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Effects of Methanol Leaf Extract of *Mucuna pruriens* on Male Anaemic Wister Rats

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ABSTRACT

Anaemia is a widespread blood condition characterised by a lack of red blood cells or a drop in haemoglobin levels, which results in decreased oxygen-carrying ability. It is a worldwide public health issue that affects people of all ages, genders, and socioeconomic situations. This study was aimed at analyzing and determining the proximate and phytochemical compositions of *Mucuna pruriens* and its antianaemic activities on male anaemic wister rats. Healthy *Mucuna pruriens* leaves were harvested in Wukari, Taraba State, Nigeria. For ten days, the plant samples were shade dried. Using a mortar and pestle, the dry sample was reduced to powder. The coarse particles were sieved, yielding a fine powder. Extraction of *M. pruriens* was done by cold maceration with 100% methanol. Twenty (20) animals were grouped into four groups (n=5), anaemic control (group 1) was left untreated, standard control (group 2) received ferrous sulphate (75mg/kg body weight) while group 3 and 4 received 100mg/kg and 200mg/kg body weight methanolic extract of *M. pruriens* respectively, treatment lasted for 2 weeks and 5 days. Haematology auto analyser was used for haematological parameters. The results showed that *M. pruriens*, has high content of organic matter, moderate content of crude fibre, low ash content and much lower moisture and lipid contents. Saponin, tannins and glycosides were also present. There was a dose-dependent increase in packed cell volume (PCV), platelet, and Red blood cells (RBC) with significance of $p < 0.05$. There was no significant $p > 0.05$ difference in White blood cells (WBC), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), lymphocyte and neutrophil. There was a significant increase weight in groups treated with aqueous extract of *M. pruriens* as compared to groups untreated and standard control which reveal a reduction in weight of rats as compared to day one. The results suggest that *M. pruriens* plant is relatively safe and possesses a significant anti-anaemic property; therefore, it may be a potential lead in the discovery of drug for treatment of hemolytic anaemia.

Keywords: *Mucuna pruriens*; Anaemia; Red blood cells; Proximate analysis; Wister rats

INTRODUCTION

Anaemia is a widespread blood condition characterised by a lack of red blood cells or a drop in haemoglobin levels, which results in decreased oxygen-carrying ability. It is a worldwide public health issue that affects people of all ages, genders, and socioeconomic situations (Fatima *et al.*, 2018; Whitney and Rolfes, 2013). It has been stated that it affects roughly 24.8% of the total global population, with approximately 1.62 billion people affected (Amao, 2018; Geurts, 2021). Nutritional deficits, chronic illnesses, genetic problems, and some drugs can all induce anaemia (Bhadra and

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Deb, 2020; Alderman and Horton, 2007). The most common types of anemia include iron-deficiency anemia, caused by inadequate iron intake or absorption, and vitamin-deficiency anemias, such as folate deficiency anemia and vitamin B12 deficiency anemia (Akindele and Busayo, 2011; Yakubu *et al.*, 2019). Iron-deficiency anemia is the most prevalent form of anemia globally and is often associated with malnutrition (Bagalkotkar *et al.*, 2006; Obioma *et al.*, 2014). Inadequate dietary intake of iron or conditions that affect iron absorption, such as gastrointestinal disorders, can lead to diminished iron stores and subsequent anemia. Similarly, deficiencies in folate and vitamin B12, often linked to malnutrition, can impair red blood cell production and result in anemia (Braunstein, 2020; Sarode, 2021).

