Original Research Article

Antidiabetic Potentials of Flour Composites of Finger Millet, Bambara Groundnut and 'Khain' (*Lecaniodiscus Cupanioides*) on Alloxan-Induced Diabetic Rats

ABSTRACT

Aims: This study is aimed at determining the effect of flour composites of Finger millet (FM), Bambara groundnut (BGN) and 'Khain' (Lecaniodiscus cupanioides) on the levels of glucose , lipids , total protein, albumin, urea, creatinine , renal and liver enzymes activity in bloods of alloxan-induced diabetic rats.
Study design: The experiment was designed using one-way analysis of variance (ANOVA).
Place and Duration of Study: Department of Food Science and Technology and Department of Home Science Nutrition and Dietetics, between May 2019 and July 2019
Methodology: Forty-eight alloxan-induced diabetic rats housed individually in metabolic cages were fed with the composite flours (Mixture of the three composites or each composite separately) while the controls were placed on commercial rat chow for six weeks. The blood glucose response, lipid profile, renal and liver enzymes were determined in diabetic rats.
Results: The diabetic rats groups fed with composite flours had significant ($p<0.05$) reduced blood glucose level (60-86 %) compared to the diabetic rats control group (35 %), significant reduced renal and liver enzymes levels compared to diabetic rats (control), weight loss, TCH (149.62 – 235.3mg/dl), TGL (97.96 – 153.72mg/dl), HDL-C (46.91 – 72.53mg/dl) and LDL-C (73.45 – 153.38mg/dl) values. Urea and creatinine values were within acceptable range.
Conclusion: Reduced renal and liver enzymes levels were observed in blood of diabetic rats indicated rats' livers were not damaged. reduced blood glucose level suggests that composite flours of Finger millet, Bambara groundnut and 'Khain' have the potentials to be used as health foods for the prevention and management of diabetes mellitus.

Keywords: *Antidiabetic, Lipids, Composite flours, Diabetes mellitus.*

1. INTRODUCTION

Diabetes mellitus is a chronic, incurable disease caused by insulin deficiency in the pancreases which affects the utilization of energy found in food by the human body. It can be genetic or gotten from wrong lifestyle choices [1] [2]. Diabetes mellitus poses health threat challenges to both developing and developed countries and is the fourth among the leading causes of mortality and ranked third of death caused by risk factor [3]. According to a compiled data of WHO, about 150 million people are diabetic globally which may double its size by the year 2025. It is evident that people in developing countries are most vulnerable due to over population, ageing, overweight, improper diet and sedentary lifestyle [2]. Owing to the high prevalence of this health challenge, the inaccessibility of insulin to the majority of the

population and ineffectiveness of conventional drugs in controlling the adverse side effects, research studies suggest that development of appropriate diets could help reduce the risk of diabetes complication and extend life expectancy [4] (Reformulate). Compounding health diets for such diet therapy can be obtained by diversifying the use of locally available low glycemic index cereals, legumes and plants fruits with the right composition and functional properties.

Finger-millet (*Eleusine coracana*) is used as whole grain, its rich dietary fibre and phenolics confer nutritional and health beneficial effects such as anti-diabetic, antioxidant and antimicrobial properties [5]. It is a millet variety cultivated in India and Africa. Finger millet is a staple for some tribes of the upland plateau of Nigeria [6] and is known as *ragi* (India) and in Nigeria as *tamba* (Hausa), *kpana* (Birom), *Sarga* (Kanuri) [7] [6].

Bambara groundnut (*Vigna subterranea* (L.) Verdc) is a less utilized legume crop widely cultivated in African countries [8]. Its dietary fibre has great potential for food and other applications [9].

Lecaniodiscus cupanioides is one of the underutilized large tropical perennial herbaceous vegetable plants widely distributed in Africa and Asia. It is of the *sapindaceae* family and is known by different names in Nigeria, such as *Ukpo/Ukpocha* (Igbo), *Utantan* (Edo), *Kafi-nama-zaki* (Hausa) and *Akika* (Yoruba) [10] [11] and also *Khain* (Jukun, middle belt) (Personal interview). It is a medicinal plant and a good source of nutrients especially of calcium, micro minerals, vitamins, fibre and disease fighting phytochemicals. These nutrients contributed to the esteemed ethno medical benefits of the plant in the treatment of wounds and sores, abdominal swelling caused by liver abscess, fevers, measles, hepatomegaly and burns [12] and also confer to its antioxidant, anti-inflammatory and anti-diabetic properties [13] [14] [15] [16].

Several studies have utilized blends of Finger millet/African Yam Bean/Wheat and Pearl millet/Bambara groundnut [17] [18] in the production of food, but there is paucity of information on the combination of Finger millet, Bambara groundnut and *'Khain'* (*Lecaniodiscus cupanioides)* seeds flours. This study therefore, seeks to investigate the anti-diabetic potential of flour blends of Finger millet, Bambara groundnut and *'Khain'* seed on (i) blood glucose response (ii) lipid profile and (iii) renal and liver enzymes of diabetic rats.

2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY

2.1 Procurement of raw materials

The raw materials Finger millet grains (*Eleusine coracana*) was purchased from Bukuru market in Jos, Plateau State Nigeria; Bambara groundnut seeds (*Vigna subterrenea*) was obtained from Mayo Lope market in Jalingo, Taraba State Nigeria; 'Khain' (*Lecaniodiscus cupanioides*) fruits were harvested from a forest in Lissam Sambo, Ussa, Taraba State Nigeria. Commercial rat chow (TOPFEEDS Broiler Finisher pellets, Batch no. 1661537, Premier feed mills CO. LTD., RC:791117, 1 Eagle flour Road, Lagos-Ibadan Expressway Toll point, Ibadan, Oyo State, Nigeria. Protein 18%, fat/oil 5%, crude fibre 5%, calcium 1%, lysine 0.85%, methionine 0.35%, salt (min.) 0.30% and metabolized Energy 2900Kcal/Kg) was obtained from Ogige market Nsukka, Enugu state Nigeria. Chemicals and reagents were procured from accredited chemical dealers.

2.2 Sample preparation

Finger millet grains and Bambara groundnut seeds were cleaned, sorted to remove stones, dirt, chaffs, weeviled seeds and other extraneous matters. Khain fruits were sorted to remove immature ones.

