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Effect of Ethanol Leaf Extract of Acacia nilotica on Phenyl hydrazine Induced Anemia in Rats

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Abstract: *The study was designed to investigate the effect of ethanolic leaf extract of A. nilotica in phenylhydrazine induced anemia in rats. The animals were divided into six groups of five animals each. Group I served as a normal control, group II as anemic control, group III as standard drug control, groups IV and V were treated with 100mg/kg and 200mg/kg of ethanolic leaf extract of A. nilotica respectively and group VI served as extract control group. Phenylhydrazine was administered intraperitoneally for two days to induce anemia in rats. All animals were allowed free access to food and water pre and post treatment. The administration of the extract lasted for 14 days period after which the animals were sacrificed and blood was obtained through cardiac puncture for haematological and liver biomarkers analysis. The effect of oral administration of ethanolic leaf extract of A. nilotica on some haematological parameters and liver biomarkers was assayed for in this study. The proximate and iron content of A. nilotica leaf were also analysed. Our findings revealed that proximate constituents of A.nilotica shows it can be used as food supplement. Results also showed that there was some significant ($p<0.05$) increase in some blood parameters (Red blood cell, Haemoglobin, White blood cell, Hematocrit, and Platelet count) in the extract treated group compared to the anemic group, suggesting that A.nilotica extract possess some anti aneamic properties. The liver biomarkers such as Aspartate amino transferase, Alanine amino transferase, Alkaline phosphatase and total protein assayed showed that there were no significant change in liver marker papameters revealing that the ethanolic leaf extract of A. nilotica is non toxic to the liver. Findings from this study suggest that ethanolic leaf extract A. nilotica is safe and can be used in management of anemia.*

Key words: *Acacia nilotica,anemia,proximate analysis,liver enzymes and blood*

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I. Introduction

Anemia is a blood disorder that is defined as either red blood cells (RBC) count below normal, red blood cells which are smaller in size than normal or a level of hemoglobin below normal. The various forms of anemia include iron deficiency anemia; hemolytic anemia; vitamin B_{12} deficiency anemia; folic acid deficiency anemia; anemia caused by inherited abnormalities of RBCs such as sickle cell anemia and thelassemia; and anemia caused by chronic ongoing disease,[1]. Anemia is a common blood disorder that affects people of all ages, although the people at greater risk are the elderly, young women of child-bearing age and the infants. This condition is not a disease but could develop as a result of various diseases. There are over 400 types of anemia, many which are rare but in all cases there is lower than normal number of circulating red blood cells, [2]. The great loss in terms of clinical diagnosis and treatment and even depletion in human resources as a result of anemia could be prevented with adequate knowledge. The incidence of anemia is higher in the third world than in the developed countries due to poor nutrition, high prevalence of blood parasites, example, plasmodium, trypanosomes and helminthes infestation. It also known that women are susceptible to anemia during pregnancy due to high demand from the developing foetus [3]. Although there are various drugs used for the treatment of anemia, they are not affordable to many poor people, especially those in the developing countries such as Nigeria. In addition, the rural populations in various parts of the world do not have adequate access to high quality drugs for the treatment of anemia, so they depend heavily on plants and herbal products for the treatment of diseases and anemia. It remains a major public health concern in many developing and under developed countries with all age groups at risk, because it causes varying degrees of lowered work capacity, impairment in cognitive performance, lowered immunity to infections, pregnancy complications, reduced psychomotor skills, and poor learning capacity [4]. A good number of medicinal

plants are traditionally employed to alleviate anemia. Some of these plants include *Telfeira occidentallis, Combretum dolichopetalum, Psorospermum ferbrifugum,Jjatropha curcas, Flacourtia flavenscens, Brillantasia* was reported to be effective in the treatment of sickle cell anemia [5].

Although modern medicine may be available in these countries, herbal medicines (phytomedicines) have often maintained popularity for historical and cultural reasons. Medicinal plants frequently used as raw materials for extraction of active ingredients which used in the synthesis of different drugs. Like in case of laxatives, blood thinners, antibiotics and anti-malarial medications, contain ingredients from plants, [6].

