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Full Length Research Paper

Antianaemic potentials of some plant extracts on phenyl hydrazine-induced anaemia in rabbits

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The antianaemic potential of three plant extracts on phenyl hydrazine-induced anaemia in rabbits was investigated. Anaemia was induced in rabbits with phenyl hydrazine hydrochloride at a dose of 30 mg kg^{-1} b.wt by subcutaneous administration. Treatment of anaemia was done with ethanolic extract of *Mangifera indica* stem bark, aqueous leaves extract of *Telfairia occidentalis* and *Amaranthus hybridus*. *In vivo* investigation showed that oral daily dose of 20 mg kg^{-1} b.wt of the ethanolic extract of *M. indica* stem bark and aqueous leaves extract of *T. occidentalis* produced a significant ($P < 0.05$) antianaemic effect. The aqueous leaves extract of *A. hybridus* only produced a minimal antianaemic effect, reflected by a significant increase ($P < 0.05$) in haemogloin concentration. Phytochemical analysis of the plant extracts detected saponins, tannins, cardiac glycosides, flavonoids and alkaloids in the 3 extracts. This study therefore, shows that *M. indica* and *T. occidentalis* extracts have antianaemic potential.

Key words: Anaemia, phenyl hydrazine, *Mangifera indica, Telfairia occidentalis, Amaranthus hybridus*.

INTRODUCTION

Anaemia is a common blood disorder that affects people of all ages, although the people at greater risk are the elderly, young women of child-bearing age and the infants. This condition is not a disease but could develop as a result of various diseases. There are over 400 types of anaemia, many of which are rare but in all cases there is lower than normal number of circulating red blood cells. Presently, more than half of the world's population will experience some forms of anaemia in their life time (Duff, 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 knowledge. The incidence of anaemia is higher in the third world than in developed countries due to the presence of many aggravating factors such as poor nutrition, high prevalence of blood parasites example, plasmodium, trypanosomes and helminthes infestation. It is also known that women are susceptible to anaemia during pregnancy due to high demand from the developing foetus (Orna, 1991; Ong, 1973). Although there are various drugs for the treatment of anaemia, they are not affordable to many poor people especially those in the developing countries such as Nigeria. In addition, the rural populations in various parts of the world do not have adequate access to high quality drugs for the treatment of anaemia, so they depend heavily on plants and herbal products for the treatment of diseases and anaemia. As a result of the fact that anaemia is very common and the incidence is likely to increase in future (Duff, 2008), there is need to prevent it or seek for more cost-effective and better treatment strategies.

Anaemia is one of the numerous ailments claimed to have been successfully treated with plant materials by traditional medicine practitioners. In China for instance, blood diseases such as malformation of blood circulatory system, anaemia, varicose veins and haemorrhages have been treated with plant materials (Richard, 1978). The crude extract of *Fagara zanthoxylum* was reported to be effective in the treatment of sickle cell anaemia (Sofowora, 1979). It was also reported that the aqueous crude extract of *Telfairia occidentalis* leaves has haematinic activity (Dina et al., 2000). It is well established that man consumes a wide variety of local

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crops and vegetables, which are believed to contribute significantly to the improvement of human health in terms of disease prevention and therapy (Breazile, 1971; Burkill, 1985). There are also claims by traditional medicine practitioners that the stem bark extract of *Mangifera indica* is used for the treatment of anaemia but it appears there is no scientific research and publication to verify this claim. Therefore, this study was initiated to investigate the antianaemic potential of *M. indica* stem bark extract, the leaves extract of *T. occidentalis* and *Amaranthus hybridus.*

MATERIALS AND METHODS

Experimental animals

Twelve healthy rabbits (Oryctolagus cuniculi) of both sexes, ranging in age from weaners to adults were used for this research. They were purchased from the animal house unit, University of Jos and kept in the Animal house experimental room. They were fed with pelleted chick mash purchased from Grand cereals and oil mills limited (Vital feeds) Jos and clean drinking water *ad libitum*. They were allowed to stabilize for two weeks before being used for the experiment. The rabbits were divided into 4 groups, each consisting of 3 rabbits and were gently handled to avoid stress. The baseline values were determined before the rabbits were induced with anaemia. Group A rabbits were given only distilled water and served as control. Groups B, C and D rabbits were treated with *T. occidentalis*, *M. indica* and *A. hybridus* plant extracts respectively.

