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Effectiveness of Fresh and Shade-Dried *Mucuna pruriens* Leaf Extract in Controlling Anaemia in Adult Male Albino Rats

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Abstract: This study evaluated effectiveness of fresh and shade-dried *Mucuna pruriens* leaf extract in managing anaemia in adult male albino rats. Fresh leaves of *Mucuna pruriens* were harvested from Enugu-Ezike, Enugu state, Nigeria. The leaves were used to prepare fresh and shade-dried leaf extracts which were subjected to chemical analysis using standard analytical methods. Fifteen adult male albino rats weighing 180-250g, grouped into three groups (A, B and C) of five rats each were used for the study. All groups received rat chow and water *ad-libitum*. Group B and C received in addition, the fresh and shade-dried *Mucuna pruriens* leaf extracts respectively after anaemia induction. Blood samples were collected from the rats for determination of haemoglobin, PCV, RBC and WBC after a 5-day acclimatization, after anaemia induction and at the end of the study. Statistical Package for Social Sciences (SPSS) for windows version 18 was used to analyze the data obtained. $p < 0.05$ was accepted as a cut-off for significant level. Fresh and shade-dried *Mucuna pruriens* leaf extracts contained iron (9.00 ± 0.28 and 3.50 ± 0.00 mg/100 mL), vitamin C (18.45 ± 2.19 and 31.35 ± 0.35 mg/100 mL) and pro-vitamin A (100.21 ± 0.28 and 170.21 ± 0.28 mg/100 mL). Haemoglobin, packed cell volume and white blood cell of rats fed fresh *Mucuna pruriens* leaf extract significantly ($p < 0.05$) increased after treatment. Shade-dried *Mucuna pruriens* leaf extract significantly ($p < 0.05$) increased red blood cell and white blood cell of the rats after treatment. Lymphocytes of the anaemic rats fed fresh and shade-dried *Mucuna pruriens* leaf extracts was significantly ($p < 0.05$) increased whereas there was no significant ($p > 0.05$) increase in the eosinophils of the anaemic rats.

Key words: Anaemia, *Mucuna pruriens*, extracts

INTRODUCTION

Anaemia is known to be one of the most dreadful diseases that is widely spread in the world (Uboh *et al.*, 2007). It (anaemia) is one of the clinical conditions that constitute a serious health problem in many tropical countries as a result of the prevalence of different forms of parasitic infections including malaria (Dacie *et al.*, 1994). Anaemic condition is characterized by a decrease in the level of circulating haemoglobin less than 13 g/dL in male and 12 g/dL in females (Okochi *et al.*, 2003). Worldwide, anaemia is the commonest red blood cell disorder (Olaniyan and Adeleke, 2005). Blood is the medium in which vital body nutrients, drugs, hormones and waste products of metabolism are conveyed (Ode and Nwaehujor, 2010).

It is generally known that the consumption of a variety of local herbs and vegetables by man contributes significantly to the improvement of human health, in terms of prevention and cure of diseases because plant have long served as a useful and natural source of the therapeutic agents (Chevellier, 1996). Through the ages, man has learnt to take advantage of the many resources placed at his disposal by nature to meet essential needs in all fields (Agbor *et al.*, 2005). The use of medicinal plants as food supplements and in the

treatment of specific diseases dates back to antiquities (Saba *et al.*, 2010). According to Saba *et al.* (2010) several plants are now known to have medicinal effects across the different regions of the world. Some of which has been shown to have haematinic properties. One of such plant is *Mucuna pruriens* whose leaf extract has been acclaimed to possess haematinic property. The study was undertaken to verify the acclaimed haematinic property of *Mucuna pruriens* leaf extract.

MATERIALS AND METHODS

Source of *Mucuna pruriens* leaves: Fresh leaves of *Mucuna pruriens* were harvested from a farm in Umachi, Enugu-Ezike, Igbo-Eze North LGA of Enugu state.