While malnutrition itself does not directly cause hemolytic anemia, malnutrition, including deficiencies in key nutrients can contribute to the development and severity of anemia by affecting red blood cell production or increasing their destruction (Rivella, 2019; Ejeh *et al.*, 2022; Ekele, 2023). Malnutrition, particularly deficiencies in essential nutrients such as iron, folate, and vitamin B12, can impair the synthesis and maturation of red blood cells, leading to anemia (Stefanova *et al.*, 2017; Edogbanya *et al.*, 2023). Iron deficiency is a common nutritional deficiency globally, affecting an estimated 1.62 billion people worldwide (Nai *et al.*, 2015; Rauner *et al.*, 2019). In developing nations like Nigeria, nutritional iron deficiency is estimated to two-thirds of children and of women of childbearing age to have iron deficiency without anemia; one-third of them have iron deficiency with anemia (Abu *et al.*, 2023; Ayo *et al.*, 2023). Inadequate dietary intake of iron or poor absorption due to malnutrition can compromise hemoglobin synthesis, leading to reduced red blood cell production (Soundarya, 2016; Cappellini *et al.*, 2017). Severe iron deficiency can increase the susceptibility to hemolytic anemia, particularly in vulnerable populations such as pregnant women and young children. Folate and vitamin B12 deficiencies are also significant contributors to anemia (Lill *et al.*, 2015; Braymer *et al.*, 2017). Globally, it is estimated that 1.6 billion people suffer from folate deficiency, while approximately 250 million people have vitamin B12 deficiency (Rohner *et al.*, 2007; Atkinson *et al.*, 2014). Inadequate intake or poor absorption of these nutrients can impair DNA synthesis and result in impaired red blood cell production or abnormal red blood cell function, increasing the risk of anemia, including hemolytic anemia (Tabasum *et al.*, 2018). Furthermore, malnutrition weakens the immune system, making individuals more susceptible to infections that can cause hemolytic anemia (Harper *et al.*, 2015). For example, malaria is a significant cause of hemolytic anemia globally, particularly in sub-Saharan Africa (Green *et al.*, 2017; Miller, 2018). Malnourished individuals are more vulnerable to malaria infection due to compromised immune function and are at a higher risk of developing hemolytic anemia as a result (Le *et al.*, 2010; Paulson *et al.*, 2011; Kautz *et al.*, 2014; Koury, 2015). Treatment for anemia depends on cause and severity, accompanying these factors are the side effect that clearly sets in, but the dependence on natural remedies (herbal medicine: the use of active ingredients in plant with ameliorating potentials) has not been given the wave as an all-round accepted means for treatment of anemia (Nairz *et al.*, 2018; Ganz, 2019; Weiss *et al.*, 2019). There is an urgent need to provide alternative and cheaper means for the management and treatment of anemia (Akindele and Busayo, 2011; Ogamba *et al.*, 2011). Unlike most pharmaceuticals which are based on a single active ingredient targeting just one haematological component, plant derived agents are rich in mixture of therapeutically active phytochemicals whose activities positively restore a normal physiological state (Kavitha and Thangamani, 2014; Eze *et al.*, 2017) (Rayavarapu and Kaladhar, 2011). It is therefore, important to try and establish if the extracts of *M. pruriens* exerts its activities through alteration of haematological parameters. This study is designed to assess the effect of the extracts of *M. pruriens* on anemic albino rat as well as proximate analysis of the study plant to ascertain the validity of the information contained in literatures.

MATERIALS AND METHODS

Plant Material

In November 2021, healthy *Mucuna pruriens* leaves were harvested in Wukari, Taraba State, Nigeria. For ten days, the plant samples were shade dried. Using a mortar and pestle, the dry sample was reduced to powder. The coarse particles were sieved, yielding a fine powder.

Preparation of Plant Extracts

For the extraction, the cold maceration process was used. The Trease and Evans (2009) method was used with minor changes.

Extraction Procedure

500mL of 100% methanol was added to 250g of the roughly powdered crude material (*M. pruriens*). The mixture was wrapped in foil paper and left at the ambient temperature for 7 days, with continuous agitation every 6 hours. The mixture was then sieved, strained, and the marc (damp compact substance) was pressed. As fine solvent extract was produced, the solvent extract was passed through Whatman No 1 filter paper. To generate the oily-sticky extract, the methanolic extract was purified by evaporating the solvent (100% methanol) at 50 C using a rotary evaporator and vacuum oven. It was subsequently immersed in 80% methanol for aqueous extract of *Mucuna pruriens* (AEMP), which was kept in the refrigerator prior to treatment.

Proximate Analysis

The AOAC (2005) technique was used to estimate the moisture content, crude fibre, ash content, crude fat, and protein content of the powdered sample.

Determination of Moisture Content

The AOAC (2005) technique was used to assess moisture content. Washed porcelain plates were dried for roughly 2 hours in a gallenkemp oven at 100°C before being cooked in desiccators and reweighed. 2g of the material was put into the weighted dishes and baked for 24 hours at 100°C. The sample dish was chilled in desiccators, weighed, and dried again until a stable weight was achieved. The % moisture was estimated using the following formula:

$$\text{Moisture content} = \frac{X - Y}{Y} \times \frac{100}{1}$$

Where;

X=Initial weight of sample; Y=Final weight of sample

Determination of ash content

The AOAC (2005) technique was used to assess moisture content. Washed porcelain plates were dried for roughly 2 hours in a gallenkemp oven at 100°C before being heated in desiccators and reweighed. 2g of the material was put into the weighted dishes and baked for 24 hours at 100°C. The sample dish was chilled in desiccators, weighed, and dried again until a stable weight was achieved. The % moisture was estimated using the following formula;

$$\frac{\text{Weight of ash}}{\text{Weight of sample}} \times \frac{100}{1}$$

Determination of nitrogen content

The nitrogen content of the sample was determined using the Micro-Kjeldahl technique. A 100 centimetre Kjeldahl digestion flask was filled with two grammes of dried powdered material. The material was gently digested using a Kjeldahl digestion tablet and 10 cm of concentrated tetraoxosulphate (VI) acid until foaming ceased. The digest was cooked till it became clear. The liquid was filtered into a 100 cm volumetric flask and filled to the top with distilled water. 10 cm of the aliquot solution and 20 cm of 45% sodium hydroxide solution were steam distilled in a distillation flask. The freed ammonia was collected using a 50 cm 20% boric acid mixed indicator solution, chilled, and titrated with a standard 0.01M HCl solution. A similar procedure was used for blank determination. T 1.75%, where T is the titre value, was used to compute the percentage crude protein.

