Kombucha modulates the gut Microbiota and promotes Insulin secretion and Pancreatic Beta cell recovery during Diabetes.

Emmanuel Edem Adade[1, 2], Nathaniel L. Lartey[1,3], Eric NY Nyarko[4], Winfred-Peck Dorleku[1, 2], Olga Quasie[5], Peter Karuri Gathumbi[6], Laud N.A. Okine[1, 2], Winfred Seth K. Gbewonyo[1, 2], and Elmer Nayra Ametefe[1, 2*]

[1]Department of Biochemistry, Cell and Molecular Biology, University of Ghana, Accra, Ghana

[2]West African Centre Cell Biology of Infectious Pathogens, University of Ghana, Ghana.

[3]Department of Health and Allied Sciences, Baldwin University College, Osu-Accra, Ghana.

[4]Department of Chemical Pathology, University of Ghana,

[5]Center for Plant Medicine Research, Mampong-Akuapem, Ghana.

[6]Department of Veterinary Pathology, Microbiology and Parasitology, University of Nairobi, Kangemi, Nairobi, Kenya.

*Corresponding Author: eametefe@ug.edu.gh

ABSTRACT

Kombucha is fermented tea brewed by the introduction of a symbiotic culture of bacteria and yeasts (SCOBY) into a sweetened tea substrate. Kombucha consumption has been anecdotally linked to the treatment of multiple diseases including diabetes. Even though the medicinal capabilities of Kombucha have been associated to its anti-inflammatory and antioxidant properties, the precise mode of action is not fully understood. The aim of this study was to investigate the antidiabetic effect of Kombucha in rat models. Diabetes was induced in adult male Wistar rats by a single intraperitoneal injection of 150 mg/kg body weight of alloxan monohydrate. Rats were grouped and administered different doses of Kombucha (5, 25 and 100 mg/kg), and standard drugs (glibenclamide and metformin). Blood samples were collected weekly to determine glucose and insulin levels. To determine the physiological effect of Kombucha treatment on beta pancreatic cells, we performed histological analysis on the pancreas after hematoxylin-eosin staining. Finally, 16S metagenomics sequencing was performed on stool samples to evaluate the temporal gut microbiota changes. We showed that alloxan injection induced diabetes characterized by hyperglycemia, hypoinsulinemia and pancreatic beta cells damage. Notably, treatment with kombucha significantly reduced blood glucose levels, increased insulin secretion and showed better-preserved pancreatic beta cells characterized by reduced inflammation and increased viable cell mass. Additionally, our analysis of gut microbiome revealed shifts in microbial taxonomical composition

characterized by the relatively high abundance of Lactobacillaceae in kombucha treated rats, in contrast, these bacteria populations were diminished in the alloxan control. Altogether, our data provide evidence that the antidiabetic mode of action of kombucha involves restoration of proper glycemic control by ameliorating pancreatic beta cell damage, improving insulin secretion and modulating the gut microbiome. Thus, kombucha could offer a positive outcome for the management of diabetes mellitus.
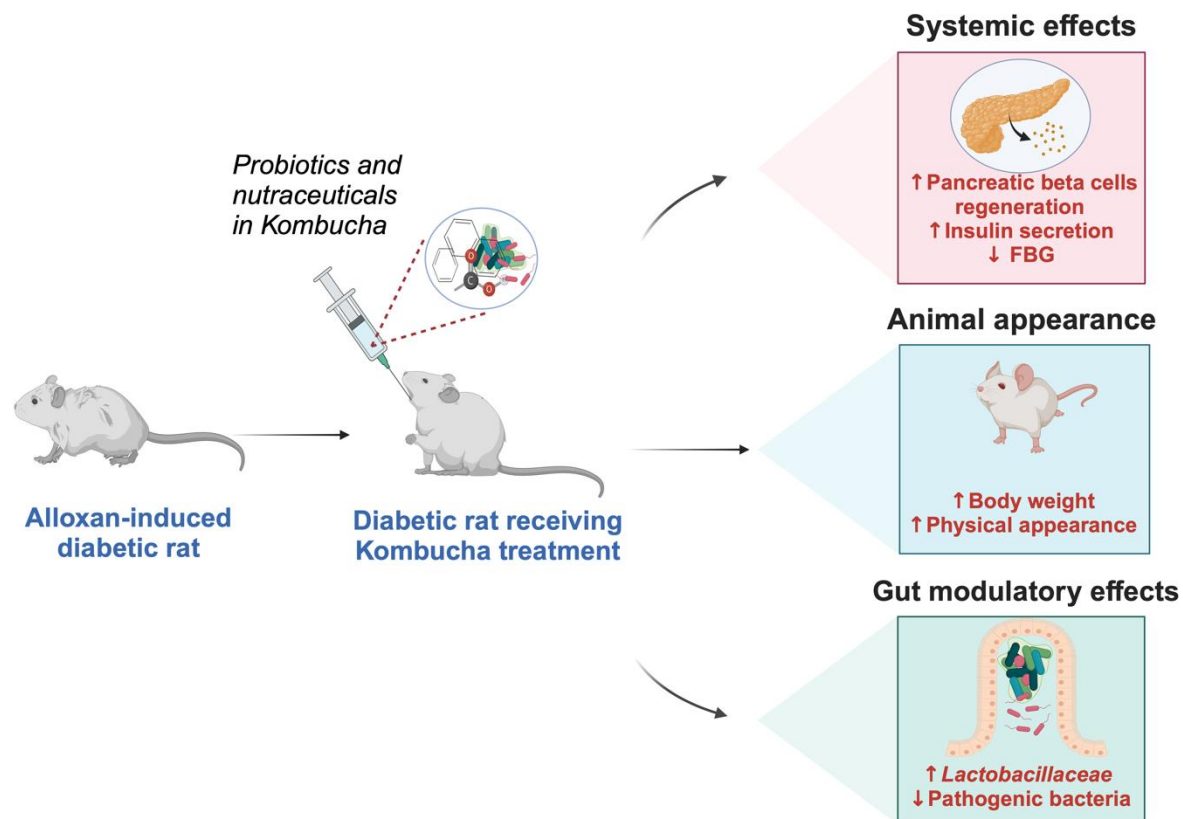
Keywords: Kombucha, alloxan monohydrate, diabetes, pancreatic beta cell, gut microbiome

1.0 INTRODUCTION

Diabetes mellitus (DM) is a complex metabolic condition that arises from dysregulation of blood glucose resulting in hyperglycemia (1,2). There are two types of DM: type 1 DM which arises from the deficiency in insulin production due to autoimmune destruction of pancreatic beta cells (3,4); and type 2 DM which constitutes about 90-95% of all cases and characterized by insulin resistance (5). Current intervention therapies for controlling the abnormally

increased blood glucose concentrations associated with this condition include subcutaneous injection of insulin peptides and administrations of oral anti-hyperglycemic agents such as metformin and other sulfonylureas (6). Consequently, the adverse effects associated with the usage of these choice drugs are inexorable (7). As a result, more and more patients are turning to traditional remedies such as Kombucha and herbal preparations to manage their diabetic conditions.

