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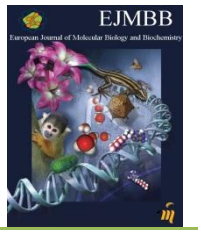

GROWTH AND TOXICOLOGICAL EFFECTS OF SWEET DETAR, *Detarium microcarpum* FRUIT SUPPLEMENT ON ALBINO RAT.

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ABSTRACT
Growth and toxicological effects of sweet detar, *Detarium microcarpum* was investigated on albino rat for four (4) weeks. Thirty five (35) rats of four weeks old were randomly distributed into five dietary treatment groups of seven per group and fed with *Detarium microcarpum* mesocarp and seed as supplement in their meal in the following ratio: Mesocarp 1:2 Rat feed (M1:2R), Mesocarp 2:1 Rat feed (M2:1R), Seed 1:2 Rat feed (S1:2R), Seed 2:1 Rat feed (S2:1R) and control (Rat feed only) for four weeks. At the end of the experiment, growth of rats was compared. Furthermore, the rats' liver and kidney function indices tests were carried out to ascertain its safety. The results revealed that *D. microcarpum* fruit supplement, did not support growth except in group A (M1:2R), which had little growth while the liver and kidney indices tests showed that the fruit is not save for consumption.

INTRODUCTION

Nutrition (nourishment or aliment) is the provision, to cells and organisms, of the materials necessary (in the form of food) to support life. The diet of an organism is what it eats, which is largely determined by the perceived palatability of foods [1]. Malnutrition in Nigeria and many other developing countries is as a result of limited sources of protein, vitamins and minerals of high biological values, particularly those of animal origin [2]. The quantity and quality of nutrients present or available in food samples imply the nutritional status of those foods. Although the quantity of food is as important as its quality, but in developing countries like Nigeria, the tendency or emphasis is now drifting from the quality (adequate proportions of nutrients) of food taken to the quantity [3]. This has led to some diseases which could have been prevented by effective feeding in such countries. Human body needs nutrients to help the body for the production of energy to meet the demands of everyday physical activities, ensure mental efficiency and protect the body against diseases. Through the ages, plants have been used by humans as sources of food, cosmetics and medicine.

Leguminosae *Detarium microcarpum* Guill. (Perr) commonly known as sweet detar is catalogued as a major African medicinal plant [4], which belongs to the family caesalpiniceae, phylum spermatophyte and the order fabacea. It is a multipurpose species, with a wide range of uses due to its medicinal properties. The roots, stems, bark, leaves and fruits are all used to treat ailments such as tuberculosis, meningitis, itching, syphilis and diarrhoea [5-7]. It is also used to treat ailments such as tuberculosis, meningitis, itching. The extract also showed moderate antitumor activity against breast cancer cells. Its fruits are fleshy [8] and quite edible (eaten raw, cooked or made into flour with many uses of its own) and hardwood used as fuel-wood. Its many uses make it a valuable and appreciated species to local communities. It is particularly associated with dry savannah countries. It is also found in Nigeria. It has an irregular distribution, but it can be locally very common. The plant is known to flower throughout the wet season and fruits between November and January.

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The fruits that are drupe-like, circular and disc-shaped, containing fibers are rich in vitamin C, potassium and calcium. Nutritionally, the seed is used as traditional soup thickener [9], contain lipids, carbohydrates, proteins, crude fibre and the essential elements: Na, K, Mg, Ca, S, P and Fe [10]. The seeds are singly embedded within the hard fruits.

In view of the claimed medicinal and nutritional importance of this plant, there is need to study the efficacy and effects of *Detarium microcarpum* fruit in albino rats. This research is therefore meant to study the nutritional status and any harmful effect when the fruit of *D. microcarpum* is consumed.

MATERIALS AND METHODS

Materials

The Plant

The plants were identified at the Department of Plant Biology, Kwara State University using botanical field guides [11-13].

The Plant Fruits

Fruits of *Detarium microcarpum* were collected from Gbugudu village in Moro Local Government Area of Kwara State, Western Nigeria. The fruit mesocarp was separated from the seed by scrapping and both were air-dried and milled separately.

