Original Research Article

Effect of Sorghum-Tigernut *Ibyer* (A Traditional Gruel) on the Fasting Blood Glucose Levels and Body Weight of Alloxan-Induced Diabetic Rats

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ABSTRACT

Background: There is growing interest in the use of natural foods in the management of chronic diseases like diabetes*.* *Ibyer* is a fibre rich gruel consumed amongst the Tiv people of Benue State made from whole sorghum or millet flours.

Aim: The aim of the study was to evaluate the effect of sorghum-tigernut *ibyer* on the fasting blood glucose levels and body weight of alloxan monohydrate-induced diabetic rats.

Methods: Sorghum flour (SF) and tigernut flour (TNF) were blended at different proportions (100:00; 90:10; 80:20; 70:30) for the purpose of *ibyer* production. The flour samples were subjected to proximate analysis using standard analytical procedures, the sensory attributes of *ibyer* produced from the different flour samples was evaluated on a 9-point hedonic scale. Thirty (30) male Wistar rats (100–180 g body weight) were grouped into five (1-5) each group containing six rats. They were induced with diabetes by injecting them with 150ml/kg of body weight with alloxan monohydrate dissolved in saline water (0.9% NaCl) except for group 1. Blood samples were collected from the tail of the rats, prior to induction, 48hrs after induction and 72hrs after three days of continuous feeding with test diet. Fasting blood glucose was measured using a standard glucometer and test strips.

RESULTS: The proximate composition of the flour samples showed a reduction in moisture (10.12-4.46), crude protein (7.45-6.25), and carbohydrate (76.10-70.53) but with a significant enhancement in the fibre content (1.00-3.00%), crude fat (3.22-12.86%) and crude ash (2.11-2.93%) depending on the level of substitution. The sensory attributes indicated that *ibyer* produced from the flour samples were generally acceptable though that produced from 10% tigernut flour addition was most preferred. Fasting blood glucose levels after 72hrs of feeding ranged from 5.52mml/L in group through to 5.45mml/L in group 5 with group 2 having the highest average fasting blood glucose levels of 17.60mml/L. the body weight changes were irregular in all diabetic rat groups.

CONCLUSION: The results indicated that sorghum-tigernut *ibyer* exerted hypoglycaemic effect on the experimental animals.

Keywords: Sorghum, Tigernut, Ibyer, Diabetic rats, Blood glucose level

1. INTRODUCTION

Diabetes is a chronic disease in which food (particularly the nutrient – carbohydrate) is not properly absorbed in the body to be used as energy [1]. Diabetes and hypertension are global health disorders afflicting millions of people worldwide with an ever-increasing incidence and prevalence the upsurge of diabetes in Africa has been linked with rapid urbanization and changing dietary habits [2, 3]. Use of indigenous foods has been advocated to reduce the incidence of chronic, diet-related non-communicable diseases such as obesity, diabetes, cardiovascular diseases and stroke [4]. The development of diabetes mellitus, obesity, cancer and cardiovascular disease (CVD) has been reported to be linked to the intake of high glycaemic index (GI) foods while intake of low GI foods has been shown to play a positive role in the management of these diseases [5].

Ibyer is an indigenous non-alcoholic gruel made from cereals (maize, sorghum and millet), consumed in Nigeria especially in Benue state by Tiv people [6]. It is prepared by cooking reconstituted whole cereal flour or paste in water and is often served with beans products such as *akpukpa* (native bread) and beans cake. Sour type (*ibyer-i-angen*) arises as result of the fermentation step that is undertaken before the porridge is prepared. The flour or wet milled paste is usually reconstituted with water to form slurry or paste after which it is left for a given time to enable fermentative microbial organisms to act on the product, producing the characteristic sourness associated with the product. Traditionally, the fermentation time lasts overnight.

