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Systematic Review

Effects of Kombucha in Diabetes Induced Animal Models: A Systematic Review

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Abstract

This study aimed to systematically review the literature to identify the effects of KB in animal models of diabetes induction. A search was carried out in the following databases: PubMed, Scopus, Scielo, ScienceDirect, and ISI Web of Knowledge, using the descriptors "(Kombucha [MeSH])" and "(Kombucha tea [MeSH])". From the articles found, two independent and previously calibrated reviewers, using the EndNote X7 (Thomson Reuters, New York, US), selected those that investigated the effects of KB in animal models of diabetes induction. Of the 1214 studies found, 7 were included in the systematic review. All studies used male rats and induced diabetes with alloxan or streptozotocin. The most prevalent substrate applied in the KB fermentation was sweetened black tea (BT). The included studies focused on biochemical analysis, mainly in markers for diabetes (glucose, insulin and glycated hemoglobin), lipid profile, antioxidant molecules, and histological analyses of the pancreas and the liver, demonstrating a reverse in damages caused by the chemical induction of diabetes in animal models. In this study, a panel of KB effects in parameters altered by diabetes induction in rats was created, which could contribute to understanding the benefits of KB administration.

Keywords: Kombucha; Polyphenols; Antioxidant; Animal Model; Diabetes Induction; Systematic Review

Abbreviations

ALP: Aspartate Transaminase; ALT: Alanine Transaminase; ALX: Alloxan; AMPK: 5' Adenosine Monophosphate-Activated Protein Kinase; AST: Alkaline Phosphatase; BT: Black Tea; DNA: Desoxyribonucleic Acid; DSL: D-Saccharic Acid 1,4-Lactone F-1,6-BA, Fructose-1,6-Bisphosphatase; g/L: Gram Per Liter; GGT, Gamma-Glutamyl Transpeptidase; G-6-Pase, Glucose-6-Phosphatase; GT, Green Tea; HbA_{1c}: Glycated Hemoglobin; KB: Kombucha Beverage; KB-BT: Kombucha Beverage was Prepared with Black Tea; KB-GT: Kombucha Beverage was Prepared with Green Tea; KB-SF: Kombucha Beverage was Prepared with Snake Fruit Juice; MDA: Malondialdehyde; mg/kg: Milligram Per Kilogram; mL/kg: Milliliter Per Kilogram; OGTT: Oral Glucose Tolerance Test; OS: Oxidative Stress; ROS: Reactive Oxygen Species; SCOBY: Symbiotic Culture of Bac-

teria and Yeast; SF: Snake Fruit; SOD: Superoxide Dismutase; STZ: Streptozotocin; KB: Kombucha Beverage; SCOBY: Symbiotic Bacteria and Yeast Colony

Introduction

Fermented beverages are nowadays used worldwide and kombucha is one of the most studied in scientific research, especially concerning its chemical and antimicrobial properties, and the treatment of several diseases and health promotion in animal models [1,6]. Kombucha beverage (KB) is originated from northeast China about 220 B.C, where it was known as "divine Che" for being an energizing and detoxifying drink tasting like sparkling apple cider or sour vinegary, depending on the period of fermentation [7-9]. In 414 A.D. a Korean physician named Kombu introduced the "divine Che" in Japan to treat the digestive problems of a Japanese king. Subsequently, kombucha was disseminated by trade routes to Russia and eastern Europe with fluctuating popularity since World War II, when it was extended from Russia to western Europe, particularly France, Italy and Switzerland, and North Africa [1,9-11].

