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Short communication

Haematological effects of aqueous extract of *Vernonia amygdalina* in wistar rats

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ABSTRACT

The present study assessed the haematological effects of aqueous extract of *(Vernonia amygdalina)* in wistar rats. Fifteen (15) adult Wistar rats were used in this study. The animals were divided into three groups of five rats each. Group 1: were given 1ml of distilled water and served as the control, Group 2: received 250mg/kg b w of *V. amygdalina* and Group 3: received 500mg/kg b w of *V. amygdalina* orally once daily for a period of twenty one days. A day after the last treatment the tail end of rats were disinfected using cotton wool soaked in methylated spirit and blood sample was collected for determination of haematological indices The result obtained from this study showed that *V. amygdalina* at all doses administered to animals produced no significant change ($p>0.05$) on the blood levels of red cell count, packed cell volume and total white blood cell count when compared to the control group. On the other hand, treatment of the animals with 250 and 500 mg/kg b w of *V. amygdalina* caused significantly decreased ($p<0.05$) blood level of neutrophil count when compared to the control group. However, *V. amygdalina* at tested doses of 250 and 500mg/kg b w produced a significantly increased ($p<0.05$) blood levels of lymphocyte, basophil, eosinophil and monocyte counts with a non significant change ($p>0.05$) observed only on the blood level of monocyte count at tested dose of 250mg/kg b w of *V. amygdalina* when compared to control group. In conclusion, the present work showed that aqueous

extract of *V. amygdalina* produced significant effect on the differential white blood cell count especially on the blood level of lymphocyte count.However, the plant extract did not produce erythropoietic effects; hence the non significant effect observed on the red cells count and packed cell volume respectively.

1. Introduction

Blood is a specialized bodily fluid that delivers necessary substances to the body's cells such as nutrients and oxygen and transports waste products away from those same cells (Cheeke and Shull, 1999). Blood accounts for 7% of human body weight with an average density of approximately 1060 kg/m^3. Blood composed of plasma and several kinds of cells; these formed elements of the blood are erythrocytes, leucocytes and thrombocytes (Austin and Perkins, 2006). Phytomedicine is the use of plant and plant materials for human and animal medicines. Plants have always been the source of important medicines since time immemorial and there is the need for better understanding of the biological effects of medicinal plants in vivo before formulation into dosage. These activities involve biological testing of plants extracts and the isolation of their active components and ultimately, their clinical validations (Kaufman *et al.,* 1999). *Vernonia amygdalina (V. amygdalina)* is a small shrub that grows predominantly in the tropical Africa. In Nigeria, the plant is locally called bitter leaf due to its bitter taste. The macerated leaves of the plant are used to make soup while the water extract are serves as a tonic drink for the prevention of certain illnesses (Izevbigie, 2003). *Vernonia amygdalina,* a member of the Asteraceae family. Several species of *Vernonia* including *V. calvoana, V. amaygdalina and V. colorata* are eaten as leaf vegetables. In Nigeria, common names for *V. amygdalina* include: bitter leaf, in English, ewuro in Yoruba, Shuwaka in Hausa and Onugbu in Igbo (Nwanjo, 2005). Many herbalists and naturopathic doctors recommend aqueous extracts of this plant for their patients as treatment for emesis, nausea, diabetes, loss of appetite-induced ambrosia, dysentery and other gastrointestinal tract problems. Until the last decade or so, there were only anecdotal reports and claims to support the health benefits. The anecdotal reports are now being supported by scientific evidence that *V. amygdalina* regimen or consumption as dietary supplements may provide multiple health benefits and boosting the immune system (Jisaka *et al.,* 1999). *V. amygdalina* is a shrub or small tree of 2-5m with petiolate of about 6mm in diameter and elliptical in shape. The leaves are green with characteristic odour and a bitter taste. No seeds are produced and therefore to be distributed through cutting. It grows under a range of ecological zones in Africaand produce large mass of foliage and drought tolerant (Bonsi *et al.,* 1995). The bitter taste is due to anti-nutritional fsctors such as alkaloids, saponins, tannins and glycosides (Ologunde *et al.,* 1999). Studies have shown that ethanol leaf extract of *V. amygdalina* has an immunomodulatory properties and it is able to inhibit proliferation of Clostridium sporogenes as seen in humoral and cell mediated response of some treated albino rats (Brinski, 2000). Many studies have shown that *V. amygdalna* extracts may strengthen the immune system through many cytokines (Sweeney *et al.,* 2005). Pharmacological studies have also shown that the leaf extract of *V. amygdalina* has both hypoglycaemic and hypolipidaemic properties in experimental animals and so could be used in managing diabetes mellitus (Izevbigie *et al.,* 2004). Therefore, the present study was designed to evaluate the haematological effects of aqueous extract of *(Vernonia amygdalina)* in wistar rats.

