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Nutritional and hematological benefits of sub-chronic feeding of processed *Icacina senegalensis* tuber flours in experimental animals

Esien David-Oku[1], Oluwatosin Elizabeth Ntaji[1], Roseline Okokon Edide[1], Juliet Ifeoma Obiajunwa-Otteh[1], Godwin Christian Akuodor[2]

[1]Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, Calabar, Nigeria

[2]Depertment of Pharmacology and Therapeutics, Faculty of Medicine, Ebonyi State University, Abakaliki, Nigeria

ABSTRACT

Objective: *Icacina senegalensis* tuber is eaten during famine in some parts of Nigeria. We investigated the nutritional and hematological benefits of consuming this tuber flours.
Method: The tuber was processed by fermenting in water at room temperature (1:3 w/v), for 3 days, thereafter rinsed in clean water and divided into two portions. The first portion was oven dried at 40°C for 48 hours and blended into flour [soaked-dried (SD)]; the second was further boiled in water (1:3, w/v) for 1 hour, strained, oven dried at 40°C for 48 hours and blended into flour [soaked-boiled-dried (SB)]. Six diets containing 10%, 20%, and 30% of these two flours were formulated with normal rat chow (SD10, SD20, and SD30; SB10, SB20, and SB30). Forty-two young Wister rats were divided into seven groups (n = 6). Group 1 was fed with normal rat chow whereas groups 2–7 were fed with the respective formulated diets, for 30 days. Body weight of the animals was taken before and at the end of the feeding experiment. The animals were sacrificed; serum and whole blood samples obtained for biochemical and hematological analyses respectively, using standard methods.
Results: Average body weight of SD groups increased (9–31 g) whereas that of SB groups reduced (−10 to −14 g). There were significant improvements ($p < 0.05$) in blood glucose and total protein concentrations in all the test groups. Also, there were improvements (non significant) in red blood cell (RBC) counts and RBC indices; whereas the Platelet and white blood cell count decreased significantly ($p < 0.05$) in all experimental diet groups compared to the control group.
Conclusion: *Icacina senegalensis* tuber flours processed by fermentation and a combination of fermentation and boiling, improved the nutritional status and RBC indices of Wister rat. Further investigations are needed to confirm the safety of the flours in mammalian systems.

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Nutritional; hematological-indices; *Icacina senegalensis*; tuber flours

Introduction

Food insecurity is a major challenge in most African countries. It is a condition whereby there is inadequate or limited food for a continuously increasing population [1,2]. There are so many edible and nutritious plant crops in farmlands and forest reserves that are neither discovered nor maximally utilized. *Icacina senegalensis* is one of such under-exploited plants [3]. Belonging to the family of *Icacinacaea* [4], it grows naturally on both farm and fallow lands. It is cultivated especially in Senegal, Nigeria, and other parts of Africa [5], where it is eaten as emergency food in famine seasons. Every part of the plant is useful; the fleshy tuberous roots, seeds, and fruits are utilized as food, whereas the leaves are used as medicine [6–8]. Flour from the tuberous root is reported to contain protein (10.3%), carbohydrates (74.5%), fat (0.7%), calcium (150 mg/100 g), and iron (7 mg/100 g) among other nutrients [6]. The high starch content

Contact Esien David-Oku drdavidoku@gmail.com Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, Calabar, Nigeria.

Table 1. Feeding experiment design.

Experimental Group	Number of animals (*n*)	Percentage supplementation of *Icacina sengalensis* tuber flour
Normal control	6	Animal feed (100%)
SD10	6	10% of soaked-dried
SD20	6	20% of soaked-dried
SD30	6	30% of soaked-dried
SB10	6	10% of soaked-boiled
SB20	6	20% of soaked-boiled
SB30	6	30% of soaked-boiled

of this plant tuber provides an alternative source of dietary energy for animal feed [3] and broiler chickens [6,9]. The low toxic levels of some anti-nutrients (phytates, cyanates, and tannins) found in *Icacina senegalensis* tuber can be effectively removed by hot water [10]. Incorporation of up to 120 g/kg of processed (soaked/boiled) *Icacina senegalensis* into broiler (chickens) finisher diets was found to improve their feed value without adverse effects on their performance [6,9]. No such study has been reported for any mammalian model. This study was therefore designed to investigate the nutritional as well as hematological benefits of consuming graded doses of processed *Icacina senegalensis* tuber flours in experimental rat model.