Traditionally the KB is prepared through fermentation for 7 to 10 days, by sweetened tea of a symbiotic culture of bacteria and yeast (SCOBY) [12-14]. The SCOBY is generally compound by acetic acid bacteria (*Komagataeibacter*, *Gluconobacter* and *Acetobacter* species), yeasts *(Schizosaccharomyces pombe*, *Saccharomycodes ludwigii*, *Kloeckera apiculata*, *Saccharomyces cerevisiae*, *Zygosaccharomyces bailii*, *Torulaspora delbrueckii*, and *Brettanomyces bruxellensis*), and often lactic acid bacteria (*Lactobacillus* and *Lactococcus*) [12-14]. Although black tea sweetened with sucrose is considered the ideal substrate for KB fermentation [9], formulations with other types of tea, such as green tea (GT), red tea [15], oolong tea [14], peppermint tea [16], lemon balm tea [16], and rooibos tea [17], and also with fruit juices, like pomegranate [18], cherry [19], and snake fruit (SF) [20], has become commonly used.

The beverage resulting from the *SCOBY* fermentation in tea or juice fruit is rich in chemical components, among which organic acids (acetic acid, glucuronic acid, gluconic acid, citric acid, L-lactic acid, malic acid, tartaric acid, malonic acid, oxalic acid, succinic acid, pyruvic acid, and usnic acid), D-saccharic acid 1,4-lactone (DSL), sugars (sucrose, glucose, and fructose), vitamins (B_1, B_2, B_6, B_{12} e C), amino acids, biogenic amines, purines, pigments, lipids, proteins hydrolytic enzymes, ethanol, carbon dioxide, minerals (ions of zinc, copper, iron, and manganese), anions (fluoride, chloride, bromide, iodide, nitrate, phosphate, and sulfate), and metabolic products of yeasts and bacteria are the most frequently reported [1,6,14]. Furthermore, other substances derived from the mixture used as a substrate to produce KB are significantly increased during fermentation, such as polyphenols, flavonoids, and tannin [15,20-22].

This set of chemicals could be related to the anti-inflammatory and antioxidant properties of KB. Two properties that once modulated can significantly impact the negative outcomes of various chronic conditions, including diabetes [2,6]. Therefore, studies have been carried out to evaluate the potential of KB in minimizing the damage caused by diabetes induction [22-24].

Diabetes is a metabolic disorder that represents a global public health problem with an estimated prevalence of 9.3% in 2019 (463 million people) and is expected to enhance to 10.2% (578 million) in 2030 and 10.9% (700 million) in 2045 [25]. Diabetes, especially type 2, is related to high morbidity and mortality. It can cause kidney insufficiency, blindness, non-traumatic amputation, and cardiovascular disease [26], and long-term hyperglycemia can lead to damage of vital organs culminating in cardiovascular disease, neuropathy, nephropathy, and retinopathy [27], therefore compromising the life quality and life expectancy of diabetic patients. Consequently, there is a growing desire among scientists who investigate this outcome to establish new approaches in association with the already available therapies, as an attempt to achieve adequate blood glucose control. In this sense, kombucha has been showing a promising opportunity in modulating altered parameters in diabetes considering animal models. Thus, we aimed to systematically review the literature to identify the effects of KB in diabetes induced animal models.

Materials and Methods

Review question

What are the effects of KB in diabetes induced animal models?

Inclusion and exclusion criteria

The inclusion criterion was studies that used animal models to investigate the effects of KB in diabetes. The use of animal models was conducted to control confounding factors in the variables of interest.

The exclusion criteria were: studies unrelated to KB, studies related to KB but unrelated to health areas, reviews, congress summaries, patent descriptions, book section, hypothesis articles, commentaries, opinion articles, previews, articles published in different languages than Portuguese, English and Spanish, letters, articles that were not fully available even after attempting to contact the authors.

Search strategy

The electronic search was conducted without initial date restriction up to and including July 2019 in PubMed, Scopus, Scielo, ScienceDirect, and ISI Web of Knowledge databases. The initial search was conducted using the MeSH and relevant entry terms: (Kombucha) OR (Kombucha tea). All references were managed in the EndNote X7 software (Thomson Reuters, New York, NY, US). Initially, duplicate references were excluded. Titles, abstracts, and study methodologies were screened based on the inclusion and exclusion criteria by two independent reviewers (GDS and CCdoA). Lists were compared and in case of disagreement, a consensus was reached by discussion. When a consensus was not achieved, a third reviewer decided if the article should be included (FN). This systematic review followed the PRISMA statements, with some adjustments [28].

