

The Effects of *Sorghum bicolor* Aqueous Leaf Sheaths Extract on Some Selected Biochemical Parameters of Phenylhydrazine-Induced Hemolytic Anemic Male Wistar Rats

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Abstract:-

Aims: In this study the effect of *Sorghum bicolor* was tested on some selected biochemical parameters (direct, total and indirect bilirubin, creatinine, total protein, serum albumin and urea) was appraised to determine if the plant extract can reverse the aberrance in the values of these parameters.

Study design: 40 experimental animals were divided into four groups and hemolytic anemia was induced in three of the groups with two of the groups given the herbal extract in varying degree (low and high dosage) to assess the efficacy of the drug on alleviating selected biochemical parameters, to ultimately investigate the curative effect of the botanical extract on hemolytic anemia.

Place and Duration of Study: Cell and Tissue Culture Research Laboratory (Drug Discovery Unit), Department of Biochemistry, Lagos State University between September 2020 and October 2020.

Methodology: 40 male Wistar rats were divided into four equal groups: normal, anemic untreated, anemic low dosed and anemic high dosed. The low dosed and high dosed rats were given the extract at 100mg/kg and 500mg/kg respectively. After 7 days of extract administration, the rats were sacrificed and blood samples taken through heart puncture were centrifuged for sera which was assayed using Randox Laboratories kit.

Result: The highest levels of bilirubin, creatinine and urea level and lowest levels of total protein and albumin were recorded in the untreated rats, indicating hemolysis. The treated rats recorded a significant dose-dependent reduction in the bilirubin levels and a significant increase in the total protein levels of the two treated rats groups.

Conclusion: this study revealed *S. bicolor* extract to be a potential amelioration of hemolytic anemia.

Keywords:- Hemolytic Anemia, Creatinine, Albumin, Bilirubin, Hemoglobin.

I. INTRODUCTION

Anemia is a general term used to define decrease in the amount of systemic red blood cells and as such the ineffective distribution of oxygen throughout the body due to impaired hemoglobin activity [1]. A global menace that has affected over 1.62 billion people worldwide [2], anemia rarely exists alone but comes as a concomitant of illnesses (malaria), hemorrhaging which can be as a result of severe menstrual flow or deep cuts, parasitic infections and nutritional deficiency of building blocks needed for red cells (such as iron, vitamin B12, erythropoietin) which causes decreased red cells production, hemoglobinopathies which causes increased destruction of red blood cell (hemolytic anemia) which is being considered in this study[3].

Hemolytic anemia occurs when erythropoiesis cannot match the pace of red cell destruction; characterized by reticulocytosis, increased unconjugated bilirubin and lactate dehydrogenase, decreased haptoglobin and peripheral blood smear finding [4]. Hemolytic anemia can be caused by various factors inclusive of: hereditary disorders of the membranes of red blood cells which causes changes to the shape (e.g spherocytosis, ovalocytosis), red cell enzyme deficiencies, [5], consumption of fava beans or taking anti-malarial drug, primaquine in glucose-6-phosphate dehydrogenase deficient individuals [6], infections such as malaria, clostridium infections, immune hypersensitivity [4] and phenylhydrazine which is used in the laboratory for experimental induction of hemolytic anemia to study erythropoietin regenerative response through clinical, pathological, and morphological studies [7]. Phenylhydrazine causes hemolytic injury by inducing reactive oxygen species formation, peroxidation of lipids and oxidative degradation of spectrin in the membrane skeleton [8].

The need for botanical alternative to conventional treatment of anemia cannot be but overemphasized as the conventional treatments though effective are expensive and largely inaccessible to the poor masses that die from this otherwise treatable disorder. *Sorghum bicolor*, commonly known as millet is a grass plant grown for food, feed for livestock and also for the brewing of alcohol. Ethnobotanical reports have shown that extracts taken from the leaf sheaths of *S. bicolor* possess hematopoietic [9] and hepatoprotective activities [10] including the enhancement of immunity by regulating splenocytes formation [11]. The leaf sheaths of *sorghum bicolor* have also been shown to have the antinoinceptive properties (blocking of the sensation of pain) and anti-inflammatory potential [12]. This plant extract besides all these benefits also have an effect on some biomarkers in the blood. These parameters: bilirubin, urea, creatinine, albumin, total protein are markedly analogous to hemolytic anemia hence the need for them to be studied.