Preparation of the *Mucuna pruriens* leaf extract

Fresh *Mucuna pruriens* leaf extract: Fresh *Mucuna pruriens* leaves were harvested. One hundred grams of the fresh leaves were washed with clean water and was ground. Two hundred millilitres of water was added to the ground sample and mixed. The extract was filtered out with a muslin cloth and the residue discarded.

Shade-dried *Mucuna pruriens* leaf: Thirty-five grams of fresh *Mucuna pruriens* leaves were washed and shade-dried for four days. The shade-dried leaves were ground

into fine powder with an electric blender. The ground sample was mixed in 200 mL of water, sieved with a muslin cloth to obtain the extract (filtrate).

Chemical analysis

Proximate analysis: Duplicate samples of the fresh and shade-dried *Mucuna pruriens* leaf extracts were used for proximate analysis. The standard method of AOAC (2010) was used for moisture determination. Crude protein determination was done using the standard method of AOAC (2000). Crude fats, ash and fibre determinations were done using the standard method of AOAC (2005). Carbohydrate was obtained by difference.

Micronutrient determination: Calcium determination was done using the standard method of AOAC (2010). Iron determination was done using the standard phenanthroline method of AOAC (2010). Vitamin C (ascorbate) determination was done using the standard procedure described by the DCIP titrimetric method of AOAC (2010) for ascorbic acid determination. Pro-vitamin A determination was done according to Jakutowicz *et al.* (1977) procedure.

Phytochemical determination: Alkaloid determination was done using the Harborne (1973) method. Oxalate determination was done using the method of Munro and Bassir (1969).

Animal study: Fifteen adult male albino rats weighing between 180-250 g were bought from Zoology Department of the University of Nigeria, Nsukka (UNN), Nigeria. The rats were grouped into three of five rats each in a way that the difference in weight between rats in a group did not exceed 5 g. The rats were acclimatized for the first five days to the diet and environment. During this period, they were fed rat chow and water *ad libitum*.

Assessment of haematological parameters: Five millilitres of blood samples were collected from the medial canthus of the rats. Haemoglobin determination was done using the cyamet method. Packed cell volume (PCV) or haematocrit determination was done using the microhaematocrit method. Red blood cell (RBC) determination was done using the Thoma (manual counting) method. White blood cell (WBC) determination was done using the manual WBC counting method. The haematological parameters of the rats were assessed after acclimatization, after anaemia induction and at the end of the experimental period.

Anaemia induction: Two millilitres of blood was obtained from the medial canthus of the rats for four days. Treatment started after anaemia was established.

Treatment of the rats: The rats were grouped into three (A, B and C) of five rats each. Group A (control) received

rat chow and water *ad-libitum*. Group B received rat chow and fresh *Mucuna pruriens* leaf extract. Group C received rat chow and shade-dried *Mucuna pruriens* leaf extract.

Statistical analysis: The computer program, Statistical Package for Social Sciences (SPSS) for windows version 18 was used to analyze data obtained from the study. Main analysis included means and standard error of mean. ANOVA was used to compare and separate means. Duncan's multiple range test was used to test the level of significance at $p < 0.05$.

RESULTS

Table 1 shows the proximate, micronutrient and phytochemical composition of the fresh and shade-dried *Mucuna pruriens* leaf extract. Proximate composition of the fresh and shade-dried *Mucuna pruriens* leaf extract showed 0.99 ± 0.01 and 0.02 ± 0.00 g/100 mL for crude protein, 0.16 ± 0.03 and 0.19 ± 0.01 g/100 mL for crude fat; and 0.84 ± 0.84 and 0.02 ± 0.02 g/100 mL for ash. Micronutrient content of fresh and shade-dried *Mucuna pruriens* leaf extracts showed iron (9.00 ± 0.28 and 3.50 ± 0.00 mg/100 mL), calcium (3.20 ± 0.42 and 2.40 ± 0.42 mg/100 mL), vitamin C (18.45 ± 2.19 and 31.35 ± 0.35 mg/100 mL) and pro-vitamin A (100.21 ± 0.28 and 170.21 ± 0.28 mg/100 mL), respectively. The alkaloid content of the extracts varied. The oxalate content of fresh and shade-dried *Mucuna pruriens* leaf extracts showed, 1.34 ± 0.60 and 1.01 ± 0.14 mg/100 mL, respectively.

