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SERVICES FOR SCIENCE AND EDUCATION

Studies on the Haematinic Potentials of *Mucuna pruriens* in Mice

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ABSTRACT

Background: Ethnopharmacological study of *Mucuna pruriens*, a creeping and leguminous plant used in the treatment of *anaemia* in the eastern part of Kogi State, Nigeria was carried out. The aim was to evaluate the effect of aqueous extract of the leaves of *M. pruriens* on haematinic activities in mice. Method: Leaves of *M. pruriens* was extracted by maceration in water. Phenyl hydrazine 4 mg/kg was administered for four days. Mice were grouped and treated with normal saline 10 ml/kg (normal and anemic control), *M. pruriens* (100, 200 and 400 mg/kg) and Fersolate (0.0214 mg/kg) p.o for 7 days. Hematological parameters measurements were carried out before treatments, days 1, 7, 14 and 21. The red blood cell count (RBC), haemoglobin concentration (Hb), white blood cell count (WBC) and hematocrit (HCT) were analyzed as indices of anemia. Phytochemical fingerprint using HPLC chromatography and mineral contents were also carried out. Results: Plant extraction percentage yield was 15%. *M. pruriens (aq*) significantly ($p<0.01$) produced a 61% recovery from anemia at 400 mg/kg after 7 days of treatment and between 94 to 146.52 % 1 and 2 weeks after treatment at all test doses. Hb, and HCT levels also significantly increased especially at 100 and 200 mg/kg doses of *M. pruriens* compared to the anemic control both at 1 and 2 weeks after treatment. HPLC analysis showed the presence of alkaloids, glycosides, saponin, terpernoids, carbohydrates, chlorogenic acid, caaffeic acid, rutin, ferulic acid and resins. The extract also contained substantial amounts of vitamins B6, C and E, as well as folic acid and iron. Conclusion: These results provide some evidence to support the traditional use of *M. pruriens* leaves in the treatment of anaemia.

Keyword: *Mucuna pruriens,* Anaemia, hematological parameters, mice, Extract.

INTRODUCTION

The World Health Organization (WHO) defines herbal medicines as herbs, herbal materials, herbal preparations and finished herbal products that contain, as active ingredients, parts of plants, other plant materials and/or its combinations. The content may vary from one place to another. Herbal medicines may contain, by tradition, natural organic or inorganic active ingredients that are not of plant origin (e.g. animal and mineral materials). (WHO, 2019; 2005). Medicinal plants, since time immemorial, have been used virtually in all cultures as food supplements and in the treatment of myriads of diseases mainly due to their acclaimed safety with chronic use (Raju *et al.,* 2017). Although, synthetic medicines abound, the emergence of drug resistance, adverse effects, cost and accessibility issues with orthodox drugs have forced attention to shift towards the use of herbal drugs in the prevention, treatment and mitigation of diseases in man (Odugbemi, 2008). Also, herbal medicines are increasingly acceptable globally due to its natural sources of origin and the general belief that they are safe and less toxic to the body. (WHO, 2019; 2005). Other advantages of this type of therapeutics include good availability, increasing demand for natural and organic products, already validated synergistic effects of herbal medicines, among others (Fabio and Ana, 2013). Several plants are now known to have medicinal effects across the different regions of the world. Some of these have been demonstrated scientifically to be of significant value in the treatment of various diseases ().

Anemia is a general term for a large number of conditions marked by a reduction in the oxygen-carrying capacity of the blood. It is a condition where the body does not have adequate healthy erythrocytes, usually measured as a decrease in the amount of haemoglobin, which is the

