Scientific Research Publishing

Effect of Kombucha and Its Non-Polar Components on Morphological Aspects of the Pancreas of Diabetic Rats with Streptozotocin

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Abstract

Objective: to evaluate the protective effect of kombucha and its components not polar, in pancreas of diabetic rats treated with streptozotocin. **Material and methods:** ninety-six male Wistar rats of 120 to 170 g of PC were used; four groups of 24 animals were formed: control (GC), control treatment (GCTx), kombucha treatment (GTxK) and treatment with non-polar components of kombucha (GTxCNP). Diabetes was induced in the last three groups with an intraperitoneal injection of streptozotocin at a dose of 65 mg/kg BW. At the same time, treatment was started in the different groups; GC and GCTx groups were administered, orally, 1 ml of sterile saline solution; to the GTxK group 324 mg/kg of kombucha; and to GTxCNP 0.006 mg/kg of the non-polar components. For the histopathological study, pancreas samples were obtained on days 1, 7, 14 and 21 and fixed in formalin. They were processed with the histological technique, 5-micron-thick sections were made and stained with the hematoxylin-eosin technique. Finally, the number of islets of Langerhans per field observed with the 10x objective was quantified. The results were subjected to a one-way analysis of variance and Tukey's test ($p < 0.05$). **Results:** Significant changes in the number of islets were observed in histological sections of the pancreas. The groups treated with streptozotocin showed a significant decrease in the number of islets 7 days after starting treatment; Likewise, their number was lower in the GCTx group compared to the GTxK and GTxCNP groups ($p \leq 0.05$). At 14 and 21 days, the lowest number of islets was maintained, although only in the GCTx and GTxK groups ($p \leq 0.05$). Streptozotocin is a drug that can be used in rats to induce an experimental model of diabetes with an effect that can last up to

21 days. **Conclusions:** This fermented beverage and its non-polar components can be a complementary treatment alternative for diabetes mellitus, since it prevents further damage to the β cells of the pancreas and the Islets of Langerhans. Specific additional studies on the main, non-polar components of kombucha are suggested to know the mechanism of protection on pancreatic islets.

Keywords

Diabetes, Kombucha, Rats, Streptozotocin, Non-Polar Components of Kombucha

1. Introduction

Diabetes is one of the most studied diseases today and considered by various authors as a metabolic problem [1] or as a metabolic syndrome [2] that chronically affects the concentration of carbohydrates, lipids and proteins in the body [3]. In this disease, the central disorder is the resistance of all peripheral tissues to the action of insulin [4], due to defects in its synthesis due to modifications of the receptor, alterations in the mechanisms intracellular action of this hormone or because it is produced in low concentrations. The result is an excess of glucose in the blood, which causes damage to blood vessels and different organs such as the eyes, kidneys, nerves, and heart [3] [5].

Diabetes is commonly classified according to the cause that originates it and from this point in: diabetes mellitus type 1 (DM1), diabetes mellitus type 2 (DM2), gestational diabetes and type MODY diabetes, being the most common type 1 and 2 [6] [7].

The diagnosis of this disease can be made in two ways: the first is based on the presence of signs and symptoms. For DM1, polyphagia, polyuria [8], polydipsia, blurred vision, weakness, weight loss, and irritability are characteristic. While DM2 manifests itself with marked weight loss, edema of the feet and hands, coagulation delay, tiredness, sleepiness, polyphagia, polydipsia and sexual weakness. In a second moment, analyzes of the concentration of glucose, insulin and glycosylated hemoglobin are performed [9]. In gestational diabetes, some of the same symptoms are present, and blood glucose concentrations that are slightly elevated (126 mg/dL) [10].

The prevalence of this disease and its complications in the body cause the health systems of the countries to have great economic and health repercussions [11].

Diabetes is a disease that affects a large number of people around the world [12], reaching epidemic proportions [13]. In the latest report by the WHO, it was estimated that the prevalence of this disease has increased in people over 18 years of age from 108 million in 1980 to 422 million in 2014, and causes the death of 1.5 million people a year. Currently the number of patients with di-

abetes ranges between 282 million worldwide, while in Latin America it is approximately 62.8 million and in Mexico, there are 8.7 million patients. Therefore, it is considered the main cause of death per year [14], establishing alterations in the functioning of the pancreas as the main cause.

The pancreas is one of the glands that plays an important role in diabetes, since it secretes insulin [15]. Embryological, it is formed from the primitive intestine [16] from a dorsal bud and a ventral bud that, when fused, give rise to the complete pancreas, it is an accessory gland [17], mixed covered by a layer of connective tissue made up of mesothelial cells [18]. From the histological point of view, the pancreas is made up of islets of Langerhans, of which there are 1 to 2 million [19]. And made up of four types of cells: β in 70%, synthesize insulin; α in 20%, are responsible for the production of glucagon, δ that make it up between 5% and 10%, produce somatostatin and PP only in 2% that produce pancreatic polypeptide [18] [20].