Sorghum (*Sorghum Bicolor L. Moench*) is the fifth most important cereal after wheat, rice, maize and barley in terms of production and utilization. The total world annual sorghum production is over 60 million tons from cultivated area of 45 mile for food, alcoholic beverages and the grain is one of the staple foods for poor and rural people. Sorghum is gluten-free thus can be consumed by people with celiac diseases [7]. Other important nutrients of sorghum include; dietary fibre, fat-soluble and B-vitamins and minerals [8]. Sorghum flour is used for flours, porridge and side dishes, malted and distilled beverages and special food such as popped grain, its protein content is higher than many grains. Sorghum is rich in antioxidant which is believed to help lower the risk of cancer, diabetes, heart diseases and other neurological diseases.

Tigernut has been used extensively mainly for human consumption in Spain [9]. It was an important food in ancient Egypt [10]. Nowadays tigernuts are cultivated in Northern Nigeria, Mali, Senegal, Ahana and Togo where they are used primarily uncooked as a side dish [11]. The flour is a good alternative for other flour like wheat flour, as it is gluten free and good for people who cannot take gluten in their diets. It is also used to make cakes and biscuits and the oil is used for cooking [12]. The dietary fibre content of tigernut is effective in the treatment and prevention of diseases such as colon cancer, coronary heart diseases, obesity, diabetes and gastro-intestinal disorders [13]. This research was aimed at determining the effect of sorghum-tigernut '*Ibyer*' on fasting blood glucose levels of alloxan monohydrate induced diabetic rats.

2. 0 MATERIAL AND METHODS

2.1 Sample Preparation

The red sorghum grain variety and dried tigernuts were purchased from Wadata Market in Makurdi, Benue State, Nigeria. Alloxan monohydrate was purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2 Sample Preparation

2.2.1 Sorghum Flour: The preparation of sorghum flour was done according to the method described by [14]. Sorghum grains were dry-cleaned by handpicking, washed in a large volume of water to remove impurities and sun-dried. The dried sorghum grains were milled into sorghum flour using hammer mill.

2.2.2 Tigernut Flour: Tigernut flour was prepared according to the method described by [15]. Dry tigernuts (brown variety) were sorted to remove unwanted materials like stones, pebbles and other foreign seeds before washing with tap water. The cleaned nuts were sun dried to a moisture content of about 13% then milled and sieved.

2.3 Formulation of Flour Blends: the two flours (SF and TNF) were blended in a ratio of 100: 0, 90:10, 80:20 and 70:30 respectively.

2.4 Preparation of *Ibyer-i-angen*: After the preparation of the sorghum-tigernut flour blends, the flour samples were mixed with 200ml of water in five different containers with a cover and kept at ambient temperature (30 ± 2^{0}C) for 24hrs to allow natural fermentation take place. The fermented paste from the two flour blends was diluted with water and cooked in a tower aluminium pot containing about 80cl of water on a gas cooker. The mixture was stirred for about 7-8min to achieve desired consistency. For feeding to the animals, the prepared/cooked *ibyer* was oven dried and milled.
Flow chat for production of *ibyer-i-angen* is shown in figure.1

Flour blends (sorghum-tigernut flour blends)
↓
Mix with cold water
↓
Stir
↓
Fermentation (12-15hrs)
↓
Dilute with water
↓
Paste
↓
Mix paste in boiling water
↓
Stir
↓
Ibyer-i-angen

Fig.1: Traditional method of production of *Ibyer-i-angen*

2.5 Determination of Proximate Composition

The samples were separately analysed for moisture, ash, fat, and fibre contents respectively using standard methods of the Association of Official Analytical Chemists [16]. Crude protein contents (N × 6.25) were estimated from the crude nitrogen contents of the samples, as determined using Micro Kjeldahl method [17]. Carbohydrate content of the sample was determined by difference as described by [18].

2.6 Sensory Analysis

Sensory characteristics of *ibyer-i-angen* prepared from sorghum and tigernut was evaluated and assessed by 20 semi-trained panellists of Department of Food Science and Technology, University of Agriculture, Makurdi who are familiar with *ibyer-i-angen*. Fresh samples of cooked porridge/gruel were assessed for appearance, taste, mouth feel, flavour and general acceptability. The judges recorded the quality characteristics of each sample using nine-point hedonic scale, where 1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely as described by [19].

