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Anti-anaemic Activity of Ethanol Extract of *Mangifera indica* L. Stem Bark in Phenylhydrazin-induced Anaemia in Rabbits

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Abstract: There is high prevalence of anaemia in Africa to which medicinal plants may provide remedy. This study was therefore undertaken to evaluate the anti-anaemic effect of ethanol extract of *Mangifera indica* stem bark in phenylhydrazine (PHZ)-induced anaemia in rabbits (*Oryctolagus cuniculi*). Twelve rabbits (1.91±0.35 kg) were divided into four groups: A, B, C and D. Rabbits in Group A were given pelleted feed and drinking water only while each rabbit in groups B, C and D was also given 30 mg/kg body weight (bwt) of PHZ by subcutaneous administration, followed by a maintenance dose of 15 mg/kg bwt, four times, at interval of 2 days. Rabbits in Group B were given distilled water, group C was treated with 50 mg/kg bwt of *M. indica* extract while group D was treated with 100 mg/kg bwt of Multivet, all by daily oral gavage for 12 days. Blood was collected for evaluation of packed cell volume (PCV) using microhaematocrit, red blood cell (RBC) counts using haemocytometer and haemoglobin (Hb) levels using cyanomethemoglobin colorimetric assay. PHZ produced an average of 53% reduction in the levels of PCV, RBC and Hb on day 2 in groups B, C and D. Treatment of the rabbits in groups B, C and D with ethanol extract of *M. indica* stem bark produced significant ($p<0.05$) increase in the levels of PCV on day 14, RBC counts and Hb levels on day 8 when compared with the PHZ treated control rabbits; these alterations in the haematological parameters compared favourably ($p>0.05$) with the Multivet. This study suggests that ethanol extract of *M. indica* stem bark have anti-anaemic effect on PHZ-induced anaemia in rabbits.

KEYWORDS: Anti-anaemia, Anacardiacea, Haematocrit, Haemoglobin, *Mangifera indica*, Multivet, *Oryctolagus cuniculi*

1.0 Introduction

Anaemia is reduction in the circulating erythrocytes mass to below age-specific and gender-specific limits (Brill and Baumgardner, 2000). It is a condition whereby there is lower than normal number of circulating red blood cells in the body; i.e. less than 4.7 million red cells per microlitre of adult male blood and < 4.2 million red cells per microlitre of adult female blood or low haematocrit levels i.e. < 47 ml/100 ml for males and < 42 ml/100 ml for females or a decrease below normal in the haemoglobin concentration of red cells of the body; i.e. < 12 g/100 ml for adult male and < 10 g/100 ml for adult female (Vasudevan and Sreekumari, 2007; WHO, 2008).

Different types of anaemia that have been characterized include pernicious anaemia caused by deficiency of vitamin B_{12}, megaloblastic anaemia due to deficiency of Vitamin B_{12} and folic acid, normocytic or aplastic anaemia caused by chronic diseases, iron deficiency anaemia caused by deficiency of iron and excessive loss of blood, haemolytic anaemia caused by haemolysis and many others (Vasudevan and Sreekumari, 2007). Thus, the causes of anaemia include but not limited to acute blood loss, sickle cell disease, thalassaemia, haemolytic disorders, endocrine insufficiency, RBC enzyme deficiency, haemoglobinopathies, acute/chronic infections such as hookworms, malaria, schistosomiasis, tuberculosis, and Human Immunodeficiency Virus (Lee *et al.*, 1999; Brill and Baumgardner, 2000; Schnall *et al.*, 2000; WHO, 2015). The form of anaemia under the current investigation is haemolytic anaemia, which is the type of

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anaemia caused by excessive haemolysis; i.e. an abnormally rapid destruction (or decreased survival) of red blood cells of the body (Vasudevan and Sreekumari, 2007).

