Anti-anaemic potentials of aqueous extract of *Sorghum bicolor* (L.) moench stem bark in rats

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Abstract

The effects of oral administration of aqueous extract of *Sorghum bicolor* (L.) Moench stem bark at the doses of 200, 400 and 800 mg/kg body weight on iron sufficient and iron deficient weaning rats were investigated. Weaning rats of 21 days old were maintained on iron sufficient and iron deficient diets for 6 weeks before the administration of the aqueous extract of *Sorghum bicolor* stem bark at various doses for 7 days. Proximate analysis of the iron sufficient and iron deficient diets showed that they were similar except in the amount of iron. Phytochemical screening of the extract revealed the presence of alkaloids and saponins. Extract administration produced significant increase in haemoglobin, packed cell volume and red blood cells in iron sufficient and iron deficient groups (*P* < 0.05). There was also significant increase (*P* < 0.05) in the catalase activity of the rat liver and kidney without any significant change (*P* > 0.05) in the serum catalase activity. The results revealed that extract administration has restored the anaemic condition in the iron deficient group and thus lend credence to its use in folklore medicine in the management of anaemia.

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Keywords: *Sorghum bicolor*; Iron deficient; Iron sufficient; Anaemia; Aqueous; Catalase

1. Introduction

Micronutrient deficiencies are still a major public health problem in many developing countries with infants and pregnant women usually at risk. Infants warrant extra concern because they require extra micronutrients to maintain optimal growth and development (Batra and Seth, 2002). The micronutrient deficiencies which are of greatest public health significance include iron deficiency and this causes varying degree of impairment in cognitive performance, lowered work capacity, lowered immunity to infections, pregnancy complications, e.g. low birth weight babies, poor learning capacity and reduced psychomotor skills.

Iron deficiency is the most common single-nutrient deficiency disease in the world (Buzina-Suboticanea et al., 1998), affecting about 15% of the world population (Eftekhari et al., 2003), 35% of women and 43% of young children (Sharmanov, 1998). It occurs when the body’s iron stores become depleted and a restricted supply of iron to various tissues becomes apparent. This may result in depletion of haemoglobin and iron-dependent intra-cellular enzymes participating in many metabolic pathways (Eftekhari et al., 2003). Therefore, there is the need for proper management of micronutrient deficiencies most especially iron deficiency.

Over the years, medicinal plants have been recognized to be of great importance to the health of individuals and communities. In many developing countries, herbal medicines are assuming greater importance in primary health care and their international trade has increased. *Sorghum bicolor* (L.) Moench (Poaceae) known by a variety of names such as great millet and guinea corn in West Africa, Kafir corn in South Africa, dura in Sudan, mtama in Eastern Africa, jowar in India and Kaoliang in China (Lupien, 1990), is an annual grass characterized by spikelets borne in pairs. It forms an important staple in the semi-arid tropics of Asia and Africa for centuries and is still a principal source of energy, protein, vitamins and minerals for millions of the poorest people in these regions. It is grown in some developed nations for animal feed (Manzelli et al., 2005).

The stem barks of ‘Karandasi’ or ‘poroporo baba’ as is popularly called within many localities in Nigeria are claimed by a reasonable number of herb sellers to cure anaemia. The blood “tonic” is usually prepared by boiling the karandasi in water for 20–30 min and taken for between 5 and 7 days.
This study was carried out to lend scientific evidence to the efficacious claim of the use of *Sorghum bicolor* stem bark in the management of iron deficiency anaemia in folklore medicine. The stem bark was used in this study since it is the part used in folklore medicine. Several workers (Adams, 1953; Beutler and Blaisdell, 1958) have used biochemical indices like haemoglobin concentration, red blood cell count (RBC), packed cell volume (PCV) and catalase activity to evaluate the state of iron deficiency anaemia, hence, the use of these indices in this study.

2. Materials and methods

2.1. Laboratory animals

Twenty-one days old weaning albino rats of both sexes (*Rattus norvegicus*) with mean weight of 28.0 g ± 2.0 g were obtained from the small animal holding unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. This study was approved by the Departmental Ethical Committee on the Use of Laboratory Animals.

2.2. Feed components

Yellow maize (*Zea mays*) and locust bean [*Parkia biglobosa* (A.) Jacq] seeds were obtained locally from Baboko Market, Ilorin, Nigeria while the soybean oil used was a product of a Grand Cereals and Oil Mills Limited, Bukuru, Jos, Nigeria. The vitamin mix was a product of BASF Aktiengesellschaft, Germany. Component chemicals of the mineral mix used were products of Sigma Chemical Company Limited, London.

