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THE EFFECT OF (*Treculia Africana*) AFRICAN BREADFRUIT ON SERUM ELECTROLYTE ENZYME AND SOME HAEMATOLOGICAL PARAMETER IN RAT: A SUB-CHRONIC STUDY

O. O OKWARI, E.E. EDET AND F. E.UBOH

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ABSTRACT

A sub-chronic effect of diet of *Treculia africana (*Bread fruit BF) on serum electrolyte, enzyme and some haematological parameters in rats was studied. The test-diets were administered for 21 days after which, serum electrolyte enzyme and heamatological parameters were assayed. The control group were similarly treated but given normal diet The result showed that serum sodium (Na+) of rats given bread fruit diet of 10% and 20% respectively were significantly (P<0.001) and P<0.05) lower, but significantly (P<0.00) higher for those given BF of 100% when compared with control. The potassium and chloride ion levels in test and control groups were not significantly different. Calcium ion levels in the animals 10% and 20% respectively were not significantly different but the Ca^{2+} levels in those given BF 100% was significantly (P<0.01) lower compared with control. The bicarbonate ion levels were significantly (P<0.01) higher in test than control. Serum levels of ALT and ALP were significantly (P<0.001) higher in test than the control. The AST levels in animals given 10% and 20% BF were not significantly different but those given BF 100% was significantly (P<0.001) higher compared with control. The breadfruit diet when consumed without enriching food supplement decreased RBC, HB, PCV, MCHC indicating microcytic hypo-chronic anaemia and possible hepato cellular damage as shown in increase liver enzymes.

KEY WORD: Bread Fruit, enzyme, blood parameter and rats.

INTRODUCTION

African breadfruit (*Treculia Africana* Decne Var Africana) family Moraceae is one of the four members of the genera Treculia found in many parts of West and Tropical Africa around evergreen and deciduous forest, by streams and sometimes planted (Hutchison *et al*, 1958; Woodland, 1991). In Nigeria it is very common in the West and Eastern States. *Treculia africana* is an unbuttressed large tree 24 – 37m high with fluted bole up to 3m in girth. The fruits are yellow and spongy in texture up to 45.72cm inches in diameter and containing many small seeds 1.27cm long like orange-pips embebed in various depths in meshy elongated bract (Irvine, 1961).

Some of the local names of breadfruit in Nigeria are Yorubas – ofon; Benis – Ize; Igbos – Ukwa; Hausa – barafuta; Efik/Ibibios/Anangs called it ediang. The Ashanti in Ghana called it totin (Keay *et al*, 1964). Breadfruit seed have groundnut flavour when toasted; sometimes they are made into refreshing drinks or used as soup condiments or thickening, breadfruit cakes, snacks and cockies (Irvine, 1961). There is scanty literature on breadfruit. Edet and his colleages (1982) reported on the proximate fatty acid composition and nutritional value of the seeds and Akubor (1997) reported on the seed composition, while Okwari *et al,* (2007) reported on the antihistaminergic activity of the breadfruit seed diet on gastric acid secretion in rat.

It is a scientific knowledge that many substances taken into the body influence secretion and excretion of electrolyte and blood cell production. There have not been reports on how breadfruit affects serum electrolytes, enzyme and its impact on haematological parameters. The aim of this study is to find out the effect of breadfruit diet on electrolyte and enzyme profile and some haematological parameters in rats.

MATERIALS AND METHOD

Animals

Albino Wistar rats (120 – 180g) from the Department of Physiology, Faculty of Basic Medical Sciences, University of Calabar, Nigeria were randomly assigned into four groups of 6 rats in cages and maintained under standard laboratory conditions of humidity and temperature 28°C and 12 hour light and dark cycle. They were fed with rat chow and allowed drinking water for seven days before the start of the experiment. At the start of the experiment, group 1 received standard diet for 21 days and serves as control, Group II, III and VI received 10%, 20% and 100% breadfruit diet respectively and also for 21 days. The feeding regiment and cleaning were done daily between the hours of 9am and 10am. At the end of the study the animals were sacrified by an overdose of either and chloroform mixture and the blood was obtained by cardiac puncture. The obtained blood samples were stored in EDTA bottle and in plain sample ottle for serum and used for electrolyte and some enzymes assay and haematological findings.

