INTRODUCTION

*Mucuna pruriens* is a wild tropical legume native to Africa and Asia (Duke 1981 in Nweze et al., 2017), it belong to the family Fabaceae, and is among the various under-utilized wild legumes. *Mucuna pruriens* is commonly called ‘velvet bean’, ‘cowhage’ or ‘cowitch’ in English, Eni in Hausa, Agbala or Aghaloko in Ibo and Werepe in Yoruba. The plant is known for the intense irritation and itching, it produces on contact with the skin when the fruit is mature and dries (Andersen et al., 2015). The itch is caused by mucunain and serotonin contained in the hairs lining the seed pods called trichomes (Reddy et al., 2008).

It has nutritional potential as a rich source of protein (23-35%) (Teixeira et al., 2003), which is comparable to those of other pulses such as soybean, rice bean and lima bean (Gurumoorthi et al., 2003). In South East Asia, the immature pods and leaves of mucuna beans are used as vegetables. The ethnic groups of Eastern Nigeria use the seeds of *Mucuna sloanet* and *Mucuna pruriens* as a condiment or as garnishing for the main dish (Ukachukwu et al., 2000). All parts of *M. pruriens* has been reported to possess valuable medicinal properties and it has been investigated in various contexts. The seeds have laxative, anthelmintic, alexipharmic and tonic effects (Taylor, 2005). The L-dopa content of the seeds is responsible for its efficacy in the management of Parkinson’s disease (Manyam et al., 2004). The dried crushed root is applied for relief of toothaches (Hishika et al., 1981). The leaves possess aphrodisiac and antimicrobial activities (Warrier et al., 1996) and are used in the treatment of ulcers, cephalgia and general debility. It is reported that people suffering from debilitating disease conditions, acute blood loss and blood deficiency diseases consume leaves of *M. pruriens* (Akindele and Busayo, 2011).

Anaemia is a condition in which haemoglobin (Hb) concentration and/or red blood cell (RBC) numbers are lower than normal and insufficient to meet an individual’s physiological needs (WHO, 2011). Alternately, World health organization (WHO) defined anaemia as a haemoglobin (Hb) concentration less than 130g/l in men and 120g/l in women. Anaemia develops when the rate of red blood cell production by the bone marrow fails to keep pace with loss or destruction (Reid et al., 2008). Haemoglobin is the oxygen carrying part of red blood cells. The role of Hb in carrying oxygen to the tissues explains the most common clinical symptoms of anaemia, which include fatigue, shortness of breath, bounding pulses or palpitations, and conjunctival and palmar pallor (WHO, 2011).

Approximately one-third of the world’s population (32.9%) was estimated to suffer from anaemia in 2010 (Kassebaum et al., 2014). The population groups most vulnerable to anaemia as of 2016 include: (42%) children under 5 years of age, particularly infants and children under 2 years of age; (39%); women of reproductive age; and (46%) pregnant women (WHO, 2016).

Anaemia is one of the most widespread public health problems, especially in developing countries due to nutritional problems (such as iron or vitamin deficiency), low socioeconomic status, inherited disorders, recurrent infections and an increased prevalence of blood parasites especially Plasmodium, Trypanosomes and helminthic infestation (Sanni et al., 2005).
It has an important health, welfare, social, and economic consequences, these include impaired cognitive development, reduced physical work capacity and in severe cases increased risk of mortality, particularly during the perinatal period (Van den Broek et al., 2000).

In the tropics, due to prevalence of malaria and other parasitic infections, between 10 to 20% of the population is reported to have less than 100 g/l of haemoglobin in the blood (Diallo et al., 2008). The great loss in terms of clinical diagnosis and treatment and even depletion in human resources as a result of anaemia could be prevented with adequate exploration into medicinal plant that can naturally boost blood level. Hence, this study investigated the haematological effects of Methanol leaf extract of *Macuna pruriens* on Phenylhydrazine (PHZ) induced anaemic Wistar strain of albino rats.

