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# Bitter gourd flavored Non-Alcoholic Wheat Beer (NAWB) exhibited antidiabetic properties by modulating carbohydrate metabolizing enzymes and upregulates insulin and GLUT-2 mRNA expressions in High Fat Diet/Streptozotocin (HFD/STZ) induced diabetic rats

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## Abstract

To improve the control of Type 2 diabetes (T2D), this study investigated the potential benefits of an alcohol-free beer flavored with bitter gourd leaves, a plant with proven hypoglycemic properties. The high fat/streptozotocin (HFD/STZ) model was used to induce diabetes in Wistar rats as test subjects. The rats were divided into eight groups ($n=5$) as follows: HP (STZ + 100% Hops); BG (STZ + 100% Bitter Gourd); 75:25BG (STZ + 75% Hops; 25%BG); 50:50BG (STZ + 50%Hops50%Bitter Gourd); 25:75BG (STZ + 25%Hops75%Bitter Gourd); Acarbose (STZ + Acarbose); DC (STZ-diabetic control group); NC (Normal Control group). Following a 14-day treatment, there was a significant ($p<0.05$) reduction in blood sugar, serum glucose, α-amylase activity, α-glucosidase activity, and lipase activity. As the percentage of bitter gourd inclusion increased, the expression of GLUT-2 and insulin genes was upregulated. The beer sample with the lowest percentage inclusion of Hops (25:75BG) had the lowest glycemic index (GI). The study suggested that bitter gourd-flavored alcohol-free beer reduces blood glucose through muptiple pathways and could be a useful dietary intervention in the management of type 2 diabetes.

**Keywords** Type 2 diabetes, Non-alcoholic beer, Glycemic index, Insulin, GLUT-2

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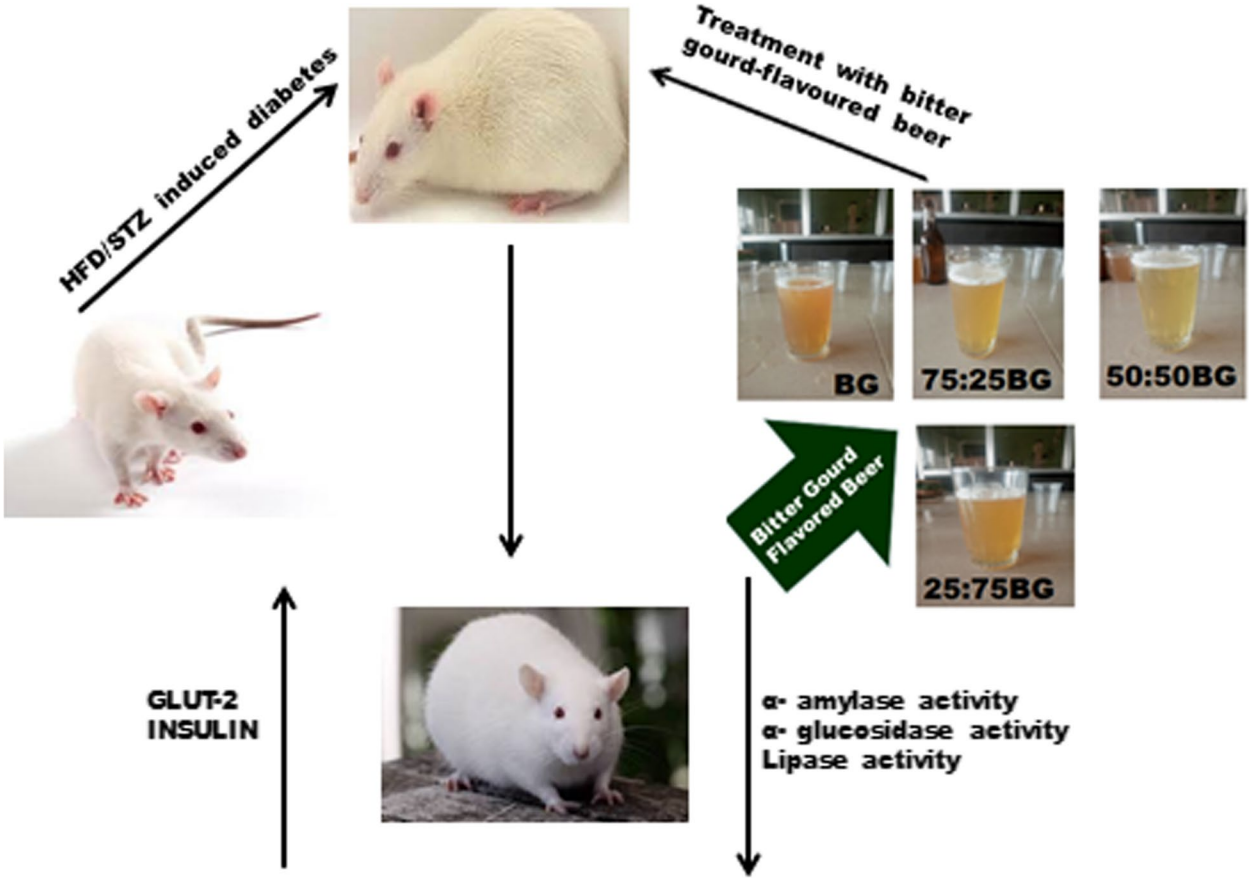
**Graphical Abstract**