The inordinate breakdown of red blood cells in circulation leads to the proliferation of bilirubin, a tetrapyrrole molecule in the blood which is a byproduct of heme breakdown. Bilirubin is usually measured as a function of both direct bilirubin and total value bilirubin [13]. Indirect or unconjugated bilirubin formed from the breakdown of hemoglobin in red cells is transported to the liver in an albumin-bilirubin complex and converted to direct bilirubin.

A high level of direct bilirubin in with high total bilirubin is generally implicative of high turnover of red blood cells and aberrant hepatic activity [13, 14]. Urea is a byproduct formed from the breakdown of proteins ingested or proteins due to tissue breakdown [15] and Creatinine is a metabolite formed from the breakdown of creatine phosphate in muscles and also from consumption of meat [16]. These two biomarkers are used to assess renal functioning as impairment of the kidneys leads to the

accumulation of these products in the blood [17] which (in this case) is brought about by the accumulation of heme and iron in the proximal glomerular tubules and has caused impaired glomerular filtration activity and reabsorption. In short, urea and creatinine are used to assess the glomerular filtration rate.

Total protein is an aggregation of the two major proteins in the blood, albumin and globulins. Albumin is one of the most abundant proteins in the blood released by the liver as part of its normal functioning. Albumin maintains fluid balance in the body by preventing leakage of blood vessels. It also helps in the repairing of worn-out tissues and transport of hormones and nutrients throughout the body [18]. Globulins are made up of hundreds of serum proteins which consists of enzymes, carrier proteins, immunoglobulins and complements. Most of which are synthesized by the liver and can be divided into four groups: alpha (1 and 2), beta and gamma [19]. Total protein assay in conjunction with serum albumin assay is of clinical significance to this study as the decrease in the levels of total protein/albumin can be as a result of renal impairment amongst other factors [19].

➤ *Reagents And Equipment*

Phenylhydrazine, Distilled water, Normal saline, Bilirubin kit, Albumin kit, Blood urea nitrogen kit, Creatinine kit, Heparin, 0.1M potassium phosphate buffer, Lithium-heparin bottles, Oral canacula, Intraperitoneal syringe, Syringes, Dissecting kit, Cotton wool, Gloves, Spatula, Micro pipette, Dissecting kit, UV vis spectrophotometer (bioway®), Centrifuge (Gulfex medical and scientific), Refrigerator (Haier thermocool), Water bath (medifield medical)

➤ *Plant Identification*

Dry leaf sheaths of *Sorghum bicolor* were purchased from herb sellers at Ojo market, Lagos and were botanically identified and authenticated by the Department of Botany; Faculty of Science. University of Lagos.

➤ *Preparation Of Extracts*

The leaves were blended with a blender into fine powder. The powder was decocted by boiling 100g per 5 liters of distilled water. The solution was then filtered and the filtrate lyophilized to obtain a concentrate of *sorghum bicolor*.

➤ *Lethal Dose Toxicity*

Toxic dose of the plant extract was determined by determining the LD₅₀ values. This was carried out by administering to the rats, 800mg/kg, 2500mg/kg, and 5000 mg/kg *Sorghum bicolor* extract.

➤ *Preliminary Examination Of Phenylhydrazine Effects On Rats*

A total of four rats were randomly selected and used for phenylhydrazine pre-induction process (0.5g phenylhydrazine was mixed in 5 ml of phosphate buffer). They were given intraperitoneal injections of the

phenylhydrazine solution of 60mg/kg for two days after which the rats were killed and the hemoglobin level was measured to be <15% proving the rats were having hemolytic anemia due to the fall in the hemoglobin level.

➤ *Grouping Of Experimental Animals*

Twenty-four healthy male Wistar rats weighing between 160g- 240g were used for this experiment. The animals were kept in the Faculty of Science animal house in cages and allowed to acclimatize for 28 days under standard conditions (ambient temperature, $29.0 \pm 2.0^\circ\text{C}$ and humidity 46%, with a 12 h light/darkness cycle) with *ad libitum* access to feed pellets and water. The rats were divided into four groups of five animals each. The animals were administered various agents as follows: the control group was not given the extract and PHZ, the second group were given PHZ only and were not treated with the *Sorghum bicolor* extract while the third, fourth groups were treated with 100mg/kg (low dose), 500mg/kg (high dose) of the extract respectively orally for 7 days. All the animal experiments were carried out in accordance with the guidelines of the Institutions Animal Ethical Committee.