**MATERIALS AND METHODS**

**Plant Collection and Identification**
The fresh leaves of velvet bean plant were collected locally from Zaria and identified by Mallam Galla at the Herbarium of Kaduna State University.

**Preparation of the Methanolic Plant Extract**
The leaves of *M. pruriens* were washed and air dried at room temperature. Then it was pulverized and macerated in 2 L of methanol for 72 hrs. The mixture was decanted through a glass funnel fitted with Whatmann No. 1 filter paper. The filtrate was then allowed to evaporate to dryness via open air evaporation. The dried extract was afterwards weighed and stored until when required.

**Experimental Animals**
Wistar rats of both sexes and weighing between 70g and 130 g were purchased from the animal house unit of the Nigerian Institute for Trypanosomiasis Research (NITR) Kaduna State. The animals were housed in locally fabricated cages within the Zoology Laboratory of Department of Biological Sciences Kaduna State University and were allowed to a seven days acclimatization period before the commencement of the study. The experimental animals were maintained on animal feed and given water *ad libitum*. Beddings were changed twice weekly.

**Haematological Parameters**
Before administration of phenylhydrazine in the rats, the baseline values of two haematological parameters were recorded, namely packed cell volume and haemoglobin concentration. Packed Cell Volume (PCV) was determined by the Microhaematocrit method, while the Haemoglobin (Hb) concentration of each animal was estimated by Cyanomethaemoglobin method using a spectrophotometer.

**Anti-anaemic activity**
For the evaluation of anti-anaemic effect of the plant extract, the methods of Joshi et al. (2018) and Gupta et al. (2018) were adopted with some modifications. Briefly, anemia was induced by intra-peritoneal injection of phenylhydrazine at 60 mg/kg body weight (Pandey et al., 2016; Ragupathy, 2008). Rats that developed anaemia with PCV level below 39% and Haemoglobin concentration lower than 13 g/dl were recruited for the study. The anaemic rats were divided into five groups of three (3) rats each as follows:

- **Group 1:** (Phenylhydrazine + Low dose extract, 250 mg/kg);
- **Group 2:** (Phenylhydrazine + High dose extract, 500 mg/kg);
- **Group 3:** (Phenylhydrazine +10 mg/kg Vitamin B12 – positive control);
- **Group 4:** (Phenylhydrazine + 1ml/kg normal saline - negative control);
- **Group 5:** (non-anemic + 1ml/kg normal saline - Normal control).

The dose of the extract was based on the work of Nweze et al. (2016) who reported a median lethal dose (LD₅₀) greater than 5000 mg/kg for the methanol leaf extract of *Macuna pruriens*. Treatment with methanol leaf extract of *M. pruriens*, vitamin B12, or normal saline was given orally throughout the duration of the study.

**STATISTICAL ANALYSIS**
The results of the haematological estimations were presented as mean ± SD of three animals per group. Total variations present in a set of data were estimated by One Way Analysis of Variance (ANOVA) and *p* value less than 0.05 was considered statistically significant.

**RESULTS**
Prior to the administration of phenylhydrazine (PHZ), the packed cell volumes (PCV) of rats were between 49 and 55 % (Table 1), with no significant difference (*p* > 0.05) in the mean PCV values of rats in the various experimental groups. Upon administration of phenylhydrazine (60 mg/kg body weight) there was an acute decline in mean PCV with values ranging from 31-35 percent in all the experimental groups except in the normal control. Between the PHZ treated groups, there was no statistically significant difference in the packed cell volumes in all the anaemic rats (*p* > 0.05). However, the mean packed cell volumes of anaemic rats were significantly lower (*p* < 0.05) than that of the normal control group (Table 1).