Anaemia is a common blood disorder which affects people of all ages, but the elderly, young women of child-bearing age and infants are at greater risk (WHO, 2015). The incidence of anaemia is higher in the developing countries like Africa and South-East Asia than in the developed countries, due to the presence of many aggravating factors such as poor nutrition and sanitation facilities, sickle cell traits, high prevalence of blood parasites such as *Plasmodium falciparum*, hookworms and helminthic infestations (WHO, 2015). Women of reproductive age may be susceptible to anaemia due to frequent blood loss during regular menstruations and childbirths (Smith and Brooker, 2010). In the developing regions of the world, infants and pregnant women are the most affected by anaemia, hence maternal and neonatal mortality was responsible for 3 million deaths in 2013 and these are the important contributors to overall global mortality (UNICEF, 2014; WHO, 2015). It has been estimated that 90,000 deaths per annum in both sexes and all age groups are due to iron deficiency anaemia alone (WHO, 2014).

Globally, the South-East Asia, Eastern Mediterranean and African regions have the highest prevalence of anaemia across the population groups; with more than half of the children in South East Asia and African regions (53.8% or more) classified as having anaemia (WHO, 2015). The global prevalence of anaemia among children (6-59 months) was 42.6%, and in women of reproductive age (15-49 years) was 29.4%, while the prevalence among pregnant women (15-49 years) was 38.2% (WHO, 2015). The incidence of anaemia in Africa among children (6-59 months) was 62.3%, women of reproductive age was 38.6% and pregnant women was 46.3%. The incidence of anaemia in Nigeria among children (6-59 months) was 71.0%, and for women of reproductive age was 49.0% while the prevalence among pregnant women (15-49 years) was 58.0% (Stevens *et al.*, 2013; WHO, 2015).

Many people in several countries of the world including Nigeria have resorted to the use of medicinal plants for the management of their ailments (Rajesh *et al.*, 2009; Adebayo *et al.*, 2010; Besong *et al.*, 2016). This is because several people believe that natural products from plants are less toxic to humans than the orthodox medicines (Adewunmi and Ojewole, 2004; Adeyemi *et al.*, 2010; Kolawole *et al.*, 2013). In addition, some biologically active plant secondary metabolites have found application as drugs or as model compounds for drug synthesis (Edema and Alaga, 2012; Mugweru *et al.*, 2016). Hence, several pharmaceutical drugs used today for the treatment of various diseases are derived from plants or plant-based products (Kamba and Hassan, 2010; Besong *et al.*, 2016).

Mangifera indica Linn (common name: Mango) which belongs to the family Anacardiaceae, is a popular perennial tree that grows in many parts of the world including Nigeria. The mango tree is an evergreen tree of varying shapes and sizes with a deep tap root and a profuse surface roots (Litz, 2009). It has a stout trunk of 90 cm, which may grow up to 1.0 m in diameter, and an umbrella-shaped crown that may reach 20-40 m high (Litz, 2009; Orwa *et al.*, 2009). The leaves are simple, alternate, borne on 1.0-12.5 cm long petioles. The leaves are 16-30 cm long by 3-7 cm broad on flowering branches and up to 50 cm long on sterile branches. The flowers are fragrant, pentameric, greenish-white or pinkish, while the fruits are large fleshy drupe of variable sizes, shapes, colours and tastes (Litz, 2009; Orwa *et al.*, 2009).

The different parts of *M. indica* such as the leaves and stem barks have been used in traditional medicines to treat several ailments such as asthma, cough, diarrhoea, dysentery, jaundice, pains and malaria fever (Gill, 1992). Scientific reports have shown that *M. indica* extracts have anti-diabetic (Aderibigbe *et al.*, 2001; Ojewole, 2005; Bhowmik *et al.*, 2009), anti-inflammatory (Garrido *et al.*, 2004; Ojewole, 2005), antibacterial activities (Bbosa *et al.*, 2007) and may promote increased production of erythrocytes in animals (Nwinuka *et al.*, 2008).

The crude extract of *M. indica* stem bark has been used in folklore medicine for the treatment of anaemia in human (Gill, 1992), however there appears to be paucity of scientific evidence to

substantiate these claims. This study was therefore initiated to evaluate the anti-anaemic activity of ethanol extract of *M. indica* stem bark in phenylhydrazine-induced anaemia in rabbits.