Experimental Animals

A total of thirty five (four weeks old) albino rats were purchased from the university of Ilorin animal house, and were acclimatized for one week in Kwara State University animal house inside wire mashed cages, fed with commercial rat chow (from Bendel feeds Nigeria Ltd) and water was supplied *ad libitum*.

Chemicals and Reagents

Urea, Creatinine, Electrolyte concentration (for calcium, potassium, chloride and sodium determination), bilirubin, ALP (Alakaline Phosphatase), ALT (Alanine Amino Transferase), AST (Aspartate Amino Transferase), GGT (Gamma Glutamyl Transferase) and albumin Kits were obtained from Randox Laboratories Ltd, Antrim UK. All other chemicals and reagents were of analytical grade and of high commercially available purity obtained from British drug house; poole, UK.

Methods

Animal Grouping

Thirty five (35) of four weeks old albino rats were randomly distributed into five dietary treatment groups of seven per group, acclimatized for one week, while food and water were supplied *ad libitum*. Below is the feeding ratio/formula for the four weeks:

Group A = Mesocarp + Rat feed* (ratio 1:2)
Group B = Mesocarp + Rat feed (ratio 2:1)
Group C = Seed + Rat feed (ratio 1:2)
Group D = Seed + Rat feed (ratio 2:1)
Group E = Rat feed

- = commercial rat feed from Bendel feeds Nigeria Ltd.

Determination of Organ: Body Weight Ratio

The experimental subjects were treated for four weeks after which they were weighed before sacrificing and then the organs (liver, kidney, and heart) were excised and weighed. The organ: body weight ratio for each animal was calculated as:

$$\frac{\text{Organ weight}}{\text{Body weight}}$$

Collection of Blood Samples

Blood samples of rats (at least 5ml from each rat) were collected through cardiac puncture after they have been anaesthesized by diethyl ether. The blood collected in syringe was released into lithium heparin bottles in other to prevent coagulation. Plasma was prepared by centrifuging the whole blood samples for 10mins at 4000rpm in a cold centrifuge. The clear supernatant (plasma) obtained was separated and kept frozen for estimation of renal and liver function tests.

Preparation of Tissue Homogenates (liver and kidney)

The liver and kidney were excised from each animal, rinsed in ice-cold 0.9% NaCl, blotted and weighed for Sub-Cellular fraction preparation.

Preparation of Sub-Cellular fraction

The liver and the kidney was weighed, macerated and homogenized with 5ml of ice – cold 0.25M Sucrose solution (pH 7.0), the homogenates were centrifuge at 12,500rpm for 15mins at 4^{o}c using an Eppendorf refrigerated centrifuge. The supernatant, termed the post mitochondria fractions (PMF) was obtained and stored frozen for subsequent analysis.

Renal Function Test

Plasma Creatinine Determination

Colorimetric Method of Bartels and Bohmer [14] was used.

Plasma Urea Determination

Colorimetric method used by Fawcett and Scott [15] was employed.

Determination of Electrolyte Concentrations

Calcium Concentration Determination

Colorimetetric method of Barnett [16] was used.

Sodium Concentration Determination

Sodium in serum and plasma is determined colorimetrically using the method of Henry [17].

Potassium Concentration Determination

Colorimeter method of Henry [17] was used.

Chloride Concentration Determination

Colorimetry method of Henry [17] was used.

LIVER FUNCTION TEST

Determination of Alanine Aminotransferase (ALT) Activity

Alanine amino transferase activity was determined by the colorimetric method of Reitman and Frankel [18].

Determination of Aspartate Amino Transferase Activity

Aspartate amino transferase activity was determined by the colorimetric method of Reitman and Frankel [18].

Determination of Alkaline Phosphatase Activity

Colorimetric method of Reitman and Frankel [18] was used.

Determination of Gamma glutamyl Transferase Activity

Gamma glutamyl transferase activity was determined by the colorimetric method of Tietz [19].

Determination of Albumin Concentration

Colorimetric method was used for the determination of Albumin concentration in the serum [19].

Determination of Direct Bilirubin Concentration

Colorimetric method of Reitman and Frankel [18] was used.