On day 21, the pattern of changes in the haematological parameters under investigation amongst group 3 (PHZ + 10mg/kg vitamin B12; positive control) rats were observed to be essentially similar to the pattern obtained amongst group 1 (PHZ + low dose extract) rats and group 2 (PHZ + high dose extract). Furthermore, when the extract treated groups were compared with the Normal control (Group 5), there was no significant difference (*p* > 0.05) with PCV values of the extract treated group approaching those of normal control. However, when compared with group 4 (PHZ + 1ml/kg normal saline: negative control) there was significant difference (*p* < 0.05) with elevations in PCV among the extract treated groups.
Table 1: Effect of plant extracts on packed cell volume of phenyl hydrazine induced anaemic rats

| Treatment groups                                      | Baseline  | D0      | D7       | D14     | D21     |
|-------------------------------------------------------|-----------|---------|----------|---------|---------|
| PHZ + 250 mg/kg extract                                | 49.6±3.0a | 32.0±3.46a | 40.7±4.51b | 42.9±2.11c | 53.6±2.15b |
| PHZ + 500 mg/kg extract                                | 51.7±2.9a | 31.7±2.61a | 31.3±1.53a | 36.8±0.46b | 50.8±0.39b |
| PHZ + 10 mg/kg Vitamin B12                            | 49.3±2.0a | 33.7±3.22a | 44.0±4.51b | 48.3±0.19d | 56.4±0.03b |
| PHZ + 1ml/kg normal saline                            | 52.7±2.5a | 35.3±2.08a | 27.3±2.0a  | 23.5±0.32a | 38.8±0.46a |
| Normal control                                         | 55.0±3.0a | 55.0±1.0b  | 54.3±2.52c | 55.8±3.01e | 55.2±1.41b |

p value: 0.2210, 0.0023*, 0.0345*, 0.0026*, 0.0138*

Values are given as mean ± standard error of mean (SEM). In each column, values with differing superscripts have statistically significant difference (p < 0.05)

The effect of treatment with methanolic leaf extract of Mucuna pruriens on Haemoglobin Concentration (Hb) of rats is shown in Table 2. Haemoglobin Concentration of anaemic rats treated with 250 and 500 mg/kg of the methanolic leaf extract recorded steady increase in mean Hb concentration compared to group 4 (PHZ + 1ml/kg normal saline: negative control). At Day 21, the Hb values of rats that received the extracts and Vitamins B12 had return to normal values, resulting to no significant difference in the mean values when compared with the normal control group (p > 0.05). But when compared with group 4 (PHZ + 1ml/kg normal saline: negative control) there was high significant difference (p < 0.05).

Table 2. Effect of plant extracts on haemoglobin concentration in phenyl hydrazine induced anaemic rats

| Treatment groups          | Baseline  | D0      | D7       | D14     | D21     |
|---------------------------|-----------|---------|----------|---------|---------|
| Anaemic + 250 mg/kg extract | 16.5±1.8a | 10.7±1.1a | 13.6±0.6b | 14.3±1.6cd | 17.9±2.3b |
| Anaemic + 500 mg/kg extract | 17.2±1.6a | 10.6±0.9a | 10.4±0.3a | 12.3±0.2b | 16.9±0.4b |
| Anaemic + 10 mg/kg Vitamin B12 | 16.4±0.2a | 11.2±0.1a | 14.7±0.7b | 16.1±2.1cd | 18.8±0.6b |
| Anaemic + 1ml/kg normal saline | 17.6±0.4a | 11.8±0.5a | 9.1±1.3a  | 7.8±0.6a  | 12.8±1.2a |
| Normal control             | 18.3±1.15a | 18.3±0.2b  | 17.8±1.1c | 17.6±1.5d | 18.4±1.7b |

p value: 0.1262, 0.0328*, 0.0023*, 0.0007*, 0.0007*

Values are given as mean ± standard error of mean (SEM). In each column, values with differing superscripts have statistically significant difference (p < 0.05)
significant difference (p < 0.05)

**DISCUSSION**

The results of this study indicate that phenylhydrazine induced anaemia in rats. These findings agree with those of Dholi et al. (2016), Drrouchula et al. (2017) and Umara et al. (2018) who also reported that administration of PHZ induces acute anaemia in rats. Phenylhydrazine induces haemolytic anaemia though oxidative alternations to red cell membrane proteins resulting in red cell injury and lysis (Berger, 2007).