Materials and Methods

Collection and identification of Icacina senegalensis *tuber*

The tubers were obtained from the wild in Akpabuyo local government, Cross River State. Identification and authentication were done by a Taxonomist in Botany Department, University of Calabar; a voucher specimen number 0620 was issued.

Preparation and formulation of Icacina senegalensis tuber flour diets

Icacina senegalensis tubers used for this study were peeled, washed, and cut into tiny pieces (about 2 cm in size). The pieces were soaked in water for 3 days with daily change of water, after which it was divided into two portions; Soaked-Dried (SD) and Soaked-Boiled-Dried (SB). SD portion was drained and oven dried at 40°C, while SB portion was boiled for 1 hour, thereafter strained and oven dried at 40°C.

Both SD and SB samples were then pulverized into respective flours and were formulated into diets containing 10%, 20%, and 30% of the respective flours by adding 90%, 80%, and 70% respectively of normal rat chow. For each supplemented portion, the flour and rat chow were blended together and prepared into a thick homogenous paste by adding boiled water. The resulting paste was pelletized manually and oven dried at 40°C. Each pelletized feed was stored in a capped container and labeled appropriately awaiting the feeding experiment.

Proximate composition of tuber flours

Proximate composition of the tuber flours was evaluated using standard methods of AOAC [11].

Animal grouping/feeding experiment

Permission for the use of Wistar rats in this study was obtained from the Animal Ethical Committee of Faculty of Basic Medical Sciences, University of Calabar. A total of 42 Albino Wistar rats used for this research were purchased from Department of Biochemistry Animal House, University of Calabar. The animals were allowed to acclimatize for 2 weeks in the animal house with a room temperature of about 23°C. The rats were then divided into seven groups (n = 6). Group 1 (normal/control) was fed with 100% normal rat chow, groups 2–7 were placed on the respective graded diets according to Table 1. The feeding was done for 30 days. The animals were given free access to food and water.

Weight change

The weight of each rat used for this experiment was taken on day 0 (before the beginning of feeding), days 15 and 30 (just before sacrifice). The mean weight and the body weight change were obtained.

Blood sample collection

At the end of 30-days experimental feeding period, the rats were fasted overnight and sacrificed the following morning by cervical dislocation method; blood samples were obtained from the heart by cardiac puncture into Ethylenediaminetetraacetic acid (EDTA) bottles (for hematological analyses) and plain tubes (for serum) using sterile needle and syringe.

Estimation of serum total protein and glucose concentrations

Serum Total protein concentration was estimated using the Biuret method described by Tietz [12]. Serum Glucose concentration was estimated using the enzymatic colorimetric test [glucose

Table 2. Proximate composition of processed *Icacina senegalensis* tuber flours.

Parameters	SD	SB
Moisture (%)	10.59 ± 0.7	6.28 ± 0.8*
Crude protein (%)	3.64 ± 0.6	5.03 ± 0.2*
Crude fat (%)	3.85 ± 0.04	1.56 ± 0.03*
Crude fiber (%)	8.27 ± 0.02	5.96 ± 0.52*
Dry matter (%)	88.41 ± 0.44	93.27 ± 0.91*
Crude ash (%)	3.82 ± 0.64	4.56 ± 0.02*
CHO (%)	70.5 ± 0.53	82.30 ± 0.75*
Energy (%)	328.71 ± 1.12	350.84 ± 0.22

Values are expressed as mean ± standard deviation. *Significantly different from SB ($p < 0.05$).

oxidase-phenol and 4 aminophenazone (GOD-PAP) reagent kit from Agappe Diagnostics, Switzerland] [13,14].

