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Research Article

Mucuna Pruriens (Karara) Leaf Extracts Enhance Certain Haematological Parameters in Albino Rats

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Abstract

Background: This study is aimed at investigating the effects of *Mucuna pruriens* on the haematology of albino rats. *Mucuna pruriens* is a plant with medicinal properties that have been used in traditional medicine for the treatment of various ailments.

Methods: The study was conducted using albino rats as the animal model. The rats were divided into three groups: a control group and two treatment groups; group 1 (control), received 5.0 ml/kg body weight of distilled water per animal; while groups 2 and 3 received cold and warm aqueous leaf extract of *M. pruriens* at 200 mg/kg body weight respectively. The extract was administered daily for 14 days. Haematological parameters were measured before and after the treatment period.

Results: *Mucuna pruriens* had a significant effect on the haematological parameters of the treated rats showing higher values of haemoglobin concentration,Packed cell volume (PCV) and red blood cell count when compared to the control ($p < 0.05$). Total white blood cell count was however, significantly decreased, when compared with the control group ($p < 0.05$). The values of lymphocytes as well as those of neutrophils were lower compared to those of the control group. There was however, no statistically significant difference between the groups administered the cold extract and the one administered the warm extract. The phytochemical analysis of both extracts revealed the presence of phytochemicals including alkaloids, flavonoids, saponins, cardiac glycosides and carbohydrates.

Conclusion: Findings from this study suggests that *M. pruriens* may have beneficial effects on the haematology parameters in albino rats. Our findings indicated that Mucuna pruriens has the potentials of increasing the haemoglobin levels of the body. Its potentials use as an anti-anaemic option is limited by the impaired effect on white blood cell production. Further studies are needed to determine the mechanisms underlying these effects and to evaluate the safety and efficacy of *M. pruriens* for use in humans.

Keywords: *Mucuna Pruriens*; Haematology; Biochemical Parameters; Albino Rats

Introduction

The significance of blood in maintaining good health cannot be over emphasized. In ancient Chinese medicine, blood is referred to as the 'mother of energy', as it provides the essential building blocks and fluids required to provide nourishment and vitality to the body [1]. Natural herbs and herbal preparations such as ugwu" (*Telfairia occidentalis)* are used in Nigeria as blood boosters, and in addressing various blood deficiency syndromes; especially in de-

bilitating disease conditions and conditions leading to acute blood losses [2]. The physiological functions of blood are numerous, and include providing material nourishment and necessary moisture required for proper organ function. Insufficient blood or blood deficiencies can lead to various problems such as weakness, lethargy, inability to concentrate, hot flushes, increased susceptibility to infection, shortness of breath, fatigue, dizziness, palpitation, anxiety, depression, insomnia, nervousness, headache, and diminished sex drive. Women are particularly susceptible to blood deficiencies due to their monthly menstrual cycle, and because the lifespan of red blood cells is relatively short, blood needs to be constantly replenished [1].

Mucuna pruriens, commonly known as velvet bean or cowhage, is a tropical legume that is widely distributed in tropical regions of the world such as in India, Africa, Central and South America. It is a climbing vine that can grow up to 15 meters in height and produces pods that contain seeds that are used for medicinal and nutritional purposes [3]. Taxonomically, *M. pruriens* belongs to the Fabaceae family, it is classified under the subfamily Papilionoideae, it belongs to the tribe Phaseoleae, and genus *Mucuna*. The plant is notorious for its extreme itches and skin irritation, particularly the young foliage and seed pods. When in contact with the skin, it produces severe irritations and many medium-sized red swollen areas on the skin that are actively itchy [4]. It has been traditionally used in Ayurvedic medicine for the treatment of a wide range of ailments [5].

All parts of *M. pruriens* possess valuable medicinal properties. In the south-eastern geopolitical zone of Nigeria *M. pruriens* is popularly known as 'agbala'and 'karara' in the Hausa language speaking region. It is used as vegetable for making soup [6,7]. It has been pharmacologically studied for various activities, including aphrodisiac, anti-diabetic, antimicrobial, and anti-epileptic activities [8,9].

A plethora of recent studies have investigated the potential therapeutic effects of *M. pruriens* for Parkinson's disease. In a review by Lieu., *et al*. [10], it was reported that during a clinical trial, extracts of *M. pruriens* were effective in reducing symptoms of Parkinson's Disease, including tremors, rigidity, and it is well-tolerated by the patients [10]. In addition extracts of *M. Pruriens* have been found to improve dopamine function and reduce oxidative stress in the brains of mice with Parkinson's disease, suggesting its potential neuro-protective effect or neuro-regenerative effects [3].