2.2.1 Preparation of finger millet (fm) flour

The preparation of Finger millet flour was done using the method of Jideani [19]. Finger millet seeds (10 Kg) were thoroughly washed using warm (65 ^{O}C) water, sun dried for 48 h, milled using attrition mill (Arttrition mill, De-Demark brand, model De-Demark super Gx 160.55), and passed through 600 µm sieve to obtain fine Finger millet flour. The flour was heat sealed in polyethylene pouches and stored at room temperature until used for analysis.

2.2.2 Preparation of de-hulled bambara groundnuts flour

Bambara groundnut flour was produced using the method described by Abdualrahman *et al.* [20]. The cleaned Bambara groundnuts (10 kg) seeds were soaked for 48 h, manually decorticated, sun dried, milled into fine flour (Arttrition mill, De-Demark brand, model De-Demark super Gx 160.55) and sieved using 600 µm mesh sieve to obtain uniform particle size flour. The flour was heat sealed in polyethylene pouches and stored at room temperature until used.

2.2.3 Preparation of 'khain' seed flour

The sorted cleaned 'Khain' fruits (10 kg) were sun dried (36 ± 2 ^{O}C), cracked to remove the seeds. The seeds were further sun dried, milled in an attrition mill (Attrition mill, De-Demark brand, model De-Demark super Gx 160.55) and sieved through a 600 µm mesh sieve (Reference). The flour was heat sealed in polyethylene pouches and stored until used for analysis and product formulation.

2.3 Blending of finger millet (fm), bambara groundnut (bgn) and khain (kh) flours

The following blends were formulated based on high water absorption capacity, swelling capacity and good pasting properties for dumpling production and were used for Bioassay. **FK_1** (95% Fm/5% Kh), **FK_2** (90% Fm/10% Kh), **FB_1K_1** (95% [90% Fm/10% BGN]/5% Kh), **FB_1K_2** (90% [90% Fm/10% BGN]/10% Kh), **FB_2K_1** (95% [80% Fm/20%bBGN]/5% Kh), **FB_2K_2** (90% [80% Fm/20% BGN]/10% Kh), **FB_3K_1** (95% [70% Fm/30% BGN]/5% Kh), **FB_3K_2** (90% [70% Fm/30% BGN]/10% Kh) (Reformulation, clear and write all abbreviations complete in the first time)

2.4 Experimental procedure

Eighty mature male albino rats of the Wistar strain of known body weights obtained from the Department of Zoology and Environmental Biology, University of Nigeria Nsukka were housed individually in metabolic cages (Animal house, Department of Nutrition and Dietetics, University of Nigeria, Nsukka). The arrival of the rats complied strictly to the arrive guidelines and all the experimental procedures were conducted in accordance with the U.K Animals (Scientific procedures) Act, 1986 and associated guidelines, E.U Directive 2010/63/EU for animal experiments. All the animals were fed with commercial rat chow (TOPFEEDS) and tap water for the first week for acclimatization, after which they were weighed again.

2.4.1 Inducement of diabetes mellitus in rats

The baseline blood glucose levels of the animals were determined before the inducement of diabetes with alloxan drug. The rat groups fasted overnight and were induced by administering alloxan monohydrate (150 mg/kg body weight, intraperitoneal) in normal saline. The rats were allowed to drink 5% glucose solution to avoid hypoglycemic effect of the drug. Blood samples were measured after three days of alloxan administration through tail tipping using glucometer (Accu-chek Active' Diabetes monitoring kit; Roche Diabetes care, Mannheim, Germany). The fifty-four rats with fasting blood glucose levels above 200 mg/dl were considered diabetic and they were divided into nine groups (6 rats / group). Eight diabetic rats groups (FK_1, FK_2, FB_1K_1, FB_1K_2, FB_2K_1, FB_2K_2, FB_3K_1 and FB_3K_2) were fed the formulated feed samples (Table 1), while a diabetic rats group (D-Control) and a non-diabetic rats group (N-Control) were fed commercial rat chow (TOPFEEDS). The animals were fed for four weeks and blood sample was obtained from each rat through tail tipping at a weekly interval and blood glucose were measured using

glucometer (Accu-chek Active' Diabetes monitoring kit; Roche Diabetes care, Mannheim, Germany). Weekly average body weights of the rats in each group (separately or collected) were measured using a weighing balance (Furi Electronic Scale, FEJ-600, Capacity 600 g).

2.5 Biochemical analysis

2.5.1 Collection of blood and preparation of serum

At the end of the experimental period (4 or 6 weeks or what), all rats were starved over-night and weighed. Each rat was anaesthetized with chloroform (Preferable diethyle ether) inside a desiccator before being sacrificed (How and need ethical approval). Blood sample was collected from each rat with a 5 ml syringe and needle by cardiac puncture and was transferred into clean EDTA and plain centrifuge tube as soon as it was collected, to prevent lysing. Part of the blood sample were centrifuged at 3000 rpm for 10 minutes (using centrifuge model what and how many rpm made in any country and clear at room temperature or cooling centrifuge), and then the serum (supernatant) was transferred into labeled sample bottles and stored at 4 ºC till used for the determination of enzymes activity. (Where the other part of blood and why devided)

Table 1: Composition of experimental diet for Bioassay (g/100g) (Reference)

Diet groups	Casein	Vit/Min. mix	Oil	Starch	Experimental diet	Total
FK_1	15	5	5	---	75	100
FK_2	15	5	5	---	75	100
FB_1K_1	15	5	5	---	75	100
FB_1K_2	15	5	5	---	75	100
FB_2K_1	15	5	5	---	75	100
FB_2K_2	15	5	5	---	75	100
FB_3K_1	15	5	5	---	75	100
FB_3K_2	15	5	5	---	75	100

key: fk1 = 95% fm/5% kh, fk2 = 90% fm/10% kh, fb1k1 = 95% (90% fm/10% bgn)/5% kh, fb1k2 = 90% (90% fm/10% bgn)/10% kh, fb2k1= 95% (80% fm/20% bgn)/5% kh, fb2k2 = 90% (80% fm/20% bgn)/10% kh, fb3k1 = 95% (70% fm/30% bgn)/5% kh, fb3k2 = 90% (70% fm/30% bgn)/10% kh (Not clear and need good presentation)

2.5.2 Determination of serum biochemistry (liver and renal function enzymes)

Blood serum was used for the evaluation of biochemical parameters, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), urea and creatinine, using commercial kits (Randox Laboratories, UK), according to the manufacturer's protocol.