2. Materials and methods

Methylated spirit, microscope, counting chamber, petri dish, cotton wool, scissors, glass slides, cover slips, heparinsed capillary tube, Hawskey microhaematocrit centrifuge, WBC and RBC pipettes, spirit lamp, staining rack, immersion oil, Leishmann stain RBC and WBC dilution fluid

2.1. Plant material collection

Fresh leaves of *Vernonia amygdalina* were harvested from Area BZ, Ahmadu Bello University, Zaria, Staff Quarters Zaria, Kaduna State Nigeria. The leaves were identified by a taxonomist in the Herbarium unit of the Department of Biological Science, Ahmadu Bello University, Zaria, Kaduna State, where a voucher specimen

number was deposited. Fresh leaves of *Vernonia amygdalina* were washed water and air dried under shade and ground into fine powder. The powder (150g) was macerated in 2.5 L of distilled water with intermittent shaking at room temperature for 24 h. It was then filtered using a filter paper (Whatmann size 1). The filtrate was evaporated to dryness in an oven at 30°C. A brownish residue weighing 50.3g was obtained and kept in a sealed container until it was reconstituted in appropriate solvent.

2.2. Animals

Fifteen (15) strains of Albino wistar rats of both sexes weighing 150 to 200 g were used for the experiments. They were procured from the Animal House of the Department of Pharmacology and Therapeutics Ahmadu Bello University Zaria, Kaduna Nigeria. They were allowed to acclimatized and maintained under standard photoperiodic condition for two weeks before the commencement of the study.

2.3. Experimental procedure

The animals were randomly assigned into three groups of six rats each as follows:
Group 1: Received 1ml of distilled water orally
Group 2: Received 250mg/kg b w body weight of *V. amygdalina* orally.
Group 3: Received 500mg/kg body weight of *V. amygdalina* orally.
All regimens were given to the animals orally once daily for a period of three (3) weeks.

2.4. Collection of blood sample and haematological parameters estimation

This was carried out by methods described by Dacie and Lewis, (2008). At the end of three weeks of administration with plant extract the tail end of rats were disinfected using cotton wool soaked in methylated spirit and blood sample was collected for the determination of some haematological indices such as red blood cells count (RBC), packed cell volume (PCV), white blood cell count (WBC) and its differential counts.

2.5. Statistical analysis

Data obtained were expressed as mean ± SEM. The data were analysis using one-way analysis of variance (ANOVA) and Tukey's post hoc test was used to determine the level of significance between control and the experimental groups. All statistical analysis was done using SPSS version 17.0 software.The value of $P<0.05$ were considered significant.

3. Results

Results showed that *V. amygdalina* at all doses administered to animals produced no significant change ($p>0.05$) on the blood levels of red cell count, packed cell volume and total white blood cell count when compared to the control group (table 1). On the other hand, treatment of the animals with 250 and 500 mg/kg b w of *V. amygdalina* caused significantly decreased ($p<0.05$) neutrophil count when compared to the control group. However, *V. amygdalina* at tested doses of 250 and 500mg/kg b w produced a significantly increased ($p<0.05$) blood levels of lymphocyte, basophil, eosinophil and monocyte count with a non significant change ($p>0.05$) observed only on the blood level of monocyte count at tested dose of 250mg/kg b w of *V. amygdalina* when compared to control group (Table 1).