II. COLLECTION OF BLOOD AND BIOCHEMICAL ANALYSIS

➤ *Blood Collection*

The rats were sacrificed the end of the 7 days of extract administration. The rats were euthanized with diethylether and vivisected after which blood samples were collected from each animal's heart into lithium heparin-treated sample bottles, respectively. The blood samples were spun in a bench top centrifuge to obtain sera at 5000 rpm for 15 minutes. The serum samples were thereafter aspirated into another set of plain sample tubes and stored in the refrigerator pending enzyme assay.

➤ *Biochemical Assay*

The assay of bilirubin, urea, creatinine, total proteins and albumins assays were carried out according to the procedures described by Randox Laboratories Ltd, United Kingdom.

➤ *Total Bilirubin Test*

The total bilirubin of the samples was determined using standard ready-to-use kits (Randox Ltd, UK) following the manufacturer's instructions which briefly entails mixing 200 μl sera samples of the sacrificed rats with 200 μl of sulphanic acid and 50 μl of nitrite, 1000 μl of caffeine and 200 μl tartate and incubated for 30 minutes at 25°C . likewise a sample blank was prepared with the same reagents (minus nitrite) and the samples' absorbance were read at 578nm..

➤ *Direct Bilirubin Test*

The direct bilirubin was determined using standard ready-to-use kits (Randox Ltd, UK) following the manufacturer's instructions which briefly entails mixing 200 μl sera samples of the sacrificed rats with 200 μl of sulphanic acid and 50 μl of nitrite, 2000 μl of 0.9% NaCl

and incubated for 10 minutes at $20-25^\circ\text{C}$. Likewise a sample blank were prepared with the same reagents (minus nitrite) and the samples' absorbance were read at 546nm with a spectrophotometer.

➤ *Indirect Bilirubin Test*

The results obtained from the total bilirubin and direct bilirubin test was used to determine the indirect bilirubin level as so: Indirect bilirubin = Total bilirubin (mg/dl) – Direct bilirubin (mg/dl)

➤ *Total Protein Test*

The total protein was determined using standard ready-to-use kits (Randox Ltd, UK) following the manufacturer's instructions by mixing reagent blank, standard and sera samples with 20 μl of distilled water, standard and sera samples mixed in with 1ml of biuret reagent respectively in different tubes and incubated for 30 minutes at $20-25^\circ\text{C}$ and the absorbance of the sample (A_{sample}) and of the standard (A_{standard}) is measured against the reagent blank with a spectrophotometer at 546nm.

➤ *Blood Urea Test*

The serum urea level was determined using standard ready-to-use kits (Randox Ltd, UK) following the manufacturer's instructions by mixing reagent blank, standard and sera samples with 5 μl of distilled water, standard and sera samples mixed in with 1ml of sodium nitroprusside and urease solution respectively in different tubes and incubated at 37°C for 10 minutes and phenol and sodium hypochlorite was mixed in immediately and incubated for 15 minutes at 37°C .

➤ *Creatinine Test*

The serum creatinine level was determined by colorimetric method manufactured by Randox Ltd, UK standard ready to use kits by mixing reagent blank, standard and sera sample with 100 μl of distilled water, standard and sera sample mixed in with 1ml of sodium hydroxide and piuric acid respectively in different test tubes and read at two different time frame to get two absorbance values (the difference between the absorbance was gotten). The readings were done at 592nm.

➤ *Albumin Test*

The albumin was determined using standard ready-to-use kits (Randox Ltd, UK) following the manufacturer's instructions by mixing reagent blank, standard and sera samples with 3 μl of distilled water, standard and sera samples mixed in with 3ml of BCG concentrate respectively in different tubes and incubated for 20 minutes at 37°C and the absorbance of the sample (A_{sample}) and of the standard (A_{standard}) is measured against the reagent blank with a spectrophotometer at 630nm.

➤ *Statistical Analysis*

Statistical analysis was done using Graph pad prism 8; results were presented as mean \pm SE and statistical evaluation of data was performed using one way analysis of

variance (ANOVA) and multiple comparison was done using Tukey’s multiple comparison test.