## Introduction

Globally, beer ranked behind water and coffee as the third most consumed drink. As industrialization spread, brewing gradually shifted from being a home-based activity to an industrial one (Pokrivčák et al. 2019). Hops are utilized as a bittering and flavoring agent as well as a preservative, while cereals serve as the sugar source. For many years, barley malt was thought to be the only cereal suitable for the production of beer, and hops as the major bittering/flavoring agent. However, over time, arious plants and fruits have been used with hops to flavor beer (Adamenko et al. 2020). A growing acceptance of wheat malt in the brewing industry has resulted from the search for alternative sources of sugar for beer production (Malomo 2015). Additionally, to maximize the health benefits of beer that have been linked to it, "special beers"—including non-alcoholic, low-alcohol, and craft beers with varying alcohol content per volume (ABV) levels depending on the laws of each country where such beer is produced—have been brewed (Salanță et al. 2020a, 2020b). The need for specialized "non-alcoholic beers" was brought on by the negative effects of alcohol consumption, including addiction and health issues including liver cirrhosis and neurodegeneration (Mellor et al. 2020). In Nigeria reports by the Nigerian National Bureau of Statistics (2019) and Uloko et al (2018) have shown a correlation between the prevalence of diabetes and the amount spent on alcohol across the different regions in Nigeria. The South-South region that spends the highest amount on alcohol also had the highest prevalence of diabetes, while the North-West region that spends the lowest amount on alcohol has the lowest recorded incidence of diabetes. Although there are several conflicting reports on the role of alcohol in diabetes prevalence, however, there is a link between alcohol usage and diabetes. Special beers are growing globally

due to their alleged health benefits, regulatory issues, alcohol consumption restrictions in some nations, and safety (Ignat et al. 2020). Additionally, to enhance the health advantages of beer, prior studies have looked into the bittering and flavorful effects of substituting other plants for hops (Adenuga et al. 2010; Zapata et al. 2019). There have been reports that herbs like a bitter leaves, bitter kola, and utazi leaves can be used in place of hops while making beer (Adenuga et al. 2010). Also, the associated difficulties in getting hops (availability and economic reasons) in parts of Africa have triggered a search for alternatives to hops in brewing.

Recent research has demonstrated that beer has health benefits, including antioxidant and anti-inflammatory properties (Salanță et al. 2020a) as well as its potential in managing diabetes (Hernández-Quiroz et al. 2020). In sub-Saharan Africa, over 25 million cases of the metabolic illness including diabetes have been reported, and the disease is expected to affect 500 million people globally. By 2045, diabetes is expected to rise by 162.5% (Saeedi et al. 2019). The use of medicinal plants and their byproducts is still a significant therapeutic approach for treating human ailments (Adamenko et al. 2020; Adenuga et al. 2010). Herbs have long been used to treat diabetes. Plants and plant extracts have been used to treat diabetes since antiquity. Among the native populations of Asia, South America, India, the Caribbean, and East Africa, *Momordica charantia (M. charantia*), also known as bitter melon, karela, balsam pear, or bitter gourd, is a well-liked herb for treating diabetes-related conditions (Cousens 2008; Joseph & Jini 2013). Because of the fruit's distinctively bitter flavor, which becomes more intense as it ripens, it is also known as a bitter melon or a bitter gourd. Numerous facts and theories explaining the anti-diabetic properties of *M. charantia* have been generated through biochemical and animal model investigations (Singh et al. 2012; Joseph & Jini 2013) with the phenolic constituents of bitter gourd such as gallic acid and epicatechin reported being involved in many pathways leading to a reduction in elevated blood glucose. The reported efficacy of bitter gourd in glycemic control of diabetes has therefore necessitated this study to see how bitter leaf could be used in substituting hops in bittering and flavoring beer to give a healthy option to diabetics as a drink.

## Materials and methods

### Sample collection

Hops were acquired from a reputed brewery in Ilesha, Osun state, Southwest Nigeria, while wheat was procured from Oja-Oba, Akure, Ondo State. Before use, the hops were kept at 4 °C. Bitter gourd leaves were sourced within Akure Metropolis and certified at the herbarium, CERAD, FUTA. The leaves were pulverized after being air-dried to a fixed weight. For later usage, the pulverized leaves were kept at room temperature in an airtight container.

### Chemicals and reagents

Analytical-grade chemicals and reagents were utilized in all experiments. P-nitrophenyl-D glucopyranoside, dinitrosalicylic acid color reagent, and streptozocin (STZ) (Art. No. S)-130 were purchased from Sigma-Aldrich, Inc. (St Louis, MO, USA). From BDH Chemicals Ltd. in Poole, Dorset, UK, we obtained the following chemicals: ethanol, acetic acid, sulfuric acid, sodium carbonate, methanol, potassium acetate, perchloric acid, phenol, and sodium hydroxide. From Matador Pharmaceutical Ltd., blood glucose test strips for the Fine-test glucometer and Acarbose were purchased (Akure, Ondo State, Nigeria).

### Beer production and characterization

#### *Production of beer*

Adenuga et al. (2010)'s methodology was used with minor adjustments. Wheat grains were soaked in water for 48 h at 20 °C, with 8-h intervals between water changes. The malting procedure, which involves germination of the wheat grains on clean trays covered with polythene nylon for five days, was followed by a 70° C. hot air kilning. The rootlets were taken out, the grain was crushed into a coarse texture, and it was kept at room temperature in sealed containers until it was needed. Then, 115 g of crushed malted wheat grains were weighed into a sieve and infused for 30 min in 5 L of warm water at 60 °C. The spent grit was removed once the wort came to a boil. The remainder of the hop pellets and bitter gourd powder were added to the wort at the 10-min mark of a two-hour boil. The hop pellets and bitter gourd powder were added to the wort in the following ratios: 100%, 75:25, 50:50, and 25:75. The wort was filled to its original volume with warm water, and it was then cooled at 12 °C. Brewer's yeast weighing 5 g was added, and it was allowed to ferment for 14 days. Following the fermentation process, the alcohol in the fermented beer was extracted using a distiller operation at 80 °C. The alcohol level was monitored using an alcohol meter. Finally, beer was bottled and allowed to age for three months.

#### *Total phenol determination*

To evaluate the total phenol content of the beer samples, 50 µL of the samples were mixed with 500 µL of 10%v/v Folin-Cioalteau reagent and 400 µL of 7.5% sodium Carbonate ($Na_2CO_3$). The reaction mixture was then incubated at 45C for 40 min, and the absorbance was

measured at 765 nm. The total phenol content was determined as the gallic acid equivalent (GAE) using gallic acid as the standard (Singleton et al. 1999).

#### *Total flavonoid determination*

The total flavonoid content of the beer samples was ascertained using the proposed method of Meda et al. (2005). The beer sample (500 µL) was diluted with 50 µL of 10% Aluminum Chloride ($AlCl_3$), 50 µL of 1 M potassium acetate ($CH_3COOK$), and 1.4 µL of distilled water. The reaction mixture was incubated at room temperature for 30 min and the absorbance was read at 415 nm. The total flavonoid content was expressed as Quercetin equivalent (QE) using quercetin as standard.