Table 2 shows the effect of the fresh and shade-dried *Mucuna pruriens* leaf extracts on the haemoglobin of the anaemic rats. The haemoglobin of the anaemic rats fed fresh *Mucuna pruriens* leaf extract was significantly ($p < 0.05$) increased from 8.80 ± 0.66 to 14.00 ± 0.63 g/dL whereas rats fed the shade-dried *Mucuna pruriens* leaf extract had an increased haemoglobin from 8.40 ± 0.68 to 11.20 ± 2.85 g/dL though the increase was not significant ($p > 0.05$).

Table 3 shows the effect of the fresh and shade-dried *Mucuna pruriens* leaf extracts on the packed cell volume (PCV) or haematocrit of the anaemic rats. The PCV of the anaemic rats fed fresh *Mucuna pruriens* leaf extract was significantly ($p < 0.05$) increased from 31.20 ± 0.86 to $44.80 \pm 0.80\%$ whereas rats fed the shade-dried *Mucuna pruriens* leaf extract had an increased PCV from 28.00 ± 0.71 to $35.80 \pm 9.55\%$ though the increase was not significant ($p > 0.05$).

Table 4 shows the effect of the fresh and shade-dried *Mucuna pruriens* leaf extracts on the red blood cell (RBC) of the anaemic rats. The RBC of the anaemic rats fed fresh *Mucuna pruriens* leaf extract was significantly ($p < 0.05$) decreased from 168.00 ± 10.20 to 146.00 ± 17.15 million/mm³ whereas rats fed the shade-dried *Mucuna pruriens* leaf extract had a significant ($p < 0.05$) increase from 282.00 ± 11.23 to 316.80 ± 85.44 million/mm³.

Table 1: Proximate, micronutrient and phytochemical composition of the fresh and shade-dried *Mucuna pruriens* leaf extracts

	Fresh leaf extract	Shade-dried leaf extract
Moisture (g/100 mL)	78.67±0.47	69.89±0.13
Crude protein (g/100 mL)	0.99±0.01	0.02±0.00
Crude fat (g/100 mL)	0.16±0.03	0.19±0.01
Ash (g/100 mL)	0.84±0.84	0.02±0.02
Crude fibre (g/100 mL)	0.30±0.00	0.20±0.00
Carbohydrate (g/100 mL)	19.31±0.50	27.73±0.14
Iron (mg/100 mL)	9.00±0.28	3.50±0.00
Calcium (mg/100 mL)	3.20±0.42	2.40±0.42
Vitamin C (mg/100 mL)	18.45±2.19	31.35±0.35
Pro-vitamin A (mg/100 mL)	100.21±0.28	170.21±0.28
Alkaloid (mg/100 mL)	3.00±0.14	0.75±0.07
Oxalate (mg/100 mL)	1.34±0.60	1.01±0.14

Table 2: Effect of the fresh and shade-dried *Mucuna pruriens* leaf extracts on the haemoglobin of the anaemic rats

	Group A	Group B	Group C
Haemoglobin (g/dL)			
Baseline	12.60±0.51 ^a	12.60±0.75 ^a	12.40±0.68 ^a
After induction	8.80±0.66 ^b	8.80±0.66 ^b	8.40±0.68 ^b
After treatment	13.20±0.37 ^a	14.00±0.63 ^a	11.20±2.85 ^b

Group A (control), Group B (rats fed the fresh leaf extract), Group C (rats fed the shade-dried leaf extract)

Table 3: Effect of the fresh and shade-dried *Mucuna pruriens* leaf extracts on the packed cell volume (PCV) or haematocrit of the anaemic rats