Data extraction

Data were extracted and tabulated independently by three reviewers (GDS, CcdoA, and CPF) to be submitted to descriptive analysis. Cases of disagreement were handled as described above. A meta-analysis of the data was not feasible, given the absence of agreement in the literature to date.

Quality assessment

The Review Manager 5.3 software was used to assess the quality of the included study in the systematic review. The checklist was composed of nine domains: (1) description of the animals used and the gender, (2) description of the animal's age at the outset of diabetes, (3) description of the animal's weight at the outset of diabetes, (4) description of the substance and dose used for diabetes intraperitoneal induction, (5) description of the KB preparation, (6) description of the KB application to the animal model, (7) biological analysis related to KB effects, (8) comparison of results between positive and negative control and (9) comparison between results concerning KB effects.

Two independent researchers (CCdoA and GPC) assessed the quality of the studies based on criteria previously established. Cases of disagreement were discussed until a consensus was reached. When a consensus was not obtained, a third reviewer participated in the discussion (FN). Studies risk of bias was evaluated according to the presence (yes, Y) or absence (no, N) of the nine domains. Studies with up to 30% of Ys had a high risk of bias, above 30% and lower than 65% had a medium risk, and above 65% had a low risk of bias.

Results and Discussion

The initial search yielded 1214 articles. After removing 430 duplicated titles, a total of 784 articles were included for the title and the abstract screening, remaining 54 articles. After reading the full text, 47 studies were excluded and 7 articles remained, satisfying the inclusion criteria. Figure 1 displays the PRISMA flowchart for the study selection process.

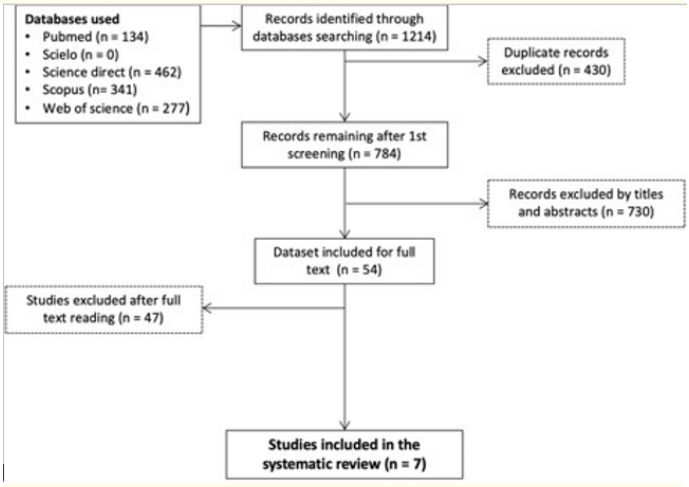

Figure 1: Flowchart of study selection.

Table 1 describes the animal characteristics, the substance and dose used to induce diabetes, and the main methodology used for KB preparation and treatment of diabetes induction.

All of the articles used male rats and six chose the Wistar strain. To induce diabetes all studies used intraperitoneal injection, 4 used

Author, Year	Animal	Gender	Animal age -outset diabetes	Animal weight - outset diabetes	Substance and dose for diabetes intraperitoneal induction	KB preparation applied to animal model				KB
						Tea or juice concentration	Sugar concentration	Fermentationperiod	Exposure period	Route/method of KB administration
Aloulou et al., 2012	Wistar rats	Male	-	179 ± 10 g	ALX at 150 mg/kg bw	Black tea at 12 g/L	100 g/L	12 d	30 d	Oral/Gavage
Bhattacharya., *et al*. 2013	Swiss rats	Male	-	6 w	180 to 200 g ALX at 120 mg/kg bw	Black tea at 4.2 g/L	100 g/L	14 d	14 d	Oral/-
Hosseini., et al. 2015	Wistar rats	Male	-	200 to 220 g	ALX at 120mg/kg bw	Green tea at 12 g/L	100 g/L	-	28 d	Oral/Gavage
Srihari., et al. 2013	Wistar rats	Male	30w	180 to 220 g	STZ at 45 mg/kg bw	Black tea at 4.5 g/L	100 g/L	14 d	45 d	Oral/Intragastric tube
Zubaidah., et al. 2018	Wistar rats	Male	3m	-	STZ at 47.5 mg/kg bw	Snake fruit juice at 1000 g/L	100 g/L	14 d	28 d	Oral/-

