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Effect of Aqueous Extract of *Spondiasmombin* (Plum) Leaf on Haematological Parameters in Iron Deficient Rats.

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Abstract: *This study was aimed at investigating the antianeamic of Spondiasmombin on iron deprived rats. The effects of oral administration of aqueous extract of Spondiasmombin leaf at doses of 100, 200 and 300mg/kg body weight on haematological parameters, of iron sufficient and iron deprived rats were investigated. Forty (40) albino rats were used for the study. Ten (10) rats were fed with iron sufficient diet (control group) and thirty (30) rats were made iron deficient by feeding them with iron deprived diet. After four weeks of feeding the rats,the haemoglobin concentration (Hb), packed cell volume (PCV), red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular volume (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cell (WBC) and serum iron (Fe) were determined. The mean PCV, Hb, MCH, serum Fe of the iron deprived rats were found to be significantly ($p<0.05$) reduced when compared with rats fed on iron sufficient feed. The anaemic rats (iron deficient) were then treated with various doses of the extract, iron standard drug (iron sulphate) and iron sufficient diets for two weeks. Proximate analysis of the iron sufficient and iron depriveddiets showed that they are similar. Phytochemical screening of the extract revealed the presence of alkaloids, tannins, flavonoids, saponins and cardiac glycosides. The mineral analysis of the extract showed the leaf contained Na (190.08 mg/100g), K (3.45 mg/100g), Fe (109.33 mg/100g), Zn(226.67 mg/100g) and Ca(17.77 mg/100g). Compared with the control Iron sufficient group (ISG), extract administration produced significant increase ($P<0.05$) in Hb, PCVand MCH at all the dose levels. The results obtained suggest that the administration of aqueous extract of Spondiasmombin leaf might have reversed the anaemic condition.*

Keywords:*Spondiasmombin, haematological parameters, iron deficient, iron sufficient,*

I. Introduction

Iron deficiency anaemia (IDA) is a global health issue with disproportionately high prevalence in developing countries.According to the National Food Consumption and Nutrition Survey in 2004, 43.7% of pregnant women in Nigeria are iron deficient. In addition to being an independent risk factor for decreased quality of life and increased morbidity and mortality, IDA in women has been linked to unfavorable outcomes of pregnancy. It is the most common nutritional disorder in the world affecting 2 billion people worldwide. The main cause of iron deficiency anaemia is iron loss due to heavy or persistent bleeding and menstruation is the most common cause in women of child bearing age. Other causes include blood loss from the digestive tract due to disorders such as erosive gastritis, peptic ulcer, stomach ulcer, inflammatory bowel disease, hemorrhoids, and blood tumours. Iron deficiency anaemiais characterized by reduction in circulating red blood cell, haemoglobin and haematocrit per unit of peripheral blood and can result in low resistance to infection, impaired psychomotor development, impaired cognitive function in children, poor academic performance, fatigue, fetal resorption, low productivity and increased risk of maternal mortality.

Spondiasmombin (Plum) is a fructiferous tree that thrives in the rainforest and coastal areas of Africa. It belongs to the family *Anacardiaceae* and readily used in folk medicine. The fruit is called *Iyeye* or *Yeye*in the Yoruba language, *Ngulungu* in Igbo and *sadaa* in Hausa . The fruits are popular for eating and the extracted juice is used to prepare ice cream, cool beverages, jam and wine like Viro de Taperiba. The decoction of the astringent bark serves as an emetic, a remedy for diarrhoea, dysentery, haemorrhoids and a treatment for gonorrhoea and leucorrhoea. Its uses show that it is a medicinal plant with a lot of potential and valuable untapped resource of active agents for treating diseases.There are many drugs used for the treatment of iron deficiency anaemia. However, some of these drugs have been reported to have side effects (such as diarrhea, constipation, epigastric abdominal discomfort) while others are not available to many poor people in the developing countries. In addition, rural populations in various parts of the world do not have adequate access to high quality drugs for the treatment of iron deficiency anaemia. They therefore, depend heavily on plants and herbal products for the treatment of diseases including iron deficiency anemia. Iron deficiency aneamiais claimed to have been successfully treated with plant materials by traditional medicine practitioners and many authors.The current study is aimed to access the putative assumption for using this plant in the treatment of iron deficiency anemia.

II. Materials And Methods

2.1. Feed Materials

Maize (*Zea may*), locust bean [*Parkiabiglobosa (A.)*Jacq] seeds were obtained locally from Janguza Market, Kano, while the soybean oil used was a product of Grand Cereal and Oil Mills Limited, Bukuru, Jos, Nigeria. The vitamin-mix were obtained from PHED AQUACULTURE (GidanKaji) Farm Airport Road, Kano. Other chemicals of the mineral mix used were obtained from Biochemistry Department Laboratory.

