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The effect of methanol leaf extract of *Amaranthus spinosus* on kidney and liver function parameters in phenylhydrazine induced anemia in wistar rats.

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ABSTRACT

This study was carried out to investigate the effect of methanol leaf extract of *Amaranthus spinosus* on kidney and liver function parameters in phenylhydrazine induced anemia in wistar rats. 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 of liver function profile level revealed significant increase (P>0.05) in alkaline Phosphatase (ALP), Alanine amino transferase (ALT), Aspartate amino transferase (AST), levels in rats treated with the plant extract when compared to normal control group. The kidney function parameters revealed no significant difference (P>0.05) in creatinine and urea level in the treated rats and normal control group. The result of the study therefore, suggests that the leaf extracts are not toxic to the liver and kidney and could be helpful in treatment of anemia.
Keywords: *Amaranthus spinosus,* kidney, liver, phenylhydrazine and anemia

INTRODUCTION

Amaranthus spinosus, commonly known as the spiny amaranth spiny pigweed, prickly amaranth or thorny amaranth, is a plant native to the tropical Americas, but is present on most continents as an introduced species and sometimes a noxious weed [1,2,3]. It can be a serious weed of rice cultivation in Asia. Amaranths has many species and they are used for different economic purposes when processed and dried [4,5,6]. The vegetable type is a herbaceous annual with upright growth habit, cultivated for both its seeds which are used as grain and its leaves which are used as a vegetable or green. Both leaves and seeds contain protein of an unusually high quality [7,8]. The grain is milled for flour or popped like popcorn. It may be eaten raw or cooked. Amaranths grown principally for vegetable use have better tasting leaves. In recent research, it was noticed that the species, *Amaranthus spinosus* has many nutritional values in the body, and also little biochemical effect in some vital organs like the kidney, heart, and the liver when eaten in large quantity [9,10]. According to world health organisation about 80% of the world's populations depend mainly on traditional medicine and the use of plant extracts are mainly involved in the traditional treatment

[11,12]. Medicinal plants constitute the major component of the traditional medicine practiced worldwide due to the economic viability, accessibility and ancestral experience [13]. Therefore, the search for safe and more effective agents from plants origin has continued to be an important area of active research [14]. *Amaranthus spinosus* are used as anti-inflammatory, anti-malarial, antibacterial, antimicrobial, antidiuretic, anti-viral and in hepatic disorders [15]. Water extract of plant showed significant immuno-stimulating activity and stem extract showed antimalarial activities [16]. *A. spinosus* have several active constituents like alkaloids, flavonoids, glycosides, phenolic acids, steroids, amino acids, terpenoids, lipids, saponins, betalains, b-sitosterol, stigmasterol, linoleicacid, rutin, catechuic tannins and carotenoids [17,18,19]. The betalains in stem bark of *Amaranthus spinosus* were identified as amaranthine, isoamaranthine, hydroxycinnamates, quercetin and kaempferol glycosides [20]. It also contains amaranthoside, a lignan glycoside, amaricin, a coumaroyl adenosine along with stigmasterol glycoside, betaine such as glycinebetaine and trigonelline [21]. Betalains are well known for their antioxidant, anticancer, antiviral and antiparasitosis properties. Many betalain containing species are used as popular medicinal plants to treat various kinds of ailments such as hepatic disorders, malaria, jaundice and scanty urine or to cure wounds [22]. The ash of fruits of *Amaranthus spinosus* is used for jaundice [23]. Water extracts from its roots and leaves have been used as a diuretic in [24]. *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 [25]. It also contains a high level of flavonoids and carbohydrate that help in energy supply of the body.

Aim of Study

The aim of this study was to investigate the effect of methanol leaf extract of *Amaranthus spinosus* on kidney and liver function parameters in phenylhydrazine induced anemia in wistar 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 leaf) 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 Kidney Function Test

Determination of Creatinine

Principle

The concentration of serum Creatinine was determined using standard method of [26] as outlined in Randox kit, UK. Creatinine in alkaline solution reacts with picric acid to form a coloured complex. The amount of the complex formed is

directly proportional to the creatinine concentration.

Procedure

Into three test tubes labeled test, standard and blank 50µl of serunn, 50µl of standard and 10ml of NaOH were added respectively. Then, 50µl of the working reagents were pipetted into each of the test tubes and was calculated using the equation below:

$$\textit{Creatinine concentration (mg/l)} = \frac{\textit{Abs of sample x concentration of standard}}{\textit{Abs of standard}}$$

Determination of Urea

The concentration of serum urea was determined using the method of [26] as outlined in Randox kit, UK.

Principle

The principle behind this method is that the ammonia produced by the action of urease enzyme on urea is estimated by measuring the blue colour of indophenols formed with phenol and hypochlorite at 578*nm* while sodium nitroprusside is used as a catalyst in this method.