### Experimental design

A few alterations were made to the Oboh and Ogunruku (2010) methodology for feed composition. From the Animal House, Department of Biochemistry, Federal University of Technology, Akure, 40 mature male Wistar rats (150–175 g), 5 weeks old, were acquired and given a two-week acclimatization period. The rats were housed in sterile plastic cages with a 12-h light/dark cycle under typical laboratory settings before being fed commercially available feed pellets and given water for the acclimation period.

### Animal care and handling

The ethical committee of the Centre for Research and Development (CERAD), Federal University of Technology, Akure authorized the protocol used and handling (Ethical no.: FUTA/ETH/20/25) and handling of the animals, which follows the guidelines published by National Institutes of Health (NIH), USA, for the care of experimental animals. The study was conducted in the Functional Food, Nutraceuticals, and Phytomedicine Laboratory at the Federal University of Technology in Akure, Nigeria.

### Glycemic index

Five (5) groups of five (5) rats each were established from the twenty-five (25) male albino adult Wistar rats that were purchased from the Animal House, Department of Biochemistry, Federal University of Technology, Akure (FUTA). The rats had unlimited access to water and were fed commercially available feed pellets. After two weeks of acclimatization, food was withheld from them, and allowed to fast overnight before receiving the beer samples. Rats' blood glucose levels were checked using an Accu-Chek Fine test glucometer at intervals of 0, 30, 60, 90, 120, 150, and 180 min while the samples were given via oral intubation. The trapezoidal formula was then used to calculate the glycemic response (Dona et al. 2010; Wolever et al. 1991).

### Induction of diabetes with STZ in HFD-fed Wistar rats (type 2 diabetic rat model)

Diabetes was induced using a modified procedure of Srinivasan and Ramarao (2007). Adult Wistar rats were given access to commercially available rat pellets (with a crude protein content of 15%, a crude fat content of 4%, a crude fiber content of 1.1%, and calcium, phosphorus, and lysin content of 0.38%) before being given a high-fat diet. These pellets were obtained from Farm Support in Akure. Following acclimation, the rats were divided into two diet groups: the normal control (NC) and the high-fat diet (HFD). To induce diabetes in the rats fed with HFD, STZ was injected intraperitoneally after two weeks of HFD dietary manipulation at a single dose of 35 mg/kg body weight. The rats' blood glucose levels were measured 72 h after induction using tail vein punctures to collect blood samples and fasting blood glucose levels were determined using an automatic auto-analyzer (Fine-test Auto-coding TM). The study utilized rats with fasting blood glucose levels of > 200 mg/dL. Intraperitoneally, the control group received 1 mL of 0.1molL citrate buffer. Seven groups of five diabetic rats each were created at random from the diabetic rats. The various groups were then subjected to various treatment plans using the beer samples. The rats were grouped as follows:

Group I: Normal control (citrate buffer pH 4.5; 1 mL/kg intraperitoneally).—NC.
Group II: Type-2 diabetic control group—DC.
Group III: Type-2 diabetic rats administered 25 $mgkg^{-1}$ body weight oral dose of Acarbose—Acarbose.
Group IV: Type-2 diabetic rats administered 15 ml $kg^{-1}$ body weight oral dose of beer samples flavored with only hops—HP.
Group V: Type-2 diabetic rats administered 15 ml $kg^{-1}$ body weight oral dose of beer samples flavored with only bitter gourd.—BG.
Group VI: Type-2 diabetic rats administered 15 ml $kg^{-1}$ body weight oral dose of beer samples flavored with 75% hops and 25% bitter gourd-75:25BG.
Group VII: Type-2 diabetic rats administered 15 ml $kg^{-1}$ body weight oral dose of beer samples flavored with 50% hops and 50% bitter gourd – 50:50BG.
Group VIII: Type-2 diabetic rats administered 15 ml $kg^{-1}$ body weight oral dose of beer samples flavored with 25% hops and 75% bitter gourd – 25:75 BG.

The animal were allowed to fast overnight, and on the $15^{th}$day, the animals were sacrificed, and the pancreas tissue was immediately isolated. A small portion was cut

into Trizol® for the RNA extraction, while the remaining tissue was rinsed with cold saline solution before homogenized with phosphate buffer (0.1 M pH 7.4) and centrifuged at 5,000 g for 10 min to collect the supernatant used for the determination of biochemical assays. Cardiac blood was collected into the plain bottle and was prepared into serum, which was used for the insulin determination using Calbiotech® ELISA kit.

#### Determination of blood glucose level

A Fine-test Auto-coding TM glucometer was used to measure the blood glucose levels using the glucose oxidase–peroxidase method. Throughout the 14-day treatment period, fasting blood glucose levels were checked every three days. After two weeks of treatment, the serum glucose level was assessed using a Randox test kit.

#### Determination of homeostatic model assessment for insulin resistance (HOMA-IR) levels

Fasting Insulin levels were determined using a Calbiotech®ELISA kit. HOMA-IR levels were calculated using the formula:

$$\frac{\text{Fasting blood sugar } (\text{mg/dL}) * \text{Fasting Insulin}}{405}$$

#### Determination of α-amylase activity

The method outlined by Worthington Biochemical Corporation (1993) was slightly modified to measure the pancreatic α-amylase activity. Saline phosphate buffer and 50 µL of pancreatic homogenate were incubated at 25 °C for 10 min. To the reaction mixture, 50 µL of 1% starch was then added, and it was incubated at 25 °C for an additional 10 min. The reaction was halted using a 200µL solution of dinitrosalicylic acid (DNSA), and the mixture was then heated in a water bath for 5 min to 100 °C. After cooling to room temperature and further diluting the reaction mixture with 2 ml of distilled water, the absorbance at 540 nm was measured.

#### Determination of α-glucosidase activity

Briefly, 430 µL of phosphate buffer, 15 µL of GSH, and 15 µL of supernatant (intestine homogenate) were pipetted into test tubes. A 40 µL solution of p-nitrophenyl-D-glucopyranoside was then added after the mixture had been incubated at 37 °C for 10 min. 2 mL of $Na_2CO_3$ was added to the solutions after another 20 min of incubation at 37 °C. Absorbance was read at 400 nm (Apostolidis et al. 2007).