2.3. Enzyme assay kit

The assay kit for catalase was a product of Sigma Chemical Company, London.

2.4. Other reagents

All other reagents used were of analytical grade and were prepared in all glass-distilled water. The reagents were stored in reagent bottles except biuret reagent which was stored in plastic container (Plummer, 1978).

2.5. Plant identification and preparation of extract

The whole plant obtained from a herb seller at Baboko Market, Ilorin, Nigeria was authenticated by Prof. F.A. Oladele of the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria where voucher specimen was deposited in the herbarium.

The method described by Yakubu et al. (2005) was used in the preparation of the plant extract. Briefly, the stem bark of the plant was dried in the oven at 40°C until a constant weight was obtained. The dried pieces were then pulverized using an electric blender (Blender/Miller III, model MS-223, Taiwan, China) and the powder obtained was stocked in a plastic container. A 15 g of the powder was poured into 100 ml of distilled water and immediately boiled for 25 min. The resulting solution was left to cool and thereafter filtered using filter paper (Whatman No. 1). The filtrate was concentrated in stem bath until a constant weight was obtained to give 2.32 ± 0.03 of the residue (brownish black slurry) which correspond to % yield of 15.47. The residues which were then pooled together was reconstituted in distilled water to give the doses of 200 (value arrived at from information obtained during ethnobotanical survey), and higher doses of 400 and 800 mg/kg body weight (were employed to account for abuse of the plant material). The reconstituted aqueous extract was administered orally using oropharyngeal cannula to all the animals in different groups.

2.6. Composition of diet

The composition of iron deficient and iron sufficient diets per kg diet is shown in Table 1. The components of the diets were thoroughly mixed and made into pellets to ensure good handling by the animals. The feeds were packed into air-tight polythene bags and stored in the freezer to prevent rancidity, auto-oxidation of the oil and microbial contamination. The proximate analyses of the compounded feeds were also carried out as described in Section 2.10.

2.7. Animal grouping and extract administration

The animals were individually housed in metabolic cages of dimensions 33 cm × 20.5 cm × 19 cm under standard conditions (12-h light:12-h dark, 28°C ± 3°C and 40–55% humidity). They were allowed free access to normal rat chow and distilled water. The acclimatization was done for seven days before the start of the experiment after which they were then fasted for 24 h (food except water was removed) prior to the commencement of the experiment. The animal grouping consisted of an initial two groups of 25 animals each as follows:

<table>
<thead>
<tr>
<th>Feed components</th>
<th>Iron sufficient (g/kg)</th>
<th>Iron deficient (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locust bean seed</td>
<td>750</td>
<td>750</td>
</tr>
<tr>
<td>Corn starch</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Sucrose</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Methionine</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Lysine</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Vitamin mix^a</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Mineral mix^b</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>FeSO4·7H2O (mg/kg)</td>
<td>35.06</td>
<td>8.50</td>
</tr>
</tbody>
</table>

Soybean oil: polyunsaturated fatty acids (58%), monounsaturated fatty acids (29%), saturated fatty acids (13%).

^a Vitamin mix (per kg of diet): vitamin A, 100,000 IU; vitamin D3, 10,000 IU; vitamin E, 100 mg; vitamin B1, 20 mg; vitamin B2, 40 mg; d-calcium pantothenate, 100 mg; vitamin B6, 15 mg; vitamin C, 250 mg; vitamin K3, 15 mg; folic acid, 5000 mcg; nicotinic acid, 200 mg; biotin, 150 mcg; inositol, 80 mg.

^b Mineral mix (g/kg): CaCl2·6H2O (0.0001), CuSO4·5H2O (0.079), MnSO4·7H2O (0.178), KI (0.032), NaCl (3.573), ZnCO3 (1.60), CaSO4·11H2O (11.610), MgSO4·7H2O (2.292), K2HPO4 (10.559). Control diet contained 1.078 g FeSO4·7H2O.
A - Rats maintained on iron sufficient diet designated as IS.
B - Rats maintained on iron deficient diet designated as ID.

Animals in groups A and B were maintained on their respective diets for 6 weeks. At the end of the 6 weeks feeding period, 5 rats each from IS and ID groups were sacrificed and their haemoglobin levels determined. The remaining rats in groups A and B were further grouped into four with five rats in each group as follows:

A1- Iron sufficient rats orally administered on daily basis for seven days with 200 mg/kg body weight of aqueous extract of *Sorghum bicolor* stem bark designated IS200.
A2- Iron sufficient rats orally administered on daily basis for seven days with 400 mg/kg body weight of aqueous extract of *Sorghum bicolor* stem bark designated IS400.
A3- Iron sufficient rats orally administered on daily basis for seven days with 800 mg/kg body weight of aqueous extract of *Sorghum bicolor* stem bark designated IS800.
A4- Iron sufficient rats orally administered on daily basis for seven days with 1 ml of the vehicle (distilled water) designated as IScontrol.