Mucuna pruriens has also been studied for its effects on male fertility. Ahmad., *et al*. [11], reported that *M. pruriens* extracts improved sperm quality and reduced oxidative stress in infertile men. It is alluded that this activity may be due to the high levels of L-DOPA (l-3, 4-dihydroxyphenylalanine) also known as "levodopa" in the plant, which has been shown to increase testosterone levels and improve sperm quality in animal studies [12].

The anti-depressive and the anxiolytic potential of *M. pruriens* have been reported. Seed extracts of *M. pruriens* have been shown to improve the symptoms of anxiety and depression in rats [13]. Other studies attributed the antidepressant effect of *M. pruriens* to its ability to increase dopamine levels in the brain [14].

In addition to its potential benefits in the management of Parkinson's disease, *M. pruriens* has also been reported to have neuroprotective properties. Kumar and Singh [15], reported that *M. pruriens* extract improved cognitive function and reduced oxidative stress in the brains of rats with Alzheimer's disease (AD). Extracts of *M. pruriens* also reduced neuro-inflammation and improved neuronal survival in mice with traumatic brain injury [16].

The anti-inflammatory activities of *M. pruriens* have been reported [17]. It has been demonstrated that seed extracts of *M. pruriens* reduced inflammation and oxidative stress in rats with colitis [17]. Another study published found that *M. pruriens* extract reduced inflammation and improved insulin sensitivity in rats with Type 2 diabetes [18]. *Mucuna pruriens* have also been found to possess antioxidant and anti-inflammatory properties [19].

Research into the haematological benefits of *Mucuna pruriens* has been neglected due to the popularity of another common herb; "ugwu" (*Telfairia occidentalis*) used as a blood tonic [2]. This study therefore seeks to determine the effects of *M. pruriens* cold and warm leaf extracts on some haematological parameters in albino rats.

Materials and Methods

Study area

The study was conducted at the National Veterinary Research Institute in the Central Diagnostic Division, located in Jos South Local Government Area of Plateau State, Nigeria.

Preparation of aqueous plant extract

To prepare the aqueous extract from *Mucuna pruriens*, the collected fresh leaves were first washed and weighed. One hundred grams (100g) of leaves were then soaked in 1000 milliliter (1 liter) of distilled water and filtered using a manual cheesecloth sieve. The resulting extract was divided into two equal parts: one

was heated to boiling point for 5 minutes, while the other was left unheated. This is to see whether there could be any difference in constituents and effect between the two groups. After heating, the extract was allowed to cool to room temperature. Both the heated and unheated extracts were measured and administered daily to the rats, with each dose consisting of 100 mL of extract as their drinking water *ad libitum* [20].

Phytochemical analysis

The secondary metabolites present in the *Mucuna pruriens* crude extracts were qualitatively determined using the methods of Harborne [21].

Acute toxicity study (Determination of LD_{50})

The median lethal dose (LD_{50}) of the plant extract was determined using the Lorke's method [20] and Harborne method [21]. A total of nine albino rats weighing between 45-66g were used in the study. Albino rats were used because they are good experimental animal model as they have similar physiology as human and they are can be housed in a small space for convenience. In the first phase of the experiment, nine albino rats were divided into three Groups and administered varying doses of the extract at 10mg/kg, 100mg/kg and 1000 mg/kg, respectively. The rats were then observed for 24 hours for signs of toxicity, behavioural changes or mortality. In the second phase, three rats were divided into three groups, with each group receiving higher doses of the extract at 1900mg/kg, 2600mg/kg and 5000mg/kg, respectively. The rats were observed for 24 hours for any changes in behaviour or mortality. The median lethal dose (LD_{50}) was calculated using the data obtained from the second phase of the experiment [22].

Experimental animals

Apparently healthy albino rats of both sexes weighing between 45-66g were obtained from the small animal house of the National Veterinary Research Institute in Vom, Plateau State, Nigeria for the study. The rats were divided into three main groups: Group 1 served as the control group and received 5.0 ml/kg body weight of distilled water, while Groups 2 and 3 each received daily oral administration of 200 mg/kg body weight of cold and warm *M. pruriens* extractrespectively for 14 days. The animals had ad libitum access to rat chow and water. Ethical approval for the use of experimental animals was issued by the animal use and care committee (AUCC), National Veterinary Research Institute Vom, Plateau State, with Reference No. NVRI/AEC/02/129/23.

Experimental design

Nine (9) albino rats were randomly divided into three groups of three animals in each group. The animals were allowed to acclimatize for two weeks prior to the commencement of the experiment. Group A served as the control and received only water and chow, while Group B received 100 ml of cold extracts and Group C received 100 ml of warm extracts containing 200mg/kg of the extract? Water and extracts were changed daily.