Carbohydrate Contents

Total carbohydrate content was calculated by deducting crude protein, moisture, fat, fibre, and ash content from 100%. The overall carbohydrate content of each sample differed.

Carbohydrate = 100 - (% protein + % fat + % ash + % Crude fibre + % moisture).

Determination of crude lipid content

2 g of dry specimens were measured into a porous thimble, whose mouth was sealed with cotton. The thimble was positioned above a receiving flask holding petroleum ether (B.P 40-60°C) and below a condenser in an extraction chamber. To extract the crude lipid, the flask was heated on a hot mantle for eight hours. Following the procedure of extraction, the thimble was taken away from the Soxhlet and the device was reassembled and heated over a water bath to recover the solvent. The crude lipid flask was removed, cleaned with a dry towel, oven dried at 100°C for 30 minutes, cooled in a dessicator, and weighed. The weight difference is given as a percentage of crude lipid content.

Determination of Crude Fibre Content

Acid-base digestion with 1.25% H₂SO₄ (w/v) and 1.25% NaOH (w/v) solutions was used to estimate crude fibre. After crude lipid extraction, a 60 cm³ beaker was filled with 200 cm³ of boiling 1.25% H₂SO₄. After boiling for 30 minutes, the contents were cooled, filtered through filter paper, and the residue rinsed with three 50 cm³ volumes of hot water. Returning the drained residue to the original beaker, 200 cm³ of boiling 1.25% NaOH was added. The content was cooked for 30 minutes, purified, cleaned as described above, and the residue was drained. The calculations involved were as follows;

$$\% \text{ Crude fibre} = \frac{X_2 - X_3}{X_1} \times \frac{100}{1}$$

Where; X₁= Weight of sample, X₂= Weight of dried sample and, X₃= Weight of ashed sample.

PHYTOCHEMICAL SCREENING

Phenols

Total phenols were determined using a spectrophotometric technique (AOAC, 2005). For 15 minutes, the fat-free sample was boiled in 50 ml of ether to extract the phenolic component. 5 mL of the extract was pipetted into a 50mL flask, followed by 10 mL of distilled water. There was also 2 mL of ammonium hydroxide solution and 5 mL of strong amylalcohol added. The samples were prepared to the specification and left to react for 30 minutes to allow for colour development. The wavelength was measured at 505 nm.

Alkaloid

The Harborne (1983) approach was used to determine this. 5g of the material was weighed into a 250 ml beaker, and 200 ml of 10% acetic acid in ethanol was poured in and sealed for 4 hours. This was filtered, and the extract was concentrated to one-quarter of its original volume in a water bath. Drop by drop, concentrated ammonium hydroxide was added to the extract until the precipitation was finished. Allowing the situation to settle, the precipitated was collected, washed with weak ammonium hydroxide, and then filtered. The residual is the dried and weighed alkaloid.

Saponin

Obadoni and Ochuko's (2001) approach was employed. The samples were pulverised, and 20g of each was placed in a conical flask with 100 cm³ of 20% aqueous ethanol. The samples were heated in a hot water bath approximately 90°C. The concentrate was placed in a 250 ml separatory funnel, then 20 ml of diethyl ether was poured into it and vigorously agitated. The aqueous layer was saved, but the ether layer was not. The purifying procedure was carried out once again. N - butanol (60 mL) was added. The n-butanol extracts were rinsed twice with 10 ml of 5% aqueous sodium chloride. In a waterbath, the residual solution was heated. Following evaporation, the samples were oven dried to a consistent weight, and the saponin concentration was measured as a percentage.

Flavonoid

The flavonoid was identified using the Bohm and Kocipai - Abyazan (1994) technique. At room temperature, 100 ml of 80% aqueous methanol was used to extract around 10g of the plant material. The entire solution was filtered using Whatman No 42 (125 mm) filter paper. The filtrate was then transferred to a crucible, dried over a water bath, and measured to a consistent weight.

Tannins

Tannin was determined using the Van - Burden and Robinson (1981) technique. A 50 ml plastic container was filled with 500mg of the sample. In a mechanical shaker, 50 cc of distilled water was inserted and agitated for 1 hour. This was filtered into a 50 ml volumetric flask and adjusted to the correct volume. The filtered sample was pipetted into a test tube along with 2 ml of 0.1 M FeCh in 0.1 NHC1 and 0.008 M potassium Ferro-cyanide. Within 10 minutes, the absorbance was determined at 720 nm.

Experimental Animal and Design

A total of twenty-four male albino rats were collected. The animals were kept in regular laboratory settings and had free consumption of standard finisher nourishment and water for one week prior to the start of the trials. Twenty (20) Albino rats ranging 130-191g (eight weeks old) were divided into four groups (n=4).

Induction of Anaemia

Anaemia was generated using Cyril *et al.* (2017)'s approach of intraperitoneal phenylhydrazine injection (40 mg/kg/body weight) over two days (Day 0 and Day 1).

