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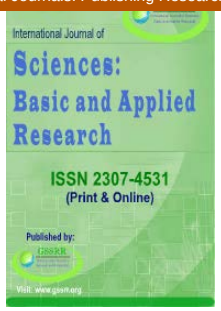

Toxicity Studies of *Amaranth viridis* Linn (Spiny Amaranth) Using Albino Rats

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Abstract

The study examined the effect of feeding albino rats with 75% Spiny amaranths leaves with respect to their body weight, liver and kidney indices, haematological and histological response. The results showed that rats fed with the leaves experienced decreased in body weight compared to the control group. The Packed Cell Volume (PCV), Haemoglobin concentration (Hb), lymphocytes were significantly elevated ($p>0.05$) compared to the control group, while neutophils was significantly lower ($p>0.05$) compared to the control. Red Blood Cells (RBC), White Blood Cells (WBC), Platelets, Mean Carpuscular Volume (MCV), Mean Carpuscular Haemoglobin (MCH), Mean Carpuscular Haemoglobin Concentration (MCHC) and some Leukocytes (monocytes, eosinophils and basophils) differential counts were not significantly ($p>0.05$) different between the control and the treated group.

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Similarly, serum total protein, albumin, globulin, glucose and bilirubin were not significantly different (p>0.05) compared to the control. The serum enzymes activities i.e asphatate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatise (ALP) were significantly (p>0.05) elevated compared to the control, which suggested toxicity induced by some of the phytocompounds present in the feed. Serum creatinine was significantly (p>0.05) elevated compared to the control, while the serum urea, uric acid, and electrolytes were not significantly (p>0.05) different between the control and those treated with the sample. The results showed that Spiny amaranths leaves have a relatively low or no toxicity.

***Keywords*:** Spiny amaranths; Amaranths *viridis*; Albino rats; Biochemical parameters

1. Introduction

During the time of natural or man-made disaster, human populations suffer from severe food shortages; thus, they become heavily reliant on wild food plants for survival [1]. Naturally, these plants, also known as "famine food " are neglected and only considered for consumption in times of nutritional stress [2]. The neglect of wild food plants has been attributed to the inadequacy of information on their nutritional contents and potential to serve as food security.

Literatures have indicated that some wild leafy vegetables are rich in both macro and micro nutrients. For examples a study carried out in South Africa shows that *Chenopodium album* leaves had 26.44% protein and *Urtica urens* leaves had 18.63%protein on dry weight basis (DW) [3]. Research conducted in Northern Nigeria and Southern Niger indicated that wild plants from the regions had considerable amount of protein [4]. The leaves of *Hibiscus canabinus*, *Fiscus dekdekenna* had protein content within the range of 11 – 28%DW. However, the leaves of *Moringa olifera* and *Adansonia digitata* was reported to have protein content of 12.12%DW and 20.72%DW respectively [5,6].

Carbohydrate is important in the supply of energy and its composition varies depending on the species [7]. For example Afolayan and Jimoh [3] reported 29.41%DW carbohydrate in *Chenopodium album* leaves, 41.95%DW carbohydrate in *Sonchus asper* leaves and 30.29%DW carbohydrate in *Urtica urens* leaves. Research conducted also shows that leaves of *Tribulus terrestis*, *Gynandropsis gynandra* contain 56%DW carbohydrate and 43%DW carbohydrate respectively [7].

Dietary fibre plays an important role in decreasing the risks of many disorders such as constipation, diabetis, cardiovascular diseases and obesity [8]. Wild leafy vegetables are also good sources of dietary fibre; and Afolayan and Jimoh [3] reported that leaves of *Chenopodium album*,*Sonchus asper* and *Urtica urens* have an appreciable dietary fibre content of 16.65%DW, 18.33%DW and 16.08%DW respectively. Wild leafy vegetables are also reported as rich sources of both macro and micro minerals [8].

Spiny amaranth (*Amaranthus viridis* Linn) also called *Rukubu* in Hausa Language belongs to the family of *Amaranthaceae* is an erect (about 60 cm tall) annual or perennial herbs widely found throughout African continent. The leaves are oval (7 cm by 5 cm), which is broad near base, obtuse, acuminate petiole long and reddish [9]. The leaves of Spiny amaranth were reported to contain 35.11%DW crude protein, 5.26%DW crude

lipids, 14.04%DW crude fibre and 24.54%DW available carbohydrate [7]. Similarly, the leaves contain appreciable quantity of both macro and micro minerals of which potassium, iron, manganese and copper are predominantly high [7]. The leaves were also reported to contain anti-nutrients and phytocompounds of medicinal importance [7].