Kombucha is a fermented tea brewed by the introduction of Kombucha culture (symbiotic culture of bacteria and yeasts (SCOBY)) to sweetened tea broth and allowed to ferment over time under controlled manufacturing procedures (8–10). The SCOBY is a symbiotic culture of bacteria and yeasts comprised of a plethora of probiotics which carry out the fermentation of sugared tea. Notably, Kombucha contains a diverse array of nutraceuticals including flavonoids, saponins and organic acids from the tea and excreted microbial metabolites arising from the fermentation procedures. Previously reported studies have suggested that the therapeutic effects of Kombucha stems from its phytochemical and probiotics composition (11). Interestingly, there are several anecdotal evidences of Kombucha's health benefits, including antidiabetic property dating back to several decades (11,12). Notwithstanding, there have been very few scientific studies to ascertain the therapeutic properties of Kombucha. Claims of the therapeutic effects of Kombucha therefore have remained essentially and largely anecdotal (11,13).

Kombucha may potentially exert its therapeutic effect through the SCOBY-derived mixture of distinct metabolites, fibers, organic acids, amino acids and antioxidants (8,11). The microbial constituent may also exert a direct effect on the gut microbiome, although the mechanism through which this may occur is not yet fully understood. Gut microbiota composition and changes in microbiome communities have been associated with the occurrence of obesity, which is a risk factor that predisposes individuals to the incidence of type 2 diabetes (14). The ability to enrich gut microbiota with probiotics, introducing specific families of beneficial microbes might provide insights into microbiome enhanced diabetes management (15,16). These probiotic organisms can synthesize short chain fatty acids including butyrates and propionates, which are essential for sustaining intestinal homeostasis (17,18). There exists great inter-individual diversity within the gut microbiota, however, some notable phyla (such as Firmicutes, which is predominant), can be associated with protection against diseases. Several factors including gut inflammation, dietary intake and gastrointestinal infections affect gut homeostasis leading to dysbiosis and potentially other disease outcomes including diabetes (19–22). In this study we demonstrated that Kombucha exerts anti-hyperglycemic activity by averting pancreatic beta cell damage and improving insulin secretion. Also, Kombucha modulates specific phyla and family of bacteria in gut microbiome of diabetic rats.

2.0. MATERIALS AND METHODS

2.1. Kombucha preparation

Kombucha was made from two (2) grams of black tea infused in a liter of sugared water (16.1 g/L) boiled for 2 minutes. The sugared tea-infused water was cooled to room temperature and seeded with a tenth volume of previous culture containing a baby mat (SCOBY) (wet weight ~ 30 g, pH = 2.74). The seeded culture was fermented for 14 days, harvested, and freeze-dried with LABCONCO freeze dryer (LABCONCO Corp. USA).

2.2. Ethical clearance and animal experimentation

Adult male Wistar rats aged between 4-6 weeks and weighing 120 ± 20 g were obtained from the animal housing facility of the Center for Plant Medicine Research (CPMR). All animals were reared and maintained in plastic metabolic cages under standard room temperature of 25°C, relative humidity of 60-70% and a consistent 12-h light-dark cycle. The animals were fed with standard powdered feed and had access to sterile drinking water. All institutional and national guidelines for the care and utilization of experimental laboratory animals were strictly followed. Ethical approval (number: CPMR/MIO-PT/8/2018) was obtained from the Institutional Ethics Committee of the CPMR, Mampong, Ghana. Care and handling of experimental animals were done according to protocol by The Foundation for Biomedical Research rules and methods on the application of animals.

2.3. Induction of diabetes in rats and Kombucha treatment

Diabetes was induced in rats as previously described (23). Briefly, a total of 36 male Wistar rats were injected intraperitoneally with a single dose of 150 mg/kg body weight of freshly prepared alloxan monohydrate (Sigma-Aldrich, USA) solution resuspended in normal saline (24). Animals were grouped into six different experimental groups (to receive different treatments) with each group made up of six animals. The diabetic status was assessed 3-days post injection by measuring fasting blood glucose (FBG) levels with a URIT G26 glucometer (Brussels, Belgium), as previously described (25). Moderately diabetic rats post induction, characterized by hyperglycemia (blood glucose levels ≥ 11 mmol/l) and glycosuria and were selected for further experiments. Diabetic rats were treated with varying dosages (5, 25 and 100 mg/kg of body weight) of reconstituted freeze-dried sterile Kombucha and control groups received 10 mg/kg metformin and 5 mg/kg glibenclamide by oral gavage once daily for 28 days. The diabetic control group was given an equal volume of water. The mean body weights of animals in each experimental group were determined weekly and at termination of treatments. At termination of treatment, animals were

sacrificed by cervical dislocation and the pancreas was excised for histological analysis.

2.4. Determination of blood insulin levels

About 1 ml of venous blood was collected from the tail vein of each animal for analysis. Prior to collection of blood samples, animals were fasted overnight. Samples were collected before treatment and at biweekly intervals for the subsequent four weeks. A small portion of the weekly sample was utilized for the determination of the FBG immediately.

At termination of treatment, a 5 ml volume of blood was obtained from each animal by cardiac puncture for determination of blood and serum biochemical indices. Sera were prepared by collection of blood into serum collection tubes without anticoagulants. The blood was allowed to clot and centrifuged at 15,000 rpm for 10 minutes with modification using the MicroCL 17 Centrifuge (ThermoFisher Scientific, Germany). Serum insulin level was determined using the Mercodia Rat Insulin ELISA kit (Sylveniusgatan 8A, Sweden). Briefly, 10 µl of each serum sample was pipetted into the appropriate well along with the standards and the analyses performed as per manufacturer instructions.

