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Evaluation of the Effect of *Sorghum bicolor* Aqueous Extract on the Haematological, Renal and Hepatic Parameters in Rats Fed with Low and High Iron Diet

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Authors' contributions

This work was carried out in collaboration between all authors. Author SOS designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author YAS managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: To determine the effect of *Sorghum bicolor* aqueous leaf sheath extract on the hematological, renal and hepatic parameters in rats fed with low and high iron diets.
Study Design: Phytochemical screening of leaf sheath of *Sorghum bicolor* and effect of the leaf extract on hematological, hepatic and renal indices of rats fed with iron deficient and iron sufficient diets.
Results: The result of the phytochemical screening indicated the presence of cardiac glycosides, alkaloids, flavonoids, glycosides, saponins and terpenoids. The evaluated hematological parameters (PCV, Hb, RBC,MCV,MCH, MCHC) prior the administration of the leaf sheath extracts (control) in the iron sufficient fed rats revealed a higher value compared to the result obtained in iron deficient fed rats, with the exception of MCHC. Subsequently, the administration of the leaf sheath extracts at different concentration (200,400,800,1600mg/kg body weight of the sample) for both iron sufficient and iron deficient fed rats revealed increased values of the hematological parameters, with the highest values recorded upon the administration of 1600mg extract/kg, Similarly, the results of the evaluated liver biomarkers for the control (albumin, AST, ALT, alkaline phosphatase and total protein) in the iron sufficient fed rats showed a higher value

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compared to the result obtained in iron deficient fed rats. The result of the liver biomarkers after administration of leaf sheath showed a slight elevation in both iron sufficient and iron deficient fed diet. Furthermore, the result revealed that aqueous sorghum leaf sheath extracts showed a slight increase in the renal function indices at varying concentration of the extracts.

Conclusion: The present study showed that administration of sorghum leaf sheath extract enhanced the hematological parameters in rats fed with iron deficient diet thereby supporting the claim that the leaf sheath extract could be used in alleviating anemic condition. The studies further established that the extract is non toxic to the liver and also that the integrity of the kidney is maintained after administration. This on the overall confirms the safety of the extract upon consumption.

Keywords: Sorghum bicolor; phytochemicals; Iron; hematology; renal function; hepatic function.

1. INTRODUCTION

Sorghum bicolor (Linn.) Pers. (Family: Gramineae; Poaceae) is the fifth most important cereal crop in the world after wheat, rice, corn and barley. *Sorghum bicolor* is an annual plant which constitute a major food crop in Africa, Europe, Asia and America, and serve as a major source of proteins, calories and mineral [1], sorghum also contains phenolic compounds [2-3], a plant secondary metabolite which serve as antioxidant which are useful in the prevention of free radical mediated diseases. Sorghum is considered as subsistence crop because of its unique tolerance to drought and adaptation to dry tropical and subtropical ecosystems throughout the world [4].

Historically, sorghum has been used in West Africa to color leather goods which include suitcases, shoes, baskets, hats and book covers. In most African countries, sorghum is processed into food and beverages, which are important sources of nutrients. These include whole grain rice-type products, breads and pancakes, dumplings and couscous, porridges, gruels, opaque and cloudy beers, and non-alcoholic fermented beverages [5-6]. Sorghum, with large juicy stems containing as much as 10% sucrose is used in manufacture of syrup. Traditionally, bundles of leaf sheaths of sorghum are extracted in a laborious cottage-industry process. Other uses of sorghum include the production of industrial alcohol from the seeds, vegetable oil, broom-making, adhesives, waxes, sizing papers and cloth [7].

Generally, the cereal is very rich in minerals, with bioavailability varying from less than 1% for iron to greater than 90% for sodium and potassium. The reasons for this are varied and complex, since many factors interact to determine the ultimate bioavailability of a nutrient [8]. Deficiencies of micronutrients are still a major public health problem in many developing countries with infants and pregnant women usually at risk. One of such deficiency disorder is anemia which is a common blood disorder that affects people of all ages, with the elderly, young women of child-bearing age, and the infants at greater risk. Anemia is a condition in which the oxygen carrying capacity of blood is reduced and many kinds of anemia exist; all characterized by reduced number of red blood cells (RBCs) or decrease amount of hemoglobin (Hb) in blood. In other terms, anemia is defined as Hb concentration in blood below the lower limit of the normal range depending on the age group and sex of the individual [9].

