



Anti-anaemic activity of aqueous extract of *Abrus precatorius* (L.) leaves in iron-deficient anaemic Wistar rats

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ABSTRACT

Abrus precatorius leaves have been claimed to be used to manage anaemia without any substantive scientific study. Therefore, this study investigated the effect of aqueous extract of *A. precatorius* leaves (AEAPL) in iron-deficient diet-induced anaemia in Wistar rats. Fifty Wistar rats (50.07 ± 3.25 g) were completely randomised into three groups, CF (commercial feed; $n = 10$), ISD (iron sufficient diet; $n = 10$) and IDD (iron deficient diet, ID; $n = 30$). After 8 weeks of feeding on their respective diets, the levels of serum iron (SFe), haemoglobin (Hb), packed cell volume (PCV), red blood cell (RBC), and soluble transferrin receptors (sTfR1) were determined. Thereafter, the animals in group CF and ISD were oral gavaged with 0.5 ml of distilled water, each, for 14 days, while the iron deficient rats in group IDD were re-assigned into IDD100, IDD200 and IDD400 and orally administered AEAPL at 100, 200 and 400 mg/kg body weight (BW) for 14 days. The ISD and IDS were subjected to proximate analysis whilst BW, haematological parameters, gastric pH and sTfR1 levels were determined in the animals. The moisture contents, crude lipids, crude protein, crude fibre, nitrogen free extract of the IDD and ISD were not significantly ($p > 0.05$) different from each other whereas the ash contents of ISD was significantly ($p < 0.05$) higher than IDD. By the first three weeks, the BW of the ISD, IDD and CF exposed animals were not significantly ($p > 0.05$) different from each other whereas, afterwards, the BW of the IDD-exposed animals had slow growth when compared with ISD and CF. The 8-weeks exposure of the rats to IDD significantly ($p < 0.05$) reduced SFe, Hb, PCV and RBC by 130.68%, 33.21%, 36.75%, and 59.18% and increased sTfR1 by 43.43% respectively. The 400 mg/kg BW of AEAPL restored ($p < 0.05$) the anaemic-related BW of the animals. Furthermore, the IDD-related 37.18%, 33.98%, 34.01%, 26.94% and 25.77% significant ($p < 0.05$) reductions in RBC, Hb, PCV, mean corpuscular volume and platelets were significantly ($p < 0.05$) and dose-dependently increased by the 100, 200 and 400 mg/kg BW of AEAPL. The IDD-treatment related increases ($p < 0.05$) in sTfR1 and pH of gastric acid compared well ($p > 0.05$) with those of AEAPL-, ISD- and CF-treated animals. The study concluded that *A. precatorius* leaf extract exhibited anti-anaemic activity, with the 400 mg/kg BW exhibiting the most pronounced activity.

1.0 Introduction

Anaemia, a condition in which the haemoglobin and the number of red blood cells are insufficient to meet the physiological needs of the individual, can adversely affect their oxygen-carrying capacity [1]. It is one of the most prevalent nutritional disorders

globally, affecting one-third of the population [1]. According to WHO [1], 40% of all children aged 6-59 months, 37% of pregnant women, and 30% of women in their reproductive age of 15-49 are affected by anaemia globally. Furthermore, the prevalence of anaemia is rising alarmingly in Africa from 38.9 to 48.7% for pregnant women and

from 37.7 to 41.5% for non-pregnant women [2]. In West Africa, the prevalence of anaemia is put at 38.61%, whilst in Nigeria, it is 8.61% [2]. Anaemia is caused by several conditions that include, but are not limited to, hookworm infestation, malaria, HIV/AIDS, and haemoglobinopathies. Stevens *et al.* [3] have also reported that, among other nutritional deficiencies like folic acid, vitamin B₁₂ and Vitamin A, iron deficiency is the most common cause of anaemia globally, accounting for 50-60% of cases. Globally, about half of the 25% of the world population that is anaemic is attributable to iron deficiency anaemia [4].

Iron deficiency is the most common nutritional deficiency in the world [1]. Iron is present in all cells in the human body and has several vital functions, such as carrying oxygen to the tissues from the lungs as a key component of the haemoglobin protein; acting as a transport medium for electrons within the cells in the form of cytochromes, facilitating oxygen use and storage in the muscles as a component of myoglobin [5]. Too little iron (as low as 20 mg/kg) can interfere with these vital functions and lead to morbidity and death in an adult human being. Iron deficiency anaemia (IDA) develops when haemoglobin concentration is depleted due to the production of iron-limited red blood cells [6].

