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Toxicity studies of African Palmyrah palm (*Borassus Aethiopum*) shoots

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The study examined the effect of feeding albino rats with 25, 50, and 75% Palmyrah Palm (*Borassus aethiopum*) shoots with respect to their body weight, liver and kidney function indices. Standard methods of biochemical analyses were employed using albino rats as the experimental animals. No mortality was observed in the LD_{50} test throughout the period of 48 h. Rats fed with 25 and 50% *B. aethiopum* shoots showed a gradual increase in the body weight throughout the period of treatment, but those fed with 75% of the shoots experienced a significant (p>0.05) decrease in body weight at the 3rd and 4th week of treatment compared to the control group. The results also showed changes in the serum total protein, albumin, globulin, glucose and bilirubin but were not significantly different (p>0.05) compared to the control group. The serum enzymes activities that is aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were significantly (p>0.05) elevated compared to the control group which suggested toxicity induced by some of the phytocompounds present in the feed. Serum creatinine, urea, uric acid, and electrolytes (Na^+, K^+) of rats fed with 75% *B. aethiopum* shoots showed significant (p>0.05) changes compared to the control group. The results showed that *B. aethiopum* shoots have a relatively low or no toxicity.

Key words: *Borassus aethiopum*, shoots, albino rats, liver function, kidney function.

INTRODUCTION

Food is no doubt the most basic necessity for one to effectively function in his ecosystem. It is a substance that often composed of carbohydrates, lipids, proteins, vitamins and water, which are eaten or drunk by animals or humans for nutrition (Wasagu et al., 2013). The constituent in food contains important chemical substances known as nutrients. These constituents are ingested, digested, absorbed and circulated in the blood streams to feed the cells that constitute body building blocks. Consequently, the increase in body resistance to diseases and faster recovery of illnesses is witnessed (Worthinghton-Roberts, 2008).

Most of the foods consumed by humans are sourced from plants and animals; the former has been grouped

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Figure 1. Palmyrah Palm (*Borassus aethiopum*) tree.

Figure 2. Palmyrah Palm (*Borassus aethiopum*) shoots "Muruchi "

into leafy vegetables, seeds, tubers and fruits (Oyiza, 2005). There are over 30,000 known edible plants, from which only 300 were domesticated accounting for more than 95% of the required human plant food (Oyiza, 2005). The part of plant responsible for bearing of seeds is known as fruit and is considered a healthy food supplement because it composed of an appreciable amount of water, carbohydrates, lipids, proteins, vitamins and minerals such as calcium, magnesium, potassium, sodium, zinc, copper and iron (Umar, 2010).

The plant *Borassus aethiopum* (Figure 1) has been described as a palm tree with huge fan shaped leaves (Ahmed et al., 2010). In Nigeria, the Hausas call it *Giginya*, the Yorubas call it *Agbonolodu*, and the Igbos call it *Ubiri*. The plant is a dioecious plant and can reach up to 20 m high on average and 1 m in diameter (Muller, 1988). The shoots of *B. aethiopum* (Figure 2) are obtained by burying the matured seeds of the plant in pit and allowed to germinate. The young germinating shoot or hypocotyls known as *Muruchi* or *Gazari*, is usually harvested after 7 to 8 weeks of planting (Ahmed et al., 2010).

Palmyrah palm *(B. aethiopum)* shoots "Muruchi" is an important source of food for the rural people in Northern Nigeria. The people consume it either raw or boiled and claimed that it enhances libido in women and aphrodisiac in men (Akinniyi and Waziri, 2011). The shoots are potential source of starch in Cote d'Ivoire, which is an important raw material in industry. Akinnnyi and Waziri (2011) reported high concentration of carbohydrate (83.00%) and crude fiber (3.96%) and low crude protein (6.9%) and fat (1.49%) in the shoot of *B. aethiopum* plant on dry weight basis. Similarly, the shoots contained an appreciable amount of both macro and micro mineral elements of which Magnesium (640 mg/100 g), Calcium (433.30 mg/100 g), Potassium (236.70 mg/100 g), Manganese (12.30 mg/100 g), Zinc (12.74 mg/100 g) and Iron (11.50 mg/100 g) are the predominant elements[7]. The research on the nutritional composition of Palmyrah palm *(B. aethiopum)* shoots "Muruchi" conducted by Umar et al. (2015) is generally in agreement with that reported by Akinnnyi and Waziri (Akinniyi and Waziri, 2011).