In recent times, herbal remedies form an integral part of the primary health care system of many nations [10]. Malted *Sorghum bicolor* grain is higher in protein and lower in fat content than corn and this is partly responsible for its haemopoietic ability [11]. It has been reported that sorghum can be used as anti-abortive, cyanogenetic, demulcent, diuretic, emollient, intoxicant and poison. Sorghum is a folk remedy for cancer, epilepsy, flux and stomach ache. The root is used for malaria in Southern Rhodesia; the seed has been employed for the treatment of breast disease and diarrhea while the stem has been used for tubercular swellings treatment [12].

Many plants have been reported in some countries of the world to have anti-anemic property like, Snake flower *Lamium album*, *Justicia secunda* and *Khaya senegalensis* [13-16]. However, the use of the sorghum leaf sheath as a remedy against anaemia (reduction of red blood cells or its function) by traditional medicine healers is common in Nigeria mostly within the local people of the Yoruba and Hausa tribes [17].

The present study therefore, seeks to evaluate the effect of *Sorghum bicolor* aqueous extract on the hematological parameters of rat with varied levels of dietary iron and also to determine the biochemical effect on the kidney and liver after administration.

2. MATERIALS AND METHOD

2.1 Materials

Dry leaves of *Sorghum bicolor* leaf sheath were purchased in December 2012 from herb sellers at Apata market Ibadan, Oyo State, Nigeria and were authenticated at the department of crop soil and pest management of the Federal University of Technology, Akure, Nigeria**.** The dry leaves were air dried for another 3 weeks, after which the lightly colored part of the leaves were cut off leaving the dark red part of leaves and ground into fine powder using electric dry milling machine. A total of 480g of the ground powder was divided into 240g each, boiled in a 4.5 litre of distilled water for 15mins. This was allowed to cool, then sieved to remove the shafts. The liquid extract was then concentrated using a freeze dryer. All reagents used were of analytical grade and were prepared in all glass-distilled water.

2.2 Feed Components

The feed composition as shown in Table 1 contained the following; maize (Zea may), locust bean [Parkia biglobosa (A.) Jacq] seeds, coconut, butter and beans and were obtained from a local market in Ibadan, Oyo State Nigeria. The amino acid and mineral- vitamin mix used were products of Sigma Chemical Company Limited, London.

2.3 Care and Treatment of Animal

A total number of 40 female Albino rats weighing 100-197g were collected from the laboratory Animal centre, Federal University of Technology, Akure. They were housed in metal cages inside one room under a standard condition with 12hrs light and darkness cycle.

2.4 Animal Grouping and Extract Administration

The 40 female Albino rats were grouped into major groups; Group A (Rats maintained on iron sufficient diet designated as IS**)** and Group B (Rats maintained on iron deficient diet designated as ID**)**, each group was further divided into 5 subgroups of 4 animals in each group respectively. The rats were administered with various concentration of the extract as follows: The first subgroup, second, third and fourth were treated with 1.0ml of 200,400, 800,1600mg/kg of the extract of *Sorghum bicolor* leaf sheath while the fifth subgroup which serve as the control received orally 0.5ml of 0.9% of Normal Saline daily for 10 days using oral pharyngeal cannula.

2.4.1 Group a (a-e) iron sufficient (IS)

Aa) Iron sufficient rats orally administered on daily basis for 10 days with 1.0ml of 200mg/kg body weight of aqueous extract *Sorghum bicolor* IS 200.
Ab) Iron sufficient rats orally administered on daily basis for 10 days with 1.0ml of 400mg/kg body weight of aqueous extract of *Sorghum bicolor* IS 400.
Ac) Iron sufficient rats orally administered on daily basis for 10 days with 1.0ml of 800mg/kg body weight of aqueous extract of *Sorghum bicolor* as IS 800.
Ad) Iron sufficient rats orally administered on daily basis for 10 days with 1.0ml of 1600mg/kg body weight of aqueous extract of *Sorghum bicolor* as IS 1600.
Ae) Iron sufficient rats orally administered on daily basis for 10 days with 0.5ml of the 0.9% of normal saline as control designated as IS control.