Acacia nilotica is multipurpose nitrogen fixing tree legume that is widespread in Africa, Asia, and Australia. It is a complex specie with nine sub-species, of which six are native to the Indian subcontinent. It occurs from sea dryness but is frost sensitive when young. It is considered as a very important economic plant since early times as a *source* of tannins, gum, timber, fuel, fodder and medicine [7]. *Acacia nilotica* is one of the plants used by traditional medicinal practitioner in Nigeria and many other African countries [8]. Although modern medicine are available in these countries in north eastern Nigeria herbal medicine has become part of the people(s) culture with greater percentage of the natives and local people depend mostly on the herbs to treat various diseases ailments, [9]. *Acacia nilotica* is an imperative multipurpose plant that has been used broadly for the treatment of various diseases [10]. It was reported that *Acacia nilotica* has anti-typhiod activities against the specie Salmonella typhi [11]. The whole plant has Anti platelet aggregatory activity [12]. Methanol extract of leaf of *Acacia nilotica* possess hypotensive and hypospasmodic activities[13]. The leaves are also reported to be a good hypocholesterolemic agent [14].

It's known that when Western medicine is often absent or hard to access or too expensive, the use of remedies from plant becomes particularly significant. The prevalence of anemia increases with age and is associated with chronic diseases, nutritional deficiencies and other conditions such as infection. Due to the cost and lack of adequate access to high quality drugs especially in rural areas, and the fact that anemia is very common and is likely to increase in future, that is why the need to study medicinal plants with anti-anemic properties will help limit the prevalence of anemia. There are claims that the leaf extract of *acacia nilotica* is use for the treatment of anemia but it appears there is no scientific research and publication to verify this claim. Therefore, this study is aimed at investigating the anti-anemic potentials of the leaf extracts of *Acacia nilotica*.The aim of the research is to investigate the effect of ethanolic leaf extract of *Acacia nilotica* on phenyl hydraxine induced anemia in rats.

II. Materials and methods

2.1Materials

2.2 Plant material

The sample was collected around Girei road, Yola, Adamawa State, and air dried at room temperature, the dried leaves was then pounded to powdered form using mortar and pestle and properly stored until required.

2,3 Equipment

Equipment used were Atomic absorption spectrophotometer (AAS), Auto haematology analyzer, Macro kjeldahl distillation unit, Muffle furnace.

Reagents : Chemicals and Reagents used were of analytical grade

2.4 Experimental animal

Adult rats of either sex were used for this research .They were procured from the animal breeding unit of the national veterinary Research institute (NVRI), Vom, Plateau State, Nigeria. They were kept in a clean cage and fed on chow diets and water for 2 weeks in order to be acclimatized to room temperature.

2.5 Methods

2.5.1 Preparation of Plant Material

The sample collected was air dried at room temperature. The dried leaves were pounded using a mortar and pestle, into powdered form and was stocked in a plastic container.

2.5.2 Preparation of Ethanol Extract

Ethanol extraction was carried out in a soxhlet apparatus. Briefly, two hundred grams portions of the dried powdered *acacia* leaves was defatted with 60/80 petroleum ether using soxhlet extractor. The petroleum ether mack

was allowed to dry, and then extracted with ethanol .The extract was concentrated in rotary evaporator. The concentrate was dried and weighed.

2.5.3 Determination of Proximate Compositions

The proximate compositions (carbohydrate, protein, lipid, ash, moisture, and fibre) of *Acacia nilotica* leaves was carried out as follows;

III. Determination of crude proteins

Procedure:

Three grams of the defatted sample was weighed separately on a filter paper and was placed in micro-Kjeldahl digestion flask. Two grams of catalyst mixture ($CuSO_4$: Na_2SO_4: SeO_2, 5:1:02 w/w) was added to the flask and then 10 mL nitrogen free concentrated H_2SO_4 was added to the flask. This flask was placed in an inclined position in a heating mantle in a fume cupboard for digestion to commence which was allowed to boil until a clear solution was obtained. Simmering was continued below boiling point for another 30 mins to ensure complete digestion and conversion of nitrogen to ammonium sulphate. After digestion was completed, the sample was allowed to cool and then Ten millitres of each digested sample was pippetted separately into the Markham steam distillation apparatus, followed by the addition of 20 mL 40% NaOH solution. Distillation was started and the liberated ammonia was trapped into 2% boric acid in a 100 mL conical flask containing 4 drops of mixed indicator (0.1% BCG and 0.1 methyl red in 95% alcohol) to a volume of 50 mL. The trapped ammonia in the boric acid was titrated against 0.01M HCl to an end point of light gray color.