Plant materials

The bark of *M. indica* stem was freshly collected from a farm in Otukpa, Ogbadibo Local Government Area of Benue while the fresh leaves of *T. occidentalis* and *A. hybridus* were purchased from Jos main market, Jos, Plateau State; all in North Central Nigeria. The plant materials were identified and authenticated by plant taxonomist, Mr. O. E. Agyeno, Department of Plant Science and Technology, University of Jos. The plant materials were thoroughly washed and dried at room temperature and 40 g of *M. indica, T. occidentalis* and *A. hybridus* each were Soxhlet extracted. The choice of water as the solvent for *T. occidentalis* and *A. hybridus* was to mimic the traditional method of preparing crude plant extracts and that of ethanol for *M. indica* was to enhance the extraction of more constituents. Besides, our pilot study revealed that *M. indica* powder was not readily soluble in water. The yield for *M. indica, T. occidentalis* and *A. hybridus* were 26.95, 17.1 and 11.67% (w/w) respectively.

Phytochemical analysis

The presence of phytochemical components in *M. indica*, *T. occidentalis* and *A. hybridus* was determined by methods earlier described (Adesina, 1983; Allen, 1974; AOAC, 1980; Trease and Evans, 1984).

Induction of anaemia

This was done as described by Harris and Kugler (1971) and modified by Sanni et al. (2005). Briefly, anaemia was induced in all the rabbits by subcutaneous administration of 2.5% neutralized phenyl hydrazine hydrochloride (Fisher Scientific Company, New Jersey, USA) as described by Harris and Kugler, (1971), at a dose of 30 mg kg^{-1} body weight, with a maintenance dose of 10, 15 and 20 mg kg^{-1} body weight of the same drug at interval of 3 days, within the duration of the experiment.

Blood collection and analysis

About 1 and 4 ml of blood was collected from each rabbit into bijou bottles with EDTA or without EDTA (for serum) by puncturing the prominent ear vein with syringe needles. The 1 ml blood was thoroughly mixed with EDTA to avoid coagulation and used for haematological tests. The 4 ml blood was allowed to clot at room temperature and then serum was collected after centrifugation at 1000 rpm for 10 min. Serum samples were stored at -20°C until when used for biochemical assays. The 1 ml blood was collected before and after anaemia induction and during treatment, but 4 ml blood was collected at the end of treatment.

Treatment of phenyl hydrazine-induced anaemia with plant extracts

Groups B, C and D rabbits were treated by daily oral administration of plant extracts, after diagnosis of anaemia, that is, 2 days after phenyl hydrazine administration, at a dose of 30 mg kg^{-1} body weight for 14 days. The plant crude extracts were reconstituted with distilled water and given to the animals orally: groups B. C and D rabbits were administered *T. occidentalis*, *M. indica* and *A. hybridus* extracts respectively.

Haematological evaluation

Packed cell volume (PCV) was determined by the microhaematocrit method, red blood cell (RBC) counts was done by improved Neubauer haemocytometer and haemoglobin (Hb) estimation was carried out by Cyanomethaemoglobin method using a spectrophotometer.

Biochemical assays

Bilirubin (Direct and Total) concentration was determined using Diazo reagents according to Van den Bergh's reaction while total protein was estimated by the Biuret method using a spectrophotometer.

Statistical analysis

The means and standard deviations (SD) of the data were calculated. The results were analyzed by one-way analysis of variance (ANOVA) and where applicable, least significant difference (LSD) was used to determine significant results. The differences between groups were considered significant at $P < 0.05$.

RESULTS

The results of phytochemical screening (Table 1) for the three plant extracts showed that *M. indica* is positive for tannins, cardiac glycosides and flavonoids; *A. hybridus* is positive for saponins, alkaloids and flavonoids while *T. occidentalis* is positive for saponins, alkaloids, cardiac

Table 1. Phytochemical components of *M. indica, A. hybridus* and *T. occidentalis* crude extracts.