The β cells are the most important, since they are in charge of secreting insulin, an anabolic polypeptide hormone with a pleiotropic effect, which is mediated by a membrane receptor with tyrosine kinase activity [21], responsible for lowering blood glucose levels. The production of this hormone is dependent on the concentration of glucose in the blood [7].

There are a large number of treatments for this disease with the sole objective of safeguarding the physiological conditions of patients who suffer from it and offering a better quality of life. Traditional medicine treatments consist of the use of hypoglycemic drugs, achieving biochemical control to prevent cardiovascular and other organ complications, regardless of body conditions [22].

The main allopathic drugs are from four groups: sulfonylureas (glibenclamide, gliclazide, glimepiride, and glipizide [23] [24], are administered orally, are metabolized in liver, with inactive metabolite products that are eliminated in the urine or through the bile [25]. The biguanides (metformin, phenformin and buformin), originate from the gaudiniana, an active ingredient that is obtained from the *Galega officinalis* [26] [27]. The thiazolidinediones or glitazones: troglitazone, rosiglitazone, glitazone and pioglitazone [28] [29]), of which only the last two are still used as safe hypoglycemic agents [24]. Finally, α-glucosidase inhibitors [28], is an enzyme that acts by hydrolysis to reduce carbohydrates to smaller sugars so that they are subsequently absorbed [30].

Alternative and/or complementary medicine or therapies constitute a therapeutic group that includes traditional medicine based on the use of natural products (NP). Some specialists mention that it is difficult to calculate the percentage of the population that uses them due to their unconventional distribution, but it is considered to be growing, both in the developing world and in the West [31]. According to the WHO, 80% of the world's population, that is, more than four billion people, use plants as their main medicinal remedy and it continues to increase in the general population, as well as in patients with chronic-degenerative diseases, such as diabetes mellitus [32] [33].

Fermented beverages, such as kombucha, are also considered alternative me-

dicines, since they contain a large number of microorganisms with probiotic properties and prebiotic compounds [34]. It's a fermented drink resulting from a group of bacteria and yeasts, sweetened with sucrose to which 20% of an infusion is added [35], with a pH of 2.7 - 3 [36] and with a history of thousands of years in eastern regions. Today it is quite popular in Western countries, it is traditionally prepared by fermenting sweetened black or green tea [37], which is inoculated with a film formed during the previous cultivation, popularly known as tea fungus, and incubated statically under aerobic conditions for 7 - 10 days [38].

Kombucha is a drink made up of two main elements: on the one hand, it contains a group of bacteria and yeasts [39], while on the other; there are a large number of chemical components. Either element depend on different factors such as the type of tea (black or green), the fermentation time, since the longer, the fermentation lasts, the more acidic the solution becomes or the source of the inoculum with which it is made [37].

The kombucha fermentation process gives rise to different compounds: acids (lactic, acetic, gluconic, glucuronic, citric, L-lactic, malic, tartaric, malonic, oxalic, succinic, pyruvic and usnic acids can also be found), ethanol, glycerol, vitamins, antibiotics, amino acids and minerals. The concentration of these products will depend on the time and the equipment in which it is fermented [37] [39] [40].

Unlike the side effects caused by allopathic medicine, the active ingredients of kombucha have the ability to reach all parts of the body due to its metabolic characteristics to restore cell membranes without any side effects, thus promoting well-being body [41].

The health benefits of consuming kombucha have been extensively studied in a large number of diseases, mainly those considered chronic-degenerative: arteriosclerosis [42], hypertension, hyperlipidemia, diabetes [39] [43] and obesity [29]. Likewise, hepato-protective properties have been attributed to it [44]. Due to the aforementioned, the objective of this study was to evaluate the protective effect of kombucha and non-polar components in the pancreas of diabetic rats with streptozotocin.

2. Material and Methods

2.1. Experimental Model for the Induction of Diabetes

This study was carried out in the Department of Veterinary Medicine of the Division of Veterinary Sciences of the University Center of Biological and Agricultural Sciences of the University of Guadalajara. Ninety-six young adult male Wistar rats weighing 120 to 170 g were used, which were housed in animal facility conditions with *ad-libitum* 12-hour light/dark cycles with climate and controlled ventilation, safeguarding the regulations, in the surgical handling and slaughter of animals [45].