2.5. Histopathological analysis of the Pancreas

The excised pancreas was fixed in a 10 % buffered formalin solution, processed through a series of washing, dehydration, clearing and infiltration steps and then finally embedded in paraffin. The formalin-fixed paraffin-embedded tissues were sectioned (5 µm thickness). The sections were subsequently stained with hematoxylin-eosin stain. Representative images were taken using a camera-fitted Leica microscope with image processing and analysis done using computer-based Leica digital pathology software. Slides were examined in a blinded fashion by a pathologist and histopathological scores given. The histological scores considered the presence of inflammatory changes in the pancreatic beta cells including leukocyte infiltration, fibrosis, and edema. A score of 0 - 3 was given with 0 indicating absence of tissue damage and 3 indicating severe inflammation/tissue damage.

2.6. 16S–Metagenomics Analysis of Rat Microbiome

Fecal samples were obtained from individual rats for DNA extraction. For each rat in a group, a sample was collected before treatment, and another collected after 28 days of treatment. Total bacteria DNA was extracted from the fecal samples using the Zymo Quick-DNATM Fecal/Soil Microbe Miniprep Kit in accordance with the manufacturer's protocol (Claassen *et al*., 2013) and prepared for sequencing. To analyze the taxonomic composition of the bacterial communities, the Ion 16STM metagenomic

Kits (Thermofisher Scientific, USA) was used to selectively amplify the V2-4-8 regions of the 16S ribosomal RNA gene of the bacteria genomes in the fecal DNA isolate on the Ion PGMTM system prior to the Ion 16S Metagenomics sequencing carried out at the WACCBIP-University of Ghana NGS Facility using paired-end sequencing on an Ion-Torrent sequencing platform. Sequence processing and analysis was performed using the ThermoScientific Ion Reporters Software.

2.7. Statistical Analysis

Statistical analysis and graphing were performed using the GraphPad prism v9.0 and RStudio (2023.06.0+421). One-way analysis of variance (ANOVA) followed by Dunnet post hoc test for significance were used to determine the statistical differences between groups. The alpha (α) for significance was set at $p < 0.05$ (95 % confidence interval).

3.0. RESULTS

3.1. Kombucha exerts anti-hyperglycemic effect on alloxan-induced diabetic rats.

To determine the antidiabetic effect of Kombucha, we used a rat model of diabetes that closes mimics diabetes in humans by the administration of alloxan monohydrate. Alloxan monohydrate administration to rats causes damage to pancreatic beta cells affecting their function (26). Critical features of alloxan induced diabetes include persistent hyperglycemia. We showed that the normal control rats maintained normal FBG (5.53 ± 0.24 mmol/L) levels till the 28-day period of study. As expected, the alloxan group showed significantly higher FBG (24.47 ± 2.54 mmol/L) when compared to normal controls, from day 14 till day 28. Conversely, treatment of diabetic rats with Kombucha showed significant reduction in FBG (7.40 ± 1.07 mmol/L for 5 mg/kg Kombucha, 4.93 ± 0.17 mmol/L for 25 mg/kg Kombucha, 6.17 ± 0.76 mmol/L for 100 mg/kg Kombucha), similar to standard drugs, metformin and glibenclamide. This suggests that kombucha possesses a blood glucose lowering effect in alloxan-induced diabetic rats.

3.2. Kombucha prevents weight loss in diabetic rats.

Diabetes induced by alloxan monohydrate is also characterized by progressive loss of body weight (27,28). We could also show in our model that, while the normal control group showed a steady increase in body weights, there was a significant decrease in body weight of alloxan diabetic group over time. In contrast, the treatment of diabetic rats with standard drugs glibenclamide and metformin reversed the body weight loss. Similarly, treatment with Kombucha at the 3 dose levels (5, 25, and 100 mg/kg Kombucha) led to a significant progressive gain in body weight. Therefore, Kombucha treatment averts diabetes-induced weight loss.
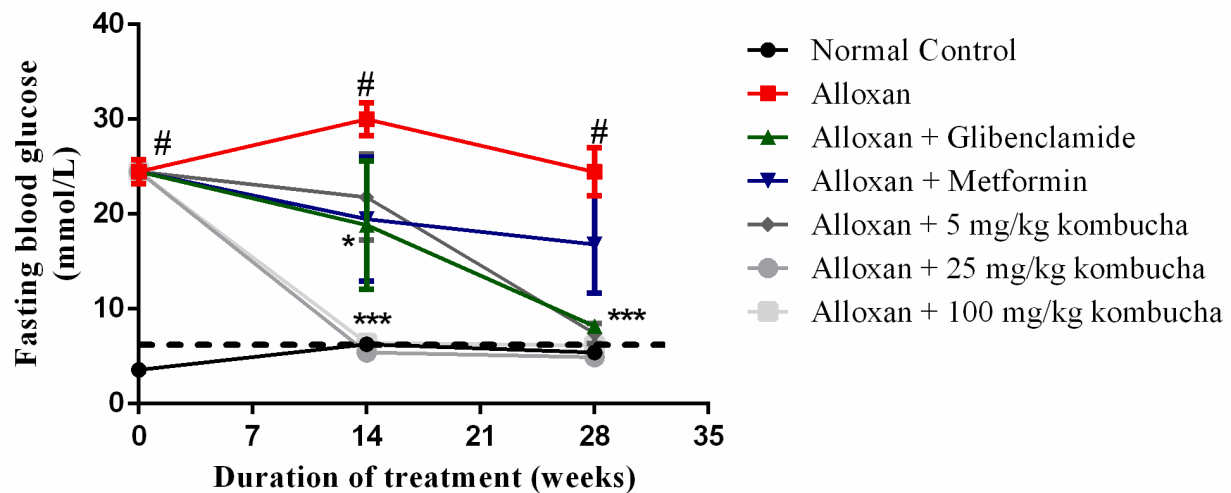
Figure 1. Kombucha treatment ameliorates hyperglycemia in diabetic rats. Effect of kombucha treatment on fasting blood glucose determined after an overnight fast on days 0, 14 and 28. Values mean ± SEM of n=4-6. (#) indicates statistical significance between normal control and alloxan group. *Indicates statistical significance between alloxan group and treatment groups.
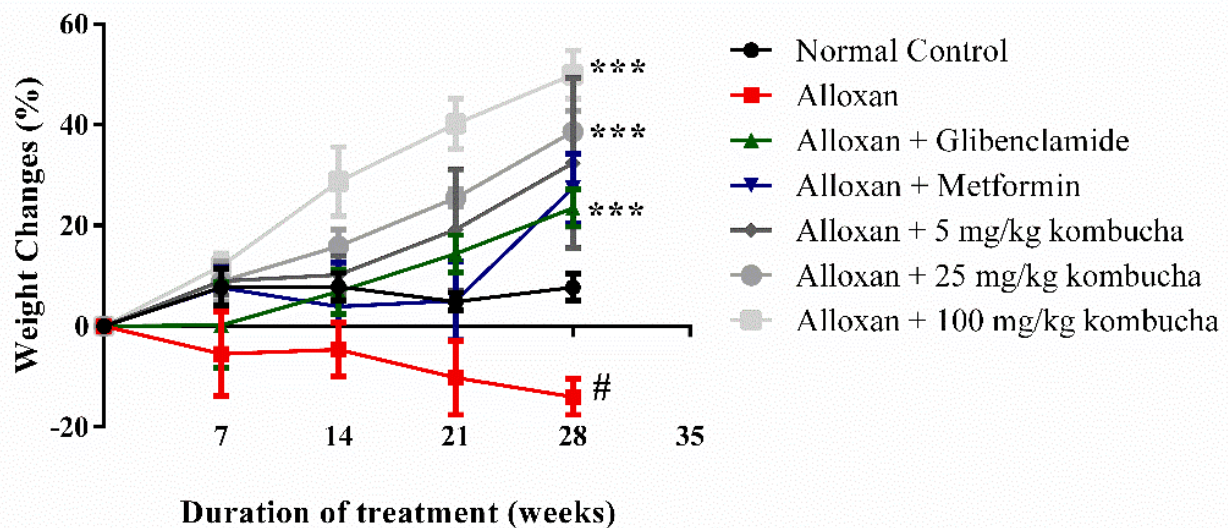