III. RESULTS

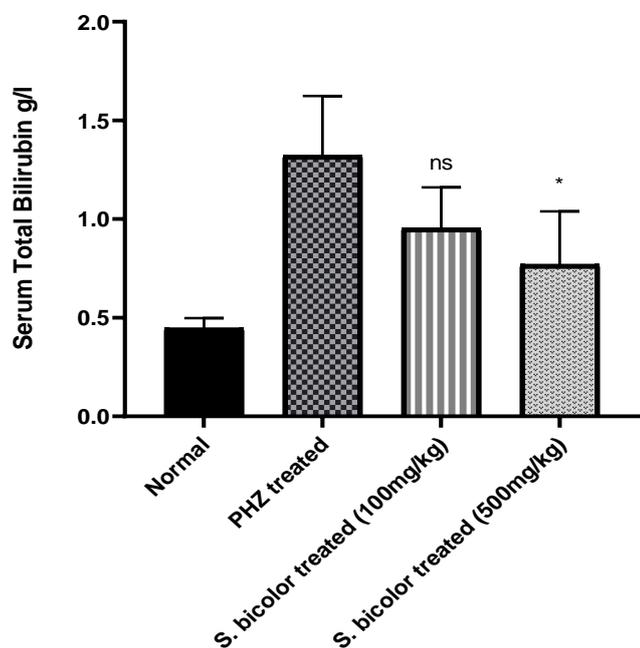


Figure 1.0: Serum Total Bilirubin in Phenylhydrazine Induced Anemic Rats (t test p value < 0.05)

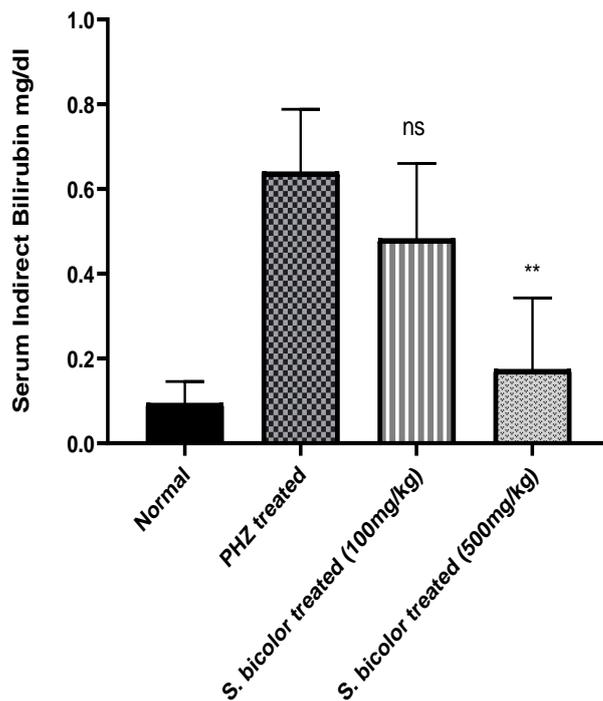


Figure 1.2: Serum Indirect Bilirubin in Phenylhydrazine Induced Anemic Rats (t test p value < 0.05)

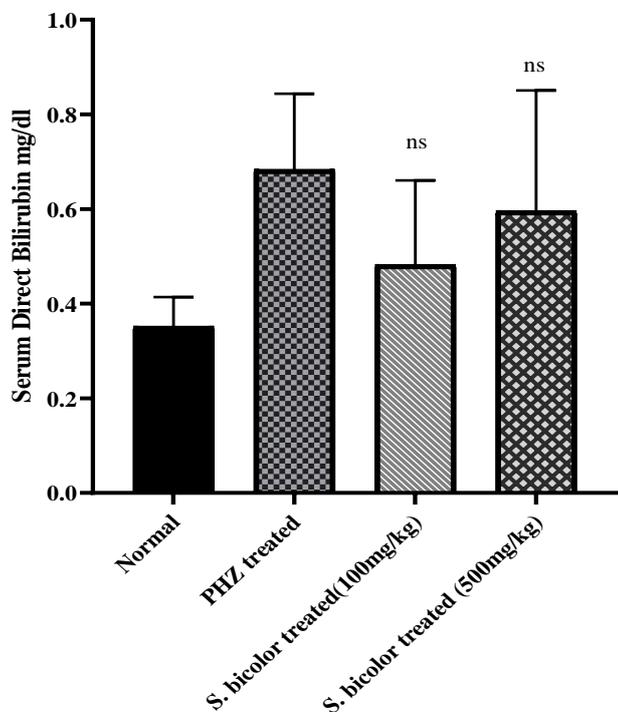


Figure 1.1: Serum Direct Bilirubin in Phenylhydrazine Induced Anemic Rats (t test p value < 0.05)