The IDA can result in dizziness, lightheadedness, chest pain, tachycardia, and dyspnea [7]. Continued iron deficiency may progress to anaemia and worsening fatigue [8]. Iron deficiency may be due to malnutrition, menstruation, pregnancy, non-steroidal anti-inflammatory drugs, gastrointestinal blood loss, and chronic kidney diseases, amongst others. Iron replacement therapy including oral supplements (ferrous sulphate, ferrous gluconate, ferric maitol, ferric citrate, heme iron polypeptide, carbonyl iron) and intravenous (parenteral) iron therapy (ferric carboxymaltose, ferric derisomaltose, iron sucrose low-molecular-weight iron dextran) as well as blood transfusion [9] are management options for severe iron deficiency anemia. Phytotherapy is another common management option aimed at relieving the condition of iron deficiency anaemia.

Over the years, medicinal plants have been recognized to be of great importance to the health of individuals and communities over the years. Herbal medicines are assuming greater importance in primary health care in many developing countries, and their international trade has increased [10, 11].

Abrus precatorius, commonly known as jequirity bean or rosary pea (English), *Ojuologbo* (Yoruba), *Oto-berebere* (Igbo), and *Idonzakara* (Hausa), is an herbaceous flowering plant in the Fabaceae family [12]. It is a weed that grows throughout the tropics and neotropics. It is a slender, perennial climber that twines around trees,

shrubs, and hedges. The pinnately compound leaves are arranged alternately, up to 15 cm long [12]. The fruit (pod) is flat, oblong, and truncate-shaped with a sharp deflexed beak of about 3-5cm long, 1.2 cm wide, and silky-textured [12]. The seedpod curls back when it opens to reveal the seeds [12]. The folkloric uses of the plant include in the treatment of inflammation, ulcer, wound, throat scratches, sores, itching, urinary disorders, infertility, cough, flu, jaundice, snake bite, hepatitis, bronchitis and worm infestation [13]. The seeds or leaves are sometimes used to 'build blood' for the management of anaemia [13]. *Abrus precatorius* has been reported to possess antidiabetic, antioxidant, neuroprotective, anticonvulsant, and antimicrobial activities [14-18]. The absence of scientific study, in the open scientific literature, that has substantiated the acclaimed use of *A. precatorius* leaves in the management/treatment of *ile eje* (Yoruba), *obara nkeke* (Igbo) and *karancin jin* (Hausa) meaning in each case, lack of blood, by the Yoruba, Igbo and Hausa people of Nigeria, has necessitated the current study. This study was therefore carried out to lend scientific evidence to the efficacious claim of the use of *Abrus precatorius* leaves in the treatment of anaemia in folklore medicine.

2.0 Materials and Methods

2.1 Laboratory animals

Forty weanling Wistar rats of both sexes (*Rattus norvegicus*) with a mean weight of 50.0 g ± 3.72 g were obtained from the animal holding unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria.

2.2 Feed components

Yellow maize (*Zea mays*), corn cob and locust bean (*Parkia biglobosa* (A.) Jacq) seeds were obtained locally from Oja Oba Market, Ilorin, Nigeria, while the vegetable oil used was a product of Grand Cereals and Oil Mills Limited, Bukuru, Jos, Nigeria. The vitamin mix was a product of BASF Aktiengesellschaft, Germany, Pantex, Holland. Component chemicals of the mineral mix used were products of Sigma Chemical Company Limited, London. Sucrose and FeSO₄·7H₂O were products of Sigma-Aldrich Co., UK.

2.3 Receptor assay kit and other reagents

The assay kit for soluble transferrin receptor (sTfR1) was a product of Elabscience Biotechnology Co., Ltd. United States of America. All other reagents used were of analytical grade and obtained from Sigma-Aldrich, Mannheim, Germany.

2.4 Plant material and authentication

Fresh leaves of *A. precatorius* were obtained from Shao town, Moro Local Government of Kwara State, Nigeria, and authenticated at the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria, where voucher specimen (UILH/001/448) was deposited in the herbarium.

2.5 Preparation of extract

The method described by Yakubu *et al.* [19] was used in the preparation of the plant extract. Briefly, the leaves of the plant were 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 stored in a plastic container. Exactly 15 g of the powder was extracted in 100 ml of distilled water (1:6.7) for 48 hours. The resulting solution was filtered using filter paper (Whatman No. 1, Maidstone, United Kingdom). The filtrate was lyophilized to give 1.92 ± 0.03 g of the residue, which corresponds to the percentage yield of 12.8%. The residue was then reconstituted in distilled water to give the doses of 100 mg/kg BW (value arrived at from information obtained during ethnobotanical survey), and higher doses of 200 and 400 mg/kg BW (were employed to account for abuse of the plant material). The reconstituted aqueous extract was administered orally using an oropharyngeal cannula to all the animals in different groups.