Procedure

Into three test tubes labeled test, standard and blank 10 µl serum, standard and distilled water were added respectively. Then, 100 µl of sodium nitroprusside and urease (Ri) were added to each of the tubes. The tubes were mixed and incubated at 37 °C for 10 minutes. Then, reagent R2 (2.50 ml) was added to each of the test tubes. After that, 2.50 ml of R_3 was also added mixed immediately and incubated at 37 °C for 15 minutes. The absorbance was measured at 546 nm. Urea concentration (mg/l) was calculated using the equation below:

Urea concentration (mg/1). $= \frac{\textit{Abs of sample x concentration of standard}}{\textit{Abs of standard}}$

Determination of Liver function test

Determination of the aspartate aminotransferase (AST) activity

Principle

The activity of aspartate aminotransferase was assayed by the method of [27] as outlined in the Randox kit used. Aspartate aminotransferase activity was measured by monitoring the formation of oxaloacetate hydrazine with 2,4-dinitrophenylhydrazine.

Kit Reagents

S/N	Content	Initial Concentration of Reagents
R1.	Phosphate Buffer	200 mmol/l, pH 7.4
	L – Aspartate	200 mmol/l
	Beta – oxoglutarate	2 mmol/l
R2.	2,4-dinitrophenylhydrazine	2 mmol/l

Procedure

Serum samples of 0.1ml was pipetted into the samples test tubes and 0.1 ml of distilled water was pipetted into the blank test tube. The, 0.5 ml of Reagent one (R_1) containing phosphate buffer, L – aspartate and beta – oxoglutarate was pipetted into both the blank and serum sample test tubes respectively. The entire reaction medium was well mixed and incubated for 30 minutes in a water bath at 37 ^{0}C. Immediately after incubation, 0.5 ml of Reagent two (R_2) containing 2,4-dinitrophenylhydrazine was added to the blank and the serum sample test tubes and allowed to stand for exactly 20 minutes at 25 ^{0}C. After, the mixtures were loaded in the semi-auto analyzer and the readings were taken.

Calculation:

To get the AST activity of the samples, the transmittance obtained for samples were extrapolated from the calibration curve made from standards, and the results were expressed in SI unit (IU/L).

Determination of the alanine aminotransferase (ALT) activity

Principle

The activity of alanine aminotransferase was assayed by the method of [27] as outlined in Randox Kit.

S/N	Kit Reagents Content	Initial Concentration of Reagents
R1.	Phosphate Buffer	100 mmol/l, pH 7.4
	L-alanine	100 mmol/l
	Beta – oxoglutarate	2 mmol/l
R2.	2, 4-dinitrophenylhydrazine	2 mmol/l

Procedure

The serum samples (0.1 ml) was pipetted into the sample test tubes and 0.1 ml of distilled water was pipetted into blank test tube, 500 µl of the ALT substrate buffer solution containing phosphate buffer, L-alanine and α-oxoglutarate (R_1) were added. The entire reaction media were well mixed and incubated for 30 minutes in a water bath at 37 ^{0}C and pH 7.4. Immediately after incubation, 0.5 ml of Reagent two (R_2) containing 2, 4-dinitrophenylhydrazine was added to the blank and sample test tubes. These were thoroughly mixed and allowed to stand for exactly 20 minutes at 25 ^{0}C. Finally, 0.5 ml of sodium hydroxide solution was added to both the blank and serum samples test tubes respectively and mixed thoroughly. After, the mixtures were loaded in the semi-auto analyzer and the readings were taken.

Calculation

To get the AST activity of the samples, the transmittance obtained for samples were extrapolated from the calibration curve made from standards, and the results were expressed in SI unit (IU/L).

Determination of the alkaline phosphatase (ALP) activity

Principle

The activity of alkaline phosphatase was assayed using standard methods as outlined in Randox kit. The alkaline phosphatase acts upon the AMP-buffered sodium thymolphthalein monophosphate. The addition of an alkaline reagent stops enzyme activity and simultaneously develops a blue chromogen, which is measured photometrically.

Procedure

Into 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, 0.05 ml (50 µl) of alkaline phsophatase substrate was added into labeled test tubes for 3 minutes, it was mixed gently and incubated for exactly 10 minutes at 37 ^{0}C. After that, alkaline phosphatase color developer (2.5 ml) was added at timed intervals and mixed well. After, the mixtures were loaded in the semi-auto analyzer and the readings were taken.

Calculation:

The alkaline phosphate (ALP) concentration in the serum was calculated thus:

$$\frac{Absorbance\,of\,sample}{Absorbance\,of\,standards} \times \frac{30}{1} = \text{IU/L of alkaline phosphatase}$$

Determination of Albumin activity

Principle

The method is based on the specific binding of bromocresol green (BCG), an anionic dye, and the protein at acid pH produce a color change of the indicator from yellow-green to green-blue with the resulting shift in the absorption wavelength of the complex. The intensity of the color formed is proportion to the concentration of albumin in the sample.