Studies on the toxicity of most wild edible plants were neglected possibly due to their nutritional value and cultural acceptance. Some of the phytocompounds are phytotoxins if ingested may have some detrimental effect on the liver or and kidney. The study is aimed at investigating the toxic effect of Spiny amaranth leaves using Albino rats as experimental animals.

2. Materials and Methods

2.1 Samples Collection and Transportation

Tender leaves of Spiny amaranth were randomly collected from different locations along River Zamfara at Jega, Kebbi State, Nigeria. Prior to analyses, the samples were identified and authenticated at the Herbarium of the Botany Unit, Usmanu Danfodiyo University, Sokoto, Nigeria. The leaves were separated from the stalk, washed with distilled water, put in separate large study envelopes and oven dried at 60^0C to constant weight [10]. The dried leaves were pulverized in a porcelain mortar, sieved through 20-mesh sieve and stored in plastic containers. The powdered samples were used for the analyses.

2.2 ToxicityStudy

2.2.1 Experimental animals and Feed preparation

Ten male albino rats weighing between 140g – 145g used in the study were obtained from Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria. The animals were housed in stainless steel cages and allowed to acclimatize to the laboratory conditions for two weeks. During the period, clean tap-water and feed (Poultry grower's mash) were supplied to the animals *ad libitum*. The animals were divided into two groups (five rats/group) based on balance weight. Group one serves as control and were fed with 100% poultry growers mash, while group two were fed with 25% poultry growers mash and 75% Spiny amaranth pulverized leaves.

All the animals were weighed before the beginning and after the experimental period which lasted for 21days. During the feeding trial, water was supplied *ad libitum.* During the feeding trial feed intake was calculated from the refusal and offered feed.

2.2.2 Collected of Blood and Organs

At the end of the 21st day, feed and water were withdrawn from the animal overnight and weighed. The animals were anaesthesised in a container saturated with chloroform vapour, slaughtered and blood collected into labelled bottles coated with Ethylene Diamine Tetraacetic Acid (EDTA) as anti-coagulants. Another sample of the blood was placed in a labelled bottle without anti-corgulant. The former and latter were used for

haematological and biochemical assays respectively. The liver and kidney of the scarified animals were fixed in 4% formalin-saline for histolgical examinations.

2.2.3 Biochemical Analyses

The blood collected for biochemical assay was centrifuged at 2000 rpm for 10 minutes and serum decanted into clean $5cm^3$ sample bottles and kept at -20^0C until analysis. The sera were used to analyse serum total protein, albumin, alkaline phosphatate, asphatate amino transferase activity, alanine aminotransferase activity, creatinine, urea, uric acid, glucose and electrolytes.

Serum total protein (Biuret method), albumin (Bromocresol Green method), creatinine, glucose, bilirubin (total and direct), urea, uric acid (phosphotungstate method), aspatate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatate (ALP) activities were determined by assay kits obtained from Randox laboratories Ltd., UK following manufacturer's instructions. The globulin concentration was obtained by subtracting albumin from the total protein. Serum Na^+ and K^+ was measured using flame emission spectrophotometer as described by [11].

2.2.4 Haematological Analyses

Anti-corgulated blood was used to evaluate haematological indices. Parked Cell Volume (PCV), Red Blood Cell count (RBC), White Blood Cell count (WBC), Platelets counts and Haemoglobin (Hb) were determined using Wintrobes micro-haemtocrit, improved Newbauer haematometer and cyano-methaemoglobin method [12,13]. The erythrocytic indices; Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were computed according to Lentowsky and Ciesia[13] methods. Serum AST, ALP and ALT were determined using the method reported by Reitman and Frankel [14].

2.2.5 Histopathological Tests

Samples of the liver and kidney from each animal were fixed in buffered 4% formalin-saline. After dehydration and embedding in paraffin wax, 5μm sections were cut and stained with Myers hematoxylin and eosin for examination under Ortodux light microscope (Acu-scope 3012 LED Series) for any changes in the tissues due to the consumption of Spiny amaranth leaves [15].

2.2.6 Statistical Analysis

The data obtained was statistically analyzed using one-way analysis of variance (ANOVA) with SPSS version 10.0 statistical package and the results were reported as mean ± standard deviation of the values. Significant difference between the means was determined using LSD at 5% level.

3. Results and Discussion

3.1. Growth Performance

The results of growth performance on rats fed with Spiny amaranths leaves were presented in Table 1. The results showed that rats fed with Spiny amaranths leaves experienced decreased in body weight compared to the control group. The leaves have met all the nutritional requirements in terms of crude protein, available carbohydrate and dietary fibre as reported by [7]. Although mortality was observed in some of the animal, but palatability and antinutritional factors could be the main causes of lower feed intake and loss in body weight of the animals. For example the leaves of Spiny amaranths had high tannin content, which might hinder protein bioavailability [7]. High oxalate contents which may lead to kidney stone if ingested may also accounts for the mortality recorded during the treatment [7]. James *et al*. [16] reported that palatability might also be responsible for the lower feed intake by the rats.

