Effect of ginger flour supplementation on fermented millet flour ‘ibyer’ anti-diabetic and biochemical properties

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Abstract

COVID-19 is a mucoso-respiratory highly contagious disease that has leaded to a tremendous globFermented millet flour (ibyer) is an indigenous non-alcoholic gruel made from cereals either (maize, sorghum and millet). It is prepared by cooking reconstituted cereal flour or wet milled paste with water. In this study, fermented millet flour supplemented with ginger powder blends were formulated in the ratio 100:0, 95:5, 90:10, 85:15, 80:20, 75:25 and 70:30 for the production of gruel. The blends were subjected to feeding trial experiment using wistar albino rat. Results analysis revealed that Serum cholesterol was less than 200 mg/dl. The fasting blood glucose was also within the recommended range (67.7 - 125.0 mg/dl). The biochemical parameters were within recommended range, total serum protein ranged from 5.82-7.06 g/L, Alanine aminotransferase ranged from 28.53 to 41.13 iu/L, Aspartate aminotransferase ranged from 28.50 to 48.66 iu/L. The albino rats showed slight increase in body weight throughout the experimental period, ranging from 78.67 -103.80 g. The experiment shows that the diet did not have any adverse effect on the experimental animals and were within the recommended range hence a good anti diabetic blend and has excellent biochemical profile properties for homes use and applications.

Introduction

Diabetes mellitus is a serious health hazard currently affecting more than 220 million people worldwide and is expected to afflict 400 million by 2030 [1]. It is a common health problem and a serious metabolic disorder associated with many functional and structural complications [2]. Clinical definitions of disease often obscure different mechanistic subtypes. It is a chronic lifelong condition that affects the body’s ability to use the energy found in food. It is caused by the body not producing enough insulin or not being able to utilize it for the absorption of glucose to provide the needed energy or may be a combination of both conditions [3]. It is a clinical syndrome characterized by hyperglycemia due to absolute or relative deficiency of insulin and/or insulin sensitivity [4].

Chronic hyperglycemia leads to long term damage, dysfunction and eventually the failure of organs, especially the eyes, kidneys, nerves and cardiovascular system [4,5]. When diabetes is left untreated it could result into heart disease, stroke, blindness and nerve damage to the legs. According to World Health Organization, the diabetes population is likely to increase to 300 million or more by the year 2025. Currently available therapies for diabetes include insulin and various oral antidiabetic agents such as sulfonylureas, metformin, meglitinides, biguanides, glinides and recently stem cell implants, which are used as monotherapy or in combination to achieve better glycemic regulation. Many of these oral antidiabetic agents have a number of serious adverse effects. Thus, the management of diabetes without any side effects is still a challenge [6].
Millets are known to have a low glycemic index as suggested by some in vivo studies however all of these studies have mainly focused on millet products from composite flour [7,8]. Starch digestibility studies on the 100% cooked millet flour have been rarely done. Dietary fibre, phenolics and lipids which are mainly lost during decortication may also affect in vitro starch digestibility [9,10]. Removal of protein and lipid or both has shown to significantly increase the expected glycemic index (eGI) [11].

Ginger has been used for thousands of years for the treatment of numerous ailments, such as colds, nausea, arthritis, migraines, and hypertension. The medicinal, chemical, and pharmacological properties of ginger have been extensively reviewed [12], as preventive or therapeutic agents [13].

There are no studies to compare the in vitro starch digestibility of cooked millet flour from whole and decorticated grains hence the study limitations. Due to difference in the grain morphology between millets and other cereals, the efforts to decorticate millet by known cereal milling methodologies including abrasion, friction mills or other dehulling techniques without biofunctional losses have not been successful. The present study was aimed at assessing the quality of fermented millet (Pennisetum glaucum) flour supplemented with ginger (Zingiber officinalis) powder its anti-diabetic properties and serum biochemistry profiles using albino rats.

Materials and methodology

Sample preparation

The most common flour production is to convert the grain/tubers in the form of flour which is achieved by milling, using hammer mills.

Preparation of fermented millet flour

The method described by Sengev et al. [14] was used with slight modifications for the production of millet flour. Pearl millet flour was prepared as shown in figure 1. The grains were sorted and cleaned to remove unwanted materials like stones, pebbles and other foreign seeds, before washing with tap water and steeping (72 h). Therefore, the grains were drained, dried, milled and sieved to get whole pearl millet flour.