2.4.2 Group b (a-e) iron deficient (ID)

Ba) Iron deficient rats administered orally on daily basis for 10 days with 1.0ml of 200mg/kg body weight of aqueous extract of *Sorghum bicolor* designated as ID200.
Bb) Iron deficient rats administered orally on daily basis for 10days with 1.0ml of 400mg/kg body weight of aqueous extract of *Sorghum bicolor* designated as ID 400.
Bc) Iron deficient rats administered orally on daily basis for 10 days with 1.0ml of 800mg/kg body weight of aqueous extract of *Sorghum bicolor* designated as ID 800.
Bd) Iron deficient rats administered orally on daily basis for 10 days with 1.0ml of 1600mg/kg body weight of extract of *Sorghum bicolor* designated as ID 1600.
Be) Iron deficient rats administered orally on daily basis with 0.5ml of 0.9% of normal saline for 10 days designated as ID control.

Table 1. Feed composition of iron sufficient and iron deficient diet

Feed Component	Iron Sufficient (g/kg)	Iron Deficient (g/kg)
Locust bean and Fish	180.0	180.0
Coconut and butter	60.0	60.0
Beans	50.0	50.0
Maize	290.0	290.0
Calcium	10.0	10.0
Lysine	8.5	8.5
Methionine	3.5	3.5
NaCl	3.0	3.0
Iron	30.5	10.5

2.5 Phytochemical Screening

Phytochemical screening involves performing simple chemical tests on the sample for the purpose of detecting different phytochemicals present. Chemical tests were carried out on the aqueous extract and on the powdered samples to identify the constituents using standard procedures [18-20].

2.5.1 Test for cardiac glycosides (keller-killani test)

Five milliliter (5ml) of the extracts was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1ml of concentrated sulphuric acid. A brown ring at the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout the thin layer.

2.5.2 Test for flavonoids

Five milliliter (5ml) of dilute ammonia solution was added to a portion of the aqueous filtrate of the extract followed by addition of concentrated H_2SO_4. A yellow coloration observed in the each extract indicated the presence of flavonoid.

2.5.3 Test for glycoside

5.0ml of extract was treated with 2.0ml of glacial acetic acid containing 1 drop of 0.1% ferric chloride, and then mixed with 1.0ml concentrated sulphuric acid. A brown ring at the interface indicates a deoxysugar characteristic of cardenolides.

2.5.4 Test for saponnins

Two grams (2g) of the powdered sample was boiled in 20ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent forth. The frothing was mixed with 3 drops of olive oil and shaken vigorously then observed for the formation of emulsion.

2.5.5 Test for tannins

0.5g of the dried powdered samples was boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration as evidence for the presence of tannins.

2.6 Collection of Blood Sample

The rats were anaesthetized in chloroform vapor. When they became unconscious, the neck area was quickly cleared off and the 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 scapulae blade and 2.5ml of blood was collected into $_{k3}$ EDTA sample bottle which was used for the haematological tests while the remaining blood was collected into lithium heparinized bottle and was used to evaluate the liver enzyme, total protein, albumin sodium (Na^+), potasium (K^+), chloride (Cl-) and bicarbonate (HCO_3) [13]. The remnant rats were quickly buried to avoid infection.

2.7 Hematological Test

The hemoglobin concentration and red blood count was determined colorimetrically at 540nm. PCV was measured using the micro-haematocrit reader after centrifuging blood containing non-heparinised capillary at 2/3 of the capillary [21]. Red blood count was determined using the method described by Woblewski [22].

2.8 Liver Enzymes, Total Protein and Albumin

Liver enzymes such as Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), total protein, and albumin were carried out within 24hrs of sample collection using colorimetric method by Randox laboratories limited, 55 Diamond Road, United Kingdom.

