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Efficacy of The Aqueous Root Extract of *Phyllanthus Muellerianus* In Alleviating Anemia in Rats

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Abstract

Introduction: *Different parts of Phyllanthus muellerianus (P. muellerianus) are used to treat a number of diseases in Zambian traditional medicine.*

Aim: *To evaluate the effect of the aqueous root extract of* P. muellerianus *on the hematological indices of albino rats and determined its phytochemical profile.*

Methods/Design: *Thirty-six rats in six groups were used for the study. The groups comprised 100 mg/kg, 200 mg/kg, and 400 mg/kg plant extract, a group on ranferon, a normal (non-anemic) group and a control (anemic) group. Anemia, induced through bleeding of the rats, was defined as hemoglobin (Hb) < 12 g/dL. The anti-anemic potential of the plant was determined by comparing its effect on the hematological parameters of rats on treatment to that of the control group. The phytochemical profile was determined using standard methods. The results were analysed using SPSS at a significance of P < 0.05*

Results: *After medication, rats on 400 mg/kg dosage showed the greatest increase in the mean values for Hb, Packed Cell Volume (PCV) and Red Blood Cell count of 23.1 %, 23.0 % and 22.2 % respectively, when compared to the control group (P < 0.05). Phytochemical screening revealed positive results for alkaloids, flavonoids, saponins, glycosides, steroids, triterpenoids and tannins.*

Conclusion: *The aqueous root extract of P. muellerianus was efficacious against anemia in a dose-dependent manner and the 400 mg/kg dosage was useful. The phytochemical compositions seem to be responsible for its hematopoietic properties. Thus, the root decoction of the plant is useful in alleviating anemia and the results lend credence to its use in traditional medicine in the management of anemia.*

Key words: *Albino rats, Phytochemicals, Traditional medicine.*

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Introduction

Anemia is a condition in which the oxygen carrying capacity of blood decreases due to reduction in red blood cells (RBCs) or hemoglobin (Hb) concentration in RBCs. It is a common blood disorder that affects people of all ethnicity and ages; although people at greater risk are the elderly, young women of child bearing age and infants (1).

It is among the top 10 causes of morbidity and mortality in Zambia (2). The prevalence of anemia in Zambia is 46 %, which is a severe public health problem based on the World Health Organization (WHO) standards (3, 4). Such high prevalence rate is expected on account that the diet of majority Zambians is mainly composed of cereals (maize) and starchy roots with little micronutrient-dense foods such as animal products and fruits (5, 6). This implies that there is a great loss of man hours of healthy adults who find themselves off work to nurse anemic patients (6, 7).

The causes of anemia are patho-physiologically diverse and multi-factorial. Thus, there are more than 400 types, some are mild while others are severe or even life threatening if not treated (8).

In rodents, symptoms include; rapid or labored respiration, anorexia, immobility, abnormal appearance or posture periocular and nasal porphyrin discharge (9).

Anemia associated with a serious disease is treated by treating the underlying disorder. In some cases, when symptoms persist or worsen, additional medications that boost RBCs may be necessary to avoid life threatening conditions and improve quality of life. For instance, Physicians prescribe iron pills or iron supplements and iron fortifications, synthetically manufactured erythropoietin, blood transfusions and removal of the spleen. Nutritionists also recommend plant products such as legumes, groundnuts, tomatoes and spinach as well as animal products such as liver and red meat (10-12) .

Interventions to prevent or treat Anemia are insufficient in Zambia because of; - inadequate qualified human resources, high disease burden and inadequate emergency facilities (2, 13).

In Africa and most Asian countries, anemia is treated using herbs such as; - *Khaya senegalensis, Justicia secunda,* and *Amaranthus spinosus* (14).

The potential of the root extract of *Phyllanthus muellerianus (P. muellerianus)* to alleviate anemia in albino rats and its phytochemical profile were investigated.