Determination of Hematological Parameters

Animals (albino rats) were put to death under chloroform at the end of the experiment. A blood sample was taken through heart puncture. For each of the four groups, 3mL of blood was obtained into an EDTA sample bottle for haematological analysis, and sample bottles were labelled accordingly. Using an auto Hema analyzer, test PCV, MCHC, RBC, MCH, MCV, platelets, WBC, neutrophil, eosinophil, and lymphocyte.

Data Analysis

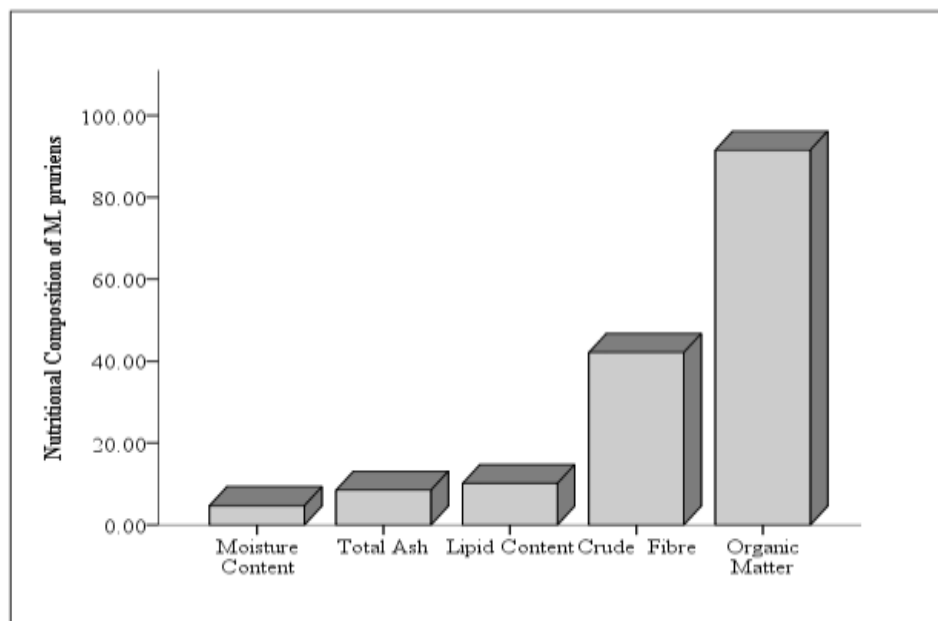
The data are provided as mean standard deviation (SD) from four animals. The varied means in each group were compared to those of day 0 (D0) and day 2 (D2) using one-way analysis of variance (ANOVA) followed by Dunnett's test. The statistical difference was judged significant at P 0.05.

RESULTS AND DISCUSSION

Proximate and Phytochemical Composition, and Percentage Yield of *M. pruriens*

The percentage yield of *Mucuna pruriens* extracted following cold maceration with methanol as the extraction solvent and the use of a rotary evaporator and vacuum oven was 17.6%. The approximate composition of *M. pruriens* is presented in Figure 4.1 below. The proximate composition of *Mucuna pruriens* indicated an extremely high level of organic matter, a moderate quantity of crude fibre, low quantities of lipid and total ash, and a little amount of moisture (Figure 4.1).

Mucuna pruriens has a small amount of saponin and flavonoid, as well as steroid, glycoside, and tannins, according to the qualitative phytochemical contents found (Table 4.1).

Figure 4.1: Proximate composition of *M. pruriens*.Table 4.1: Phytochemical constituents in *Mucuna pruriens*.

Phytochemical	Result
Flavonoids	+
Glycosides	++
Saponin	+
Steroid	++
Tannins	++

Key: - = absent, + = slightly present, ++ = Present, +++ = Heavily present.

Effect of Aqueous extract of *M.pruriens* on PHZ induced Anaemic Wistar Rats Body weight and PCV

The effect of *M. pruriens* aqueous extract on body weight and changes in PCV during and after therapy was studied. When the test groups were compared to the control groups on day one, there was no significant change in the weight of the rats ($F(3, 8) = [2.27]$, $p = 0.158$). On day nineteen, however, there was a substantial ($F(3, 8) = [14.52]$, $p = 0.001$) difference in body weight between at least two groups. One-way ANOVA was used to analyse the data, with significance set at $p < 0.05$. Dunnett's t-test indicated a significant difference in rat body weight between groups administered with 100mg/kg AEMP ($p = 0.009$) and 200mg/kg AEMP ($p = 0.006$) (Figure 4.2).

On day one, a one-way ANOVA revealed that the mean difference in PCV levels between groups was significant ($F(3, 8) = [9.06]$, $p = 0.006$), whereas PCV levels in groups treated with ferrous sulphate (SC), 100mg/kg AEMP, and 200mg/kg AEMP were significantly different from the untreated at $p = 0.006$, $p = 0.016$, and $p = 0.023$, respectively. On day seven nonetheless, there was no statistically significant ($F(3, 8) = [1.50]$, $p = 0.287$) difference in PCV levels

between at least two groups. Finally, PCV levels on day 19 were statistically different across groups ($F(3, 8) = [28.03]$, $p = 0.001$). Finally, PCV levels on day 19 between groups were statistically different ($F(3, 8) = [28.03]$, $p = 0.001$), Dunnett's t-test showed that standard control and groups that received 100mg/kg and 200mg/kg of aqueous extract of *Mucuna pruriens* were significantly different with $p = 0.002$, $p = 0.001$ and $p = 0.001$ respectively as compared to the untreated group at a dose-dependent increase accordingly (Figure 4.3).

