Effect of Composite Flours of Finger Millet, Bambara Groundnut and 'Khain' (*Lecaniodiscus Cupanioides*) on Blood Glucose Response, Lipids, and Liver Enzymes of Alloxan-Induced Diabetic Rats

Doris G. Ibrahim [a*], Ifeyinwa S. Asogwa [a], Eucheria U. Onwurafor [a] and Jane C. Ani [a]

[a] Department of Food Science and Technology, University of Nigeria Nsukka, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author DGI designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author JCA supervised the study. Authors ISA and EUO managed the analyses of the study, managed the literature searches, reviewed and edited the manuscript. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Aims: This study is aimed at determining the effect of composite flours of Finger millet (Fm), Bambara groundnut (BGN) and 'Khain' (Kh) (*Lecaniodiscus cupanioides*) on the blood glucose response, lipids and liver enzymes levels 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,University of Nigeria, Nsukka. Enugu State. May 2019 and July 2019
Methodology: Sixty rats were grouped into ten groups (six rats per group), housed individually in metabolic cages and were fed commercial rat chow for two weeks. After inducement with alloxan, eight groups of diabetic rats were fed with the composite flours [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%

*Corresponding author: Email: idoris170@gmail.com;

[70% Fm/30% BGN]/5% Kh), FB_3K_2 (90% [70% Fm/30% BGN]/10% Kh)] for four weeks while the two control (positive and negative) groups continued on commercial rat chow for four weeks also. The blood glucose response, blood lipids, total serum protein, albumin, urea, creatinine content, and liver enzymes were determined in the rats. The flours of Finger millet, Bambara groundnut and 'Khain' were analyzed for dietary fibre (DF) and phytochemical content.

Results: The total DF was higher (12.615 %) in finger millet and lower (5.375 %) in BGN. *Khain* had a value of 10.31 % total DF. Phenol content of samples ranged from 37.99 mg/100g for *khain* seed flour to 36.09 mg/100g in finger millet and tannin content of the samples ranged from 2.22 - 2.28 mg/100g. The diabetic rats groups fed with composite flours had significant ($p<0.05$) reduced blood glucose levels (60-86 %) compared to the positive control group (35 %), significantly reduced liver enzymes levels were observed in blood of diabetic rats groups fed with composite flours when compared to positive control group, diabetic rats groups fed with composite flours also showed weight loss, Total Cholesterol values of 149.62 – 235.3mg/dl, Triglycerides (97.96 – 153.72mg/dl), High Density Lipoprotein-Cholesterol (46.91 – 72.53mg/dl) and Low Density Lipoprotein-Cholesterol (73.45 – 153.38mg/dl) values. Urea and creatinine values of the diabetic rats groups fed with composite flours were within acceptable range.

Conclusion: The reduced blood glucose and liver enzymes levels in blood of diabetic rats fed with composite flours showed that composite flours of Finger millet, Bambara groundnut and 'Khain' have the potentials to be used as dietetics for the prevention and management of hyperglycemia.

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 among 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, research studies have suggested that developing appropriate diets could help reduce the risk of diabetes complication and extend life expectancy [4]. 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 [13-16].

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 effect of composite flours of Finger millet, Bambara groundnut and *'Khain'* seed on (i) blood glucose response (ii) lipid profile and (iii) liver enzymes of alloxan-induced diabetic rats.

2. MATERIALS AND METHODS

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. Chemicals and reagents were procured from accredited chemical dealers: Accu-chek active, (50 strips, Ref 07124112019 code 333, Lot 2020-5, 24695033, Roche Diabetes Care, GmtH Sandhofer Strasse 116 68305 Mannheim. Germany); Kermel Casein [(Soluble, light, white) AR 500g, MF C6H5*CH2*NH2 MW: 107.16; CASR No. (9000-71-9); Nitrogen content (Dried); Min. 12.0 %, LOD (105oC) 12%].

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

Freshly harvested 'Khain' fruits were sorted cleaned to remove dirts and immature fruits. About (10 kg) of the fruits were sun dried (36 ± 2 ^{O}C) for 3 days, the dried fruits were manually cracked to remove the seeds. The seeds were further sun dried and 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 (Personal interview). The flour was packaged and heat sealed in polyethylene pouches and stored at room temperature until used for analysis and product formulation.

2.3 Composite Flours of Finger Millet (Fm), Bambara Groundnut (BGN) and Khain (KH) Seeds

Composite flours of finger millet and Bambara groundnut (Fm [100% finger millet], FB$_1$ [90% finger millet/10% BGN], FB$_2$ [80% finger millet/20% BGN], and FB$_3$ [70% finger millet/30%BGN]) were blended with Khain at varing levels of 0 – 15 % using a 4x4 factorial in complete randomized design (CRD). This will not be discussed in this paper. 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% Finger millet/5% Khain), FK$_2$ (90% Finger millet /10% Khain), FB$_1$K$_1$ (95% [90% Finger millet/10% BGN]/5% Khain), FB$_1$K$_2$ (90% [90% Finger millet /10%

BGN]/10% Khain), FB_2K_1 (95% [80% Finger millet /20%Bambara Groundnut]/5% Khain), FB_2K_2 (90% [80% Finger millet /20% Bambara Groundnut]/]/10% Khain), FB_3K_1 (95% [70% Finger millet /30% Bambara Groundnut]/]/5% Khain), FB_3K_2 (90% [70% Finger millet /30% Bambara Groundnut]/]/10% Khain)

2.4 Experimental Procedure

Sixty 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

Fifty-four out of the sixty rats were induced with diabetes, while six rats were used as non-diabetic control (Negative-Control) group. 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 (Positive-Control) and non-diabetic rats group (Negative-Control) were fed commercial rat chow (TOPFEEDS). The animals were fed composite flours 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 were measured using a weighing balance (Furi Electronic Scale, FEJ-600, Capacity 600 g).

2.5 Experimental Diet Formulation

The diet was formulated to achieve at least 15 % protein, 5 % of minerals and 5 % fats as shown in Table 1. Casein, Vitamins/Minerals mixture and vegetable oil were used to supplement deficiencies in the composite flours.

2.6 Biochemical Analysis

2.6.1 Collection of blood and preparation of serum

At the end of the 6 weeks experimental period, all rats were starved over-night and weighed. Each rat was anaesthetized with diethyle ether inside a desiccator before being sacrificed. Blood sample was collected from each rat with a 5 ml syringe and needle by cardiac puncture (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) and was transferred into clean EDTA and plain centrifuge tube as soon as it was collected, to prevent lysing. The blood sample was centrifuged (800-1 lower speed centrifugal machine) at 3000 rpm for 10 minutes, and the serum (supernatant) transferred into labeled sample bottles and stored at 4 ^{O}C till used for the determination of enzymes activity.