Parameters	M. *indica*	A. *hybridus* i	*T. occidentalis*
Saponins	_	+	+
Tannins	+	_	_
Anthraquinones	_	_	_
Alkaloids	_	+	+
Steroids	_	_	_
Cardiac glycosides	+	_	+
Flavonoids	+	+	+

+ = present.
- = Absent.

Table 2. Effect of plant extracts on packed cell volume (%) of phenyl hydrazine-induced anaemia in rabbits.

Duration of experiment (days)	Groups of Rabbits			
	(A) Treatment with distilled water	(B) Treatment with *T. occidentalis*	(C) Treatment with *M. indica*	(D) Treatment with with *A. hybridus*
0	29.0± 3.0	31.7±0.5	29.7 ± 0.59	29.0±2.0
2	10.0±1.0	13.7 ±4.	12.0± 2.0	13.7± 0.59
6	19.3±2.1	25.7 ±2.5	19.7 ± 1.5	22.7 ±5.5
9	26.0±1.0[b]	23.3± 1.2	31.0 ± 1.0[b]	20.3± 1.5
12	26.0±1.0	21.7 ± 0.59	26.7± 0.6	22.02±.0
16	17.7± 0.59[a,b]	20.0 ± 2.0[a]	19.7±0.59[b]	17.0 ± 2.0

Values are expressed as means ± SD, n = 3. Values with the same superscripts on a row are significantly different at P < 0.05.

Table 3. Effect of plant extracts on red cell counts ($X10^{12}$/L) of phenyl hydrazine-induced anaemia in rabbits.

Duration of experiment (days)	Groups of rabbits			
	(A) Treatment with distilled water	(B) Treatment with *T. occidentalis*	(C) Treatment with *M. indica*	(D) Treatment with *A. hybridus*
0	3.8 ± 0.25	4.3 ± 0.52	3.4 ± 0.56	3.5 ± 0.67
2	2.9 ± 0.24	2.9 ± 0.36	2.6 ± 0.27	2.7 ± 0.49
6	1.8 ± 0.27	1.9 ± 0.45	2.1 ± 0.28	1.9 ± 0.61
9	2.7± 0.1[a,b]	3.0 ± 0.25[a]	3.4 ± 0.14[b]	2.6 ± 0.10
12	3.2 ± 0.32	3.2 ± 0.40	3.6 ± 0.10	3.10± 0.51
16	2.2 ± 0.3[b]	2.3 ± 0.29	3.1 ± 0.1[b]	2.2 ± 0.42

Values are expressed as means ± SD, n = 3. Values with the same superscripts on a row are significantly different at P < 0.05.

glycosides and flavonoids. The reduction of PCV values by more than 50% of the baseline values in all rabbits day 2 after phenyl hydrazine administration is an indication of anaemia. Table 2 shows that *M. indica* and *T. occidentalis* extracts significantly increase (P < 0.05) the PCV values of treated rabbits. Table 3 shows that after anaemia have been induced in rabbits, daily oral treatment with extract of *M. indica* and *T. occidentalis* significantly increase (P < 0.05) the RBC counts over the control. Table 4 shows that daily oral treatment with *T. occidentalis*, *M. indica* and *A. hybridus* extracts significantly increase (P < 0.05) the haemoglobin concentrations of treated rabbits over the control. Table 5 indicates that there is a significant elevation (P < 0.05) in the serum bilirubin of all the treated rabbits when compared with the control.

DISCUSSION

Anaemia was diagnosed by a more than 50% reduction in PCV values of all rabbits groups from the baseline

Table 4. Effect of plant extracts on haemoglobin concentration (gldl) of phenyl hydrazine-induced anaemia in rabbits.