2.0 Materials and Methods

2.1 Materials

2.1.1 Plant Material and Authentication

The stem bark of *Mangifera indica* was harvested from a farmland at Otukpa, Ogbadibo Local Government Area of Benue State, Nigeria. It was authenticated in the Department of Forest Production and Products, College of Forestry, University of Agriculture, Makurdi, Nigeria. A voucher specimen has been deposited in the College Herbarium, and was assigned a voucher number FH/0201.

2.1.2 Chemicals and Equipment

Multivet and Hot air oven (MINI/75/SS/F) were products of Arab Pesticides and Veterinary Drugs Mfg. Co., Irbid, Jordan and Gen Lab Ltd, Cheshire, UK. Phenylhydrazine hydrochloride (PHZ) and Soxhlet extraction apparatus (UNSPSC: 41104812) were manufactured by Fisher Scientific Company, New Jersey, USA respectively. Spectrophotometer and Atomic absorption spectrophotometer [AAS] were products of PSC Biotech, Dublin, Ireland, while other laboratory reagents were of analytical grade made by British Drug House Chemicals Ltd, Poole, England.

2.1.3 Experimental Animals

Twelve rabbits, *Oryctolagus cuniculi*, (1.91 ± 0.35 kg) of both sexes used for the study were purchased from the Animal House Unit of the University of Jos, Jos, Plateau State, Nigeria.

2.2 Methods

2.2.1 Preparation of Extract

The stem bark of *M. indica* was sun-dried for one week, and pulverized to a fine powder. A metallic sieve was used to separate the fibers from the powder. The powder was stored in a dry container for one week. Ethanol extract of *M. indica* stem bark was obtained using Soxhlet extraction apparatus. In this method, 40 g of pulverized sample was placed in a Soxhlet apparatus and extracted for 72 hours using absolute ethanol as the solvent. Ethanol was evaporated from the mixture and the extract was dried to a constant weight in a hot air oven maintained at 50 ^{0}C. The percentage yield of extract was calculated as:

$$\text{Yield of extract (\%)} = \frac{\text{Weight of extract} \times 100}{\text{Weight of sample}}$$

2.2.2 Plant Secondary Metabolites and Mineral Element Analyses

Ethanol extract of *M. indica* stem bark was qualitatively analyzed for the presence of alkaloids, tannins, saponins, flavonoids, anthraquinones, cardiac glycosides and steroids by adopting the methods described by Trease and Evans (1992). The plant extract was also quantitatively analyzed for the presence of iron (Fe), sodium (Na), potassium (K), zinc (Zn), copper (Cu), magnesium (Mg) and calcium (Ca) with Atomic Absorption Spectrophotometer according to the procedure described by Horwitz (1975).

2.2.3 Induction of Anaemia and Treatment with M. indica Extract and Multivet

Twelve healthy rabbits were divided into 4 groups consisting of three rabbits per group. Rabbits in Group A were administered water only and served as the control. Rabbits in Groups B-D were each injected subcutaneously with 30 mg/kg body weight (bwt) of 2.5% neutralized PHZ to induce anaemia. Thereafter, 15 mg/kg bwt of PHZ were administered four times to each rabbit, as maintenance doses on days 3, 6, 9 and 12 of the experiment as described by Sanni *et al.* (2005).

Anaemia was confirmed in rabbits in Groups B, C and D on day 2 of the experiment when there was an average of 53% decrease in the Packed Cell Volume (PCV), Red Blood Cell (RBC) and Haemoglobin (Hb) levels. Thereafter, rabbits in group B were treated with distilled water and served as PHZ control while

those in group C were treated with 50 mg/kg bwt of ethanol extract of *M. indica* stem bark. Rabbits in group D were treated with 100 mg/kg bwt of Multivet, all by daily oral gavage for 12 days, using oral canula.