Table 1: Description of animal characteristics, substance and dose used to induce diabetes, and the main methodology used for KB preparation and treatment of diabetes induction by author and year of publication.

ALX: Alloxan Monohydrate; d: Days; g: Gram; g/L: Gram Per Liter; m: Month; mg/kg bw: Milligram Per Kilogram of Body Weight; STZ: Streptozotocin; w: Week

streptozotocin (STZ) (three articles a 45 mg/kg dosage and one a 47.5 mg/kg dosage) and 3 used alloxan (ALX) dissolved in saline (two studies a 120 mg/kg dosage and one a 150 mg/kg dosage).

Based on the results of this systematic review, the KB has shown an important potential to modulate the damages caused by the diabetic induction and consequences of exposure to hyperglycemia in preclinical rodent models, reversing the negative results reported in the included studies. From the findings reported by these studies, we create a panel of KB effects in parameters altered by diabetes induction in rats, which could contribute to understanding the benefit of KB administration (Figure 3).

The animal used in all studies comprise male rats and the diabetes was induced by STZ or ALX. Chemical induction of diabetes in preclinical rodent models is usually performed with these two substances, despite others that are available for this purpose. The active principle of STZ and ALX is related to there cytotoxicity to pancreatic β-cells, producing a disruption in homeostasis and leading to the development of hyperglycemia due to pathways that included OS, established by a reduction of antioxidants and increase of pro-oxidants biomarkers, which are generated from the overproduction of reactive oxygen species (ROS) [29]. The DNA of pancreatic islets is one of the ROS targets after exposure to ALX and STZ, and the consequence is DNA fragmentation in pancreatic β-cells with subsequent cell apoptosis [30].

In the presence of an unbalanced cellular environment due to ROS, an augmentation in insulin resistance and an impairment in insulin secretion from β-cells can be observed. This leads to the oxidation of proteins and carbohydrates with the production of adverse compounds such as MDA, an end product of polyunsaturated fatty acids peroxidation [31]. On the other hand, prolonged exposure to hyperglycemia induces protein glycation also attributable to OS, more specifically to ROS, increasing intracellular and extracellular free radical circulation, with disturbance in cellular core and triggering signaling pathways for several molecular mechanisms [32,33]. The cell injury caused by ROS in both conditions can be minimized by the enzymatic catalyze of harmful oxidants, such as superoxide formed in oxygen metabolism. SOD is the enzyme that can convert this very damaging compound into molecular oxygen peroxide, a less toxic substance, and this makes it extremely relevant in the presence of OS [34].

The KB was prepared with black tea (KB-BT) (4 studies, using 4.2, 4.5, 12, and 20 g/L concentrations) or green tea (KB-GT) (1 study, using 12 g/L concentration) or snake fruit juice (KB-SF) (3 studies, using 20 g/L and 1000 g/L), sugar at 100 g/L (all studies) and most used 14 days of fermentation (5 studies). Regarding the application of KB for treatment of diabetic induction, the exposure period occurred between 14 to 45 days (14 days - 1 study - KB-BT; 28 days - 4 studies - KB-GT and KB-SF; 30 days - 1 study - KB-BT; 45 days - 1 study - KB-BT) and all studies used the oral route to administration the beverage, with 2 studies applying by gavage (KB-BT and KB-GT), 2 studies by intragastric tube (KB-BT and KB-SF) and 3 not revealing the method of KB administration (KB-BT and KB-SF).