2.5.2.1 Determination of serum alanine aminotransferase (alt) activity

Serum alanine aminotransferase (ALT) activity was determined by Reitman and Frankel [21] method.

2.5.2.2 Determination of serum aspartate aminotransferase (ast) activity

The activity of aspartate aminotransferase (AST) was determined by Reitman and Frankel [21] and Schmidt and Schmidt [22].

2.5.2.3 Determination of serum alkaline phosphatase (alp) activity

The alkaline phosphatase (ALP) activity was determined according to the Deutsche Gesellschaft fùr Klinische Chemie [23] method.

2.5.3 Determination of serum total protein (tp) concentration

Serum total protein (TP) concentration was determined using Tietz [24] method.

2.5.4 Determination of serum albumin (alb) concentration

Serum albumin (ALB) concentration was determined using the method of Grant *et al.* [25] and Doumas *et al.* [26].

2.5.5 Determination of urea in serum

Urea in serum was determined using the method of Orsonneau *et al.* [27].

2.5.6 Determination of creatinine in serum

Creatinine determination in serum was carried out by Jaffe's reaction as documented by Toora and Rajagopal, [28].

2.5.7 Total serum cholesterol

This was determined using the method described by Allain *et al.* [29].

2.5.8 Serum triglyceride

This was determined using glycerol phosphate oxidase method (enzymatic test) described by Jacobs and Van Denmark [30].

2.5.9 Serum high density lipoprotein cholesterol (hdl-c)

This was determined using dextran-sulphate method as described by Albers *et al.* [31].

2.5.10 Serum low-density lipoprotein cholesterol (ldl-c)

This was done using Friedewald formula as reported by Sood [32].

2.6 Statistical analysis

The data were analyzed using IBM SPSS Statistics version 23.0, the mean and standard deviation of the triplicate analyses was calculated. The one-way analysis of variance (ANOVA) was used to determine significant differences between the means, while the means were separated using the New Duncan Multiple Range Test (NDMRT) at $p<0.05$.

3. RESULTS AND DISCUSSION

3.1 Blood glucose response of diabetic rats fed with finger millet, bambara groundnut and *khain* (*Lecaniodiscus cupanioides*) composite flours

The blood glucose responses of diabetic rats fed with finger millet (FM), bambara groundnut (BGN) and *khain* (*Lecaniodiscus cupanioides*) composite flours for 6 weeks are shown in Figure 1.

The average blood glucose of all the rats before inducement with alloxan ranged from 91 – 130 mg/dl (week 1). With exception of the non- diabetic rats (N-control) group, significant ($p<0.05$) increases were observed in the blood glucose of the other rats groups 72 hours after inducement with alloxan at week 2, values ranged from 248 - 555 mg/dl indicating hyperglycemic condition. This was owned to the activity of alloxan which spanned up enormous amounts of free radicals (ROS) in the pancreatic beta cells, thereby destroying the insulin secreting cells and resulting to hyperglycemic condition [33].

At week 3, the blood glucose of diabetic rat groups fed with the composite flours (FK_1 to FB_3K_2) decreased significantly ($p<0.05$) from 207 – 101 mg/dl and continued in the same trend to the sixth week of the study (Figure 1). On the other hand, the diabetic rats (D-control) group showed significant ($p<0.05$) increase in blood glucose (266.3 - 472.3 mg/dl) at week 3. This group was not fed with the composite flours and exhibited hyperglycemic condition up to the sixth week contrary to the non-diabetic rats (N-control) group which were fed the same rat chow (Figure 1).

The lowering of the blood glucose levels of the diabetic rats fed with composite flours (FK_1 to FB_3K_2) could be due to the presence of fibre and phytochemicals in the composite flours (Where the chemical composition of Finger Millet, Bambara Groundnut and 'Khain' (Lecaniodiscus Cupanioides) to correlate their dietary fibre and or constituents effect on glucose response, lipids and enzyme). Since fibre cannot be broken down into sugar molecules, it therefore, passes through the body undigested and does not contribute to the body nourishment but promote health in diverse ways. The soluble fibre binds to fatty substances in the intestines and carries them out as waste, thus lowering low-density lipoprotein (LDL-C). It also helps in regulating the body's use of sugars, promote satiety and keep the blood sugar level in check. The insoluble fibre also helps in bowel movement, promoting regularity and helping prevent constipation. These make fibre important in weight management and control of diabetes [34].

Phytochemicals (phenolics, tannins among others) have hypoglycemic properties. Phenolics act as antioxidant, its activity enhances the pancreatic beta cell viability of alloxan induced diabetic rats [35]. Phytochemicals can reduce hyperglycemia by inhibition of enzymes activities. Tannins inhibit alpha-amylase activity while phenolics inhibit alpha-glucosidase activity thereby slowing down starch digestion and as a result control postprandial hyperglycemia [36] [37]. (Where the chemical composition of Finger Millet, Bambara Groundnut and 'Khain' (Lecaniodiscus Cupanioides) to correlate their phytochemical and or constituents (phenolic, flavonoids and fatty acid effects on hyperglycemia or glucose response, lipids and enzyme).