Preparation of ginger powder

Ginger flour will be prepared according to the method of Sekwati-Monang, [15] with slight modification as shown in figure 2. Fresh ginger roots were sorted by soaking in water to get rid of dirts and to remove unwanted materials, before washing with tap water. The cleaned roots were drained, sliced, sundried, milled using hammer mill and sieved through 600 μm aperture size.

Preparation of “ibyer” from fermented millet flour and ginger powder blends

Ibyer was produced as described by Kure and Wyasu [16] with slight modification. Each sample weighing 100:0, 95:5, 90:10, 85:15, 80:20, 75:25, 70:30 (Table 1) of both fermented millet flour and ginger powder were mixed each with 10 ml of distilled water to form a slurry. It was allowed to ferment for 12 h. 200 ml of boiling water was added to the slurry which was heated for 10 minutes with continuous stirring to avoid the formation of lumps. The gruel was allowed to cool to 40 °C. The production flow chart is as shown in figure 3.
Experimental animals’ model and their maintenance

Twenty-one (21) healthy wistar albino rats aged 3 weeks (21 days), weighing 100 g to 130 g were obtained from Benue state university, college of health science. Three rats were kept in animal cages in an animal house Department of Home Science and Management, Federal University of Agriculture, Makurdi. The rats were allowed to acclimatize with the laboratory condition for 7 days in well ventilated cages. They were divided into 7 groups of 3 rats each. Each of the rats was given an identification mark in form of an indelible mark on tail, head and back. During the acclimatization period, the rats were also provided access to food and water. Each group was provided with 100 g of the respective diet on daily basis for 28 days and the left over was collected each next morning and weighed. The animals were also provided with clean tap water ad libitum throughout the experimental period. The rats were weighed before and after acclimatization and on weekly basis throughout the experimental feeding period. The mean weekly weight gain was computed.

Induction of experimental diabetes

A total of 21 rats (surviving from the normal feeding trial) were used in the experiment. Diabetes was induced by a single intraperitoneal injection at 130 mg/kg body weight with alloxan monohydrate after the animals were fasted for 24 hours. Blood was collected from the tail vein of the rats after 72 hours of alloxan injection by measuring the initial glucose level using glucometer. Rats having fasting blood glucose (FBG) level greater than 200 mg/dl were confirmed diabetic. The fasting blood glucose was determined at day 17, 24 and 31 respectively for 2 weeks using one touch digital glucometer using one touch digital glucometer.

Measurement of body weight

The body weight of each animal was assessed using an electronic weighing scale once before commencement of the feeding and then every 7 day interval for 28 days.

Blend formulation (%) of fermented millet flour supplemented with ginger powder for experimental feeding

Diets were prepared using vitalyte (5%), sucrose (10%), corn starch (70%), rice husk (5%) and fermented millet flour and ginger powder (in different proportion) as shown in the Table 2.

Treatment of the animals

Each group was provided with 100 g of the respective diet as per daily portion provided in the Table 2.

Table 1: Blend Formulation (%) of fermented millet flour supplemented with ginger powder for "ibyer" production.

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Millet</th>
<th>Ginger</th>
</tr>
</thead>
<tbody>
<tr>
<td>716</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>924</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>839</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>746</td>
<td>85</td>
<td>15</td>
</tr>
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<td>958</td>
<td>80</td>
<td>20</td>
</tr>
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<td>469</td>
<td>75</td>
<td>25</td>
</tr>
<tr>
<td>577</td>
<td>57</td>
<td>30</td>
</tr>
</tbody>
</table>

KEY: 716 = M100(Control), 924 = M95G5, 839 = M90G10, 746 = M85G15, 958 = M80G20, 469 = M75G25, 577 = M70G30. Where, M = Millet, G = Ginger

Table 2: Blend formulation for experimental feeding.