2.2. Plant Materials and Authentication

The leaf of the plant was obtained from Igboho Local GovernmentArea in Oyo State, Nigeria and was authenticated in the Department of Plant Biology, Bayero University Kano with the v;oucher number: BUKHAN 0315.

2.3. Preparation of Aqueous Extract of *Spondiasmombin* leaf

The method described by Yakubu*et al*.,was adopted for the preparation of the aqueous extract of*mombin* leaves. The leaves were separated from the stem and dried under the shade for 72 hrs to achieve a constant weight. The dried pieces were ground into fine powder. About 1.5kg of the powder was weight and soaked in 1.5L of cold distilled water for 48hrs. The mixture was stirred and filtered. The filtrate was subjected to a slow but complete evaporation using well labeled petridishes. The extract in the filterate yielded 17.30gand this was then package in an air-tight container, labeled and stored below 4^0C in a freezer.

2.4. Phytochemical Screening (Qualitative) and Determination of total minerals

The aqueous extracts of *Spondiasmombin*were screened for some phytochemical constituents using standard proceduresWhile the total amounts of Na, K andCa, Fe, Zn, in the digested samples were determined by colorimetric method.

2.5. Experimental Animals

Albino rats (*Rattusnorvegicus*) of both sexes were obtained and kept in the animal holding unit of the Department of Biological Sciences, Bayero University Kano, Nigeria.

2.6. Composition of Diet

The components of the feed were thoroughly mixed and made into pellet. The feed was produced weekly and packed into air-tight polythene bags.

Table 1: Feed Components of Iron Sufficient and Iron Deprived Diets

Feed Composition	Fe sufficient (g/kg)	Fe deprived (g/kg)
Locust beans	710	710
Corn Starch	40	40
Soybean oil	40	40
Sucrose	100	100
Methionine	20	20
Lysine	10	10
Vitamin mix	10	10
Mineral mix	30	30
Fibre	40	40
$FeSO_4 \cdot 7H_2O$	1.078	-

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

Mineral mix (g/kg diet): $CoCl_2.6H_2O$ (0.001), $CuSO_4.5H_2O$ (0.078), $MnSO_4.2H_2O$ (0.178), KI (0.032), KH_2PO_4 (10.559), NaCl (3.573), $MgSO_4.7H_2O$ (2.292), $ZnCO_3$ (1.6), $CaSO_4$ (11.61).Control diet contained 1.078g $FeSO_4 \cdot 7H_2O$ while Iron deficient diet contains no additional $FeSO_4.7H_2O$.

Vitamin mix (per kg of diet): vitamin A, 100,000 lU; vitamin D_3, 10,000 lU; vitamin E, 100 mg; vitamin B_1, 20 mg; vitamin B_2, 40 mg; d-calcium pantothenate, 100 mg; vitamin B6, 15 mg; vitamin B_{12}, 10µg; vitamin C, 250 mg; vitamin K_3, 15 mg; folic acid, 5000 mg; nicotinic acid, 200 mg; biotin, 150 mg; choline chloride, 400µg; inositol, 80 mg.

2.8. Animal grouping

Permission was obtained for the study from the departmental ethical committee and the study was carried out according to the principles prescribed for laboratory animal use. A total number of forty (40) albino rats were used. They were maintained under standard laboratory situations and allowed free access to normal rat feed and water. The acclimatization was done for seven days before the start of the experiment after which they were fasted for 24 hours (food except water was removed) prior to the commencement of the experiment. The animals were grouped into two

A: Ten (10) rats were maintained on iron sufficient diet designated as IS

B: Thirty (30) rats were maintained on iron deprived diet designated as ID

At the end of the 4 weeks feeding period, 4 rats from each group were sacrificed and their haematological indices determined. The remaining rats in groups B were further grouped into six groups with four rats in each group as follows:

B1- Iron deprived feed all through designated as IDG (iron deprived group)

B2- Change of feed designated as CFG (change of feed group). The animals in this group were fed with iron sufficient feed to see if the presence of iron in the diet can restore back their anemic status.

B3-Administered with Iron sulphate designated as SDG (standard drug group)

B4- Administered with 100mg/kg b/wt of the aqueous extract leaf designated as SM 100mg.

B5- Administered with 200mg/kg b/wt of the aqueous extract leaf designated as SM 200mg

B6- Administered with 300mg/kg b/wt of the aqueous extract leaf designated as SM 300mg

The rest of the animals in group **A**maintained their normal feed for the two weeks.

2.9.Collection of Blood Samples

The method described by Yakubu*et al.* was used. At weeks four and six, the animals were sacrificed by jagular decapitation and the blood sampleswere collected in an EDTA containing bottle which was then used for haematological assay.