2.9 Renal Function Test

Renal function tests such as sodium (Na^+), potasium (K^+), chloride (Cl^-) and bicarbonate (HCO_3^-) were carried out using colorimetric method by TECO Diagnostics 1268 N- Lakeview Ave. United State of America (USA). Also urea and creatinine level were determined by colorimetric method manufactured by Randox laboratories limited 55 Diamond Road, United Kingdom.

2.10 Statistical Analysis

All analysis was run in triplicate. The mean value and standard deviation were calculated using the Microsoft Excel software (Microsoft Corporation, Redmond, WA)

3. RESULTS AND DISCUSSION

Foods and nutrients play a vital role in maintaining the health of the individual and in reducing the risk of various diseases. There is a global acceptance of this fact and this formed a relationship between "nutrition" and "health" which is known as “nutraceuticals". Nutraceuticals are medicinal foods that play a role in maintaining well being, enhancing health, modulating immunity and thereby preventing as well as treating specific diseases. Thus the field of nutraceuticals can be envisioned as one of the missing blocks in the health benefit of an individual.

Anemia is a chronic disease characterized by reduced concentrations of serum iron, transferrin, and total iron binding capacity with high erythrocyte sedimentation rate. Anemia sometimes is a silent killer and if left untreated, in severe cases it is life threatening. In recent time, the use of nutraceuticals in the management and prevention of anemic condition have been reported [23].

The result showing the presence of some phytochemical constituents is as shown in Table 2. The results indicated the presence of cardiac glycosides, alkaloids, flavonoids, glycosides, saponins and terpenoids. These phytochemicals have been reported to perform many functions in plants with different biochemical and pharmacological actions in animal species when ingested [24].

Table 2. Phytochemical constituents of aqueous extract of *Sorghum bicolor* leaf sheath

Phtochemicals	Occurence
Cardiac Glycoside	+ve
Alkaloids	-ve
Flavonoids	+ve
Glucosides	+ve
Saponins	-ve
Tannins	+ve

Note: +ve= present; -ve= absent

The effect of the aqueous leaf sheath extract on some hematological parameters (PCV,Hb, RBC,MCV,MCH,MCHC) of iron sufficient and iron deficient fed rats were as presented on Tables 3 and 4. The results of the evaluated hematological parameters before the administration of the leaf sheath extracts in the iron sufficient fed rats (PCV:38.00, Hb:12.68,RBC:3.95,MCV:9.62,MCH:30.46,MCHC:33.31) revealed a higher value compared to the result obtained in iron deficient fed rats (PCV:32.00,Hb:10.62,RBC:3.60,MCV:8.50, MCH:28.24,MCHC:33.31), with the exception of MCHC (Mean Cell hemoglobin concentration), which is not significantly different from the value obtained for iron deficient fed rats. The result is as expected since iron have should have a positive correlation to the hematological parameters because low level of iron normally bring about an induction of iron deficiency anemia [13,25,26].

On a general note, the administration of the leaf sheath extracts at different concentration (200,400,800,1600mg/kg body weight of the sample) for both iron sufficient and iron deficient fed rats revealed increased values of the hematological parameters, with the highest values recorded upon the administration of 1600mg/kg. The results of this study also indicate that administration of leaf sheath extract of *S. bicolor* may be useful in alleviating anaemic condition. This is in agreement with previous reports and this possibly could be as a result of its direct effect on the hematopoietic systems [27,28].

The result of liver biomarkers (albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase and total protein) in rat fed with sufficient and deficient iron diet is as presented on Tables 5 and 6. The evaluated liver biomarkers before the administration of the leaf sheath extracts in the iron sufficient fed rats (AST:10.75, ALT:11.25, ALP:77.00 Total protein:61.33, Serum albumin:38.00) showed higher values compared to the result obtained in iron deficient fed rats (AST:8.50, ALT:9.25, ALP:69.50, Total protein:58.25, Serum albumin:31.25). The higher level of the liver biomarkers in the iron sufficient diet prior to the administration of varying concentration of aqueous sorghum leaf sheath extract might be due to lipid peroxidation in the liver tissue that is induced by iron. This is in agreement with the result of Yeun et al. [29], who observed an increase in oxidative stress due to exposure to high dietary iron, which will ultimately bring about an increase in the markers of oxidative stress in the liver. The result of the liver biomarkers after administration of leaf sheath showed a slight elevation of the evaluated biomarkers (albumin, AST, ALT, alkaline phosphatase and total protein) in both iron sufficient and iron deficient fed diet. The slight increase after the administration of the extract is an indication that aqueous sorghum leaf sheath extract is non-toxic to the liver and would therefore not affect adversely the iron storing capacity of the liver. This observation further established the hematopoietic potential of aqueous sorghum leaf sheath extract [26,27].

