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Research Article

Combined Effect of Sorghum Bicolor Formulated Diet and Glibenclamide on Some Biochemical Changes in Alloxan-Induced Diabetic Rats

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ABSTRACT

Food-drug synergy and safety are primarily focused on the potential benefits of using foods to lower the risk of chronic diseases. For the past few years, reports have shown that whole grains, particularly sorghum (Sorghum bicolor), may be potential alternatives to ameliorate type 2 diabetes mellitus (T2DM) symptoms. This study, evaluated the combined effect of sorghum formulated diet and glibenclamide on alloxan induced diabetic rats. Fifty-five (55) albino rats (Wistar strain) were randomly divided into eleven groups of five rats each. The animals received different dosage of the drug (1 mg/kg, 0.5 mg/kg and 0.25 mg/kg), formulated diet (20 g/kg, 15 g/kg and 10 g/kg b.w) and the combination for 28 days. The glycemic index (GI) and glycemic load (GL) of the formulated diet were determined. Fasting blood glucose of rats was measured weekly while lipid profiles were estimated. The GI and GL were found to be 38.02% and 18.39% respectively. There were significant ($p<0.05$) reductions in the FBG concentrations of the groups treated with diet + glibenclamide compared to the negative control throughout the treatment period with a pronounced at third and fourth week. A significant reduction was observed in LDL-cholesterol ($p<0.001$), total cholesterol ($p<0.05$), and triglycerides ($p<0.05$) levels in groups given a combined treatment as compared to groups given single treatments of diet or glibenclamide. It can be concluded that a combination of the formulated diet and glibenclamide may be useful in the management of diabetes mellitus.

Keywords: *Sorghum, Glycemic index, Glycemic load, Diabetes, Formulated diet*

INTRODUCTION

Diabetes mellitus (DM) is a chronic, lifelong progressive metabolic disease characterized by hyperglycemia due to absolute or relative insulinopenia. There are several different types of DM and each is caused by a complex interplay between genetic predisposition and environmental factors. The metabolic dysregulation that contributes to hyperglycemia includes diminished insulin secretion, impaired glucose utilization, or increased glucose production, and eventually causes pathophysiological changes in multiple organs and organ systems (Pinti *et al.*, 2019).

World health organization has categorized DM as the 7th leading cause of death in the USA while it was estimated that 422 million adults present diabetes in 2014, 4 times higher than the recorded cases in 1980 (Gojka, 2016). Clinicians also believe that DM may occur by the carbohydrates and fat existence in the daily diet given that

starch digestion in mammals is accomplished by α-amylase and α-glucosidase. Inhibition of starch digestive enzymes or glucose transporters can reduce glucose release and absorption in the small intestine. This decrease could help to manage DM (Lascar *et al*., 2018). It is estimated that in 2017, there were 451 million people (ages 18-99 years) with diabetes worldwide (Cho *et al.*, 2018), and this number is expected to rise, mostly due to type 2 DM. Individuals with type-2 DM are usually dyslipidemic even when they are under relatively good glycemic control. They exhibit several lipid abnormalities including elevated plasma triglycerides, high low density lipoprotein cholesterol (LDL-C), and decreased high density lipoprotein cholesterol (HDL-C). Among the established risk factors for coronary heart disease (CHD), the lipid triad (elevated triglycerides, decreased high-density lipoprotein cholesterol, and elevated small dense low-density lipoprotein cholesterol) is a powerful risk factor for atherosclerosis in type 2 diabetes. An abnormal lipid profile is more common in diabetics and gets aggravated with poor glycemic control. Thus, the analysis of the lipid profile is needed to investigate how the lipid metabolism, especially HDL and LDL cholesterol, is affected by diabetes (Solano and Goldberg, 2006). However, the anti-diabetic drug pathways include stimulating insulin synthesis, inhibiting the production of endogenous glucose, and blocking carbohydrate absorption from the intestine (Rines *et al.*, 2016; Vieira *et al.*, 2019).

For the past few years, reports have shown that whole grains including sorghum [*Sorghum bicolor* (L.) Moench] may be a potential alternative to ameliorate T2DM symptoms (Della *et al.*, 2018). *Sorghum bicolor* (SB) contains several secondary metabolites which are reported to be effective in preventing various metabolic diseases, such as cancers, T2DM, obesity, and hyperglycemia (Althwab *et al.*, 2015; Awika *et al*. 2018).

Epidemiological and intervention studies have increased both public and expert awareness of the possible importance of blood sugar regulation and the varying glycemic index (GI) of foods in the etiology and treatment of chronic diseases (NNR, 2004). For instance, a low GI diet (LGID) decreases the risk of chronic diseases, such as Type 2 diabetes and coronary heart disease, and decreases the developing risk factors for these diseases as well as decreases the risk for cancer, especially among those who are overweight or obese (Wolever and Bolognesi, 1996).

