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Evaluation of the effect of methanol leaf extract of *Amaranthus spinosus* on hematological and serum electrolytes level in phenylhydrazine induced anemia in wistar albino rats.

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ABSTRACT

The study was carried out to evaluate the effect of methanol leaf extract of *Amaranthus spinosus* on hematological and serum electrolytes level in phenylhydrazine induced anemia in wistar albino rats. The twenty five (25) albino rats weighing 155-160g were used for the study. They were allowed to acclimatize for one week with free access to feed and water. After acclimatization, they were randomly distributed into five groups (A, B, C, D and E) of 5 rats each. The administration of the leaf extract was oral with the aid of an intubation tube. The groups received different doses of methanol extract of the plant, *Amaranthus spinosus* as follows: Group A: was normal control group administered normal saline and feed. Group B: was (positive control) group received 10mg/kg body weight of phenylhydrazine (untreated). Group C: was (standard control) group administered with phenylhydrazine and treated with Hemoglobin 12 (standard drug). Group D: was induced anaemia and were treated with 200mg/kg body weight of methanol extract of *Amaranthus spinosus* leaves, and Group E: was induced anaemia and were treated with 400mg/kg body weight of methanol extract of *Amaranthus spinosus* leaves. At the end of 28 days the blood samples were collected from rats through the ocular vein for biochemical and haematological assays using standard biochemical methods. The results also revealed no significant difference (P>0.05) in Potassium (K^+) and Sodium (Na^+) concentration of rats treated with methanol leaf of *Amaranthus spinosus* when compared to normal control group. However, there was significant increase (P>0.05) in chloride (Cl^-) with the rats treated with the plant extract when compared to the normal control group. The results of haematological studies showed no significant difference (P>0.05) in Packed cell volume (PCV),and Haemoglobin (HB) levels in treated rats when compared to normal control groups. The result of the study therefore, suggests that the leaf extracts are not toxic and could be helpful in treatment of anemia

Keywords: *Amaranthus spinosus,* hematological, serum electrolytes and anemia

INTRODUCTION

Amaranthus spinosus originates probably from lowland tropical South and Central America and was introduced into other warmer parts of the world from about 1700 AD onwards [1,2,3]. At present it occurs in all tropical and subtropical regions, including tropical Africa, often gregariously and as a weed [4,5,6]. It is sometimes collected for home consumption as a cooked, steamed or fried vegetable, especially during periods of drought [7,8]. The leaves are occasionally found for sale on the market [9,10]. In Uganda and Kenya, it demands a lower price, for example, because of its spines and because it is not much liked [11,12]. Its use is declining, and it is acquiring the status of a famine food. It has a bitter taste and is usually eaten in small quantities as a substitute when no other vegetables are available. *Amaranthus spinosus* is also used as forage and said to increase the yield of milk in cattle [13,14]. *Amaranthus spinosus* contains a high amount of alkaloids, which show that they can take part in nitrogen fixation in the body system, and also could be effective in the treatment of malaria [15,16]. It also contains a high level of flavonoids and

carbohydrate that help in energy supply of the body. Previous study has shown that A. spinosus causes a decrease in PCV and an increase in RBC level in the blood and that it has the ability to reverse anaemia and other blood related diseases in the rat, *Amaranthus spinosus* is used for jaundice [17,18]. Anaemia is the blood disorder characterized by a decrease in the number of red blood cells [19,20]. It also arise as a result of decrease in haemoglobin concentration and packed cell volume [21,22]. Haemoglobin is a critical part of RBC that ensures that the blood cells are red before binding with oxygen. There is a defect in oxygen supply to the body in an individual affected with a case of anaemia. These decrease in Red Blood Cells leads to insufficient oxygen supply to tissues, which leads to some physiologic consequences such as tissue hypoxia, fatigue, weakness, dizziness, headache, numbness of hand and feet, low body temperature, pale skin, rapid or irregular heartbeat, shortness of breath, chest pain and irritability [23,24]. Indigenous systems of medicine like Siddha, Ayurveda and Unani are mainly use medicinal plants for treatment of various ailments of the human beings and animals. With the development of these systems, the herbal plants are being sought after, both by Clinicians and patients in search for cure of diseases. Herbal medicine is a form of complementary and alternative medicine and is becoming increasingly popular in both developing and developed countries [25,26]. WHO has described traditional medicine as one of the surest means to achieve total health care coverage of the world's population .In pursuance of its goal of providing accessible and culturally acceptable health care for the global population, WHO has encouraged the rational use of traditional plant based medicines by member states and has developed technical guidelines for the assessment of herbal medicines [27,28]. The Herbal drugs have been used throughout the world and have raised greater attention in recent times, because of their diverse nature of curing diseases, safety and well tolerated remedies compare to the conventional medicines. Moreover the herbs with natural combinations of constituents as a whole are naturally occurring remedies which have proved to be more effective and safer than conventional medicines [29,30].