Sample collection and analysis

Determination of the PCV values using the micro-haematocrit analysis

At the end of the 14 days experimental period, blood samples were collected from both the rats administered the aqueous extracts (cold and warm) and from the control group via the retro-orbital vein, using a capillary tube inserted into the eye through the media canthus. Blood was allowed to flow through the tube by capillary attraction up to ¾ of its capacity. The lower end of the tube was sealed with plasticine to prevent the blood from spilling during centrifuging. The blood samples were collected into EDTA bottles and gently mixed by rocking or repeated inversion to ensure proper mixing of the sample and the anticoagulant. The collected blood samples were analyzed for changes for both haematological and biochemical parameters. The blood samples were centrifuged using a haematocrit centrifuge at 3000rpm for 5 minutes. The PCV was read using a haematocrit reader. The result was reported in percentage (%) [23].

Total white blood cell analysis using the haemocytometer method

Three hundred and eighty microliter (380 µl) of Turk's solution was taken into clean test tubes using a pipette. After which twenty microliter (20 µl) of the collected blood samples were added into the test tubes and mixed gently. The counting chamber was filled using a Pasteur pipette and allowed to settle for about 2 minutes. After which it was viewed in the chamber using the low power objective (x10) of a microscope, all the 16 small square boxes were counted of the 4 large outer squares.

Calculation: $N \times DF \times 10^6/A \times D$

Where: N = Number of cells counted DF = Dilution factor A = Area of the chamber D = Depth of the chamber 10^6 = Conversion factor

White blood cells differential count using the thin smear and Leishman's staining technique

A clean and grease-free microscope glass slide was used for making the thin blood film. A drop of well-mixed blood of about 1 cm in diameter was placed on the slide, away from the edge. At a 45-degree angle, a spreader was placed in front of the blood drop, allowing the blood to spread across the edge of the spreader. The spreader was moved quickly and swiftly in a single motion across

the slide. The smear was allowed to air dry before proceeding with staining. The slide was appropriately labelled with the laboratory number and date and was stained.

Statistical analysis

The results were subjected to statistical analysis using the one-way Analysis of variance (ANOVA), where $p < 0.05$ is considered statistically significant.

Results

Phytochemical screening

Phytoconstituent	Presence in *Mucuna pruriens* (Cold extract)	*Mucuna pruriens* (Warm extract)
Alkaloids	++	++
Saponins	++	++
Flavonoids	++	++
Glycosides	++	++
Carbohydrates	+	++
Tannins	-	+

Table 1: Phyto-constituent profile of cold and warm extracts of *Mucuna pruriens*.

Keys: ++ (High), + (Moderate), - (Not detected).

Table 1 above shows the phyto-constituent profile of *Mucuna pruriens*. The presence of various phyto-constituents is denoted by plus (+) signs, with the intensity of presence indicated by the number of plus signs. The absence of a phyto-constituent has been denoted by a minus (-) sign. From Table 1, *M. pruriens* contains alkaloids, saponins, flavonoids, and glycosides in significant amounts, as indicated by the presence of two plus signs (++). The presence of carbohydrates is indicated by one plus sign (+), suggesting a moderate presence. Tannins, however, are absent in *M. pruriens*, as indicated by the minus sign (-). Overall, the phyto-constituent profile of *M. pruriens* suggests that it may have several potential health benefits, as these phyto-constituents are known to possess a wide range of biological activities such as antioxidant, anti-inflammatory, and antimicrobial properties.

Acute toxicity study (Determination of LD_{50})

LD_{50} is above 5,000 mg/kgfor both cold and warm extracts of *Mucuna pruriens.*

Haematological result

Table 2 presents the mean standard deviation of three haematological parameters (PCV%, RBC count, and WBC count) of the albino rats administered the cold and warm aqueous extract of *M. pruriens* and the control group. The results show that the PCV% and RBC values were higher in both the group administered cold extract and the group administered warm extract when compared to the control group. The result also showed that the values of the WBC count were higher in the group administered the cold extract when compared to both the group that had the warm extract and the control group. The total white blood cells (TWBC) count were within the normal range. However, the standard deviation for the WBC count in the cold extract group was much greater than the other groups. The "a" after the standard deviation values indicates that there was a statistically significant difference between the treatment groups and the control group. These findings suggest that the leaf extract of *Mucuna pruriens* have the potentials of being used for treating anemia.