Determination of Total Bilirubin

The method described by Nwanjo [20] was adopted for the determination of bilirubin content in the animals.

Determination Protein Concentration

The method described by Plummer [21] was used to determine the protein concentration of the homogenates.

Statistical Analysis

Results were expressed as Mean ± SD. The data were analyzed by one way analysis of variance (ANOVA) and Duncan Multiple Range Test to separate treatment means. The p values of < 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Results

Only group A animals that was fed with M1:2R formula had significant growth while other groups (B, C and D) fed with different quantities of the fruit formula had a significant reduction in their growth compared with the control (group E) at the end of the experiment (Table 1).

The heart: body weight ratios of group E rats were significantly different with those of groups A and B whereas, it is not significantly different with groups C and D. However, the kidney: body weight ratio of group E rats was significantly different with all other groups (A-D) that fed with *D. microcarpum* fruit supplement. Furthermore, the liver: body weight ratio of group E rats was significantly different with those of groups B-D but there was no significant difference with group A (Table 2).

There was a significant decrease in the albumin concentration of all treated animal groups compared with the control, except group A animals, which was not significantly different. The bilirubin concentrations of all treated groups (A-D) were significantly higher than the control group E, whereas the bilirubin concentrations of groups A and B were significantly reduced compared with groups C and D (Table 3).

Furthermore, there was a significant decrease in the activities of AST, ALP, ALT, and GGT in the plasma of control group E compared with the treated groups A-D except the activities of ALP in group B and GGT of group C, which were not significantly different with the control (Table 3). There was no significant difference in urea concentration of animals in group A whereas there was a significant difference in groups B, C and D compared with the control group E. The creatinine concentrations of all the treated animals (groups A-D) were significantly different compared with the control group E. Moreover, there was a significant difference in the electrolyte concentrations in the treated animals, except group C, which had no significant difference in their potassium ion concentration compared with the control group E (Table 4). The protein concentrations in the liver of the treated groups B-D were significantly different from control group E, except group A which was not significantly different. Similarly, the protein concentrations in the kidney of all treated groups (A-D) were significantly different compared with the control group E (Table 5).

Table 1. Growth effect of *Detarium microcarpum* fruit supplement on albino rats (g)

Week	Groups				
	A (M1:2R)	B (M2:1R)	C (S1:2R)	D (S2:1R)	E (CONTROL)
0	99.5±13.26 [a]	111.0±9.48[a]	118.9±5.79[a]	118.9±10.79[a]	116.7±14.24[a]
1	101.0±1.48 [a]	111.4±0.33[a]	115±3.16 [a]	115.3±4.99 [a]	118.5±2.86[a]
2	105.2±3.48 [e]	97.9±3.21 [d]	96.8±1.47 [c]	90.4±3.67[b]	141.5±3.00 [a]
3	112.0±3.21[b]	88.1±1.23 [d]	87.5±1.67 [e]	99.2±3.56 [c]	161.4±4.77 [a]
4	126.1±6.57[b]	96.3±8.20[c]	78.9±5.52 [e]	90.8±12.29 [d]	173.9±6.43 [a]
Weight gained	26.6	-14.7	-40.0	-28.1	57.2

Results in Mean ± SD: of seven determinations.

M1:2R = Mesocarp 1:2 Rat mash

M2:1R = Mesocarp 2:1 Rat mash

S1:2R = Seed 1:2 Rat mash

S2:1R = Seed 2:1 Rat mash

Control = Commercial Rat feed only

Table 2. Organ: body weight ratios of rats fed with *Detarium microcarpum* fruit supplement

Groups	Organ: body Weight Ratio		
	Heart	Kidney	Liver
A (M1:2R)	0.004 ± 0.0005[b]	0.008 ± 0.0010 [b]	0.041 ± 0.0030 [a]
B (M2:1R)	0.004 ± 0.0007 [b]	0.008 ± 0.0018 [b]	0.050 ± 0.0040 [d]
C (S1:2R)	0.003 ± 0.0004 [a]	0.008 ± 0.0018 [b]	0.038 ± 0.0071[c]
D (S2:1R)	0.003 ± 0.0004 [a]	0.008 ± 0.0018 [b]	0.036 ± 0.0064[b]
E (Control)	0.003 ± 0.0003 [a]	0.007 ± 0.0010 [a]	0.040 ± 0.0030[a]

Results are Mean ± SD: of seven determinations.