#### Determination of lipase activity

Using the Lucia et al. (2006) methodology, the activity of pancreatic lipase was assessed in a reaction mixture containing 1 mL of a substrate, 0.5 mL of phosphate buffer, and 100 µL of tissue that had been diluted to 3 mL with distilled water. 200 µL of isopropanol was added after 5 min of incubation at 40 °C, and the absorbance was measured at 410 nm. The substrate contained 19 mL of solution B and 1 mL of solution A (40 mg of p-nitrophenyl palmitate in 12 mL isopropanol) (0.1 g of gum Arabic and 0.4 mL of Triton X).

#### RNA extraction and reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted from the pancreas of the rats in each group using Trizol®. RNA in the samples was measured using Nanodrop2000TM, visualized with 1.5% agarose gel, and treated with DNase I. (Invitrogen). 1 g of cDNA was created using the iScriptTM cDNA Synthesis kit. For all expression levels, two reference genes—--Insulin and GLUT-2—were used as the benchmark. It took 40 thermal cycles of 15 s at 94 °C, 10 s at 60 °C, and 30 s at 72 °C to complete the reactions, which were carried out in a final volume of 20:l with 2.5 ng/L of cDNA, 1 × PCR buffer, 0.2 M of each primer, 0.2 mM dNTP, 2 mM $MgCl_2$, 0.1 × SYBR® Green, and 0.25 U platinum Taq DNA (Olagoke et al. 2021). Using a single cycle of 94° C for 10 s, 55° C for 1 min, and 94° C for 15 s, dissociation curves were obtained to validate the amplification of a single unique product for each reaction. Utilizing SYBR fluorescence, the design and analysis tool StepOne™ was used. The reactions were carried out in triplicate in three to six different experiments. The values of gene expression were determined using the 2CT approach (Livak & Schmittgen 2001).

#### Data analysis

The aggregate data from studies done in triplicate were expressed as mean and standard deviation. Using IBM SPSS Statistics for Windows, version 20 (IBM Corp., Armonk, N.Y., USA), means were compared using one-way analysis of variance, followed by Duncan's multiple range testing and least significant differences. Results were accepted at $p < 0.05$ (Yalta 2008).

## Results and discussion

In a diabetic condition, the body's capacity to use or retain glucose declines after the consumption of carbs, and the liver produces more glucose during the time between meals, which leads to hyperglycemia (Szablewski 2011). Hyperglycemia is brought on by either a decrease in insulin synthesis, a decrease in insulin action, or a combination of the two disorders (Czech 2017). One major way of managing hyperglycemia is through dietary intervention. This study focuses on how produced beer could be exploited as a dietary intervention for managing hyperglycemia.

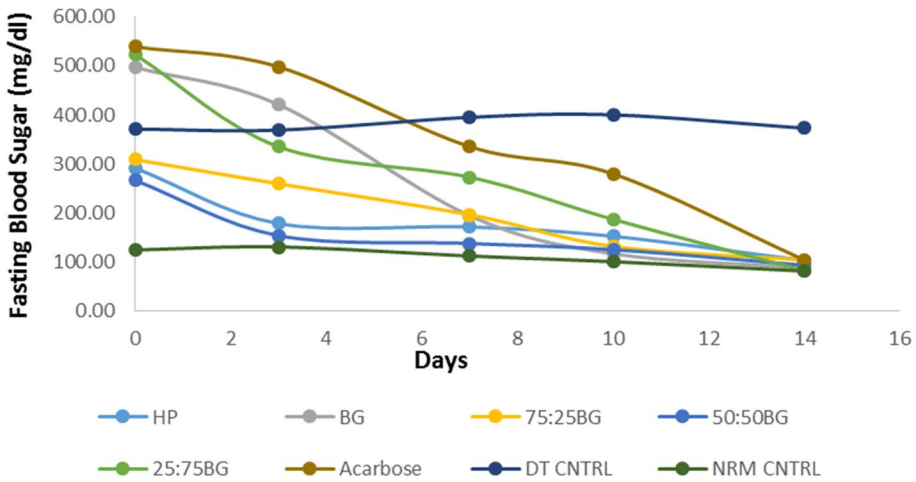



**Fig. 1** Effect of Non-alcoholic Wheat Beer Flavored with Bitter Gourd on Fasting Blood Sugar Level of Type 2 Diabetic rats. HP (STZ + 100%Hops); BG (STZ + 100% Bitter gourd); 75:25BG (STZ + 75%Hops25%Bitter Gourd); 50:50BG (STZ + 50%Hops50%Bitter Gourd); 25:75BG (STZ + 25%Hops75%Bitter Gourd); Acarbose (STZ + Acarbose); DC (STZ-diabetic control group); NC (Normal Control group)

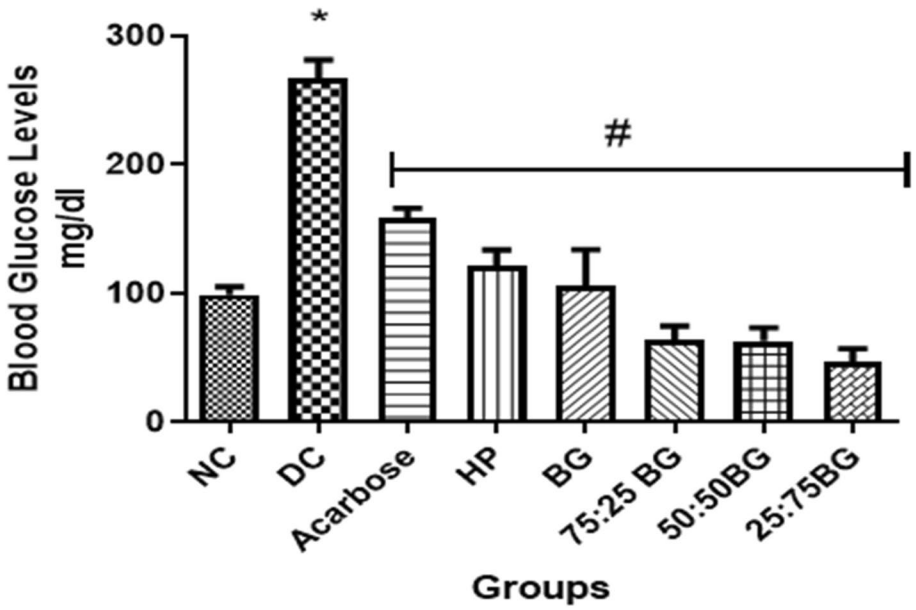



**Fig. 2** Effect of Non-alcoholic Wheat Beer Flavored with Bitter Gourd Flavored on Blood Glucose Levels of Type 2 Diabetic rats. HP (STZ + 100%Hops); BG (STZ + 100% Bitter gourd); 75:25BG (STZ + 75%Hops25%Bitter Gourd); 50:50BG (STZ + 50%Hops50%Bitter Gourd); 25:75BG (STZ + 25%Hops75%Bitter Gourd); Acarbose (STZ + Acarbose); DC (STZ-diabetic control group); NC (Normal Control group)