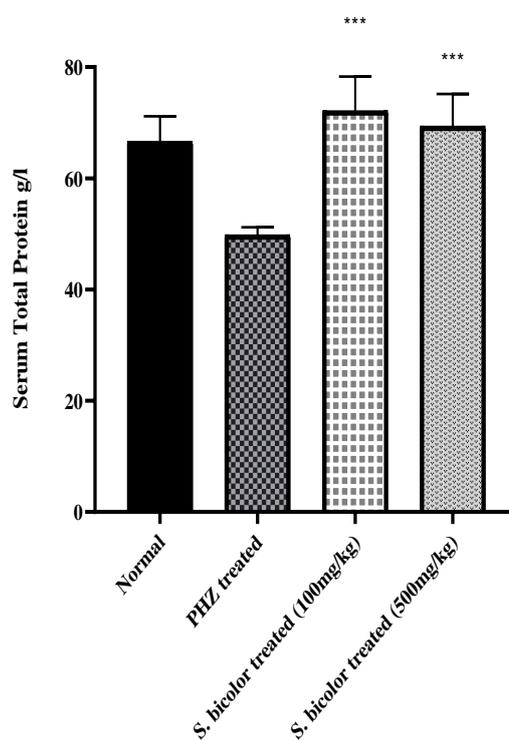


Figure 1.3: Serum Total Protein in Phenylhydrazine Induced Anemic Rats (t test p value < 0.05)

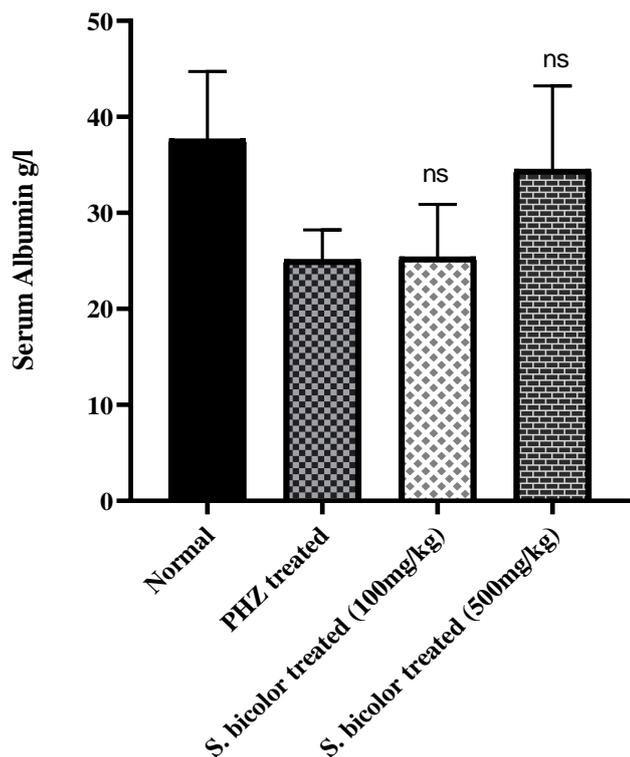


Figure 1.4: Serum Albumin in Phenylhydrazine Induced Anemic Rats (t test p value < 0.05)

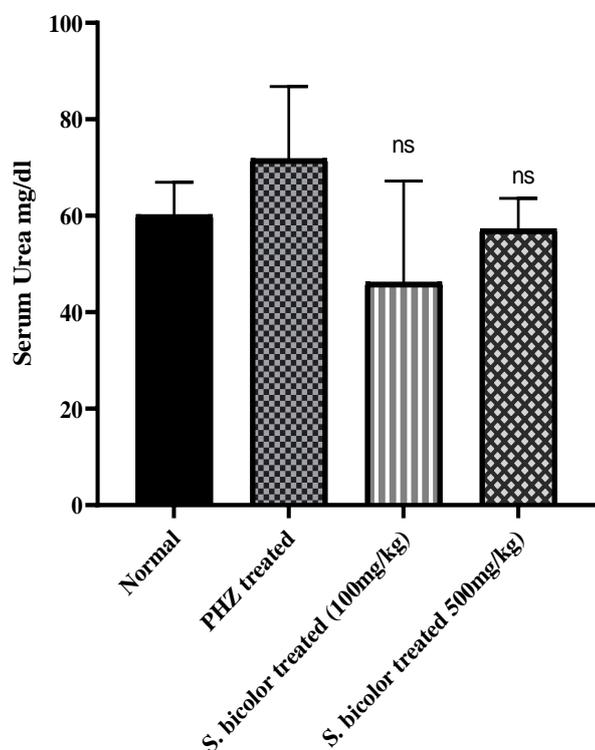


Figure 1.5: Serum Urea in Phenylhydrazine Induced Anemic Rats (t test p value < 0.05)