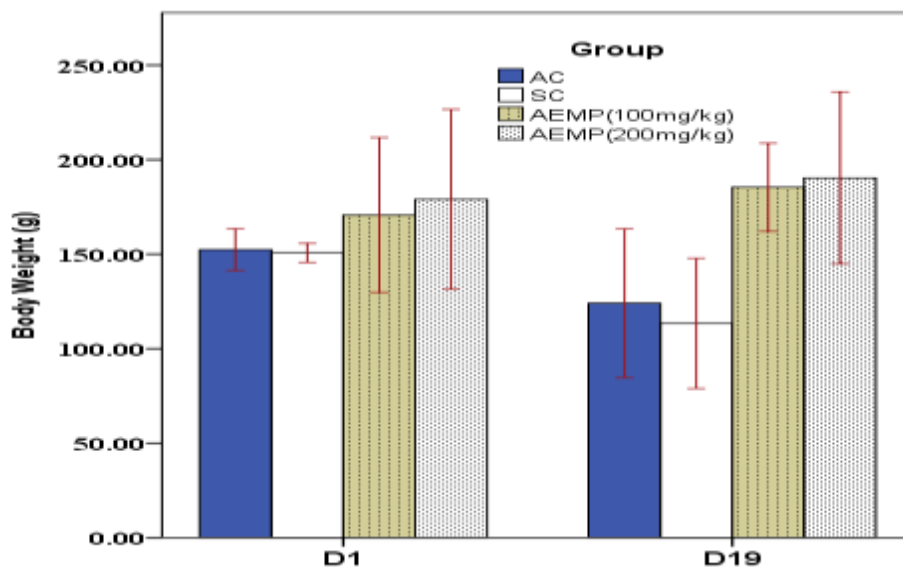


Figure 4.2: Effect of Aqueous extract of *M.pruriens* on PHZ induced Anaemic Wistar Rats body weight.

Values are mean \pm SD, $p < 0.05$ is considered significant. D1 = First day before commencement of treatment, D19 = Nineteen day, before animals were sacrificed. AC = Anaemic control: received no treatment, SC = Standard control: received ferrous sulphate, AEMP = groups that received 100mg/kg and 200mg/kg of aqueous extract of *Mucuna pruriens* (AEMP). Body weight in gram scale. $P < 0.05$ is considered significant.

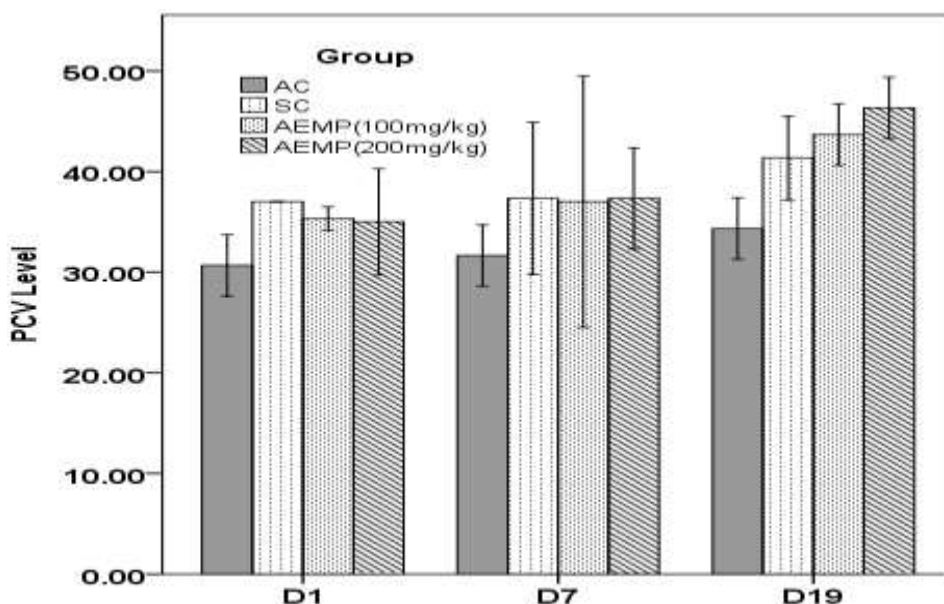


Figure 4.3: Effect of Aqueous extract of *M. pruriens* on PHZ induced anaemic Wistar rats PCV.

Values are Mean \pm SD. AC = Anaemic control group; SC = Standard control group: received ferrous sulphate; AEMP(100mg/kg) = Group treated with 100mg/gk aqueous extract *Mucuna pruriens*; AEMP(200mg/kg) = Group treated with 200mg/kg aqueous extract of *M. pruriens*. D1 = Day one, before extract was administered; D7 = Seventh days of treatment; D19 = Day 19, after animals were sacrificed. $P < 0.05$ is considered significant.