Evaluation of Hematological indices

Serum white blood cell (WBC) count, red blood cell (RBC) count, platelet count, hemoglobin count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and packed cell volume (PCV) were analyzed using an automated hematology analyzer (Mythic 22CT from Orphee) [15,16].

Statistical analysis

The results of this study were presented as mean ± Standard error of mean (SEM) and statistically analyzed using one-way analysis of variance with SPSS (version 20) window statistical software program. Student "*t*" test was used for pair-wise comparison, and differences were considered significant at $p < 0.05$.

Results

The result of proximate evaluation as shown in Table 2 shows that SD flour had significantly higher moisture, crude fat, and crude fiber content than SB. SB on the other hand was significantly higher in protein, dry matter, crude ash, Carbohydrate (CHO), and energy content, than SD flour.

The percentage body weight change of the animals at the end of the feeding experiment is as shown in Figure 1. There was a dose-dependent increase in body weight among the SD groups, with SD30 significantly higher ($p < 0.05$) than the control group. The animals fed with SB supplemented diets (except SB20) recorded significant weight losses.

The effects of dietary supplementation of processed *Icacina senegalensis* tuber flours (SB and SD) on serum glucose and total protein of experimental animals are as shown in Table 3. There were significant improvements in serum glucose of the SD and SB groups except for SD10 whose level was not significantly higher than the control group. All the dietary groups recorded improvements in serum

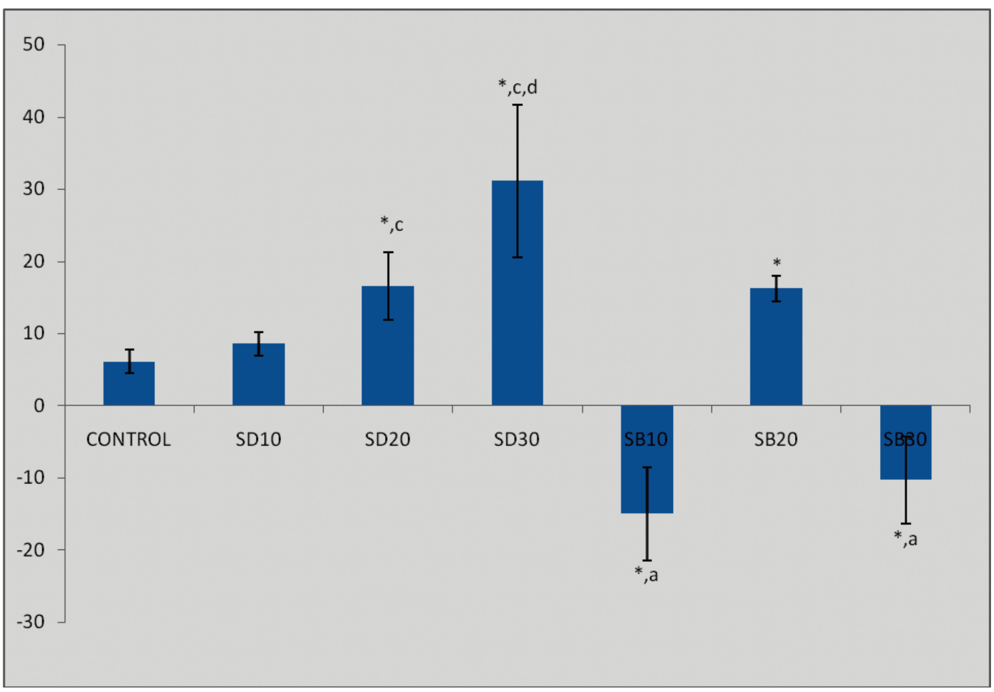

Figure 1. Percentage body weight changes in SD and SB groups. Values are mean ± SEM, $n = 5$; $^{a}p < 0.05$ *vs.* Control; $^{b}p < 0.05$ *vs.* SD10; $^{c}p < 0.05$ *vs.* SD20.

Table 3. Effect of SD and SB on blood glucose and total protein concentrations in the different experimental groups.