<table>
<thead>
<tr>
<th>Sample code</th>
<th>716</th>
<th>924</th>
<th>839</th>
<th>746</th>
<th>958</th>
<th>469</th>
<th>577</th>
</tr>
</thead>
<tbody>
<tr>
<td>Millet</td>
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<td>9.5</td>
<td>9</td>
<td>8.5</td>
<td>8</td>
<td>7.5</td>
<td>7</td>
</tr>
<tr>
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<td>-</td>
<td>0.5</td>
<td>1</td>
<td>1.5</td>
<td>2</td>
<td>2.5</td>
<td>3</td>
</tr>
<tr>
<td>Corn starch</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>Vitalyte</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
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<tr>
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<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

KEY: 716 = M100(Control), 924 = M95G5, 839 = M90G10, 746 = M85G15, 958 = M80G20, 469 = M75G25, 577 = M70G30. Where, M = Millet, G = Ginger

Quantitative determination of total protein concentration in serum was measured by biuret method using Randox Diagnostic Assay kits.

**Principle:** Protein react with cupric ions in alkaline solution to form a colored chelate. The absorbance of final color is proportional to the concentration of total protein in the serum sample at 546 nm.

**Procedure:**

1. The reagent (1.0 ml) of reagent was pipetted into three different clean dry tubes as blank, standard and test sample. After that, 20 μl of protein standard and 20 μl of serum sample were added in respective tube then mixed properly. The mixture was incubated at 37 °C for 5 minutes and absorbance was measured at 546 nm against the reagent blank in a spectrophotometer and calculated as follows.

\[
\text{Total protein concentration (mg/dL)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 6
\]

Total cholesterol determination

Quantitative determination of total cholesterol in blood was measured by CHOD/PAP methods using TECO Diagnostic kit USA.

**Assay principle:** For total cholesterol, esters were hydrolyzed by cholesterol esterase to give free cholesterol and fatty acids. In subsequent reaction, cholesterol oxidase oxidizes the 3-OH group of free cholesterol to liberate cholest-4-en-3-one and hydrogen peroxide. In the presence of peroxidase, hydrogen peroxidase couples with 4-aminoanipyrine (4-AAP) and phenol to produce red quinonine dye. Absorbance colored dye is measured at 505 nm and is proportional to amount of total cholesterol concentration in serum sample.

**Procedure:** The reagent (1.0 ml) of reagent was pipetted...
into three clean dry tubes labelled as blank, standard and test. After that, 10 μL of cholesterol standard and 10 μL of serum sample was added in respective tubes labelled with standard test. The mixture was incubated at 37 °C for 10 minutes. The absorbance of the test sample as well as standard was measured immediately against their respective blank at 505 nm in a UV-Visible spectrophotometer and the results were calculated using the formula given below.

\[
\text{Total Cholesterol (mg/dL)} = \frac{\text{Absorbance of test sample} \times 200 \text{ cholesterol standard absorbance}}{\text{Absorbance of standard}}
\]

**Serum aspartate aminotransferase (AST) determinations**

The serum AST was estimated colorimetrically by the 2,4-dinitrophenylhydrazine (2,4-DNPH) method using Randox Diagnostic Kit (Randox Laboratories Ltd, Crumlin, UK).

**Principle:** The enzyme aspartate aminotransferase (glutamate oxaloacetate transaminase-GOT) catalyzes an exchange of amino acid group of aspartate for an α-keto group (i.e. converts L-aspartate and α-ketoglutarate to oxaloacetate and L-glutamate). The oxaloacetate formed reacts with 2,4-dinitrophenylhydrazine to produce a hydrazine derivative, which in an alkaline medium (sodium hydroxide) produces a brown coloured complex whose intensity can be read by a spectrophotometer.

\[
\text{L-aspartate} + \alpha\text{-ketoglutarate AST} \rightarrow \text{Oxaloacetate} \rightarrow + L\text{-glutamate}\OXALOACETATE + 2,4,\text{DNPH NaOH} 2,4, \text{Dinitrophenyl hydrazone (Brown colored complex)}
\]

**Procedure:** Two (2) test tubes labelled “blank” and “sample” were prepared. Procedure 1 (measurement against a reagent blank) was adopted for the determination of serum AST. The solution (0.5 ml) R1 (L-aspartate, buffer, α-ketoglutarate) was placed in the two test tubes each. Thereafter, 0.1 ml of serum (representing 21 samples of the 7 groups) was placed in the marked “sample”. Also 0.1 ml of distilled water was added to the “blank” test tube. The content of the tubes were mixed thoroughly and incubated in a water bath at 37 °C for 30 minutes. After 30 minutes of incubation, 0.5 ml solution of R2 (2,4, DNPH) was added to both test tubes, mixed and allowed to stand for 20 minutes at room temperature and the content was measured at 540 nm using spectrophotometer.