Effect of Aqueous extract of *M. pruriens* on Haematological Parameters of PHZ induced anaemic Rats

Haematological auto analyzer was used to screen for the haematological indices reported below. Results are from blood sample obtained after sacrificing the rats. The effect of methanolic extract of *Mucuna pruriens* on RBC, WBC and platelet count showed that there was not a statistically significant difference ($F(3,8) = [2.32]$, $p = 0.153$), ($F(3,8) = [2.05]$, $p = 0.185$) and ($F(3,8) = [1.83]$, $p = 0.220$) respectively between groups as shown in Figure 4.4 and Figure 4.5.

Results for MCH, MCV and MCHC respectively showed no significant difference ($F(3,8) = [1.30]$, $p = 0.342$), ($F(3,8) = [0.933]$, $p = 0.468$) and ($F(3,8) = [2.67]$, $p = 0.118$) between at least two groups treated according to one-way ANOVA (Figure 4.6).

Results for neutrophil, eosinophil and lymphocyte from one-way analysis of variance showed that there is not a statistically significant difference ($F(3,8) = [1.04]$, $p = 0.427$), ($F(3,8) = [1.04]$, $p = 0.427$) and ($F(3,8) = [0.89]$, $p = 0.485$) respectively, in neither of neutrophil, eosinophil nor lymphocyte between groups of such indices (Figure 4.7).

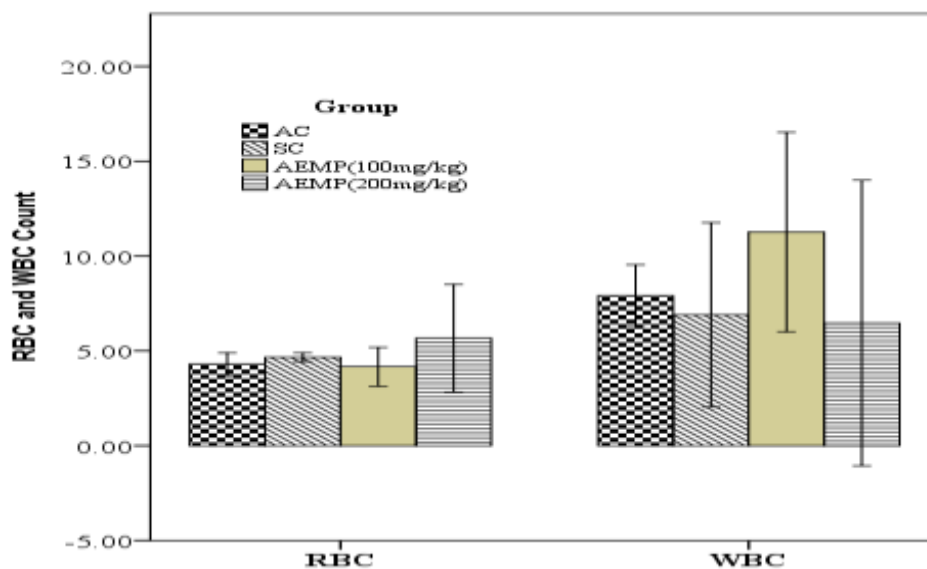


Figure 4.4: Effect of Aqueous extract of *M. pruriens* on RBC and WBC count in PHZ induced anaemic Rats.

Values are Mean \pm SD. AC = Anaemic control group; SC = Standard control group: received ferrous sulphate; AEMP(100mg/kg) = Group treated with 100mg/gk aqueous extract *Mucuna pruriens*; AEMP(200mg/kg) = Group treated with 200mg/kg aqueous extract of *M. pruriens*. $P < 0.05$ is considered significant. RBC = Red blood cell count, WBC = White blood cell count.

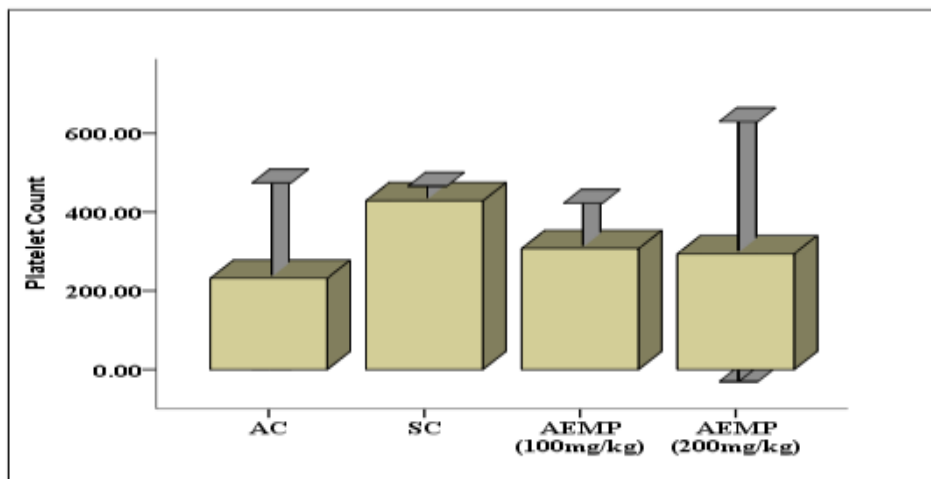


Figure 4.5: Effect of Aqueous extract of *M. pruriens* on Platelet count in PHZ induced anaemic Rats.