2.10. **Haematological Parameters**

Hb, PCV, RBC, MCV, MCV, MCHC and WBC were analysed using automated heamatologic analyzerSysmexkx 2, haemoglobin, packed cell volume, red blood cells.

2.13. Statistical Analysis

Data were presented as Mean ± SEM of 4 replicates. The data were analyzed using. Analysis of Variance (ANOVA). Significant difference between the treatment means was determined at 95% confidence level using INSTAT Multiple Range Test.

III. Results

Results of quatitativephytochemical screening of *Spondiasmombin*

Phytochemical screening of aqueous extract of *Spondiasmombin* leaf revealed the presence of tannins,saponins, flavonoids, cardiac glycosides and alkaloids.Mineral analysis of aqueous extract of *spondiasmombin* leaf revealed the presence of Na, K, Ca, Zn and Fe in various amounts as shown below in table (2).

Table 2: Mineral composition of *Spondiasmombin*(mg/100g)

Minerals	Composition
Na	190.08 ± 2.45
K	3.45 ± 0.20
Ca	17.77 ± 0.15

Fe	109.33 ± 3.06
Zn	226.67 ± 9.50

Values are expressed as Mean ± SEM (n =3).
Proximate composition of both iron sufficient (IS) and iron deficient (ID) formulated feeds showed that the components in the iron sufficient feed (ISF) are similar to those in the iron deficient feed(IDF) (Table 2).

Table 3: Proximate composition of Iron sufficient and Iron deprived Diets

Component (%)						
Grp	**M.C**	**A**	**C.P**	**L**	**C.F**	**CHO**
ISF	4.26 ± 0.15 [a]	3.67 ± 0.06 [a]	40.46 ± 0.67 [a]	10.26 ± 0.42 [a]	3.05 ± 0.10 [a]	38.28 ± 0.99 [a]
IDF	4.10 ± 0.10 [b]	3.72 ± 0.24 [b]	42.17 ± 0.99 [b]	8.00 ± 0.87 [b]	3.06 ± 0.26 [b]	38.95 ± 0.48 [b]

Values are expressed as Mean ± SEM (n = 3). Values in each column with same alphabet superscript are significantly different (P < 0.05). *Spondiasmombin*: SM, Iron Sufficient Feed: ISF, Iron Deprived Feed: IDF, M.C: moisture content, A: ash content, C.P: crude protein, L: lipid C.F: crude fibre CHO: carbohydrate.

At the end of the four (4) weeks of feeding with iron deficient and sufficient diet, the level of PCV, HB, RBC, MCV, MCH, and WBC of the animals placed on iron deprived feed (IDF) decreased significantly (p>0.005) when compared with rats fed with iron sufficient feed (ISF) (Table 4).

Table 4: Haematological parameters of rats fed with iron deprived and sufficient diets after four weeks of feeding.

Parameters	ISG	IDG
PCV (%)	46.25 ± 2.30 [a]	40.40 ± 5.20 [b]
Hb (g/dL)	12.28 ± 0.36 [a]	6.96 ± 1.70 [a]
RBC ($10^6/\mu L^3$)	6.82 ± 0.20 [a]	4.06 ± 1.16 [b]
MCVfL (μm^3)	67.8 ± 2.60 [a]	66.25 ± 4.15 [b]
MCH (pg)	18.00 ± 0.50 [a]	11.46 ± 1.10 [a]
MCHC (g/dL)	16.63 ± 1.50 [a]	15.15 ± 2.10 [b]
WBC ($10^3/\mu L$)	26.55 ± 1.10 [a]	24.93 ± 0.80 [b]
Serum Fe (µg/dL)	55. 80 ± 1.60 [a]	39.54 ± 1.20 [a]

Results are presented as Mean ± standard deviation, (n=4). Values in each rows with same a superscript are significantly different (P < 0.05). ISG: Iron Sufficient Group, IDG: Iron Deprived Group; PCV: packed cell volume; Hb: haemoglobin; RBC: red blood cell; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; WBC: white blood cell.

Table 5: Haematological parameters of iron sufficient and iron deprived rats administered with different doses of aqueous extract of *Spondiasmombin* leaf after six weeks of feeding.