2.7 Animal Treatments

Thirty (30) male Wistar rats (100–180 g body weight) were obtained from the small Animal Experimental Unit of the National Veterinary Research Institute Vom, Plateau State. The rats were housed under standard hygienic conditions in metal cages with wood shavings as bedding. Rats were also kept under natural thermal environmental conditions with ambient temperature of 24 C–26 C and relative humidity of 70%–80%, and approximately alternating 12hr light/dark cycles. They were given access to a standard pelletized rat chow and water *ad libitum*. The animal experiments adhered to the Guide for the Care and Use of Laboratory Animals.

2.8 Induction of Experimental Diabetes

Diabetes mellitus (DM) was induced in overnight-fasted rats by a single i.p. injection of freshly-prepared alloxan monohydrate, dissolved in a cold physiological saline (0.9% NaCl) solution at the dose rate of 150 mg/kg body weight. The animals were given free access to 5% glucose solution in order to overcome the alloxan-induced hypoglycaemia for the first one-hour post-treatment with alloxan monohydrate. Blood glucose concentration of the rats was estimated 48hours after alloxan administration and DM was confirmed by analysis of blood samples, collected from the vein at the tip of the tail, using a portable blood glucometer and glucose test strips (On Call®Plus, Hannover, Germany). Animals with blood glucose concentration equal or more than 14 mmol/L were considered diabetic and used in the entire experimental group. Animals were weighed and randomly assigned to six groups and treated as follows:

Group 1, Control group: (normal saline only);
Group 2, Diabetic + Treated with Sample A (alloxan 150 mg/kg, i.p);
Group 3, Diabetic + Treated with Sample B (alloxan 150 mg/kg, i.p);
Group 4, Diabetic + Sample C (alloxan 150 mg/kg, i.p);
Group 5, Diabetic + Sample D (alloxan 150 mg/kg, i.p);

Experimental animals were fed thirty grams (30g) each of the test diet, after 48hrs of verification of diabetes, daily for 3 days.

2.9 Measurement of Blood Glucose

Experimental animals were rearranged according to the blood glucose concentration, except the control group, before commencement of treatment. Blood glucose concentration in all experimental groups were recorded following overnight fasting 72h after commencing the feed trials, using a portable glucometer (On Call®Plus, Hannover, Germany) and glucose test strips.

2.10 Measurements of Body Weight

Rats were weighed individually at Day 0 (before induction of DM), day 2 (post induction of DM) and 72 h (post-treatment with feed samples) using a digital precision weighing balance, and the body weights were recorded to calculate the body weight gains.

2.11 Statistical Analysis

Data collected were subjected to Analysis of Variance (ANOVA). Means were separated with Fisher's LSD using SPSS software (2009 model) and judged significantly different at 95% confidence level ($p<0.05$).

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Proximate composition of blends of sorghum and tigernut flours

Table 1 shows the results obtained for the proximate composition of blends of sorghum and tigernut flours. The values of the proximate composition expressed in percentage showed significant difference at $p<0.05$ between all the samples, however sample B (2.05), C (2.67) and also sample D (3.18) had significant difference in the fibre content, there was a difference in sample A (1.00) with the lowest value. Some of the parameters had a decreasing trend across the samples from A - D with moisture content ranging from 5.49 to 10.12, crude protein ranged from 6.28 to 7.45, and carbohydrate ranged from 72.11 to 76.10 whereas crude fat showed an increasing trend of 3.22 to 10.22 and crude ash increased from 2.11 to 2.72 having the lowest values of all the parameters with significant difference between the samples.