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oxygen carrying pigment of the red blood cells (Williams, 2006; Priyanka *et al.,* 2015). There are over 400 types of anaemia, many of which are rare, with a few clinically significant ones; but in all, there is usually fall in haemoglobin concentration below normal values for a person's age, gender and environment, but anaemia may not always be accompanied by a fall of the red cell count below normal values. Anaemia, throughout the world, is the most observed red cell disorder associated with several conditions such as nutritional deficiencies, genetic or acquired defects, parasitic infections, blood loss, as well as drug toxicity. It affects people of all ages although the people at greater risk are the elderly, young women of child bearing age and infants (Adebayo *et al,* 2017). In Nigeria, several plants are used either in whole or parts to manage anaemia. Some documented plants include *Parqueting nigrescens, Sorghum bicolor, Terminalia catappa, Trema orientalis, Mangifera indica, Waltheria indica, Theobroma cacao* etc. (Ozioma and Okaka, 2019; Gbadamosi *et al.,* 2012).

Mucuna pruriens commonly called velvet beans is a tropical climbing legume that belongs to the family Fabaceae. The plant is believed to have originated from India where it is a popular medicinal plant and is uses as constituent of more than 200 of their indigenous drug formulations (Raju *et al.,* 2017). All parts of *Mucuna pruriens* possess valuable medicinal properties; the leaves are used in bone fractures, cough, dog-bite, madness, pain, pleuritis (pleurisy), ring worm, scorpion sting, snake-bite, sores and syphilis; the roots are said to be useful to cure cholera, elephantiasis, diuretic and purgative (Raju *et al.,* 2017). The dried leaves have been reported to contain crude protein, vitamins A, C, E, B6, B9, B12, iron, copper and zinc (Nweze et al., 2017). *Mucuna pruriens* is notorious for the extreme itchiness it produces on contact, particularly with the young foliage and seed pods. This property is attributed to the presence of serotonin and the protein *mucuna* in the hair on the pods (Andersen et al., 2015; Joglekar, 1963). The plant has been studied for various activities like anti-diabetic, aphrodisiac, antineoplastic, antiepileptic, antimicrobial activities, learning and memory enhancement, anti-venom, anthelmintic, and anti-inflammatory activities. The phytochemical screening has revealed the presence of alkaloids, reducing sugar, anthraquinones, flavonoids, saponins, tannins, cardiac glycosides, phenols and steroids (Raju *et al.,* 2017).

Mucuna pruriens is used in folklore medicine to treat anaemia and other diseases in several parts Nigeria. The liquid content from the fresh leaves is used either alone or in combination with other herbs for the treatment of anaemia among the Igala speaking people of Kogi state of Nigeria, where the plant is called "Alumaje." In the Eastern part of Nigeria, particularly among the Igbos, the liquid content of the leaves of *Mucuna pruriens* commonly called "Agbala" is a very common remedy for the treatment of anaemia. The liquid is usually obtained from fresh leaves by manual collection, washed with clean water and squeezed to remove the liquid content of the leaves. This can be consumed directly or boiled for about five minutes and taken orally as blood tonic or booster (Katzenshlager *et al*., 2004). This study was therefore, designed to evaluate the effects of the aqueous extract of the leaf of *Mucuna pruriens* in laboratory models of anaemia to ascertain the claims of the traditional users of the leaf of *Mucuna pruriens* for the treatment of anemic conditions. In a study carried out by Obioma *et al* (2014) to demonstrate the anti-anaemic potential of aqueous leaf extract of *Mucuna pruriens*, Wister albino rats that have not been previously induced with anaemia were used as pharmacological models for the study. The authors compared the hematological parameters of the treated groups to the negative control group as a yardstick for establishing the anti-anaemic potential of the plant

extract. In this present study, however, the ameliorative effect of *Mucuna pruriens* on phenylhydrazine-induced anaemia in laboratory mice was investigated.