Grps	**PCV (%)**	**Hb (g/dL)**	**RBC($10^6/\mu L^3$)**	**MCVfL(□)**	**MCH (pg)**	**MCHC (g/dL)**	**WBC($10^3/\mu L$)**
ISG	43.05 ± 3.90[a]	10.83 ± 0.98 [a]	6.39 ± 0.57	68.78 ± 2.90	18.80 ± 0.48[a]	25.4 ± 0.30	15.73 ± 0.13
IDG	36.05 ± 0.92[ab]	6.42 ± 0.28[ab]	4.34 ± 0.33 [b]	67.15 ± 4.00	13.10 ± 0.43[ab]	23.95 ± 0.83	14.76 ± 0.44
CFG	42.73 ± 5.60 [b]	10.53 ± 1.12 [b]	6.46 ± 0.74	67.6 ± 1.30	16.30 ± 0.50[b]	24.13 ± 0.87	16.05 ± 1.72
SDG	43.06 ± 2.60 [b]	12.00 ± 0.39 [b]	6.93 ± 0.55	66.6 ± 2.20	16.40 ± 0.76[b]	24.73 ± 0.25	14.05 ± 2.50

SM 100	41.50 ± 4.70 [b]	9.78 ± 0.97 [b]	5.03 ± 0.89	65.68 ± 3.37	17.38 ± 0.87[b]	24.93±0.88	14.38 ± 4.60
SM 200	40.68 ± 6.33 [b]	9.05 ± 1.18[b]	5.94 ± 0.94	66.75 ± 1.67	17.43 ± 0.87[b]	24.63 ± 1.76	14.12 ± 1.76
SM 300	40.80 ± 1.85 [b]	10.00 ± 1.85[b]	6.96 ± 0.44 [b]	68.06 ± 3.00	19.95 ± 4.98 [b]	24.33 ± 1.28	14.15 ±3.60

Values are expressed as Mean ± SD (n = 4). Values in each column with same superscript are significantly different (P < 0.05). ID: iron deprived rats; IS: iron sufficient rats; CF: change of feed; SD: standard drug; SM: *SpondiasMombin*; PCV: packed cell volume; HB: haemoglobin; RBC: red blood cell; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; WBC: white blood cell.

IV. Discussions

The deficiency of iron has been described as the most prevalent nutritional deficiency and iron deficiency anemia is estimated to affect more than one billion people worldwide.From table 2, iron which spondiasmombin contains play a significant role in hematopoiesis. However, the therapeutic potential of the herb cannot be established on the basis of availability of iron content alone as other factors play a role in the absorbtion of iron in the body. Such factors are alkaloids, flavonoids, saponins, tannins, Ca, Zn and K. For example the flavonoids have veinotonic properties and protect capillaries.From the result in Table 3, it shows that there was no significant difference in the proximate analysis of Iron sufficient and Iron deprived diet. The result shows that the compositions of the two diets are essentially similar except in the iron content. This is an indication that the diets only differ in the amount of iron.

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 . Result from table 4 showed the establishment of iron deficiency anaemia in the first four weeks of this study. The significant decrease in the heamoglobin concentration of the iron deficient group when compared with iron sufficient group is sufficient to conclude that the feed induced intended condition of this study i.e. iron deficiency anemia. The result shows that there was a significant ($p<0.05$) decrease in Hb and MCH level in iron deprived animals; this may be attributed to induction of IDA when the animals were placed on iron-deprived diet. This is supported by a previous study in which rats placed on iron poor ration (5–8 mg iron/kg ration) for 5 weeks were iron deficient and anaemic (when haemoglobin concentration was less than 7 g/dl)..

Medicinal plants such as Aegelmarmelos, Carissa congesta, Eugenia jambolana, Ficuscarica, Phoenix sylvestris, Phyllanthusemblica, Vitisvanifera, Moringaoleifera and Telfariaoccidentalis experimentally tried on rats have been found to significantly increase heamological parameters.From table 5, Administrationofaqueousextractof*Spondiasmombin* leaf extract, Change of feed and standard drug (Table 5) resulted in significant increases in haemoglobin (i.ewhen haemoglobin concentration was greater than 7g/dl)and PCV 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-deficient rats were also increased by treatment with all the three doses of the aqueous extract.The significant increase in the blood parameters, Hb and PCV of the iron deprived groups following the administration of *Spondiasmombin* leaf could be as a result of the presence of phytochemical such assaponins as seen exhibited by spondiasmombin leaves extract and some mineral elements such as iron. Saponin containing herbs have been successfully used to promote and vitalize blood circulationwhile iron is important in the production of Hb and it plays an important role in flavor protein – cytochrome system activities hence it is used to treat iron deficiency anemia.

V. Conclusion

The study shows that aqueous leaf extract of *Spondiasmombin* at different doses from 100mg/kgbody weight to 300mg/kg body weight can be used as traditional medicine in the management of iron deficiency anemia because it contains some phytochemicals such as saponoinsthat can produce iron which is needed in the restoration of the deficiency state. This is because dietary iron deficiency led to a decrease in the iron-containing protein haemoglobin, and by extension reduction in red blood cells and PCV and RBC levels.

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