The elevations in haematological parameters recorded in anaemic rats treated with either Vitamin B12 or the leaf extract of *Mucuna pruriens* are indicative of their haematopoietic properties. Vitamin B12 (cobalamin) is an essential nutrient required in blood cell formation and maturation. When in insufficient quantity, vitamin B12 deficiency results in Megaloblastic anaemia, a condition characterized by proliferation of immature red cell with reduced lifespan (Castellanos-Sinco et al., 2015; Koury and Ponka, 2004). There anti-anaemic activity demonstrated with the doses of the methanol leaf extract of *Mucuna pruriens* agrees with those of Obioma et al. (2014) who investigated the anti-anaemic potentials of the aqueous extract and raw leaves of *Mucuna pruriens*. Similarly, Madukwe et al. (2014) also reported that the fresh and shade-dried leaf extracts of *Mucuna pruriens* significantly increased haemoglobin, packed volume and white cell counts in anaemic rats. In a review article, Ravindra and Ashvini (2019) also mentioned *Mucuna pruriens* (Agbala) as a plant having anti-anaemic potentials. Furthermore, Nweze et al. (2017) reported that the fresh leaves of *M. pruriens* are washed and macerated in water to make a decoction which is drunk to boost blood supply. The anti-anaemic activity as demonstrated by *Mucuna pruriens* may be due to the presence of blood forming moieties in the leaf extract. Proximate analysis of the leaves showed the presence of iron, vitamin C and pro-vitamin A (Akomas et al., 2014; Madukwe et al., 2014). Iron is an essential haematopoietic factor required in the formation of the haem component of haemoglobin found in red cells. Vitamin C has also been reported to participate in haemopoiesis (Musyoka et al., 2016).

Phytochemical screening of the leaf extracts has also revealed the presence of saponins, flavonoids and alkaloids (Nweze et al., 2016). Certain class flavonoids and alkaloids have been reported to possessing antioxidant activity. Thus, the presence of these antioxidants in the leaf extract of *M. pruriens* may facilitate the reversal of the damaging effect of phenylhydrazine on red cells. Phenyl hydrazine is reported to induce anaemia through oxidative damage to red cell membrane by increasing the formation of reactive oxygen species (Lavanya et al., 2018). However, alkaloids and flavonoids protect cells as powerful antioxidants which prevent or repair damage done to red cells by free radicals or highly reactive oxygen species (Beack et al., 2020).

In conclusion, the results of the analysis carried out on Phenylhydrazine (PHZ) induced anaemia in Wistar strain of albino rats orally treated with methanolic leaf extract of *M. pruriens* revealed that the extracts boosted blood production, thereby suggesting its antiaemic potentials which can be used in folklore medicine for the management of anaemia.

**REFERENCE**

Akindele, A.J., and Busavo, F.L. (2011). Effects of the hydroethanolic extract of *Mucuna pruriens* on haematological profile in normal and haloperidol treated rats. *Nigeria Quarterly Journal of Hospital Medicine*, 212: 8-93.

Akomas S.C., Okafor A.I., and Ijioma S.N. (2014). Hypoglycemic, hematologic and hypolipidemic activity of *Mucuna pruriens* ethanol leaf extract in alloxan induced diabetic rats. *Annual Research and Review in Biology*, 4(24): 4284-4292.

Andersen, H.H., Elberling, J.P. and, Arendt-Nielsen, L. (2015). Human surrogate models of histaminergic and non-histaminergic itch. *Acta Derm Venereol*, 95(7): 771-777 doi:10.2340/00015555-2146

Beack B.S.S., Fant Y.S.A., Kopa K.T., Hadidjatou D., Kojom L.P., Kognou M.A., Ndomou M., Agbor A.G., Ngono N.R.A., Tchiegang C. (2020). Hemopoietic effects of some herbal extracts used in treatment of infantile anemia in Cameroon. *World Journal of Pharmaceutical and Medical Research*, 6(1): 147-155.