MATERIALS AND METHOD

Plant Material

Fresh leaves of *Mucuna pruriens* were collected in May, 2019 from Idah, Kogi State, Nigeria and were identified by Mr. Hakeem Lateef of the Department of Traditional Medicine and Medicinal Plant Research (TM & MPR) of National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja, Nigeria. It was assigned voucher number NIPRD/H/7108.The fresh leaves were air dried under shade for 2 weeks and pulverized into fine powder using pestle and mortar. A 200 g of the powdered leaves was extracted with 1 L of water by cold maceration with occasional shaking for 48 h (Trease and Evans, 2002). The filtrate was evaporated at 45^{o} C in a water bath to obtain the extract. The percentage yield of the aqueous extract of *Mucuna pruriens* is calculated be to 15.48 % $^{w}/_{w}$).

High Performance Liquid Chromatography Analysis

The bioactive constituents of the extracts were analyzed by high performance liquid chromatography. The HPLC consisted of Ultra-Fast LC-20AB equipped with SIL-20AC auto-sampler; DGU-20A3 degasser; SPD-M20A UV-diode array detector (UV-DAD, wavelength of 190 – 800 nm); column oven CTO-20AC, system controller CBM-20A lite and Windows LC solution software (Shimadzu Corporation, Kyoto Japan); column, 5µm VP-ODS C_{18} and dimensions (4.6 x 150 mm). The chromatographic conditions included mobile phase: 0.2% v/v formic acid and acetonitrile (20:80); isocratic mode; flow rate 0.6 ml/min; injection volume 10 µl of 50 mg/ml solution of extract in the mobile phase; detection UV 254 nm. Reference standards of rutin, caffeic acid, ferulic acid and chlorogenic acid were analyzed under the same condition as the extract. The HPLC operating conditions were programmed to give the following: solvent A: 80%, solvent B: 20%, column oven temperature was 40^{o}C and total run time of 15 minutes. (Krishna and Manohar 2014).

Analysis of Mineral Contents

The sample (0.52g) was digested with a mixture of concentrated nitric acid and Perchloric acid (7:3) on a regulated heating mantle in a fume hood. The digested sample solution was filtered into 50 cm^3 volumetric flask through Whatman filter paper, made-up to mark with deionized water and transferred into caped plastic bottle. The same process of digestion was adopted for blank sample preparation, without the analyte. The sample solution was analyzed for Calcium (Carmona & Soares Pereira), Copper (Cu), Manganese (Mn), Magnesium (Mg), Iron (Tijani, Okhale, Oga, Salawu, & Chindo), Lead (Pb) using Atomic Absorption Spectrometer (AAS) after optimization and calibration of the equipment with the standards solution of these elements followed by blank and the sample. The data obtained were processed the formulae:

$$\text{Metal } (\mu g/g) = \frac{C \times V \times D.f}{W}$$

where, C is the concentration obtained from the AAS machine (mg/L); V is the volume of the ample solutions in mL; W is the sample's weight in grams and D.f is the dilution factor used (Samali *et al*, 2017).

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Animals

Swiss albino mice of both sexes weighing 25 -30 g were obtained from the Animal Facility Centre, NIPRD. Animals were housed in propylene cages with saw-dust as bedding under standard environmental condition of temperature (26 ± 2 ^{o}C), and 12-hour light/dark cycle. They were fed with standard rodent diet and allowed free access to water. All experiments were performed in accordance with the "Principles of Laboratory Animal Care" (NIH, 2011) Publication No. 85; rev.1985) and NIPRD Standard Operating Procedures for Studies involving whole animal.

Acute Toxicity Study

The oral acute toxicity test was carried out in accordance to the OECD guideline (OECD Test No. 425, 2001) on oral acute toxicity testing. Female mice were collected and fasted overnight. On the test day 4 mice were weighed, 3 were given *Mucuna pruriens* 2000 mg/kg body weight at a volume of 10 ml/kg, one mouse served as control. Mice were observed individually during the first 8 hours with special attention given to the first critical four hours, and periodically for the next 24 hours and then daily for 14 days. All observations were systematically recorded with individual record maintained for each animal. Parameters observed in each mouse include general signs and symptoms of toxicity such as abdominal writhing, hyperactivity, sedation, convulsion, tremors, diarrhea, grooming, paralysis and death.