Kit Reagent	
Content	**Initial Concentration of Reagents**
Bromocresol green reagent	pH 4.2 (0.12mmol/L)
Albumin standard, Albumin Aqueous primary standard	5g/dL

Procedure

Into three test tubes labeled blank, sample test and standard were added 1.0ml of bromocresol green reagent respectively. Into the sample test tubes was added 5µL of serum sample. Into the test tube labeled standard was added 5µL of the standard reagent. The mixtures were gently shaken and incubated for 10minutes at room temperature. Afterwards they were loaded into the semi-auto analyzer and their readings were taken.

Calculation:

Total Bilirubin was calculated thus:

Total Bilirubin/dl = absorbance of sample x 43.2

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

Liver functions Parameters of Phenylhydrazine Induced Anemia in Rats Administered *Amaranthus spinosus* Methanol Leaf Extract .

Results in Table 1 revealed that there was significant increase ($P>0.05$) observed in ALP, ALT, AST, in anaemic rats induced when compared with the normal control group. It was also observed that there was a significant decrease ($P<0.05$) in ALP, ALT, AST and Albumin in all treated rats with methanol *Amaranthus spinosus* when compared to theiranaemic counterparts.The result also showed a significant decrease ($P<0.05$) in rats administered with Hemoglobin 12 when compared to positive control group .

Table 1: Liver functions Parameters of Phenylhydrazine Induced Anemia in Rats Administered *Amaranthus spinosus* Methanol Leaf Extract .

Group	ALT (u/l)	AST (u/l)	ALP (u/l)	Albumin
A	9.70^{c}±1.89	4.14^{c}±2.11	100.98^{b}±16.92	4.42^{a}±0.49
B	27.94^{a}±5.38	20.42^{a}±3.85	215.16^{a}±46.98	3.76^{b}±0.44
C	14.12^{b}±1.68	12.35^{b}±4.06	107.58^{b}±25.19	4.35^{ab}±0.38
D	13.20^{bc}±2.84	11.14^{b}±1.89	87.78^{b}±20.55	3.99^{ab}±0.44
E	16.33^{b}±1.78	15.07^{ab}±8.46	105.60^{b}±24.91	4.11^{ab}±0.33

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.

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

The kidney function parameters in Table 2 revealed that there was no significant difference (P>0.05) in creatinine and urea level in the treated rats when compared to normal control group. 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: Kidney Functions Parameters and Electrolyte Level Concentration of Phenylhydrazine Induced Anemia in Wistar Rats Administered *Amaranthus spinosus* Methanol Leaf Extract.

Group	Creatinine (umol/l)	Urea (mmol/l)	K^+ (mmol/L)	Na^+ (mmol/L)	Cl^- (mmol/L)
A	146.47^{ab}±5.43	10.83^{c}±3.28	6.24±0.9	145^{c}.10±2.27	87.24^{b}±1.68
B	238.85^{a}±75.32	29.80^{a}±2.96	12.44^{a}±1.48	148.0^{a}±0.16	98.42^{a}±1.68
C	123.93^{b}±47.13	18.17^{b}±3.47	10.24^{b}±0.37	142.85^{b}±0.25	91.14^{b}±0.21
D	146.47^{ab}±50.38	9.48^{c}±3.05	6.26^{bc}±0.38	144.70^{b}±0.82	92.80^{b}±2.21
E	173.51^{ab}±69.72	10.93^{c}±3.40	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 the liver function parameters revealed that there was significant increase (P>0.05) in ALP, ALT, AST in anaemic rats when compared with the normal control group. It was also observed that there was a significant decrease (p<0.05) in ALP, ALT, AST and albumin in all rats treated with methanol extract of *Amaranthus spinosus leaves* when compared to positive group. The result also showed a significant decrease (p<0.05) in rats administered with hemoglobin 12 when compared to positive control group. This was in agreement with findings of [28,29]: they respectively reported a reduction in liver function parameters of phenylhydrazine induced anemic rats administered varying doses of aqueous leaf extract of fluted pumpkin and *Acrido carpus smeathmannic* root extract. This study suggests that administration of *Amaranthus spinosus* methanol leaf extract in appropriate dose is safe and could have protective and restorative potential in cases of liver damage or injury. The kidney function parameters results revealed that there was no significant difference (p<0.05) in creatinine and urea level in the treated rats with the plant extract when compared to normal control group. 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 kidney [30] 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-untreated group. The study also suggest that the Inability of the plant to increase urea level indicates that this plant may have effect in reducing the urea level in kidney disease condition especially in diabetic condition where the urine levels increases and as well help in the maintenance of kidney function.

CONCLUSION

This study revealed that treatment of anaemia with methanol leaf extract of *Amaranthus spinosus* improved liver function (ALT, AST, and ALP) and kidney function (Creatinine and Urea) of the wistar albino rats.

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