B1- Iron deficient rats orally administered on daily basis for seven days with 200 mg/kg body weight of aqueous extract of *Sorghum bicolor* stem bark designated ID200.
B2- Iron deficient rats orally administered on daily basis for seven days with 400 mg/kg body weight of aqueous extract of *Sorghum bicolor* stem bark designated ID400.
B3- Iron deficient rats orally administered on daily basis for seven days with 800 mg/kg body weight of aqueous extract of *Sorghum bicolor* stem bark designated ID800.
B4- Iron deficient rats administered with 1 ml of the vehicle (distilled water) on daily basis for seven days designated as IDcontrol.

The extracts and the distilled water were administered to the various groups between 10.00 and 10.30 h using oro-pharyngeal cannula.

2.8. Preparation of serum and tissue homogenates

The rats were anaesthetized in chloroform vapour. When they became unconscious, the neck area was quickly cleared of fur and skin to expose the jugular veins. The veins after being slightly displaced (to avoid contamination with interstitial fluid) were then sharply cut with a sterile scalpel blade and about 3 cm$^3$ of blood was collected into EDTA sample bottles for the haematology tests while the remaining blood was collected into clean and dry centrifuge tubes and allowed to clot for 30 min. This was then centrifuged at 33.5 × g for 15 min (Yakubu et al., 2005) using uniscope Laboratory Centrifuge (model SM800B, Surgifriend Medicals, England). The sera were thereafter aspirated using Pasteur pipettes into clean, dry, sample bottles and were then stored frozen overnight before being used for the enzyme assay.

The animals were thereafter quickly dissected and the organs of interest (liver and kidney) removed. The kidney was decapsulated after which the organs were blotted with clean tissue paper and weighed. The tissues were homogenized in 0.25 M sucrose solution (1:5 w/v). The homogenates were transferred into specimen bottles and kept frozen for 24 h before being used for the analyses.

2.9. Phytochemical screening

The presence of alkaloids and phlobatannins were determined according to the method described by Harborne (1973) while the method described by Odebiyi and Sofowora (1978) was used for flavonoids and tannins. Steroids and phenolics were qualitatively determined by the method of Trease and Evans (1989) while glycosides and saponins were determined by the methods of Sofowora (1993) and Wall et al. (1954) respectively.

2.10. Proximate analysis of formulated diet

Proximate analysis carried out on the formulated diet included ash and organic mineral content based on the principle that inorganic residues are always left behind after the organic matter must have been burnt away, fat (ether extraction) (Heldrich, 1990) based on the principle that non-polar components of the sample are easily extracted into organic solvent. The crude fibre was carried out by the method described by Heldrich (1990) was estimated as the bulk of roughage in foods referred to as crude fibre. The crude protein based on the use of nitrogen as an index of the protein termed ‘crude protein’ and was obtained using the expression, nitrogen × $6.25$ (conversion factor) while carbohydrate was by estimation by difference as described by Oyeleke (1984).

2.11. Haematological test

The haemoglobin concentration was determined colorimetrically at 540 nm as previously described (Wroblewski et al., 1949). PCV was measured using the microhaematocrit reader after centrifuging blood containing heparinised capillary tubes at 6.7 × g for 5 min (Bull et al., 1958). Red blood count was determined using the method described by Wroblewski et al. (1949).

2.12. Determination of catalase activity and protein concentration

Catalase (EC 1.11.1.6) activities in the rats’ liver and kidney homogenates as well as the serum were measured colorimetrically at 570 nm according to the method described by Sinha (1972). Briefly, the homogenized tissues were centrifuged at 569.5 × g for 20 min and the supernatant diluted × 50. A 1 cm$^3$ of appropriately diluted enzyme preparation was rapidly mixed with reaction mixture containing 4 cm$^3$ of hydrogen peroxide and 5 cm$^3$ of phosphate buffer in a conical flask at room temperature. A 1 cm$^3$ portion of the reaction mixture was withdrawn and blown into 2 cm$^3$ dichromate/acetic reagent at 60 s interval and absorbance read at 570 nm. Protein content was estimated using the Biuret method as described by Gornal et al. (1949).
Table 2
Proximate composition of iron deficient and iron sufficient diets