Table 3. Liver Function Indices in Experimental Rats fed with *D. microcarpum* fruit supplement.

Biomarkers	Groups				
	A (M1:2R)	B (M2:1R)	C (S1:2R)	D (S2:1R)	E (Control)
ALB (g/dl)	0.9±0.1[a]	0.7±0.1[b]	0.6±0.2[b]	0.8±0.2[b]	1.0±0.1[a]
BIL (mg/l)	0.4±0.1[b]	0.4±0.1[b]	1.0±0.1[c]	1.1±0.1[c]	0.1±0.1[a]
AST (U/l)	34.4±7.1[b]	35.8±6.8[b]	51.8±3.3[d]	76.4±3.3[c]	34.4±14.0[a]
ALP (U/l)	12.4±2.2[b]	11.6±2.2[a]	20.4±7.1[c]	12.4±2.2[b]	11.6±2.2[a]
ALT (U/l)	17.6±5.9[b]	21.0±1.2[c]	33.0±2.7[e]	44.0±2.7[d]	14.4±1.8[a]
GGT (U/l)	63.0±2.7[b]	52.4±40.5[c]	8.0±2.5[a]	25.8±15.3[d]	18.6±2.2[a]

Values are expressed in Mean ± SD: of seven determinations.

Table 4. Renal Function Indices in Experimental Rats fed with *D. microcarpum* fruit supplement.

Biomarkers	Groups				
	A(M1:2R)	B(M2:1R)	C(S1:2R)	D(S2:1R)	E(Control)
Urea (g/l)	6.7±1.5[a]	5.3±0.5[d]	11.5±2.2[b]	14.4±2.6[c]	6.1±1.5[a]
Creatinine (mg/dl)	0.81±0.24[b]	0.83±0.67[c]	0.28±0.15[d]	0.46±0.30[e]	0.51±0.39[a]
Calcium (mg/dl)	19.2±17.7[b]	27.9±17.6[d]	25.2±7.8[c]	52.9±5.0[e]	21.1±11.1[a]
Sodium (mEq/L)	201±35.6[b]	125±78.7[c]	121±29.0[d]	100±44.0[c]	106±37.2[a]
Potassium (mEq/L)	4.0±0.8[b]	1.3±0.4[c]	2.3±1.2[a]	2.9±1.6[b]	2.2±1.2[a]
Chloride (mEq/L)	163±31[c]	123±20[d]	133±47[b]	116±36[b]	133±38[a]

Values are expressed in Mean ± SD: of seven determinations.

Table 5. Protein concentration Indices on Experimental Rat fed with *D. microcarpum* fruit supplement.

Organs	Groups				
	A(M1:2R)	B(M2:1R)	C(S1:2R)	D(S2:1R)	E(Control)
Liver	4.0±0.7[a]	3.4±0.7[d]	3.8±1.2[c]	4.9±2.2[b]	4.2±0.7[a]
Kidney	1.4±1.1[c]	1.4±0.7[b]	2.6±1.6[d]	4.2±3.1[e]	1.5±0.4[a]

Values are expressed in Mean ± SD: of seven determinations.

DISCUSSION

The observed significant reduction in the growth of rats fed with *D. microcarpum* fruit supplement may be due to the fact that the fruit may contain anti nutrients. Anti-nutrients can prevent the digestion and absorption of the food as expected, or the nutrient in the quantity consumed may not be made available for the growth of these rats, more so that the fruit was not cooked. Heat is capable of destroying some of the anti-nutrients that might be present in foods.Bilirubin is bounded to albumin and transported in the blood to the liver [22]. The observed reduction in the albumin of the rat fed with groups B, C and D may adversely affect the bounding and transportation of bilirubin in the blood of animals in the groups to their liver thereby reducing the amount of bilirubin in the liver. Martin [23] has reported that when the concentration of albumin is significantly reduced, the plasma osmotic pressure will be insufficient to draw water from the tissue spaces back into the plasma. Bilirubin is formed from the breakdown of erythrocytes and other haem-containing proteins. The more rapid the destruction of the red blood cell and degradation of haemoglobin, the greater the amount of bilirubin in the body fluid [24]. The significant increase in the concentration of bilirubin in the plasma of rats fed with fruit supplement may be due to hepatocellular jaundice, which may have been caused by the fruit supplement.

