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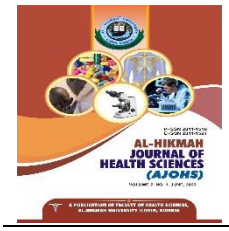

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Original Article

Haemopoietic Potentials of the Aqueous Leaf Extract of *Vernonia Amygdalina* on Haematological Parameters in Phenylhydrazine Induced Anaemic Wistar Rats

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ABSTRACT

Anaemia is the most prevalent nutritional deficiency disorder in the world; it is defined as a condition by which the haemoglobin content of blood is lower than normal as a result of deficiency of one or more essential nutrients.

O**bjective:** This study examined haemopoietic potentials of aqueous leaf extract of *Vernonia amygdalina* on haematological parameters in phenylhydrazine induced albino wistar rats.

Materials and Methods: Thirty (30) wistar rats were grouped into five (5) groups of six (6) rats per group. The phenylhydrazine was used to induce anaemia intra-peritonially and aqueous extract of *Vernonia amygdalina* was administered orally for 14 days at different concentrations of 100 mg/ kg, 200 mg/kg and 400 mg/kg with Normal and Negative controls). A volume of 5mls of blood sample was collected and analysed for haematological parameters using Haematology Autoanalyzer.

Results: The mean and standard deviation of HGB g/dl, PCV %, RBC x 10^{12}/L and WBC x 10^{9}/L of the treated groups were expressed as (8.45 ± 0.54 to 13.20 ± 0.32), (23.50 ± 2.38 to 40.50 ± 4.65), (4.09 ± 0.26 to 7.33 ± 0.76) and (24.90 ± 8.08 to 12.43 ± 1.63) respectively in relation with the control groups which showed significant difference ($p<0.05$) in their profile when compared with the control groups.

Conclusion: It was revealed that the plant possesses erythropoietic properties according to the results of some haematological parameters.

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Introduction

The consumption of plant materials is believed to contribute immensely to the improvement of the health of man and animals. It is estimated that 80% of the population of Africa depends on medicinal plants to satisfy their healthcare requirements (Yedjou *et al.*, 2008). *Vernonia amygdalina* commonly called "Bitter leaf" is a medium sized shrub with petiolate green leaf of about 6mm diameter and elliptic in shape, it has approximately more than 23,600 species (Vicki *et al.*, 2009). Vernonia is a genus of about 1,000 species of forbs and shrubs of which *Vernonia amygdalina* is the most prominent specie and one of the pan tropical tribes of the family Asteraceae (Johri and Singh 1997).

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The genus vernonia is named by English botanist William Vernon. Several species of vernonia including *Vernonia calvoana, Vernonia amygdalina*, and *Vernonia colorata* are eaten as leaf vegetables (Akah and Okafor 2004). It grows predominantly in tropical Africa especially in Nigeria, Zimbabwe and South Africa and it is domesticated in parts of West Africa (Johri and Singh, 1997).

In Nigeria, it is known by several local names such as ''Ewuro'' in Yoruba language, ''Onugbu'' in Igbo language, "Oriwo" in Benin language, "Ityuna" in Tiv language, "Chusar doki or fatefate" in Hausa language and "Etidot" in Ibiobio. (Egedigwe 2010), "uzi" in Ebira and elsewhere in Africa, it is called "muop or ndole" in Cameroon, "tuntwano" in Tanzania, and "mululuza"in Uganda (Mbang *et al*., 2008). 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 illnesses. *Vernonia amygdalina* have been shown to be valuable nutritionally. It contains significant quantities of lipids (Ejoh *et al.,* 2007) and 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 (Bonsai *et al.,*1995). Oguntola (2013) pointed out that the leaves of *Vernonia amygdalina* if squeezed and placed on cuts would stop bleeding of injured vessels. It is rich in calcium, vitamin C and saponins. It was observed that calcium was a principal active ingredients present in plasma and bones. It is free calcium ions that are physiologically active in coagulation mechanism. Calcium ions are essential for the conversion of prothrombin to thrombin and for the normal action of heart muscle and for neuromuscular conduction. *Vernonia 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 (Eyo *et al.,* 2013). The roots are used for treatment of gastro-intestinal problems, malaria, tooth ache and fertility problems (Momoh *et al.,*2010). Leaf decoctions are also used to treat diarrhea, dysentery and hepatitis (Eyong *et al.,* 2011).