	Group A	Group B	Group C
Packed cell volume (%)			
Baseline	44.20±2.13 ^a	47.20±2.66 ^a	45.40±2.68 ^a
After induction	28.60±0.80 ^b	31.20±0.86 ^b	28.00±0.71 ^b
After treatment	44.20±1.11 ^a	44.80±0.80 ^a	35.80±9.55 ^b

Group A (control), Group B (rats fed the fresh leaf extract), Group C (rats fed the shade-dried leaf extract)

Table 4: Effect of the fresh and shade-dried *Mucuna pruriens* leaf extracts on the red blood cell (RBC) of the anaemic rats

	Group A	Group B	Group C
Red blood cell (million/mm³)			
Baseline	296.00±14.35 ^a	170.00±4.90 ^b	364.00±9.27 ^a
After induction	268.00±10.00 ^b	168.00±10.20 ^b	282.00±11.23 ^b
After treatment	276.00±37.90 ^b	146.00±17.15 ^b	316.80±85.44 ^c

Group A (control), Group B (rats fed the fresh leaf extract), Group C (rats fed the shade-dried leaf extract)

Table 5 shows the effect of the fresh and shade-dried *Mucuna pruriens* leaf extracts on the white blood cell (WBC) of the anaemic rats. The WBC of the rats fed the fresh and shade-dried leaf extract significantly ($p < 0.05$) increased after treatment. The neutrophils of the rats fed fresh and shade-dried *Mucuna pruriens* leaf extracts was decreased after anaemia induction and after treatment, however, this decrease was significant ($p < 0.05$) in the rats fed shade-dried *Mucuna pruriens* leaf extracts. Lymphocytes of rats fed fresh and shade-dried *Mucuna pruriens* leaf extracts were significantly ($p < 0.05$) increased after treatment. There was no significant ($p < 0.05$) difference in the eosinophils of rats fed fresh and shade-dried *Mucuna pruriens* leaf extracts, however, rats fed fresh *Mucuna pruriens* leaf extracts showed an increase in eosinophils after treatment.

Table 5: Effect of the fresh and shade-dried *Mucuna pruriens* leaf extracts on the white blood cell (WBC) of the anaemic rats

	Group A	Group B	Group C
White blood cells (cells/mm³)			
Baseline	6.70±0.54 ^a	7.02±0.77 ^a	5.62±0.46 ^a
After induction	2.64±0.21 ^b	2.60±0.23 ^b	3.24±0.41 ^b
After treatment	8.28±0.69 ^a	9.64±0.99 ^a	7.00±1.78 ^a
Neutrophil (%)			
Baseline	67.60±4.45 ^a	67.20±1.50 ^a	70.00±1.67 ^a
After induction	49.80±2.65 ^b	48.40±3.87 ^b	51.80±2.54 ^b
After treatment	32.40±2.79 ^b	37.20±3.50 ^b	23.60±4.99 ^b
Lymphocyte (%)			
Baseline	32.40±4.83 ^a	31.60±1.37 ^a	29.60±1.72 ^a
After induction	32.60±2.04 ^a	39.20±2.13 ^a	27.40±2.40 ^a
After treatment	65.60±0.40 ^b	58.00±0.50 ^b	54.80±0.00 ^b
Eosinophils (%)			
Baseline	2.00±0.00 ^a	2.00±0.00 ^a	2.00±0.00 ^a
After induction	2.00±0.00 ^a	2.00±0.00 ^a	2.00±0.00 ^a
After treatment	1.60±0.40 ^a	2.50±0.50 ^a	2.00±0.00 ^a

Group A (control), Group B (rats fed the fresh leaf extract), Group C (rats fed the shade-dried leaf extract)