Despite the nutritional benefits of the plant shoots "Muruchi", anti-nutritional factors such as phytate, tannins, oxalate, hydrocyanic acid and nitrate, which blocked the bioavailability of some mineral elements, are also present in the shoots as reported by Ahmed et al.

(2010). However, the anti-nutrients to nutrients molar ratio are all below the critical level known to impair the availability of some mineral elements such as calcium, magnesium, iron and zinc (Umar et al., 2015).

The use of Palmyrah palm *(B. aethiopum)* shoots "Muruchi" as food is very common especially in areas where the plant grows. Even though *B. aethiopum* shoots are important sources of nutrients and phytocompounds that play important roles in protection against conditions such as cardiovascular diseases and cancer, they also contain other compounds that may cause hepatic/tubular necrosis. The toxicity studies of *B. aethiopum* shoots in this region of Nigeria is not fully documented and hence very scanty. The research therefore, aimed at evaluating the safety of *B. aethiopum* shoots obtained from Sokoto State, Nigeria, by acute and sub-chronic administrations in rats.

MATERIALS AND METHODS

Sample collection and treatment

The matured shoots of *B. aethiopum* plant were collected from the area of cultivation in Kware Local Government Area, Sokoto State, Nigeria. The sample was collected in black polythene bags and transported to laboratory. Prior to analyses, the sample was authenticated at the Herbarium section, Botany Unit, Usmanu Danfodiyo University, Sokoto, Nigeria. The seedlings were dehulled, washed with distilled water, chopped in pieces, milled and then air dried. The dried sample was then pulverized into powder using pestle and mortar and sieved. The powdered sample was stored in a clean polythene bag until when required for analysis.

Toxicological studies

Animals

Albino rats (males and females) weighing 165 to 250 g were purchased from the Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. The animals were kept at the animal house of the department in a wire mesh cages. They were fed with poultry grower's feed and tap water *ad libitum* for two weeks to acclimatize before starting the experiment. Animal treatment and handling was done according to the ethical guidelines reported by Zimmerman (1983) and in accordance with U.S. guidelines as contained in the National Institute of Health guide for the care and use of laboratory animals (National Academy of Science (NAS), 2011).

Acute toxicity study (determination of LD_{50})

Powdered *B. aethiopum* shoots was fed to five (5) groups of one rat each (one after the other at a grace observation period of 24 h). Another (control group) was fed with grower's feed. All the groups receive tap water *ad libitum*. Observation for toxic symptoms was made and recorded systematically at 1, 2, 4 and 6 h after administration. Finally, the number of survivors was noted after 48 h. The toxicological effect was assessed on the basis of mortality, which was expressed as LD_{50} and calculated using the limit test dose, up and down procedure of Organization for Economic and Cultural Development (OECD) (OECD, 2001).

Sub-acute toxicity studies

Twenty four female albino rats weighing between 165 – 250 g were group into six groups (five rats per group). The animals were housed in stainless steel and fed with the following diets:

Group 1: Control (100% poultry growers mash).
Group 2: fed with 75% poultry growers mash + 25% pulverized *B. aethiopum* shoots.
Group 3: fed with 50% poultry growers mash + 50% pulverized *B. aethiopum* shoots.
Group 4: fed with 25% poultry growers mash + 75% pulverized *B. aethiopum* shoots.
The body weights of all the animals before and within 28 days (weekly) of treatment were recorded.

Collection of blood and serum

At the end of the 28th day, feed and water were withdrawn from the animals overnight and weighed. The animals were anaesthetized in a container saturated with chloroform vapor and then slaughtered. Blood samples were collected at slaughter into labelled bottles and were used for biochemical assays.