Haematological Parameters	Cold Mean ± SD	Warm Mean ± SD	Control Mean ± SD
PCV%	39.00 ± 4.69^{a}	39.50 ± 3.70^{a}	37.75 ± 3.10^{a}
RBC ($x10^4/\mu L$)	9.38 ± 1.50^{a}	9.10 ± 1.49^{a}	8.70 ± 1.01^{a}
WBC ($x10^3/\mu L$)	15.45 ± 13.79^{a}	8.35 ± 5.11^{a}	6.23 ± 0.69^{a}

Table 2: Haematological parameters of albino rats given cold and warm *Mucuna pruriens* extracts and distilled water.

Note: SD; Standard Deviation; PCV: Packed Cell Volume; RBC: Red Blood Cell Count; WBC: White Blood Cell Count

Haematological Parameters	Cold Mean ± SD	Warm Mean ± SD	Control Mean ± SD
PCV%	48.50 ± 2.65^{ab}	46.00 ± 5.35^{b}	44.75 ± 1.71^{a}
RBC ($x10^4/\mu L$)	8.28 ± 1.27^{a}	8.20 ± 1.29^{ab}	7.63 ± 0.47^{b}
WBC ($x10^3/\mu L$)	15.53 ± 13.20^{a}	9.53 ± 5.77^{a}	8.73 ± 0.46^{a}

Table 3: Haematological parameters of the albino rats given cold and warm extracts of *Mucuna pruriens* after 7days of administration.

Note: SD; Standard Deviation; PCV: Packed Cell Volume; RBC: Red Blood Cell Count; WBC: White Blood Cell Count

Table 3 shows the mean standard deviation of three haematological parameters (PCV%, RBC count, and WBC count) in albino rats given cold and warm *M. pruriens* extract and distilled water on day seven of the study, compared to the control group. The results indicated that the cold extract group had the highest PCV% and RBC count among all three groups, while the warm extract group has the second highest values. The control group has the lowest values for these parameters. The standard deviation values for

PCV% and RBC count are relatively small for all groups, indicating less variability in the results. For the WBC count, the cold extract group has the highest mean value, followed by the warm extract group, and then the control group. However, the standard deviation for the WBC count is relatively high for all groups, indicating greater variability in the results. The "a" and "b" after the standard deviation values indicate that there are statistically significant differences between the treatment groups and the control group, and between the cold extract group and the warm extract group, respectively.

Haematological parameters of day 14	Cold Mean ± SD	Warm Mean ± SD	Control Mean ± SD
WBC 10^3/µL	7.20 ± 1.00^a	8.31 ± 4.07^a	6.92 ± 3.55^a
Neut 10^3/µL	46.00 ± 8.25^a	47.20 ± 8.04^a	44.00 ± 9.77^a
Lymph 10^3/µL	61.80 ± 9.15^a	61.00 ± 7.28^{ab}	48.80 ± 5.07^b
Mono%	2.80 ± 1.10^a	2.80 ± 1.30^a	2.60 ± 1.14^a
RBC 10^4/µL	7.88 ± 1.79^a	7.81 ± 1.97^a	7.73 ± 2.10^a
HGB g/Dl	134.2 ± 33.6^a	142.8 ± 42.0^a	165.6 ± 24.6^a
MCV Fl	57.00 ± 2.35^a	61.40 ± 2.07^a	64.60 ± 8.17^a
MCH pg	16.92 ± 0.88^a	18.62 ± 0.97^{ab}	22.10 ± 3.85^b
MCHC g/dL	298.80 ± 21.61^a	304.20 ± 15.72^{ab}	342.6 ± 31.7^b

Table 4: Haematological parameters of albino rat given cold and warm *Mucuna pruriens* extracts and distilled water after day 14 of administration.

Note: Means that do not share same alphabet are significantly different

Keys: PCVL Packed Cell Volume; RBC: Red Blood Cell; WBC: White Blood Cell; HCT%: Haematocrit; HGB: Haemoglobin; Neut: Neutrophil; Lymph: Lymphocyte; Mono: Monocyte; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Haemoglobin; MCHC: Mean Corpuscular Haemoglobin Concentration

Normal Range for Leprine: PCV = 35-45; RBC = 6.3-10.0; WBC = 5.0-23.0; HGB = 12.0-18.0; HGB = 8-40; Lymph = 50-80; Mono = 2-7; MCV = 58-67; MCH = 17-24; MCHC = 29-37.Source: Clinical Pathology Laboratory, National Veterinary Research Institute, Vom.