Induction of Anemia

Anaemia was induced in mice by daily intraperitoneal administration of 4 mg/kg phenylhydrazine *i.p.* (PHZ) for 4 days (Yeshoda *et al.,* 1942; Berger, 1985). On the 5th day, two drops of blood from nipping the tails of the mice were collected into crayon bottles containing diluent from the hematology analyzer (Wincom YNH7021) for hematological analysis. Mice with haemoglobin concentration lower than 13 g/dl were recruited for the study (Agbor *et al*., 2005).

Experimental Design

The anemic mice were randomized based on their haemoglobin concentrations into different treatment groups (n=6) along with a non-anemic group as described below:

- Group 1: Non-Anemic control (NAC) received 10 ml/kg normal saline p.o.
- Group 2: Anemic control (AC) received 10ml/kg normal saline PO
- Group 3: Received 100 mg/kg of *Mucunan pruriens* leaf extract PO.
- Group 4: Received 200 mg/kg of *Mucuna pruriens* leaf extract PO.
- Group 5: Received 400 mg/kg of *Mucuna pruriens leaf extract* PO.
- Group 6: Received 0.0214 mg/kg of Ferrous sulfate (FS)

All treatment was administered orally. Treatments were carried out for seven days and hematological analysis was carried on days 7, 14 and 21 respectively after the last treatment. Percentage recovery was calculated as:

$$Percentage\ Recovery = \frac{Post\ treaatment\ test - Pretreatmenttest}{Post\ treatment\ Normal - Pretreatment\ test} \times 100$$

Statistical Analysis

The results were expressed as mean ± S.E.M, n=6. The data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post-hoc test where appropriate using Graph Pad Prism 6.03 software. $P \leq 0.05$ were considered significant.

RESULTS

Oral Acute Toxicity Studies

The oral acute toxicity of the extract was determined to be relatively safe at 2000 mg/kg dose. Behavioural signs observed at 2000 mg/kg were rearing, paw licking, and sedation after two hours of administration. Rearing is the sign of anxiety in rodents whereby they raise the fore arm intermittently as they move about randomly. This effect was noticed among the mice between 30 minutes to 55 minutes after administration of the extract and stopped after 120 minutes. Paw licking is a sign of pain and this effect lasted between 30 and 45 minutes after administration of *M. pruriens.* Sedation means the mice became immobile and clustered in one location as from 90 minuts.

High Performance Liquid Chromatography (HPLC) Analysis

The HPLC chromatogram (Figure 1), showed that eleven peaks were detected with retention times in minutes of 3.024, 3.169, 3.610, 3.948, 4.551, 5.498, 5.935, 6.671, 7.263, 8.671and 9.403. Identification of the phytoconstituents of the extract was achieved by comparing their retention time with those of the reference standards analyzed under the same experimental condition as the extract. Compound with retention time of 3.948, 4.551, 5.498 and 7.263 minutes corresponded to chlorogenic acid, caffeic acid, rutin and ferulic acid respectively.