P. muellerianus is part of the indigenous knowledge used to treat anemia in the Northern part of Zambia. However, its efficacy had not been scientifically established. Local names for *P. muellerianus* in Zambia include: *Chewa-Mkuzandola, Tumbuka-Kapikanduzi* (15), *Icibemba-Umupetwalupe, Kaonde-Mulembalemba, Mambwe-Mupetwandupe.* It is classified under the family Phyllanthaceae consisting of approximately 1,000 species which are widely distributed in Africa, Asia, America and Australia. It is a monoecious, glabrous, straggling or small tree of up to 12 meters tall. It occurs in riverine forest and wooded grasslands on deep and well-drained soils. It is cheap and easy to access in Zambia hence the study was biased towards finding medical solutions coming from local flora (16). It has many medicinal uses especially to treat intestinal problems, body pain and as an antiseptic (17).

Materials and Methods

Collection and Preparation of the aqueous root extract of the plant

The roots of the plant were harvested in October 2016 from Kaunda Square area of Lusaka. They were thoroughly washed to remove debris (soil). One kilogram of the roots was boiled in 1.5 litres of distilled water. The resulting solution was allowed to cool then sieved to remove non-soluble plant matter and finally filtered through Whatman filter paper number 4. The mixture was boiled to dryness on a heating mantle, the resulting brittle powder was weighed and kept at 4 ^{o}c until further use. Various Concentrations of the extract were made by dissolving the appropriate quantity of the solid extract in distilled water to make a solution on a mass by volume basis.

Animals and induction of Anemia

UNZABREC granted ethical clearance on REF. No: 005-09-16. Thirty-six male albino rats weighing between 150 and 180 grams were used in the study. All laboratory work was done according to the Guidelines for the Care and Use of Laboratory Animals (9). In addition, they were euthanized in diethyl ether at the end of the study (18).

Anemia was induced by bleeding the rats under light anaesthesia using diethyl ether as an anaesthetic (19, 20). The formula described by Lee and Blaufox (21) was used to determine the quantity of blood removed through bleeding.

Administration of the plant extract

Medication started 24 hours after confirming induction of anemia. Ranferon, the extract and distilled water were administered by oral intubation as shown in Table I.

Collection of blood samples

Rats were anesthetised in diethyl ether, 24 hours after administration of the last extract. When they became unconscious, blood was collected from their retro-orbital plexus for hematological studies (22).

Hematological tests

Hematological tests were done 3 times; - at the baseline of the study, on day 12 to confirm that anemia had been induced and after treatment (23, 24).

Phytochemical Screening

The qualitative phytochemical screening of the plant extract was carried out at the University of Zambia, Department of Chemistry using standard procedures (25, 26).

Mineral quantification of the plant extract

The mineral content was determined by AAS using Perkin-Elmer atomic analyst 400.

Data Processing and Statistical analysis

The mean values were calculated using ANOVA followed by dunnett's test. Results were analysed using SPSS software version 21, at a significance level of $P < 0.05$.

Results

The color of solid extract was dark brown and the percentage yield was 1.6 %. The extractive weight of the plant extract was 16 g and it had the mineral composition of 230.5 mg Fe, 273.5 mg Mn, and 138 mg Zn.

Results for preliminary Phytochemical Screening

Phytochemical tests showed presence of alkaloids, saponins, flavonoids, tannins, triterpenoids and steroids as illustrated in Table II.

Effects of the plant extract on hematological indices

A one-way analysis of variance was conducted to evaluate the research question, **"is the aqueous root extract of *P. muellerianus* useful in alleviating anemia in rats?"** The rats were divided in 6 groups based on the treatment option (Group 1: Control, Group 2: Normal, Group 3: Ranferon, Group 4: 100 mg/kg, Group 5: 200 mg/kg and Group 6: 400 mg/ kg plant extract). The data was normally distributed and the assumption of homogeneity of variances was tenable based upon the results of Levene's test, $P< 0.05$.

Effects of the plant extract on Hemoglobin

The study defined anemia as Hb < 12 g/dL. Post-hoc comparisons using dunnett's test indicated the statistically significant increase in mean Hb values between the control group and the rats on plant extract treatment as follows; group 4, M=1.7833, group 5, M=2.6167, group 6, M=3.5333. The differences were dose-dependent (F 63.948=5, p= 0.000, SEM=.2065, Fig 1.0) while the partial eta squared value was 0.914.