2.6 Feed formulation

The method adopted for the formulation of the iron-sufficient and iron-deficient feed was as described by Modupe and Oladiji [20]. Locust beans seed was used as the main source of protein. The components (Table 1) 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.

2.7 Proximate analysis of formulated diets

The diets (ISD and IDD) were screened for their proximate constituents as highlighted by AOAC [21]. The proximate analyses included moisture that centers on drying (100°C) a sample to a constant weight, 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) based on the principle that non-polar components of the sample are easily extracted into organic solvent.

The crude fibre was estimated as the bulk of roughage in the diets. The crude protein, based on the use of nitrogen as an index of the protein termed 'crude protein', was obtained using the expression, nitrogen \times 6.25 (conversion factor), while carbohydrate was estimated by difference.

2.8 Animal grouping, confirmation of induction of anaemia and extract administration

Forty, weanling Wistar rats were housed in cages under standard conditions ($25 \pm 3^\circ\text{C}$, 12 hours light/dark; 45-50% relative humidity). The animals were also allowed free access to normal rat chow and tap water. They were acclimatized for 7 days before the start of the experiment. After acclimatization, the animals were fasted for 24 hours and their serum iron levels determined (from the blood obtained from ocular puncture) using standard methods prior to the commencement of the experiment. The animal grouping consisted of an initial three groups as follows:

ISD – Ten rats were maintained on an iron-sufficient diet.

IDD - Twenty-five rats were maintained on an iron-deficient diet.

CF- Five rats were maintained on commercial feed.

The animals in ISD and IDD were maintained on IS- and ID-diets, respectively for 8 weeks. To confirm the induction of iron deficiency at the end of the 8-week feeding period, blood samples were collected through ocular puncture from 5 rats each from the ISD and IDD groups, after which haemoglobin (Hb), packed cell volume (PCV) and red blood cell counts (RBC) were determined [22]. In addition, the serum was prepared after which iron levels (SFe) using Forrozine colorimetric method [23] and soluble transferrin receptors (sTfR1) were also investigated [24]. The remaining rats in the IDD group were further sub-grouped into four (IDD, IDD100, IDD200 and IDD400) of five rats ($n = 5$) as follows:

IDD- Iron-deficient rats orally administered 1ml of the vehicle (distilled water) on daily basis for 2 weeks.

IDD100- Iron-deficient rats orally administered daily, 100 mg/kg BW of aqueous extract of *A. precatorius* leaves for 2 weeks.

IDD200- Iron-deficient rats orally administered daily 200 mg/kg BW of aqueous extract of *A. precatorius* leaves for 2 weeks.

IDD400- Iron-deficient rats orally administered daily, 400 mg/kg BW of aqueous extract of *A. precatorius* leaves for 2 weeks.

The extracts and the distilled water were administered to the various groups between 0800 GMT and 0830 GMT, using an oro-pharyngeal cannula.

Table 1: Feed components of iron-sufficient and iron-deficient diets

Feed components	Iron sufficient diet (ISD; g/kg)	Iron deficient diet (IDD; g/kg)
Locust bean seed	750	750
Maize flour	40	40
Corn husk	20	20
Soybean oil	40	40
Sucrose	100	100
Methionine	5	5
Lysine	5	5
^a Vitamin mix	10	10
^b Mineral mix	30	30
FeSO ₄ ·7H ₂ O (mg/g)	35.06	8.05

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): CoCl₂·6H₂O (0.001), CuSO₄·5H₂O (0.079), MnSO₄·7H₂O (0.178), KI (0.032), NaCl (3.573), ZnCO₃ (1.60), CaSO₄ (11.610), MgSO₄·7H₂O (2.292), K₂HPO₄ (10.559). Control diet contained 1.078 g FeSO₄·7H₂O.

ISD = Iron sufficient diet; IDD = Iron deficient diet

2.9 Ethical statement

This study was approved by the Departmental Ethical Committee on the Use of Laboratory Animals of the Department of Biochemistry, with the approval number BCH/UIL/05/2016.

2.10 Collection of gastric juice and preparation of serum

The rats were fasted overnight (with free access to water) for the collection of pure gastric juice. Under diethyl ether anesthesia, the pyloric sphincter is tied off to allow gastric secretions to accumulate in the stomach after which the abdominal wall was incised. The pyloric end of the stomach is then gently ligated with silk thread, avoiding damage to the blood vessels; the gastric juice is then aspirated and centrifuged at 4000 rpm for 20 minutes. While still under the diethyl ether vapour, the abdomen was then sutured closed [25].