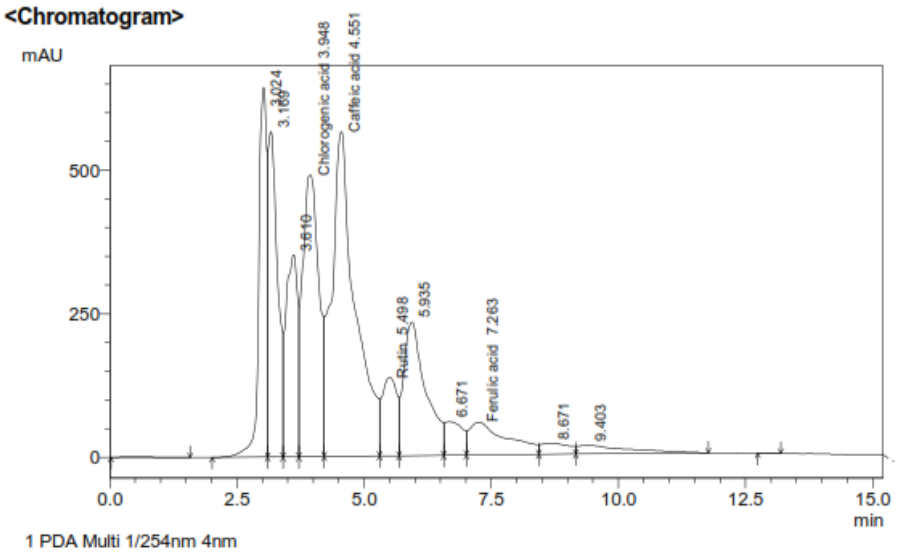

Figure 1: HPLC chromatogram of Mucuna extract.

Effects on Hematological Parameters

Administration of Phenylhydrazine (4 mg/kg) to mice significantly ($p<0.05$) decreased the red blood cells count There were also decrease in the level of Hemoglobin and Hematocrit in all treated animals.

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Treatment of animals for seven days with extract of *Mucuna pruriens* caused increase in the RBC in all extract treated animals. The increase was significant ($p<0.05$) at 400 mg/kg when compared to the anaemic control group. The increase in RBC was progressive at all doses of *Mucuna pruriens.* Significant increase was recorded at 7 days and 14 days' post treatment with extract. The positive control group treated with ferrous sulphate showed similar increases in RBC after 7 days' treatment with significant increase at 7 days and 14 days post treatment. By the 3rd week of experiment, the Hb, RBC and HCT returned to normal (Table 1,2 and 3). Figure1 presents the finger prints of ethanolic extract of *Mucuna pruriens*. The Hb of anaemic mice increased drastically within the first week of the experiment, though the increase was higher for the groups treated with *M. pruriens* extract than the anemic control at week 3 and 4 in an inverse dose related manner.

After an initial increase in the WBC of the various groups administered with phenyl hydrazine, there was a steady increase in the percentage recovery at all the groups. However, all groups treated with the extract significantly caused greater recovery percentage as with the standard reference drug (Table 4).

Induced changes in the hematological parameters of the mice during the study are presented in Tables 1, 2, 3 and 4. Daily (4 days) oral administration of phenylhydrazine (PHZ) significantly ($p<0.05$.) decreased the level of RBC, Hb, HCT compared to the non-anaemic control group (Tables 1,2 and 3). Mice in both anaemic control and treated (extract) groups showed significant increase ($p<0.05$) in the level of white blood cells (WBC) in the pre-treatment and post – treatment up to the second week of the study with only the normal saline group mice normalizing to same level with mice in zero control group by the third week of the study (Table 4). Two weeks post treatments the Hb, RBC and HCT return to normal with no further increases (Table 1,2 and 3). The mineral content of the *M. pruriens* extract shown in Table 5 indicated the presence of calcium, Manganese, Magnesium, Iron and Lead (Ca, Mn, Mg, Fe and Pb). Mineral element such as Ca, Fe, Mn and Mg are required by the body for various metabolic processes.

Table 1: Effect of *Mucuna pruriens* extract on Red Blood Cell (RBC)

Treatments	Pre-treatment Week 0	After 7 days Treatment		Week 1 Post-Treatment		Week 2 Post-treatment	
	RBC (g/dl)	RBC (g/dl)	% Recovery	RBC (g/dl)	% Recovery	RBC (g/dl)	% Recovery
NAC	8.99 ± 0.76	8.62 ± 0.44**		8.23 ± 0.67		8.24 ± 0.59*	
AC	4.64 ± 0.30[a]	6.17 ± 0.55	25.49	6.16 ± 0.13	42.41	6.29 ± 0.17	45.88
Mp 100 mg/kg	5.71 ± 0.66[a]	7.66 ± 0.55	39.70	8.85 ± 0.76*	124.64	11.83 ± 0.76**	.254.49
Mp 200 mg/kg	5.91 ± 0.50[a]	7.43 ± 1.07	32.34	9.31 ± 0.32**	146.52	11.75 ± 0.43**	217.93
Mp 400 mg/kg	5.26 ± 0.55[a]	8.54 ± 0.97*	61.12	8.06 ± 0.95	94.11	9.52 ± 1.02*	143.18
FS 0.0214 mg/kg	5.41 ± 0.17[a]	7.58 ± 0.70	41.68	10.39 ± 0.66**	176.68	13.18 ± 0.65***	295.88