Aim of Study

This study is aimed at evaluating the effect of methanol leaf extract of *Amaranthus spinosus* on hematological and serum electrolytes level in phenylhydrazine induced anemia in wistar albino rats.

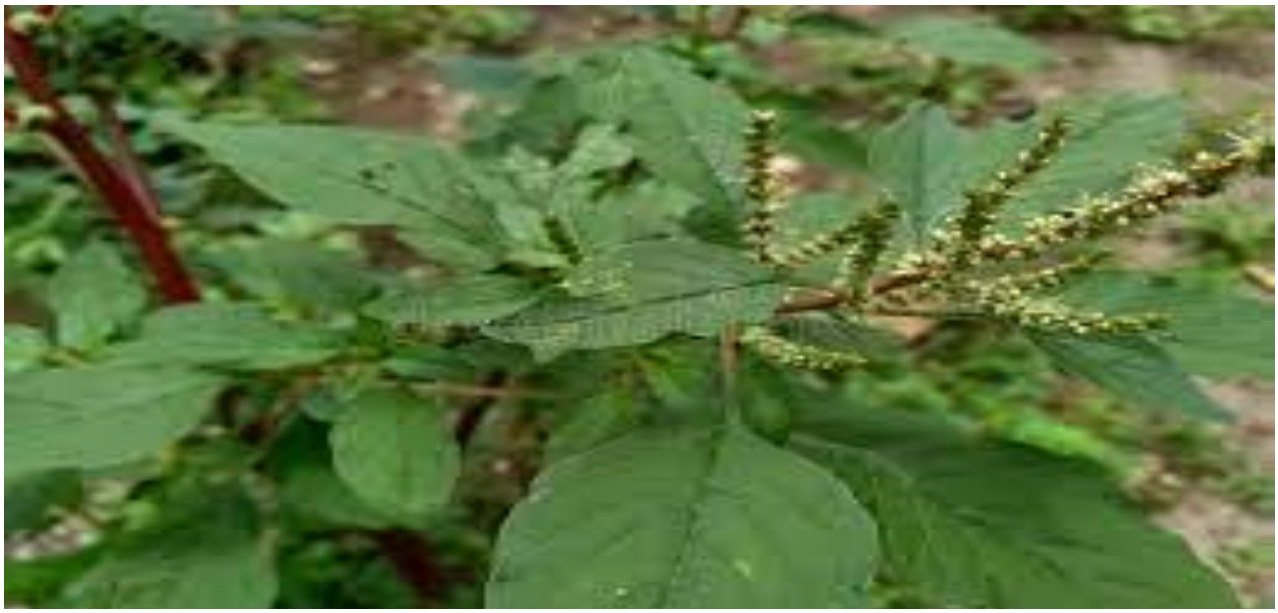

Figure 1: *Amaranthus spinosus* (Oba village in Idemilli South L.G.A, Anambra State)

MATERIALS AND METHODS

Methodology

Collection of Plant Samples

Amaranthus spinosus(Bush green leave) used in this research work were freshly obtained from the garden in Oba village in Idemilli South L.G.A, Anambra State and were botanically identified and authenticated by a botanist as *Amaranthus spinosus* before usage at the laboratory.

Extraction of Plant Materials

The collected plant samples were rinsed in clean water and spread under ambient temperature for 24 hours. The fresh plant samples were ground into powder using mortar and pestles, the powder obtained were then used to prepare the extract.

Preparation and Induction of Experimental Anaemia in Rats

Experimental Animals

The twenty-five (25) wistar albino rats weighing 155 – 160g were used for this study. The rats were obtained from the animal house of Faculty of Veterinary Medicine, University of Nigeria, Nsukka, and were transported to the Biochemistry Department of Chukwuemeka Odimegwu Ojukwu University, Uli, Anambra State of Nigeria. They were acclimatized for 7 days in steel cages. The albino rats were fed with standard commercial feed and water throughout the experimental period.