Table 4 shows the haematological parameters of albino rats given cold and warm *Mucuna pruriens* extracts and distilled water. The mean and standard deviation (SD) of each parameter is presented for each treatment group: cold, warm, and control. The haematological parameters measured on day fourteenth are WBC (white blood cell count), Neut (neutrophil count), Lymph (lymphocyte count), Mono% (monocyte percentage), RBC (red blood cell count), HGB (haemoglobin), HTC% (haematocrit percentage), MCV (mean corpuscular volume), MCH (mean corpuscular haemoglobin), and MCHC (mean corpuscular haemoglobin concentration). The results show that the cold and warm extract groups have higher WBC and Neutrophil counts compared to the control group, but there is no significant difference between the cold and warm extract groups. This could be attributed to the possible similarities of their phytochemical composition. The Lymphocyte count is significantly higher in the cold and warm extract groups compared to the control group. The Monocyte percentage (%) is not significantly different among the three groups. For the RBC parameters, there is no significant difference between the three groups. However, for the HGB, HTC%, MCV, MCH, and MCHC parameters, the control group has significantly higher mean values compared to the cold and warm extract groups. The results suggest that *M. pruriens* extract in cold and warm water does not significantly affect the RBC parameters but may have an impact on the WBC parameters and HGB, HTC%, MCV, MCH, and MCHC parameters. Further studies may be needed to investigate the effects of different doses and duration of administration of *M. pruriens* extract on these parameters.

Discussion

The phytochemical screening of the leaf extract of the plant revealed the presence of alkaloids, flavonoids, saponins, cardiac glycosides, and carbohydrates, which are known to have various biological functions. Flavonoids are known to offer protection against allergies, free radicals, ulcers, hepatotoxins, and tumours, while saponins have properties such as protein precipitation, cholesterol-binding, and haemolysis [2,23]. However, other phytocomponents such as alkaloids and glycosides found in this plant do not have properties related to increased haematopoiesis. The chemical constituents present in *M. pruriens* leaf were carbohydrate, flavonoids, and glycosides, which are believed to be responsible for the observed therapeutic (haematopoietic effect) [24]. This result is consistent with a previous study, which found alkaloids present in the leaf [25].

The acute toxicity study was conducted to establish the therapeutic index of the drug and to determine the range of the drug that could be toxic to the animal. The results showed no behavioural changes, toxicity, or mortality at the maximum oral dose level of 8g/kg body weight [26]. Thus, the *M. pruriens* leaf extract is not toxic according to the acute toxicity study for the cold and warm extracts.

The results of the haematological parameters of albino rats were presented in table 2, 3 and 4. There was no statistically sig-

nificant correlation between the test groups and the control, which agrees with the study of Tende., *et al*. [27]. Descriptive statistics were used to describe the means for each parameter, indicating that test parameters had higher means compared to controls for most of them. This supports the understanding that the active substance increased the haematology parameters in the test group. An increase in the white blood cells (WBC), haemoglobin concentrations (HGB), haematocrit (HCT), and lymphocytes (LYM) count was observed across the test groups as shown in the descriptive statistics in Table 4, indicating that those test parameters had higher means compared to the controls. This supports the findings of Madukwe., *et al*., [28] that the active substance of M*. pruriens* increased the haematology parameters, but is contrary to the findings of Ndukwe., *et al*., [29]. A relative increase was observed in the red blood cell counts (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) as shown in Table 4. There was an observed difference in Monocytes (MON) and Neutrophil (NEU) tests when compared to the control (Table 4).

Conclusion

The study demonstrated that the aqueous leaf extract of *M. pruriens* can significantly increase the total red blood cell count, particularly in the parameters of PCV, HB, MCV, and MCHC, but a decrease in the total white cell count and lymphocytes values. Therefore, *Mucuna pruriens* has the potentials to increase haemoglobin levels and may be used for the treatment of anemia. However, the impaired decreased white blood cell production levels seen in the result could limit its use as an herbal blood booster despite its popularity, therefore necessitating further studies to eliminate this negative effect. Also, further studies are therefore, needed to determine the mechanisms underlying the observed haematopoietic effect of the extracts and to evaluate the safety and efficacy of *M. pruriens* for use in humans.

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Authors Contribution

Barde Israel Joshua, Ishaku Leo Elisha, Ibrahim Emmanuel Arin and Abubakar Sadiq Abubakardesigned the research work and drafted the manuscript; Kabantiyok Dennis, Budaye James, Habibu Haliru, Oguche Moses Ojonugwa, Leo Shedua Nyam and Bakam Judith Dizot carried out the research work; Makama Sunday,Makoshi Micah Shehu, Ngulukun Sati Samuel and Muhammad Maryam carried out the data analysis and edited the manuscript. All authors read and approved the final version of the manuscript before submission.

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Conflict of Interest

The authors declare no conflict of interest.

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