Materials and Methods

Plant Identification and Authentication

Fresh leaves of *Vernonia amygdalina* used were purchased at a local market (Ganmo market) in Ilorin South Local Government Area, Kwara State. Nigeria. The plants were detached from its stem and it was identified and authenticated by Mr. Bolu Ajayi of the Department of Plant Biology, Faculty of Life Sciences, University of Ilorin, Nigeria. Voucher number, UILH / 001/ 2020 / 1023 was assigned and archived at Herbarium Unit of the University,

Aqueous Plant extraction.

The plant was allowed to air dried at room temperature of 27 ^{0}C, ground into a powder and sieved to regrind the coarse samples. A measure of 200 gram of the powdered sample was soaked in 2 liters of distilled water and the mixture allowed to stand for 48 hours with occasional shaking. The resulting mixture was filtered using cheese cloth *(Nwaoguikpe, 2010).* The filtrate was concentrated at University of Ilorin General Research Laboratory using water bath method at 45^{0}C for the period of one week.

Experimental Design

Thirty (30) Wistar rats weighing between 180-200g per body weight were purchased from Animal House, Department of Biochemitry, University of Ilorin (Unilorin) Kwara state. The rats were kept in animal cage, allowed free access to water and fed for three (3) weeks (Chick Growers Mesh, Guinea Feed, Nigeria). The rats were divided into 5 groups A, B, C, D and E of 6 rats per group. Group A and group B served as normal and negative control respectively, while group C, group D and group E were the treated groups. Rats in each group were placed in separate cages with free access to normal rat meal and water and allowing two weeks of acclimatization to their environment. After acclimatization, the rats in all groups were induced with phenylhydrazine except Group A, via intra-peritoneal injection of 45mg/kg (0.5ml) on day 1 at 9am and 6pm and left for 24hours. After 24 hours, group C, group D and group E were given 100mg/kg, 200mg/kg and 400mg/kg of the plant extracts of *Vernonia amygdalina*, respectively on a daily basis for a period of 14 days, as previously described by Ashour (2014). The rats were subsequently treated as follows:

Group A: Normal control group. Rats in this group received rat meal and water only *ad libitum*

Group B: Phenylhydrazine only - Negative control group. Rats in this group were not treated after administration of the doses of phenylhydrazine but had access to animal feeds and water *ad libitum*.

Group C: Phenylhydrazine plus plant extract. Rats in this group were treated with 100 mg/kg body weight of the extract of the *Vernonia amygdalina* after administration of phenylhydrazine.

Group D: Phenylhydrazine plus Plant extract. Rats in this group were treated with 200 mg/kg body weight

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of the plant extract of the *Vernonia amygdalina* after administration of phenylhydrazine.

Group E: Phenylhydrazine plus plant extract. Rats in this group were treated with 400 mg/kg body weight of plant extract of the *Vernonia amygdalina* after administration of phenylhydrazine (Zara *et al*., 2014).

Blood sample collection

A volume of 5ml of blood sample was collected from each of the rats into EDTA bottles after 2 weeks of the experimental study for laboratory analysis using standardized technique (Joseph *et al.,* 2013).

3.8 Laboratory analysis of samples

Peripheral blood sample was collected in to heparinised capillaries tube for packed cell volume (PCV).

Peripheral blood sample was smeared on the glass slide, fixed and stained with Leishman stain and examine for differential count.

Blood samples was analyzed for haematological indices such as, HGB, RBC, PCV, WBC, MCV, MCH, MCHC, NEUT, LYM and PLT using Haematology Autoanalyzer (Sysmex Haematology Autoanalyzer).

Results

The results were presented as a mean ± standard deviation

Table 1 showed the Mean and Standard Deviation of differences in weight of rats before and after administration of the extract.

There was no significant difference in all groups as regard to their initial and final weights of rats as p-values were higher (p>0.05).