Table 1: Body weight changes of Rats fed with Spiny amaranths leaves

Parameter	Control	Sample
Initial weight (g)	142.10	141.50
Final weight (g)	152.90	106.99
Gain/loss in weight (g)	10.80	-34.51
Average Daily Growth (g/day)	0.51	-1.64
Feed intake (g/day)	13.33	4.07
Feed: Weight gain/ Weight lost (g)	25.92	-2.48
Mortality	0	2

3.2 Haematological Studies

The results were presented in Table 2. The results indicate that, the index of cellular immunity the MCHC, MCH, MCV, WBC and Platelets shows no significant difference ($p<0.05$) between the control and the treated group. Similarly, the erythrocyte counts (RBC) was not significantly difference ($p>0.05$) between the control and the treated group and is within the normal range of $6.76 – 9.75x10^{12}L^{-1}$ set for rat [17]. The results generally suggested that, the plant analysed may not cause anaemia and have no effect on the body immune system.

3.3 Liver Function Tests

Liver plays an important role in the body considering its function in detoxification of metabolic processes [18]. The detoxification process disturbs the integrity of cell membrane which may damage the liver function. Therefore change in the concentration of total protein, albumin, globulin and enzymes (ALP, AST and ALT) in the serum may indicate the state of the liver and the type of damage [19].

The result of the liver function is presented in Table 3. Total protein is associated with evaluation of hydration status or possible haemorrhage and is a marker for acute and chronic inflammation [20]. From the result, the concentration of serum total protein shows no significant difference ($p>0.05$) between the control and the sample treatment and is within the rat normal values of 5.6 – 7.6gdL^{-1} [17]. Albumin is synthesized by the liver and is a major form of protein present in blood and is a marker of liver damage [21]. The concentration of serum albumin and globulin show no significant difference ($p>0.05$) between the control and the sample treatment. The decrease in the concentration of serum total protein, albumin and globulin are indication of tissue injury and reflection of hepatic toxicity [22].

Table 2: Haematological Indices of Rat Fed with Spiny amaranths

Parameter	Control	Sample
WBC (10^9dL)	12.75 ± 0.72	8.65 ± 1.55
RBC (10^{12}dL)	6.51 ± 0.61	8.48 ± 1.18
Haemoglobin (g/L)	12.08 ± 0.28	14.90 ± 2.10*
PCV (%)	44.75 ± 0.85	54.50 ± 6.50*
MCV (fL)	68.78 ± 1.75	64.48 ± 1.27
MCH (pg)	18.56 ± 0.57	17.58 ± 0.42
MCHC (g/dL)	26.98 ± 0.34	27.27 ± 0.60
Platelet (10^9dL)	417.25 ± 27.42	506.00 ± 77.00
Lymphocyte (%)	80.50 ± 3.59	88.00 ± 3.00*
Neutophils (%)	14.25 ± 4.10	8.00 ± 1.00*
Monocytes (%)	4.50 ± 0.87	ND*
Eosinophils (%)	0.75 ± 0.48	0.50 ± 0.00
Basophils (%)	ND	ND

Values with asterisk (*) within the same row are significantly different at $p<0.05$.

WBC = White Blood Cells; **RBC** = Red Blood Cells; **PCV** = Packed Cell Volume; **MCV** = Mean Cell Volume; **MCH** = Mean Cell Haemoglobin; **MCHC** = Mean Cell Haemoglobin Concentration; **fL** = Femtoliters (10^{-15}); **pg** = Pictogramme (10^{-12}), **ND** = Not Detected.

Bilirubin is another marker of liver dysfunction as elevation in bilirubin concentration suggests increase in haemolysis. The serum total and direct bilirubin show no significant difference ($p>0.05$) between the control and those treated with the sample and are within the normal rat values of 0.2 – 0.55gmgdL^{-1} [17]. The results obtained indicate that, the feed may not cause haemolysis.

The liver enzymes, aspartate and alanine amino transferases (AST and ALT) are involved in amino acid metabolism and are present in liver and kidney [11]. Serum ALT and AST are levels were always found to increases in liver cell damage and the greater the degree of liver damage the higher the activities of both enzymes [11]. The results showed a significant increase ($p>0.05$) in all the enzymes activities between the control and the treated group, which indicate that the sample may cause cytotoxic effect on the liver and may also affect the permeability of the cell membrane making it leaky [22].