2.6.2 Determination of serum biochemistry (liver 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.

2.6.2.1 Determination of serum alanine aminotransferase (ALT) activity

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

2.6.2.2 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].

2.6.2.3 Determination of serum alkaline phosphatase (alp) activity

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

2.6.3 Determination of serum total protein (TP) concentration

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

2.6.4 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].

2.6.5 Determination of urea in serum

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

2.6.6 Determination of creatinine in serum

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

2.6.7 Total serum cholesterol

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

2.6.8 Serum triglyceride

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

2.6.9 Serum high density lipoprotein cholesterol (HDL-C)

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

2.6.10 Serum low-density lipoprotein cholesterol (LDL-C)

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

2.7 Determination of Phytochemicals

2.7.1 Determination of alkaloid

The Gravimetric method of Harborne [33] was used;

2.7.2 Determination of Saponin

Saponin content of the sample was determined by the method of AOAC [34].

2.7.3 Determination of tannin

The Folin-Denis colorimetric method as described by Kirk and Sawyer [35] was used for the determination of tannin.

2.7.4 Determination of oxalate

The titration method of AOAC [34] was used.

2.7.5 Determination of total phenols

Total phenolic content was determined according to the Folin-Ciocaltaeu assay (Sadasivam and Manickam [36].

2.7.6 Determination of phytic acid

The colorimetric method described by Haug and Lantzseh [37] was used to determine phytic acid content of the samples.

2.8 Determination of Dietary Fibre

The method of AOAC [34] was employed to determine the dietary fibre content of the flour samples.

2.9 Statistical Analysis

All analyses were carried out in triplicate. Statistical significance established using one-way analysis of variance (ANOVA), and data were reported as the mean and standard deviation. Means separation was done using the New Duncan Multiple Range Test (NDMRT) at $p < 0.05$. Statistical analysis was carried out using IBM SPSS Statistics version 23.0.

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

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$_1$K$_1$	15	5	5	---	75	100
FB$_1$K$_2$	15	5	5	---	75	100
FB$_2$K$_1$	15	5	5	---	75	100
FB$_2$K$_2$	15	5	5	---	75	100
FB$_3$K$_1$	15	5	5	---	75	100
FB$_3$K$_2$	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

3. RESULTS AND DISCUSSION

3.1 Dietary fibre and Phytochemical Content of Finger Millet, Bambara Groundnut and *Khain* (*Lecaniodiscus cupanioides*) Seed Flours

Dietary fibre and Phytochemical content of finger millet, bambara groundnut and *khain* (*Lecaniodiscus cupanioides*) seed flours are presented in Table 2.

Statistically, significant (p<0.05) differences were seen in the dietary fibre content of all the parameters measured among samples as shown in Table 2. Soluble dietary fibre content of the samples ranged from 0.53 - 1.71 % with finger millet recording the highest value while *khain* had the lowest. Bambara groundnut recorded value of 1.15 % for soluble DF which was quite higher than 0.5 % reported for same [38]. Soluble Dietary Fibre (SDF) fractions are valued components in foods because they trap fatty substances in the gastro-intestinal tract thereby, reducing cholesterol level in the blood, lower the risk of heart disease, reduce postprandial blood glucose, and insulin contents in the human body [39]. The values for insoluble dietary fibre ranged from 4.225 % for BGN to 10.905 % for finger millet. *Khain* recorded a value of 10.310 %. The total DF was higher (12.615 %) in finger millet and lower (5.375 %) in BGN. *Khain* had a value of 10.31 % total DF which was higher than what was contained in BGN.

Phenol content of samples ranged from 37.99 mg/100g for *khain* seed flour to 36.09 mg/100g in finger millet as shown in Table 2. BGN and *khain* seed flour have similar (p>0.05) phenol content. Phenols possess anti-diabetic, anti-microbial and anti-oxidant properties [40-42]. Phenols are responsible for the partial inhibition of amylase and α-glucosidase during enzymatic hydrolysis of complex CHO and delay the absorption of glucose which eventually controls the post prandial blood glucose levels [41]. Tannin content of the samples ranged from 2.22 - 2.28 mg/100g as shown in Table 2. BGN recorded the highest tannin value while finger millet had the lowest. Significant (p<0.05) differences were seen among the tannin content of the samples. Tannin acts as antioxidants in protecting the body against cell damage by neutralizing chemicals. Tannin also has antimicrobial, anti-cancer and positive effect on cardiovascular system [43]. Phytate content of the flours ranged from 5.56 - 5.63 mg/100g with BGN having the lowest value which differ significantly (p<0.05) from finger millet and khain seed flour in their phytate contents. Saponin content of samples ranged from 2.09- 2.98 mg/100g with *khain* seed flour recording the highest while BGN recorded lowest value. Results are significant statistically (p<0.05) among samples. Saponin may reduce occurrences of cancer and can lower cholesterol and post-prandial blood glucose response [44]. BGN had the highest oxalate content (11.29 mg/100g) followed by finger millet (11.11 mg/100g) while *khain* seed flour recorded the lowest (10.73 mg/100g). These values differ significantly (p<0.05) from each other. Alkaloids content of the samples ranged from 0.31 - 0.32 mg/100g. BGN recorded the highest while *khain* seed flour and finger millet have similar value. Samples were not significantly (p>0.05) different from each other.

Table 2. Phytochemical and Dietary fibre content (mg/100g) of finger millet, bambara groundnuts and *khain* (*Lecaniodiscus cupanioides*) seed flour

PARAMETERS	FM	BGN	*KHAIN*
Alkaloids	0.31 [a] ±0.01	0.32 [a] ±0.00	0.31 [a] ±0.01
Saponins	2.38 [b] ±0.01	2.09 [c] ±0.01	2.98 [a] ±0.01
Tannins	2.22 [c] ±0.00	2.28 [a] ±0.01	2.26 [b] ±0.01
Oxalates	11.11 [b] ±0.05	11.29 [a] ±0.02	10.73 [c] ±0.1
Phytates	5.63 [a] ±0.01	5.56 [b] ±0.01	5.63 [a] ±0.02
Phenols	36.09 [b] ±0.59	37.64 [a] ±0.47	37.99 [a] ±0.11
Soluble DF (%)	1.71 [a] ± 0.01	1.15 [b] ± 0.05	0.53 [c] ± 0.03
Insoluble DF (%)	10.905 [a] ± 0.005	4.225 [c] ± 0.025	10.31 [b] ± 0.01
Total DF (%)	12.15 [a] ± 0.015	5.375 [c] ± 0.075	10.84 [b] ± 0.04

Key: Value with the same super script on the same row were not significantly (p>0.05) different. FM – Finger millet, BGN – Bambara groundnut, KHAIN – Lecaniodiscus cupanioides, DF = Dietary fibre

3.2 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 during the 6 weeks study period are shown in Fig. 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 (Negative-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 [45].