Duration of experiment (days)	Groups of Rabbits			
	(A) Treatment with distilled water	(B) Treatment with *T. occidentalis*	(C) Treatment with *M. indica*	(D) Treatment with *A. hybridus*
0	12.58 ± 0.8	13.15 ± 1.12	12.41 ± 0.99	12.71 ± 0.88
2	16.61 ± 3.74	17.58 ± 2.90	17.86 ± 1.87	17.38 ± 0.77
6	12.5 ± 2.29	11.2 ± 1.65	11.2 ± 1.39	9.1 ± 2.35
9	$12.43 \pm 1.18^{a,b,c}$	14.74 ± 0.6^{a}	14.21 ± 0.17^{b}	14.14 ± 0.1^{c}
12	$15.68 \pm 0.44^{a,b,c}$	17.67 ± 1.57^{a}	17.38 ± 0.17^{b}	17.05 ± 0.1^{c}
16	11.96 ± 1.85	14.45 ± 1.8	14.15 ± 1.05	13.35 ± 0.28

Values are expressed as means ± SD, n = 3. Values with the same superscripts on a row are significantly different at P < 0.05.

Table 5. Effect of plant extracts on serum bilirubin and total protein of phenyl hydrazine-induced anaemia in rabbits.

Groups of rabbits	Conjugated bilirubin (mg dl^{-1})	Total bilirubin (mg dl^{-1})	Unconjugated bilirubin (mg dl^{-1})	Total protein (g dl^{-1})
(A) Treatment with distilled water	$0.17 \pm 0.05^{b,c}$	$0.81 \pm 0.05^{a,b,c}$	$0.65 \pm 0.9^{a,}$	12.79 ± 3.63
(B) Treatment with *T. occidentalis*	0.22 ± 0.03	1.2 ± 0.13^{a}	0.98 ± 0.16	14.58 ± 0.92
(C) Treatment with *M. indica*	0.36 ± 0.02^{b}	1.27 ± 0.19^{b}	0.91 ± 0.17	12.11 ± 0.27
(D) Treatment with *A. hybridus*	0.47 ± 0.1^{c}	1.35 ± 0.14^{c}	0.88 ± 0.05^{c}	11.88 ± 2.86

Values are expressed as means ± SD, n = 3. Values with the same superscripts in a column are significantly different at P < 0.05.

values. Similarly, the PCV values and RBC counts were significantly decreased from the baseline values 2 days after administration of phenyl hydrazine hydrochloride, indicating that the chemical could effectively induce anaemia in rabbits at the given dosage. Phenyl hydrazine hydrochloride has earlier been used to induce anaemia in rats (Bowman and Rand, 1980). These authors reported that anaemia was observed after 6 days of injection. There seems to be recovery from anaemia in day 9 by the control group given only distilled water, probably due to decreasing effect of the drug before a boaster dose was given. Phenyl hydrazine was reported to induce the development of Henz bodies on RBC membranes after six days of exposure, which protect them against further destruction by the chemical (Bowman and Rand, 1980; Gordon-Smith, 1980).

The results of haematological tests indicated that treatment with ethanolic extract of *M. indica* stem bark and aqueous leaves extracts of *T. occidentalis* significantly elevates ($P < 0.05$) all the parameters of the anaemic rabbits on day 9 (Tables 2 - 4). On the other hand, the aqueous leaves extracts of *A. hybridus* produced a significant elevation ($P < 0.05$) only in haemoglobin concentration (Table 4). This result shows that the stem bark extract of *M. indica* and leaves extract of *T. occidentalis* have antianaemic potentials while the leaves extract of *A. hybridus* has little potential. These findings agree with previous report that aqueous extract of *T. occidentalis* leaves has haematinic effect (Dina et al., 2000). Other investigators also reported that the extract of *Khaya senegalensis* stem bark has antianaemic activity in phenyl hydrazine-induced anaemic rats (Sanni et al., 2005). Another report indicates that oral administration of *Tectona grandis* extract to rats, previously treated with phenyl hydrazine, increased the concentration of haemoglobin, red blood cells number, haematocrit and reticulocytes counts (Diallo et al., 2008). The differences in antianaemic potentials of the plant extracts might be due to the different phytochemicals present especially, the polyphenols (flavonoids).

It has been reported that phenyl hydrazine causes oxidative damage to red cells by increasing the formation of reactive oxygen species (Clemens et al., 1984; Hill and Thornalley, 1982). 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. Phytochemical screening of the extracts revealed the presence of saponins, flavonoids and alkaloids. Thus, it appears that the presence of these antioxidants in the plant extracts reverses the damaging effect of phenyl hydrazine. A study on *M. indica* (Stoilova et al., 2005) showed that mangiferin, a normal metabolite in the plant's leaves and stem, has strong antioxidant property.