The fact that low glycemic index diets have been associated with anti-diabetic potential and are also commonly used for edible purposes suggests the possibility of therapeutic interaction when used together with a conventional anti-diabetic agent either consciously or inadvertently. The interaction may be synergistic, additive, or antagonistic. There is a need, therefore, to carry out a systematic investigation into the effect of such combinations. This study is designed to examine the synergistic effect of a sorghum-based diet with low glycemic index and oral hypoglycemic drug, glibenclamide in Alloxan-induced diabetic rats of the Wistar strain.

MATERIALS AND METHODS

Chemicals/Reagents

All chemicals and reagents used in this study were of analytical grade.

Experimental animals

Fifty-five (55) male albino rats (Wistar strain), weighing between 100 to 110 g were obtained from the Department of Biological Sciences, Bayero University, Kano. They were maintained under standard conditions of temperature (28±2 0C) and relative humidity (46 ± 6%) and housed 5/cage with 12 hours light-dark cycle and adequate ventilation for two weeks before the experiments to acclimatize. The animals were fed with a commercial diet (Vital Feed Nigeria Limited) and water, *ad libitium*. The animals were deprived of food for 12 hours before the commencement of the study.

Food sample

Whole Sorghum (*Sorghum bicolor*) was purchased from Dawanau market, Kano, Kano State Nigeria. Smooth Spinach (*Amaranthus hybridus*), dried crayfish, and olive oil were purchased from Tarauni market, Kano. Sorghum, crayfish, and spinach leaves are locally available and commonly consumed in Kano state, Nigeria.

The vegetables and grain were identified and certified in the Department of Biological Sciences, Bayero University, Kano, with the following voucher numbers: *Amaranthus hybdridus* (BUKHAN 0020), *Sorghum bicolor* (BUKHAN 0475).

Proximate analysis of formulated diet

The proximate compositions of the diet were determined using conventional standard methods of the Association of Official Analytical Chemists, AOAC (2000).

Determination of crude protein content

Kjeldahl's (1883) method was used to determine the protein content. Each of the samples 0.15g was weighed into the digestion tubes. Concentrated H_2SO_4 (2 mL) was added to each tube and swirled gently until the samples and the acid were thoroughly mixed. Kjeldahl catalyst (8 g) was added to each tube. The tubes were heated until the solution clears and boiled for 2 hours then allowed to cool. The content of

each tube was transferred into 100 mL volumetric flasks and diluted with distilled water up to the mark. Then 2% boric acid (10 mL) and 5 drops of the mixed indicator were measured into a 250 mL Erlenmeyer flask. An aliquot of the digest (10 mL) was transferred into the distillation flask and the flask was attached to the distillation apparatus. Sodium hydroxide (15 mL) was added to the distillation flask containing the digest. Nitrogen was distilled into a boric acid/mixed indicator receiver flask until the 150 mark was reached. The condenser tip was washed with distilled water and the distillate was titrated with 0.025 N H_2SO_4 until a pink endpoint was attained.

$$\%\ Nitrogen = \frac{0.014\ (\frac{MeN}{100g})\ \times\ T_V\ \times V_D\ \times N}{W_S V_A}\ \times\ 100$$

Where;
W_S = Weight of sample analyzed,
T_V = Titre value blank,
V_D = Total volume of digest,
N = Concentration of H_2SO_4,
V_A = Volume of digest distilled.

The calculation of the total protein was done through the multiplication of the percentage of nitrogen with 6.25.

Determination of crude fibre

Crude fibre or insoluble fibre includes cellulose, some hemicelluloses, and lignin. The crude fibre content of the formulated feed was determined using the method described by AOAC (1990). Two grams (2g) of the feed were dissolved in 50 mL of sulfuric acid (0.25 N) contained in a beaker which was then boiled for 30 minutes. Fifty milliliters (50mL) of sodium hydroxide (0.3N) was then added to this mixture and boiled for 30 minutes. Fifty milliliters (50mL) of sodium hydroxide (0.3N) was then added to the mixture and boiled for 30 minutes. The thermal treatment was followed by filtration using Whatman Filter Paper No. 42. The residue obtained was washed several times with hot distilled water until complete alkalis eradication. The insoluble material obtained was dried at 150 °C for 8 hours, weighed, and then incinerated at 550 °C in the self-regulating muffle furnace, preheated at 550 °C for 3 h. The ash was weighed with the same precision scale. The crude fibre content (g / 100 g) was obtained by the following relationship:

$$Crude\ fibre(\%) = \frac{(M1 - M2)}{M} \times 100$$

M = Taken sample mass (g);
M1 = Sample mass after lasting 8 hours in the oven (g)
M2 = Dry residue mass after incineration at 550 ° C for 3 h (g).