O. O. Okwari, Department of Physiology, Faculty of Basic Medical Sciences, University of Calabar – Calabar, Nigeria
E.E. Edet, Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar – Calabar, Nigeria
F. E. Uboh, Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar – Calabar, Nigeria

Preparation of Breadfruit Diet

The breadfruit was purchase in Calabar, Cross River State and was identified in the Department of Botany, University of Calabar, Nigeria. The fruit was fermented and the seeds were freed from the slimy meshy mass and washed. The seed 1.5kg were then boiled for a few minutes 10 minutes and then transfer to a drying oven (Astell Hearson, England) set at 60°C. The dried fruits (1.2kg) were blended to power using (Philip Blender/Grinder England).

Table I: The composition of standard diet and test are as shown

Mineral content	Group I Control	Group II 10% BF	Group III 20% BF	Group IV 100% BF
Corn starch	150.00	150	150	-
African breadfruit	-	100	200	1000
Casilan	100.00			-
Sucrose	571.00	470	470	-
Glucose	50.00	50.00	50.00	-
Corn oil	50.00	50	50	-
Cellulose	18.00	80	80	-
Mineral mixture	40	40	40	-
Vitamin mixture	10	10	10	-
Total	1000	1000	1000	1000

Source: ICN Biochemical 1996.

Table 2: Composition of mineral and vitamin mixture

Mineral oil composition	Mg/100g	Vitamin mixture	Mg/kg
Ca^{2+} phosphate dibasic	500	Thiamine hydrochloride	600mg
Sodium chloride	74	Riboflavin	600mg
Potassium chloride	220	Pyrodoxin HCl	700mg
Potassium sulphate	52	Nicotinic acid	3mg
Magnesium oxide	24	D-Ca^{2+} panthothenate	1.6mg
Maganous carbonate	3.50	Folic Acid	200mg
Fenic citrate	6	D-Biotin	20mg
Zinc carbonate	1.6	Cynocobolamine	1.0mg
Potassium iodate	0.01	Vitamin D-3	200mg
Sodium selemite	0.01	Vitamin K	50mg
Chronum potassium	0.55		
Cupric carbonate	0.30		

Source: ICN Biochemical 1996* For Vit. Mixture riboflavin was added last and was thoroughly mixed to allow uniformity.

Electrolyte assay

The assay of sodium (Na^+) potassium (K^+) calcium (Ca^{2+}) was carried out using flame photometer (Petracourt model 410c England) while the chloride (Cl^-) and Bicarbonate (HCO_3^-) meter were used to determine these anions in the University of Calabar Teaching Hospital, UTCH, Calabar.

Haematology

The red blood cell and platelets counts were carried out by the method described by Baker, *et al* (1998). The improved neubauer was charge with 1:200 dilution and the cells were counted in 80 small squares, observing strictly the margin rule. Haemoglobin (Hb) content was done by cyanmethaemoglobin (Baker and Silverton, 1985). The result of RBC, Hb and PCV were employed for the determination of absolute value as described by (Baker and Silverton, 1985).

Statistical Analysis

The results were analysed by one way analysis of variance (ANOVA) followed by student t-test to evaluate the significance of difference between the mean values of measured parameters in respect of test and control groups. A significant difference was accepted at $P<0.05$.

RESULTS

The serum electrolytes (Table 1) shows that sodium (Na^+) levels of control, at 10%, 20% and 100% **BF** meal . The result show that the serum Na^+ levels of animals fed with 10% and 20% BF respectively were significantly ($P<0.001$) and ($P<0.05$) lower but the Na^+ levels in the 100% BF meal was significantly ($P<0.001$) higher compared with control. The potassium levels in test and control were not significant different. The calcium levels in control BF 10%, BF 20% and BF 100% were shown in the table respectively. Both BF 10% and BF 20% were not significantly different but the BF 100% was significantly ($P<0.01$) lower compared with control. The bicarbonate (HCO^-_3) ion level were significantly ($P<0.001$) higher in test than control while the chloride (Cl^-) ion levels were not significantly different.