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 (Fig. 1). On the other hand, the diabetic rats (Positive-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 (FK_1 to FB_3K_2) and exhibited hyperglycemic condition up to the sixth week contrary to the non-diabetic rats (Negative-control) group which were fed the same rat chow (TOPFEEDS) (Fig. 1).
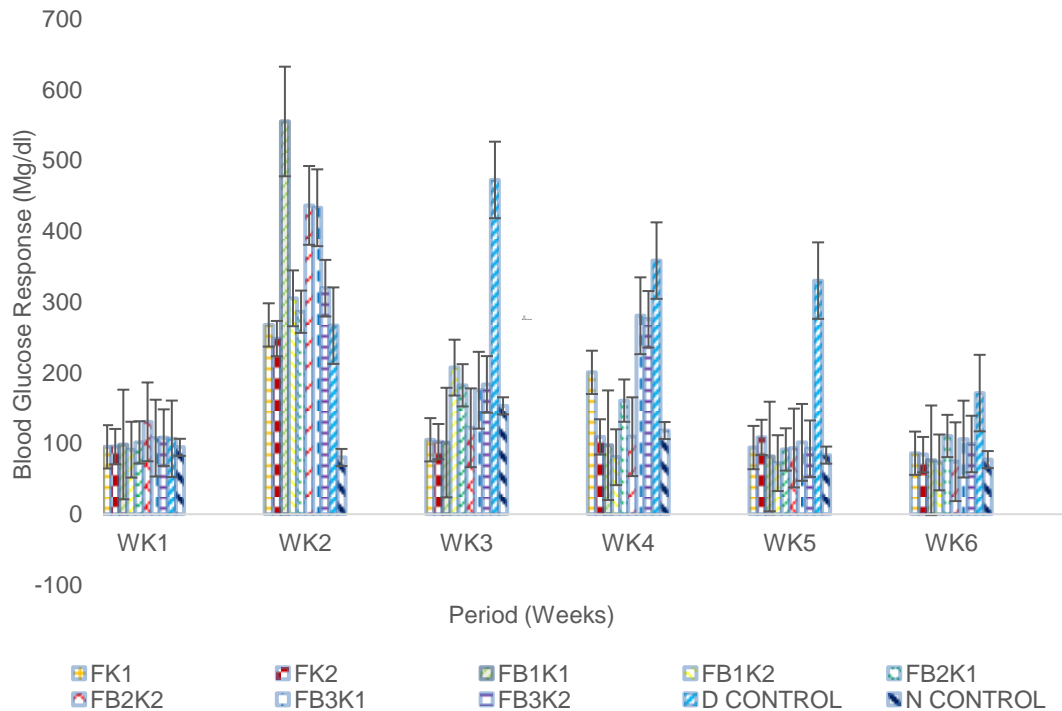
The lowering of the blood glucose levels of the diabetic rats fed with composite flours (FK_1 to FB_3K_2) could be due to the presence of fibre and phytochemicals in the flours (Table 2). Since fibre cannot be broken down into sugar molecules, it therefore, passes through the body undigested and does 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 [46].

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 [47]. 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 controlled the postprandial hyperglycemia (Fig. 1) [48,49].

3.3 Effect of Finger Millet, Bambara Groundnuts and *khain* (*Lecaniodiscus cupanioides*) Composite Flours on Blood Glucose Reduction (%) in Alloxan Induced Diabetic Rats

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

Fig. 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, Positive D-control = Diabetic rats control, Negative control = Non-diabetic rats
control

Composite flour FB_1K_1 (95 % [90 % finger millet/10 % BGN] and 5 % *khain*) could reduce the blood glucose levels of diabetic rats group FB_1K_1 by 86.31 %, followed by composite flour FB_2K_2 (90 % [80 % finger millet/20 % BGN] and 10 % *khain*) on diabetic rats group FB_2K_2 (83.04 %), while commercial rat chow (TOPFEEDS) could reduce the blood glucose of positive D-control group by 35.79 %.

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 (Positive 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 (Table 2) when compared to the commercial 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. [50] reported >60 % reduction in blood glucose for plantain-base functional dough meal on diabetic rats.

3.4 Changes in the Weights of the Diabetic Rats during the Six Weeks Period of Study

Changes in the weights of the diabetic rats during the six weeks period of study are presented in Fig. 3. The average weights of rats on arrival ranged from 72 – 137.2 g (Week 0). The weights of all the rats increased significantly ($p<0.05$) to 100 – 157.13 g after one week of acclimatization period (Week 1).

At week 2, 72 hours after inducement with alloxan, the weights of all the diabetic rats were not significantly ($p>0.05$) different from their

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 all 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. As a result the body starts burning fats and muscles for energy, causing a reduction in the overall body weight. Also excessive passing of urine could result when blood sugars are very high, leading to dehydration and consequently weight loss [51]. Weight loss of all 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 during week 5 and 6 of Fig. 1 [52,53]. On the other hand, the non-diabetic rats group (Negative-Control) continued to gain weights throughout the study period (Fig. 3).

3.5 Lipids of Diabetic Rats Fed with Finger Millet, Bambara Groundnut and *khain* (*Lecaniodiscus cupanioides*) Composite Flours

Table 3 showed lipids of diabetic rat groups fed with finger millet, bambara groundnut and *khain* (*Lecaniodiscus cupanioides*) composite flours. There were significant ($p<0.05$) differences between the diabetic rats groups fed with the composite flours and the positive and negative 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 diabetic rats groups fed composite flours reflecting the effects of the presence of dietary fibre and phytochemicals in bambara groundnut