Determination of crude fat content

The crude fat content of the feed was determined using the method of AOAC (1980)**.** Petroleum ether (300 mL) was transferred into a clean, dried round bottom flask fitted to the soxhlet extraction unit. Three grams (3 g) of the ground sample (W1) was carefully weighed into a fat-free filter paper and then re-weighed (W2). It was folded properly and placed in the extraction thimble which in turn was then fixed into the soxhlet extraction unit with a retort stand serving as forceps and cold-water circulation made constant. The heating mantle was switched on and the temperature was adjusted to 70 ^oC until the solvent was refluxing at a steady rate. The extraction was carried out for 2 hours and 30 minutes before the heating mantle was switched off. The thimble was dismantled and the filter paper bearing the sample was air-dried and placed in an oven to get a constant weight. It was then reweighed (W3).

$$\%\ Crude\ fat = \frac{(W2 - W3)}{W1} \times 100$$

Where;
W1 = weight of the sample
W2 = weight of the sample + filter paper (before extraction)
W3 = weight of the sample + filter paper (after extraction)

Determination of ash content

The ash content was determined by the method of AOAC (1980)**.** Crucibles were dried in an ovum and a desiccator was used to cool the crucibles and weighed (W1) before use. Five grams (5 g) of the sample was weighed. The crucibles with the samples (W2) were introduced into a muffle furnace, set at 600 ^0C, and then ignited in the furnace for 8 hours. The crucibles with the ash were taken out and cooled in a desiccator and weighed (W3).

Calculation:

$$\%\ Ash = \frac{weight\ of\ ash}{weight\ of\ sample} \times 100$$

$$= \frac{(W3 - W1)}{(W2 - W1)} \times 100$$

Where;
W1= weight of the crucible
W2= weight of crucible + sample
W3= weight of crucible + ash residue

Determination of moisture content

The moisture content of the feed was determined by weighing the samples into moisture cans and placed in an oven at 80°C until the constant weight of the sample was obtained (dried to constant weight). They were brought out and allowed to cool in a desiccator and re-weighed (AOAC, 2005).

Calculation:

$$\%\ Moisture = \frac{weight\ of\ wet\ sample - weight\ of\ dried\ sample}{weight\ of\ wet\ sample} \times 100$$

Determination of crude carbohydrate content

The calculation of total carbohydrate contents in the formulated feed was done according to the relationship given by FAO (1998).

100 % Crude Carbohydrate = 100% - (% Protein + % Fiber +% Fat + % Moisture + % Ash)

Estimation of energy value

The calculation of the energy value of the formulated feed was done according to the relationship given by Crisan and Sands (1978).

Energy value (kcal/100 g) = (2.62 x % Protein) + (8.37 x % Fat) + (4.2 x % Carbohydrate).

Mineral analysis

Mineral contents were determined by the Association of Official Analytical Chemists methods (AOAC, 1990). The wet digested samples were taken for instrumental analysis using Varian Instrument (AA240FS) for the atomic absorption spectrophotometry (AAS).

Determination of vitamin C content

The amount of vitamin C was determined by titration using the method described by Pongracz *et al*. (1971). Ten grams (10 g) of the ground sample were soaked for 10 min in 40 mL of a mixture of 2% metaphosphoric acid and 8% acetic acid (1:1 ratio). The mixture was centrifuged at 3000 rpm for 20 min and the supernatant obtained was diluted and adjusted with 50 mL of bi-distilled water. Ten (10) mL of this mixture was titrated to the endpoint with 0.5 g/L dichlorophenol-indophenol (DCPIP).

Preparation of experimental diet and animal feeding design

The diet used in this study was formulated using whole sorghum grain (*Sorghum bicolor*) as the major source of carbohydrate and other ingredients; crayfish, smooth spinach, and olive oil in the ratio of 5:3:1.5:0.5 respectively (Table 1). Whole sorghum was washed, dried, and coarsely ground using a grinding machine. A fresh sample of smooth spinach was washed, shade-dried and coarsely ground. Dried crayfish were also coarsely ground using a RIGCHINA laboratory blender (MD-326S). Whole sorghum (50%), 30% crayfish, 15% smooth spinach, and 5% olive oil were measured and formulated in a ratio of 5:3:1.5:0.5, respectively, and then reconstituted into solid pastes with hot water under the supervision of a dietician to ensure consistency (Table 1). The paste was modified into pellets for easy consumption of the animals and shade-dried. The animals were fed 20 g/kg.bw/day, 15 g/kg.bw/day, and 10 g/kg.bw/day of the feed as the high, moderate, and low doses, respectively (Thorbek *et al*., 1982).