Effects of the plant extract on RBCs

Post-hoc comparisons using dunnett's test indicated statistically significant mean difference at the 400 mg/kg dosage (F 2.975, =5, p=0.024, SEM=.4412, Fig 2.0).

Effects of the plant extract on MCV, MCH and MCHC

Post-hoc comparisons using dunnett's test indicated that the differences in mean values for MCV, MCH and MCHC between the control group and the rats on plant extract treatment were not statistically significant ($p > 0.05$). As for MCHC, the mean value (33.3 g/dL), remained constant at all stages of the study in all groups.

Discussion

Effect of *P. muellerianus* on the hematological parameters

Dosages of 100, 200, and 400 mg/kg of the root extract and ranferon were administered orally to anemic albino rats to monitor their effect compared to that of the control group that did not receive any drug but distilled water. The results demonstrated that the root extract and ranferon were able to restore the hematological indices of experimental animals to normal levels and the significant ($P< 0.05$) effect was found to be dose-dependent. In addition, the partial eta squared values for PCV and Hb were close to 1 suggesting that the aqueous root extract of the plant was both statistically significant and biologically/clinically efficacious against anemia. The Phytochemical and mineral compositions of the root extract seem likely to be responsible for the hematinic effect of *P. muellerianus* and their presence in the plant extract agrees with previous studies (27, 28).

The blood parameters, Hb, PCV, and RBCs together with the level of iron are indicative indices of anemia that could be used to indicate nutritional values of ingested diets as well. MCV, MCH and MCHC are constants for typing anemia hence they were not statistically different ($P>0.05$) when experimental groups were compared to the control group after treatment. They, however, decreased after inducing anemia, indicating microcytosis as their decrease reflects a release of RBCs, which are less saturated in Hb (hypochromia). Therefore, occurrence of anemia observed in this study is attributable to lowered values of these indices and related to the report by Osman et al.,(29). The observed increase in Hb, PCV, and RBCs in experimental rats might be due to the stimulating effect of *P. muellerianus* on hematopoiesis and consequently the production of RBCs into the blood stream, which in turn compensated for anemia.

Moreover, the increase in these hematological parameters was dose-dependent and could be due to high nutritional values of this plant particularly in minerals such as Fe (230.5 mg). Fe plays a significant role in erythropoiesis. It is required for the synthesis of Hb and myoglobin while its deficiency causes anemia. However, the therapeutic potential of *P. muellerianus* could not be established based on available Fe content alone as other factors play a role in its absorption in the body. In this context, Fe is a necessity for the formation of the heme part of Hb as reported by others (8). The high Fe content of the plant under investigation justifies and partly supports the traditional use of its roots in treating anemia. The results also place it under a group of plants with ant-anemic potential, which are richest in Fe content according to the related findings of Koné et al.,(14). However, Schmelzer et al.,(17) reported an insignificant Fe content of 15 mg per gram of dry fruits of *P. muellerianus.*

The plant also had high content of Zinc (138 mg) and Manganese (273.5 mg). Zn is required for the function of over 200 enzymes and is important in growth and sexual development in man. Mn is both nutritionally essential and potentially toxic. It is important for brain and nerve function (can bind with neurotransmitters and stimulate faster or more efficient transmission of electrical impulses throughout the body, in effect, speeding up cognitive function (8).

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The methanolic and ethylacetate leaf extracts of *P. muellerianus* were shown by Assob et al.,(30) to have the minimum inhibition concentration (MIC) of between 0.07 and 1.25 mg/mL and a lethal dose (LD_{50}) of more than 4 g/kg body weight of male and female rats. Based on their study, they concluded that *P. muellerianus* is not toxic, taking into consideration the 5 g/kg threshold of toxic substances. On the other hand, Adedapo et al.,(31) showed that the leaves of *P. muellerianus* significantly ($P< 0.05$) reduced the hematological parameters, had toxic potential and were therefore, poisonous to the animals.