The animals were kept in clean cages placed in the Animal Experimental Room of University of Jos, with 12 hours light and 12 hours dark cycle, and acclimatised for two weeks before being used for the experiment. The animals were also fed *ad libitum* pelletized feed (Grand Cereals and Oil Mills Limited, Jos, Nigeria) and clean drinking water.

Ethical approval for the study was obtained from the Animal Welfare Committee of the Faculty of Pharmaceutical Sciences, University of Jos, Jos, Nigeria. The animals were also handled with care according to the Guidelines and Principles for Biomedical Research Involving Animals (CIOMS, 1985; Suckow *et al.*, 2012).

2.2.4 Blood Collection and Analysis

Blood was withdrawn from the rabbits on day 0 (before anaemia induction), then on day 2 (post-injection with PHZ) and on days 4, 8 and 14 of the experiment (Sanni *et al.*, 2005). One milliliter of blood was collected from each rabbit by puncturing the prominent ear vein with syringes and needles (Dina *et al.*, 2000), into a Bijou bottle containing an anticoagulant (EDTA). The blood was thoroughly mixed with the EDTA and used for determination of the levels of Red Blood Cells (RBC), Packed Cell Volume (PCV) and haemoglobin (Hb). PCV was determined with Microhaematocrit, RBC with Haemocytometer using Hayem's fluid while Haemoglobin was done with Cyanmethaemoglobin using Drabkin's diluting fluid (Baker *et al.*, 2000). The percentage decreases in the levels of PCV, RBC and Hb from their respective baseline values were calculated using the following expression:

$$\text{Change (\%)} = \frac{A - B \times 100}{A}$$

Where A represents the value of blood parameter on day 0 (baseline value) of the experiment while B represents the value of the blood parameter on day 2 of the experiment.

2.2.5 Determination of Body Weight

The body weight of the rabbits were determined with a weighing balance, on the first day (day 0), before the administration of PHZ and then on days 3, 10 and 15 of the experiment.

2.2.6 Statistical Analysis

Results were presented as means ± standard deviation (SD) of three determinations. The data were analyzed by One-Way Analysis of Variance. Least Significant Difference was used to determine the level of significance. The differences between the group means were considered significant at $p<0.05$.

3.0 Results

The weight of ethanol extract of *M. indica* stem bark after the extraction and drying was 14.8 g whereas that of the pulverized sample was 40.0 g. Therefore, the percentage yield of the ethanol extract of *M. indica* stem bark was calculated as 37.0 (w/w). The ethanol extract of *M. indica* stem bark contained tannins, cardiac glycosides and flavonoids. The ethanol extract of *M. inidca* stem bark also contained iron, sodium, potassium, copper, zinc, calcium and magnesium at different concentrations (Table 1).

The four groups of rabbits had similar average body weights throughout the duration of the experiment. There was no significant ($p>0.05$) difference in the mean body weight of the rabbits in groups C and D when compared with the PHZ control and the distilled water treated control rabbits (Table 2).

The PCV values of rabbits in groups B, C and D decreased by 64 , 56 and 42% respectively from their baseline values on day 2, after the administration of PHZ whereas the PCV of the distilled water treated control rabbits increased (Table 3). However, treatment with ethanol extract of *M. indica* stem bark significantly ($p<0.05$) increased the PCV values of rabbits in group C when compared with the PHZ-treated control rabbits (group B), and compared favourably ($p>0.05$) well with those of group D rabbits that received the Multivet (the reference drug) on day 14 of the experiment (Table 3).

Similarly, PHZ decreased RBC counts in rabbits in groups B, C and D by 47, 55 and 45% respectively on day 2 of treatment (Table 4). Treatment of rabbits in group C with the ethanol extract of *M. indica* stem bark produced significant ($p<0.05$) increase in RBC counts when compared with the PHZ control, on days 8 and 14 of the experiment, which was comparable to those of the PHZ treated rabbits that received the Multivet (Table 4).