Table 1: Proximate composition of Blends of sorghum and tigernut flours

	Samples				
Parameters (%)	A	B	C	D	LSD
Moisture	10.12^{a}±0.02	8.23±0.01^{b}	6.23^{c}±0.01	5.49^{d}±0.01	0.02
Protein	7.45^{a}±0.00	7.19^{b}±0.00	7.01^{c}±0.00	6.28^{d}±0.01	0.02
Fat	3.22^{e}±0.01	4.84^{d}±0.00	8.43^{c}±0.00	10.22^{b}±0.00	0.01
Fibre	1.00^{d}±0.03	2.05^{c}±o.03	2.67^{b}±0.01	3.18^{a}±0.02	0.04
Ash	2.11^{d}±0.01	2.39^{c}±0.00	2.65^{b}±0.01	2.72^{a}±0.00	0.01
Carbohydrate	76.10^{a}±0.02	75.32^{b}±0.01	73.01^{c}±0.02	72.11^{d}±0.01	0.03

Mean with different superscript within the same column are significantly different at (P<0.05).
LSD: Least Significant Difference SF: Sorghum Flour; TNF: Tigernut flour.
A: 100% SF. B: 90% SF and 10% TNF C: 80% SF and 20% TNF D: 70% SF and 30% TNF

3.1.2 Sensory properties of Ibyer produced from blends of sorghum and tigernut flours.

The results of the sensory evaluation of *Ibyer* produced from blends of sorghum and tigernut flours are shown in table 2. The sensory qualities measured were appearance, mouth feel, taste, flavour and general acceptability. The results showed that the appearance ranged from 6.20 to 7.15 across the samples with sample D having the lowest, samples A, B and C having similar mean rating. Flavour properties ranged from 6.05 to 6.75, taste ranged from 6.50 to 6.90 and general acceptability ranged from 6.45 to 7.40 with no significant difference in all the samples. There was significant difference in sample C, and D but no difference in sample A and B with respect to mouth feel. All the samples were generally acceptable though sample B was most preferred.

Table 2: Results of the Sensory scores of *"Ibyer"* produced from blends of sorghum and tigernuts flour.

Sample	A	B	C	D	LSD
Appearance	7.150^{a}	7.050^{a}	6.850^{a}	6.200^{ab}	0.943
Flavour	6.650^{a}	6.750^{a}	6.750^{a}	6.050^{a}	1.171
Mouth feel	7.150^{a}	6.950^{a}	6.300^{ab}	5.600^{bc}	0.958
Taste	6.900^{a}	6.750^{a}	6.650^{a}	6.500^{a}	1.168
General acceptability	7.400^{a}	7.150^{a}	6.750^{a}	6.450^{a}	1.015

Mean with different superscript within the same column are significantly different at (P<0.05).

LSD: Least Significant Difference; SF: Sorghum Flour; TNF: Tigernut flour.
A: 100% SF. B: 90% SF and 10% TNF C: 80% SF and 20% TNF D: 70% SF and 30% TNF

3.1.3 Effect of Sorghum-tigernut Ibyer on the Fasting Blood glucose levels of alloxan induced diabetic rats.

The results of the fasting blood glucose levels of alloxan induced diabetic rats before and after feeding with test diets are presented in figure 2. The results showed that the average fasting glucose levels of the diabetic rats before feeding with the test diets ranged from 22.30mml/L to 32.00mml/L and 3.15mm/L to 6.05mm/L in the non-diabetic rats (control group). However, there was considerable reduction in average fasting blood glucose levels of the diabetic rats 0hrs through to 72hrs post treatment. The results ranged from 5.52mml/L from group 1 (control group) through to 5.45mml/L in group 5 with rats' group 2 having the highest average fasting blood glucose levels of 17.60mml/L

3.1.4 Body Weight of Rats before and after Feeding with Test Diet

Figure 3 presents results of body weight changes during the experimental period. Results showed steady weight increase in the non-diabetic rat group from 98.717 to 118.450g from 0hrs to 72hrs post feeding. However, the diabetic rat groups showed slight variations in body weight changes with groups 2 and 4 showing steady decrease in weight ranging from 151.075g to 175.017 and 154.100g to 171.700 in groups 2 and 4 respectively. However, groups 3 and 5 had body weight changes in no particular order. Group 4 showed an initial average body weight decrease from 165.94 to 147.72g 48hrs after induction and a subsequent increase from 147.72 to 153.660g 72hrs after feeding. While rat groups 5 showed an initial average weight gain from 166.925g at 0hrs to 175.500g 48hrs after induction but decreased after 72hrs to 155.025g.