The blood collected was centrifuged at 2000 rpm for 10 min and serum decanted into clean 5 cm^3-sample bottle and kept at -20°C until analysis. The sera was used to analyze serum total protein, albumin, aspartate aminotransferase activity, alanine amino-transferase activity, alkaline phosphatase activity, creatinine, bilirubin, urea, uric acid, glucose, electrolytes; while anti-coagulated blood was used for red blood cells count, white blood cells count, platelets count, hemoglobin, packed cell volume and red blood cell indices.

Biochemical analyses

Liver function indices (Serum total protein, albumin, globulins, enzyme activities, total bilirubin, and direct bilirubin) and kidney function indices (Serum creatinine, urea, uric acid, Na^+ and K^+) were determined to follow the methods reported by Saidu (2005).

Statistical analysis

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

RESULTS

Acute toxicity study (LD_{50})

Acute toxicity test of *B. aethiopum* shoots produced no mortality after 48 h of observation. However, the sample did not produce any gross negative behavioural changes such as excitement, restlessness, convulsions or coma in the rat, instead reduced reaction to noise was observed.

Body weight (growth performance)

The growth performance of rats fed with control and *B.*

Table 1. Body weight changes of Rats fed with *Borassus aethiopum* shoots.

Dose (%)	Initial weight	1st week	2nd week	3rd week	4th week
0.00 (control)	166.82±1.48	167.81±1.03	167.91±1.77	168.16±1.92	168.97±1.06
25.00	193.28±0.93	193.74±0.71	194.06±0.91	195.00±0.67	195.74±0.74
50.00	203.53±3.11	204.02±3.44	204.50±3.46	205.32±2.87	205.73±2.61
75.00	205.21±2.75	206.04±2.60	206.93±2.56	205.66±2.51*	205.29±2.61*

Values are mean ± standard deviation. *= Significantly different from the control (P < 0.05) using one way analysis of variance (6).

Table 2. Liver function indices and serum glucose level of rats fed with *Borassus aethiopum* shoots.

Parameters	0% (Control)	25%	50%	75%
TP (g/dl)	6.50 ± 0.66	5.99 ± 0.50	5.73 ± 0.87	5.64 ± 0.24
ALB (g/dl)	4.10 ± 0.77	3.87 ± 0.21	3.62 ± 0.21	3.54 ± 0.24
GB (g/dl)	2.40 ± 1.23	2.12 ± 1.00	2.11 ± 0.12	2.10 ± 0.34
TB (mg/dl)	0.49 ± 0.10	0.51 ± 0.77	0.58 ± 0.49	0.69 ± 0.46
DB (g/dl)	0.33 ± 0.12	0.37 ± 0.29	0.39 ± 0.45	0.48 ± 0.11
TG (mMol/L)	5.12 ± 0.17	4.56 ± 1.88	3.99 ± 1.45	3.58 ± 1.76
ALP (IU/L)	101.7 ± 2.70	319.2 ± 2.9*	406.6 ± 5.9*	453.6 ± 3.0*
ALT (IU/L)	9.33 ± 3.12	13.67 ± 0.58*	23.77 ± 0.68*	31.0 ± 1.00*
AST (IU/L)	26.33±2.93	35.0±3.46*	39.50±3.04*	59.03±0.21*

Values are mean ± standard deviation.TP=Total protein. ALB=Albumin; TB=Total bilirubin. GB=Globulins; DB=Direct bilirubin; TG=Total glucose; ALP=Alkaline phosphatase; ALT=Alanine aminotransferase; AST=Asphatate aminotransferase. *= Significantly different from the control (P < 0.05) using one way analysis of variance (6).

aethiopum shoots (treatment diets) is presented in Tables 1. The result indicates a gradual increase in the body weight throughout the period of treatments at a dose of 25 and 50% not significantly (p<0.05) different compared with the control group. However, there is a noticeable increase in the body weights of rats treated with 75% of the sample at the 1st and 2nd week of the treatment, but at the 3rd and 4th week of the treatment there was a decrease in the body weights which is significantly different (p<0.05) compared to the control group.