Table 1. Composition of the Experimental Diet (Thorbek *et al.*, 1982)

Ingredient	% Composition
Whole Sorghum	50
Cray Fish	30
Smooth Spinach	15
Olive oil	5
Total	100

The experimental design comprises eleven (11) groups of five (5) rats each. Alloxan (84 mg/kg.bw.) was used to induce diabetes in the negative control (Group 2) and other experimental groups except for the normal control (Group 1). Group 3 animals were further subdivided into 3A, 3B, and 3C and were administered, in addition to alloxan, high dose (HD), moderate dose (MD), and low dose (LD) of glibenclamide (reference anti-diabetic drug) respectively. Group (4) was also subdivided into 4A, 4B, and 4C and received HD, MD, and LD of a low glycemic index diet (LGID). Group five (5) animals were subdivided into 5A, 5B, and 5C and received HD, MD, and LD of glibenclamide and LGID combination. The fasting blood glucose (FBG) of all the rats in each group was measured on weekly basis for 4 weeks.

Glycemic index and glycemic load determination

Meal training

The rats were acclimatized for one week on a standard chow diet (Vita feed). Following acclimatization, rats were fed at 7 a.m. and 4 p.m. every day. Initially, they were exposed to the test diet for one day, 7 a.m.–4 p.m., with chow provided at 4 p.m. until the following day. To start the training, rats were placed in individual cages for both meals and allowed to eat chow for two hours. It quickly became apparent that rats were not eating sufficiently, so the 4 p.m. feedings were changed to group feedings of three mice per cage, to induce competition between the rats. Thereafter, the 7 a.m. (beginning of dark phase) meal was referred to as the 'individual' feeding time, and the 4 p.m. (end of dark phase) meal was referred to as the 'group' feeding time. After three days, the meal time was halved and rats were allowed to eat for 1 h at both time intervals. An additional three days later, the available time for food was reduced to 30 min. It was determined that 1 h total of food access was the minimum amount of time to ensure sufficient feed intake. After another three days, the individual meal time was dropped for

a final time to 15 min, and correspondingly, the group meal time was increased to 45 min.

Determination of glycemic index (GI)

The glycemic index (GI) testing was performed once the rats were trained to reliably eat individually within 15 min at the start of the dark phase. The rats were placed individually in a clean cage without bedding. A drop of blood was obtained from the end of the rat's tails cut with a scalpel blade. The blood droplet was tested using an Accu-Chek Performa glucometer. The rats were allowed to settle for 15 min and then blood glucose levels were measured; this blood glucose level was considered the basal level and the test began. The rats were randomly provided 0.3 g of available carbohydrate from the test diet on a petri dish (which was obtained by subtracting the total fiber content from the carbohydrate content of the diet). The petri dish and any remnant food were removed after 15 min. Blood glucose levels were again estimated with a glucometer at 15, 30, 45, 60, 90, and 120 min intervals after provision of the food. If all food was consumed, the results were calculated. Otherwise, the test for that rat with that test food was repeated at least once. The control groups were orally administered with 0.3g of standard glucose dissolved in distilled water. Blood glucose levels were measured before oral administration of the glucose solution. Blood glucose levels were again measured with the aid of a glucometer at 15, 30, 45, 60, 90, and 120 min intervals after ingestion of the standard glucose solution.

Glycemic index calculation

Blood glucose curves were constructed from blood glucose values of animals in each group at time 0, after 15, 30, 45, 60, 90, and 120 minutes' intervals after consumption of the glucose (control) and test food. The incremental area under the curve (IAUC) was calculated for reference food (glucose) by the trapezoidal rule (Gibaldi and Perrier, 1982) as the sum of the surface of trapezoids between the blood glucose curve and horizontal baseline going parallel to the x-axis from the beginning of blood glucose curve at time 0 to the point at time 120 min to reflect the total rise in blood glucose concentration after eating the reference food (glucose). The IAUC from the animals fed with the test food was similarly obtained. The incremental area under the blood glucose response curve (AUC), was calculated using the formula **$\Delta x = (b-a)/n$.**

Such that $a = x_0 < x_1 < x_2 < x_3 < \ldots . < x_n = b$, x = equal subintervals between a and b, as reported by Wolever *et al.* (1991).