Figure 2. Weight loss in diabetic rats was averted upon kombucha treatment. Values mean ± SEM of n=4-6. (#) indicates statistical significance between normal control and alloxan group. * Indicates statistical significance between alloxan group and treatment groups.

3.3. Kombucha reverses alloxan-induced reduction in blood insulin.

Another feature of alloxan diabetes is hypoinsulinaemia, similar to clinical diabetes (29). Insulin is a hormone that regulates glucose homeostasis by causing glucose to enter cells during a state of hyperglycemia. Hence, we determined whether the attenuation of hyperglycemia in kombucha-treated groups would be consistent with serum insulin levels. The normal control group showed a normal insulin concentration of 0.55 ± 0.88 µg/L. Expectedly in alloxan-treated rats, there was significantly reduced levels of serum insulin compared to normal control rats (0.38 ± 0.01 µg/L). Compared to the alloxan-treated group, the group that received standard drug glibenclamide showed a significantly higher serum insulin level (0.44 ± 0.03 µg/L). Administration of another standard drug, metformin, appeared to increase blood insulin levels, albeit not to significant levels (0.40 ± 0.01 µg/L). Importantly, kombucha treatment increased serum insulin levels significantly compared to alloxan-treated group (0.45 ± 0.01 µg/L for 5 mg/kg Kombucha, 0.50 ± 0.03 µg/L for 25 mg/kg Kombucha, 0.52 ± 0.01 µg/L for 100 mg/kg Kombucha). Therefore, kombucha treatment prevents hypoinsulinaemia in diabetics rats.