Serum analysis by biochemical indices is often employed to ascertain organs integrity following exposure to toxicological agents [25]. The significant elevation in the activities of plasma ALT, AST, ALP, and GGT following the administration of the fruit supplement may be an indication of liver injury. This may be attributed to the generation of free radicals which trigger chains of

reaction resulting in liver damage. The increase in serum activities of these enzymes might be due to their leakage into the circulatory system following alteration and permeability of hepatocyte membrane, reflecting a severe damage to the structural architecture of the liver [26-28]. These results are in conformity with earlier reports by Ghouri *et al* [29] who reported that elevated ALT may also be caused by dietary choline deficiency.

Electrolytes, urea, and creatinine are markers of kidney functions; and renal damage has often been associated with alteration in the levels of these parameters [30]. These indices also evaluate the functional capacity of the nephrons at the glomerular and tubular levels [31]. Creatinine, uric acid and urea are major catabolic products of muscle, protein and purine metabolism respectively. Urea and creatinine are waste products which are passed into the blood stream to be removed by the kidney. Increase in the levels of these waste products in the blood is an indication of renal dysfunction [32]. Therefore the high levels of creatinine and urea in the plasma of rats fed with *D. microcarpum* fruit supplement imply possible glomerular dysfunction. This result also agrees with the report of Ashafa *et al* [33], in which ingestion of aqueous leaf and berry extracts of *Phytolacca dioica* caused elevation in creatinine and urea concentration and this was linked with glomerular dysfunctions. This may cause a reduction in glomerular filtration rate leading to urea and/or creatinine retention. It may also be an indication of adversity in muscle creatinine.

The most common cause of electrolyte imbalance or disturbance is associated with renal failure [34]. Calcium ion plays a vital role in muscle contraction and serves as an intracellular second messenger for hormones. It is also important in nerve cells for effective transfer of nerve impulses and also for blood clotting [35]. Calcium ion concentration in the plasma of rats administered with *D. microcarpum* fruit (groups A-D) shows a significant increase compared with the control. This may adversely affect these vital roles play by Ca^{2+}.

Sodium and potassium are the major extracellular and intracellular cations respectively in living systems. Sodium regulates the total amount of water in the body and its transmission across cells plays roles critical to body functions while adequate level of potassium ions is essential for normal cell function. Many processes in the body, especially in the nervous system, muscles and renal selective reabsorption, require electrical signals for communication. The movements of these ions are critical in generation of these electrical signals. The observed significant increase in the plasma levels of sodium and potassium ions of the rat administered with *D. microcarpum* fruits supplement is suggestive of a relative increase in the amount of body water to sodium, a probable consequence of impaired selective reabsorption capability of the nephron of these rats. Chloride ion which is found in the fluid outside the cells and in the blood also plays a role in helping the body maintain a normal balance of fluids. Elevations in chloride ion may be seen in diarrhoea, certain kidney diseases, and sometimes in over activity of the parathyroid glands and chloride is normally lost in the urine, sweat, and stomach secretions. Excessive loss can occur from heavy sweating, vomiting, and adrenal gland and kidney disease. However, the observed significant increase in chloride ion level following the administration of the *D. microcarpum* fruit supplement might have resulted from adrenal gland and kidney dysfunctioning of rats fed with it.

CONCLUSION

The administration of *D. microcarpum* fruit supplement in rats, most especially groups B - D (M2:1R, S1:2R, S2:1R), significantly reduced their growth. Furthermore, the liver and kidney of these rats also compromised with this fruit supplement. This research therefore concluded that the eating of the fruits of *D. microcarpum* may not be completely saved and therefore, care should be taken on the consumption of the fruits.

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