Estimation of glycemic load

Glycemic load (GL) was determined by the method of Salmeron *et al.* (1997). Glycemic load was calculated by taking the percentage of the food's carbohydrate content in a typical serving food and multiplying it by its glycemic index value. The following formula was used:

$$GL = \frac{Net\ Carbohydrate(g) \times GI}{100}$$

Net Carbs = Total carbohydrates in the food sample served.

Fasting blood glucose determination

Blood was collected from the tail of the rats by nipping with a sharp razor blade. Blood sugar was estimated from a drop of blood on a glucometer stripe attached to a glucometer (ACCU-CHEK® ACTIVE GU, Roche®, Mannheim Germany). Glucose estimation was done weekly throughout the study period (Ibrahim *et al*., 2016).

Lipid profile determination

Total Cholesterol, HDL cholesterol, triglycerides, and LDL cholesterol were measured with Cardio Check Professional Analyzer using PTS lipid panels test strips. PTS Panels® lipid panel test strips measure total cholesterol, HDL cholesterol, and triglycerides in whole blood with the cardioChek PA or the cardioChek Plus professional analyzer, and provide a quantitative result. PTS panels test strips are designed for use with fresh, capillary (fingerstick) whole blood, or fresh venous whole blood collected in EDTA or heparin tubes. A MEMo Chip® was provided with each package of test strips and must be properly inserted into the analyzer before any test can be run. The MEMo Chip contains the test name, calibration curve, lot number, and test strip expiration date. After the test strip is inserted into the analyzer and blood is applied to the test strip, test results are displayed in as little as 90 seconds (Ibrahim *et al*., 2016).

Combination index analysis

The combination index (CI) was obtained for fasting blood glucose, total cholesterol, triglycerides, HDL cholesterol, and LDL cholesterol using the method of Chou and Talalay (2008) by employing the derived equation:

$$CI = \frac{(D)1}{(Dx)1} + \frac{(D)2}{(Dx)2}$$

Where $(Dx)_1$ is for $(D)_1$ "alone" that inhibits a system x%, and $(Dx)_2$ is for $(D)_2$ "alone" that inhibits a system x% whereas in the numerator, $(D)_1 + (D)_2$, "in combination" also inhibit x%. Note that the denominators of the last two terms are the expression of MEE. The CI value quantitatively defines synergism (CI<1), additive effect (CI=1), and antagonism (CI>1).

Based on the above CI algorithms, a plot of CI values at different effect levels (fa's) was determined using CompuSyn Software, ComboSyn, Inc. PD Science LLC (USA).

Statistical analysis

The data were analyzed using SPSS version 23.0. The mean and standard deviation (SD) of the triplicate analyses were calculated. Repeated measure of analysis of variance (ANOVA) was performed to determine significant differences between the means at $p<0.05$.

RESULTS AND DISCUSSION

The nutrient compositions of the formulated diet are presented in Table 2. The diet was found to be rich in, protein (19.60±0.234%), fiber (9.63±0.061%), crude fat (7.80±0.021%), and some vital minerals like magnesium, potassium, and calcium of 280.48±0.624 mg/100g, 200.28±0.624 mg/100g, and 126.34±0.24 mg/100g respectively. There was a significant amount of vitamin C (13.42±0.126 mg/100g) in the diet. However, a relatively lower zinc concentration was observed in the formulated diet.

Table 2. Nutritional Composition of the Formulated Diet

Parameter	Nutrient value
Proximate composition (%)	
Total Carbohydrate	58.06±1.352
Crude Protein	19.60±0.234
Crude Fat	7.80±0.021
Moisture	3.70±0.021
Dietary fiber	9.63±0.061
Ash	2.37±0.172
Energy (kcal)	381.66±21.870
Minerals (mg/100g)	
Calcium	126.34±0.240
Magnesium	280.48±0.624
Potassium	200.28±0.624
Zinc	2.13±0.169
Sodium	28.69±0.126
Vitamin (mg)	
Vitamin C	13.42±0.076

Values are expressed as mean ± SD (n = 3 determinants)

The glycemic index and glycemic load of the diet were calculated to be 38.02% and 18.39% respectively as presented in Table 3.