Fig. 2. Fasting blood glucose levels of alloxan induced diabetic rats before and after feeding with test diet.
Group 1(control; normal saline) Group 2(sample A at 100%SF) Group 3(sample B at 90%SF and 10%TNF) Group 4(sample C at 80%SF and 20% TNF) and Group 5 (sample D at

70%SF and 30%TNF)
key:
SF; Sorghum flour
TNF: Tigernut flour

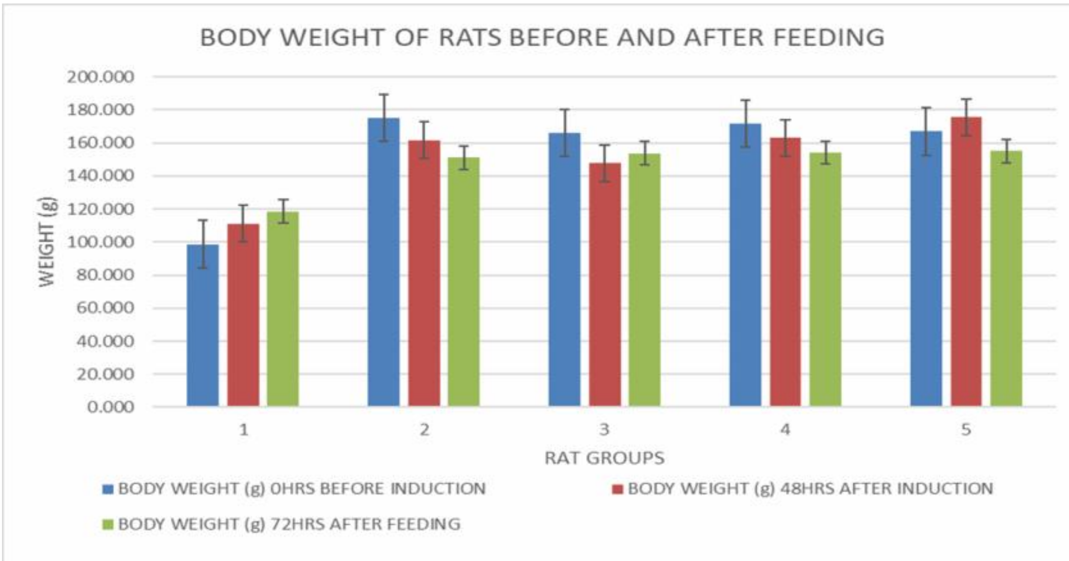

Fig. 3. The average body weight of the rats (grams).

Group 1(control; normal saline) Group 2(sample A at 100%SF) Group 3(sample A at 90%SF and 10%TNF) Group 4(sample C at 80%SF and 20% TNF) and Group 5 (sample D at 70%SF and 30%TNF)
Key: SF; Sorghum flour
TNF: Tigernut flour

3.2 Discussion

3.2.1 Proximate composition of sorghum and tigernut flour blends.

There was a significant (*P*<.05) difference in the moisture content of all the samples. The moisture content of the sample flour decreased with increase in the tigernut flour substitution. The gradual decrease in moisture content might be due to the low moisture content found in tigernuts. The result is in agreement with those of [20] who reported a decrease in moisture content in flour. Low moisture content is an indication of good keeping quality of any food product; it also reduces microbial growth thereby extending the shelf life of the product.

The study also showed that there was significant (*P*<.05) difference in protein content of the samples. The protein content decreased with increase in tigernut flour. This may be attributed to the low protein content of tigernut as reported by [21]. Protein content may also vary due to the genotype, temperature, soil and fertility and environmental conditions during plants development. The protein content of the four samples meets the recommended dietary allowance (RDA) of 0.8g of protein per kilogram of body weight per day [22].

The fat content of the samples increased steadily from samples A to D with increase in tigernut substitution. There was significant (*P*<.05) difference in the fat content of all the samples. Sorghum flour has been reported to contain fats of up to 4.25%, [18, 23]. The increase in the fat content could be attributed to the increase in the tigernut substitution as tigernut has been reported to be a rich source of fats [21, 24, 25, 26].