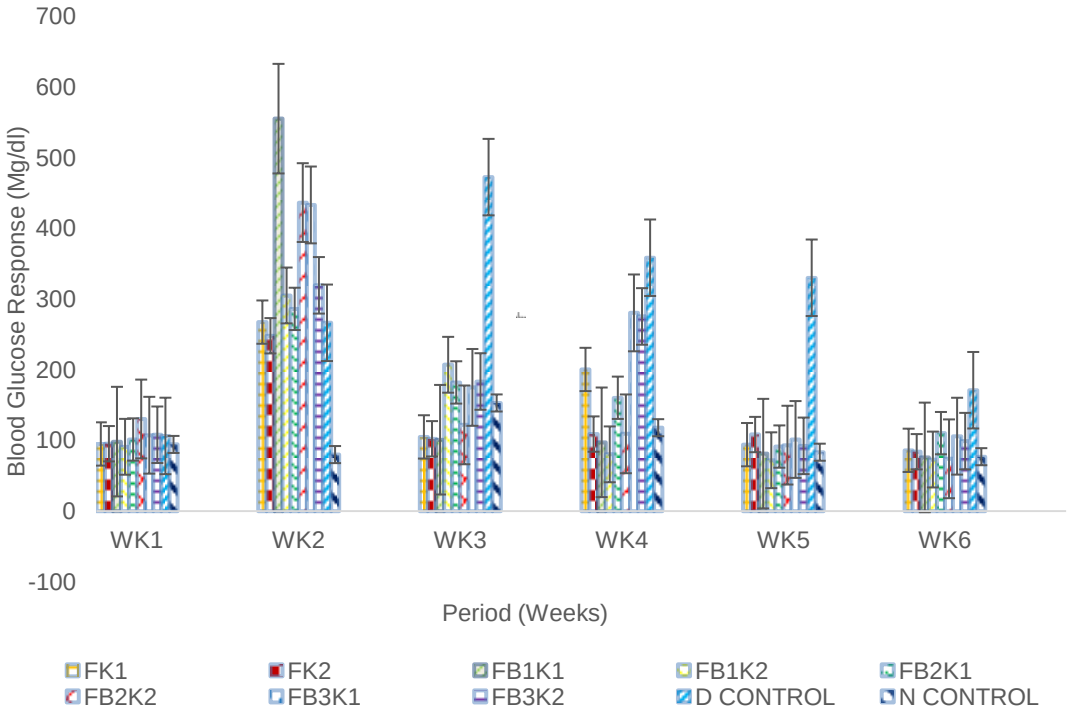

Figure 1: Blood glucose response of diabetic rats fed with finger millet, bambara groundnut and *khain* (*Lecaniodiscus cupanioides*) composite flours with standard error bars.

Key: FK1 = 95%FM/5%Kh, FK2 = 90%FM/10%Kh, FB1K1 = 95%(90%FM/10%BGN)/5%Kh, FB1K2 = 90%(90%FM/10%BGN)/10%Kh, FB2K1= 95%(80%FM/20%BGN)/5%Kh, FB2K2 = 90%(80%FM/20%BGN)/10%Kh, FB3K1 = 95%(70%FM/30%BGN)/5%Kh, FB3K2 = 90%(70%FM/30%BGN)/10%Kh, D control=Diabetic rats control, N control=Non-diabetic rats control (Not clear and need good presentation)

3.2 Blood glucose reduction (%) effect of finger millet, bambara groundnuts and *khain* (*Lecaniodiscus cupanioides*) composite flours on alloxan induced diabetic rats

The blood glucose reduction (%) effect of composite flours of finger millet (FM), bambara groundnuts (BGN) and *khain* (*Lecaniodiscus cupanioides*) on alloxan induced diabetic rats are presented in Figure 2.

Diabetic rats group FB_1K_1 (95 % [90 % finger millet/10 % BGN] and 5 % *khain*) had the highest percent blood glucose reduction (86.31 %), followed by diabetic rats group FB_2K_2 (90 % [80 % finger millet/20 % BGN] and 10 % *khain*) (83.04 %), while the D-control group (diabetic rats fed with rat chow) had the lowest (35.79 %) blood glucose reduction.

It was observed that significant ($p<0.05$) difference existed between the percentages of blood glucose reduction of diabetic rats fed with composite flours when compared with the diabetic rats (D-control) fed with commercial rat chow. This observation could be due to the presence and composition of fibre and phytochemicals in the composite flours when compared to the rat chow. However, there was also

significant ($p<0.05$) difference in the blood glucose reduction among the diabetic rats groups fed with the composite flours. This may be attributed to the varying phytochemical and fibre contents of the composite flours. Fibres and phytochemicals contributed to the hypoglycemic potentials of the composite flours of finger millet, bambara groundnuts and *khain*. Famakin *et al*. [38] reported >60 % reduction in blood glucose for plantain-base functional dough meal on diabetic rats.

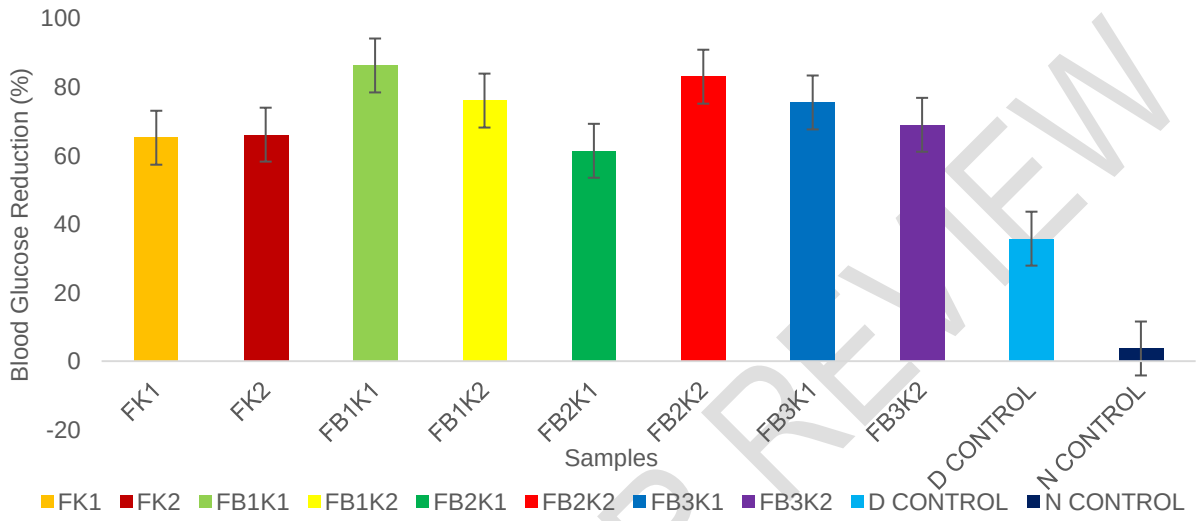

Figure 2: Blood glucose reduction (%) effect of composite flours of finger millet, bambara groundnuts and *khain* (*Lecaniodiscus cupanioides*) on alloxan induce diabetic rats with standard error bars