Table 3. Incremental Area Under the Curve and GI Classification of the Formulated Diet

Test food	Available CHO (g)	iAUC (mmol.min/l)	GI (%)	GL	Class
Whole sorghum diet	0.3	197.54	38.02	18.39	Low

Table 4 shows the effect of a low glycemic index diet and glibenclamide on fasting blood glucose in alloxan-induced diabetic rats. The administration of alloxan (84mg/kg) caused a significant ($p < 0.01$) increase in the fasting blood glucose of the experimental animals when compared with the negative control. It was observed that in the second week of treatment, i.e. week two, the group treated with a combination of glibenclamide (0.5mg/kg.bw) and low glycemic index diet (LGID) (15g/kg.bw Group 5B) had a significant ($p < 0.05$) decrease in their fasting blood glucose concentration as compared to the negative control (Group 2) and other experimental groups. At the last week of treatment (week 4), it was observed that there was a significant ($p < 0.05$) decrease in the fasting blood glucose of groups treated with glibenclamide (1.0mg/kg.bw) + LGID (20g/kg.bw/day) and glibenclamide (0.5mg/kg.bw) + LGID (15g/kg.bw/day) as compared to the negative control (Group 2) and other experimental groups. A synergistic effect was observed at weeks three and four of treatment with high, moderate, and low doses of the combined treatment with glibenclamide and LGID with combination index values of less than 1.

Table 5 shows the lipid profile of alloxan-induced diabetic rats fed with a low glycemic index diet. There was a significant ($p<0.05$) increase in total cholesterol and triglyceride levels in diabetic control rats (Group 2) in contrast to the normal control (Group 1) and animals treated with a low dose of glibenclamide + LGID (Group 5C). Both groups treated with glibenclamide, LGID, and their combination (glibenclamide + LGID) showed significantly ($p<0.05$) reduced triglyceride level when compared with the diabetic controls. However, groups treated with LGID only, and combination of LGID and glibenclamide increased HDL and reduced LDL-C significantly compared with diabetic control to values similar to that seen in the normal control rats. HDL-cholesterol showed synergistic effect at all three doses with CI<1.

Table 4. Effect of a Low Glycemic Index Diet and Glibenclamide on Fasting Blood Glucose in Alloxan Induced Diabetic Rats

Group	WK0(mmol/l)	WK1(mmol/l)	WK2(mmol/l)	WK3(mmol/l)	WK4(mmol/l)
1	3.73±0.492[b]	3.93±0.535[b]	4.03±0.467[b]	4.18±0.610[b]	4.39±0.380[a]
2	15.49±1.584[a]	13.24±2.197[a]	13.18±2.454[a]	12.58±2.303[a]	9.01±0.831[ab]
3A	20.11±2.044[a]	17.37±3.919[a]	13.35±2.154[a]	10.46±0.650[a]	6.39±0.497[b]
3B	16.48±2.232[a]	14.49±1.741[a]	11.93±1.137[a]	10.09±1.112[a]	6.67±0.455[b]
3C	13.14±4.391[a]	12.10±3.795[a]	10.86±3.101[a]	9.03±1.250[a]	7.42±0.282[b]
4A	13.61±2.850[a]	12.09±1.967[a]	9.97±0.531[a]	7.65±0.500[ab]	6.38±0.522[b]
4B	13.56±2.301[a]	11.63±0.560[a]	9.93±0.908[a]	7.72±1.091[ab]	6.50±0.548[b]
4C	15.09±1.903[a]	13.54±2.376[a]	12.06±2.240[a]	10.20±1.335[a]	6.93±0.497[b]
5A	18.54±7.428[a]	13.38±3.685[a] CI=1.47	11.14±3.504[a] CI=1.50	8.01±1.444[ab] CI=0.27	5.42±0.564[a] CI=0.39
5B	13.85±2.752[a]	11.68±1.462[a] CI=1.09	8.89±0.937[b] CI=0.81	6.63±0.409[b] CI=0.21	4.86±0.328[a] CI=0.35
5C	16.72±3.783[a]	14.79±3.001[a] CI=1.00	12.36±2.726[a] CI=1.12	9.65±2.757[a] CI=0.31	6.50±0.867[b] CI=0.55

Values are expressed as mean±SD, (n=4). Mean values within the same column with different superscripts are statistically significant at $p<0.05$. CI- combination index.

CI<1= synergy which indicates that two or more components are mixed, and the effect is greater than the sum of the effects of the individual components when applied alone. CI>1= antagonism i.e. an interaction between two or more drugs that have opposite effects on the body, drug antagonism may block or reduce the effectiveness of one or more of the drugs. CI=1, additive i.e. this action occurs when the combined effect of two or more chemicals is equal to the sum of the effect of each agent given alone (they do not interact in a direct way). LGID= Low Glycemic Index Diet 1- Normal Control- Non-diabetic without receiving LGID and glibenclamide, 2- Negative Control- Diabetic without receiving LGID and glibenclamide, G3A- 1mg/kg.bw Glibenclamide, G3B- 0.5/mg/kg.bw Glibenclamide, G3C- 0.25mg/kg.bw Glibenclamide, G4A- 20g/kg.bw/day Diet, G4B- 15g/kg.bw/day Diet, G4C- 10g/kg.bw/day Diet, G5A- 1mg/kg.bw Glibenclamide + 20g/kg.bw/day Diet, G5B- 0.5mg/kg.bw Glibenclamide + 15g/kg.bw /day Diet, G5C- 0.25mg/kg.bw Glibenclamide + 10g/kg.bw/day Diet.