Administration of PHZ to rabbits in groups B and C reduced the level of Hb by 59% when compared with their baseline values. The Hb levels of rabbits in group D was also reduced by 48% when compared to their baseline values. All these changes in the Hb levels became prominent on day 2 post-PHZ administration. However, rabbits in group A fed with animal feed and water produced slight decrease of 3.16% in Hb levels from the baseline values (Table 5). Furthermore, treatment of rabbits in group C with ethanol extract of *M. indica* stem bark significantly ($p<0.05$) increased their haemoglobin concentrations when compared with the PHZ-treated control, on day 8 of the experiment and thus compared favourably ($p>0.05$) with the Multivet-treated group (Table 5).

4.0 Discussion

Anaemia is commonly diagnosed by decrease in the levels of Hb, PCV and RBC of animals (Dina *et al.*, 2000; Sanni *et al.*, 2005; Nwinuka *et al.*, 2008). The decrease in the levels of PCV, Hb and RBC from the baseline (day 0) values on day 2 of experiment, after injection of rabbits with PHZ, may be an indication that anaemia has been induced in the rabbits in groups B, C and D animals. These findings agrees with those of Bowman and Rand (1980), who reported that a more than 50% reduction in PCV values after injection of PHZ into experimental animals was an indication of anaemia. Berger (1985) also reported induction of anaemia after the administration of PHZ to 8 week old rats with a decrease of 55% from the normal erythrocyte concentration and 47% reduction of PCV values within 3 days. Similarly, a 62% decrease in PCV values from the baseline values, after the administration of PHZ to rats, has previously been reported to be an indication of anaemia (Sanni *et al.*, 2005).

PHZ has earlier been reported to induce anaemia by oxidative break down of RBCs, i.e. by increasing the formation of reactive oxygen species such as nitric oxide, resulting in haemolytic anaemia (Hill and Thornalley, 1982; Clemens *et al.*, 1984; Amer *et al.*, 2004; Borley *et al.*, 2010). The significant elevation of RBC counts, haemoglobin concentrations and PCV values after treatment of anaemic rabbits with ethanol extract of *M. indica* stem bark confers anti-anaemic activity on *M. indica* stem bark extract, which was comparable to the reference haematinic drug, Multivet. Dina *et al.* (2000), Sanni *et al.* (2005), and Diallo *et al.* (2008), have in separate studies reported that similar plants extracts also have anti-anaemic activities in animals intoxicated with PHZ as evident form the increases in the values of their PCV, haemoglobin concentrations and RBC counts.

Studies have earlier shown that the aqueous extract of *M. indica* stem bark when administered to rats (Nwinuka *et al.*, 2008), and when the plant leaves were also fed to goats (Ajayi *et al.*, 2005), were safe and elevated the levels of PCV, WBC and RBC of the animals. These authors did not induce anaemia with PHZ or any other drug but only investigated the toxic effect of *M. indica* extract in animals and found that it increased their haematological parameters. However, the current study evaluated the effect of *M. indica* extract in PHZ- induced anaemia in rabbits and its capacity to protect their red cells against several doses of the drug.

The secondary metabolites such as tannins, cardiac glycosides and flavonoids found in the extract of *M. indica* stem bark were similar to that previously reported by Madunagu *et al.* (1990). The presence of elements like iron, copper, zinc, magnesium, calcium, sodium and potassium also agreed with an earlier report (Nũñez-Sellẽs *et al.*, 2007). Thus, these organic and inorganic chemical constituents of *M. indica* stem bark extract may be responsible for its *in vivo* anti-anaemic activity. Flavonoids belong to the polyphenols group of secondary metabolites reported to act as powerful antioxidants which protect red cell membranes against oxidative damage by reactive oxygen species and free radicals (Youdim *et al.*, 2000; Adedayo *et al.*,

Table 1: Some Mineral Element Constituents of Ethanol Extract of *M. indica* Stem Bark

Mineral element	Concentration (ppm)	Relative composition
Iron (Fe)	96.47	High
Sodium (Na)	354.09	High
Potassium (K)	4561.95	Very high
Copper (Cu)	0.78	Very low
Zinc (Zn)	5.34	Very low
Calcium (Ca)	10,543.70	Very high
Magnesium (Mg)	1976.05	Very high