This work is divided into four experimental phases: In the first phase, 96 male Wistar rats weighing 120 to 170 g were distributed in four groups of 24 animals each: control (CG), treatment (GCTx), kombucha treatment (GTxK) and non-

polar component treatment (GTxCNP), subsequently the basal blood glucose and insulin levels of the total animals were determined. In the second phase, a state of type 1 diabetes was induced in three of the four groups with the administration of streptozotocin at a dose of 65 mg/kg of body weight, intraperitoneally. After 24 h, the concentration of glucose and insulin in intracardiac blood samples was quantified [46] and 6 animals from each group were sacrificed for the extraction of the pancreas and its subsequent histopathological analysis. At the same time, the administration of kombucha was started at a dose of 324 mg/kg, and of the non-polar components at a dose of 0.006 mg/kg, orally during the 21 days of the experiment. In phase three, after 7 days, blood samples were obtained from 6 animals of each group, which were sacrificed to obtain the pancreas for histopathological study. In phase four, on days 14 and 21 of the experiment, intracardiac blood samples were obtained from 6 animals, which were sacrificed to obtain the pancreas.

2.2. Preparation of Kombucha Tea

Kombucha tea was prepared from a zooglea mother, to which was added a liter of green tea, already prepared and cooled to room temperature and mixed with 150 g of refined sucrose, covered with a semi-permeable cloth to the passage of air. Left to rest for 14 days in a jar away from direct light to allow fermentation, then the liquid or ferment, called kombucha tea, was extracted and refrigerated at 6˚C [47]. The dose of kombucha for the present work was determined based on the studies carried out by Aloulou *et al.* [12] who established the amount of 5 ml/kg body weight orally as a dose of kombucha. Complete kombucha was rotary evaporated to remove water and quantify solute content, 324 mg/5 ml [46].

2.3. Preparation of Non-Polar Components

To obtain the non-polar components of the kombucha, 100 ml of the kombucha tea solution and 200 ml of hexane. They were placed in a decantation bulb, stirred and allowed to stand for 3 min, from this solution the first fraction was obtained, later at 100 ml hexane (100 ml) was added to the first fraction, they were placed in the decantation bulb and 100 ml were finally obtained. Finally, the fractions were placed separately in 500 ml Erlenmeyer flasks under refrigeration. For the non-polar fractions, the total volume was weighed together with the ball flask on a balance, and then it was placed on a rotary evaporator to remove the hexane. Finally, the ball flask was weighed; 400 microliters (µl) of dimethyl sulfoxide (DMSO) were added and calibrated with a sterile solution to a total volume of 210 ml of solution to supply the animals [48].

2.4. Preparation of Streptozotocin in Citrate Buffer Solution pH 4.5

For the preparation of the citrate buffer solution, two reagents were used: dehydrated trisodium citrate with a molecular weight of 294.10 and monohydrate citric acid with a molecular weight of 210.14 with a volume of 25 ml., up to obtain

a 0.1 mol/L citrate buffer solution at pH 4.5 [48]. For the dilution of the strepto-zotocin, 1 ml was used of solution for every 100 mg of the drug.

2.5. Histopathological Study of the Pancreas

Six animals from each group were anesthetized with sodium pentobarbital at a dose of 40 mg/kg intraperitoneal, on days 1, 7, 14 and 21, and were euthanized to obtain the pancreas of each of the rats. The tissue was fixed in stabilized formalin, samples were blocked in paraffin, five-micron sections were cut on a rotary microtome, and they were stained with the Hematoxylin-Eosin technique [49].

2.6. Quantification of the Number of Islets

For the quantification of the number of islets of Langerhans, four fields were randomly chosen from each cut, using the 10x objective with a Zeiss microscope and the Future Winjoe program.

2.7. Statistical Analysis

The data obtained were subjected to an analysis of variance to compare the differences between groups, followed by a *post hoc* test at a significance level of 0.05, using the SigmaStat 3.1 software [50].

3. Results

3.1. Histopathological Analysis

From the histological point of view, in rat pancreas samples stained with HE from day 1, a characteristic structure of this organ was observed, that is, the presence of islets of Langerhans with a typical number and distribution, with a population differentiated from α, β, δ and pp cells. The pancreatic tissues of the four groups do not present changes in their morphological structure (Figures 1(a)-(d)).