Streptozotocin (STZ) impairs insulin secretion by triggering necrosis and destroying the β-cells of the pancreas resulting in a similar situation seen in Type-1 diabetes. However, low doses of STZ mildly impair insulin secretion by triggering apoptosis rather than necrosis in the affected β-cells, allowing for the regeneration of impaired pancreatic β-cells; a condition similar to Type-2 diabetes mellitus (T2DM). However, the mild impairment of insulin secretion does not address the insulin resistance associated with T2DM; various research however has revealed that rats fed with a HFD become insulin resistant, therefore, creating an HFD/STZ model that causes a mild impairment of insulin secretion as well as insulin resistance closely mimic a T2DM condition (Nath et al. 2017; Sahin et al. 2007; Srinivasan et al. 2005). Figure 1 shows the various groups of the experimental animals having > 200 mg/dl fasting blood glucose except for the normal control

**Table 1** The glycemic index of bitter gourd flavored beer samples

| Sample | Glycemic Index (GI) |
|---|---|
| HP | 55 |
| BG | 62 |
| 75:25 BG | 51 |
| 50:50 BG | 47 |
| 25:75 BG | 37 |

*Abbreviations: HP* Hops alone, *BG* Bitter Gourd alone, *75:25BG, Hops* Bitter Gourd (75:25%), *50:50BG, Hops* Bitter Gourd (50:50%), *25:75BG, Hops* Bitter Gourd (25:75%)

group that wasn't induced. Over the 14-day treatment period, the fasting blood glucose level of the treated animals reduced, except for the diabetic control. After the 14-day treatment the serum glucose levels were analyzed using a Randox glucose kit, the result is presented in Fig. 2. All the treated groups were significantly different from the diabetic control group. The groups treated with beer samples of varied inclusion of bitter gourd had lesser serum glucose when compared with the other treated groups. More importantly, 25:75BG had the lowest glucose level though it is not significantly different from the other groups.

In managing T2DM, one of the proposed means is by promoting the consumption of food/food products with a low glycemic index (GI) (Oboh et al. 2021). Table 1 shows the GI of the beer samples. GI is a measure of postprandial blood glucose response. The outcome of various research has shown a close-knit relationship between GI and diabetes management (Akerele et al. 2021; Bell et al. 2015; Eleazu 2016). Food products that have been labeled to have high GI tend to raise postprandial blood glucose faster and higher than those labeled to have low GI. Food/food products with GI 55 and below are classified as having low GI, and these products tend to make the consumer have a feeling of being full for a longer period than when foods with a high GI are consumed. Food with GI of 56–70 is classified as food with medium GI while food with 70 and above is classified as having high GI (Foster-Powell et al. 2002). The beer samples had GIs ranging from 37—62. The beer sample with the highest inclusion of bitter gourd:hops (25:75BG) had the lowest GI of 37 while the beer sample with only Bitter Gourd (BG) had the highest GI (62). This observation might be due to the phenolic (phenol and flavonoid) content of the beer samples, which showed that higher percentage inclusion of bitter gourd in the beer samples in ratio to hops had higher phenolic and flavonoid content. The total phenol and flavonoid contents presented in Table 2 shows that the beer sample with 25% Hops and 75% bitter gourd had the highest phenolic contents. There might be a reaction between the phenolic contents of hops and bitter gourd that could have brought about the increase in the flavonoid and phenolic content as the percentage of bitter gourd included is increased. This is in contrast to earlier findings of Akerele et al. (2022) which showed that the higher the inclusion of bitter leaf in ratio to hops in the beer samples, the higher the GI. However, the higher the percentage inclusion of bitter gourd in ratio to hops in the beer samples in this study, the lower the GI.

In managing T2DM, various mechanisms have been proposed by various researchers. One such is the inhibition of carbohydrate metabolizing enzyme (α-amylase and α-glucosidase) activities. α-amylase hydrolyses starch polymers into small-chain dextrins by acting on the α-1,4 glycosidic bonds in the starch molecule, while α-glucosidase breaks down disaccharides into glucose. The inhibition of these enzymes thus reduces a rise in postprandial blood glucose (Kazeem et al. 2013; Teng & Chen 2017). The inhibition of these enzymes has been linked by different reports with the (poly) phenols present in plants (AL-Ishaq et al. 2019). The result presented in Fig. 3 showed that the treated samples had a lower enzyme activity when compared to the diabetic control. The samples with differing ratios of Hops:Bitter Gourd however had a higher inhibition on α-amylase activity when compared to the other treated groups. Furthermore, in Fig. 4, the effect of the beer samples on α-glucosidase activity in diabetic rats also showed that the beer samples with various percentage inclusions of Hops:Bitter gourd also had a higher inhibitory effect on α-glucosidase activity. The inhibitory effect of the beer samples on α-amylase and α-glucosidase activity might likely be due to the phenolic constituents of both

**Table 2** Total Flavonoid (mg QE/mL) and Total Phenol (mg GAE/mL) Contents of Bitter Gourd Flavored Non-alcoholic Wheat Beer

| Sample | HP | BG | 75:25BG | 50:50BG | 25:75BG |
|---|---|---|---|---|---|
| Total Flavonoid | $1.55 \pm 0.02^{a}$ | $1.71 \pm 0.01^{a}$ | $1.40 \pm 0.01^{a}$ | $1.65 \pm 0.01^{a}$ | $1.90 \pm 0.01^{b}$ |
| Total Phenol | $14.07 \pm 0.03^{a}$ | $12.00 \pm 0.06^{a}$ | $15.71 \pm 0.03^{a}$ | $17.00 \pm 0.03^{b}$ | $17.27 \pm 0.03^{b}$ |