Values are given in parts per million (ppm)

Table 2: Body weights of Phenylhydrazine-induced Anaemic Rabbits Treated with Ethanol Extract of *M. indica* Stem Bark and Multivet

	Body Weight (kg)			
Duration of experiment (days)	**Groups of rabbits**			
	Normal control	Phenylhydrazine + distilled water	Phenylhydrazine + 50 mg/kg bwt of *M. indica* extract	Phenylhydrazine + 100 mg/kg bwt of Multivet
0	1.83 ± 0.30^{a}	1.73 ± 0.64^{a}	1.60 ± 0.35^{a}	2.18 ± 0.14^{a}
3	1.92 ± 0.25^{a}	1.87 ± 0.64^{a}	1.65 ± 0.44^{a}	2.22 ± 0.10^{a}
10	1.83 ± 0.23^{a}	1.73 ± 0.45^{a}	1.87 ± 0.48^{a}	2.05 ± 0.22^{a}
15	2.02 ± 0.27^{a}	1.78 ± 0.58^{a}	2.02 ± 0.45^{a}	2.30 ± 0.10^{a}

Values are means ± SD; values with different superscripts across the same row are significantly different from the control at $p<0.05$.

Table 3: Effect of Ethanol Extract of *M. indica* Stem Bark and Multivet on PCV of Phenylhydrazine-induced Anaemic Rabbits

	Packed Cell Volume (%)			
Duration of experiment (days)	**Groups of rabbits**			
	Normal control	Phenylhydrazine + distilled water	Phenylhydrazine + 50 mg/kg bwt of *M. indica* extract	Phenylhydrazine + 100 mg/kg bwt of Multivet
0	34.3 ± 5.10^{a}	31.0 ± 3.00^{a}	30.0 ± 3.00^{a}	32.3 ± 4.20^{a}
2	43.3 ± 9.00^{b}	11.0 ± 5.20^{a} (64.52)	13.0 ± 3.60^{a} (56.67)	18.7 ± 1.20^{a} (42.11)
4	41.3 ± 9.60^{b}	12.3 ± 4.00^{a}	17.0 ± 1.00^{a}	14.7 ± 3.50^{a}
8	43.0 ± 4.00^{b}	20.3 ± 0.59^{a}	22.0 ± 2.00^{a}	23.0 ± 1.00^{b}
14	41.7 ± 1.5^{b}	20.7 ± 0.59^{a}	26.0 ± 1.00^{b}	28.7 ± 0.59^{c}

Values are means ± SD; values with different superscripts a, b or c in the same row are significantly different from PHZ control at $p<0.05$. The values in parenthesis are percentage (%) decreases in PCV values on day 2 after PHZ administration.

Table 4: Effect of Ethanol Extract of *M. indica* Stem Bark and Multivet on RBC Counts of Phenylhydrazine-induced Anaemic Rabbits

Duration of experiment (days)	RBC Counts ($x10^{12}$ cells. L^{-1}) Groups of rabbits			
	Normal control	Phenylhydrazine + distilled water	Phenylhydrazine + 50 mg/kg bwt of *M. indica* extract	Phenylhydrazine + 100 mg/kg bwt of Multivet
0	4.6±0.26[a]	4.40±0.41[a]	4.98±0.27[a]	4.60±0.26[a]
2	5.9±1.09[b]	2.30±1.10[a] (47.72)	2.20±0.47[a] (55.82)	2.50±0.35[a] (45.65)
4	5.8±0.99[b]	0.98±0.14[a]	1.40±0.17[a]	1.50±0.68[a]
8	6.1±0.43[b]	1.37±0.10[a]	2.10±0.17[b]	1.80±0.14[a]
14	6.2±0.19[b]	2.60±0.35[a]	3.50±0.19[b]	3.30±0.10[c]

Values are means ± SD; values with different superscripts a, b or c in the same row are significantly different from PHZ control at $p<0.05$. The values in parenthesis are percentage (%) decreases in RBC counts on day 2 after phenylhydrazine hydrochloride administration.