(Table 2). Reports also showed that BGN contains mono and polyunsaturated fatty acid, which helps to decrease level of LDL-C and increase levels of high density lipoprotein (HDL-C) in blood [54]. 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 [55].
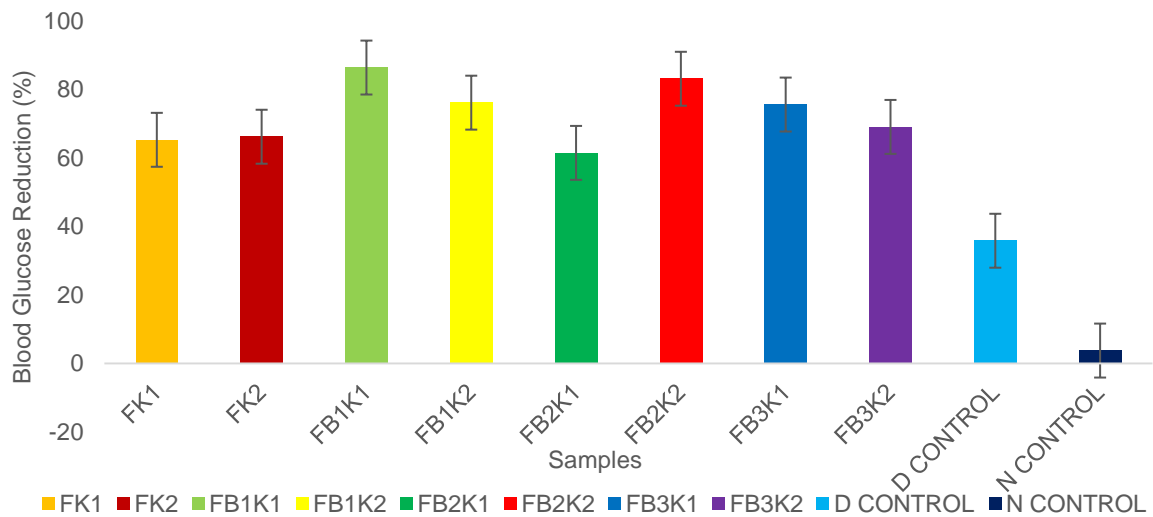
On the other hand, diabetic rat groups fed with composite flours containing higher *khain* (K_2) incorporation showed decreased high density lipoprotein (HDL-C) and Triglyceride (TGL) levels in blood. This was attributed to the antioxidant activity of the phenolics in *khain* (Kh) (Table 2). Phenolics had lipid lowering effect on HDL-C and the other serum lipids [56]. 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 3). 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 [57]. 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 (Negative-Control) had the highest TGL value of 168.87 mg/dl (Table 3). Veeramalla and Madas, [55] 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 [55].

3.6 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 4. 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 (Negative-control) rat group (6.20 g/dl), this is because chronically elevated blood glucose is associated with kidney problems resulting in low TSP levels [58]. This is evident in the diabetic (Positive 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 [58]. Hathama and Aymen [59] 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 [60]. This was owned to the quick intervention of the dietary fibre and phytochemicals (Table 2) in the composite flours to prevent prolonged hyperglycemia as earlier reported in Fig. 1.
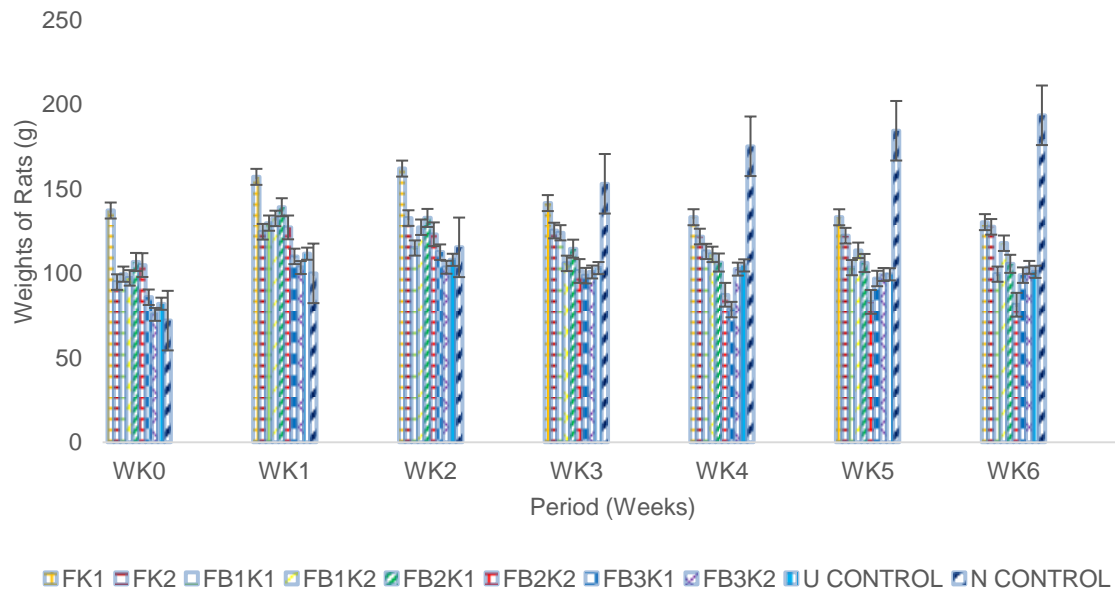

Fig. 2. Blood glucose reduction (%) effect of composite flours of finger millet, bambara groundnuts and *khain* (*Lecaniodiscus cupanioides*) on alloxan induce diabetic rats with standard error bars
Key: FK1 = 95%FM/5%Kh, FK2 = 90%FM/10%Kh, FB1K1 = 95%(90%FM/10%BGN)/5%Kh, FB1K2 = 90%(90%FM/10%BGN)/10%Kh, 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, Positive D control = Diabetic rats control, Negative control = Non-diabetic rats control

Table 3. 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 ± 3.26	151.34^{bc} ±1.25	72.53^a±0.50	122.33^b±2.62
FK$_2$	237.85^a ± 3.15	116.24^f ±5.63	63.55^c±0.76	151.06^a±5.04
FB$_1$K$_1$	210.83^c ±1.18	135.49^e ±3.15	59.37^d ±0.69	124.36^b±1.12
FB$_1$K$_2$	235.35^a ± 2.52	119.54^f ±2.19	58.06^e ±0.27	153.38^a± 2.93
FB$_2$K$_1$	190.15^d ± 1.36	146.37^{cd} ±1.69	53.44^f ±0.77	89.27^c±1.65
FB$_2$K$_2$	222.12^b ±2.01	97.90^h ±5.19	50.94^g±0.21	151.6^a±2.11
FB$_3$K$_1$	149.62^f ± 1. 31	153.72^b ±2.63	70.14^b ±.0.13	73.45^d±0.36
FB$_3$K$_2$	169.94^e ± 2.81	141.84^d ±2.16	46.91^i ±1.33	88.13^c±2.08
D-Control	124.66^g ± 4.21	107.47^g ± 3.26	48.44^h ± 1.25	54.73^f ± 3.0
N-Control	151.06^f ± 1.7	168.87^a ± 3.06	49.05^h ± 0.41	68.23^e ± 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, Positive D control = Diabetic rats control, Negative control = Non-diabetic rats control HDL-C = high density lipoprotein, TGL = Triglyceride, TCH = Total cholesterol, LDL-C = low density lipoprotein

Fig. 3. Changes in the weights of the diabetic rats during the six weeks period of study 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, Positive D control = Diabetic rats control, Negative control = Non-diabetic rats control

There was no significant (P=.05) difference in the albumin levels of all the rat groups contrary to the observation of Hathama and Aymen [59] 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 (Negative-control) recorded albumin values that are within the recommended limit of 3.5 – 5.5 g/dl [61]. These normal albumin values indicate that the liver of the diabetic rats was not damaged due to diabetes condition. This achievement was accrued to the anti-diabetic potentials of fibre and phytochemicals in the flours (Table 2). Uncontrolled diabetes can lead to liver damage which in turn causes hypoproteinemia by decreasing synthesis of plasma proteins like albumin [58,59].