Experimental Design

The twenty five (25) albino rats weighing 155-160g were used for the study. They were allowed to acclimatize for one week with free access to feed and water. After acclimatization, they were randomly distributed into fivegroups (A,B,C,D and E) of 5 rats each. Divided into five (5) groups of five (5) rats each. The administration of the leaf extract was oral with the aid of an intubation tube. The groups received different doses of methanol extract of the plant, *Amaranthus spinosus* as follows:

- **Group A:** was normal control group administered normal saline and feed.
- **Group B:** was (positive control) group received 10mg/kg body weight of phenylhydrazine (untreated) and
- **Group C:**was (standard control) group administered with phenylhydrazine and treated with Hemoglobin 12 (standard drug).

- **Group D:** was induced anaemia and were treated with 200mg/kg body weight of methanol extract of *Amaranthus spinosus* leaves.
- **Group E:** was induced anaemia and were treated with 400mg/kg body weight of methanol extract of *Amaranthus spinosus* leaves.

Treatment was by daily oral administration for 28 consecutive days. All the groups had free access to feed and drinking water *ad-libium*, while the rat feed used in the study was Growers Mash of Vital Feed Limited, Jos, Plateau state, Nigeria.

Induction of Experimental (Phenylhydrazine) Anaemia in Rats

The wistar albino rat was induced 10mg/kg body weight of phenylhydrazine by intra-peritoneal injection each for three (3) days to develop hemolytic anaemia respectively. The body weights of rats were taken at weekly intervals with electronic weighing balance. All the protocols as approved by Institutional Animal Ethics Committee (IAEC) were observed in this study.

Collection of Blood Sample from animals for Biochemical Analysis

Blood samples were collected from the animals through medial canthus of the eye of the rat. The serum samples were put into a specimen plain bottles without anticoagulant. Serum sample were separated from the clot by centrifugation at 3000rpm for 5minutes using bench top centrifuge (MSE Minor, England). Serum samples were separated into plain tubes and stored in the refrigerator until analyses. All the analyses were completed within 24hours of sample collection.

Determination of serum electrolytes

Estimation of Sodium

Principle

This was done according to the method of [37].The method is based on the modification of those first describe by Maruna and Trinder in which sodium was precipitated as the triple salt, sodium magnesium uranyl acetate, with the excess uranium then being reacted with ferrocyanide, producing a chromophore whose absorbance varied inversely as the concentration of sodium in the test specimen.

Procedure

Into three test tubes labeled test, serum, standard and blank were added 50 µl of serum, 50 µl of standard and 50 µl distilled water respectively. Then, 1.0 ml filtrate reagent was added into the test tubes. All test tubes were vigorously shaken and mixed continuously for 3 minutes. The tubes were centrifuged at (1500rpm) for 10 minutes. The supernatant was analyzed, taking care not to disturb the protein precipitate. A labelled test tubes corresponding to the above filtrate was added 50 µl of supernatant to respective tubes. Then, 1.0 ml acid reagent was added into the tubes and 50 µl of color reagent was added and also mixed. Zero spectrophotometer with distilled water at 550 nm and read the absorbance of all test tubes.

$$Sodium\ conc. = \frac{Abs\ of\ blank\ - Abs\ of\ sample\ \ x\ standard\ \ concentration}{Abs\ of\ blank\ - Abs\ of\ Std}$$

Estimation of the potassium

Principle

This was done according to method of [37]. The amount of potassium is determined by using sodium tetraphenylboron in a specifically prepared mixture to produce a colloidal suspension. The turbidity of which is proportional to potassium concentration in the range of 2-7 mEq/L.

Procedure

Into three test tubes labeled test, serum, standard and blank were added 10 µl of serum, 10 µl of standard and 10 µl distilled water respectively. Then, 1.0 ml potassium reagent was added into the test tubes. It was mixed and left to stand at room temperature for 3 minutes. After 3 minutes, the wavelength of spectrophotometer were read at 500 nm, zero spectrophotometer with reagent

blank. Read and record the absorbance of all tubes.

$$Potassium\ conc = \frac{Absorbance\ of\ sample\ x\ standard\ conc}{Absorbance\ of\ standard}$$

Estimation of the Chloride

This was done according to the method of [37].

Principle

$Hg(SCN)_2 + 2Cl^- \longrightarrow HgCl_2 + 2SCN^-$

$3SCN^- + Fe^{3+} \longrightarrow 4Fe(SCN)_3$ red complex

Chloride ions form a soluble, non-ionized compound, with mercuric ion and was displaced thiocyanate. The released thiocyanate ions reacted with ferric ions to form a color complex that absorbs light at 480 nm, the intensity of the color produced is directly proportional to the chloride concentration.