**Serum alanine aminotransferase (ALT) determination**

The serum ALT was estimated colorimetrically by the 2,4-dinitrophenylhydrazine (DNPH) method using Randox Diagnostic Kit (Randox Laboratories Ltd., Crumlin, UK).

**Principle:** The enzyme alanine aminotransferase (glutamate pyruvate transaminase- GPT) catalyzes an exchange of amino acid group of alanine for an α-keto group of α-ketoglutarate (i.e. converts L-alanine and α-ketoglutarate to pyruvate and L-glutamate). The pyruvate formed reacts with 2,4-dinitrophenylhydrazine (2,4-DNPH) to produce a hydrazine derivative, which in an alkaline medium (sodium hydroxide) produces a brown coloured complex whose intensity can be read by a spectrophotometer.

\[
\text{L-alanine} + \alpha\text{-ketoglutarate ALT} \rightarrow \text{pyruvate} + L\text{-glutamate}
\]

\[
\text{Pyruvate} + 2,4, \text{DNPH NaOH} \rightarrow 2,4, \text{dinitrophenyl hydrazone (Brown colored complex)}
\]

**Procedure:** Two (2) test tubes labelled “blank” and “sample” were prepared. Procedure 1 (measurement against a reagent blank) was adopted for the determination of serum ALT. The solution (0.5 ml) solution R1 (phosphate buffer, L-alanine and α-ketoglutarate) was placed in the two test tubes each. Thereafter, 0.1 ml of serum (representing 21 samples of the 7 groups) was placed in the marked “sample”. Also 0.1 ml of distilled water was added to the “blank” test tube. The content of the tubes were mixed thoroughly and incubated in a water bath at 37 °C for 30 minutes. After 30 minutes of incubation, 0.5 ml solution of R2 (2,4, DNPH) was added to both test tubes, mixed and allowed to stand for 20 minutes at room temperature and the content was measured at 540 nm using spectrophotometer.

**Statistical analysis**

The mean and standard deviation of the result data from the experiment was calculated and analyzed using single factor analysis of variance (ANOVA) in the statistical package for social science (SPSS, 2003) Software (SPSS version 12.0.1 for windows). The Duncan’s New Multiple Range Test was used to determine the significance difference between mean values.

**Result**

Fasting blood sugar level (mg/dL) of the experimental alloxan induced albino rats fed fermented millet flour supplemented with ginger powder is shown on table 3. The experimental albino rats were considered diabetic when the glucose level got above 200 mg/dL. The Table 3 shows that the fasting blood glucose of the experimental animals increased significantly on day 17 which shows the albino rats were hyperglycemic after alloxan induction in all the groups. There was a slight decrease in glucose level of the experimental albino rats on day 24 and day 31 in all the groups apart from the control group (716) which was unstable due to 0% ginger.

Serum cholesterol level (mg/dL) of experimental albino rats fed fermented millet flour supplemented with ginger powder is shown on table 4. The serum cholesterol level of experimental albino rats fed fermented millet flour spiced with ginger powder. The serum cholesterol level decreased from day 7 to day 14 while an increase on day 21 due to the effect of the alloxan monohydrate and a decrease on day 28. The control group was unstable due to the absence of ginger in the diet. The values were within the recommended range for cholesterol level (< 200 mg/dL).
Serum total protein content (g/L) of the experimental albino rats fed fermented millet flour supplemented with ginger powder is shown on Table 5. The serum total protein of the albino rats fed fermented millet flour supplemented with ginger powder. The serum total protein increased from day 7 to day 14 in all the groups while a decrease in day 21 when alloxan was introduced because alloxan caused a defect in the secretion of protein. On day 28 the serum total protein increased while the control group was unstable from the initial day to day 28 due to the absence of ginger. The values obtained were within the recommended range 3.5-8 g/L.

Aspartate aminotransferase (iu/L) of experimental albino rats fed fermented millet flour supplemented with ginger powder is shown on Table 6. The AST value of the experimental albino rats fed fermented millet flour supplemented with ginger powder reveals an enzyme found in the muscle tissue and other organs such as pancreas, kidney, heart cells and liver. The AST value was within the recommended range reported by Al-Amin, et al. [17]. The AST decreased from day 7 to day 14 and increased on day 21 due to the effect of alloxan. On day 28 the AST reduced which indicate that the animals were utilizing their diet well. The values were within the recommended range (10-55 iu/L).