3.4. Kombucha attenuates alloxan-induced pancreatic beta cell damage.

Alloxan causes diabetes by a mechanism which involves pancreatic beta cell damage which results in reduced quantity and quality of insulin (30). Our observation that Kombucha treatment prevents hypoinsulinaema could imply lower or no damage to the beta cells of the pancreas. Thus, we examined the H&E-stained sections of pancreas histopathologically. Analysis of the sections revealed normal morphology in the normal control group characterized by high cellular density in several multifocal islets of Langerhans including the pancreatic beta cells, as well as the absence of inflammation and leukocyte infiltration and edema. However, in the alloxan-treated group, pancreatic sections revealed almost no beta cells with extensive fibrosis occurring in the foci previously occupied by the beta cells, leukocytes infiltration and edema (Fig 4A). Of note, the pancreatic sections of kombucha-treated groups showed no evidence of tissue damage, similar to the normal control groups (Fig 4A). These impressions were further confirmed by histological scoring. Consistent with no visible damage, the control group had a low histological score (0.08 ± 0.08). The alloxan group showed a significantly increased score (2.60 ± 0.25), and Kombucha treatment reduced the pancreatic beta cell histological score (0.25 ± 0.18 for 5 mg/kg Kombucha, 0.14 ± 0.15 for 25 mg/kg Kombucha, and 0.17 ± 0.16 for 100 mg/kg Kombucha) (Fig 4B). Thus, treatment with kombucha likely improves alloxan-induced diabetes in rats by averting pancreatic beta cell damage thus maintaining insulin production and consequently preventing hyperglycemia.
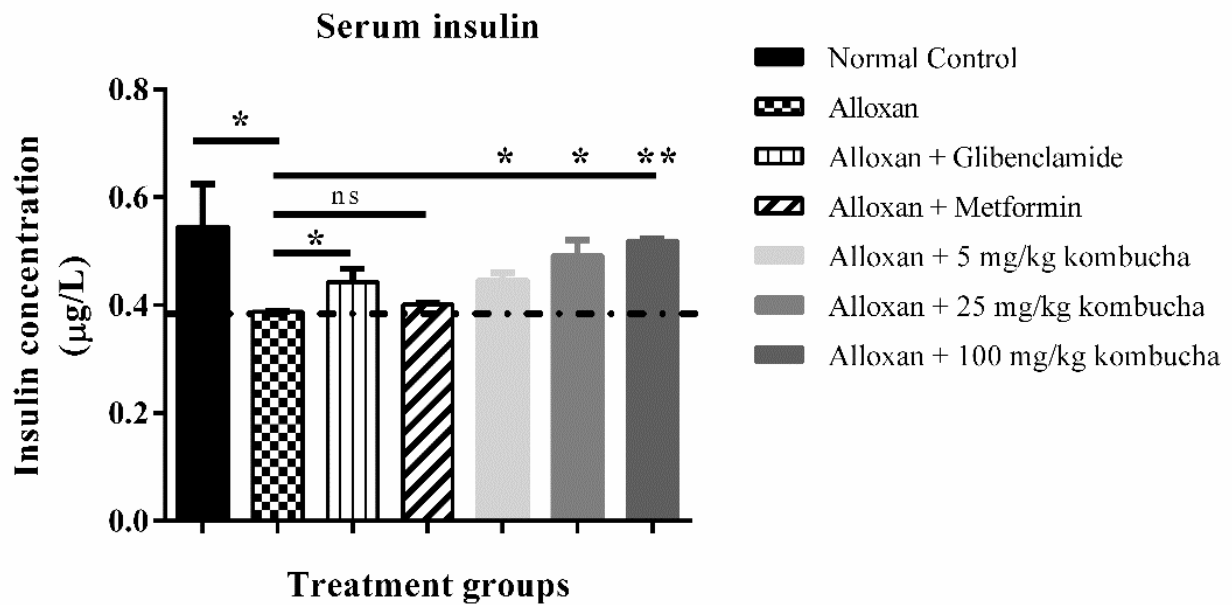
Figure 3. Kombucha treatment promotes insulin secretion in alloxan-induced diabetic rats.   serum insulin levels determined at day 28 following treatment of diabetic rats with standard drugs and kombucha. Values mean ± SEM of n=4-6. (#) indicates statistical significance between normal control and alloxan group. * Indicates statistical significance between alloxan group and treatment groups.
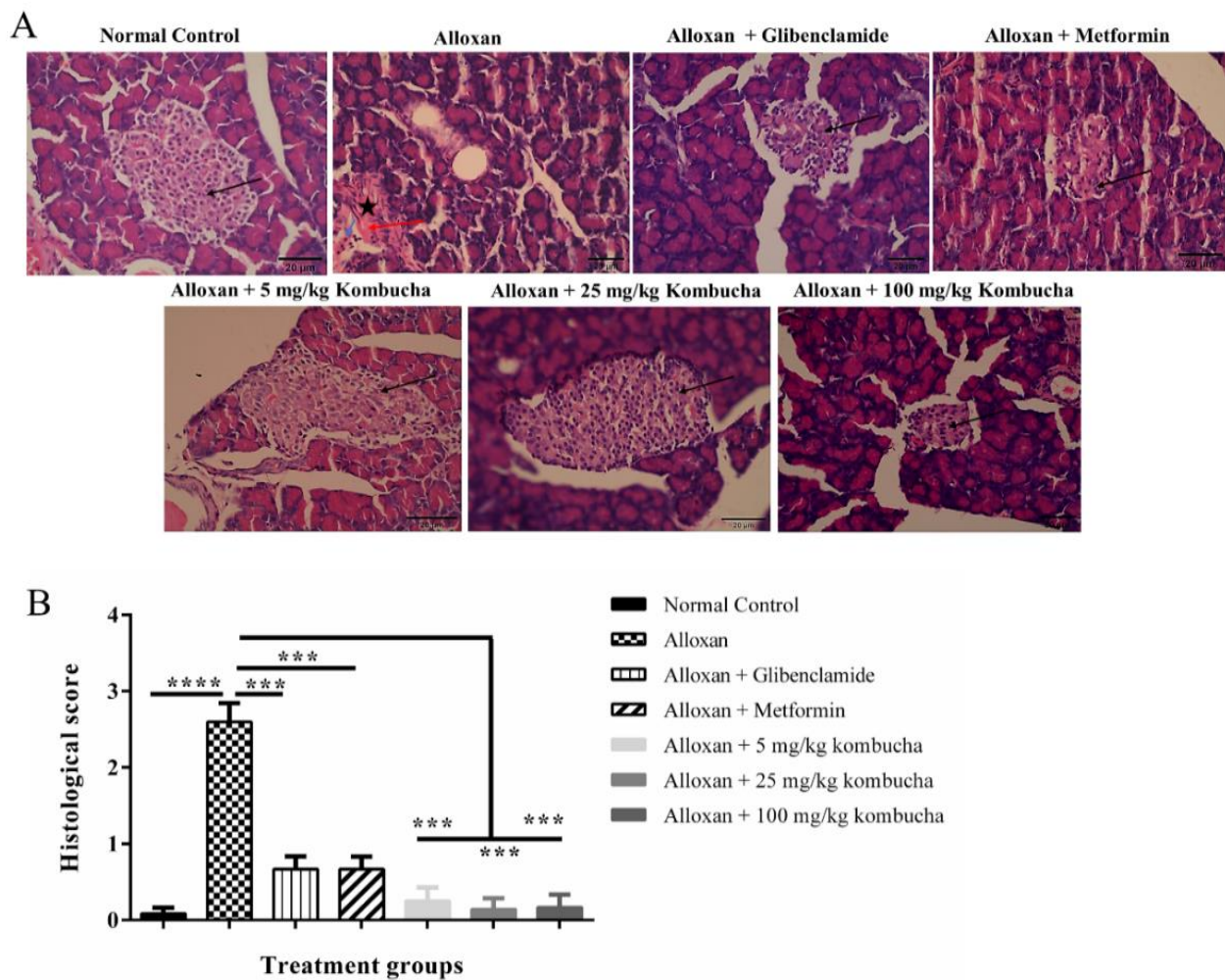
Figure 4. Alloxan-induced pancreatic beta cell damage in diabetic rats is diminished by Kombucha treatment. (A) Histopathology of paraffin cross sections of pancreas tissues after hematoxylin-eosin staining. Representative images of pancreas at 40× magnification. Normal control rat shows a normal tissue morphology characterized by the presence of pancreatic beta cells without the presence of inflammation in the beta cells of the pancreas. The alloxan group shows significant signs of tissue damage characterized by leukocyte infiltration (blue arrow), extensive fibrosis (red arrow) and destruction of the pancreatic beta cell (star). Treatment with standard drugs glibenclamide and metformin show various degrees of pancreatic beta cell regeneration. Also, treatment with kombucha at the dose levels showed the absence of any tissue damage and a much more intact pancreatic tissue. (B) Histological scores of inflammatory changes in the pancreas showed a significant protective effect of kombucha treatment of pancreas histomorphology. Values mean ± SEM of n=4-6. * Indicates statistical significance between alloxan group and treatment groups.

3.5. Kombucha modulates species diversity of the gut microbiota in diabetic rats.

Several factors including antibiotic administration, gut inflammation, dietary patterns, and gastrointestinal infections affect gut microbiota homeostasis and results in dysbiosis (19,20). We showed in this study that the dominant gut bacteria belonged to the Lactobacillaceae family in the normal control group. In contrast, bacteria of this family were in relatively lower abundance in the untreated diabetic control (Fig 5A). The level of Lactobacillaceae abundance found in the Kombucha treated diabetic rats was near control levels. Interestingly, a relatively higher bacteria richness was observed in the alloxan group, this shift in microbial community over the 28 days experimental duration resulted in a diverse microbiota with the potential ecological succession of other organisms that emerge because of a diminished level of the Lactobacillaceae family of bacteria.