Key: Value with the same super script on the same column were not significantly ($p>0.05$) different. FK1 = 95%FM/5%Kh, FK2 = 90%FM/10%Kh, FB1K1 = 95%(90%FM/10%BGN)/5%Kh, FB1K2 = 90%(90%FM/10%BGN)/10%Kh, FB2K1= 95%(80%FM/20%BGN)/5%Kh, FB2K2 = 90%(80%FM/20%BGN)/10%Kh, FB3K1 = 95%(70%FM/30%BGN)/5%Kh, FB3K2 = 90%(70%FM/30%BGN)/10%Kh, D control=Diabetic rats control, N control=Non-diabetic rats control (Not clear and need good presentation)

3.3 Changes in the weights of the diabetic rats during the period of study

Changes in the weights of the diabetic rats during the period of study (What is the period how many weeks or months) are presented in Figure 3. The average weights of rats on arrival ranged from 72 – 137.2 g (Week 0). The weights of all the rats increased significantly ($p<0.05$) from 100 – 157.13 g after acclimatization period (Week 1).

At week 2, 72 hours after inducement with alloxan, the weights of the diabetic rats were not significantly ($p>0.05$) different from the previous weights at week 1. However, some of the rats gained slight weights while some showed slight weight lost. The slight loss of weights could be as a result of ill-health condition of the rats owned to alloxan inducement, leading to loss of appetite. The loss of weight of the diabetic rats continued significantly ($p<0.05$) to the third week. This was attributed to the association of diabetes with weight loss. Due to insufficient insulin, the body is prevented from getting glucose from the blood into the body's cells to use as energy. When this happens the body starts burning fats and muscles for energy, causing a reduction in the overall body weight. Also when blood sugars are very high, diabetics tend to pass excess urine and this leads to dehydration and consequently weight loss [39]. Weight loss of the diabetic rats continued to the sixth week but did not show significant ($p>0.05$) difference. Average weight loss of 5 – 33.8 % was observed among the various diabetic rats groups.

Loss of weight in diabetes is good, because losing 5 – 10 % of body weight can help the diabetic to reach and hold normal blood sugar levels without medication as observed in week 5 and 6 of Figure 1 [40] [41]. On the other hand, the non-diabetic rats group (N-Control) continued to gain weights throughout the study period (Figure 3).

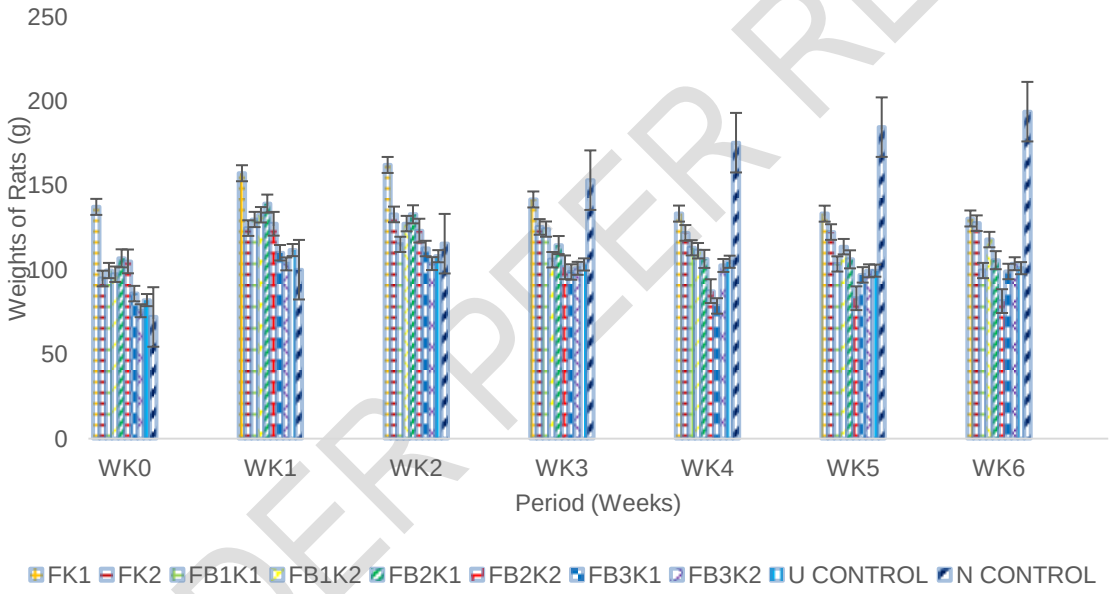

Figure 3: Changes in the weights of the diabetic rats during the period of study with standard error bars.

Key: Value with the same super script within the same group were not significantly ($p>0.05$) different. FK1 = 95%FM/5%Kh, FK2 = 90%FM/10%Kh, FB1K1=95%(90%FM/10%BGN)/5%Kh, FB1K2 = 90%(90%FM/10%BGN)/10%Kh, FB2K1= 95%(80%FM/20%BGN)/5%Kh, FB2K2 = 90%(80%FM/20%BGN)/10%Kh, FB3K1 = 95%(70%FM/30%BGN)/5%Kh, FB3K2 = 90%(70%FM/30%BGN)/10%Kh, D control=Diabetic rats control, N control=Non-diabetic rats control(Not clear and need good presentation)

3.4 Lipids (Due to not measures the all lipid parameters for eexample VLDL-C, PUSFA……etc) of diabetic rats fed with finger millet, bambara groundnut and *khain* (*Lecaniodiscus cupanioides*) composite flours

Table 2 showed the lipid profile of diabetic rat groups fed with finger millet, bambara groundnut and *khain* (*Lecaniodiscus cupanioides*) composite flours. There were significant ($p<0.05$) differences among the diabetic rats groups fed with the composite flours and the control groups fed with rat chow in all the parameters tested. Total cholesterol (TCH) values ranged from 124.66 - 235.35 mg/dl while low density lipoprotein (LDL-C) values recorded 54.73 - 153.38 mg/dl. Lower TCH and LDL-C values were observed in samples with higher bambara groundnut incorporation. Rats group FB_3K_1 (95 % [70 % finger millet/30 % BGN] and 5 % *khain*] recorded the lowest TCH (149.62 mg/dl) and LDL-C (73.45 mg/dl) values among the rats groups reflecting the effects of the presence of mono and polyunsaturated fatty acid in bambara groundnut (Where in the chemical composition of Finger Millet, Bambara Groundnut and 'Khain' (Lecaniodiscus Cupanioides) to correlate their phytochemical and or constituents (phenolic, flavonoids and fatty acid effects on hyperglycemia or glucose response, lipids and enzyme). Monounsaturated fats help to decrease level of LDL-C and increase levels of high density lipoprotein (HDL-C) in blood [42]. The levels of TCH and LDL-C in diabetic rats blood obtained in this study were within the acceptable level (200-239 mg/dl) and (100-159 mg/dl) respectively [43].