Results are expressed as mean ± standard deviations (SD) of three determinations. Averages followed by different letters on the same row differ by Tukey test at $p < 0.05$

HP-100% Hops; BG-100% Bitter gourd; 75:25 BG- 75% Hops:25% Bitter gourd; 50:50BG-50% Hops:50% Bitter gourd; 25:75BG- 25%Hops:75%Bitter gourd

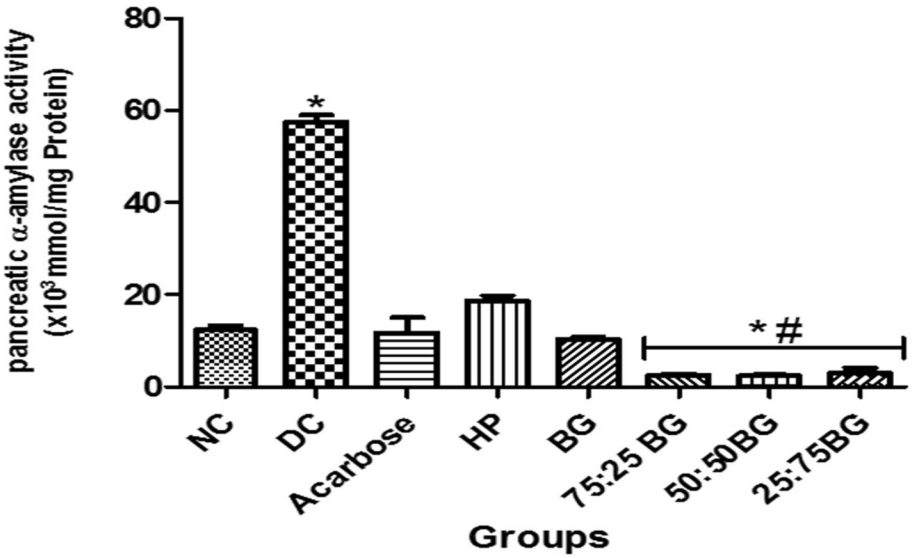



**Fig. 3** Pancreatic α-amylase Activity of Type 2 Diabetic Rats Treated with Non-Alcoholic Wheat Beer Flavored with Bitter Gourd. HP (STZ + 100%Hops); BG (STZ + 100% Bitter gourd); 75:25BG (STZ + 75%Hops25%Bitter Gourd); 50:50BG (STZ + 50%Hops50%Bitter Gourd); 25:75BG (STZ + 25%Hops75%Bitter Gourd); Acarbose (STZ + Acarbose); DC (STZ-diabetic control group); NC (Normal Control group)

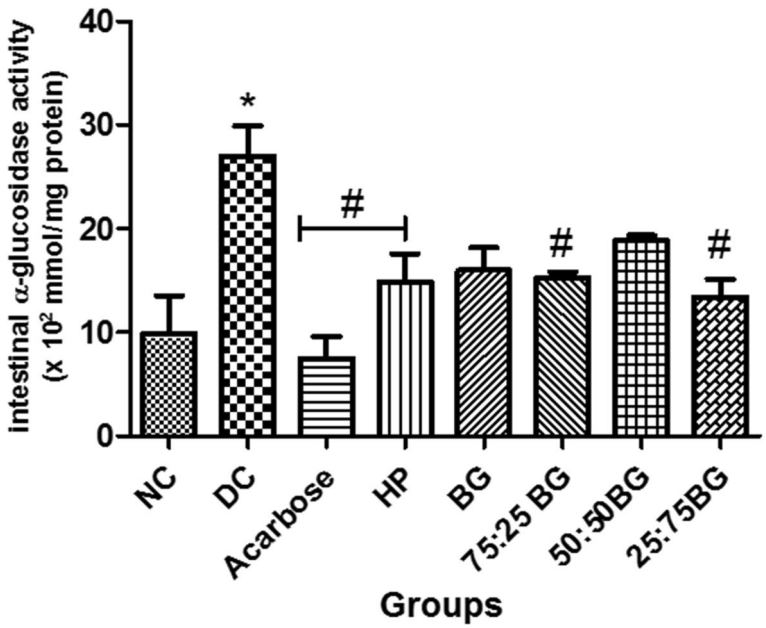



**Fig. 4** Intestinal α-glucosidase Activity of Type 2 Diabetic Rats Treated with Bitter Gourd Flavored Non-Alcoholic Wheat Beer. HP (STZ + 100%Hops); BG (STZ + 100% Bitter gourd); 75:25BG (STZ + 75%Hops25%Bitter Gourd); 50:50BG (STZ + 50%Hops50%Bitter Gourd); 25:75BG (STZ + 25%Hops75%Bitter Gourd); Acarbose (STZ + Acarbose); DC (STZ-diabetic control group); NC (Normal Control group)

the hops and bitter gourd leaves. This is in agreement with several past studies (AL-Ishaq et al. 2019; Kazeem et al. 2013) that have linked the inhibition of these starch hydrolyzing enzymes with the phenolic constituents of plants. This shows that there is a relationship between carbohydrate metabolizing enzymes and GI.

Inhibition of lipase activity has also been proposed as another mechanism through which T2DM can be managed or controlled. Lipase catalyzes the breakdown of triacylglycerols (TAGs) into glycerol and fatty acids. Increased plasma fatty acids however have been linked with insulin resistance, therefore inhibition of

