FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 4 No. 3, September, 2020, pp 370 – 374
DOI: https://doi.org/10.33003/fjs-2020-0403-399

ANTI-ANAEMIC POTENTIAL OF METHANOLIC LEAF EXTRACT OF *MUCUNA PRURIENS* ON PHENYLHYDRAZINE (PHZ) INDUCED ANAEMIC ALBINO WISTAR RATS.

***[1]Kasang Naman, [1]Habibat Oseni and [2]Emmanuel Enoh**

[1]Department of Biological Science, Kaduna State University, Tafawa Balewa Way, Kaduna, Kaduna State, Nigeria.
[2]Nigerian Institute for Tryponosomiasis Research (NITR) Kaduna State, Nigeria

Corresponding Author's email: kasang.naman@kasu.edu.ng 08065667859

ABSTRACT

The antianaemic potential of methanolic leaf extracts of *Mucuna pruriens* was investigated using phenylhydrazine (PHZ) induced anaemic albino Wistar rats. Fifteen rats used for the study were randomized into five experimental groups. To induced anaemia, the rats (except the normal control, Group E), received 60 mg/kg of the haemolytic agent Phenylhydrazine intraperitoneally (*i.p*) for two consecutive days. Anaemic Wistar rats in groups A and B received a daily oral dose of 500 and 250 mg/kg of the methanolic leaf extract of *Mucuna pruriens*. Nweze *et al*. (2016) had reported a median lethal dose greater than 5000 mg/kg for the methanol leaf extract of *Mucuna pruriens*. Groups C and D received Vitamin B12 (10 mg/kg) and normal saline (1 ml/kg), respectively. Normal control rats also received normal saline (1 ml/kg). Extract or normal saline was administered *per os* (*p.o*) while vitamin B12 was administered *i.p*. for a duration of 21 days. Packed cell volume (PCV) and haemoglobin concentration were determined weekly for three weeks. The result of the study indicated that both the methanolic leaf extract of *Mucuna pruriens* and Vitamin B12 significantly ($p < 0.05$) increased the packed cell volume and haemoglobin concentrations in treated rats compared to the negative control group of rats. This indicated that the methanolic leaf extract of *Mucuna pruriens* has anti-anaemic properties and could be utilized in the management of anaemia.

Keywords: Phenylhydrazine, Anaemia, Anti-anaemic properties, Vitamin B12, *Mucuna pruriens*.

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 Agbaloko 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 sloanei* and *Mucuna puriens* 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, between10 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 anemia 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 *Mucuna 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 heamatological 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 (LD_{50}) greater than 5000 mg/kg for the methanol leaf extract of *Mucuna 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	D_0	D_7	D_{14}	D_{21}
PHZ + 250 mg/kg extract	49.6±3.0^a	32.0±3.46^a	40.7±4.51^b	42.9±2.11^c	53.6±2.15^b
PHZ + **500** mg/kg extract	51.7±2.9^a	31.7±2.61^a	31.3±1.53^a	36.8±0.46^b	50.8±0.39^b
PHZ + **10** mg/kg Vitamin B_{12}	49.3±2.0^a	33.7±3.22^a	44.0±4.51^b	48.3±0.19^d	56.4±0.03^b
PHZ + 1ml/kg normal saline	52.7±2.5^a	35.3±2.08^a	27.3±2.0^a	23.5±0.32^a	38.8±0.46^a
Normal control	55.0±3.0^a	55.0±1.0^b	54.3±2.52^c	55.8±3.01^e	55.2±1.41^b
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	D_0	D_7	D_{14}	D_{21}
Anaemic + **250** mg/kg extract	16.5±1.8^a	10.7±1.1^a	13.6±0.6^b	14.3±1.6^{bc}	17.9±2.3^b
Anaemic + **500** mg/kg extract	17.2±1.6^a	10.6±0.9^a	10.4±0.3^a	12.3±0.2^b	16.9±0.4^b
Anaemic + **10** mg/kg Vitamin B_{12}	16.4±0.2^a	11.2±0.1^a	14.7±0.7^b	16.1±2.1^{cd}	18.8±0.6^b
Anaemic + 1ml/kg normal saline	17.6±0.4^a	11.8±0.5^a	9.1±1.3^a	7.83±0.6^a	12.8±1.2^a
Normal control	18.3±1.15^a	18.3±0.2^b	17.8±1.1^c	17.6±1.5^d	18.4±1.7^b
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 ($p < 0.05$)

DISCUSSION

The results of this study indicate that phenylhydrazine induced anaemia in rats. These findings agree with those of Dholi *et al*. (2016), Droucoula *et al*. (2017) and Umaru *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 cell 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 haematopoiesis (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 phenyl hydrazine 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 antianaemic 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: folic 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.

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

Droucoula, G.C., Kouakou, S.L., Miezan, B.A.P., Tchimon, D.P., and Yapi, H.F. (2017). Antianaemic properties of *Trema guineensi* 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., Pugalenthi, 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., Shastry, S., Shinde, S., and Guptal, S.S. (1981). Preliminary phytochemical and anti-inflammatory activity of seeds of *Mucuna pruriens. Indian Journal of pharmacology,* 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.*PharmaTutor.,* 6(7): 32-34.

Kassebaum, N. J., Jasrasaria, 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 B_{12}, 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 bettle* 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 V.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*. *Phytotheraphtic 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. Throbo 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 anaemic rats. *Compound Clinical Pathology,* 25:797–803.

Obioma B.E., Okechukwu P.C.U., Emmanuel I.O., Ifemeje 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 Ethnomedicine*, 4-8

Ravindra R.P., and Ashvini A.N. (2019). Medicinal plants for treatment of anaemia: 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 hydrazineinduced 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.

Umaru, 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 cochinchinensis*. *Global Journal of Pure and Applied Science*, 6:11-15.

Van den Broek, 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.