On the other hand, diabetic rat groups fed with composite flours containing higher *khain* (K_2) incorporation showed decreased high density lipoprotein (HDL-C) and Triglyceride (TGL) levels in blood. This was attributed to the antioxidant activity of the phenolics in *khain* (Kh). Phenolics had lipid lowering effect on HDL-C and the other serum lipids [44]. HDL-C and TGL values obtained in this study ranged from 46.91 - 72.53 mg/dl and 97.9 - 168.87 mg/dl respectively (Table 2) (Where in the chemical composition of Finger Millet, Bambara Groundnut and 'Khain' (Lecaniodiscus Cupanioides) to correlate their phenolic effects on hyperglycemia or glucose response, lipids and enzyme). Report showed that an HDL-C value of 60 mg/dl and above protects against heart disease while a value below 40 mg/dl is a sure risk of coronary heart disease [45]. TGL values below 150 mg/dl are within the normal range, while values between 150 - 199 mg/dl fall within the risk level. The non-diabetic rats group (N-Control) had the highest TGL value of 168.87 mg/dl (Table 2). Veeramalla and Madas, [43] also observed higher TGL levels in non-diabetic patients. Low TGL levels of the diabetic rat groups could be attributed to weight loss or possibly genetic [43].

Table 2: Lipids (mg/dl) of diabetic rats fed with finger millet, bambara groundnut and *khain* (*Lecaniodiscus cupanioides*) composite flours

Rat Groups	TCH	TGL	HDL-C	LDL-C
FK_1	$225.13^{b} \pm 3.26$	$151.34^{bc} \pm 1.25$	$72.53^{a} \pm 0.50$	$122.33^{b} \pm 2.62$
FK_2	$237.85^{a} \pm 3.15$	$116.24^{f} \pm 5.63$	$63.55^{c} \pm 0.76$	$151.06^{a} \pm 5.04$
FB_1K_1	$210.83^{c} \pm 1.18$	$135.49^{e} \pm 3.15$	$59.37^{d} \pm 0.69$	$124.36^{b} \pm 1.12$

FB_1K_2	$235.35^{a} \pm 2.52$	$119.54^{f} \pm 2.19$	$58.06^{e} \pm 0.27$	$153.38^{a} \pm 2.93$
FB_2K_1	$190.15^{d} \pm 1.36$	$146.37^{cd} \pm 1.69$	$53.44^{f} \pm 0.77$	$89.27^{c} \pm 1.65$
FB_2K_2	$222.12^{b} \pm 2.01$	$97.90^{h} \pm 5.19$	$50.94^{g} \pm 0.21$	$151.6^{a} \pm 2.11$
FB_3K_1	$149.62^{t} \pm 1.31$	$153.72^{b} \pm 2.63$	$70.14^{b} \pm 0.13$	$73.45^{d} \pm 0.36$
FB_3K_2	$169.94^{e} \pm 2.81$	$141.84^{d} \pm 2.16$	$46.91^{i} \pm 1.33$	$88.13^{c} \pm 2.08$
D-Control	$124.66^{g} \pm 4.21$	$107.47^{g} \pm 3.26$	$48.44^{h} \pm 1.25$	$54.73^{f} \pm 3.0$
N-Control	$151.06^{t} \pm 1.7$	$168.87^{a} \pm 3.06$	$49.05^{h} \pm 0.41$	$68.23^{e} \pm 1.49$

Key: Value with the same super script on the same column were not significantly ($p>0.05$) different. FK1 = 95%FM/5%Kh, FK2 = 90%FM/10%Kh, FB1K1 = 95%(90%FM/10%BGN)/5%Kh, FB1K2 = 90%(90%FM/10%BGN)/10%Kh, FB2K1= 95%(80%FM/20%BGN)/5%Kh, FB2K2 = 90%(80%FM/20%BGN)/10%Kh, FB3K1 = 95%(70%FM/30%BGN)/5%Kh, FB3K2 = 90%(70%FM/30%BGN)/10%Kh, D control=Diabetic rats control, N control=Non-diabetic rats control(Not clear and need good presentation)

3.5 Total serum protein, albumen, creatinine and blood urea nitrogen concentration in serum of the diabetic rats fed with finger millet, bambara groundnut and *khain* (*Lecaniodiscus cupanioides*) composite flours

Significant (P=.05) differences were observed in the total serum protein (TSP) of diabetic rats fed with composite flours of finger millet, bambara groundnut and *khain* (*Lecaniodiscus cupanioides*) as shown in Table 3. The TSP values ranged from 5.16 – 6.20 g/dl. Low TSP values were seen in the diabetic rat groups when compared with the non-diabetic (N-control) rat group (6.20 g/dl), this is because chronically elevated blood glucose is associated with kidney problems resulting in low TSP levels [46]. This is evident in the diabetic (D-control) group which had the lowest TSP value (5.16 g/dl), this could be due to prolonged hyperglycemia which could lead to kidney malfunction resulting to hyperfiltration or hypoproteinemia because plasma proteins are lost in the urine [46]. Hathama and Aymen [47] also reported significant decreased in TSP level of type 2 diabetes mellitus, indicating that lower TSP levels was associated with type 2 diabetes mellitus. Diabetic rat groups fed with the composite flours had TSP values within the recommended limits of 5.5 – 8.0 g/dl [48]. This was owned to the quick intervention of the dietary fibre and anti-nutrients in the composite flours to prevent prolonged hyperglycemia as earlier reported in figure 1.