IV. Calculations

$$\%\text{Nitrogen} = \frac{(a-b) \times 0.01 \times 14.005 \times c \times 100}{D \times E \times 1000}$$

Where,

a= Average titre value for sample

b= Titre value for blank

c= Volume to which the digest was made up to

D= Aliquot taken for distillation

E= Weight of dried samples taken for digestion

% Crude protein = %Nitrogen × 6.25 [15]

Determination of crude fat

The method of AOAC [16] was employed; this method was based on the principle that non-polar components of samples are easily extracted into organic solvents.

Procedure:

Three grams of the sample (moist free) was placed into a fat free thimbles. This was then weighed plugged with glass wool and then was introduced into soxlet extractors containing 160 mL petroleum ether. A clean dry receiver flask was weighed and then fitted to the extractors. The extraction unit was then assembled and cold water was allowed to circultate, while the temperature of the water bath was maintained at 60^{o}C. Extraction was carried out for 8 hrs. At the end of this time, the thimble containing the sample was removed and placed in an oven at 70^{o}C for 3 hrs and dried to constant weight. The weight of the dish and the content was obtained using a standard analytical balance.

V. Calculation

$$\text{Fat}(\%) = \frac{x-y}{z} \times 100$$

Where,

x= Weight and thimble and oil

y= Weight and empty thimble

Z= Weight of sample

Determination of moisture content

The moisture content was determined using the standard method of AOAC [16].

Procedure:

Clean crucible with lid was weighed using an analytical balance. The sample grinded to fine powder was mixed to obtain a homogenous sample of a large surface. Two grams of the sample was placed in each of the crucible and was dried in an oven at 100 ^{0}C for 17 hrs. The sample was removed from the oven and was weighed. The process was continued until a constant weighed was obtained.

VI. Calculation

Moisture (100%) = $\frac{t-u}{s} \times 100$

Where,

s = Weight of sample for analysis
t = Weight of sample + crucible before drying
u = Weight of sample + crucible after drying
t-u = Loss of weight or moisture content

Determination of Ash content

The ash content was determined using the method by AOAC [17].

Procedure:

A porcelain crucible with lid was weighed after been ignited in a muffle furnance (M-525) for 5 min at 550^{0}C and cooled in a dessicator. Two grams of the sample was placed in the crucible with appropriate labelling and weighed again. The crucible with the contents in it was ignited again in the furnance (0M-525) at 550^{0}C for 18 h to light grey ash. Thereafter, the crucibles was removed, cooled in the dessicator immediately and then was weighed.

VII. Calculation

The difference in weight or loss in weight of the crucible and sample before ashing gave the organic matter content of the diet, while the difference between the weight of the crucibles alone and the crucible and ash gave the weight of the ash of each sample.

Ash (100%) = $\frac{100\ (x - y)}{z}$

Where,

x = Weight of crucible and content after drying
y = Weight of empty crucible
x-y = Weight of ash
z = Weight of sample

Determination of crude fibre

Determination of crude fibre was carried out according to the procedure of AOAC [18].

Procedure:

With the use of a weighing balance, 4 g of the moisture-free sample was weighted and transferred into a beaker, 50 mL of H_2SO_4 was added into the beaker followed by distilled water to a volume of 200 Ml. This was heated to boiling and kept boiling for 30 min while maintaining constant volume by addition of hot distilled water. Thereafter, the content was poured into a prepared butchner funnel and was connected to a vacuum pump. The insoluble matter in the beaker was washed severally with hot distilled water until the filtrate is acid-free as was indicated by litmus paper. The filter paper containing the acid-free residue was transferred into a beaker and was boiled for 30 min while maintaining constant volume by addition of hot distilled water. The mixture was then filtered and washed as earlier described until it is alkaline free. Finally, the resultant residue was washed with two portions of 2 mL 95% alcohol. The residues on the filter paper was transferred to a proclain crucible which was dried in an oven maintained at 100^{0}C to a constant weight after cooling in a desiccator. The crucible was then placed in a muffle furnance at 550^{0}C for 8 h, it was cooled and weighed.

Calculation: fibre (%) = $\frac{x-y}{z} \times 100$
Where,
x = Weight and thimble and oil
y = Weight of empty thimble
z = Weight of sample

Estimation of total carbohydrate

The total carbohydrate content of the diet samples was obtained by subtracting the sum percentage crude Protein, crude Fat, Moisture, Fibre and Ash from 100.

Determination of iron

Atomic absorption spectrophotometer (AAS) was used to analyze the iron content by method described by AOAC, [18].