By day 7 of the experiment, in samples from the control group (CG) (Figure 2(a)), more than one islet of Langerhans per field (10X objective) was observed, as well as a regular population of α and β cells. While in the pancreas of rats in the control treatment group (GCTx) (Figure 2(b)) severe hypoplasia of the Islets of Langerhans was observed with a discrete presence of α and β cells. In animals treated with kombucha (GTxK) (Figure 2(c)) few islets of Langerhans per field were observed, in addition to primarily α cells, few β cells, and islet degeneration. For the samples of animals treated with the non-polar fractions (GTxCNP) (Figure 2(d)) during the same treatment period, the pancreas presented α cells and a discrete population of β cells in the Islets of Langerhans. On days 14 and 21, the pancreas of the control group remained intact, with no apparent damage (Figure 3(a), Figure 4(a)). On the contrary, in the control group treatment (GCTx) the decrease in the number of islets of Langerhans was maintained (Figure 3(b), Figure 4(b)). In the pancreatic sections of the animals treated with

kombucha (GTxK) (Figure 3(c), Figure 4(c)) and with the non-polar components (GTxCNP) (Figure 4(c), Figure 4(d)), islets were observed with the presence of regular to abundant and with a discrete population of cells β.
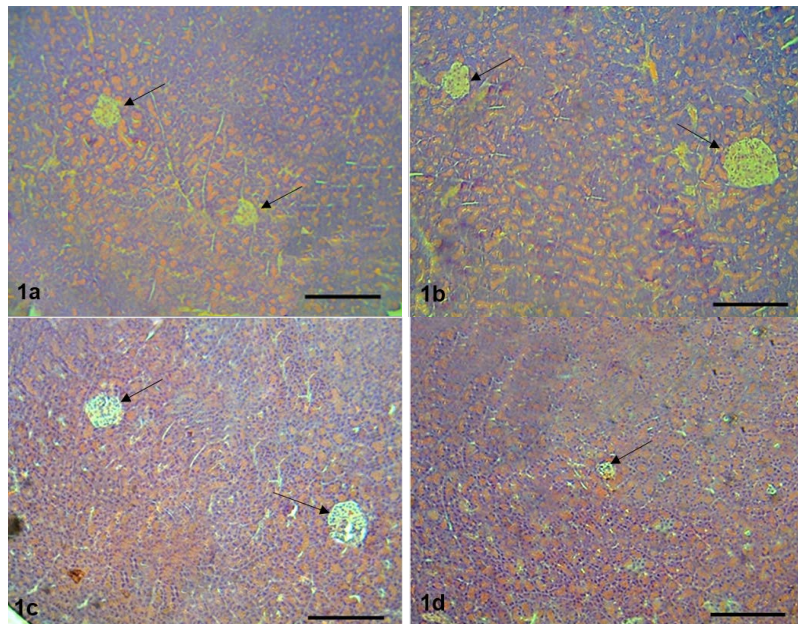

Figure 1. Photomicrographs of pancreatic sections stained with H&E. from the control group shows apparently normal islets of Langerhans of different sizes [(1a, 1 day), (1b, 7 days), (1c, 14 days) and (1d, 21 days)]. Scale bar = 200 μm, 10x objective.
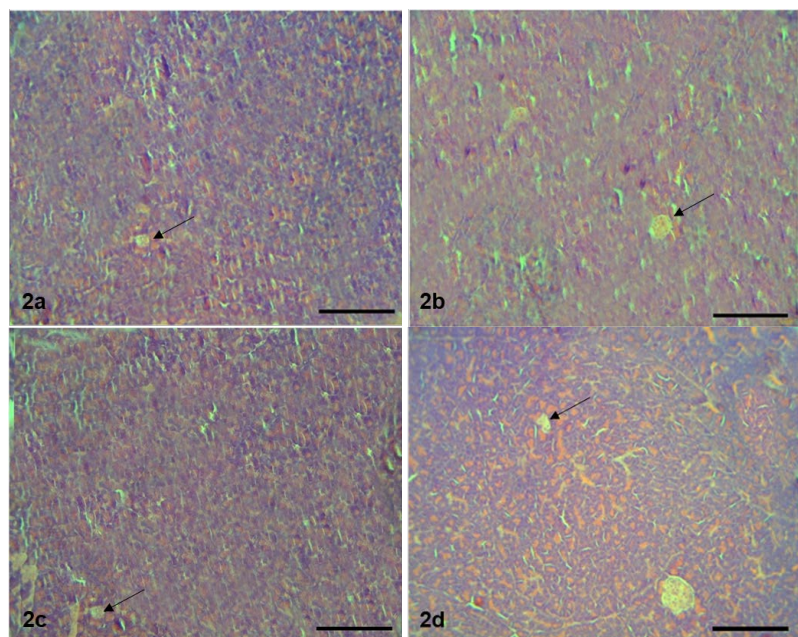