The three plant extracts significantly increase ($P < 0.05$) the serum bilirubin concentrations when compared with the control (Table 5). This suggests that all the extracts may increase the metabolism of bilirubin beyond the common level observed with haemolytic breakdown of red cells by phenyl hydrazine hydrochloride. More

studies need to be carried out to determine if the plant extracts have toxic effect on the liver of animals. All the three plant extracts did not produce significant effect ($P > 0.05$) on the total protein concentration (Table 4). Thus, it appears that the plant extracts did not interfere with the animal's protein metabolism; and that they were healthy and fed normally. This study shows that *M. indica* and *T. occidentalis* extracts have antianaemic potentials lending credence to the use of these plant extracts in folklore medicine for the management of anaemia.

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REFERENCES

Adesina SK (1983). Chemical examination of *K. senegalensis*. Fitoterapia 3: 41-48.

Allen ST (1974). Chemical analysis of ecological material. Blackwell Scientific publication, New York p. 313.

Association of Official Method of Analysis (AOAC) (1980). Official methods of analysis. Hornitz, W., Ed. AOAC, Washington D. C. pp. 256-380.

Bowman WC, Rand MJ (1980). Textbook of Pharmacology. Blackwell Scientific publication, New York pp. 21-31.

Breazile JE (1971). Textbook of Veterinary physiology. 1st ed. Lea and Fabiger, Philadelphia pp. 205-210.

Burkill HM (1985). The useful plants of West Tropical Africa. Vol. 1 families A –D, Royal Botanical garden. 2nd ed. p. 28.

Clemens MR, Remmer H, Waller HD (1984). Phenylhydrazine-induced lipid peroxidation of red blood cells: in vitro and in vivo monitoring by the production of volatile hydrocarbons. Biochem. Pharmacol. 33: 1715-1718.

Diallo A, Gbeassor M, Vovor A, Eklu-Gadegbeku K, Aklikokon K, Agbonon A, Abena AA, de souza C, Akpagana K (2008). Effect of *Tectona grandis* on phenylhydrazine-induced anaemia in rats. Fitoterapia 79(5): 332-336.

Dina OA, Adedapo AA, Oyinloye OP, Saba AB (2000). Effect of *T. occidentalis* extract on experimentally induced anaemia in domestic rabbits. Afr. J. Biomed. Res. 3: 181-183.

Duff S (2008). Types of Anaemia. www,innvista.com.

Gordon-Smith EC (1980). Haematological effect of drug therapy. Clin. Haematol. 9: 572-576.

Harris PF, Kugler JK (1971). Unusual mononuclear cells in Guinea-pig peripheral blood during anaemia. J. Anat. 108: 1-12.

Hill HAO, Thornalley PJ (1982). Free radical production during phenylhydrazine-induced haemolysis. Can. J. Chem. 60: 1528-1531.

Ong HC (1973). Anaemia in pregnancy in an Aborigine population. Med. J. Malays. 28: 22-28.

Orna NU (1991). Iron deficiency anaemia. Clin. Pharm. Herb. Med. 7: 12-16.

Richard H (1978). Chinese Herbal Medicine. Ancient art and modern Science. Shocker Brook, New York pp. 49-52.

Sanni FS, Ibrahim S, Esievo KAN, Sanni S (2005). Effect of oral administration of aqueous extract of *khaya Senegalensis* stem bark on phenylhydrazine-induced anaemia in rats. Pak. J. Biol. Sci. 8(2): 255-258.

Sofowora A (1979). African Medicinal plants. Proceeding of a conference. University of Ife Press, Ife Nigeria. p. 3.

Stoilova S, Gargova A, Stoyanova L (2005). Antimicrobial and antioxidant activity of the polyphenol mangiferin. Kerla polonica 51: 1/2.

Trease GE, Evans WC (1984). Pharmacognosy. Bailliere Tindall, London pp. 184-186.