Experimental groups	Blood glucose (mg/dl)	Total protein (g/dl)
Control	47.0 ± 1.4	7.2 ± 0.16
SD10	52.9 ± 4.8	9.4 ± 0.24^{a}
SD20	68.1 ± 4.9^{a,b}	9.2 ± 0.18^{a}
SD30	83.1 ± 6.2^{a,b,c}	8.2 ± 0.40
SB10	74.8 ± 4.4^{a,b}	8.2 ± 0.17
SB20	68.7 ± 2.9^{a,b}	9.0 ± 0.18^{a}
SB30	74.1 ± 2.6^{a,b}	8.8 ± 0.18^{a}

Values are mean ± SEM, *n* = 5. $^{a}p < 0.05$ *vs*. Control; $^{b}p < 0.05$ *vs*. SD10; $^{c}p < 0.05$ *vs*. SD20.

Table 4. Effect of SD and SB on RBC, WBC, and PLT counts in the experimental groups.

Groups	RBC ($\times 10^6$ cell/µl)	WBC ($\times 10^3$ cell/µl)	PLT ($\times 10^3$ cell/µl)
Control	7.96 ± 0.42	24.8 ± 0.41	1175 ± 29.6
SD10	8.47 ± 0.02	20.8 ± 0.28^{a}	1052 ± 39.1^{a}
SD20	7.68 ± 0.12^{b}	15.7 ± 0.78^{a,b}	763 ± 16.4^{a,b}
SD30	7.62 ± 0.11^{b}	16.7 ± 0.83^{a,b}	961 ± 6.8^{a,b,c}
SB10	7.61 ± 0.11^{b}	16.9 ± 0.24^{a,b}	991 ± 15.6^{a,c}
SB20	7.14 ± 0.22^{a,b}	19.5 ± 0.75^{a,c}	1009 ± 32.0^{a,c}
SB30	7.46 ± 0.09^{b}	15.3 ± 0.19^{a,b}	854 ± 5.9^{a,b,c}

Values are mean ± SEM, *n* = 5. $^{a}p < 0.05$ *vs*. Control; $^{b}p < 0.05$ *vs*. SD10; $^{c}p < 0.05$ *vs*. SD20.

total protein, but SD10, SD20, SB20, and SB30 did much better.

Results of hematological assessment of experimental groups (Tables 4 and 5) show similar RBC but significant ($p < 0.05$) reduction in WBC and platelet counts when compared with the Control group. Quite understandably, the results on HB and PCV followed the trend of RBC with some improvements in the SD groups. The red cell indices, MCV, MCH, and MCHC increased significantly ($p < 0.05$) among all the experimental diet groups.

Discussion

Proximate and nutritional effects

The results of this study show that the moisture and crude fat contents of soaked-dried processed *Icacina senegalensis* flour (SD) were higher than those of soaked-boiled/dried processed flour (SB). This is attributable to the additional heat treatment (boiling), which is known to affect physicochemical properties of processed foods [17]. However, the fat content reported in this work is higher than that reported for *Icacina senegalensis* in Ghana [6]. Boiling further improved nutrient availability in SB flour as shown in its significantly higher content of carbohydrate, crude protein, fiber, ash, dry matter, and energy than SD sample. The fiber contents of SD and SB flours in this study are higher than those found in processed *Icacina senegalensis* seed [7,18]. Such levels of fiber could facilitate the reduction of blood total cholesterol while improving HDL cholesterol which is regarded as the good cholesterol [19]. The risks of bowel cancer [20,21] and gallstones formation [22,23] can also be reduced on consumption of these processed flours. The carbohydrate and energy levels of both SD and SB are higher than those earlier reported for *Icacina senegalensis* tuber and seed [7]. This implies that the tubers could be a good source of starch for human consumption, livestock feed, and industrial use [6,24]. The high protein, carbohydrate, and energy content of SD and SB flours account for significant weight gain, blood glucose, and protein observed among the experimental animals. Obesity, defined in a rat model [25] as increase in % body weight change of 25–45 over normal control, was found only in SD30 group. The mean fasting blood sugar reported in this study (53–83 mg/dl) shows that none of the dietary groups was diabetic [26]. Obesity is one of the risk factors that predisposes to diabetes [27]. But for SB20, it would seem that SB samples offer dietary treatment potential for obesity. However, the significant weight loss with concomitant increase in blood glucose observed in SB10 and SB30 fed groups (though not explained by our data) could mark the beginning of events that

Table 5. Effect of SD and SB on Hb, PCV, MCV, MCH, and MCHC in the experimental groups.