Results of the renal function parameters (sodium, potassium, chloride, bicarbonate, urea and creatinine) of iron deficient and iron sufficient fed rats before and after the administration of sorghum leaf sheath extracts are as shown on Tables 7 and 8. The results showed that the renal function parameters of iron sufficient fed rats is almost the same with that of iron deficient fed rats prior to the administration of the aqueous leaf sheath extracts. Furthermore, the result revealed that aqueous sorghum leaf sheath extracts showed a slight increase in the renal function indices at varying concentration of the extracts. This by implication is that the administration of the extract produces no toxic effect on the kidney thereby preserving the integrity of the kidney [30]. Report has shown that common complication of chronic renal disease is anemia, which results from inadequate erythropoietin or from iron deficiency as a result of inadequate absorption or mobilization. This by implication is that anemia and kidney disease are closely related, therefore hematopoietic potential of the sorghum leaf sheath extract contribute to the preservation of kidney integrity. The management of anemia in chronic renal disease patients must strike an appropriate balance between stimulating generation of erythroblasts (erythropoiesis) and maintaining sufficient iron levels for optimum hemoglobin (Hb) production [31].

Table 3. Hematological parameters (PCV, Hbc, RBC, MCV, MCH, MCHC) of iron sufficient (IS) fed Rats after administration of aqueous extract of *Sorghum bicolor* leaf sheath

Group	PCV (%)	Hb (g/L)	RBC ($x10^{12}$/L)	MCV (fl)	MCH (lg)	MCHC (g/l)
Control	38.00±0.42	12.68±0.3	3.95±2.13	9.62±1.02	30.46±0.41	33.31±0.33
200mg/kg	39.50±0.21	13.18±0.35	4.06±1.10	9.73±2.00	32.44±0.04	33.35±0.30
400mg /kg	42.50±0.48	14.18±0.36	4.25±1.13	10.00±0.34	33.35±0.42	33.35±0.13
800mg/kg	43.75±1.71	14.58±0.20	4.28±1.24	10.24±0.21	34.11±2.20	33.31±0.14
1600mg/kg	47.50±1.77	14.78±0.27	4.50±1.14	10.26±2.22	34.21±0.24	33.32±0.30

Results are expressed as mean±standard deviations (SD) of three determinations

Table 4. Hematological parameters (PCV, Hbc, RBC, MCV, MCH, MCHC) of iron deficient (IS) fed Rats after administration of aqueous extract of *Sorghum bicolor* leaf sheath

Group	PCV (%)	Hb (g/L)	RBC ($x10^{12}$/L)	MCV (fl)	MCH (lg)	MCHC (g/l)
Control	32.00±022	10.62±0.16	3.60±1.10	8.50±0.23	28.24±0.04	33.31±1.33
200mg/kg	35.75±3.11	11.35±0.21	3.83±2.02	9.03±0.01	30.10±0.07	33.31±0.30
400mg /kg	36.50±2.01	12.15±0.22	4.04±1.01	9.04±0.04	30.11±0.08	33.29±0.20
800mg/kg	37.00±0.18	12.58±0.31	4.20±0.26	8.99±0.07	29.95±0.22	33.31±0.30
1600mg/kg	39.25±1.16	13.08±0.20	4.20±0.26	8.77±0.20	29.24±1.17	33.31±0.17

Results are expressed as mean±standard deviations (SD) of three determinations

Table 5. Liver biomarkers of Iron sufficient (IS) fed Albino rats after administration of aqueous extract of Sorghum bicolor leaf sheath