Procedure

Into three test tubes labeled test, serum, standard and blank were added 10 µl of serum, 10µl of standard and 10 µl distilled water respectively. Then, 1.5 ml chloride reagent was added into each test tube and mixed gently and incubated at room temperature for 5 minutes at 37^0C. The absorbance was read and recorded at 520 nm.

$$Chloride\ conc = \frac{Absorbance\ of\ unknown\ x\ standard\ calibrateor}{Absorbance\ of\ calibrateor}$$

Determination of Hematological Analysis

Determination of Packed Cell Volume (PCV)

Packed cell volume (PCV) was estimated as described by [16]. Blood sample was taken with a heparnized capillary tube and sealed over a gas flame. The filled tubes were placed in the microhematocrit centrifuge and spun at 10,000 for 10 minutes. The tubes were placed into a specially designed reader and the PCV was read as a percentage.

$$PCV = \frac{Packed\ RB\ Ccolumn\ \ height\ x\ 100}{Total\ blood\ volume\ height}$$

Determination of the Haemoglobin (HB) concentration

Principle

Heamoglobin (HB) concentration was determined using cyanomethaglobin technique as outlined by [16]. Drabkin's solution which contains potassium ferricyanide, potassium cyanide and potassium dihydrogen phosphate was mixed with the haemoglobin. The ferricyanide forms methaemoglobin which is converted to cyanomethaemogloin by the cyanide. The cyanomethaemoglobin produces a colour which is measured colourimetrically.

Procedure

Whole blood (20 µl) was added to 4 ml of Drabkin's solution in a test tube in a 1:250 dilution. This was well mixed, allowed to stand for 10 minutes at room temperature and the absorbance was read with colourimetrically at 540 nm with Drabkin's solution as a blank.

$$Heamoglobin\ (HB) = \frac{OD/Ab}{OD/Ab} \times \frac{conc.\ standard}{4}$$

Determination of White Blood Cell (WBC) Count

Principle

The white blood cell total was determined following the method described by [16]. The blood sample was diluted with Turk solution in a ratio of 1:20 dilutions in a test tube. The Turk solution lyses all the other cells in the blood except the WBC. Turk solution is made up of acetic acid. The WBCs were counted gentian violet in the ratio of 2:1. The gentian violet stains the nuclear of the leucocyte under a low

power microscope of 10 × magnification using a NUBA counting chamber. The number of cells in the diluted blood sample was reported as the number of cells in 1ml of whole blood sample. Any number gotten was then multiplied by 100. Normal range of WBC in 100ml of whole blood sample is 3500-7000.

Procedure:

Whole blood (20ml) was added to 380ml of diluted fluid(Turk solution) and mixed properly. The counting chamber was charged with well mixed diluted blood with the aid of pipette. Cells were allowed to settle in a moist chamber for 3 minutes. All the four corners of the 16 chambers were visualized under a low power (10x) objective microscope, and the cells were counted in all the four marked corner squares. Any figure gotten was then multiplied by 100.

Total WBC can also be calculated as;

$$Total\ WBC\ (mm^3) = \frac{N\ x\ 20}{0.1\ X\ xA}$$

N = Number of cells counted
0.1 = depth of chamber
A = Area counted
20 dilution factor

Statistical Analysis

The statistical analysis of the result was analyzed with statistical package for social science (SPSS) to obtain the mean and the standard deviation of the triplicate of result data for the descriptive analysis. Duncan table was used to obtain the comparative result of the various groups of the study. $P<0.05$ (95 %) confidence interval will be considered siginficant for the statically analysis.

RESULTS AND DISCUSSION

Hematological parameters of Different Groups of Phenylhydrazine Induced Anemia in Rats Administered *Amaranthus spinosus* Methanol Leaf Extract

Results in Table 1 revealed that there was no significant difference ($P>0.05$) observed in haemoglobin level concentration (HB,) of rats treated with *Amaranthus spinosus* leaf extract when compared to normal control groups. The results also revealed that there was no significant difference ($P>0.05$) observed in PCV, and HB in anaemic rats and treated with Haemoglobin 12 when compared to normal control group. However, there was a significant increase in ($P>0.05$) observed in TWBC anaemic of rats when compared to normal groups.