Data expressed as Mean ± SEM g/dl (x 10^{12}/L); n= 6; RM One-way ANOVA followed by Dunnett's post-hoc test was done; * ($p< 0.05$); ** ($p< 0.01$) *** ($p< 0.001$) significantly different from anemic control (AC). [a] ($p< 0.05$) significantly different compared to Non-anemic control (NAC). Mp (*Mucuna puriens*) FS (Fersolate)

Table 2: Effect of *Mucuna pruiens* extract on Haemoglobin (Hb)

Treatments	Pre-treatment Week 0	After 7 days Treatment		Week 1 Post-Treatment		Week 2 Post-treatment	
	Hb (g/dl)	Hb (g/dl)	% Recovery	Hb (g/dl)	% Recovery	Hb (g/dl)	% Recovery
NAC	260.83 ± 16.41	312.17 ± 15.11		244.68 ± 13.93		244.40 ± 16.36	
AC	226.80 ± 10.18	263.25 ± 12.89	42.70	208.00 ± 6.82	-105.15	209.00 ± 11.48	-64.49
Mp 100 mg/kg	240.67 ± 19.19	260.40 ± 17.76	27.59	282.40 ± 20.44**	1040.64	344.74 ± 20.63***	1243.54
Mp 200 mg/kg	246.50 ± 15.82	254.50 ± 28.81	12.18	285.75 ± 8.19**	-2156.59	341.00 ± 9.98***	436.53
Mp 400 mg/kg	228.67 ± 13.75	277.83 ± 26.43	58.88	252.17 ± 23.77	146.75	281.17 ± 24.98*	-204.02
FS 0.0214 mg/kg	237.33 ± 8.66	249.60 ± 18.03	16.39	316.50 ± 19.17***	1077.58	378.50 ± 23.23***	1003.86

Data expressed as mean ± SEM (g/dl); n= 6; RM One-way ANOVA followed by Dunnett's post-hoc test **;** * ($p< 0.05$); ** ($p< 0.01$) *** ($p< 0.001$) represents significant difference from anemic control (AC). *M.pruriens* at all doses, significantly increased rates of recovery as from one-week post-treatment through week 2 post-treatment. NAC (Non-anemic control), Mp (*Mucuna puriens*) FS (Fersolate)

Table 3: Effect of *Mucuna pruriens* extract on Hematocrit (HCT)

Treatments	Pre-treatment Week 0	After 7 days Treatment		Week 1 Post-Treatment		Week 2 Post-treatment	
	HCT (%)	HCT (%)	% Recovery	HCT (%)	% Recovery	HCT (%)	% Recovery
NAC	36.97 ± 2.95**	51.72 ± 1.82		36.11 ± 2.86		38.36 ± 3.73	
AC	18.99 ± 1.29	44.90 ± 2.69	79.15	30.75 ± 1.77	68.68	31.97 ± 2.78	66.96
Mp 100 mg/kg	23.56 ± 3.46	43.14 ± 3.88	69.55	53.54 ± 10.09***	238.83	59.26 ± 3.56***	252.29
Mp 200 mg/kg	24.65 ± 2.22	41.18 ± 6.04	61.05	47.03 ± 1.57**	195.24	57.29 ± 1.82***	211.76
Mp 400 mg/kg	21.43 ± 2.44	45.48 ± 4.49	79.40	39.51 ± 4.44	123.16	45.24 ± 4.52*	-126.59
FS 0.0214 mg/kg	21.91 ± 1.08	40.63 ± 3.84	62.81	51.97 ± 3.68**	211.59	63.48 ± 3.97***	269.62