Disparities (P=.05) were observed in the serum creatinine levels of the diabetic rat groups and the control groups (Table 4). Creatinine values ranged from 0.88 - 1.39 mg/dl, with the non-diabetic rat (Negative-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 (Positive D-control) group fed with commercial rat chow. This may be due to the intervention of fibre and phytochemicals in the composite flours. 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 [46,62]. 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 [63]. High serum creatinine levels may be indicative of kidney disease and can be lowered by increasing dietary fibre intake [63,64]. 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 [63].

The blood urea nitrogen (BUN) showed significant differences (P=.05) among the diabetic rat groups and control groups as represented in Table 4. The BUN levels ranged

from 7.15 - 23.25 mg/dl. The diabetic rats (Positive 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 phytochemicals in the composite flours (Table 2). High BUN level of Positive D-control rat group could be as a result of prolonged hyperglycemia (Fig. 1), which leads to a decline in kidney function; this limits the ability to filter waste from the blood [65].

Higher BUN level (29.22 mg/dl) was reported for diabetic subjects in other studies [66], while Xie et al. [65] 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 (Positive 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 [67].The BUN/Creatinine ratio of test groups and control groups are presented in Table 4. The BUN/Creatinine ratio is a more accurate assessment for kidney function since both BUN and creatinine may have some limitations [68,69]. A normal BUN to creatinine ratio is usually 10:1 to 20:1 [68,69]. The BUN/creatinine ratio obtained in this study fall within the range

10:1 to 19:1, except for diabetic rats 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 as 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 [68].

3.7 Liver Enzymes Levels in the Blood of the Diabetic Rats Fed with Composite Flours of Finger Millet, bambara Groundnut and *khain* (*Lecaniodiscus cupanioides*)

The Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) levels in the blood of both diabetics and non-diabetics (Negative-control) rats groups showed significant (P=.05) differences as shown in Table 5. 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 rats (Negative-control) group, while the diabetic rats (Positive 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).

Table 4. 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 (mg/dl)	BUN (mg/dl)	BUN/Cr ratio
FK_1	$5.50^g\pm 0.25$	$4.70^{de} \pm 0.1$	$0.88^h \pm 0.01$	$7.15^g \pm 0.35$	8 : 1
FK_2	$5.70^c\pm 0.24$	$4.56^g \pm 0.2$	$1.03^f \pm 0.03$	$19.82^b \pm 1.19$	19 : 1
FB_1K_1	$5.62^d \pm 0.10$	$4.66^f \pm 0.13$	$0.95^g \pm 0.01$	$13.91^{de}\pm 0.37$	15 : 1
FB_1K_2	$5.86^b\pm 0.31$	$4.65^t \pm 0.3$	$1.02^t \pm 0.01$	$18.74^b \pm 0.81$	18 : 1
FB_2K_1	$5.49^h \pm 0.13$	$4.76^{ab}\pm 0.11$	$1.21^d \pm 0.01$	$14.81^d \pm 0.52$	12 : 1
FB_2K_2	$5.56^e \pm 0.11$	$4.68^{ef}\pm 0.05$	$1.23^c \pm 0.02$	$16.05^c \pm 0.25$	13 : 1
FB_3K_1	$5.50^g \pm 0.1$	$4.74^{bc} \pm 0.1$	$1.19^e \pm 0.01$	$9.67^t \pm 0.73$	7 : 1
FB_3K_2	$5.51^f \pm 0.15$	$4.72^{cd} \pm 0.1$	$1.23^c \pm 0.01$	$12.84^e \pm 0.53$	11 : 1
D-Control	$5.16^i \pm 0.1$	$4.78^a \pm 0.1$	$1.24^b \pm 0.004$	$23.25^a \pm 0.26$	19 : 1
N-Control	$6.20^a \pm 0.15$	$4.68^{ef} \pm 0.18$	$1.39^a \pm 0.004$	$17.15^c \pm 1.17$	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, Positive D control = Diabetic rats control, Negative control=Non-diabetic rats control, TSP = Total serum protein, ALB = albumen, BUN = blood urea nitrogen, BUN/Cr = blood urea nitrogen /Creatinine

Table 5. 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 ± 0.33	11.27^d ± 0.57	50.59^g ± 0.94
FK$_2$	10.68^f ± 0.34	7.73^g ± 0.38	47.45^h ± 0.17
FB$_1$K$_1$	14.53^c ± 0.27	10.55^e ± 0.18	68.24^e ± 0.27
FB$_1$K$_2$	13.74^d ± 0.23	8.87^f ± 0.24	58.68^f ± 0.38
FB$_2$K$_1$	16.61^b ± 0.29	15.61^b ±0.24	72.53^d ± 0.36
FB$_2$K$_2$	16.39^b ± 0.22	11.95^c ± 0.12	66.85^e ±0.37
FB$_3$K$_1$	13.64^d ± 0.26	11.45^{cd} ± 0.38	76.28^c ± 0.37
FB$_3$K$_2$	12.45^e ± 0.24	10.67^e ± 0.13	59.48^f ± 0.38
D-Control	19.75^a ± 0.61	19.85^a ± 0.06	132.07^a ± 2.44
N-Control	13.20^d ± 0.11	7.03^h ± 0.38	77.97^b ± 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 ALT = Alanine aminotransferase, AST = Aspartate aminotransferase, ALP = Alkaline phosphatase

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 (Table 2). 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 [70]. 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 [71].

The AST/ALT ratios obtained are < 2:1, signifying that the liver is not damaged. The diabetic (Positive D-control) rats group had an elevated ALP level (132.07 U/L) above the normal range. This was due to prolonged hyperglycemia among the group. Diabetes is associated with renal hyperfiltration which could result to ALP elevation [72]. 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 [73]. Renal hyperfiltration has been observed in patients with newly diagnosed type-2 diabetes [72]. Vozarova et al. [74] and Mathur et al. [75] reported elevated ALP, AST and ALT among diabetics when compared to non-diabetics.