Alkaline phosphatise (ALP) is a marker enzyme for the plasma membrane and endoplasmic reticulum. It is often used to assess the integrity of plasma membrane [23]. It is also related to the function of hepatic cell. Increase in serum level of ALP is due to increased synthesis of enzyme in the presence of increasing biliary pressure [22]. Significant elevation of serum alkaline phosphatase is an indication of cholestasis [24]. With no effective control of ALP activity towards improvement in the secretory function of the hepatic cell.

Glucose level indicates adequate energy supply to animals. Excess glucose is converted to glycogen by the liver. High level of glucose is an indication of certain liver diseases. The result shows no significant difference ($p>0.05$) between the control and sample treatment and is within the normal values for rat [17].

Table 3: Liver Function Indices and Serum Glucose Level of Rat Fed with Spiny amaranth

Parameter	Control	Sample
Total protein (mgL^{-1})	7.54 ± 0.35	6.66 ± 0.67
Albumin (mgL^{-1})	3.28 ± 0.20	3.05 ± 0.16
Globulin (mgL^{-1})	4.27 ± 0.42	3.60 ± 0.81
Total Bilirubin (mgL^{-1})	0.38 ± 0.05	0.33 ± 0.00
Direct Bilirubin (mgL^{-1})	0.22 ± 0.07	0.14 ± 0.01
ALT (IU^{-1})	3.60 ± 0.26	8.90 ± 0.15*
AST (IU^{-1})	14.50 ± 0.54	61.75 ± 16.75*
ALP (IU^{-1})	106.26 ± 1.78	168.36 ± 0.72*
Glucose ($mmoleL^{-1}$)	5.12 ± 0.21	3.57 ± 0.95

Values with asterisk (*) within the same row are significantly different at $p<0.05$. **AST** = Aspartate aminotransferase; **ALT** = Alanine aminotransferase; **ALP** = Alkaline phosphatise, **IUL^{-1}** = International Unit per Litre.

3.4 Kidney Function Tests

The results of kidney function tests are presented in Table 4. The indices (creatinine, urea, uric acid and electrolytes) are required to assess the normal functioning of different parts of the neprons and are the significant markers of renal dysfunction by the feed consumed [7].

The serum creatinine of the rat fed with the sample is significantly ($p>0.05$) higher than the control and also higher compared to rat normal values of $0.2 – 0.8mgdL^{-1}$ [17]. This indicates that the sample contained some active compounds that may cause kidney related malfunction.

Urea is a nutritional pointer connected to protein intake and is use in assessing kidney function in conjunction with creatinine which originates from the non-enzymatic conversion of creatinine in muscle and is filtered by the kidney [20]. The sample serum urea level is not significantly difference ($p>0.05$) compared to control and is within the normal values of $2.5 – 22.5mmolL^{-1}$[17]. Similarly, the serum uric acid is not significantly difference ($p>0.05$) between the control and those treated with the sample. These signified no renal dysfunction as far urea and uric acid associated with the sample.

Electrolytes in the body are of great importance for osmotic balance in the body. Sodium and potassium ions are the major extra cellular and intracellular fluid regulating acid-base balance [22]. The results show no significant difference ($p>0.05$) between the control and the sample treated group, which suggest that the sample fed to the animal may not have significant effect on the variation of body acid-base balance and hence cause no renal dysfunction [15].

Table 4: Kidney Function Indices of Rats fed with Spiny amaranths

Parameter	Control	Sample
Creatinine ($mgdL^{-1}$)	0.79 ± 0.09	1.32 ± 0.09*
Urea ($mmoleL^{-1}$)	5.99 ± 0.45	7.61 ± 0.69
Uric acid (mgL^{-1})	1.84 ± 0.25	1.83 ± 0.02
K^+ ($mmoleL^{-1}$)	5.08 ± 0.34	4.75 ± 0.75
Na^+ ($mmoleL^{-1}$)	139.50 ± 2.02	132.50 ± 12.50

Values with asterisk (*) within the same row are significantly different at $p<0.05$

3.5 Histopathological Studies

From the result, the histological sections of the livers and the kidneys of rats fed with the sample showed features consistent with inflammation and congestion, which are nonspecific and are likely to result from mild tissue reaction during the period of treatment [15]. This suggests that the liver and the kidney show no sign of hepatic and tubular necrosis respectively.

4. Conclusion

From the present findings, it is clear that rats fed with Spiny amaranths leave experienced decrease in body weight compared to those fed with normal diet which was attributed to the present of antinutritional factors and non-palatability of the feed. Haematological indices showed that the leaves may not cause anaemia. Some biochemical parameters showed that the leaves may have effect on liver and kidney indices. Histological studies indicated features consistent with inflammation and congestion, which are nonspecific and hence no sign of hepatic and tubular necroses. The results suggests that Spiny amaranth leaves have a relatively low or no toxicity profile, however the mechanism(s) of toxicity of this plant is still being investigated.

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