Alanine aminotransferase (iu/L) of experimental animals fed fermented millet flour supplemented with ginger powder is shown on table 7. The ALT is a liver test. It is a test used to detect whether a liver is functioning.

The Table 7 shows the ALT value of the experimental albino rats fed fermented millet flour supplemented with ginger powder. The ALT decreased from day 7 to day 14 during the normal feeding trial and increased on day 21 due to the effect of alloxan monohydrate. There was an increase on day 28 in all the groups apart from the control group which was unstable from the initial to the day 28. The values obtained were within recommended range (7-56 iu/L).

### Discussion

Fasting Blood Glucose level of experimental alloxan monohydrate induced albino rats fed fermented millet flour supplemented with ginger powder is shown in table one. From the table 1, the FBG of the experimental albino rats showed that at day 14 when the alloxan monohydrate was introduced, the FBG was checked after 72 H of induction and the values were high showing that the animals were diabetic. At day 24 the glucose level reduced indicating that the diet was able to bring down the glucose level. The feeding continued to day 31 and when the glucose level was checked it reduced more than the day 24 and this is due to the presence of Zingiberene which worked on Neuroprotective effect of ginger on antioxidant chemicals.

Cholesterol is a waxy, fat like substance that is found in all the cells in the body. Cholesterol is a naturally occurring fat that the body needs to produce important structure and chemicals.

From table 4, the cholesterol level decreased from day 7 to day 14. After alloxan induction at day 21, the cholesterol level increased due to the effect of alloxan. At day 28 the cholesterol level reduced due to the effect of ginger and millet.
The experimental animals were utilizing their diet well. The cholesterol level increased from day 7 to day 28 due to the absence of ginger.

The Serum total protein is a biochemical test for measuring the total amount of protein in serum. Protein in serum is made up of albumin and globulin. From Table 5, the serum total protein increased from day 7 to day 14 during the normal feeding trial.

After alloxan was induced at day 21 the serum total protein decreased due to the effect of the alloxan. At day 28, the serum total protein increased which means the animals were utilizing their diet well. The control was unstable from day 7 to day 28 because the diet does not contain ginger.

AST are enzymes found mainly in the muscle tissues and other organs such as pancreas, kidney, heart cells and liver. From Table 6, the AST reduced from day 7 to day 14. After alloxan induction at day 21, the AST increased because of increased conditions of cell damage and also due to the effect of alloxan. At day 28, the AST reduced showing that ginger powder at various concentration did not have any damage to the heart, muscle, brain and kidney that are vital organs in the body because the values were within range reported by Al-Amin, et al. [17] that worked on Antidiabetic and hypolipidaemic properties of ginger (Zingiber officinale) in streptozotocin induced diabetic rats. The control sample was unstable from day 7 to day 28 of the feeding trial.

Alloxan aminotransferase ALT is a liver test. It is known as the main liver marker. It is an enzyme found in the cells of the liver. From Table 7, the ALT reduced from day 7 to day 14 and the reduction shows that ginger powder at various concentrations did not have any damage to the liver, heart, muscle, brain and kidney that are vital organs in the body because the values were within range reported by Al-Amin, et al. [16] that worked on Antidiabetic and hypolipidaemic properties of ginger (Zingiber officinale) in streptozotocin induced diabetic rats. After alloxan induction, the ALT increased at day 21 and reduced at day 28. The control experienced increment from day 7 to day 28 due to the absence of ginger.

**Conclusion**

Fermented millet flour spiced with ginger flour has favourable impact on measurements of glucose in rats with
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diabetes. Ginger has effects on carbohydrate metabolism and insulin sensitivity. Ginger was significantly effective in lowering serum glucose, serum cholesterol in the diabetic rats compared with the control. The biochemical parameters showed that cholesterol level, AST, ALT decreased. There was a fluctuation in the control sample however, during alloxan induction, there was a slight decrease in cholesterol level, AST and ALT from day 21 to day 28 while the total serum protein increased from day 21 to day 228.

Acknowledgement

The authors acknowledged the department of Food science and Technology for support in the course of this research.

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