DISCUSSION

The iron content of the fresh leaf extract was higher than the shade-dried leaf extract. Iron is a haematinic substance that is an essential component of red blood cells and the muscles that assist in the transportation of oxygen throughout the body (Arollado and Osi, 2010). Iron is needed for haemoglobin synthesis (George-Gay and Parker, 2003). Calcium which functions in the mineralization of bones (Groppper *et al.*, 2005) was present in the fresh and shade-dried extracts. Oxalic acid, which is a plant toxicant (Muchoki *et al.*, 2010) was more in the fresh than shade-dried leaf extract. However, shade-drying possibly had little or no effect on the oxalate content of the leaves since oxalates are easily vapourized organic compounds (Muchoki *et al.*, 2010). Oxalic acid is known to cause calcium deficiency in man and in non-ruminants because oxalates of calcium are insoluble (Ene-Obong, 2001), thus inhibiting calcium absorption (Hodgkinson, 1977) and increasing fecal calcium excretion (Groppper *et al.*, 2005). The oxalic acid/calcium ratio of the fresh and shade-dried leaf extracts were 0.42 showing that consumption of these extracts will not have a negative effect on calcium. Ene-Obong (2001) reported that in foods where oxalic acid/calcium ratio is more than 3, it is necessary to avoid an excessive intake of these foods and supplement them with milk products which are capable of compensating for the calcium that is chelated by the excess oxalic acid. Oxalic acid also inhibits the absorption of iron forming insoluble oxalate mineral (iron) complex which are insoluble and poorly absorbed (Groppper *et al.*, 2005).

Vitamin C or ascorbate was found in the fresh and shade-dried leaf extracts. Ascorbate enhances the intestinal absorption of non-heme iron either by reducing iron to a ferrous (Fe²⁺) form from a ferric (Fe³⁺) form or by forming a soluble complex with iron in the alkaline pH of the small intestine thereby enhancing iron absorption

(Groppper *et al.*, 2005). The fresh and shade-dried leaf extracts contained pro-vitamin A. Pro-vitamin A carotenoids represent a group of compounds that are precursors of vitamin A. They are synthesized by a wide variety of plants and thus are found naturally in many fruits and vegetables (Groppper *et al.*, 2005). Alkaloids were present in the extracts with the fresh leaf extract having more alkaloid than the shade-dried leaf extract. A wide variety of alkaloids are found in plants and some are very toxic (Ene-Obong, 2001). However, the type and toxicity of the alkaloids were not determined in this study. The haemoglobin of rats fed fresh *Mucuna pruriens* leaf extract significantly ($p < 0.05$) increased after treatment. This might be due to the iron and protein content of the fresh leaf extract. Iron is essential for haemoglobin formation. Haemoglobin is composed of haem, the non-protein portion that contains iron and globin, a simple protein (Lutz and Pryztulski, 1997). However, the type of iron found in plant sources are the non-haem iron whose absorption is slow because it is closely bound to organic molecules in food as ferric iron (Lutz and Pryztulski, 1997). The vitamin C content of the fresh leaf extract may have played a role in the significant increase in haemoglobin. This is because vitamin C is known to enhance iron absorption by forming a soluble compound with iron. Anaemic rats fed the shade-dried *Mucuna pruriens* leaf extract had an increased haemoglobin though it was not significant ($p > 0.05$). Haemoglobin is an iron-containing molecule capable of carrying oxygen and is found in red blood cells (Lee and Nieman, 2010).

The PCV (hematocrit) of the anaemic rats fed fresh *Mucuna pruriens* leaf extract was significantly ($p < 0.05$) increased. Similarly, the anaemic rats fed the shade-dried *Mucuna pruriens* leaf extract had an increased PCV though the increase was not significant ($p > 0.05$). The increase in PCV value simply means an increase in the number of red blood cells per unit volume of the suspension (Stoltz and Donner, 1991). According to George-Gay and Parker (2003) haematocrit represents the percentage of the total volume of red blood cells relative to the total volume of whole blood in a sample. There was a significant ($p < 0.05$) increase in the red blood cells of the anaemic rats fed the shade-dried *Mucuna pruriens* leaf extract. This shows that in an anaemic condition, ingestion of the shade-dried *Mucuna pruriens* leaf extract may boost blood. The main function of red blood cells is the transportation of oxygen into the tissues of the body (Agbor *et al.*, 2005). The shade-dried *Mucuna pruriens* leaf extract probably stimulated the peritubular cells of the kidney due to the low oxygen levels that occurred as a result of anaemia (low haemoglobin). This is because the production of red blood cells by the bone marrow is stimulated by low oxygen levels in peritubular cells of the kidney in a process called erythropoiesis (George-Gay and Parker, 2003). During erythropoiesis, renal erythropoietic factor