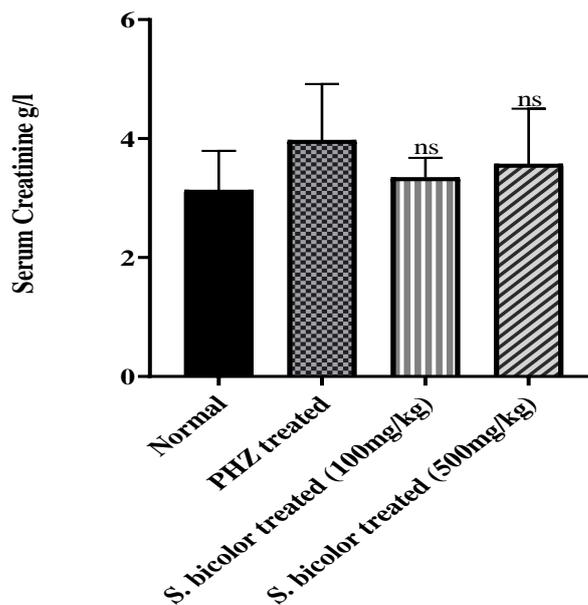


Figure 1.6: Serum Creatinine in Phenylhydrazine Induced Anemic Rats (t test p value < 0.05)

IV. DISCUSSION

Sorghum bicolor is one of the major grain crops for human and animal nutrition throughout the drier areas of Africa and India. Its leaf is also used in traditional medicine to treat anemia in these regions [20]. It has been previously demonstrated that the aqueous extract of the leaves stimulated erythropoiesis in a specific and dose dependent manner [20]. This research work focused on the testing of the feasibility of *Sorghum bicolor* as a potential anti-anemic plant to alleviate the deviant biochemical parameters caused as a result of anemia, parameters such as: bilirubin, total protein, albumin, urea and creatinine.

Bilirubin which is produced from the breakdown of hemoglobin senescent red cells to bilirubin and is carried to the liver (indirect/unconjugated bilirubin) where it undergoes conjugation to form bilirubin diglucuronide (direct/ conjugated bilirubin) and is excreted to the bile. Bilirubin level thus includes both the conjugated and unconjugated (free) forms and, if the serum is elevated, is usually indicative of liver damage or hemolysis [21]. On a general note, the administration of the extract caused a significant dose dependent reduction in bilirubin. This trend in *Sorghum bicolor* significantly reducing the total bilirubin level of the anemic rats perhaps corroborates [22] report of the rejuvenation of spleen that can purportedly lead to high clearance of the indirect bilirubin from the sera of the anemic rats and [23] report of the reduction in red cell lysis in *Sorghum bicolor* treated animals.

Low levels of the liver produced proteins: albumin and globulin (total protein) is a clear indication of compromised hepatic activity and as such, the significant elevation in the levels of total protein in both the low dosed and high dosed rats (Fig1.3) and a slight elevation in the levels of albumin (Figure 1.4) after administration of the extract shows that

the extract is non-toxic to the liver and would therefore not affect adversely the capacity of the liver and has also perhaps contributed to the reparation liver stress caused by the anemic condition induced in the rats [24]. The significant elevation in protein levels may be attributed to increase in the globulin fraction of the proteins produced by the livers. The renal function biomarkers (urea and creatinine) increased slightly but not significantly, knowing that elevated concentrations of urea and creatinine in the serum is an indication of renal failure suggests that the extract had no deleterious effect on the kidneys and probably has the potential to repair the kidneys' defective operation brought about by hemolytic disorder. Further investigation is however needed to confirm this.

V. CONCLUSION

This present study showed that the administration of sorghum leaf sheath extract remediated anemia; making its use as a potential botanical alternative to treating anemia feasible. 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.

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