For the collection of blood and preparation of serum, the procedure described by Yakubu *et al* [26] was adopted. Briefly, the jugular veins were then exposed and sharply cut with a sterile blade. Exactly 2 cm³ of the blood was collected from each rat into EDTA sample bottles for the haematology tests, while another 5 cm³ of the blood was collected into centrifuge tubes (clean and dried) and allowed to clot for 30 minutes. This was then centrifuged at 4000 rpm for 15 minutes. The sera were thereafter aspirated into sample bottles (clean

and dried) using Pasteur pipettes, and stored frozen overnight before being used to assay for the soluble transferrin receptor.

2.11 Determination of haematological parameters

The haematological parameters were analyzed using the Automated Haematology Analyser KX-21, (Sysmex Corporation, Kobe, Japan), adopting the principle of Dacie and Lewis [22].

2.12 Determination of pH of gastric juice

The pH of the gastric juice was determined using an indicator strip as outlined by Farinati *et al* [27].

2.13 Quantification of serum soluble transferrin receptors

The procedure as highlighted by Wians *et al* [24] was adopted for the quantification of soluble transferrin receptor in the serum of the animals (sTfR2) using ELISA technique. The optical value (OD) was read spectrophotometrically at a wavelength of 450 nm. The OD value is proportional to the concentration of sTfR2 in the samples by comparing the OD of the samples to the standard curve.

2.14 Data analysis

Values obtained were expressed as \pm standard error of mean (SEM). The values were subjected to Analysis of Variance (ANOVA) as well as Duncan Multiple Range test (DMRT) to determine statistical significance. Differences were considered significant at $p < 0.05$.

3.0 Results

Proximate analyses revealed that the percentages of moisture, crude lipid, crude protein, crude fibre and nitrogen free extract of the iron-deficient (ID) diet were not significantly ($p > 0.05$) different from those of the iron-sufficient (IS) diet with percentage change for the proximate constituents ranging from -2.58% to 5.50% (Table 2). In contrast, the ash content of the ID diet was significantly ($p < 0.05$) lowered by 41.36% when compared with that of the IS diet (Table 2).

The BW of the animals maintained on IDD was not significantly ($p > 0.05$) different from each other in the first three weeks of feeding whereas the BW of animals maintained on the ISD and CF progressively increased in the first three weeks of monitoring (Figure 1). By the end of the eight weeks induction period, the BW of the IDD-exposed animals had marginally increased by only a difference of 54.87 g whilst those maintained on ISD and CF increased by 130.21 g and 136.59 g respectively (Figure 1). Although, there were increases in all the treatment groups, the BW of those animals maintained on IDD and CF were not significantly ($p > 0.05$) different from each other on all the remaining 5 weeks of intervention (Figure 1).

Table 3 revealed the effect of IDD and ISD on selected biochemical and haematological parameters of rats. The 8-weeks exposure of the experimental animals to IDD significantly ($p < 0.05$) reduced the SFe, Hb, PCV and RBC by 130.68%, 33.21%, 36.75%, and 59.18% and also increased sTfR1 by 43.43% respectively.

Generally, all the treatments significantly ($p < 0.05$) increased the BW of the animals by the 9th and 10th weeks of experimental period with those of the animals maintained on the ISD and CF producing the most pronounced effects amongst the feed-related treatment group (Table 4). Furthermore, the extract at 200 and 400 mg/kg BW significantly ($p < 0.05$) and dose dependently increased the BW with the 400 mg/kg BW of the extract comparing favourably ($p > 0.05$) with their respective ISD and CF values (Table 4).

The effects of oral administration of aqueous extract of *A. precatorius* leaves on the selected haematological parameters of rats fed on iron-deficient diet are shown in Table 5. Exposure of the animals to IDD significantly ($p < 0.05$) reduced the

white blood cells (WBC), red blood cell (RBC), haemoglobin (HGB), packed cell volume (PCV) and platelets by 24.47%, 47.28%, 42.14%, 30.89% and 25.77% respectively. The exposure of the IDD-treated rats to the all the doses of the extract (100, 200 and 400 mg/kg body weight) increased the levels of RBC and HGB that compared favourably ($p > 0.05$) with those maintained on ISD. The PCV of IDD-exposed rats that received the 100 and 200 mg/kg body weight of the extract were not significantly ($p > 0.05$) different from those of the ISD whereas the 400 mg/kg body weight of the extract significantly ($p < 0.05$) raised the PCV when compared with the ISD-treated animals. Similarly, the 100, 200 and 400 mg/kg body weight of the extract significantly ($p < 0.05$) produced levels of WBC that were higher than of the ISD-treated animals. Compared with the rats maintained on the ISD, the platelets were reduced ($p < 0.05$) in the IDD rats that were administered the 100 mg/kg body of the extract whereas the platelets compared favourably ($p > 0.05$) with that of the ISD-fed rats at 200 and 400 mg/kg body weight of the extract (Table 5).