Berger J. (2007). Phenylhydrazine haemotoxicity. *J Appl Biomed.*, 5:125-130. doi:10.32725/jab.2007.017

Castellanos-Sinco. H.B., Ramos-Penafiel C.O., Santoyo-Sanchez A., Collazo-Jamola J., Martinez-Murillo C., Montano-Figueroa E., Sinco-Angeles A. (2015): Megaloblastic anaemia: folie acid and vitamin B12 metabolism. *Revista Medica Del Hospital General De Mexico.*, 78(3): 135-143

Dholi S.K., Rajeshwari V., Anusha V., Priya J.J., Ravali T., Mounika M. (2016). Anti-anaemic Activity of Ethanolic Extract of *Punica granatum* Seeds on henylhydrazine-Induced Anaemia. *Asian Journal of Ethnopharmacology and Medicinal Foods*, 2(2): 27-31.

Diaillo, A., Gbeassor, M., Vovor, A., Eklu-Gadegbeku, K., Aklikokou, K., Agbonon, A., and Akpagana, K. (2008). Effect of Tectonagrandisdom phenylhydrazine induced anaemia in rats. *Fitoterapia*, 79(5): 332-336.

Drrouchula, G.C., Kouakou, S.L., Miezan, B.A.P., Tchimon, D.P., and Yapi, H.F. (2017). Antianaemic properties of *Trema guineensis* aqueous and ethanolic extracts. *European Journal of Biotechnology and Bioscience*, 5(2): 20-24.

Gupta, D. (2018). Anti-anemic activity of hydro-alcoholic leaf extract of brassica oleracea var in phenylhydrazine induced anemic rats. *Asian Journal of Pharmaceutical Education and Research*, 7(2):12-18..

Gurumoorthi, P., Pugalenthii, M., and Janardhanan, K. (2003). Nutritional potential of five accessions of a South Indian tribal pulse, *Mucuna pruriens var. utilis*. II. Investigation on the total free phenolics, tannins, trypsin and chymotrypsin inhibitors, phytohaemagglutinins and in vitro protein digestibility. *Tropical and Subtropical Agroecosystems*, 1: 153-158.
Hishika, R., Shatraiy, S., Shinde, S., and Guptal, S.S. (1981). Preliminary phytochemical and anti-inflammatory activity of seeds of Mucuna pruriens. Indian Journal of pharmacy, 13(1):97–98.

Joshi, A., Kushwah, C., Gupta, D., Malviya, S. and, Kharia, A. (2018). Anti-Anemic Activity of Hydro-Alcoholic Extract Seeds of Foeniculum vulgare in Phenylhydrazine Induced Anemic Rats. PharamTutor, 6(7): 32-34.

Kassebaum, N. J., Ijasrasaria, R., and Naghavi, M. (2014). A systematic analysis of global anemia burden from 1990 to 2010. Blood, 123: 615–624.

Koury M.J., and Ponka P. (2004): New insights into erythropoiesis: the role of folate, vitamin B12, and iron. Annu. Rev. Nutr.; 24:105-131

Lavanya S., Swathi K., Babu V., Srivani, Metlakuna A.S., Goud B.M. (2018). Evaluation of Anti Anaemic Activity of Aqueous Piper betle Leaf extract against Phenylhydrazine Induced Anaemia in Wistar Rats. European Journal of Pharmaceutical and Medical Research, 5(12): 226-230.

Madukwe E.U., Okafor A.M., Ogbonna U.U. (2014). Effectiveness of fresh and shade-dried Mucuna pruriens leaf extract in controlling anaemia in adult albino rats. Pakistan Journal of Nutrition; 13: 579-583

Manyam, B.V., Dhanasekaran, M., and Hare, T.A. (2004). Neuroprotective effects of the antiparkinson drug Mucuna pruriens. Phytotherapeutic Research, 18:706–712.