4. CONCLUSION

Flours of Finger millet (FM), Bambara groundnut (BGN) and *Khain* (*Lecaniodiscus cupanioides*)

were found to contain dietary fibre and phytochemicals which are beneficial to health. Composite flours of Finger millet (FM), Bambara groundnut (BGN) and *Khain* (*Lecaniodiscus cupanioides*) could lower blood glucose of diabetic rats when compared with the positive D-control. The hypoglycemic potential of the flour composites resulted to reduced 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.

5. RECOMMENDATION

I recommend that the histopathological study of the diabetic rats be investigated to determine effect of the composite flours on the vital organs.

ETHICAL APPROVAL

"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"

ACKNOWLEDGEMENTS

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Michael D. Types of diabetes mellitus by Rebecca Buffum Taylor; 2017.
   Accessed 13 Nov 2020.
   Available:https://www.webmd.com/diabetes/guide/types-of-diabetes-mellitus
2. World Health Organization (WHO). Diabetes mellitus; 2017.
   Accessed 13 Nov. 2018
   Available:https://www.who.int/news-room/fact-sheets/detail/diabetes;https://www.who.int/health topics/diabetes
3. Hannah R, Max R. "Causes of death" our world in data; 2018.
   Available:https://ourworldindata.org/causes-of-death accessed 16/11/2020
4. Nestor KK, George GT, Jacko RFA, Massara CC, Georges NA. Influences of the variety and cooking method on glycemic index of yam. Pak J Nutr 2009; 8(7):993-999.
5. Devi PB, Vijayabharathi R, Sathyabama S, Malleshi NG, Priyadarisini VB. Health benefits of finger millet (*Eleusine coracana l.*) polyphenols and dietary fiber: A review. J Food Sci Technol. 2014;51(6):1021-1040.
   DOI: 10.1007/s13197-011-0584-9. Epub 2011 Nov 22. PMID: 24876635; PMCID: PMC4033754.
6. Roger Blench Finger-Millet: The contribution of vernacular names towards its prehistory Paper prepared for the RIHN Symposium 'Small millets in Africa and Asia' Tokyo September19-20th, J. Archaeol Anthropol. Sci. 2010;5.
7. Umar ID, Kwon-Ndung EH, Mailafia S. Genotypic restriction patterns of finger millet (*Eleusine coracana* [1.] Gaertn) landraces in northern Nigeria. Int J Genet Mol Biol. 2016;8(2):7-10.
   DOI:105897/IJGMB2013.0093
8. Jideani VA, Diedericks CF. Nutritional, Therapeutic, and Prophylactic Properties of Vigna subterranea and Moringa oleifera. In (Ed.), Antioxidant-Antidiabetic Agents and Human Health. Croatia: Intech Open. 2014;187.
   Available: https://doi.org/10.5772/57338

9. Fasoyiro S, Yudi W, Taiwo K. Processing and utilization of legumes in the tropics In: Elissa, A. A. (editor). Trends Food Sci Technol. Croatia; 2012.
   DOI:10.5772/36496
10. Nafiu MO, Abdulsalam TA, Akanji MA. Phytochemical Analysis and Antimalarial Activity Aqueous Extract of *Lecaniodiscus cupanioides Root.* Journal of Tropical Medicine. 2013;1- 4.
    Article ID 605393,
    Available:https://doi.org/10.1155/2013/605393
11. Oselebe HO, Nnamani CV, Okporie EO. Ethnobotanical studies of traditional leafy vegetables and spices of Ebonyi state, Nigeria: Potentials for improved nutrition, food security and poverty reduction Anim. Res. Int. 2012;9(1):1485-1496.
12. Yemitan OK, Adeyemi OO. CNS depressant activity of *Lecaniodiscus cuponiodes Fitoterapia.* 2005;76(5):412-418.
    DOI: 101016/j.fitote.2005.02.010.
    PMID: 15955638.
13. Sofidiya MO, Jimoh FO, Aliero AA, Afolayan AJ, Odukoya OA, Familoni OB. Antioxidant and antibacterial properties of *Lecaniiodiscus cupanioides.* Res J Microbiol. 2008;3(2):91-98.
    Available:https://scialert.net/abstract/?doi=jm.2008.91.98 DOI:10.3923/jm.2008.91.98
14. Simpson B, Claudie D, Smith N, Wang JP, McKinnon R, Semple S. Evaluation of the anti-inflammatory properties of Dodonaea polyandra, a Kaanju traditional medicine. J Ethnopharmacol. 2010;132(1): 340 – 343.
    DOI: 101016/j.jep.2010.07.012.
    Epub 2010 Jul 13. PMID: 20633620.
15. Veeramani C, Pushpavalli G, Pugalendi KV. In vivo antioxidant and hypolipidemic effect of *cardiospermum halicacabum* leaf extract in streptozotocin-induce diabetic rats. J Basic Clin Physiol Pharmacol. 2010; 21(2):107-125.
    Available:https://doi.org/10.1515/JBCPP.2010.21.2.107
16. Muthukumran P, Begum VH, Kalaiarasan P. Anti-diabetic activity of Dodonaea Viscosa (L) leaf extracts. *Int J Pharmtech Res.* 2011;3(1):136 – 139 CODEN (USA): IJPRIF ISSN: 0974-4304
17. Abioye VF, Olatunde SJ, Elias G. Quality attributes of cookies produced from composite flours of Wheat, germinated Finger millet and African yam bean.

Int J Res Granthaalayah 2018;6(11):172-183.

D0I:105281/ zenodo.18454271

18. Agbara GI, Masaya FA, Badau MH, Igwebe OA, Yakaka AM. Evaluation of Functional, Proximate, Microbial and Sensory Properties of Pearl Millet-Bambara Groundnut Yarsala (A Traditional deep-fried fermented puff) Asian J Agric Food Sci. 2018;6(2):69-77.
Available:https://www.ajouronline.com/index.php/AJAFS/article/view/5260

19. 19. Jideani VA. Characteristics of local Pearl millet (*Pennisetum glaucum*) grains. Niger. Food J. 2005;23(1):193-204.
DOI: 104314/nifoj.v23i1.33617

20. Abdualrahman MAY, Ali AO, Elkhalifa EA, Sulieman AE. Effect of Bambara groundnut flour (*Vigna subterranea* (L.) Verdc.) supplementation on chemical, physical, nutritional and sensory evaluation of wheat bread. Pak J Biol Sci. 2012;15 (17): 845-849
PMID: 24163969,
DOI:103923/pjbs.2012.845.849

21. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases Am J Clin Pathol. 1957; 28(1):56-63.
DOI: 101093/ajcp/28.1.56.
PMID: 13458125.

22. Schmidt E, Schmidt FW. Determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. *Enzyme Biol. Clin.* 1963;3:1-5.

23. Deutsche Gesellschaft für Klinische Chemie. Recommendations of the German Society for Clinical Chemistry. Standardisation of methods for the estimation of enzyme activities in biological fluids. Experimental basis for the optimized standard conditions. Z Klin Chem Klin Biochem. 1972;10(6):281-91.
PMID: 4681028.