Observed Phytochemicals and their action

Plants used in the treatment of disease contain a wide range of active principles with biological activity, some of which are responsible for the characteristic odours, pungencies and colours of plants while others give a particular plant its culinary, medicinal or poisonous virtues, which could be used as the base for discovering modern drugs for curing various diseases including anemia (27). These chemical principles vary in distribution within the plant parts, as well as their occurrence within plant species. The presence or absence of such compounds depends largely on the extent of accumulation, the amount of plant material used, cultivation period, season of collection, plant-to-plant variability in the medicinal content and the analytical method employed (32). That is why phytochemical screening of plants must be done constantly, even on the ones whose secondary metabolites are already known.

The phytochemical tests performed on the root extract of *P. muellerianus* showed positive results for steroids, triterpenoids, alkaloids, flavonoids, saponins, cardiac glycosides and tannins which were consistent with previous reports (17, 27, 33).

As inferred from other reports, alkaloids, the most revered of all phytochemicals are said to be pharmacologically active and their action is felt in the nervous system, blood vessels, respiratory system, and the gastrointestinal tract. They are antispasmodic, analgesic and have bactericidal effects. They inhibit cyclic adenosine monophosphate (cAMP) phosphodiesterase leading to accumulation of cAMP. This effect stimulates phosphorylation of proteins and synthesis of proteins, which improves erythropoiesis (27, 28).

Saponins are known to; inhibit platelet aggregation and thrombosis, lower the cholesterol levels, have anti-diabetic and anti-carcinogenic properties, have been successfully used in the management of liver inflammation, promote and vitalize blood circulation, as expectorants (cough suppressants) and for hemolytic activities. Since saponins are active agents that lyse the membrane of RBCs or other walls, it is likely that the plant extract used in this study first lysed RBCs. Then the cells overcame this inhibition by producing a glycosidic enzyme that cleaves some of the terminal sugars from the saponins, which detoxified it (27). This detoxification of saponins reinforced the proper use of iron contained in the plant extract allowing it to synthesize heme / hemoglobin for new RBCs, thus leading to an observed improvement of Hb, RBCs and PCV in the plant extract treated groups. Glycosides enhance the natural resistance and have the recovery powers for the body (27).

Flavonoids are significantly recognised for their anti-oxidant, anti-carcinogenic, anti-microbial, anti-tumour properties, anti-inflammatory, anti-allergic, hepato-protective, ant-thrombotic, and anti-viral activities. They are known to possess a well-established protective effect against membrane lipoperoxidative damages. The antioxidant activity of phenols and flavonoids is mainly attributed to their redox properties because of which they act as reducing agents, electron / hydrogen donators, and singlet oxygen quenchers. In addition, they have a metal chelating potential. It has also been demonstrated that the antioxidants such as flavonoids can act: either by neutralizing reactive oxygen species (ROS) by directly reacting with superoxide anion, nitric oxide and peroxynitrite thereby preserving vascular function and protecting vascular injuries from ROS and perhaps from other oxidant species, or they could stimulate erythropoiesis (34)

Tannins are well known for their anti-oxidant, anti-inflammatory, diuretics, anti diarrhoeal, anti-microbial, astringent properties, anti-tumor, anti-HIV activity as well as for soothing relief, and skin regeneration. Tannins from many plants especially Euphorbiaceae are used to treat cells that have gone neoplastic, and to stop bleeding during circumcision. They have been used traditionally as styptic and internally for the protection of inflamed surfaces of the mouth and throat. Steroids regulate carbohydrate and protein metabolism and possesses anti-inflammatory properties. This might correspond to their ethnomedicinal significance (27).

Conclusion

P. muellerianus was efficacious against anemia in a dose dependent manner, $P < 0.05$. The most useful dosage in alleviating anemia was 400 mg/kg. The rich array of phytochemicals (especially alkaloids, saponins, and flavonoids) and high iron compositions seem likely to be responsible for its hematinic effect. Further studies are needed with this plant to evaluate the anti-anemic active components, and to elucidate their mode of action.