Data expressed as mean ± SEM (g/dl); n= 6; RM One-way ANOVA followed by Dunnett's post-hoc test; * ($p< 0.05$); ** ($p< 0.01$) *** ($p< 0.001$) represents significant difference from anemic control (AC). *M.pruriens* at all doses, significantly increased rates of recovery as from one-week post-treatment through week 2 post-treatment. NAC (Non-anemic control), Mp (*Mucuna puriens*) FS (Fersolate)

Table 4: Effect of *Mucuna pruriens* extract on White Blood Cells (WBC)

Treatments	Pre-treatment Week 0	After 7 days Treatment		Week 1 Post-Treatment		Week 2 Post-treatment	
	WBC ($x10^9$g/L) WBC ($x10^9$g/L)	WBC ($x10^9$g/L)	% Recovery	WBC ($x10^9$g/L)	% Recovery	WBC ($x10^9$g/L)	% Recovery

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NAC	5.48 ± 0.68	9.77 ± 0.47		9.68 ± 1.18		2.80 ± 0.31	
AC	15.54 ± 2.85	13.28 ± 0.75	39.23	11.25 ± 1.17	73.25	2.50 ± 0.50	102.35
Mp 100 mg/kg	18.02 ± 3.24	10.23 ± 1.00	94.44	16.34 ± 1.34	20.12	8.18 ± 2.16	66.51
Mp 200 mg/kg	18.87 ± 2.91	14.90 ± 2.72	43.59	18.35 ± 2.24	5.63	9.40 ± 2.09	56.94
Mp 400 mg/kg	18.13 ± 3.68	8.73 ± 1.29	112.36	11.93 ± 2.15	73.36	8.88 ± 0.74	118.27
FS 0.0214 mg/kg	21.68 ± 1.64	8.90 ± 1.29	107.27	15.20 ± 1.64	54.03	9.75 ± 1.01	54.33

Data expressed as mean ± SEM (g/dl); n= 6; RM One-way ANOVA followed by Dunnett's post-hoc test; No significant difference from anemic control (AC). *M.pruriens* at all doses, did not significantly increased rates of recovery as from one-week post-treatment through week 2 post-treatment. NAC (Non-anemic control), Mp (*Mucuna puriens*) FS (Fersolate)

Table 5: Mean Concentration of Mineral Content (µg/g)

Sample	**Mean Concentration (mg/g)**					
	Cu	Ca	Fe	Mn	Mg	Pb
A1	ND	1.20 ± 0.11	0.06.2 ± 0.12	0.02 ± 0.00	1.70 ± 0.12	0.14 ± 0.00
WHO/RDA	0.34 - 0.90mg/day	1500 – 2000 mg	-	1.2 - 2.6mg/day	80 - 420mg/day	*0.01mg/g

*WHO = World Health Organization, RDA = Recommended daily allowance, ND means not detected.

DISCUSSION

Phenylhydrazine produces both aryl and hydroxyl radicals when incubated with mice liver microsomes (Gannett *et al*., 1997) and resulting in oxidation under normal body temperature and pH (Rehse and Shahrouri, 1998). The generated radicals caused oxidative stress on the red blood cell membrane resulting in hemolytic anemia due to lipid peroxidation (McMillan *et al*., 1998; Cighetti *et al.,* 1999; Zimmermann *et al.,* 1997; Nelson *et al.,* 1997, Unami *et al*., 1996). In the present study, administration of mice with PHZ (4 mg/kg/day for 4 days) resulted in a severe hemolytic anemia characterized by decreased RBC, Hb and HCT (Table 1).