Sub-acute toxicity studies

The liver function test

Table 2 presents the liver function indices in rats fed with *B. aethiopum* shoots. There is general decrease in the serum total protein (TP), albumin (ALB), Globulins (GB), and total glucose (TG) in all the treated groups not significantly (p<0.05) different compared with the control, but serum total bilirubin (TB) and direct bilirubin (DB) increased in all the treated groups not significantly (p<0.05) different compared with the control. In all the treated groups serum alkaline phosphatase (ALP), aspatate aminotransferase (AST) and alanine aminotransferase (ALT) showed a significant (p<0.05) increase compared with the control group.

The kidney function test

Table 3 gives details of the kidney function indices in rats fed with *B. aethiopum* shoots. Serum creatinine, urea, uric acid and potassium showed an increase not significantly (p<0.05) different compared to the control in rats fed with 25 and 50%. Those fed with 75% increase significantly (p<0.05), compared with the control. On the other hand, there is decrease in serum sodium in rats fed with 25 and 50% of the sample not significantly (p<0.05) different compared with the control group, except rats fed with 75% of the sample showed

DISCUSSION

Acute toxicity (LD_{50}) study

The mean lethal dose (LD_{50}) of the *B. aethiopum* shoots to rats did not produce any mortality and did not produce

Table 3. Kidney Function Indices of Rats fed with *Borassus aethiopum* shoots.

Parameters	0.00 (Control)	25%	50%	75%
Creatinine (µMol/L)	81.42±0.10	88.50±0.20	90.06±0.01	104.96±0.10*
Urea (mMol/L)	9.96±0.00	10.71±1.16	11.34±1.54	14.11±0.55*
Uric acid (µMol/L)	201.20±2.20	205.10±4.10	212.20±5.00	220.01±2.20*
Sodium (ppm)	31.30±3.12	31.06±0.11	30.00±0.10	28.00±0.10*
Potassium (ppm)	8.32±2.10	8.71±0.40	8.82±1.20	9.80±2.60*

Values are mean ± standard deviation. *= Significantly different from the control ($P < 0.05$) using one way analysis of variance (6).

any grossly negative behavioural changes such as excitement, restlessness, convulsions or coma in the rat. Instead, reduced reaction to noise was observed suggesting that, the *B. aethiopum* shoots may have depressant effect on the central nervous system and is relatively 'safe' in or non-toxic to rats compared to some known poisonous plants (Hassan et al., 2006).

Body weight (growth performance)

B. aethiopum shoots met al.l the nutritional requirements in terms of crude protein and available carbohydrate (Akinniyi and Waziri, 2011; Umar et al., 2015). The reduction in the body weight at high dose could be due to reduced feed and water intake observed from the animals, it could also be due to palatability, loss of appetite and more importantly anti-nutritional factors. For instance the high tannins content in the samples may hinder protein bioavailability (Ademoroti, 1996), the saponins present in the sample may have caused loss of appetite (Hassan et al., 2006).

The liver function test

Liver plays an important role in the body considering its function in detoxification of metabolic processes (Helal et al., 2009). 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 (Ashafa et al., 2009).

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 (Boonprong et al., 2007). 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 – 8.6 g/Dl (The Rat Fan Club, 2010). Albumin is synthesized by the liver and is a major form of protein present in blood and is a marker of liver damage (Oboh et al., 2005). 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, and decrease are indication of tissue injury and reflection of hepatic toxicity (Hassan et al., 2007).

Bilirubin is a major breakdown product of haemoglobin. Haemoglobin is derived from red cells that have outlived their natural life and subsequently have been removed by the spleen (Oboh and Akindahunsi, 2005). During splenic degradation of red blood cells, haem is separated out from iron and cell membrane components (Jonston, 1999). Haem is transferred to the liver where it under goes further metabolism in a process called conjugation. Conjugation allows haem to become more water-soluble (Oboh et al., 2005). The water solubility of bilirubin allows the bilirubin to be excreted into bile. As the liver becomes stimulated, the total bilirubin may rise (Oboh et al., 2005). As presented in the result, there is an increase in the serum total bilirubin and direct bilibubin in rats fed with *B. aethiopum* shoots with no significant difference ($p<0.05$) between the control and the treated groups. This is a clear indication that the sample may not interfere with or reduce the metabolism of bilirubin in the liver as well as increase in haemolysis (Oboh et al., 2005; Jonston, 1999; Anosike et al., 2008).