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Tables and Figures

Table I. Treatment options for the rats

Group (n=6)	**Condition of the rats**	**Treatment option**
1	Normal (non-anemic)	distilled water
2	Control (Anemic)	distilled water
3	Anemic	200 mg ranferon
4	Anemic	100 mg/kg
5	Anemic	200 mg/kg
6	Anemic	400 mg/kg

Table II. Results for the preliminary phytochemical screening of the extract

Test	Observation	Result
Alkaloids	Turbidity	+++
Steroids	Green-blue colour	++
Triterpenoids	Violet upper layer	++
Flavonoids	Red colour	+++
Saponins	Stable foam	+++
Glycosides	Brown ring	+++
Tannins	Green precipitate	+++

Key: Negative, + Trace, ++ Moderate, and +++ High

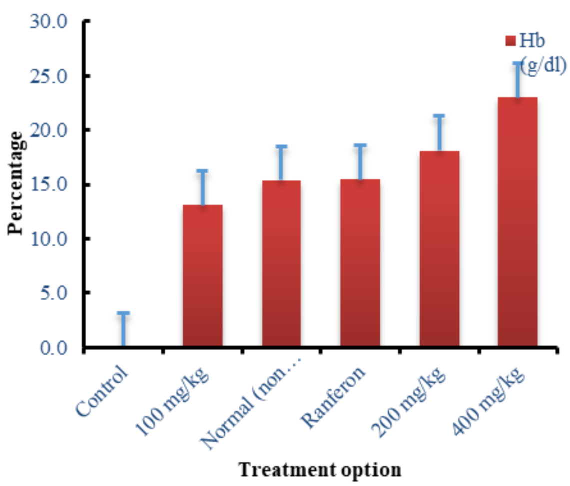

Fig 1.0 Comparisons in Hb after treatment. The percentage increase in Hb for the experimental groups on plant extract from the control group were 13.1, 18.1 and 23.1 % at the dosages of 100, 200 and 400 mg/kg respectively, $P < 0.05$. The same results were obtained for the PCV.

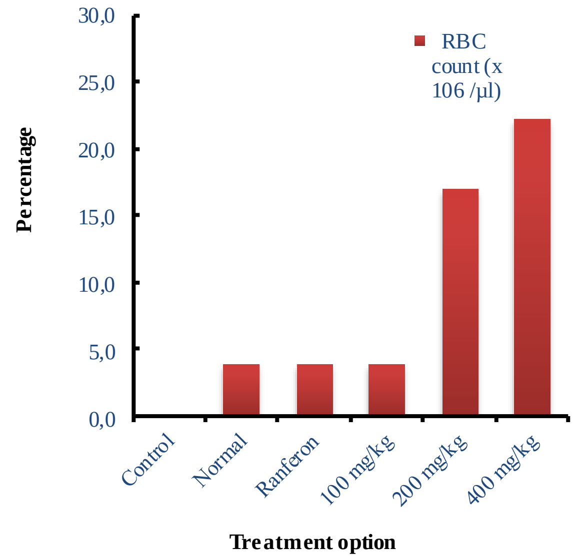

Fig 2.0 Comparisons in RBC. The percentage differences after treatment were that, there was an increase in RBCs in a dose dependent manner with the greatest increase (22.2 %) observed at the 400 mg/kg dosage, $P < 0.05$.

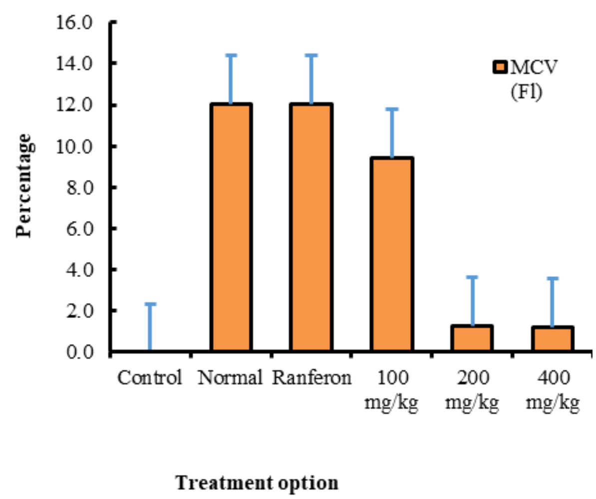

Fig 3.0 Percentage differences in MCV after treatment. The same values were obtained for MCH.