Table 6 depicts the effects of oral administration of aqueous extract of *A. precatorius* leaves on the pH of gastric acid content of rats fed on iron-deficient diet. There was a significant increase ($p < 0.05$) in the pH of the gastric acid of IDD-treated rats when compared with those fed on the ISD (41.31%) and the CF. In contrast, the administration of 100, 200 and 400 mg/kg body weight of *A. precatorius* leaves reduced ($p < 0.05$) the pH of the gastric acid contents which compared favourably ($p > 0.05$) with those fed on ISD and CF (Table 6).

Compared with the soluble transferrin receptor 1 (sTfR1) in the animals that were exposed to the ISD, the corresponding animals maintained on the IDD produced sTfR1 that was significantly ($p < 0.05$) higher by 53.31% (Table 6), as against that of 27.76% increase in sTfR2 by the CF. Furthermore, the administration of 100, 200 and 400 mg/kg body weight of *A. precatorius* leaves produced sTfR2 that compared favourably ($p > 0.05$) with those of the animals maintained on IS diet (Table 6).

4.0 Discussion

According to Kaunda *et al* [28], a well-formulated diet plays a key role in combating micronutrient nutrition, which continues to be a challenge in developing countries. Proximate analysis of the ISD and IDD revealed that the composition of crude lipid, crude protein, crude fibre, nitrogen free extract and moisture were not significantly different from each other, but the ash content. This implies that the composition of the two diets (ISD and IDD) was essentially similar except for the ash content. The ash content, a

Table 2: Proximate composition of iron-deficient and iron-sufficient feeds

Formulated Diet	Moisture Contents (%)	Ash Contents (%)	Crude Lipids (%)	Crude Protein (%)	Crude Fibre (%)	Nitrogen Free Extract (%)
ISD	3.45 ± 0.02 ^a	4.11 ± 0.03 ^a	9.01 ± 0.05 ^a	45.02 ± 0.08 ^a	1.09 ± 0.02 ^a	38.13 ± 0.06 ^a
IDD	3.35 ± 0.07 ^a	2.41 ± 0.02 ^b	8.83 ± 0.12 ^a	46.18 ± 0.01 ^a	1.03 ± 0.05 ^a	37.51 ± 0.11 ^a
Change in proximate constituents (%)	2.90	41.36	2.00	-2.58	5.50	1.63

ISD = Iron sufficient diet; IDD = Iron deficient diet

Values of IDD with the same superscript as those of ISD are not significantly different ($p > 0.05$)

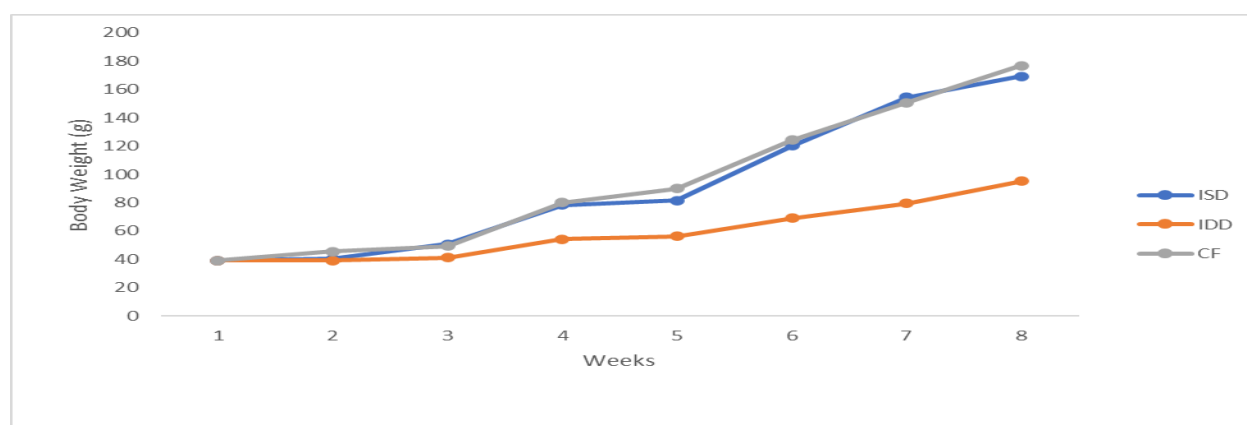


Figure 1: Body weight of Wistar rats maintained on iron-deficient diet, iron sufficient diet and commercial feed for 8 weeks