Table 5. Lipid Profile of Alloxan Induced Diabetic Rats fed with Formulated Diet from Whole Sorghum and Glibenclamide

Group	Treatments	Total Chol (mmol/L)	Triglycerides (mmol/L)	LDL-Chol (mmol/L)	HDL-Chol (mmol/L)
1	Normal Control- Non-diabetic without treatment	6.91±0.596[a]	5.09±1.435	1.74±0.302[a]	4.15±0.468[a]
2	Negative Control- Diabetic without treatment	8.27±0.602[b]	8.27±1.543[b]	3.74±0.382[b]	2.88±0.286[b]
3A	Glibenclamide 1mg/kg.bw	6.35±0.277[a]	4.57±2.050	1.48±0.236[a]	3.97±0.263
3B	Glibenclamide 0.5mg/kg.bw	6.10±0.478[a]	4.56±1.165[a]	2.34±0.810[a]	2.85±0.540[b]
3C	Glibenclamide 0.25mg/kg.bw	6.44±0.359[a]	5.59±2.126	2.26±0.439[a]	3.07±0.808[b]
4A	LGID 20g/kg.bw/day	5.98±0.531[a]	4.93±1.440	1.06±0.180[a]	4.24±0.249[a]
4B	LGID 15g/kg.bw/day	6.99±0.254[ab]	5.65±0.547	1.36±0.191[a]	4.40±0.102[a]
4C	LGID 10g/kg.bw/day	7.05±0.634[ab]	5.33±0.381	2.17±0.246[a]	3.81±0.611
5A	LGID 20g/kg.bw/day +Glibenclamide 1.0mg/kg.bw	6.52 ±0.377[a] CI=0.39	4.41 ± 1.105[a] CI=7.45	1.28 ± 0.229[a] CI=2.11	4.38 ± 0.305 [a] CI=0.59
5B	LGID 15g/kg.bw/day +Glibenclamide0.5mg/kg	6.92±0.730[ab] CI=94.56	5.42 ± 0.341 CI≫ 1	1.03 ± 0.099[a] CI=1.38	3.95 ± 0.291 CI=0.40
5C	LGID 10g/kg.bw/day +0.25mg/kg Glibenclamide	5.89 ±0.665[b] CI=100.70	4.23 ± 1.180[a] CI≫ 1	1.38 ± 0.470[a] CI=0.55	3.71 ± 0.481 CI=0.38

Values are expressed as mean ± SD, (n=4). Mean values within the same column with different superscripts are statistically significant at $p<0.05$. CI- combination index.

CI<1= synergy which indicates that two or more components are mixed, and the effect is greater than the sum of the effects of the individual components when applied alone. CI>1= antagonism i.e. interaction between two or more drugs that have opposite effects on the body, drug antagonism may block or reduce the effectiveness of one or more of the drugs. CI=1, additive i.e. this action occurs when the combined effect of two or more chemicals is equal to the sum of the effect of each agent given alone (they do not interact directly). LGID = Low Glycemic Index Diet. CI=1, additive i.e. this action occurs when the combined effect of two or more chemicals is equal to the sum of the effect of each agent given alone (they do not interact directly).