24. Tietz NW. Clinical Guide to laboratory Tests 3rd Edition. WB Saunders Company, Philadelphia PA. 1995;518-519.

25. Grant GH, Silverman LM, Christenson RH. Amino acids and proteins. In: Tietz N.W. (Ed.) Fundamentals of clinical chemistry. 3rd Ed. WB Saunders Company, Philadelphia. 1987;312-316.

26. Doumas BT, Watson WA, Biggs HG. Albumin Standards and Measurement of Serum Albumin with Bromocresol Green. *Clinica Chimica Acta.* 1971;31(1):87-96.

PMID: 5544065
DOI: 101016/0009-8981(71)90365-2

27. Orsonneau JL, Massoubre C, Cabanes M, Lustenberger P. Simple and Sensitive Determination of Urea in Serum and Urine. *Clin Chem.* 1992;38(5):619 – 623
PMID: 1582010.

28. Toora BD, Rajagopal G. Measurement of Creatinine by Jaffe's reaction– Determination of Concentration of Sodium hydroxide required for maximum color development in standard, Urine and Protein free filtrate of serum. Indian J Exp. Biol. 2002;40(3):352 – 354.
PMID: 12635710.

29. Allain CC, Poon LS, Richmond CS, Fu PC. Enzymatic determination of total cholesterol. J Clin Chem. 1974;20(4):470-475.
PMID: 4818200.

30. Jacobs NJ, Van-Denmark PJ. The purification and properties of the alpha - glycerophosphate oxidizing enzyme of streptococcus feacalis 10C1 *Arch. Biochem. Biophys.* 1960;88:250 – 255.
DOI: 10.1016/0003-9861(60)90230-7.
PMID: 14406376.

31. Albers JJ, Warnick UR, Cheung MC. Quantification of High Density Lipoprotein. Lipids. 1978;13(12): 926-932.
Available:https://doi.org/10.1007/BF02533852

32. Sood R. Text Book of Medical Laboratory Technology. Jaypee: the Health Science Publishers (P) Ltd, New Delhi. 2006.
ISBN 9788180615917 Pp 196 – 210, 598 - 690, 1225 -1247

33. Harborne JB. Phytochemical Methods: A guide to Modern Techniques of Plant Analysis. Chapman and Hall Publisher, New York. 1980;32 – 35.

34. AOAC, Official Methods of Analysis of Association of Official Analytical Chemists 18th Edition; 2010. Washington, DC.

35. Kirk RS, Sawyer R. Pearson's Composition and Analysis of Foods (9th ed). Longman Scientific and Technical, Churchill living Stone. 21998. Edingburgh. 1998;211-212.

36. Sadasivam S, Manickam A. Biochemical Methods for Agricultural Sciences. Wiley Easthern Limited, New Delhi India. 1992; 186-191.

37. Haug W, Lantzseh HJ. Sensitive method for the rapid determination of phytate in cereals and cereal products J Sci Food Agric. 1983;34(12):1423–1426.

Available:https://doi.org/10.1002/jsfa.2740 341217

38. Yao DN, Kouassi KN, Erba D, Scazzina F, Pellegrini N, Casiraghi MC. Nutritive Evaluation of the Bambara Groundnut Ci12 Landrace [*Vigna subterranea (L.)* Verdc. (Fabaceae)] Produced in Côte d'Ivoire *Int J mol sci*, 2015;16(9):21428–21441. Available:https://doi.org/10.3390/ijms16092 1428 PMID: 26370971; PMCID: PMC4613261.

39. Yue-yue Y, Sen M, Xiao-xi W, Xue-ling Z. Modification and Application of Dietary Fiber in Food Hindawi J Chem. 2017;1-8. Available:https://doi.org/10.1155/2017/934 0427

40. Chethan S, Malleshi NG. Finger Millet Polyphenols: Characterization and their Nutraceutical Potential Am J Food Technol. 2007;2(7):582-592. DOI: 103923/ajft.2007.582.592

41. Shobana S, Sreerama YN, Malleshi NG. Composition and enzyme inhibitory properties of finger millet (*Eleusine coracana* L.) seed coat phenolics: mode of inhibition of ??-glucosidase and pancreatic amylase. Food Chem. 2009;115(4):1268–1273. DOI: 101016/j.foodchem.2009.01.042

42. Viswanath V, Urooj A, Malleshi NG. Evaluation of antioxidant and antimicrobial properties of finger millet polyphenols (*Eleusine coracana*) Food Chem. 2009; 114(1):340–346. Available:https://doi.org/10.1016/j.foodche m.2008.09.053

43. Rathore T, Singh R, Kamble DB, Upadhyay A, Thangalakshmi S. Review on finger millet: processing and value addition The Pharma Innov J. 2019;8(4):283-291. Corpus ID: 224354632

44. Kumar A, Metwal M, Kaur S, Gupta AK, Puranik S, Singh S, Singh M, et al. Nutraceutical Value of Finger Millet (*Eleusine coracana* [L] Gaertn) and Their Improvement Using Omics Approaches Front plant sci. 2016 7:1-14. Available:https://doi.org/10.3389/fpls.2016. 00934

45. Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol. Res. 2001;50(6): 537–546. PMID: 11829314.

46. Johnson LW, Weinstock RS. The Metabolic syndrome: concepts and controversy. Mayo Clin. Proc. 2006;81(12): 1615-1620 DOI: 104065/81.12.1615. PMID: 17165640.

47. Yin P, Zhao S, Chen S, Liu J, Shi L, Wang X, et al. Hypoglycemic and hypolipidemic effects of polyphenols from burs of *Castanea mollissima* Blume. *Molecules.* 2011;16(11): 9764–9774. PMID: 22116046; PMCID: PMC6264564. DOI: 103390/molecules16119764.

48. Tong WY, Wang H, Waisundara VY, Huang D. Inhibiting enzymatic starch digestion by hydrolyzable tannins isolated from Eugenia Jambolana. Lebensm Wiss. Technol. 2014;59(1):389-395. DOI:101016/j.lwt.2014.04.007

49. Sakulnarmrat K, Konczak I. Composition of native Australian herbs polyphenolic-rich fractions and in vitro inhibitory activities against key enzymes relevant to metabolic syndrome. Food Chem. 2012;134:1011–1019 PMID: 23107721. Epub 2012 Mar 8. DOI:101016/j.foodchem.2012.02.217.