Musyoka T.M., Dorothy N.W., Wycliffe A.M., Juma K.K., Nzioka M.D., et al. (2016). In vivo antianaemic effect and safety of aqueous extracts of Erythrina abyssinica and Zanthoxylum usambarensis in mice models. J. Hemetol. Therbo Dis., 4:242 doi:10.4172/2329-8790.1000242

Nweze, N. E., Ezema, C., Ezema, S.A., Ifeanyi, J. E., and Ezema, W. S. (2017). Toxicity and nutritional studies on Mucuna pruriens leaves from Nsukka, South-Eastern Nigeria. Compound Clinical Pathology, 26: 1-6.

Nweze, N.E., Nwachukwu, K.A., and Adieme, I.C. (2016). The effect of Iresine herbstii Hook on some haematological parameters of experimentally induced anemic rats. Compound Clinical Pathology, 25:797–803.

Obioma B.E., Okechukwu P.C.U., Emmanuel I.O., Ifeemeje J.C. (2014). Antianaemic potential of aqueous leaf extract of Mucuna pruriens on Wistar albino rats. International Journal of Current Microbiology and Applied Sciences, 3(2): 707-712

Obioma B.E., Okechukwu P.C.U., Emmanuel I.O., Ifeemeje J.C. (2014). Antianaemic potential of aqueous leaf extract of Mucuna pruriens on Wistar albino rats. International Journal of Current Microbiology and Applied Sciences, 3(2): 707-712

Ragupathy S. (2008). Malasars medicinal utility of the flora in the Velliangiri hills. Journal of Ethnobiology and Ethnmedicine, 4-8.

Ravindra R.P., and Ashvini A.N. (2019). Medicinal plants for treatment of anemia: A brief review. World Journal of Pharmaceutical Research, 8(5): 701-717

Reddy, V.B., Luga, A.O., Shimada, S.G., LaMotte, R.H., and Lerner, E.A. (2008). Cowhage-evoked itch is mediated by a novel cysteine protease: a ligand of protease-activated receptors. Journal of Neuroscience, 28:4331–4335.

Reid, R., Burt, A., Harrison, D., David, J., Levison, D. A., and MacSween, R.N.M. (2008). Muir’s textbook of Pathology; (14th ed.). Hodder Education, United Kingdom.

Sanni, F.S., Ibrahim, S., Esievo, K.A.N., and Sanni, S. (2005). Effect of oral administration of aqueous extract of Khaya senegalensis stem bark on Phenyl hydradzineinduced anemia in rats. Pakistani Journal Biological Science, 8(2):255-258.

Taylor, L. (2005). The healing power of rainforest herbs: a guide to understanding and using herbal medicinal. Square One Publishers, Garden City Park, New York

Teixeira, A.A., Rich, E. C, and Szabo, N. J. (2003). Water extraction of L-Dopa from Mucuna bean. Tropical and Subtropical Agro-ecosystems, 1(2&3): 159-172.

Umuru, H.A., Moses, M.A., and Zailani, H.A. (2018). Effect of Solanum nigrum Methanol Leaf Extract on Phenylhydrazine Induced Anaemia in Rats. Jordan Journal of Biological Sciences, 11(1): 65-71.

Ukachukwu, S. N., and Obioha, F. C. (2000). Effect of time duration of thermal treatments on the nutritive value of Mucuna cochinensis. Global Journal of Pure and Applied Science, 6:11–15.

Van den Brock, N.R., and Letsky, E.A. (2000). Etiology of Anemia in Pregnancy in South Malawi.American Journal of Clinical Nutrition, 72 (suppl):247S–256S.

Warrier, P. K., Nambiar, V.P.K., and Mankutty, C. (1996). Indian medicinal plants. Orient Longman, Chennai

WHO; World Health Organisation. (2011). The World Traditional Medicines Situation in Traditional Medicines. World Health Organization. (2016). Global Health Observatory data repository: prevalence of anaemia in women Accessed May 2, 2018 http://apps.who.int/gho/data/view.main.GSWCAH28REG.