Figure 2. Photomicrographs of pancreas sections stained with H&E, of the control group Treatment, shows an absence (2c, 14 days) or hypoplasia [(2a, 1day), (2b, 7 days,) (2d, 21 days)] of the islets of Langerhans due to the effect of streptozotocin. Scale bar = 200 μm, 10x objective.
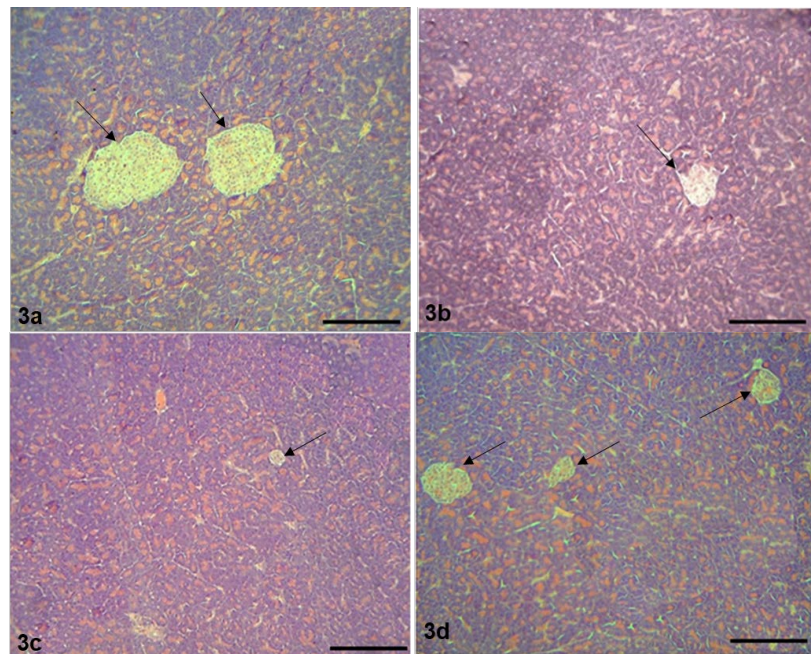
Figure 3. Photomicrographs of pancreas sections stained with H&E. of the Kombucha Treatment group shows the presence of islets of Langerhans of different sizes (3a, 3b, 3c, 3d), irregularly shaped [(3a, 1 day), (3b, 7 days), (3c, 14 days) and (3d, 21 days)]. Scale bar = 200 μm, 10x objective.
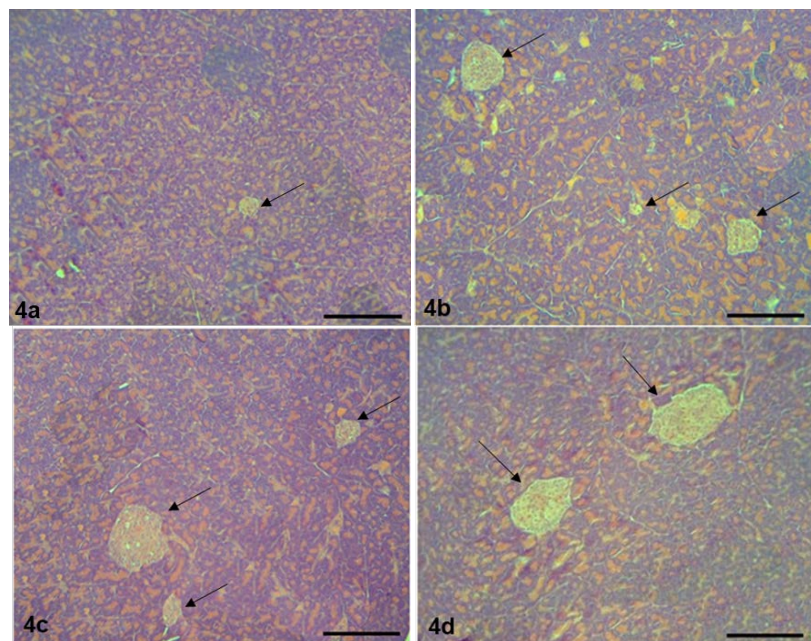

Figure 4. Photomicrographs of H&E-stained sections of the pancreas. Of the non-polar components, treatment group shows the presence of islets of Langerhans of different sizes, irregularly shaped [(4a, 1 day), (4b, 7 days), (4c, 14 days) and (4d, 21 days)]. Scale bar = 200 μm, 10x objective.