Procedure:

Five ml of the sample was pipetted into 50 ml of volumetric flask, 5 ml of sodium acetate was then measured into the digested sample in a volumetric flask followed by addition of 0.5 ml hydroxylamine hydrochloride and 5 ml of O-phenanthroline. The mixture was then made up to 50 ml with distilled water and was allowed to develop for 30 mins. The absorbance reading was then taken using AAS.

2.5.4 Induction of anemia in Rats

Anemia was induced in the rat model by administration of phenylhydrazine (PHZ) dissolved in olive oil in the ratio 1ml: 14ml, through the peritoneal route at 40mg/kg according to Roquel *et al.*, [19].

2.5.5 Experimental Design

The thirty (30) Albino rats were randomly grouped into six (6) groups. The Rats in each of the group was allowed free access to normal rat chow and water. Groups I, II, VI represent the normal control group, Negative (Anemic) control group and positive (Standard drug) control group respectively whereas groups III, IV, and V will represent the experimental anemic groups.

Table 1: Experimental Design

Group	Description	Treatment
Group I	Normal control	Normal diet + water
Group II	Negative (Anemic) control	Anemia + Normal diet + water
Group III	Positive (Std drug) Control	Anemia + Normal diet + water+ Astymin
Group IV	Experimental anemic group	Anemic + Normal diet + water + 100mg/kgbwt of *Acacia nilotica* ethanolic leaf extract
Group V	Experimental anemic group	Anemic + Normal diet + water + 200mg/kgbwt of *Acacia nilotica* ethanolic leaf extract
Group VI	Extract control group	Normal diet + water + *Acacia nilotica* leaf extract

2.5.6 Collections of Blood samples

After 48 hours of induction of anemia with phenylhydrazine solution in the rats, *Acacia nilotica* crude extract was administered as treatment for two weeks after which the rats were sacrificed. All administration was done orally using Oropharyngeal cannula once per day for 14 days. Blood samples were collected at the beginning and at the end of two weeks of the experimental period. The Albino rats were subjected under chloroform vapor anesthesia and sacrificed to collect blood sample by cardiac puncture in the entire group. About 3 ml of blood was collected into an EDTA sample bottle for haematological assay and liver enzyme and protein determination.

2.5.7 Determination of Haematological parameters

Haematological parameters were determined using an Auto Haematology Analyzer, which include: packed cell volume PCV, Haemoglobin content HGB, Red blood count cell RBC, White blood cell count WBC, Mean corpuscular volume MCV, Mean corpuscular haemoglobin concentration MCHC , Platelet count PLT, Hematocrit HCT.

Aspartate Amino Transferase (AST), Alkaline Phosphatase (ALP), Alanine Amino Transferase (ALT) and total protein determination

2.5.8 Determination of Serum Aspartate Amino Transferase (AST)

Aspartate amino transferase (AST) was estimated using colorimetric method [20]

Procedure:

GOT Buffer substrate solution was incubated in water bath at 37^0C for 5 mins, serum, pyruvate standard and distilled water was mixed and was incubated at 37^0C for 30 mins, after which it was removed and 2,4-dinitrophenylhydrazine was added, mixed and then will be allowed to stand at room temperature for 20 mins. Then 0.4 N NaOH was added and read at 510 nm.

VIII. Calculations

$\frac{\mathrm{T-TB}}{\mathrm{S-SB}} \times 100$ of std m/L

$\frac{\mathrm{ODT-ODTB}}{\mathrm{ODS-ODSB}} \times 43$ m/L

Where,
T = Test ,TB = Test blank ,S = Standard , SB = Standard blank ,43 = Concentration of standard
OD = Optical density

2.5.9 Determination of Serum Alanine Amino Transferase (ALT)

Alanine amino transferase (ALT) was estimated using colorimetric method [20].

Procedure:

GPT Buffer substrate solution was incubated in water bath at 37^0C for 5 mins, serum, pyruvate standard and distilled water was mixed and incubated at 37^0C for 30 mins, after which it was removed and 2,4-dinitrophenylhydrazine was added, mixed and then allowed to stand at room temperature for 20 mins. Then 0.4 N NaOH was added and read at 510 nm.