Table 3: Selected biochemical and haematological parameters of rats maintained on iron-deficient and iron-sufficient feeds

Formulated Diet	Serum iron (mmol/L)	Haemoglobin (g/dL)	Packed Cell Volume (%)	Red Blood Cell ($\times 10^3/\mu\text{L}$)	Soluble Transferrin Receptor (ng/ml)
ISD	4.06 ± 0.13	10.39 ± 0.10	42.05 ± 5.95	6.59 ± 0.15	11.24 ± 0.26
IDD	1.76 ± 0.07 ^a	7.80 ± 0.05 ^a	30.75 ± 0.55 ^a	4.14 ± 0.05 ^a	19.87 ± 0.22 ^a
Change in parameters (%)	130.68	33.21	36.75	59.18	43.43

ISD = Iron sufficient diet; IDD = Iron deficient diet

Values with superscripts across the IDD row are significantly different ($p < 0.05$)

Table 4: Body weight of iron-deficient Wistar rats administered different doses of *Abrus precatorius* leaf aqueous extract for the 9th and 10th weeks of experimental period

Weeks	Body weight (g)					
	ISD	IDD	CF	ID100	ID200	ID400
9	179.70 ± 6.02 ^a	102.26 ± 2.83 ^b	181.10 ± 4.00 ^a	103.93 ± 3.02 ^b	137.14 ± 2.33 ^c	182.16 ± 2.01 ^a
10	191.42 ± 4.01 ^a	110.18 ± 3.81 ^b	193.06 ± 2.05 ^a	116.06 ± 2.06 ^b	152.21 ± 3.03 ^c	193.96 ± 3.12 ^a

ISD = Iron sufficient diet; IDD = Iron deficient diet; CF = Commercial feed; ID100 = Iron-deficient rats maintained on 100 mg/kg body weight of *Abrus precatorius* leaf extract; ID200 = Iron-deficient rats maintained on 200 mg/kg body weight of *Abrus precatorius* leaf extract; ID400 = Iron-deficient rats maintained on 400 mg/kg body weight of *Abrus precatorius* leaf extract

Table 5: Haematological parameters of iron-deficient rats administered different doses of *Abrus precatorius* leaf aqueous extract

GROUPS	WBC (x10 ³ /μL)	RBC (x10 ⁶ /μL)	HGB (g/dL)	PCV (%)	PLT (x10 ³ /μL)
ISD	18.27±3.70 ^b	8.27±0.14 ^a	13.43±0.32 ^a	50.50±0.45 ^b	1063.33±16.56 ^b
CF	13.80±1.91 ^a	8.04±0.10 ^a	14.17±0.49 ^a	47.63±2.73 ^a	789.33±12.61 ^a
IDD	14.77±3.71 ^a	4.36±0.04 ^b	7.77±0.15 ^b	34.90±2.46 ^c	899.00±14.99 ^c
IDD100	21.03±0.32 ^c	7.21±0.29 ^a	13.00±0.25 ^a	49.17±2.09 ^b	763.67±21.36 ^a
IDD200	22.10±1.66 ^c	7.38±0.46 ^a	13.20±0.35 ^a	51.36±3.23 ^b	1078.00±21.38 ^b
IDD400	24.00±1.26 ^d	7.47±0.25 ^a	14.07±0.22 ^a	61.67±5.34 ^d	1090.33±23.37 ^b

Values are expressed as the mean of five determinations ± SEM. Column values with different superscript are significantly (p < 0.05) different from each other, WBC- white blood cell; RBC- red blood cell; HGB- haemoglobin; PCV- packed cell volume; PLT- platelet; ISD- iron sufficient feed rats; CF- rats fed with commercially sold feed; IDD- iron deficient feed rats; IDD100- rats administered with 100 mg/kg body weight extract of *Abrus precatorius* + iron deficient feed; IDD200- rats administered with 200 mg/kg body weight extract of *Abrus precatorius* + iron deficient feed; IDD400- rats administered with 400 mg/kg body weight extract of *Abrus precatorius* + iron deficient feed.