The RBC transports oxygen to all organs and tissues of different parts of the body. Deficiency and /or inability of the RBC to perform its oxygen distribution function to all parts of the body leads to problems related to normal body functions. In this study, PHZ altered the function of RBC as a result of the induced hemolysis characterized by decreased levels of RBC, Hb and HCT. *M.pruriens* extract at all doses used restored the levels of RBC, Hb, and HCT after one week. Hematocrit is the ratio of the RBC volume to the volume of the whole blood. Deficient hematocrit ratio indicates anemia as seen in the normal control group. The ability of *M. pruriens* to significantly improve the level of hematocrit, RBC, and Hb (tables 1,2 and 3) shows anti-anemic potential. From the mineral constituents' analysis of *M. pruriens*, micro elements such as magnesium, iron, and manganese were found to be present. These elements play important role in biosynthesis of vitamins required as co-factors in hematopoiesis. The lowest administered dose of 100 mg/kg reduced the recovery time of the blood parameters from 2 weeks in the anaemic control to 1 week. Also, the recovery was progressive such that after 2 weeks of continuous treatment, the Hb concentration and HCT were higher in the treated groups than in the normal control group (Table 2 and 3). It was also observed that the recovery of the treated groups was dose related with the dose of 100 mg/kg effecting the highest change. At the third week of the experiment, treatment of anaemic mice with *M.*

pruriens did not increase the RBC, Hb and HCT any further (Table 1,2,3). Under normal condition the body can generate new RBC to replace lost once but this will take much longer time as shown in this study. The recovery time of two weeks for untreated anaemic rats has earlier been reported when mice were bled 30% of their total blood volume to induce hemorrhagic anemia in another study (Agbor and Odetola, 2001). A significant correlation with diagnostic values has been demonstrated between RBC, Hb, PCV or HCT and the RBC indices (MCV, MCH and MCHC) in both humans and rodents (Archer, 1982; Bain, 1989). Hemoglobin (Hb) is the protein contained in red blood cells that is responsible for delivery of oxygen to the tissues and to ensure adequate tissue oxygenation, a sufficient hemoglobin level must be maintained. When the hemoglobin level is low, the patient has anemia. In this study, *M. pruriens* at all doses used significantly ($p<0.05$ –$p<0.001$) increased the recovery rate of phenylhydrazine-induced anemia (Table 2).

The hematocrit measures the volume of red blood cells compared to the total blood volume (red blood cells and plasma). Percentage level of haematocrit therefore, is the relative concentration of red blood cells to the whole blood volume. The reduced or sub-optimal percentage haematocrit value indicates dilutional anaemia and proportionate inability of oxygen delivering function of RBC. The ability of *M. pruriens* to significantly increase the recovery rates of phenylhydrazine-induced anemia in mice shows that the leaves possess haematinic and anti-anemic properties.

In this study, the hematinic potentials of the hydroethanolic leaf extract of *Mucuna pruriens* was evaluated following ethnobotanical claims of its usage to boost blood parameters and treat anaemia in some local communities in Nigeria. For such folkloric usage, the fresh leaves are collected manually, washed with clean water and then squeezed to remove the liquid content of the leaves which then can be consumed directly or boiled for about five minutes and taken orally as blood tonic to boost blood production (Katzenshlager *et al*., 2004). The results indicated the presence of phytochemicals such as alkaloids, glycosides, saponin, terpenoids, carbohydrates, chlorogenic acid, caffeic acid, rutin, ferulic acid, resins and mineral elements such as Ca, Fe, Mn and Mg (Table 5) which are required by the body for various metabolic processes. Minerals elements are of fundamental importance in agriculture and also to human health (Das and Singh, 2011).

CONCLUSION

In conclusion the aqueous extracts of *M*. *pruriens* leaves reversed anaemia induced by phenylhydrazine similar to those induced by parasite such as *Plasmodium falciparum* (Diallo *et al.,* 2008). The vitamin and mineral constituents of the leaf may be responsible for the haematinic effect of *M. pruriens* leaves. This result supports the traditional use of *M. pruriens* in the treatment of anaemia.

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