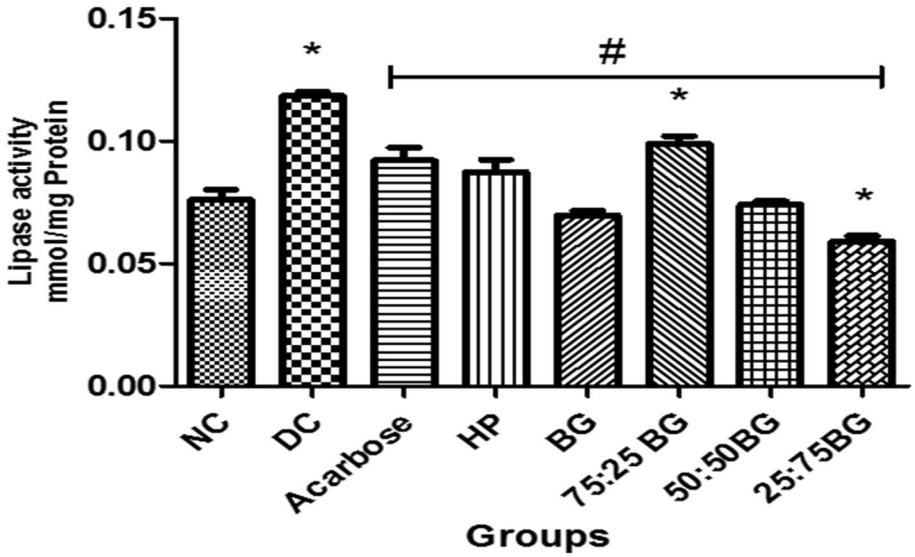



**Fig. 5** Lipase Activity of Type 2 Diabetic Rats Treated with Bitter Gourd Flavored Non-Alcoholic Wheat Beer. HP (STZ + 100%Hops); BG (STZ + 100% Bitter gourd); 75:25BG (STZ + 75%Hops25%Bitter Gourd); 50:50BG (STZ + 50%Hops50%Bitter Gourd); 25:75BG (STZ + 25%Hops75%Bitter Gourd); Acarbose (STZ + Acarbose); DC (STZ-diabetic control group); NC (Normal Control group)

**Table 3** Fasting blood sugar, Fasting Insulin and HOMA-IR levels of STZ-induced diabetic rats treated with bitter gourd flavored Non-alcoholic Wheat Beer

| Group | FBS (mg/dL) | FI (μU/ml) | HOMA-IR |
|---|---|---|---|
| NC | 81.00 ± 7.62 | 11.85 ± 1.74 | 2.37 ± 0.07 |
| DC | 372.74 ± 11.53 | 9.31 ± 0.98 | 8.55 ± 1.60 |
| Acarbose | 103.50 ± 0.71 | 8.62 ± 0.44 | 2.20 ± 0.10 |
| HP | 102.67 ± 8.74 | 10.62 ± 0.87 | 2.69 ± 0.31 |
| BG | 87.33 ± 7.77 | 9.15 ± 0.65 | 1.97 ± 0.25 |
| 75:25BG | 103.67 ± 9.19 | 9.85 ± 1.52 | 2.52 ± 0.17 |
| 50:50BG | 93.00 ± 2.89 | 9.62 ± 0.76 | 2.21 ± 0.21 |
| 25:75BG | 81.67 ± 2.89 | 10.89 ± 1.96 | 2.20 ± 0.24 |

Results are expressed as mean ± standard deviations ($n = 5$)

HP (STZ + 100%Hops); BG (STZ + 100% Bitter gourd); 75:25BG (STZ + 75%Hops25%Bitter Gourd); 50:50BG (STZ + 50%Hops50%Bitter Gourd); 25:75BG (STZ + 25%Hops75%Bitter Gourd); Acarbose (STZ + Acarbose); DC (STZ-diabetic control group); NC (Normal Control group)

*FBS* Fasting Blood Sugar, *FI* Fasting Insulin, *HOMA-IR* Homeostatic Model Assessment for Insulin Resistance

lipase activity thus helps in curbing insulin resistance (Dostálek et al. 2017; Park et al. 2020). Figure 5 shows the effect of the various beer samples on lipase activity. The treated samples all inhibited lipase activity with BG, 50:50BG, and 25:75BG having the highest inhibitory effect on lipase activity. The highest percentage inclusion of BG together with hops however seems to have a higher inhibitory effect on lipase activity. This also corresponds to the result of the GI, α-glucosidase, and plasma glucose levels. The inhibition of lipase is in tandem with the report of Rahim et al. (2015) that showed that polyphenols, most especially flavonoids possessed strong lipase inhibitory ability. The inhibition of lipase activity corresponds to the phenolic and flavonoid content of the beer samples (Table 2).

HOMA-IR was designed as an indirect measure of insulin resistance which marks an early stage of T2DM. It is derived from fasting glucose and fasting insulin levels. It tells how much insulin is needed to keep blood sugar levels in check. Although different studies have reported different ranges for HOMA-IR, however, there is a consensus that the higher the HOMA-IR levels, the more insulin resistance (Matthews et al. 1985; Gutch et al. 2015; Sinaiko & Caprio 2012). Table 3 shows the DC group having the highest HOMA-IR levels. The HOMA-IR levels decreased as the bitter gourd in ratio to hops is being increased although not significantly different. The levels of HOMA-IR might also be responsible for the upregulation of the insulin gene as the HOMA-IR levels correspond to the expression of insulin in rats treated with the beer samples.

The effect of the beer samples on diabetic rats was further explored at the molecular level. The glucose transporter GLUT-2 plays a critical role in the glucose-stimulated insulin generation from pancreatic beta-cells and the glucose metabolism in hepatocytes. In pancreatic beta-cells, GLUT-2 serves as a glucose sensor, detecting even little fluctuations in blood sugar levels and increasing the release of insulin (Ogilvy-Stuart & Beardsall 2020). This study demonstrates a relationship