There are many enzymes such as Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and alkaline phosphatase (ALP) that are found in the serum, which do not originate from the extracellular fluid. During tissue damage, some of these biomolecules find their way into the serum through the disruption of cell membranes (Ashafa et al., 2009). Serum enzyme measurement therefore, provides a valuable tool in toxicity studies. The significant ($p<0.05$) increase in enzymes activity in all the treated groups at high doses of the *B. aethiopum* shoots compared to control implied cytotoxic effect on the liver, which is an indication that the sample is not completely safe if consumed in high quantity. In this study there is a significant ($p<0.05$) increase in AST and ALT activities are sign associated with the phytotoxins, and are indicative of liver inflammation (Cheesbrough, 1991). Increase in AST level is also associated with increase catabolism of amino

acids in the liver, perhaps due to dietary cyanide (Aning et al., 1998), which is present in *B. aethiopum* shoots (Umar et al., 2015).

Alkaline phosphatase (ALP) is a marker enzyme for the plasma membrane and endoplasmic reticulum. It is often used to assess the integrity of plasma membrane (Akanji et al., 1993). 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 (Hassan et al., 2007). Significant elevation of serum alkaline phosphatase is an indication of cholestasis with no effective control of ALP activity towards improvement in the secretory function of the hepatic cell (Van and De Broe, 1994).

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 a decrease in the concentration of glucose level with no significant difference ($p>0.05$) between the control and sample treatment and is within the normal values for rat (The Rat Fan Club, 2010).

The kidney function test

The results of kidney function tests are presented in Table 3. The indices (creatinine, urea, uric acid and electrolytes) are required to assess the normal functioning of different parts of the nephrons and are the significant markers of renal dysfunction by the feed consumed (Hassan et al., 2006).

Creatinine is a chemical waste molecule that is generated from muscle metabolism and is produced from creatine, a molecule of major importance for energy production in muscle (Canani et al., 2005). The kidney filters out most of the creatinine and disposes it in the urine (Harita et al., 2008). At high dose (75%), there is a significant ($p<0.05$) increase in the serum creatinine level compared with the control group. The value is above the normal range of (70 – 95 µmol/L) for rats (Ashafa et al., 2009), which is indicative of possible kidney malfunction or renal failure, perhaps due to constituents detected in the sample such as tannins, cardiac glycosides, saponins and flavonoids (Umar et al., 2015).

Uric acid is a product of purine catabolism (Baille et al., 2007). High concentration of uric acid (hyperuricemia) in blood serum causes gout (Friedman et al., 1998). The result indicate a significant ($p<0.05$) increase in serum uric acid in rats fed with high dose of the sample compared with the control. The significant change indicates a compromised renal malfunction, which could be due to phyto-compounds present in the sample (Umar et al., 2015).

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 (Hassan et al., 2007). The results showed a significant difference ($p>0.05$) at higher dose between the control and the treated group, which suggest that the sample fed to the animal may have significant effect on the variation of body acid-base balance and hence may course some renal dysfunction (Hassan et al., 2006).

Conclusion

From the present findings, it is clear that no mortality was recorded in LD_{50} test indicating that *B. aethiopum* shoots is relatively non-toxic. The rats fed with high dose of *B. aethiopum* shoots experienced decrease in body weight compared to those fed with normal diet, which was attributed to the presence of anti-nutritional factors and non-palatability of the feed. Some biochemical parameters showed that if consumed in high quantity, *B. aethiopum* shoots might have effect on liver and kidney indices. The results suggest that, *B. aethiopum* shoots have a relatively low or no toxicity profile. However, the toxicity mechanism(s) of this plant is still undergoing investigation.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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