Group	AST(a/l)	ALT(u/l)	ALP(u/l)	Total protein	Serum albumin
Control	10.75±0.21	11.25±0.06	77.00±2.07	61.33±3.01	38.00± 0.02
200mg/kg	11.00±0.20	12.25±1.01	81.50±1.03	63.30±2.01	39.45±0.08
400mg /kg	13.25±1.07	14.00±1.07	82.50±1.05	65.28±3.09	40.80±0.10
800mg/kg	15.25±0.10	16.00±0.04	84.00±2.06	67.15±2.07	43.38±0.31
1600mg/kg	16.00±2.24	18.75±2.03	90.50±2.09	71.95+4.09	46.68+0.35

Results are expressed as mean±standard deviations (SD) of three determinations

Table 6. Liver biomarkers of Iron deficient (ID) fed Albino rats after administration of aqueous extract of *Sorghum bicolor* leaf sheath

Group	AST(a/l)	ALT(u/l)	ALP(u/l)	Total protein	Serum albumin
Control	8.50±0.04	9.25±0.02	69.50±1.01	58.25±3.00	31.25±0.05
200mg/kg	10.25±003	11.75±0.08	21.75±1.04	60.58±0.34	33.80±0.07
400mg /kg	11.75±1.08	14.50±0.03	76.25±1.03	61.78±2.05	39.33±0.04
800mg/kg	12.50±0.05	15.25±0.05	77.50±2.02	65.90±1.03	41.70±0.06
1600mg/kg	15.00±0.09	16.00±0.01	89.75±2.00	70.28+3.02	44.03+0.08

Results are expressed as mean ± standard deviations (SD) of three determinations

Table 7. Renal sodium, potassium and chloride of Iron sufficient (IS) fed albino rats after administration of aqueous extract of *Sorghum bicolor* leaf sheath

Group	Na (umol/l)	K(umol/l)	Cl (umol/l)	Bicarbonate (mmol/l)	Urea (mmol/l	Creatinine (mmol/l)
Control	126.00±3.01	3.33±0.02	88.25±2.1	20.50±1.03	4.80±0.01	59.03±2.00
200mg/kg	129.30±2.04	3.88±0.01	97.50±3.01	23.50±0.02	5.50±2.00	66.60±3.25
400mg /kg	131.10±0.07	4.18±0.07	98.00±2.09	29.25±1.02	5.80±0.07	67.85±2.34
800mg/kg	132.75±0.08	4.38±0.04	99.25±1.07	24.50±1.00	5.90±0.27	69.55±3.00
1600mg/kg	136.45+2.05	4.60+0.03	100.00+3.00	25.50±1.00	7.03±0.32	70.98±3.00

Table 8. Renal sodium, potassium and chloride of Iron Deficient (ID) fed albino rats after administration of aqueous extract of *Sorghum bicolor* leaf sheath

Group	Na (umol/l)	K(umol/l)	Cl (umol/l)	Bicarbonate (mmol/l)	Urea (mmol/l)	Creatinine (mmol/l)
Control	125.00±2.01	3.10±0.04	84.00±1.04	20.75±0.02	3.83±0.03	58.65±0.03
200mg/kg	128.75±1.09	3.85±1.00	95.25±0.09	23.00±0.07	5.40±1.01	65.05±2.00
400mg /kg	130.10±0.05	3.93±0.05	96.25±2.01	23.25±0.09	5.58±0.02	66.68±1.00
800mg/kg	131.98±0.04	4.23±0.03	96.25±2.01	24.00±1.00	5.90±0.27	68.85±2.08
1600mg/kg	136.08+0.15	4.45+0.07	100.00+3.00	24.50±1.00	6.25±1.04	70.00±3.00

Results are expressed as mean±standard deviations (SD) of three determinations

4. CONCLUSION

The present study showed that administration of sorghum leaf sheath extract enhanced the hematological parameters in rats fed with iron deficient diet thereby supporting the claim that the leaf sheath extract may be useful in alleviating anemic condition. The increase in the hematological parametrs may possibly be ascribed to the presence of a number of phytochemicals including polyphenols. The studies further established that the extract is non toxic to the liver; and also that the integrity of the kidney will be maintained after the administration of the extract.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared no competing of interest

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