Table 1: Relationship between Initial weight and Final weight

Group	A(normal control)	B (negative control)	C (100mg/kg body weight)	D (200mg/kg body weight)	E (400mg/kg body weight)	p-value
Number of Rat	6	6	6	6	6	
Initial weight(g)	182.08±6.17^{a}	189.89±5.81^{a}	189.40±11.43^{a}	184.85±9.43^{a}	185.90±12.64^{a}	0.76
Final weight(g)	187.60±5.97^{a}	170.88±7.66^{a}	178.55±10.16^{a}	171.78±8.29^{a}	174.50±10.88^{a}	0.10

Table 2, the HGB (g/dl) was significantly different among groups A, group B and group C with p<0.05 when compared to that of group D and group E. However, there was no statistical difference between group D and group E when compared to HGB (g/dl) (p > 0.05), but statistically different from that of groups A, group B and group C. Similarly, there was significant difference in the mean and standard Deviation of PCV between groups B, group C and group D with p<0.05, while the PCV of group A was not statistically different from that of group E. Also, group A, group D and group E were not statistically different from each other in terms of RBC (x10^{12}/L), but the three groups were different from group B and group C (p<0.05). With respect to WBC (x10^{9}/L), there was no significant difference between groups B, group C, group D, and group E, but the four groups were statistically different from group A.

Table 2: Relationship between Haematological Parameters and Different Concentrations

Group	A(normal control)	B (negative control)	C (100mg/kg body weight)	D (200mg/kg body weight)	E (400mg/kg body weight)	p-value
Number of Rat	6	6	6	6	6	
HGB (g/dl)	14.98 ± 1.07^{a}	8.45 ± 0.54^{b}	10.80 ± 0.73^{c}	12.60 ± 0.40^{d}	13.20 ± 0.32^{d}	0.00
PCV (%)	42.00 ± 4.69^{a}	23.50 ± 2.38^{b}	29.50 ± 2.08^{c}	35.25 ± 2.22^{d}	40.50 ± 4.65^{a}	0.00
RBC(x10^{12}/L)	7.41 ± 0.80^{a}	4.09 ± 0.26^{b}	5.11 ± 0.59^{c}	6.46 ± 0.39^{a}	7.33 ± 0.76^{a}	0.00
WBC(x10^{9}/L)	13.20 ± 0.95^{a}	24.90 ± 8.08^{b}	11.73 ± 3.68^{b}	12.48 ± 5.16^{b}	12.43 ± 1.63^{b}	0.00

RBC: Red Blood Cell, HGB: Haemoglobin, PCV: Packed cell volume, WBC: White blood cell.

Table 3 Showed the Mean and Standard Deviation of MCV (fL), MCH (pg) and MCHC (g/dl). There was no significant difference in all groups with regards to red cell indices, as p-values for the three parameters were higher (p > 0.05).

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Table 3: Relationship Between Red cell indices and Concentrations of Extract

Group	A(normal control)	B (negative control)	C (100mg/kg body weight)	D (200mg/kg body weight)	E (400mg/kg body weight)	p-value
Number of Rats	6	6	6	6	6	
[MCV (fL)	56.75 ± 0.50[a]	56.75 ± 1.50[a]	56.75 ± 3.46[a]	58.25 ± 2.06[a]	54.75 ± 0.96[a]	0.09
MCH (pg)	20.00 ± 1.15[a]	20.75 ± 0.50[a]	19.50 ± 2.65[a]	18.25 ± 3.50[a]	34.25 ± 3.54[a]	0.64
MCHC(g/dl)	35.75 ± 1.50[a]	36.50 ± 1.73[a]	31.00 ± 5.77[a]	33.50 ± 6.40[a]	26.00 ± 7.95[a]	0.08

MCV: Mean cell volume, MCH: Mean cell haemoglobin, MCHC: Mean Cell Haemoglobin Concentration

Table 4.4 Showed the Mean and Standard Deviation of LYM (%), NEUT (%), PLT (x10^9/L) in the studied rats across the groups. There was no significant difference in all the groups (Group A -E) with respect to their statistical analysis as p-values for the three parameters were greater than 0.05 (P>0.05).