The fibre content of samples also increased with increase in tigernut substitution. These results were in accordance with [21] who reported an increase in the fibre content of tigernut-wheat flour with increasing tigernut flour substitution. There was a significant (*P*<.05) difference in the fibre content of all the samples.

There was also a steady increase in the ash content of the samples. There was a significant (*P*<.05) difference in the ash content of all the samples as the level of tigernut substitution increased. The ash content result is in agreement with the reports of [27] and comparable with those of [28]. Ash content is an indication of the mineral content in flour.

There was however, a decrease in the Carbohydrate content from Samples. There was a significant (*P*<.05) difference in carbohydrate content of all the samples. The decrease in carbohydrate content is attributed to an increase in tigernut flour.

3.2.2 Quality characteristics on the sensory attributes of Ibyer produced from blends of sorghum and tigernut flour.

There was no significant (*P*<.05) difference in appearance of the sample A, B and C but there was a significant (*P*<.05) difference in sample D. This could be attributed to the fact that tigernut has a colour that is distinct from that of sorghum and so as the amount of tigernut in each sample increased across the rows there would be a considerable change in the appearance of the products. It was however noted that the sensory scores for appearance were considerable high even though with slight differences. This means all products were appealing to the eyes.

There was no significant (*P*<.05) difference in the mouth feel of sample A and B but there was a significant difference in sample C and D. The differences in mouth feel could be attributed to higher amounts of fibre in samples C and D. *Ibyer* is a gruel made from whole cereals, particularly sorghum and millet, due to this it has a coarse feel when consumed.

There was also no significant (*P*<.05) difference among the samples in terms of taste, flavour and general acceptability. All the samples were generally acceptable indicating that *ibyer* of acceptable eating qualities can be produced from flour blends and sorghum and tigernuts. These results are in agreement with the reports of [6] who reported general acceptability of *Ibyer* produced from sorghum and soy addition meaning addition of other legumes to the product may not affect the eating qualities of the product.

3.2.3 Effect of sorghum-tigernut Ibyer on the Fasting Blood Glucose Levels Alloxan Induced diabetic rats.

Ibyer made from flour blends of sorghum and tigernut at different levels of substitution. The results showed that the average fasting blood glucose of the rats in the various groups differed significantly. After three days of consuming approximately 30g of the test diets daily, their fasting blood glucose levels dropped significantly. The results showed that rat groups fed with samples containing higher amounts of tigernut had lower fasting blood glucose levels than those of the control group. This could be attributed to the fact that tigernut is a rich source of fibre [29, 30]. Fibre has been reported to exert some hypoglycaemic effects in subjects with type II diabetes. It is also reported that arginine an amino acid (not determined) found in tigernut has the potential of stimulating the release of insulin thereby ameliorating the effect of diabetes [31]. This could be one of the reasons for the lowered blood glucose levels after three days of consecutive feeding with the test diet. Several studies have shown that sorghum extracts and sorghum rich diets exert hypoglycaemic effects in either human subjects or lab animals, thus agreeing with the findings of this research [32, 33].

4. CONCLUSION

The results of the proximate composition revealed that, inclusion of tigernut flour in to sorghum flour at levels of up to 30% resulted in notable increase in the fat, fibre and ash with the substitution of both samples while protein, moisture and carbohydrate decreased. The significant increase in fibre content could be nutritionally advantageous in diabetic diets.

Results of the sensory evaluation of *ibyer* produced from sorghum and tiger flour blends showed that all the samples were generally acceptable, though sample B produced from 90:10% sorghum and tigernut flour blends was most preferred. From the results, it is evident that nutritious diets can be formulated by complementing unexploited tuber and cereals like tigernut and sorghum respectively, such blends could be used to diversify their uses to develop new products.

The test diet was observed to have some hypoglycaemic effect on the experimental animals, however, mechanism of action of achieving these results was not studied and so remains unclear. Therefore, it would be worthwhile to further investigate the effect of this diet on blood glucose levels.

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