Groups	Hb (g/dl)	PCV (%)	MCV (fL)	MCH (pg)	MCHC (g/dl)
Control	13.7 ± 0.4	41.9 ± 6.3	61.8 ± 0.0	17.4 ± 0.2	28.3 ± 0.3
SD10	14.4 ± 0.2	51.2 ± 0.5^{a}	60.6 ± 0.6	17.5 ± 0.2	28.2 ± 0.1^{c}
SD20	15.1 ± 0.2^{a}	56.3 ± 1.3^{a}	72.8 ± 0.6^{a,b}	19.8 ± 0.1^{a,b}	27.1 ± 0.2^{a}
SD30	14.2 ± 0.1^{c}	51.4 ± 0.4^{a}	67.0 ± 0.7^{a,b,c}	18.8 ± 0.1^{a,b,c}	27.6 ± 0.1^{a}
SB10	13.6 ± 0.2^{b,c}	49.1 ± 0.4^{c}	64.0 ± 0.4^{a,b,c}	17.5 ± 0.1^{c}	27.3 ± 0.3^{a,b}
SB20	13.1 ± 0.2^{b,c}	46.5 ± 1.2^{c}	64.1 ± 0.3^{a,b,c}	18.5 ± 0.2^{a,b,c}	29.1 ± 0.6^{a,b,c}
SB30	13.5 ± 0.2^{b,c}	53.3 ± 0.4^{a}	71.1 ± 0.3^{a,b,c}	18.7 ± 0.1^{a,b,c}	25.7 ± 0.2^{a,b,c,d}

Values are mean ± SEM, *n* = 5. $^{a}p < 0.05$ *vs*. Control; $^{b}p < 0.05$ *vs*. SD10%; $^{c}p < 0.05$ *vs*. SD20%.

can lead to diabetes. It has been observed in human subjects that plasma glucose response to weight loss cannot be forecasted by initial clinical parameters [28]. Therefore further investigation is needed to establish a possible role of *Icacina senegalensis* tuber in dietary weight loss and raised blood glucose reported in this study.

Hematological changes

The result of this study showed that RBC and Hb concentrations in all the experimental groups were similar to the control group. However, the RBC indices (PCV, MCH, MCV, and MCHC) of both SD and SB groups were in most cases higher than the control. This is indicative of positive improvement in volume, size, and concentration of hemoglobin in the RBCs. Fe is a cofactor in the synthesis of Hb. The processing of *Icacina senegalensis* tubers in this study may have reduced certain anti-nutrients thereby making Fe to be absorbed more efficiently [28,29,30]. An earlier study [6], reported no adverse effects of processed *Icacina senegalensis* tuber supplemented meal on PCV and hemoglobin levels of poultry. The significant decrease in WBCs and platelet counts observed in this study on mammalian system is contrary to that observed in poultry [6,19–20]. Differential WBC count could have revealed the white cell type most affected and hence an insight to underlying cause. Unfortunately, this analysis was not done in this study. Platelet count is known to fall in liver cirrhosis but abnormal results can be obtained in conditions other than liver diseases [31,32]. Since the liver plays a central role in intermediary metabolism, it is readily impacted by nutrient toxicity. Such impacts as could be made by processed *Icacina senegalensis* tuber flours (unpublished data) are being considered for another report from our studies.

Conclusion

Processing of *Icacina senegalensis* tuber by a 3-day soaking in water on one hand, and further boiling of a portion of the soaked sample on the other, improved the nutrient quality of flours made from them. Graded dietary inclusion of these flours caused significant improvement in the nutritional status, RBC, and RBC indices of mammalian (rodent) model used in this study. However, the accompanied reduction in the levels of WBC and platelets need further investigation.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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