Table 6: pH of gastric acid and soluble transferrin receptors of iron deficient rats administered aqueous extract of *Abrus precatorius* leaves

Group	pH of gastric acid	Soluble Transferrin Receptor (ng/ml)
ISD	4.27 ± 0.03 ^b	13.15 ± 0.09 ^a
CF	4.26 ± 0.02 ^b	16.80 ± 0.99 ^b
IDD	6.03 ± 0.05 ^a	22.16 ± 0.04 ^c
IDD100	4.21 ± 0.01 ^b	13.37 ± 0.01 ^a
IDD200	4.18 ± 0.02 ^b	14.03 ± 0.08 ^a
IDD400	4.19 ± 0.08 ^b	13.08 ± 0.03 ^a

Values are expressed as the mean of five determinations ± SEM. Column values with different superscripts are significantly (p < 0.05) different from each other

ISD- iron sufficient feed rats; CF- rats fed with commercially sold feed; IDD- iron deficient feed rats; IDD100- rats administered with 100 mg/kg body weight extract of *Abrus precatorius* + iron deficient feed; IDD200- rats administered with 200 mg/kg body weight extract of *Abrus precatorius* + iron deficient feed; IDD400- rats administered with 400 mg/kg body weight extract of *Abrus precatorius* + iron deficient feed.

measure of total amount of minerals present in a food, plays a vital role in various aspects of food industry and affect quality, taste and nutrition [29, 30]. An iron deficient diet is characterized by very low levels of iron (typically 2-45 mg/kg or lower) which may be accompanied by high levels of antinutritional factors (not determined in the present study; a limitation of this study), thereby inhibiting the bioavailability of iron and consequently iron absorption [29,30]. Therefore, the 41.36% reduction in the ash content of the IDD implies a decrease in total mineral content and directly correlates with decrease in total iron, which is a reflection of the 8.05 mg/g of FeSO₄.7H₂O incorporated in the IDD as against the 35.06 mg/g of FeSO₄.7H₂O. Similar iron deficient diets have been used to induce iron deficiency anaemia in both experimental and humans [29, 30].

The differences in the concentrations of iron in both the formulated feed of iron-sufficient diet and iron-deficient diet led to reduction in the haematological parameters of the rats, as the reduction in the levels of SFe, Hb, PCV and RBC were accompanied by increase in the number of sTfR1. Therefore, it is quite understandable that a deficiency of iron from the IDD and as evident from this study on SFe will lead to reduced haemoglobin production and consequently fewer number of RBC and low packed cell volume. This trend of reduction in iron and iron-related RBC and factors relating to it lend credence to the induction of iron deficiency anaemia in the animals in the present study. The increase in sTfR1, a highly sensitive marker for iron deficiency anaemia, also alluded to the induction of iron deficiency in the animals arising from tissue-level iron depletion and increased erythropoietic activity.

The low progressive body weight exhibited by the iron deficient rats when compared with the high progressive body weight by the rats maintained on ISD, CF may be due to severe metabolic inefficiency, reduced cellular energy production altered endocrine regulation. By extension, the extract at 200 and 400 mg/kg BW might have resuscitated iron deficiency in the animals maintained on the IDD probably by enhancing and promoting efficient utilization of nutrients for growth through enhancing oxygen transport (since levels of haemoglobin have increased) and supporting cellular respiration which consequently aid weight gain.

The levels of PCV, RBC, HGB and by extension the WBC and platelets were lowered in the rats maintained on iron-deficient diet as compared to those maintained on iron-sufficient diet. This trend of reduction in the levels of PCV, HGB and RBC may be adduced to limited bioavailability of iron, as evidenced from the lowered ash content of IDD, for the biosynthesis of

