 0.50^{a1}	40.50 ± 3.50^{a1}
C	300	38.50 ± 0.50^{a2}	38.00 ± 1.00^{a2}	40.50 ± 1.50^{a2}
D	Normal saline	38.00 ± 1.00^{a1}	38.87 ± 0.78^{a1}	39.00 ± 1.00^{a1}

Data expressed as Mean ± SD. Mean values in a column with different alphabets are significantly different ($p < 0.05$). Mean values in a row with different figures are significantly different ($p < 0.05$).