50. Famakin O, Fatoyinbo A, Ijarotimi OS, Badejo AA, Fagbemi TN. Assessment of nutritional quality, glycaemic index, antidiabetic and sensory properties of plantain (*Musa paradisiaca*)-based functional dough meals. J. Food Sci. Technol. 2016;53(11): 3865-3875. DOI: 101007/s13197-016-2357-y. Epub 2016 Nov 29. PMID: 28035142; PMCID: PMC5156628.

51. Ruchi M. Is weight loss caused by diabetes dangerous?; 2022. Accessed 30/03/2022 Available:https://www.medicinenet.com/is_ weight_loss_caused_by_diabetes_danger ous/ask.htm

52. 52. Johns Hopkins. Diabetes: The Johns Hopkins Endoscopic weight loss program. 2021. Accessed 03-10-2021. Available at https://www.hopkinsmedicine.org>--

53. 53. Michael D. Will weight loss help with your diabetes? 2021. Retrieved 03-10-2021. Available:https://www.webmd.com/diabete s/safe-diet-tips-for-diabetes

54. Alhassanm AJ, Sule MS, Abubakar H, Abdulmumin TM, Dangambo MA. Fatty Acids Composition of Bambara Groundnut (Vigna Subterranean (L) Verd C) Grown In Madobi, Kano State-Nigeria. Eur. Sci. J. 2014;10(24):143-151. Available:https://doi.org/10.19044/esj.2014 .v10n24p%p

55. Veeramalla V, Madas S. Comparism of lipid levels in the diabetic and non-diabetic patients: a study in a tertiary care hospital Int. J. Adv. Med. 2017;4(6):1573-1577. Available:http://dx.doi.org/10.18203/2349-3933.ijam20175169

56. Ogbunugafor HA, Okpuzor J, Igwo-Ezikpe MN, Ezekwesili CN, Nebedum JO, Ekechi AC. et al. Anti-inflammatory medicinal plants: The effect of three species on serum antioxidant system and lipids levels in rats. NISEB J. 2011. 11(1):105-113. Available:http://www.nisebj.orgNISEB 2010039/11202

57. Bravo E, Napolitano M, Botham KM. Post-prandial Lipid Metabolism. The Missing Link between Life-Style Habits and the Increasing Incidence of Metabolic Diseases in Western Countries. Open Transl Med J. 2010;2:1-13. DOI:102174/1876399501002010001

58. Mohammed NJ, Al-Gazally ME, Awadh AAM. Evaluation of the serum total protein in patients with diabetes mellitus (type I and type ii) and study genetic level of glutathione-s-transferaseu 1. Med J Babylon. 2015;12 (3):625-631

59. Hathama RH, Aymen A. Influence of Diabetes Disease on Concentration of Total Protein, Albumin and Globulums in Saliva and Serum: A comparative Study. Irq. Nat. J. Chem. 2015;15(1):1-11.

60. Jamie E. What to know about the protein test and the results: Total Protein Test: Normal levels, Uses, Results and Procedure; 2019.
Accessed 01/04/2022.
Available:https://www.medicalnewstoday.com/articles/325320

61. Sridevi D. Albumin; 2015.
Accessed 01/04/2021.
Available:https://emedicine.medscape.com/articles/2054430-overview

62. Davita. Fibre in the kidney diet; 2021.
Accessed 03-10-2021.
Available:https://www.davita.com/diet-nutrition/articles/basics/fiber-in-the-kidney-diet

63. Sherrell Z. What to know about high creatinine levels; 2021.
Accessed 01/10/2021
Available:https://www.medicalnewstoday.com/articles/when-to-worry-about-creatinine-levels

64. Salmean YA, Segal MS, Langkamp-Henken B, Canales MT, Zello GA, Dahl W. Food with added fibre lower serum creatinine levels in patients with chronic kidney disease. J Ren Nutr. 2013; 23(2):e29-e32
DOI: 101053/j.jrn.2012.04.002. Epub 2012 Jun 26. PMID: 22739658.

65. Xie Y, Bowe B, Li T, Xian H, Yan Y, Al-Aly Z. Higher blood urea nitrogen is associated with increased risk of incident diabetes mellitus. Kidney int. 2018;93:741-752.
Available:https://doi.org/10.1016/j.kint.2017.08.033

66. Bamanikar SA, Bamanikar AA, Arora A. Study of Serum urea and Creatinine in Diabetic and Non-Diabetic Patients in a Tertiary Teaching Hospital. J Med Res. 2016; 2(1):12-15.
DOI:10.31254/jmr.2016.2104

67. Chris H. Urea and the clinical value of measuring blood urea concentration 2016a; Accessed 01/04/2022.
Available:https://acutecaretesting.org/en/articles/urea-and-the-clinical-value-of-measuring-blood-urea-concentration.

68. Lewis SM, Dirksen SR, Heitkemper MM, Bucher L, Harding M. Medical- Surgical Nursing: Assessment and Management of Clinical Problems 9th ed. St. Louis, Missouri; 2013.
ISBN 978-0-323-10089-2.
OCLC 228373703

69. Chris H. Urea and creatinine concentration, the urea: creatinine ratio Available:https://acutecaretesting.org/en/articles/urea-and-creatinine-concentration-the-urea-creatinine-ratio
(2016b). Accessed 01/04/2022.

70. Tolman KG, Fonseca V, Dalpiaz A, Tan MH. Spectrum of liver disease in type 2 diabetes and management of patients with diabetes and liver disease. Diabetes Care. 2007;30(3):734-43.
DOI: 102337/dc06-1539.
PMID: 17327353.

71. Charles PD. Liver Function Tests (Normal, Low and High Levels and Results); 2022.
Available:https://www.medicinet.com Liver blood test
Accessed 04/04/2022

72. Oh SW, Han KH, Han SY. Associations between Renal Hyperfiltration and Serum Alkaline Phosphatase. *PloS One* 2015; 10(4):e0122921. PMID: 25853240; PMCID: PMC4390244.
DOI:101371/journal.pone.0122921

73. Helal I, Fick-Brosnahan GM, Reed-Gitomer B, Schrier RW. Glomerular hyperfiltration; definitions, mechanisms and clinical

implications. Nat Rev Nephrol. 2012; 8(5):293-300.
DOI:101038/nrneph.2012.19.
PMID: 22349487.

74. Vozarova B, Stefan N, Lindsay RS, Saremi A, Pratley RE, Bogardus C. et al. A. High alanine aminotransferase is associated with decreased hepatic insulin sensitivity and predicts the development of type 2 diabetes. Diabetes. 2002; 51(6):1889-1895.
DOI: 102337/diabetes. 51.6.1889. PMID: 12031978.

75. Mathur S, Mehta DK, Kapoor S, Yadav S. Liver Function in Type-2 Diabetes Mellitus Patients. Int J Sci Study. 2016; 3(10):43-47
DOI:1017354/ijss/2016/09