(an enzyme) is secreted in response to peritubular cell hypoxia. This factor interacts with a plasma protein to form erythropoietin, a hormone that circulates to the bone marrow to stimulate the stem cells to produce more red blood cells (George-Gay and Parker, 2003). However, the anaemic rats fed the fresh *Mucuna pruriens* leaf extract showed a significant ($p < 0.05$) decrease in their red blood cells. This shows that consumption of the fresh *Mucuna pruriens* leaf extract may not have an effect on the red blood cells in anaemic conditions. According to Agbor *et al.* (2005) any pathological or physiological condition that affects the red blood cell alters its function and this may be detrimental to the body.

The white blood cells of the anaemic rats fed the fresh and shade-dried *Mucuna pruriens* leaf extract were significantly ($p < 0.05$) increased. This increase could be attributed to the normal physiologic response of the defense mechanisms following the perception of a foreign body/challenge. White blood cells are the main players in infectious/inflammatory and immune responses (George-Gay and Parker, 2003). They defend the body against organisms and injury (George-Gay and Parker, 2003). There was a significant ($p < 0.05$) decrease in the neutrophils of the anaemic rats fed shade-dried *Mucuna pruriens* leaf extract. The neutrophils of the anaemic rats fed fresh *Mucuna pruriens* leaf extract decreased but it was not significant ($p > 0.05$). According to George-Gay and Parker (2003) neutrophils are highly motile and are the first to arrive in response to acute inflammation or infection. They migrate out of the capillaries and into the inflamed tissue site in a process called diapedesis or emigration. The neutrophils ingest micro-organisms and debris and then die, forming purulent exudate which is removed by the lymphatics or through the epithelium (George-Gay and Parker, 2003). This is suggestive of the anti-bacterial activity of the fresh and shade-dried *Mucuna pruriens* leaf extract. Ingestion of the extracts probably led to a reaction of the body against foreign body hence the decrease in neutrophils.

Eosinophils significantly ($p < 0.05$) increased in the anaemic rats fed the fresh *Mucuna pruriens* leaf extract, however, there was no change in the eosinophils of the anaemic rats fed the shade-dried *Mucuna pruriens* leaf extract. Ingestion of the fresh *Mucuna pruriens* leaf extract probably led to the reaction against foreign body or probably induced allergic condition in the anaemic rats though this was not ascertained in the study. Eosinophils function principally to ingest and kill multi-cellular parasites (George-Gay and Parker, 2003). They are also effective in detoxifying antigen-antibody complexes that form during allergic reactions (George-Gay and Parker, 2003).

Lymphocytes of the anaemic rats fed the fresh and shade-dried *Mucuna pruriens* leaf extract were significantly ($p < 0.05$) increased. This is suggestive of

the anti-microbial property of leaves of *Mucuna pruriens*. Lymphocytes are primarily involved in fighting chronic bacterial and viral infections.

Conclusion: The fresh and shade-dried *Mucuna pruriens* leaf extracts facilitated an increase in the haematological indices of the anaemic rats. However, the fresh *Mucuna pruriens* leaf extract did not increase the red blood cells of the anaemic rats.

Recommendation: The specific mechanism of action by which fresh and shade-dried *Mucuna pruriens* leaf extract produce its effect on increasing haemoglobin, packed cell volume and red blood cell in experimental animals need to be investigated. There is also need to ascertain the decrease in red blood cell of the anaemic rats fed fresh *Mucuna pruriens* leaf extract.

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