IX. Calculations

$\frac{\mathrm{T-TB}}{\mathrm{S-SB}} \times 100$ of std m/L

$\frac{\mathrm{ODT-ODTB}}{\mathrm{ODS-ODSB}} \times 43$ m/L

Where,
T = Test ,TB = Test blank, S = Standard, SB = Standard blank, 43 = Concentration of standard
OD = Optical density

2.5.10 Determination of Serum Alkaline Phosphatase (ALP)

Alkaline phosphatase (ALP) was determined using colorimetric method [20].

Procedure:

One cm^3 of reagent was placed in a cuvette, 0.50 cm^3 of serum was then be added. The solution was mixed and the absorbance ($A_{initial)}$ read and recorded immediately and timing simultaneously. The first, second and third absorbance was read against air blank at 405 nm.

X. Calculation

$\mu/L = 2760 \times \Delta A$ 405nm/min
Where ΔA : Difference in absorbance

2.5.11Determination of total protein
It will be determined using method by Tietz, [21].

Procedure:

The sample, 0.02 ml of the standard serum and distilled water was pipetted into different test tubes, and then 1.0 ml biuret reagent was added to each of test tubes, then it was mixed and then incubated at 25^0C for 30 min. The spectrophotometer was set to zero level using distilled water at 530 nm absorbance measured.

The Concentration of total protein = $\frac{\text{absorbance of sample}}{\text{absorbance of standard}} \times$ concentration of standard protein

XI. Results

Table 2: Proximate composition and Iron contents of leaf of *Acacia nilotica*

Macronutrients	Composition (%)
Moisture content	1.49±0.17
Crude fibre	10.73±0.05
Crude fat	3.68±0.17
Ash content	2.11±0.06
Crude protein	1.26±0.06
Carbohydrate	80.73±0.12
Iron	0.06±0.01mg/100g

Results are expressed as mean±SEM of three determinations

Table 3: *Effect of ethanolic leaf extract of A. nilotica on haematological parameters in phenylhydrazine induced anemia in rats*

Group	RBC (10^{12}/l)	WBC (10^9/l)	HGB (g/dl)	HCT (%)	PLT(10^9/l)	MCV(fl)	MCH(pg)	MCHC(g/dl)
Group I (normal Control)	8.15±0.30	13.87±0.25	14.73±0.52	58.39±0.30	533.25±14.20	61.00±1.08	19.34±0.52	25.35±0.49
GroupII (anemic Control)	3.22±0.48*	8.10±0.30*	5.53±0.61*	30.97±3.51*	376.00±9.26*	80.25±0.48*	25.13±1.24*	38.68±1.20*
Group III (standard drug)	7.30±0.38	12.49±0.78	12.90±0.25	51.16±0.27	468.75±6.22	67.75±1.10	19.36±0.45	26.23±0.30
Group IV (100mg/kg-bwt	6.14±0.30**	8.75±0.58**	8.28±0.27**	43.67±0.59**	381.00±12.91**	70.00±0.82	20.15±0.36	28.70±0.60
Group V (200mg/kg-bwt)	6.85±0.23**	11.23±0.70**	14.36±0.55**	52.37±0.38**	501.50±13.26**	71.25±1.31	19.98±0.73	27.43±0.34
GroupVI (extract Control) (200mg/kg -bwt)	6.98±0.30	13.49±0.37	14.85±0.54	53.60±0.40	539.00±17.83	70.50±2.10	19.48±0.54	26.45±0.46

Results are expressed as mean±SEM for 5 determinations. * = significant increase or decrease as compared to normal control group at p<0.05.
**= significant increase or decrease as compared to anemic group at p<0.05.

Table 4: Effect of ethanolic leaf extract of *A.nilotica* on liver biomarkers

Group	AST(IU)	ALT(IU)	ALP(IU)	Total protein(g/dl)
Group I (normal Control)	137.33±2.33	36.33±0.88	52.00±1.52	51.33±1.33
Group II(anemic Control)	170.33±1.45*	51.33±0.67*	52.33±1.33*	42.00±1.00*
Group III(standard drug)	145.67±1.76	24.33±0.67	53.67±1.67	43.67±1.76
Group IV(100mg/kg-bwt	150.00±1.15*	24.00±1.15*	59.33±0.33*	42.00±1.00*
Group V(200mg/kg-bwt)	144.67±0.67*	29.33±0.67*	53.67±1.67*	44.00±1.50*
Group VI (extract 200mg/kg -bwt)	153.67±0.88*	35.00±2.00*	53.33±2.03*	48.00±4.51*

Results are expressed as mean±SEM for 5 determinations. * = significant increase or decrease as compared to normal control group at p<0.05.