Table 2 shows the results of serum enzymes: ALT, AST, ALP and the ratio of AST/ALT for control, BF 10%, BF 20% and BF 100% groups. Both ALT and ALP were significantly ($P<0.001$) higher than control. The AST level were see table respectively. The AST levels in BF 10% and BF 20% had no significant differences but BF 100% was significantly (P<0.001) higher than control.

The result of the haematolgoical studies showed that the RBC, platelets and Hb for control BF 10%, BF no significant differences when compared with control. But MCH and MCHC were significantly lower (P<0.01) when compared with control.

Table 1: Serum electrolytes in Breadfruit diet fed rats (mmol/L)

	Control	BF 10%	BF 20%	BF (100%)
Na^+	144.83±0.43	141.00±0.49***	142.00±0.97*	148.67±0.46**
K^+	5.11±0.02	5.25±0.27^{NS}	4.65±0.27^{NS}	5.10±0.15^{ns}
HCO_3^-	24.33±0.36	26.33±0.37**	26.83±0.77*	26.67±0.49**
Cl^-	101.67±0.83	100.50±0.37^{NS}	104.00±1.89^{NS}	100.50±0.37^{NS}

Values in X ± SEM; n = 6; NS = not statistically significant;
* = (P<0.05); ** = (P<0.01); *** = (P<0.001) compared to control.
BF = Breadfruit.

Table 2: Serum enzymes in breadfruit fed rats (mmol/L)

Enzymes	Control	BF 10%	BF 20%	BF (100%)
ALT	36.50±1.71	51.33±2.49***	52.33±2.85**	51.00±1.47***
AST	60.17±2.05	60.00±1.95^{NS}	60.00±1.95^{NS}	68.50±2.20*
ALP	342.60±2.59	379.90±6.96**	374.85±5.95**	429.80±5.07
AST:ALT ratio	1.65	1.17	1.15	1.34

Value in X ± SEM * = (P<0.05); ** = (P<0.01); *** = (P<0.001) compared to control n = 6; NS = not statistically significant;
BF = Breadfruit

Table 3: Effect of breadfruit diet on some haematolgoical parameters

Parameters	Control	10% BF	20% BF	(100%) BF
RBC (10^9 cell/L)	7.13±0.07	6.67±0.13*	6.66±0.14*	5.98±0.21***
Platelets 10^3cell/mm^3	125.8±1.68	61.67±1.83**	77.08±2.28**	96.6±2.71***
Hb (g)	14.47±0.15	12.32±0.19**	12.32±0.19**	12.68±0.44**
PCV (%)	45.75±0.72	45.17±0.82^{NS}	44.17±0.52^{NS}	43.83±0.87^{NS}
MCV (fl)	64.24±0.41	66.48±0.76^{NS}	65.59±0.94^{NS}	69.45±1.15^{NS}
MCH (pg)	20.23±0.09	17.92±0.72*	17.74±0.24**	21.82±0.49*
MCHC (%)	31.64±0.26	27.23±1.27**	26.69±6.69***	30.40±0.13**

Value are in X ±SEM, n = 6; * = (P<0.05); ** = (P<0.01) and
*** = (P<0.001); compared to control NS = not statistically significant; BF = Breadfruit

DISCUSSION

The serum sodium (Na^+) levels in 10% and 20% bread fruit fed animals were significantly (P<0.001 and P<0.05) lower but was significantly (P<0.001) higher in those fed with 100% bread fruit compared with control. This suggest that 100% bread fruit may attribute to the changes in levels of sodium. It has been reported that sodium imbalance may result from a loss or gain of sodium, or from a change in water volume as sodium affect body water distribution, maintain acid-base balance as well as influence chloride and potassium levels (Loeb *et* al, 2005). The potassium K levels were not significantly different in all the groups depicting equilibrium between the intracellular compartment. Potassium is vital to homeostasis. The major force maintaining the difference in cation concentration between the intracellular fluid and the extra cellular fluid is the activity of sodium potassium pump intergral to all cell membrane (Field et al 2006) this may explain the seeming difference in the breadfruit diet.