The effect of Kombucha on the gut microbiota in diabetic state was further investigated by the metrics of diversity indices. Bacteria species richness and evenness indices are utilized to estimate the alpha diversity within different microbiota assessing temporal changes in microbiota (31–34). The Choa1 diversity index which measures species richness taking into consideration rare taxa showed that there was no statistical variation over the treatment period for normal controls, alloxan group, and alloxan diabetic animals treated with metformin and 100 mg/kg Kombucha. A significant reduction in the number of species found within the microbiome composition of the glibenclamide and 5 mg/kg Kombucha treatment groups were comparable before and after treatment (Fig 5B). There was no change in species richness, measured as the Shannon diversity index between the normal control and the glibenclamide treatment groups over the period. In the alloxan group however, there was a significant increase in species richness at the end of the treatment period. In contrast, administration of Kombucha or metformin generally decreased species richness (Fig 5C). Similarly, there were also significant changes in species evenness over the treatment period There was an increase in species evenness in the normal control and other treatment groups except in the 5 mg/kg Kombucha treatment group, where no change in species evenness was found. In the alloxan group, there was an increase in species evenness while the reverse was mostly observed for the Kombucha-treated groups (Fig 5D). However, in the 100 mg/kg Kombucha and the 10 mg/kg metformin treatment groups, species evenness significantly decreased after treatment. Thus, the dominant gut microbiota was found to belong to the Lactobacillaceae family and we also showed that kombucha treatment of diabetic rats alters richness and evenness of bacteria species.
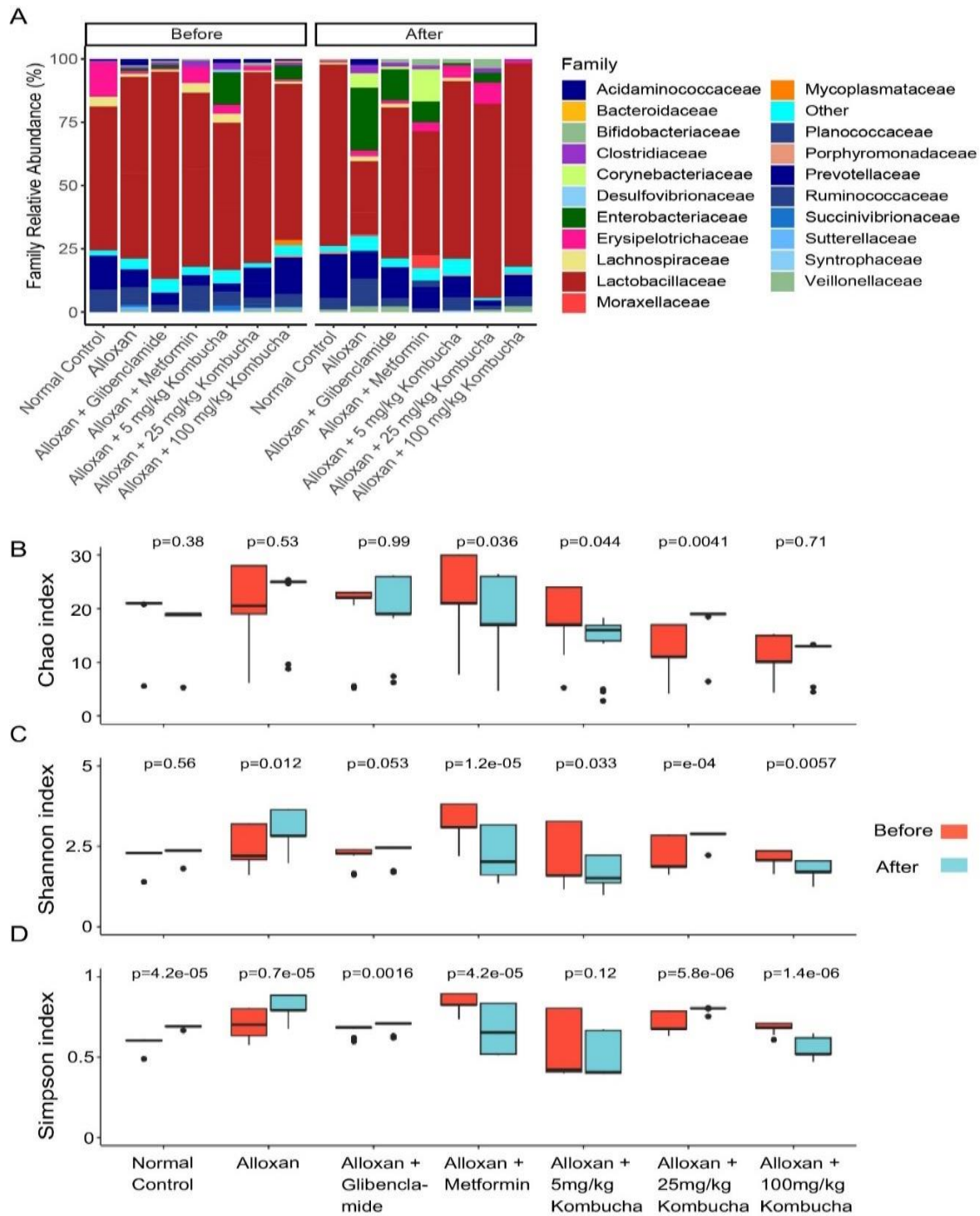
Figure

5. Gut microbiota of diabetic rats before and after different treatments. (A) Family level. Estimation of the relative abundance was done by percentage abundance of the read count to the total reads from sequencing. Analysis was done by computing the relative abundance of each phylum from the sequenced data. B-D) Alpha- and beta-diversity of microbiome in diabetic animals before and after treatment. (B) Choa1 diversity index. (C) Shannon diversity index (D) Simpson diversity index. For all panels: n = 3 per condition. T-test was used to compare the pairwise variations within the before and after treatment groups at 95 % confidence interval.

4.0. DISCUSSION

Here, we studied the antidiabetic effect of kombucha in alloxan-induced diabetic rats. Kombucha is known to possess antioxidant and anti-inflammatory properties (35). We found that Kombucha possesses an antidiabetic effect by preventing hyperglycemia. Notably, Kombucha treatment improved insulin secretion by attenuating pancreatic beta cell damage in alloxan-induced diabetic rats.

Alloxan-induced diabetes mimics clinical diabetes and therefore serves as an appropriate model for the study of potential antidiabetics drugs in animals. Alloxan is a toxic analogue of glucose that accumulates in the beta cells of the pancreas via the GLUT2 glucose transporter (26,29). Inside the cell, alloxan generates reactive oxygen species (ROS) and radicals which damage the pancreatic beta cells and ultimately results in an insulin-dependent diabetic state (29). Alloxan diabetes is therefore characterized by hyperglycemia and consequent weight loss. Generally, the reduction in blood glucose levels is the key target of most treatment methods. Thus, our finding that treatment of alloxan diabetic rats with kombucha lowers fasting blood glucose levels shows that kombucha possesses anti-hyperglycemic effects.