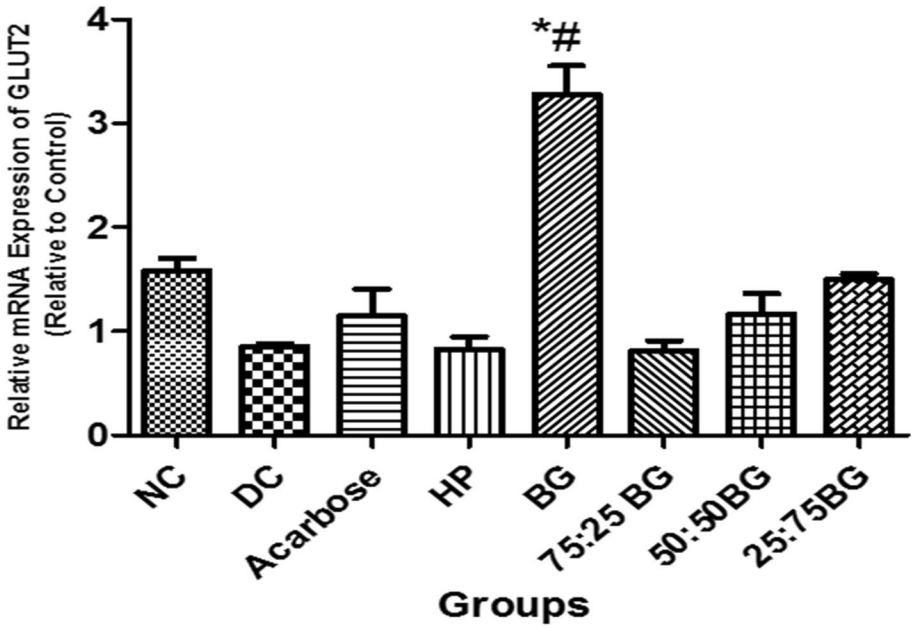



**Fig. 6** Relative mRNA expression of GLUT-2 in the pancreas of type 2 diabetic rats treated with bitter gourd flavored non-alcoholic wheat beer. HP (STZ + 100%Hops); BG (STZ + 100% Bitter gourd); 75:25BG (STZ + 75%Hops25%Bitter Gourd); 50:50BG (STZ + 50%Hops50%Bitter Gourd); 25:75BG (STZ + 25%Hops75%Bitter Gourd); Acarbose (STZ + Acarbose); DC (STZ-diabetic control group); NC (Normal Control group)

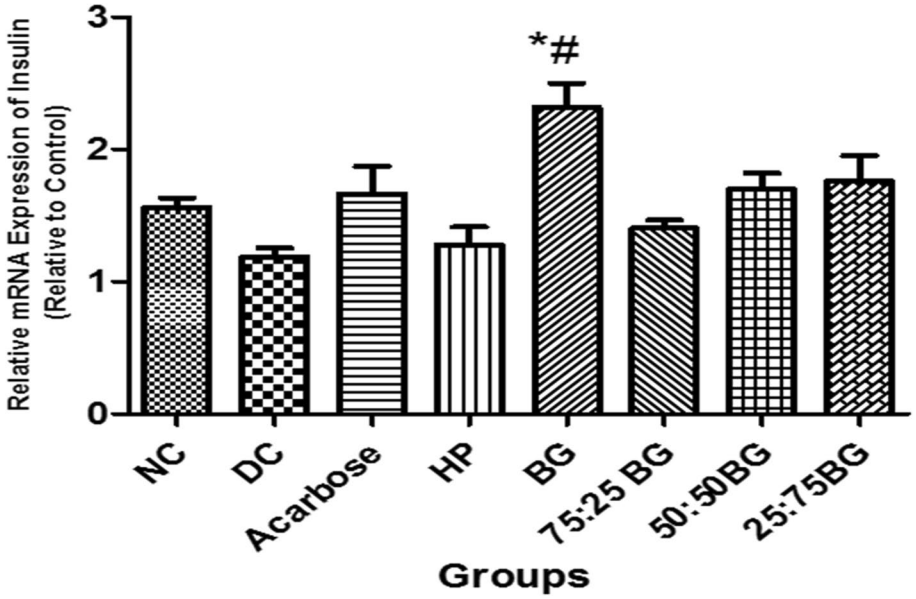



**Fig. 7** Relative mRNA expression of Insulin in the pancreas of type 2 diabetic rats treated with bitter gourd flavored non-alcoholic wheat beer. HP (STZ + 100%Hops); BG (STZ + 100% Bitter gourd); 75:25BG (STZ + 75%Hops25%Bitter Gourd); 50:50BG (STZ + 50%Hops50%Bitter Gourd); 25:75BG (STZ + 25%Hops75%Bitter Gourd); Acarbose (STZ + Acarbose); DC (STZ-diabetic control group); NC (Normal Control group)

between insulin and GLUT-2 mRNA expressions. The group treated with BG had the highest expression of GLUT-2 as presented in Fig. 6 as well as insulin as shown in Fig. 7, significantly different from the DC and NC. The insulin expression corresponds to the GLUT-2 expression. Though not significantly different, the insulin expression was upregulated as the percentage inclusion of BG increased, this also corresponds to what is obtainable in the GLUT-2 expression. This is in line with various research that has shown a link

between GLUT-2 expression and Insulin expression (Al-Shaqha et al. 2015; Sharawy et al. 2016). Furthermore, the upregulation of GLUT-2 and insulin might be due to the phenolic content of the beer samples. Quercetin a known phenolic constituent of bitter gourd (Saliu et al. 2019) has been reported to reduce glucose absorption by GLUT-2 and inhibit insulin-dependent activation of phosphoinositide 3-kinases (PI3K), thus making room for upregulation in the expression of insulin. Quercetin also has a regulatory effect on the nuclear factor kappa-light-chain-enhancer of activated β-cells (NF-κB) which helps in improving glucose-stimulated insulin secretion (AL-Ishaq et al. 2019).

## Conclusion

The results of this research have shown that alcohol-free-BG-flavored beer having better effects on all proposed mechanisms in T2DM management when compared to that flavored with hops only. The effect of the bitter gourd/hops flavored beer on all the proposed mechanisms in managing T2DM, therefore, makes it a promising drink that not only helps satiates the thirst for beer in diabetics but as well helps in lowering blood glucose. Further trials on diabetic patients could however be carried out in a bid to ascertain the effect of this drink during clinical trials.

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43014-023-00161-0.

**Additional file 1.**

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**Authors' contributions**
G. P. Akerele – Beer production, Methodology, data curation and analysis, write-up. B.C. Adedayo – Result analysis and supervision. Prof. G. Oboh – Experimental research design. O.B. Ogunsuyi – Methodology and PCR analysis. I.S Oyeleye- Data curation, Editing and Methodology. The author(s) read and approved the final manuscript.

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**Availability of data and materials**
The datasets during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

## Declarations

**Ethics approval and consent to participate**
The ethical committee of the Centre for Research and Development (CERAD) of the Federal University of Technology, Akure authorized the protocol used for the animal study, and it was issued the certificate number FUTA/ETH/20/25. The National Institutes of Health (NIH), USA, published recommendations for the care of experimental animals, were followed.

**Consent for publication**
Not applicable.

**Competing interests**
We declare that there are no competing interests either financial or non-financial for this research.

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## Publisher's Note