4. Discussion

The study investigated the effect aqueous extract of *Vernonia amygdalina* on some haematological indices in rats. The assessment of haematological parameters could be used to reveal the deleterious effect of foreign compounds, toxins, chemicals and plant extracts on the blood constituents of animals. They are also used to determine possible alterations in the levels of biomolecules such as enzymes, metabolic products, haematology and normal functioning (Magalhaes *et al.,* 2008; Oyedemi *et al.,* 2011). In this study, our results showed that *V. amygdalina* at all doses administered to animals produced no significant change ($p>0.05$) on the blood levels of red cell count and packed cell volume when compared to the control group.

Table 1
Effect aqueous extract of *Vernonia amygdalina* on some haematological indices in wistar rats.

Treatment given	RBC (10^{12}/L)	PCV (%)	WBC (10^{9}/L)	Neutrophils Count (%)	Lymphocytes (%)	Monocytes (%)	Eosinophs (%)	Basophils (%)
Control + distilled water	5.74±0.4	48.00±0.4	10.19±0.5	47.40±1.2	46.40±1.1	5.40±0.4	0.20±0.2	0.00±0.0
V. amygdalina (250mg/kg b w	4.65±0.3^{ns}	49.60±1.4^{ns}	9.61±1.81^{ns}	35.33±1.2^{a}	58.50±1.4^{a}	7.00±0.6^{ns}	3.11±0.4^{a}	0.60±0.4^{a}
V. amygdalina (500mg/kg b w)	5.33±0.3^{ns}	50.29±1.2^{ns}	11.7±0.4^{ns}	23.14±1.7^{a}	60.14±1.9^{a}	12.71±0.9^{a}	3.14±0.3^{a}	0.86±0.1^{a}

Values are presented as mean ± SEM.
Values are statistically significant compared to control group at ^{a} $p < 0.05$ while ns =not significant.

This is an indication that there was no destruction of red blood cells and no change in the rate of production of RBC (erythropoiesis) therefore, suggesting the non toxic nature of the plant extract to red blood cells. In addition, this finding in our present work also revealed that *V. amygdalina* does not have the potential to stimulate erythropoietin release from the kidneys, which is the humoral regulator of RBC production and also affect the oxygen-carrying capacity of the blood and the amount of oxygen delivered to the tissues since red blood cells and haemoglobin (Hb) are very important in transferring respiratory gases (Polenakovic and Sikole, 1996; Oyedeji *et al.,* 2013). On the other hand, the blood level of total white blood cell count produced no significant change ($p>0.05$) after twenty one days of administration of plant extract to the animals when compared to the control group. The insignificant change produced by *V. amygdalina* on the level of total white blood cell count which is involved phagocytosis (Brinski, 2000), suggests that the immune system was not compromised. However, treatment of the animals with 250 and 500 mg/kg b w of *V. amygdalina* caused significantly decreased ($p<0.05$) blood level of neutrophil count when compared to the control group. This reduced neutrophil count caused by plant extract probably indicates that the ability of the body to attack and destroy invading bacteria, viruses and other injurious agents (Phagocytosis) was compromised. However, *V. amygdalina* at tested doses of 250 and 500mg/kg b w produced a significantly increased ($p<0.05$) blood levels of lymphocyte, basophil, eosinophil and monocyte count with a non significant change ($p>0.05$) observed only on monocyte count at tested dose of 250mg/kg b w of the extract when compared to control group. This could be due to the presence of some active phytochemicals in the extract which confers the extract with immunostimulatory property as evident by significantly elevated lymphocyte count. Suggesting, that the extract may have immune boosting effect on the animals. This finding is in agreement with the work of Sweeney *et al.,* (2005) which suggest that *V.amygdalina* extract may strengthen the immune system through many cytokines regulation. Immunity is the resistance of the body against pathogenic agents. The most powerful type of immunity is the acquired immunity which could be either cellular or humoral immunity. Cellular immunity is brought out by the activation of T- lymphocytes while humoral immunity is by the activation of B- lymphocytes. Increase in the number of lymphocytes is a measure of the strength of the immune system. Thus, foreign or invading organisms can easily be identified and destroyed.

5. Conclusion

In conclusion, the present work showed that aqueous extract of *V. amygdalina* produced significant effect on the differential white blood cell count especially on the blood level of lymphocyte count. However, the plant extract did not show erythropoietic effects; hence the non significant effect observed on the red cells count and packed cell volume respectively.

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