Another feature of alloxan diabetes is hypoinsulinaemia resulting from destruction of pancreatic beta cells. Insulin is an important hormone responsible for lowering excessive levels of blood glucose. Studies involving therapeutic interventions in diabetes mostly involved the measurement of circulating levels of insulin. For example, the hypoglycemic effect of *Clitoria ternatea* Linn. (Fabaceae) is linked to its effect on serum insulin levels (36). Similarly, in our alloxan model of diabetes, we observed significantly reduced levels of insulin in the alloxan group. Accordingly, our findings that kombucha treatment improves serum levels of insulin and ameliorates alloxan-induced pancreatic beta cell injury accounts for the prevention of hyperglycemia in these rats. Kombucha possesses significant antioxidant activity by scavenging free radicals (35). Thus, one explanation for the ameliorative effect of kombucha on the alloxan-damaged pancreatic beta cells is that kombucha is able to scavenge the free radicals generated by the alloxan and prevent further damage to the beta cells and promote regeneration. Another possible explanation for the pancreatic beta cell regeneration phenotype could be the reported evidence of Kombucha's anti-inflammatory properties (37). In a randomized controlled pilot investigation in humans, it was shown that Kombucha exerts an anti-hyperglycemic effect in diabetic patients (38). Kombucha has thus

been registered for clinical trials. Another study in rats confirms the anti-hyperglycemic effect of Kombucha (12). Other studies have attempted to show the mechanism by which Kombucha exerts its antidiabetic effects (12,35,37,38). What we report differently in this study is the comprehensive examination of the pancreas histologically and functionally to determine how the better-preserved histomorphology of the pancreatic beta cells impacts insulin production and improves diabetes in rats.

The composition of the core microbiota in healthy state as demonstrated in this study is consistent with literature and reflects the usefulness of Kombucha in gut microbiota protection (31–34). The presence of Lactobacillaceae in the gut may trigger the inhibition of lipopolysaccharide production and increased tight junction formation in the epithelial cells (39). This helps in reducing inflammation in the gut hence reducing the chances of pancreatic damage and insulin production (40,41). In addition, the gut microbiome has been reported to produce short chain fatty acids and other organic acids such as acetate which are important for the maintenance of colonocyte homeostasis. The administration of Kombucha to the diabetic rats maintained and sustained their respective gut flora by enriching the Lactobacillaceae community members

resulting in the outcompeting of pathogenic bacterial from potentially colonizing the gut (Fig 5A). This is consistent with the study that reported the ability of Kombucha administration to inhibit pathogenic microbial communities for colonizing the gut in type 2 diabetic rats with subsequent improvement in gut microbiome and intestinal gut architecture (37). It has been suggested that antioxidants and probiotics found in Kombucha could potentially inhibit intestinal glucose absorption in the small intestine resulting in a decline in glucose export into the portal circulation via the glucose transporters (GLUT5 and GLUT2) and the sodium glucose transport protein 1 (42). GLUT2 transporters in the pancreatic beta cells serve as a sensor of free-flowing glucose that accurately gauge the serum glucose levels. Thus, the anti-hyperglycemic effect of Kombucha may involve Kombucha-induced recovery or regeneration of damaged pancreatic beta cells by alloxan leading to normalized insulin secretion and glucose utilization (30). While the precise mechanism of action of Kombucha is not fully clear, perhaps, a contribution of probiotics activity complements the action of antioxidants by the process of reducing gut inflammation and simultaneously stimulating pancreatic cells regeneration as shown here. Furthermore, probiotics can synthesize peptides and metabolites that can

scavenge free radicals from the body. As reported by previous studies, Kombucha demonstrates capabilities as a detoxification beverage (39). Another benefit of Kombucha is that the acetic acid and glucuronic acids present in Kombucha can conjugate toxins and eliminate them from the body. The sophisticated consortia of nutraceutical and probiotics within Kombucha makes it a potent antioxidant agent (39,43). Altogether, the consumption of probiotics including Kombucha, to reconstitute the gut microbiota in dysbiosis state to restore a healthy gut is exciting. This study provides insight into understanding the combined synergistic action of probiotics and nutraceuticals in Kombucha as plausible mechanism of the anti-diabetic action in diabetic rats particularly linking biochemical events and systemic effects to its gut modulatory properties.

In conclusion, we provide experimental evidence that Kombucha treatment improves diabetes in rats by ameliorating pancreatic beta cell damage to produce insulin and prevent hyperglycemia. Kombucha, therefore, could be further explored as a therapeutic agent for the management of diabetes.

DECLARATIONS

Competing Interests: The authors have no competing interests to declare that are relevant to the content of this article.

Ethics Approval: This Research using animal experimentation has followed International Guidelines for animal experimentation as stated in The Foundation for Biomedical Research rules and methods on the application of animals. Ethics approval was obtained from the Institutional Ethics Committee of the CPMR, Mampong, Ghana. Ethical approval (number: CPMR/MIO-PT/8/2018).

REFERENCES

1. Banday MZ, Sameer AS, Nissar S. Pathophysiology of diabetes: An overview. Avicenna Journal of Medicine. 2020.

2. Moini J. Pathophysiology of Diabetes. In: Epidemiology of Diabetes. 2019.

3. Knip M, Siljander H. Autoimmune mechanisms in type 1 diabetes. Autoimmunity Reviews. 2008.

4.   Kahaly GJ, Hansen MP. Type 1 diabetes associated autoimmunity. Autoimmunity Reviews. 2016.

5.   World Health Organization. Global Report on Diabetes. Isbn. 2016;

6.   Wang YW, He SJ, Feng X, Cheng J, Luo YT, Tian L, et al. Metformin: A review of its potential indications. Drug Design, Development and Therapy. 2017.

7.   Li D. Metformin as an antitumor agent in cancer prevention and treatment. Journal of Diabetes. 2011.

8.   Dufresne C, Farnworth E. Tea, Kombucha, and health: A review. Food Research International. 2000;

9.   Selvaraj S, Gurumurthy K. An overview of probiotic health booster-kombucha tea. Chinese Herbal Medicines. 2023.

10.   Kitwetcharoen H, Phung LT, Klanrit P, Thanonkeo S, Tippayawat P, Yamada M, et al. Kombucha Healthy Drink—Recent Advances in Production, Chemical Composition and Health Benefits. Fermentation. 2023.