Values are Mean \pm SD. AC = Anaemic control group; SC = Standard control group: received ferrous sulphate; AEMP(100mg/kg) = Group treated with 100mg/gk aqueous extract *Mucuna pruriens*; AEMP(200mg/kg) = Group treated with 200mg/kg aqueous extract of *M. pruriens*. $P < 0.05$ is considered significant.

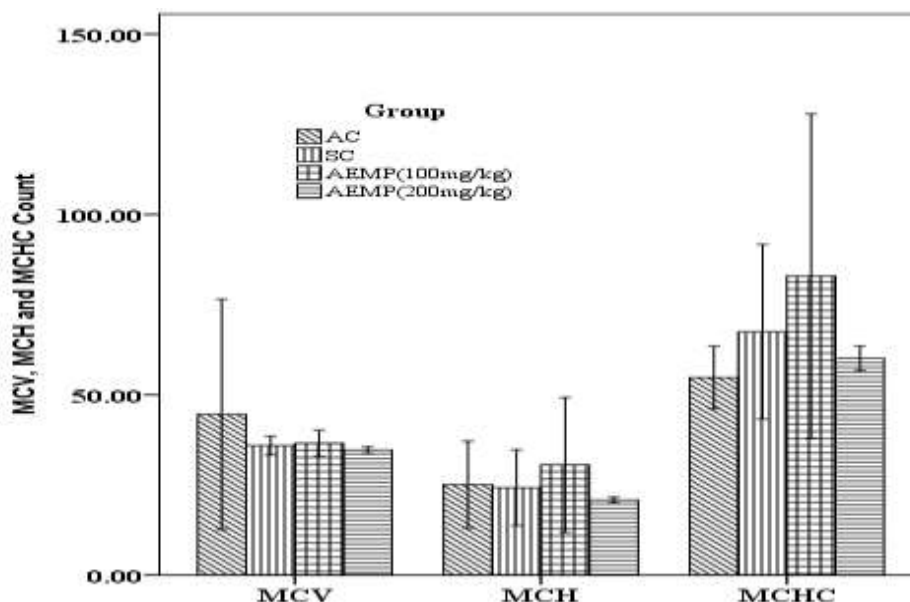


Figure 4.6: Effect of Aqueous extract of *M. pruriens* on Platelet count in PHZ induced anaemic Rats.

Values are Mean \pm SD. AC = Anaemic control group; SC = Standard control group: received ferrous sulphate; AEMP(100mg/kg) = Group treated with 100mg/gk aqueous extract *Mucuna pruriens*; AEMP(200mg/kg) = Group treated with 200mg/kg aqueous extract of *M. pruriens*. $P < 0.05$ is considered significant. MCH = Mean Corpuscular Haemoglobin, MCV = Mean Cell Volume, MCHC = Mean Cell Haemoglobin Concentration.

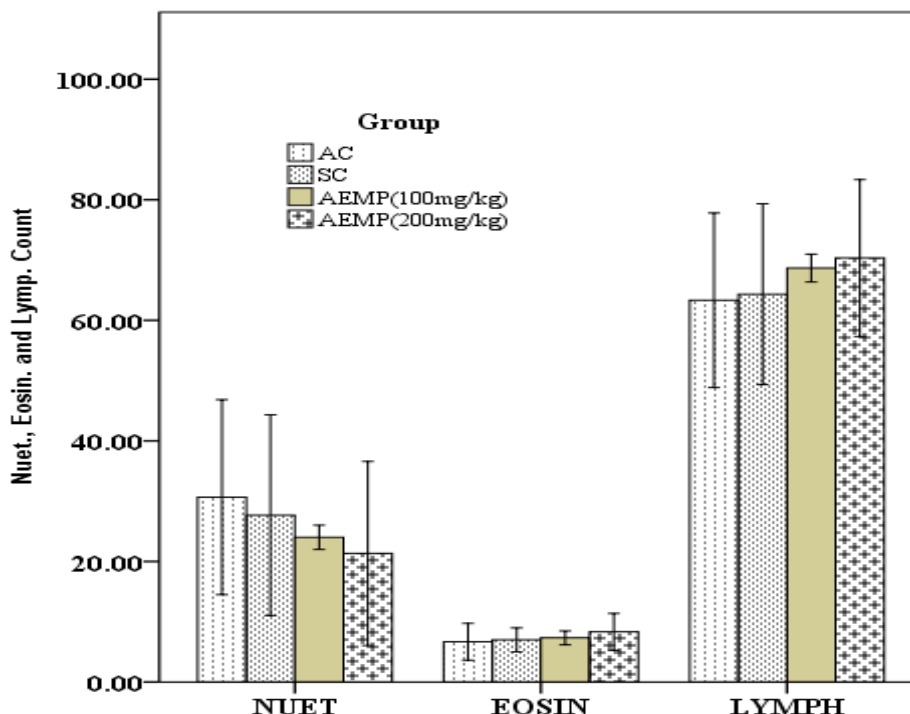


Figure 4.7: Effect of Aqueous extract of *M. pruriens* on Platelet count in PHZ induced anaemic Rats.