3.2. Quantitative Analysis of the Islets of Langerhans

The analysis of the number of islets of Langerhans in pancreatic tissue and

stained with H/E with the objective of 10x allowed observing important differences. In the Control Group, an average of 1.6 islets of Langerhans per field was identified; while in the pancreas of the Experimental Groups a gradual decrease of these was observed, this being more severe in the Control Treatment group than in the groups treated with kombucha and non-polar components. Statistical analysis showed significant differences ($p < 0.05$) between the Control Group and the treated group, corresponding to days 7, 14 and 21, respectively (**Figures 5-8**).
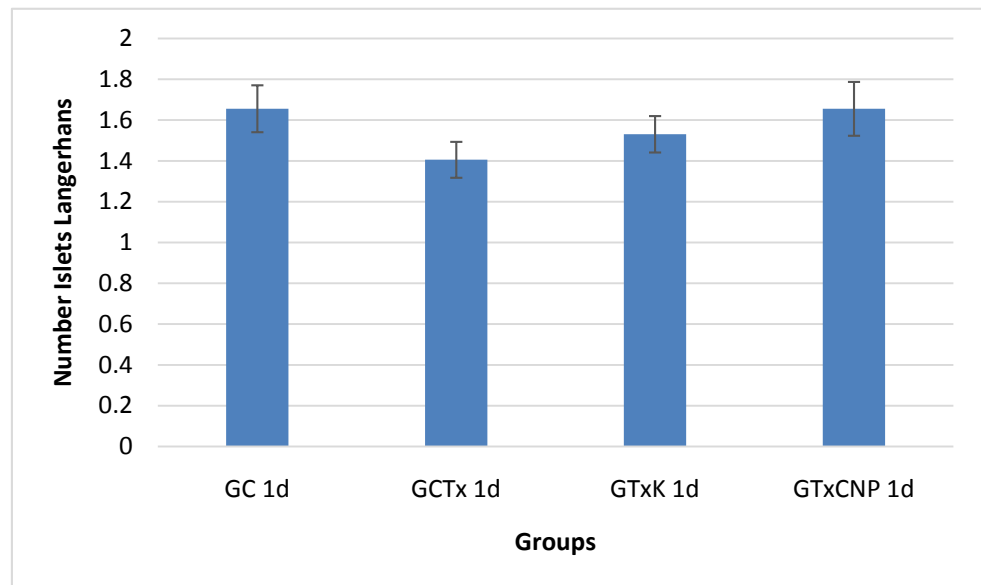

Figure 5. Average number of islets of Langerhans on day 1 of the experiment, no statistically significant difference is observed between the four groups.
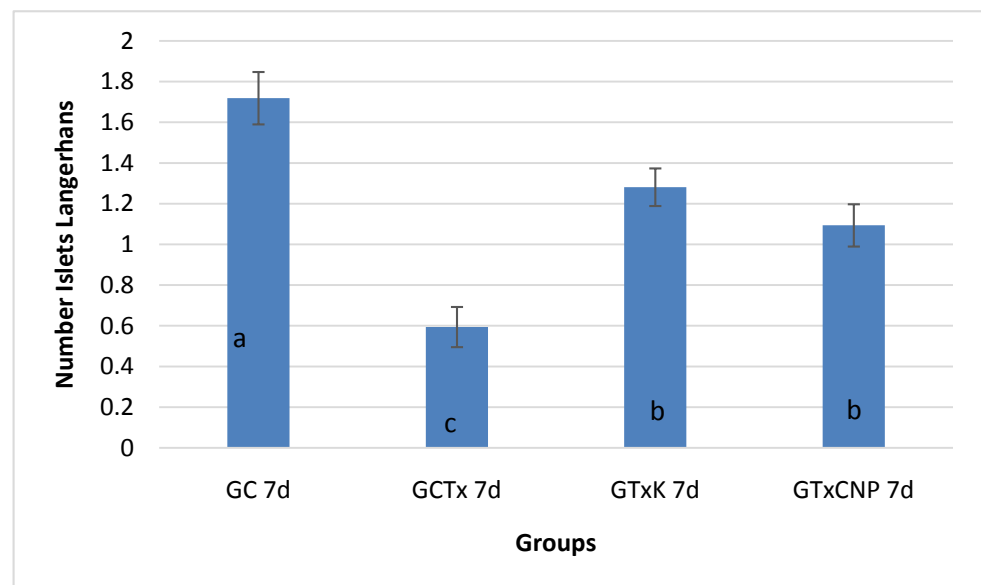