The calcium ion levels were not significantly different in 10% and 20% groups but were significantly (P<0.05) lower in 100% BF fed group. Breadfruit is rich in phosphate (Edet *et al*, 1982). The lower and the non – significance in calcium level may be due in part to intracellular adjustment, buffering and metabolic activity with phosphate present in breadfruit, although the phosphate level was not measured. The bicarbonate (HCO^-_3) ion levels were significantly higher in test groups than control groups while the chloride (Cl^-) ion level were not significantly different in the test and control groups. These ion levels among other events are responsible for the acid-base balance (Sembulingam and Sembulingam, 2007). Increase HCO^-_3 ion may be due to acute or chronic alkalosis (Edwards *et al*, 1996). The chloride and bicarbonate exchanger protein in the cell membrane is said to mediate the simultaneous movement of these two ions in opposite direction (Nelson and Cox, 2005). There exist an inverse relationship between the plasma Cl^- concentration and the rate at which the filtered HCO^-_3 is reabsorbed. The mechanism of this reciprocal relation is not well known. But one explanation may be the existence of the well known chloride shift in response to changes in HCO^-_3 to maintain the electrical neutrality between plasma and cell compartment. So as a consequence of the reciprocal relationship as plasma HCO^-_3 concentration rises, Cl^- concentration in the plasma falls. This inverse relationship serves to maintain the sum of the plasma concentration of HCO^-_3 and Cl^- constant (Nelson and Cox, 2005).

The ALT and ALP levels in the test groups were significantly (P<0.001) higher compared with control. But the AST levels were not significantly different in 10% and 20% groups but was significantly higher in 100% BF fed compared with control. Elevation of liver enzymes in serum are mostly associated with liver damage and the activities of either ALT or AST or both are often considered together (Chapman *et al*, 2006; Loeb *et al*, 2005). It has been hypothetised that in cardiac disorders, there is always a noticeable change in the levels of cardiac specific enzymes such as the amino transferases. Stroeve and Makarova (1984) reported that a high AST: ALT ration indicate pathology involving the heart and the reverse of this ratio points to the liver. hepatocellular injury such as active cirrhosis or drug induced hepatisis. Moderate rise in ALP are said to reflect biliary obstruction (Loeb *et al*, 2005).

The effect of breadfruit ingested for 21 days on haematolgoical parameter showed a reduction of RBC count when compared with the control (although within normal physiological ranges). This indicates that breadfruit has some mild influence in haemopoeisis, although the mechanisms were not investigated. Red blood cells transport oxygen to cell by haemoglobin (Hb) (Ganong, 2005; Guyton and Hall, 2000). The Hb content of the breadfruit fed rats was also significantly reduced. The reduction may be due to the decrease RBC count and possibly the mineral content of breadfruit. It may be that breadfruit has little iron content required for the Hb elevation in blood. The PCV of the breadfruit fed rats was not significantly different from the control. PCV is a measure of the number and size of the red blood cells (Lewis *et al*, 2002). The result indicates a reduction in number of RBC with corresponding decrease in size of the RBCS. This confirmed by the non-significant differences in the MCV. This may be due to the decreases in Hb, PCV and RBCs. Both MCH and MCHC were reduced significantly as result of the reduction of Hb, PCV and RBCS. Reduced MCHC indicates iron deficiency characterized by low PCV, Hb and RBC (Ganong, 2002; Cheesbrough, 2005).

Thrombocytopenic effect was observed in the test groups. The possible cause of the depressed platelet production may be diminished megakaryocytes resulting from ingestion of breadfruit (Guyton and Hall, 2001). Edet et al (1982) reported that breadfruit has low nutritional value.

In conclusion, whole breadfruit consumption may lead to increase in sodium serum level. It is known that in some diseases that affect the heart, liver and kidney, a train of events that set in often manifest clinical features of hypervolaemia. Volume expansion in some common systemic disease is associated with sodium and fluid overload resulting largely due to secondary responses to circulatory insufficiency associated with primary disorder (Field *et al*, 2006). The increase in serum enzyme points to possible hepatrophy, while decrease in RBC, Hb and MCHC points to possible microcytic hypochronic anaemia. Hence, the consumption of breadfruit should be highly regulated.

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