heme in the bone marrow. Although, serum iron, ferritin, transferrin and transferrin saturation alongside soluble transferrin receptor, zinc protoporphyrin and hepcidin constitutes established and novel markers of iron status, haemoglobin remains one of the most reliable indicators of iron deficiency anaemia. Iron deficiency anaemia occurs when the status of iron store in the body is exhausted and the levels of haemoglobin and haematocrit decline [31]. Therefore, the reduced levels of iron might have impaired erythropoiesis in the bone marrow, which consequently will reduce the quantity and size of the red blood cells produced (reduced mean corpuscular volume). The findings here are similar to the report of Modupe and Oladiji [20] on similar reductions in packed cell volume, haemoglobin and red blood cell count of iron deficient rats maintained on formulated iron deficient diet for 6 weeks. In addition, the reduction in the levels of WBC and platelets by the iron deficient diet may suggest that the diet might have impaired/inhibited the proliferation of leukocytes in the bone marrow and lymphoid tissues and the key immunotransmitter for the white blood cells (interleukin 1, in this instance), and might have also induced thymic atrophy and a decrease in megakaryopoiesis. The findings in the present study with respect to reduction in the white blood cell count is similar to the reports by Wong *et al* [32] who concluded that there exist a significant association between iron deficiency anaemia and leukopenia (abnormally low white blood cell count) and neutropenia (abnormally low levels of neutrophils). One most striking fact in the present study is that the extract of *A. precatorius* at 100 and 200 mg/kg body weight completely reversed the IDD-induced alterations in the haematological parameters whereas the 400 mg/kg body weight of the extract did not only reverse the IDD-induced trends in the haematological parameters of the animals, but also increased the indices by some folds. This school of thought contrast that of previous researchers wherein it was reported that the aqueous extract of *A. precatorius* leaves when administered at 400, 800 and 1600 mg/kg for 18 days to male rats reduced the packed cell volume, haemoglobin, red blood cells, white blood cells mean corpuscular volume and mean corpuscular haemoglobin [33]. In another study, Madaki *et al* [34] reported that administration of 600 mg/kg of the methanolic extract for 28 days significantly increased the haemoglobin, packed cell volume, red blood cells, mean corpuscular haemoglobin concentration and platelets reinforcing the hematinic property of the plant extract at this dose. Although, the differences in the pattern of results from the aforementioned studies is not immediately known, it is possible that the extract might possess hematinic property at particular doses while very

high doses might become haematotoxic in the animals. All these consistently emphasize the ameliorative effects of aqueous extract of *A. precatorius* leaves on iron deficiency in the animals and is in line with the report of Nyirenda *et al* [35] following the administration of aqueous root extract of 400 mg/kg of *Phyllanthus muellerianus* to tail-bled-induced anaemic rat model. A possibility on the mechanism of action might be that the components of *Abrus precatorius* may have restored iron deficiency via several mechanisms including enhancing the bioavailability of iron which ultimately stimulated hematopoiesis, promoting the secretion and the release of erythropoietin, a hormone necessary for the production of blood cells and increasing the resistance of the red blood cell membranes to oxidative damage, earlier reported by Falayi *et al* [36] on the same plant.

Generally, gastric acid plays a role in converting dietary iron into absorbable form in the intestine of animals and a high pH of gastric juice directly correlates with increased risk of iron deficiency and by extension, iron deficiency anaemia [31, 37, 38]. Therefore, the increased intragastric pH of the animals maintained on IDD may be related to iron deficiency anaemia [39], as it might have adversely influenced the absorption of inorganic iron. In contrast, the administration of the extract restored the pH of the gastric acid, probably due to its ability to enhance the bioavailability of iron, and hence converting it to soluble forms that can be readily absorbed by the stomach acid.

Soluble transferrin receptors (sTfR) are highly sensitive protein biomarkers found in the blood/serum of animals that can be used in the diagnosis of iron deficiency anaemia [40], elevated with iron deficiency anaemia. The increase in the soluble transferrin receptors in the rats maintained on iron-deficient diet as against those in other treatment groups confirms that when there is iron deficiency in tissues, the tissues will express more transferrin receptors in order to maximize uptake and these are then cleaved and subsequently released into the blood stream and then manifest as increase in sTfR. Such high levels of soluble transferrin receptors in the animals maintained on iron deficient diet implies a confirmation of the lack of iron in the body [41,42]. Interestingly, the fact that the levels of sTfR in the animals exposed to the aqueous extract of *Abrus precatorius* leaves compared with those of the animals that were treated with ISD and the commercial diet suggest restorative capacity of the extract and this continuously emphasizes the ability of the extract to mitigate against iron deficiency anaemia. The restorative activities of the plant extract on soluble transferrin receptors may be via up-regulating iron absorption, promoting erythropoiesis and reducing

the inflammation induced as a result of iron restriction.

5.0 Conclusion

Aqueous extract of *Abrus precatorius* leaves exerted significant restorative effects by normalizing the haematological parameters, pH of gastric acid and the soluble transferrin receptors of the iron deficient anaemic rats. Therefore, the extract could be a promising therapeutic agent for the management of iron deficiency. The study recommended they plant may be explored for the management of iron deficiency after necessary clinical trials.

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None

Authors Contributions

ATO – Conceptualization and Supervision; ATA - Data curation, Formal analysis and Resources; ROY – Methodology and Writing – original draft, review & editing; OMD - Writing – review & editing.

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