Table 1: Hematological parameters of Different Groups of Phenylhydrazine Induced Anemia in Rats Administered Amaranthus spinosus Methanol LeafExtract

Group	TWBC	PCV	Hemoglobin
A	16.15^{c}±1.23	45.80^{a}±2.36	15.26^{a}±0.78
B	11.97^{a}±2.36	38.50^{b}±5.71	12.83^{b}±1.90
C	21.52^{b}±2.32	45.90^{a}±2.16	15.30^{a}±0.71
D	22.10^{b}±3.44	46.00^{a}±2.71	15.33^{a}±0.90
E	22.37^{b}±2.87	45.80^{a}±1.35	15.26^{a}±0.44

A = Normal Control, B = Phenylhydrazine Control, C = Phenylhydrazine + Hemoglobin 12 (standard drug). D = Phenylhydrazine + 200mg/kg body weight of methanol extract of Amaranthus spinosus leaf. E = Phenylhydrazine + 400mg/kg body weight of methanol extract of Amaranthus spinosus leaf.

Electrolyte Level Concentration of Phenylhydrazine Induced Anemia in Wistar Rats Administered *Amaranthus spinosus* Methanol Leaf Extract.

The results also revealed that there was no significant difference (P>0.05) observed in Potassium (K^+), Sodium (Na^+) concentration of rats treated with methanol leaf extract of *Amaranthus spinosus* when compared to normal control group. However, there was significant increase (P>0.05) in chloride (Cl^-) with the rats treated with the plant extract when compared to the normal control group.

Table 2: Electrolyte Level Concentration of Phenylhydrazine Induced Anemia in Wistar Rats Administered *Amaranthus spinosus* Methanol Leaf Extract.

Group	K^+ (mmol/L)	Na^+ (mmol/L)	Cl^- (mmol/L)
A	6.24±0.9	145^{c}.10±2.27	87.24^{b}±1.68
B	12.44^{a}±1.48	148.0^{a}±0.16	98.42^{a}±1.68
C	10.24^{b}±0.37	142.85^{b}±0.25	91.14^{b}±0.21
D	6.26^{bc}±0.38	144.70^{b}±0.82	92.80^{b}±2.21
E	6.35^{b}±0.51	145.52^{ab}±3.16	90.21^{b}±0.26

A = Normal Control, B = Phenylhydrazine Control, C = Phenylhydrazine + Hemoglobin 12 (standard drug). D = Phenylhydrazine + 200mg/kg body weight of methanol extract of *Amaranthus spinosus* leaf. E = Phenylhydrazine + 400mg/kg body weight of methanol extract of *Amaranthus spinosus* leaf.

DISCUSSION

The results of haematological studies showed that there was no significant difference (P>0.05) observed in (PCV, HB, RBC) of anaemic rats treated with haemoglobin 12 when compared to normal control group. However, there was a significant increase in (P>0.05) observed in TWBC of rats with the plant extract when compared to normal groups. This was in agreement with the work of [31,32,33], which reported higher values for TWBC in treatment groups relative to control groups in their studies on the anti-anemic effect of dried beet green in phenylhydrazine anemic-treated rats. The increase in red blood cell count of the anemic rats following the administration of *Amaranthus spinosus* of methanol leaf extract indicates that the plant extract has ability to stimulate the erythropoietin release by the kidney which is the humoral regulator of red blood cell production [34,35,36]. The study suggests that the plant extract has anti-anaemic property and can be employed to improve bone marrow functions.

The results also revealed that there was no significant difference (p>0.05) in

potassium (k^+) and sodium (Na^+) concentration of rats treated with methanol extract of *Amaranthus spinosus* when compared to normal control group. However, there was significant increase ($p>0.05$) in chloride (Cl^-) in the rats treated with plant extract when compared to the normal control group. Higher level of serum urea and creatinine indicates an underlying condition affecting on the [37,38,39,40,41] which was not recorded in this studies. However, the level of urea and creatinine evaluated showed that there was no form of nephrotoxicity in the treatment groups as there was no significant ($p>0.05$) increase in the values of urea and creatinine compared to the anemic-untreatedgroup.

CONCLUSION

In conclusion, the study showed that methanol leaf extract of *Amaranthus spinosus* have haematological properties. The study also revealed that treatment of anaemia with methanol leaf extract of *Amaranthus spinosus* improved haematological indices of (TWBC, PCV, HB, RBC,) of the wistar albino rats. The plant extract can also be used in the maintenance of healthy body weight.

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