<table>
<thead>
<tr>
<th>Components</th>
<th>Amount (%)</th>
<th>ID</th>
<th>IS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>40.02</td>
<td>39.46</td>
<td></td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>29.00</td>
<td>28.23</td>
<td></td>
</tr>
<tr>
<td>Lipid</td>
<td>10.84</td>
<td>10.63</td>
<td></td>
</tr>
<tr>
<td>Crude fibre</td>
<td>5.79</td>
<td>5.47</td>
<td></td>
</tr>
<tr>
<td>Total ash</td>
<td>8.26</td>
<td>10.48</td>
<td></td>
</tr>
<tr>
<td>Moisture</td>
<td>6.09</td>
<td>5.73</td>
<td></td>
</tr>
</tbody>
</table>

ID, iron deficient; IS, iron sufficient; crude protein was obtained using the expression nitrogen × 6.25.

2.13. Statistical analysis

Statistical significance was determined at P<0.05 using Duncan Multiple Range Test (DMRT) to account for various treatment groups (Mahajan, 1997).

3. Results

Phytochemical screening of aqueous extract of Sorghum bicolor stem bark revealed the presence of alkaloids and saponins while other phytochemicals screened namely tannins, phenolics, glycosides, flavonoids, steroids and phlobatannins were not detected.

Proximate composition of both iron deficient (ID) and iron sufficient (IS) formulated feeds are shown in Table 2. Proximate analysis of the diets showed that the components in iron deficient formulated feed (ID) were essentially similar to those in the iron sufficient formulated feed except in the total ash content of the iron sufficient feed which was higher than in the iron deficient feed. Analysis using atomic absorption spectrophotometer revealed that the iron deficient diet contained 8.50 mg iron per kg of diet while the iron sufficient diet contained 35.06 mg iron per kg diet.

Table 3 revealed the effects of the formulated diets and oral administration of aqueous extract of Sorghum bicolor stem bark on selected haematological parameters of weaning albino rats. Maintaining the animals on the ID and IS diets for 6 weeks resulted in significant reduction (P<0.05) in the haemoglobin content of ID-animals only. However, administration of the aqueous extract of Sorghum bicolor stem bark to the ID animals at various doses (200, 400 and 800 mg/kg body weight) produced significant increase (P<0.05) in the haemoglobin content in dose dependent manner. Extract administration at all the doses to the IS group also increased significantly (P<0.05) their haemoglobin content (Table 3). This similar pattern of increase in PCV and RBC was also observed in the two diet groups following the administration of Sorghum bicolor stem bark (Table 3). These increases (P<0.05) were however not dose dependent while administration at 800 mg/kg body weight to the IS groups resulted in values of PCV and RBC that compared favorably with the control (P>0.05).

Table 4 depicts the effect of administration of aqueous extract of Sorghum bicolor stem bark at various doses on the catalase activity of the rat liver, kidney and serum of weaning albino rats. Extract administration resulted in significant increase (P<0.05) in the catalase activity in the two diet groups (ID and IS) in the rat liver and kidney. The catalase activity in the serum were not significantly different (P>0.05) from the control (Table 4).

4. Discussion

The most reliable indication of iron deficiency anaemia is haemoglobin. This is because it is the iron-containing protein found in red blood cells that allows the red blood cells to function as the oxygen transport system to the tissues of the body. Next to haemoglobin in this regard is the haematocrit (Ht) or packed cell volume which is a measure of the portion of the blood volume made up by red blood cells.

The fact that the compositions of the diets are essentially similar except in the iron content is an indication that the diets only differ in the amount of iron (Table 2).

From Table 3, the significant decrease in haemoglobin, PCV and RBC levels of iron deficient rats may be attributed to induction of iron deficiency anaemia when rats were placed on iron-deficient diet (8.50 mg/kg) as is the case in this study. This is supported by a previous study in which rats placed on iron-poor ration (5–8 mg iron/kg ration) ad libitum for 6 weeks were iron deficient and anaemic (when haemoglobin concentration was less than 7 g/dl) (Margoles, 1984; Fernandes et al., 1997).

However, administration of aqueous extract of Sorghum bicolor stem bark resulted in significant increases in haemoglobin (i.e.
when haemoglobin concentration was greater than 7 g/dl), PCV and RBC levels in all the dose groups. Such increase in the blood parameters further lend credence to the acclaimed use of the plant extract as anti-anaemic agent since the Hb, PCV and RBC levels of iron-sufficient rats were also increased by treatment with all the three doses of the aqueous extract of Sorghum bicolor stem bark (Table 3).