Discussion

Literature indicated the efficacy of low glycemic index diets in the management of diabetes. The present study shows that the nutrient composition of the formulated diet contains an appreciable amount of fiber. Jenkins *et al.* (2012) reported that high fiber and low glycemic index diet with legumes reduced blood glucose levels as compared with a wheat fiber diet in type 2 diabetic patients. Diet rich in dietary fiber is beneficial for the treatment of type 2 diabetes mellitus (ADA, 2013), as dietary fiber ameliorates postprandial hyperglycemia by delaying digestion and absorption of carbohydrates and enhancing satiety, which leads to a reduction in body weight (Post *et al*., 2012). It was recently reported that the consumption of a high fiber diet for four weeks enhanced insulin secretion in non-diabetic overweight subjects (Bodinham *et al*., 2012). Also, the result of this present study showed a high amount of magnesium in the formulated diet. The inverse relationship between cereal grains and diabetes may also be attributed to increased consumption of magnesium as a higher concentration of magnesium was shown in the cereal grains in this study. This finding may support the previously reviewed evidence by Kelley and Mandarino (2000) who reported that increased intake of magnesium can decrease the incidence of type 2 diabetes. The present study revealed a significant amount of potassium present in the formulated diet. Similarly, the literature indicates that potassium supplementation yields improved insulin sensitivity, responsiveness, and secretion. Insulin administration induces a loss of potassium; and a high potassium intake reduces the risk of heart disease, atherosclerosis, and cancer (Norbiato *et al*., 1984; Khaw and Barrett-Connor, 1984). The incorporation of low-glycemic index carbohydrate foods such as whole grains in the diet has been shown to reduce the post-prandial and 24 hours glucose response in individuals with diabetes (Simpson *et al.*, 1981). The result of glycemic index and glycemic load indicated that the formulated diet from whole sorghum is a low glycemic index and glycemic load diet. This could be attributed to the fact that intact whole grain sorghum was used and the diet was coarsely ground. Besides, it is well documented that low GI and GL of foods may be affected by factors such as variety, processing, and particle size as well as fibre, and protein content among other factors (Modu *et al.*, 2011). Numerous animal feeding studies have shown that whole grain sorghum in diet effectively improves glucose metabolism compared to sorghum-free diets (Chung *et al*., 2011). The fat and protein content of the formulated diet in this research could have been attributed to the low glycemic index value of the diet. In correspondence to the current findings, Lee *et al*. (2022) suggested that adding fat and protein to carbohydrate-containing foods has the potential to reduce the glycemic response and lower the overall GI. The mechanisms by which these nutrients affect blood glucose concentration have been proposed in many studies. For instance, high levels of protein have been shown to produce greater gastric inhibitory peptide (GIP) and insulin responses resulting in a lower postprandial glucose peak and a reduced glycemic response (Hätönen *et al*., 2011), while higher fat content has the potential to delay gastric emptying, thereby slowing digestion and the absorption of glucose (Henry *et al.*, 2006). Further affirming that the formulated feed used in this study is of low glycemic index according to WHO classification of low, moderate, and high glycemic index diets.

The anti-diabetic activity of sorghum observed in this study may be partially attributed to the presence of condensed tannins. An earlier study has shown that the tannin-rich extract from brown sorghum bran exhibits inhibitory activities against porcine pancreatic α-amylase at low concentrations (Hargrove *et al.*, 2011). It was observed in this study, that combined treatment of the animals with glibenclamide and LGID resulted in a remarkable increase in the fasting blood glucose in comparison with the rats treated separately with glibenclamide and LGID. The result of the lipid profile revealed that the hypercholesterolemia and hypertriglyceridemia observed in the alloxan-exposed rats were significantly ($p<0.05$) reduced by the combined treatment of the diabetic rats with glibenclamide and LGID. Apart from the regulation of carbohydrate metabolism, insulin plays an important role in lipid metabolism. Insulin insufficiency, in diabetes mellitus, is associated with hypercholesterolemia and hypertriglyceridemia, which have been reported to occur in experimental diabetic rats (Loci *et al.*, 1994). However, the present study revealed that LGID formulated from whole grain sorghum alone caused a significant increase in HDL cholesterol thereby, lowering LDL cholesterol levels. Furthermore, combined treatment with LGID and glibenclamide exhibited a synergistic effect in increasing the HDL cholesterol of such groups. However, Lakshmi and Vimala (1996) observed that daily consumption of 100g of unrefined sorghum in the form of pancakes over 3 weeks showed a significant reduction *($p<0.05$)* in total cholesterol, triglycerides and LDL cholesterol and an increase in HDL cholesterol. Combined treatment with LGID and glibenclamide played a better role in lowering LDL cholesterol level.

CONCLUSION

Evidence from the present study showed that the combined treatment with a low glycemic index diet (LGID) and

glibenclamide was highly effective in lowering the blood glucose concentrations in diabetic rats. The synergy observed had a significant effect in increasing the HDL cholesterol concentration thereby lowering the LDL cholesterol concentration as seen in this study. Similarly, the combined treatment was found to be highly effective in managing some of the complications associated with diabetes, especially hyperlipidemia. A low glycemic index diet (LGID) exhibited a synergistic effect on the hypoglycemic potential of glibenclamide. Therefore, the combined treatment could be useful in the management of diabetes mellitus.

AUTHORS' CONTRIBUTIONS

The conceptualization of the study was by author AIb. Author SYM drafted and revised the manuscript. Formal analysis was done by author MB; Investigation was by ZDS and Methodology was by AId. All the authors read through the final version and gave approval for its publication.

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None

CONFLICT OF INTEREST

The authors wish to declare that there is no conflict of interest.

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