Figure 6. Number of islets of Langerhans on day 7 of treatment. The literals a, b, c indicate statistical difference at a significance level of $p < 0.05$.
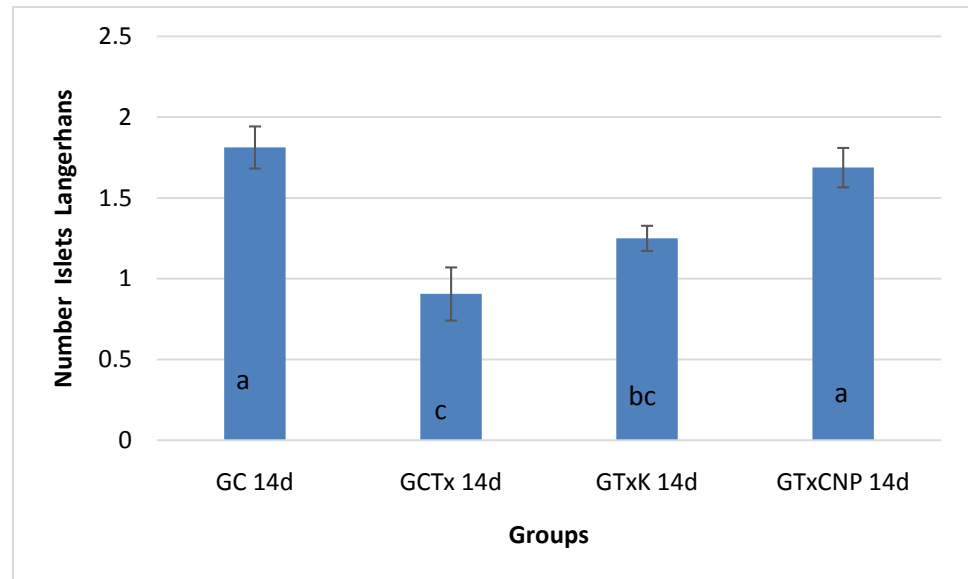
Figure 7. Average number of islets of Langerhans on day 14 of the experiment. The letters a, b, c indicate statistical difference at a significance level of $p < 0.05$.
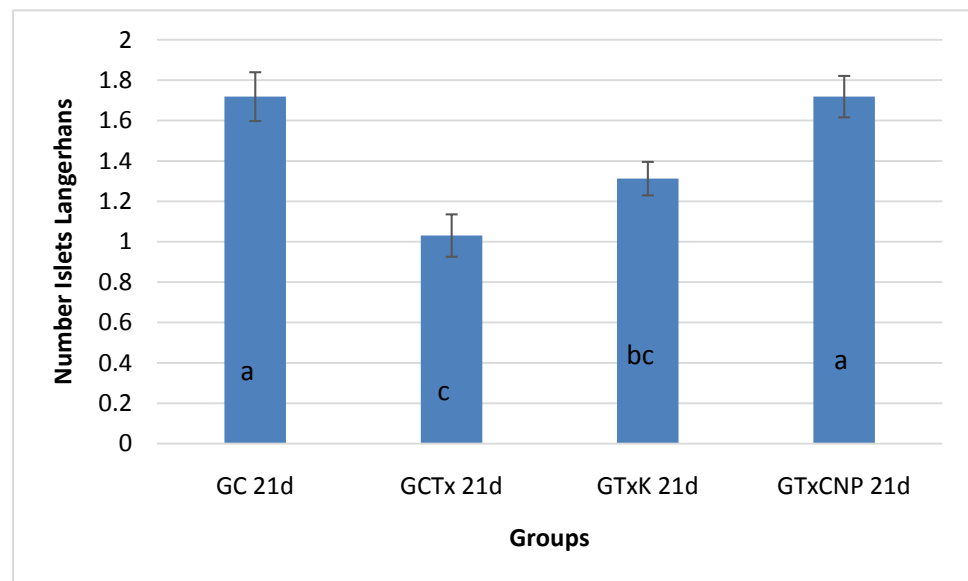
Figure 8. Average number of islets of Langerhans on day 21 of the experiment. The literals a, b, c indicate statistical difference at a significance level of $p < 0.05$.

4. Discussion

Kombucha tea has become a very popular drink worldwide for its beneficial effects in a number of ailments that range from mild, such as digestive disorders, to chronic-degenerative diseases such as hypercholesterolemia, cancer, hypertension and diabetes. In the present study, we want to show the effects of kombucha tea and its non-polar components in a rat model with diabetes mellitus, a problem that year after year continues to affect millions of people around the

world. This disorder was induced with streptozotocin administered intraperitoneal at a dose of 65 mg/kg [51]. Streptozotocin as a diabetes-inducing agent has been used since the last century [52] by several researchers in different animal models at doses ranging from 25 to 100 mg/kg of body weight, as well as different routes of administration [53] [54].

As previously reported, when applying this drug, insulin and glucose levels were modified, since the hyperglycemic effect was obtained in the first two hours after application, possibly due to a glycolic effect in the liver, declining later at ten hours, increasing after 24 hours, causing permanent hyperglycemia and consequently decrease insulin levels. The duration of the diabetic state depends on the dose of streptozotocin, since under the conditions of this study a hyperglycemic effect was demonstrated for up to 21 days [46], considering that doses of more than 100 mg cause immediate destruction of the β cells of the pancreas and even death due to diabetic coma [55].