There was no significant (P=.05) difference in the albumin levels of all the rat groups contrary to the observation of Hathama and Aymen [47] which reported decreased albumen in serum of diabetics when compared to non-diabetics. The albumin values ranged from 4.65 – 4.78 g/dl, showing that both the diabetics and non-diabetic rats groups recorded albumin values that are within the recommended limit of 3.5 – 5.5 g/dl [49]. These normal albumin values indicate that the liver was not damaged due to diabetes condition. (where the globulin content here and in methods)This achievement was accrued to the anti-diabetic potentials of fibre and phytochemicals in the composite flours (Where in the chemical composition of Finger Millet, Bambara Groundnut and 'Khain' (Lecaniodiscus Cupanioides) to correlate

their fibre or phytochemicals effect on hyperglycemia or glucose response, lipids and enzyme). Uncontrolled diabetes can lead to liver damage which in turn causes hypoproteinemia by decreasing synthesis of plasma proteins like albumin [46] [47].

Disparities (P=.05) were observed in the serum creatinine levels of the diabetic rat groups and the control groups (Table 3). Creatinine values ranged from 0.88 - 1.39 mg/dl, with the non-diabetic rat (N-control) group recording the highest serum creatinine level (1.39 mg/dl) while the diabetic rat group FK_1 (95 % finger millet/5 % *khain*) had the lowest value (0.88 mg/dl). It was observed that the diabetic rat groups fed with composite flours had lower serum creatinine levels than 1.24 mg/dl recorded for diabetic rat (D-control) group fed with rat chow. This may be due to the intervention of fibre and phytochemicals in the composite flours (Where in the chemical composition of Finger Millet, Bambara Groundnut and 'Khain' (Lecaniodiscus Cupanioides) to correlate their fibre or phytochemical constituents effect on hyperglycemia or glucose response, lipids and enzyme).... The soluble fibre binds to fatty substances in the intestines and carries them out as waste, thus lowering low-density lipoprotein (LDL) and keeping the gastrointestinal function healthy. It also adds bulk to stool and prevent constipation [34] [50]. Uncontrolled diabetes can damage the filtering system of the kidney and reduce the ability to clean waste from the blood, resulting to accumulation of creatinine in the blood [51]. High serum creatinine levels may be indicative of kidney disease and can be lowered by increasing dietary fibre intake [51] [52]. Serum creatinine values obtained in this study are within the normal serum creatinine levels (0.7 – 1.4 mg/dl) recommended for people with diabetes [51].

The blood urea nitrogen (BUN) showed significant differences (P=.05) among the diabetic rat groups and control groups as represented in Table 2. The BUN levels ranged from 7.15 - 23.25 mg/dl. The diabetic rats (D-control) group fed with rat chow recorded the highest BUN value (23.25 mg/dl), while the diabetic rat groups fed with the composite flours showed lower BUN levels. This could be attributed to the hypoglycemic potential of fibre and anti-nutrients in the composite flours. High BUN level of D-control rat group could be as a result of prolonged hyperglycemia (Figure 1), which leads to a decline in kidney function; this limits the ability to filter waste from the blood [53].

Higher BUN level (29.22 mg/dl) was reported for diabetic subjects in other studies [54], while Xie *et al*. [53] reported that a BUN over 25 mg/dl was associated with increased risk of incident diabetics mellitus. The BUN values obtained in this study fall within the acceptable range of 7 - 22 mg/dl, except for diabetic rats (D-Control) group that had a higher BUN value of 23.25 mg/dl slightly above the normal range, this signifies decrease in kidney function [55].The BUN/Creatinine ratio of test groups and control groups are presented in Table 3. The BUN/Creatinine ratio is a more accurate assessment for kidney function since both BUN and creatinine may have some limitations [56] [57]. A normal BUN to creatinine ratio is usually 10:1 to 20:1 [56] [57]. The BUN/creatinine ratio obtained in this study fall within the range 10:1 to 19:1, except for diabetic groups FK_1 (95 % finger millet and 5 % *khain*) and FB_3K_1 (95 % [70 % finger millet/30 % BGN] and 5 % *khain*) that recorded approx. 8:1. High BUN/creatinine ratios may be a result of sudden kidney failure, shock, and severe dehydration, among others, while low BUN/Creatinine ratio may be associated with liver disease (due to decrease in the formation of urea) and malnutrition [56].

Table 3: Total serum protein, albumen, creatinine and blood urea nitrogen concentration in serum of the diabetic rats fed with finger millet, bambara groundnut and *khain* (*Lecaniodiscus cupanioides*) composite flours

Test Groups	TSP (g/dl)	ALB (g/dl)	Creatinine	BUN (mg/dl)	BUN/Cr

			(mg/dl)		ratio
FK_1	5.50^{g}± 0.25	4.70^{de} ± 0.1	0.88^{h} ± 0.01	7.15^{g} ± 0.35	8 : 1
FK_2	5.70^{c}± 0.24	4.56^{g} ± 0.2	1.03^{f} ± 0.03	19.82^{b} ± 1.19	19 : 1
FB_1K_1	5.62^{d} ±0.10	4.66^{f}± 0.13	0.95^{g} ± 0.01	13.91^{de}± 0.37	15 : 1
FB_1K_2	5.86^{b}± 0.31	4.65^{f} ± 0.3	1.02^{f} ± 0.01	18.74^{b} ± 0.81	18 : 1
FB_2K_1	5.49^{h} ±0.13	4.76^{ab}± 0.11	1.21^{d} ± 0.01	14.81^{d} ± 0.52	12 : 1
FB_2K_2	5.56^{e}± 0.11	4.68^{ef}± 0.05	1.23^{c} ± 0.02	16.05^{c} ± 0.25	13 : 1
FB_3K_1	5.50^{g} ± 0.1	4.74^{bc} ± 0.1	1.19^{e} ± 0.01	9.67^{f} ± 0.73	7 : 1
FB_3K_2	5.51^{f}± 0.15	4.72^{cd} ± 0.1	1.23^{c} ± 0.01	12.84^{e} ± 0.53	11 : 1
D-Control	5.16^{i} ±0.1	4.78^{a} ± 0.1	1.24^{b} ± 0.004	23.25^{a} ± 0.26	19 : 1
N-Control	6.20^{a} ± 0.15	4.68^{et} ± 0.18	1.39^{a} ± 0.004	17.15^{c} ± 1.17	12 : 1