Values are Mean \pm SD. AC = Anaemic control group; SC = Standard control group: received ferrous sulphate; AEMP(100mg/kg) = Group treated with 100mg/gk aqueous extract *Mucuna pruriens*; AEMP(200mg/kg) = Group treated with 200mg/kg aqueous extract of *M. pruriens*. $P < 0.05$ is considered significant. NEUT = Neutrophil Count; EOSIN = Eosinophil Count; LYMPH = Lymphocyte Count.

DISCUSSION

The increases in haematological indices (PCV and Eosinophil count, RBCs, and MCH) seen in anaemic rats treated with *Mucuna pruriens* aqueous extract are indicative of its haematopoietic activities. The anti-anaemic efficacy showed with dosages of *Mucuna pruriens* methanol leaf extract corresponds with that of Obioma *et al.* (2014), who evaluated the anti-anaemic potentials of *Mucuna pruriens* aqueous extract and raw leaves. Similarly, Madukwe *et al.* (2014) found that fresh and shade-dried *Mucuna pruriens* leaf extracts significantly enhanced haemoglobin, packed cell volume, and white cell counts in anaemic rats. Ravindra and Ashvini (2019) listed *Mucuna pruriens* as a plant with anti-anaemic properties in a review paper. Furthermore, Nweze *et al.* (2017) stated that fresh *M. pruriens* leaves have been cleaned and marinated in water to create a decoction that is consumed to increase blood flow. *Mucuna pruriens*' anti-anaemic action may be related to the presence of blood-forming components in the leaf extract (Naman *et al.*, 2020). Phytochemical analysis of plant extracts indicated the presence of saponins, flavonoids, steroids, tannins, and glycosides, which coincides with the findings of Nweze *et al.* (2016) and Naman *et al.* (2020). Certain flavonoids and alkaloids have been shown to exhibit antioxidant action. Thus, the existence of these antioxidants in *M. pruriens* leaf extract may aid in the reversal of phenyl hydrazine's detrimental effect on red cells. Phenyl hydrazine has been shown to cause anaemia by increasing the generation of reactive oxygen species in the red cell membrane (Lavanya *et al.*, 2018). Alkaloids and flavonoids, on the other hand, protect cells as potent antioxidants that inhibit or correct damage to red cells caused by free radicals or oxygen species that are highly reactive (Eze *et al.*, 2017).

The body weight of laboratory animals is utilised as an objective marker of pain and suffering (Ghasemi *et al.*, 2021) and is an indicator of animal distress (Rauner *et al.*, 2019). The improvement in body weight of rats that received methanolic extract of *M. pruriens* might be attributable to the extract's nutritional impact, among other things. Body weight loss of more than 20% is considered significant suffering in animal studies and is a possible measure for human

endpoint decisions provided a terrible result is foreseen (Madukwe *et al.*, 2014). Furthermore, weight loss of up to 20% combined with food and water consumption of 40% of normal for 72 hours has been considered a moderate sign of distress and pain in laboratory animals, and weight loss of > 25% combined with food and water consumption of 40% of normal for seven days or anorexia is a significant sign of discomfort and pain in laboratory animals (Baumans *et al.*, 1994). Body weights give an objective assessment of the health and/or growth of experimental animals (Atkinson *et al.*, 2014). In adults, failure to keep a healthy body weight or failure to attain predicted body weight in developing animals implies problems.

CONCLUSION

In conclusion, the findings of the study on phenylhydrazine (PHZ)-induced anaemia in Wistar strain albino rats orally given the methanolic leaf extract of *M. pruriens* demonstrated that the extracts increased blood production, implying antianaemic potentials that can be used in folklore medicine to treat anaemia. The physical impact of *Mucuna pruriens* methanolic extract was weight increase in rats. The change was dose-dependent as those with 200mg/kg were found with more mass body index compared to those treated with 100mg/kg of the plants extract. PCV change in animals was found significant from day 7 of the treatment. Platelets increase was also dose-dependent. The activity of M. pruriens on anaemic rats showed an ameliorative property which signifies that the plant can be useful in treatment of nutritional disorder of iron deficiency.

Findings from this study showed that the plant *Mucuna pruriens* is able to restore cellular part of the blood. Anaemia is seen as a nutritional disorder characterized by decreased level of blood cells especially the RBC, with *M. pruriens* effect anaemic rats, *M. pruriens* can be used for treatment of IDA. The study was based on qualitative active metabolite in *M. pruriens* which indicate a need for its quantitative composition and activity targeted at ameliorating anaemic cases. The active ingredient responsible for the antianaemic activity was not investigated, this gives room for further research in this aspect.

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