XII. Discussions

The data on the proximate constituent of the plant sample in table 2 shows clearly that *A. nilotica* can be used as food supplement. The leaf of *A. nilotica* has a low moisture content (1.49 %). The lower moisture content will give it a storage advantage. The ash content is a measure of mineral content. The ash content (2.11 %) of leaf of *A. nilotica* indicated that the leaf contained low amount of mineral elements. The fat content of the leaf of the plant was low (3.68 %). This implies that the leaf of *A. nilotica* can function effectively in low fat diet such as those required by patients with cardiovascular diseases and obesity as reported by Gropper *et al*[22]. The leaf of *A. nilotica* can be classified as carbohydrate rich food because of its high carbohydrate content (80.73 %). This shows it could be a reliable source of energy. The data on the proximate analysis also showed that crude fibre (10.73 %) was higher than that reported for *T.occidentalis* (4.60 %) by Abolaji *et al.* [23], and Ogbe *et al.*, [24] have reported that *T. occidentalis* posses anti-anemic potentials. Nutritionally, this is of beneficial effect since it had been reported that food fibre aids absorption of cholesterol. This also makes the plant suitable for combating anemia in pregnant women [25]. However, the protein content of leaf of *A. nilotica* was low (1.26%) compared to other values reported for most shrubs grown in West Africa [25].

The value of iron content in *A. nilotica* leaf was (0.06mg/100g). Iron is important for the synthesis of red blood cells essential for formation of haemoglobin, the oxygen carrying pigment in red blood cells. Iron is used in the treatment of anemia, tuberculosis and disorder of growth. Iron is an energizer but excess can cause fatique but we hardly have excess if taken from natural source.The availability of iron in a diet may modulate the regenerative response [25].

Our findings also revealed that rats in group 1 which served as the normal control and which were not induced with anemia or treated had haematological parameters within normal range throughout the duration of the experiment. The haematological parameters, RBC, HGB, HCT, in the anemic group (group II) showed a significant decrease when compared to the normal control group (group I). This could be as a result of the toxicity induced by phenylhydrazine, by perioxidation of RBC membrane lipids and this effect may be a result of the auto-oxidation of the drug and the interaction of oxygen radicals with membrane lipids [26]. However, in the extract treated group, there was a significant ($p<0.05$) increase in the haematological parameters when compared to the anemic group. This could be due to the presence of phytochemicals [11] in the plant extract which are well known hemopoietic factors that have direct influence on the production of blood in the bone marrow and also presence of appreciable amount of Iron content. Our finding also reavealed that there was an increase in the MCV, MCH and MCHC in the anemic group when compared to the extract treated group and normal control group, which are indicators of macrocytosis, thus describing the anemia as macrocytic. Phenylhydrazine is known to decrease HGB, RBC and PCV levels, whereas it induces increase in the MCV, MCH and MCHC levels. This also supports the work of Nku-Ekpang *et al.* [27] in which phenylhydrazine decreased HGB, RBC and PCV levels but there were increased in the levels of MCV, MCH and MCHC levels. Our investigation on WBC shows that there was a decrease in the WBC levels of the anemic groups when compared to the normal control group. This may imply a reduction in the ability of the rats to resist infection. There was an increase in the levels of WBC in the extract treated group which suggest that the extract possess some potentials that are capable of boosting the immune system in rats. The results also showed a decrease in the platelet count of the anemic group when compared to the normal control group. However, there was a significant increasein platelet count in extract treated group when compared to the anemic group, indicating that the extract has a stimulating effect on platelet production.

In the results of the liver biomarkers (AST,ALT, ALP and total protein) of the extract treated group (group IV and V) and extract control group (group VI), there was no significant difference when compared to that of the normal group which was an indication that the ethanolic leaf extract of *A. nilotica* was non toxic to the liver. However, there was significant increase in the liver biomarkers of the anemic group when compared to the normal control group. This may be due to the toxicity induced by phenylhydrazine.

XIII. Conclusion

From the collective data of this study, it can be concluded that the ethanolic leaf extract of *A.nilotica* has a considerable anti-anemic activity as shown in phenylhydrazine induced anemia in rats, indicating the potential use of this plant in the management of anemia. The anti-anemic effect produced by *A. nilotica* leaf extract may be due to the presence of Iron content and also perhaps the presence of phytochemicals.

Recommendation

Further studies on this plant is required to know the exact mechanism by which it exerts its anti-anemic effects.

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