Key: Value with the same super script on the same column were not significantly ($p>0.05$) different. FK1 = 95%FM/5%Kh, FK2 = 90%FM/10%Kh, FB1K1 = 95%(90%FM/10%BGN)/5%Kh, FB1K2 = 90%(90%FM/10%BGN)/10%Kh, FB2K1= 95%(80%FM/20%BGN)/5%Kh, FB2K2 = 90%(80%FM/20%BGN)/10%Kh, FB3K1 = 95%(70%FM/30%BGN)/5%Kh, FB3K2 = 90%(70%FM/30%BGN)/10%Kh, D control = Diabetic rats control, N control=Non-diabetic rats control, U/C = Urea/Creatinine (Not clear and need good presentation)

3.6 Renal and liver enzymes levels in the blood of the diabetic rats fed with finger millet, bambara groundnut and *khain* (*Lecaniodiscus cupanioides*) composite flours

The Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) levels in the blood of both diabetic and non-diabetic rats groups showed significant (P=.05) differences as shown in Table 4. The diabetic rats groups fed with composite flours FK_1 to FB_3K_2 showed lower levels of AST (13.2 – 16.6 U/L), ALT (7.03 – 15.6 U/L) and ALP (47.45 – 77.97 U/L) alongside the non-diabetic rat (N-control) group, while the diabetic rats (D-control) group had the highest levels of the liver enzymes: AST (19.75 U/L), ALT (19.85 U/L) and ALP (132.07 U/L).

The low levels of liver enzymes among diabetic rat groups fed with composite flours could be attributed to the anti-diabetic potentials of fibre and phytochemicals (tannins, phenolics etc.) in the composite flours (Where in the chemical composition of Finger Millet, Bambara Groundnut and 'Khain' (Lecaniodiscus Cupanioides) to correlate their fibre or phytochemicals effect on hyperglycemia or glucose response, lipids and enzyme).... These had a quick intervention on the hyperglycemia of the diabetic rat groups. Diabetes is associated with a large number of liver disorders including elevated liver enzymes, acute liver failure among others [58]. Liver enzymes are released into the blood when the liver is damaged, thereby resulting to their high levels in the blood. The values of AST, ALT and ALP obtained in this study fall within the normal range for AST (5 – 40 U/L), ALT (7 – 56 U/L) and ALP (45 – 115 U/L) levels [59].

The AST/ALT ratios obtained are < 2:1, signifying that the liver is not damaged. The diabetic (D-control) rat group had an elevated ALP level (132.07 U/L) above the normal range. This was attributed to the fact that diabetes is associated with renal hyperfiltration which could result to ALP elevation [60]. Renal hyperfiltration indicates an increased glomerular filtration rate above normal values and is associated with early phases of kidney disease in the setting of various conditions such as diabetes and obesity [61]. Renal hyperfiltration has been observed in patients with newly diagnosed type-2 diabetes [60]. Vozarova *et al*. [62] and Mathur *et al.* [63] reported elevated ALP, AST and ALT among diabetics when compared to non-diabetics.

Table 4: Renal and liver enzymes levels in the blood of the diabetic rats fed with finger millet, bambara groundnut and *khain* (*Lecaniodiscus cupanioides*) composite flours

Sample	AST (U/L)	ALT (U/L)	ALP (U/L)
FK_1	$16.33^{b} \pm 0.33$	$11.27^{d} \pm 0.57$	$50.59^{g} \pm 0.94$
FK_2	$10.68^{f} \pm 0.34$	$7.73^{g} \pm 0.38$	$47.45^{h} \pm 0.17$
FB_1K_1	$14.53^{c} \pm 0.27$	$10.55^{e} \pm 0.18$	$68.24^{e} \pm 0.27$
FB_1K_2	$13.74^{d} \pm 0.23$	$8.87^{f} \pm 0.24$	$58.68^{f} \pm 0.38$
FB_2K_1	$16.61^{b} \pm 0.29$	$15.61^{b} \pm 0.24$	$72.53^{d} \pm 0.36$

FB_2K_2	$16.39^{b} \pm 0.22$	$11.95^{c} \pm 0.12$	$66.85^{e} \pm 0.37$
FB_3K_1	$13.64^{d} \pm 0.26$	$11.45^{cd} \pm 0.38$	$76.28^{c} \pm 0.37$
FB_3K_2	$12.45^{e} \pm 0.24$	$10.67^{e} \pm 0.13$	$59.48^{f} \pm 0.38$
D-Control	$19.75^{a} \pm 0.61$	$19.85^{a} \pm 0.06$	$132.07^{a} \pm 2.44$
N-Control	$13.20^{d} \pm 0.11$	$7.03^{h} \pm 0.38$	$77.97^{b} \pm 1.23$

Key: Value with the same super script on the same column were not significantly ($p>0.05$) different. FK1 = 95%FM/5%Kh, FK2 = 90%FM/10%Kh, FB1K1 = 95% (90%FM/10%BGN)/5%Kh, FB1K2 = 90% (90%FM/10%BGN)/10%Kh, FB2K1= 95% (80%FM/20%BGN)/5%Kh, FB2K2 = 90% (80%FM/20%BGN)/10%Kh, FB3K1 = 95% (70%FM/30%BGN)/5%Kh, FB3K2 = 90% (70%FM/30%BGN)/10%Kh, D control=Diabetic rats control, N control=Non-diabetic rats control (Not clear and need good presentation)

4. CONCLUSION

Composite flours of Finger millet (FM), Bambara groundnut (BGN) and *Khain* (*Lecaniodiscus cupanioides*) were found to lower blood glucose of diabetic rats when compared with the control. The hypoglycemic potential of the flour composites resulted to reduced renal and liver enzymes indicating that the livers of the rats were not damaged. Hence, composite flours of Finger millet, Bambara groundnut and *Khain* (*Lecaniodiscus cupanioides*) have potentials suitable for prevention and management of diabetes mellitus.

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

"All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee"

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