Table 4.4 Relationship between the granulocytes and Thrombocyte

Group	A(normal control)	B (negative control)	C (100mg/kg body weight)	D (200mg/kg body weight)	E (400mg/kg body weight)	p-value
Number of Rat	**6**	**6**	**6**	**6**	**6**	
LYM (%)	623.00±271[a]	334.50±62.63[a]	633.25±151.41[a]	400.75±264.16[a]	565.50±112.97[a]	0.14
NEUT (%)	95.25 ± 3.59[a]	88.00 ± 10.42[a]	95.75 ± 3.86[a]	92.50 ± 8.43[a]	78.50 ± 19.67[a]	0.20
PLT (x10^9/L)	4.75 ± 3.59[a]	12.00 ± 10.42[a]	4.25 ± 3.86[a]	6.75 ± 6.95[a]	20.50 ± 18.56[a]	0.19

LYM: Lymphocyte, NEUT: Neutrophil, PLT: Platelet

Discussion

This study examined the effects of aqueous leaf extract of *Vernonia amygdalina* on haematological parameters in phenyhydrazine induced anaemia in wistar rats and the parameters at different concentrations of the extract. This study also found that there was no significant difference in the MCV (fL), MCH (pg), MCHC (g/dl), LYM (%), NEUT (%), PLT (x10^9/L), in initial weight and final weight of the rats in the treated and control groups. This result corroborates that of *Jing and Zon* (2011) who found that aqueous leaf extract of *Vernonia amygdalina* does not have effect on MCV, MCHC and LYM in rats. However, the result of this study disagrees with that of *Medvinsky et al., (2011)* who found that aqueous leaf extract of *Vernonia amygdalina* has significant effect on MCV (fL), MCH (pg) and weight of albino rats.

The result of this study revealed that there were significant differences among groups A (normal control), group B (negative control) and group C (100mg/kg body weight) with respect to HGB (g/dl) p<0.05, and there was no statistical difference in HGB (g/dl) between group D and group E (p>0.05) This means that the aqueous leaf extract of *Vernonia amygdalina* showed different level of effect on the HGB of the rats in the different treatment groups. Similarly, there was significant difference in the PCV between groups B (negative control), group C (100 mg/kg body weight) and group D (200 mg/kg body weight), while the PCV of group A (normal control) was not significantly different from that of group E (400 mg/kg body weight). This means that the aqueous leaf extract of *Vernonia amygdalina* exerted different level of effect on the PCV of the rats at different treatment groups. Also, group A, group D (200 mg/kg body weight) and group E were not significantly different from each other with respect to RBC (x10^{12}/L), but the three groups were different from groups B (negative control) and group C. when compare with WBC (x10^9/L), there was no significant difference between groups B (negative control), group C (100 mg/kg body weight), group D (200 mg/kg body weight), and group E (400 mg/kg body weight), but the four groups were statistically different from group A (normal control). This means that the aqueous leaf extract of *Vernonia amygdalina* has no stimulatory effect on the WBC of the rats in the different treatment groups. This result supported the earlier finding of William and Krause (2008), who reported that there was significant effect of *Vernonia amygdalina* on HGB (g/dl) and WBC (x10^9/L) among animals and that the higher the dosage, the better the effect.

This study also found that there was no significant difference in the MCV (fL), MCH (pg), MCHC (g/dl), LYM (%), NEUT (%), PLT (x10^9/L), MXD, initial

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weight and final weight of the rats in the treatment and control groups. This result corroborates that of *Jing and Zon* (2011) who found that aqueous leaf extract of *Vernonia amygdalina* does not have effect on MCV, MCHC and LYM in rats. However, the result of this study disagrees with that of Medvinsky *et al*., (2011) who found that aqueous leaf extract of *Vernonia amygdalina* has significant effect on MCV (fL), MCH (pg) and weight of albino rats.

Conclusion

This study examined the effects of aqueous leaf extract of *Vernonia amygdalina* on haematological parameters in phenylhydrazine induced anaemia in wistar rats, which showed that Aqueous leaf extract of *Vernonia amygdalina* possesses haemopoietic effect on the haematological parameters in phenylhydrazine induced anaemia in wistar rats in the different treatment groups.

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