Iron serves as the core of haemoglobin molecule, which is the oxygen-carrying component of red blood cells. The ability of red blood cells to carry oxygen is attributed to the presence of iron in the haemoglobin molecule. A lack or loss of iron by any means implies reduced production of haemoglobin and subsequent reduction in the volume of red blood cells. Hence, it may be concluded that dietary iron deficiency led to a decrease in the iron-containing protein, haemoglobin, and by extension reduction in red blood cells and PCV levels. Such reduction in Hb, PCV and RBC showed that the dietary iron deficiency had left the early stages into the latter stage of iron deficiency anaemia (Batra and Seth, 2002).

Jubi formula, a herbal preparation made from three medicinal herbs (Parqueatina nigrescens, Sorghum bicolor and Harungana madagascariensis) has been successfully used in the treatment of anaemia in humans and in the treatment of Trypanosoma brucei-induced anaemia in rabbits (Erah et al., 2003). Based on the present study, it is most likely that the haemoglobin restoring and anti-anaemic effects of Jubi formula may in part be contributed by Sorghum bicolor made possible by the presence of bioactive agents like alkaloids and saponins.

Saponins, which Sorghum bicolor is composed of, are known to inhibit platelet aggregation and thrombosis. Saponin containing herbs have been successfully used in the management of liver inflammation, as tonic sedative formulas and to promote and vitalize blood circulation (Shi et al., 2004; Wang et al., 2004).

Since saponins are membrane active agents that lyse red blood cells or other wall, it is possible that the red blood cells were initially lysed by this herb; the cells overcome this inhibition by producing glycosidic enzyme which cleaves some of the terminal sugars from the saponin, thereby detoxifying it (Pathirana et al., 1990). This detoxification of saponins, thus enhanced the proper utilization of the iron contained in the aqueous extract of Sorghum bicolor to synthesize heme/haemoglobin for new red blood cells thus leading to an improved Hb, PCV and RBC.

Iron deficient (ID) group showed a significantly reduced catalase activity when compared to the iron sufficient (IS) group in the tissues studied (Table 4). However, there was induction of catalase activity, an iron dependent enzyme, probably by de novo synthesis following the administration of the aqueous extract of Sorghum bicolor stem bark. The increase in the catalase activity, a heme containing enzyme (Yip and Dallman, 1996), may be attributed to the bioactive agents in the plant extract which might have been responsible for the enhanced production of iron which is an essential constituent of its structure and activity. Since there was no significant change in the serum enzyme, the authors are of the opinion that the plant extract may not have caused any leakage of the enzyme from the tissues.

5. Conclusion

This study has lent credence to the efficacy of aqueous extract of Sorghum bicolor (L.) Moench stem bark at the dose of 200 mg/kg body weight as used in traditional medicine in the management of iron deficiency states and higher doses of 400 and 800 mg/kg body weight. This may be made possible by its ability to make readily available iron which is needed in the restoration of the deficiency state.

References


Buzina-Suboticanea, K., Buzina, R., Stauljicen, A., Tadinac-Babic, M., Juhovic-Markus, V., 1998. Effects of iron supplementation on iron nutri-

Table 4

<table>
<thead>
<tr>
<th>Group</th>
<th>Catalase activity*</th>
<th>Kidney</th>
<th>Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>IS</td>
<td>IS</td>
</tr>
<tr>
<td>Control</td>
<td>1.450 ± 0.01 a</td>
<td>2.590 ± 0.06 b</td>
<td>4.780 ± 0.04 d</td>
</tr>
<tr>
<td>Baseline</td>
<td>1.020 ± 0.01 b</td>
<td>2.370 ± 0.03 b</td>
<td>4.480 ± 0.04 b</td>
</tr>
<tr>
<td>200 (mg/kg) bw</td>
<td>1.780 ± 0.05 c</td>
<td>2.780 ± 0.01 e</td>
<td>5.130 ± 0.03 b</td>
</tr>
<tr>
<td>400 (mg/kg) bw</td>
<td>2.070 ± 0.02 d</td>
<td>2.970 ± 0.03 g</td>
<td>5.690 ± 0.01 c</td>
</tr>
<tr>
<td>800 (mg/kg) bw</td>
<td>2.210 ± 0.07 e</td>
<td>3.220 ± 0.04 f</td>
<td>5.360 ± 0.02 d</td>
</tr>
</tbody>
</table>

Values carrying different superscripts (a–e) different from the control and down the group for each feed type and for each parameter are significantly different (P < 0.05) n = 5 replicates ± S.D.

* Activity is expressed as specific enzyme activity in μmole of H2O2/sec/mg protein/ml.

The extract was administered for 7 days.

ID, iron deficient rats.

IS, iron sufficient rats.