Histological structure and functioning of the pancreas are of vital importance, since the secretion of hormones such as insulin synthesized by β cells depends on it to maintain a balance in the concentration of blood glucose [18], and the homeostasis of other organs such as the liver, kidney, eyes, adipose tissue and the nervous system itself [7].

The increase in blood glucose in the bloodstream causes the release of insulin caused by the depolarization of the β cells of the pancreas, thus allowing the entry of glucose through the GLUT2 transporter found in the kidney, liver and small intestine, and is the responsible for regulating the production of insulin, maintaining normal limits in the body [7]. On the other hand, in the liver it increases the synthesis of glucokinase, favoring the use of glucose, it increases glycolysis by stimulating glucokinase, it increases the pentose pathway that provides NADPH by stimulating glucose 6 phosphate dehydrogenase, which favors the synthesis of glycogen, reduces glycogenesis, stimulates protein and lipid synthesis, and inhibits the formation of ketone bodies. In muscle, it stimulates the entry of glucose, increases glycolysis, stimulates glycogen synthesis, stimulates the uptake and use of ketone bodies and favors the entry of amino acids into the cell. While in adipose tissue it increases the pentose pathway that produces NADPH, it favors the uptake of fatty acids by stimulating lipoprotein lipase 1 and stimulates the synthesis of triglycerides [56].

One of the most important elements identified in the present work was a lower number of islets of Langerhans when administering streptozotocin, and a moderate recovery with both kombucha and non-polar fraction treatments, no important changes were identified in the appearance of the different cell lines, mainly in β cells. Function as a glycemic sensor, which allows them to integrate nutrient signals and modulators such as the arrival of food in the digestive tract and its subsequent absorption is accompanied by numerous signals that are increased glucose levels and other plasma metabolites, secretion of some gastrointestinal hormones, activation of parasympathetic nerves, etc. All of these signals control the secretion of insulin that has receptors on the cell membrane, such as

in the liver. In the organ it increases the activity and stimulates the synthesis of glucokinase, favoring the use of glucose, in muscle it stimulates glucose entry (by translocation of GLUT 4 towards the membrane), and in adipose tissue it stimulates uptake (GLUT 4) and use of glucose by the adipocyte [56].

The pancreas has two types of secretions, one exocrine and one endocrine, the first is carried out mainly by two enzymes, amylase and lipase, which subsequently pass to the duodenum. The second is carried out by the islets of Langerhans, formed by cells (α, β, δ and pp) these are responsible for synthesizing insulin and glucagon to maintain the function of the liver, kidney and adipose tissue [57]. There are 1000 to 3000 cells in the islets of Langerhans, of which α cell make up 20%, β cells 60% [58], and islets make up 1% of all tissue. Pancreatic and exist in three sizes: small < 50 μ, medium 50 - 100 μ and large 200 - 300 μ [59]. Their function depends on stimuli triggered by blood glucose concentrations and the CNS. The results show that in the control group the number of islets did not present a statistically significant difference during the 21 days of treatment that the experiment lasted, maintaining an average of 2 islets per field. While in the group of animals that were induced diabetes with streptozotocin at a dose of 65 mg/kg, the number of islets showed a considerable decrease in number and size. Caused by the effect of this drug on DNA and cellular components such as: proteins, carbohydrates and lipids causing damage upon entering the cell by the same glucose receptor GLUT2, increasing the synthesis of nitric oxide [60] and H_2O_2 and inducing DNA fragmentation by alkylation and destruction of β cells [61]). While the groups of animals treated with kombucha and the non-polar components showed a statistically significant difference due to the damage to the cells of the pancreas. But there was a slight recovery caused by the mechanism of action of the antioxidants of the metabolites, such as tannin polyphenols [62] and flavonoids [63], which when penetrating the cell prevent DNA fragmentation and purge free radicals, restoring in this way the cells of the organism without side effects [41] [64].

5. Conclusions

1) Under the conditions of the present study, diabetes was induced with streptozotocin, based on the manifested signs, whose effect has a duration of at least 21 days.

2) This fermented beverage and its non-polar components can be a complementary treatment alternative for diabetes mellitus, since they have positive effects by lowering blood glucose levels, maintaining insulin concentration and preventing further damage to the β cells of the pancreas and Islets of Langerhans.

3) It is necessary in the future to carry out specific studies on the main components of the extract of non-polar components of kombucha, as well as chemical and microbiological studies of kombucha to interpret the effect on diabetes mellitus.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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