Table 5: Effect of Ethanol Extract of *M. indica* Stem Bark and Multivet on Haemoglobin Levels (g/L) of Phenylhydrazine-induced Anaemic Rabbits

Duration of experiment (days)	Haemoglobin Levels (g/L) Groups of rabbits			
	Normal control	Phenylhydrazine + distilled water	Phenylhydrazine + 50 mg/kg bwt of *M. indica* extract	Phenylhydrazine + 100 mg/kg bwt of Multivet
0	14.87±0.90[a]	12.89±0.80[a]	11.99±0.58[a]	13.48±1.20[a]
2	14.40±3.00[b] (3.16)	5.23±0.28[a] (59.43)	4.86±0.85[a] (59.43)	6.97±0.84[a] (48.29)
4	14.17±2.60[b]	9.53±1.30[a]	12.10±1.10[a]	11.55±1.10[a]
8	17.20±3.50[b]	9.63±0.30[a]	13.75±1.10[b]	12.10±1.10[c]
14	13.85±1.96[b]	9.08±0.39[a]	9.53±0.68[a]	10.21±0.69[a]

Values are means ± SD; values with different superscripts a, b or c in the same row are significantly different from the PHZ control at $p<0.05$. Values in parenthesis are the percentage (%) decrease in haemoglobin levels of rabbits on the day 2 after administration of PHZ.

2008; Sowunmi and Afolayan, 2015), while tannins are anti-inflammatory agents which play important role in preventing inflammatory conditions (Sowunmi and Afolayan, 2015). This protective role of flavonoids prevents haemolytic anaemia due to excessive haemolysis, mediated by the elevated level of nitric oxide, a potent ROS generated by phenylhydrazine (Borley *et al.*, 2010). The mineral elements found in *M. indica* stem bark extract are important elements required for synthesis, healthy functioning and protection of red cells (Sanni *et al.*, 2005). Iron is an essential element required for red cells production while copper and zinc are important components of enzymes which regulate cell functions and protect red cell membranes from premature destruction (Vasudevan and Sreekumari, 2007). The key enzyme, 5-aminolevulinate synthase, which controls the biosynthesis of heme (Hunter and Ferreira, 2009), and the antioxidant enzyme, catalase, which protects red cells against oxidative destruction, require iron as their essential element (Chelikani *et al.*, 2004).

Calcium, potassium and sodium are important elements involved in the physiological and metabolic processes required for healthy functions of animal cells. Sodium and potassium are electrolytes which maintain acid-base balance and are also involved in membrane function that are essential for the survival of red cells (Bender and Mayes, 2006). Copper and zinc protect the red cells from reactive oxygen species or free radicals damage and they are important component of superoxide dismutase (SOD), a copper/zinc-dependent enzyme which eliminates superoxide radicals (Tainer *et al.*, 1983; Davis, 2003). Magnesium is an important component of red blood cells. It influences the electrical properties of red cell membranes and their permeability characteristics (Swaminathan, 2003). It was earlier shown that magnesium-deficient diet leads to significant decrease in the levels of RBCs and haemoglobin of rats, with a resultant decrease in whole blood iron (Sanchez-morito *et al.*, 2000). Thus, the presence of these elements in *M. indica* stem bark extract might have promoted the synthesis of red blood cells and their protection against drug-induced haemolysis, hence the accelerated rate of recovery from PHZ-induced anaemia in the treated rabbits.

Conclusion

In conclusion, this study has shown that ethanol extract of *M. indica* stem bark has anti-anaemic effect on phenylhydrazine-induced anaemia in rabbits. This activity may be due to the presence of flavonoids and essential elements such as iron, copper, magnesium and zinc. This therefore gives credence to the acclaimed use of *M. indica* stem bark extract in traditional medicine for the management of anaemia in humans. However, further studies are required to isolate, purify and characterize the bioactive agent(s) responsible for this health benefit.

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