11.   Jayabalan R, Malbaša R V., Lončar ES, Vitas JS, Sathishkumar M. A review on kombucha tea-microbiology, composition, fermentation, beneficial effects, toxicity, and tea fungus. Comprehensive Reviews in Food Science and Food Safety. 2014.

12.   Aloulou A, Hamden K, Elloumi D, Ali MB, Hargafi K, Jaouadi B, et al. Hypoglycemic and antilipidemic properties of kombucha tea in alloxan-induced diabetic rats. BMC Complementary and Alternative Medicine. 2012;

13.   Greenwalt CJ, Steinkraus KH, Ledford RA. Kombucha, the fermented tea: Microbiology, composition, and claimed health effects. Journal of Food Protection. 2000.

14.   Chen J, He X, Huang J. Diet Effects in Gut Microbiome and Obesity. Journal of Food Science. 2014;

15.   Goodrich JK, Waters JL, Poole AC, Sutter JL, Koren O, Blekhman R, et al. Human genetics shape the gut microbiome. Cell. 2014;

16.   Marco ML, Heeney D, Binda S, Cifelli CJ, Cotter PD, Foligné B, et al. Health benefits of fermented foods: microbiota and beyond. Current Opinion in Biotechnology. 2017.

17.   Flint HJ. The impact of nutrition on the human microbiome. Nutrition Reviews. 2012;

18.   Wang J, Qin J, Li Y, Cai Z, Li S, Zhu J, et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature. 2012;

19.   Bevins CL, Salzman NH. Paneth cells, antimicrobial peptides and maintenance of intestinal homeostasis. Nature Reviews Microbiology. 2011.

20.   Donaldson GP, Lee SM, Mazmanian SK. Gut biogeography of the bacterial microbiota. Nature Reviews Microbiology. 2015.

21.   Jovel J, Patterson J, Wang W, Hotte N, O'Keefe S, Mitchel T, et al. Characterization of the gut microbiome using 16S or shotgun metagenomics. Frontiers in Microbiology. 2016;

22.   Marsh AJ, O'Sullivan O, Hill C, Ross RP, Cotter PD. Sequence-based analysis of the bacterial and fungal compositions of multiple kombucha (tea fungus) samples. Food Microbiology. 2014;

23.   Lartey NL, Asare-Anane H, Ofori EK, Antwi S, Asiedu-Larbi J, Ayertey F, et al. Antidiabetic activity of aqueous stem bark extract of Annickia polycarpa in alloxan-induced diabetic mice. Journal of Traditional and Complementary Medicine. 2021;11(2):109–16.

24.   Aruna R V., Ramesh B, Kartha VNR. Effect of betacarotene on protein glycosylation in alloxan induced diabetic rats. Indian Journal of Experimental Biology. 1999;

25.   Radenkovi M, Stojanovi M, Prostran M. Journal of Pharmacological and Toxicological Methods Experimental diabetes induced by alloxan and streptozotocin: The current state of the art. Journal of Pharmacological and Toxicological Methods. 2016;

26.   Rohilla A, Ali S. Alloxan Induced Diabetes: Mechanisms and Effects. International Journal of Research in Pharmaceutical and Biomedical Science. 2012;

27.   Mallhi IY, Sohaib M, Khan AU, Rabbani I. Antidiabetic, Antioxidative and Antihyperlipidemic Effects of Strawberry Fruit Extract in Alloxan-Induced Diabetic Rats. Foods. 2023;

28.   Ibrahim RM, Abdelhafez HM, EL-Shamy SAEM, Eid FA, Mashaal A. Arabic gum ameliorates systemic modulation in Alloxan monohydrate-induced diabetic rats. Scientific Reports. 2023;

29.   Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia. 2008.

30.   Radenković M, Stojanović M, Prostran M. Experimental diabetes induced by alloxan and streptozotocin: The current state of the art. Journal of Pharmacological and Toxicological Methods. 2016;

31.   Chao A, Chazdon RL, Colwell RK, Shen TJ. Abundance-based similarity indices and their estimation when there are unseen species in samples. Biometrics. 2006;

32.   Lemos LN, Fulthorpe RR, Triplett EW, Roesch LFW. Rethinking microbial diversity analysis in the high throughput sequencing era. Journal of Microbiological Methods. 2011;

33.   Magurran AE. Measuring biological diversity. Current Biology. 2021;

34.   Simpson EH. Measurement of diversity [16]. Nature. 1949.

35.   Bhattacharya S, Gachhui R, Sil PC. Effect of Kombucha, a fermented black tea in attenuating oxidative stress mediated tissue damage in alloxan induced diabetic rats. Food and Chemical Toxicology. 2013;

36.   Daisy P, Rajathi M. Hypoglycemic effects of Clitoria ternatea Linn. (Fabaceae) in alloxan-induced diabetes in rats. Tropical Journal of Pharmaceutical Research. 2009;

37.   Xu S, Wang Y, Wang J, Geng W. Kombucha Reduces Hyperglycemia in Type 2 Diabetes of Mice by Regulating Gut Microbiota and Its Metabolites. Foods. 2022 Jan;11(5):754.

38.   Mendelson C, Sparkes S, Merenstein DJ, Christensen C, Sharma V, Desale S, et al. Kombucha tea as an anti-hyperglycemic agent in humans with diabetes – a randomized controlled pilot investigation. Frontiers in Nutrition. 2023;

39.   Wen L, Duffy A. Factors influencing the gut microbiota, inflammation, and type 2 diabetes. Journal of Nutrition. 2017;

40.   Everard A, Belzer C, Geurts L, Ouwerkerk JP, Druart C, Bindels LB, et al. Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity. Proceedings of the National Academy of Sciences of the United States of America. 2013;

41.   Wander PL, Boyko EJ, Leonetti DL, McNeely MJ, Kahn SE, Fujimoto WY. Change in visceral adiposity independently predicts a greater risk of developing type 2 diabetes over 10 years in Japanese Americans. Diabetes Care. 2013;

42.   Kwon O, Eck P, Chen S, Corpe CP, Lee J, Kruhlak M, et al. Inhibition of the intestinal glucose transporter GLUT2 by flavonoids. The FASEB Journal. 2007;

43.   Everard A, Belzer C, Geurts L, Ouwerkerk JP, Druart C, Bindels LB, et al. Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity. Proc Natl Acad Sci U S A. 2013 May 28;110(22):9066–71.