To prepare the KB the included studies used sucrose in a concentration of 100 g/L and BT, GT, or SF in varying concentrations. These variations seemed to cause little impact when considering a positive or no reversion of the adverse consequences of diabetes induction in rats since all studies showed the KB potential to reverse diabetic parameters. Nevertheless, it is important to mention that when comparing 3 mg/kg, 6 mg/kg, and 12 mg/kg concentration of KB-BT, the intermediary dosage was more effective in decreasing the glycemia, HbA_{1c} blood level, and G-6-Pase and F-1,6-BA activity in the liver, and increasing the insulin and total hemoglobin blood levels and hexokinase activity in the liver [35]. Contrarily, analyzing KB-SF in concentrations of 5 mL/kg, 10 mL/kg, and 15 mL/kg revealed better results for the highest dosage in reversing OS biomarkers, with a significant increase in SOD blood level and decrease in MDA blood level [20]. These results may reflect the differences between the substrate used to produce KB, the SCOBYs, the KB preparation, the animal exposure to KB, and/or the ideal concentration of KB for a certain parameter modulation.

The KB fermentation usually increases the polyphenol content in teas or juices, such as total flavonoids in KB-BT [15], KB-GT [15], and KB-SF [20], the tannin in KB-BT and KB-SF [22], quercetin in KB-BT, KB-GT [21], and KB-SF [22], and catechins [21], theaflavin, and thearubigin in KB-BT and KB-GT [1,15,21]. The polyphenols are widely recognized due to their capacity to improve human and animal health by minimizing the OS state through their antioxidant activity, stimulating the restoration of the unbalanced cellular environment [39-41]. Therefore, the disturbance in oxidant and antioxidant balance promoted by diabetes induction (showed in four studies), could have been diminished by the polyphenols present in KB, as all studies demonstrated an increase in SOD and a reduction in MDA levels in the blood and the pancreas.

Table 2 describes the aim, treatment groups, biological analysis and collection site, and results of the included studies. All studies that conducted glucose analysis (5 studies - KT-BT and KB-SF) identified a statistically significant decrease in blood levels in the diabetic group treated with KB. Two studies (KB-BT) verified the blood levels of insulin and glycated hemoglobin (HbA_{1c}) and identified, respectively, a significant increase and decrease in these diabetes biomarkers.

The decreased activity of hexokinase (a glycolytic enzyme responsible for the adequate use of hepatic glucose) is observed in insulin deficiency associated with altered carbohydrate metabolism, which occurs in diabetes, leading to an impairment in glucose utilization by the liver [36,37]. Instead, the hepatic activities of G-6-Pase and F-1,6-BA are increased in diabetes, favoring gluconeogenesis, and contributing to fasting hyperglycemia. Insulin deficiency associated with hyperglycemia can stimulate the activity of these enzymes, favoring the storage of glycogen in the liver [38]. In rats that suffered induction by diabetic drugs, this is also observed, but the KB-BT showed the capability to increase the activity of hexokinase and decrease the activity of G-6-Pase and F-1,6-BA, which probably contributes to the reduction of glucose levels in the blood [35].

Author/year	Studies aim	Groups (n)	Biological analysis (site)	Results (p value)
Aloulou., *et al.* 2012	Evaluated the biological activities of KT towards pancreatic lipase and α-amylase as well as its effects on liver-kidney function.	G1: Normal control - Con (n = 8) G2: Diabetes (n = 8) G3: Con + BT by gastric gavage (5 ml/kg bw) every d (n = 8) G4: Con + kT by gastric gavage (5 ml/kg bw) every d (n = 8) G5: Diabetes + BT by gastric gavage (5 ml/kg bw) every d (n = 8) G6: Diabetes + KT by gastric gavage (5 ml/kg bw) every d (n = 8)	AST (blood)	Decreased (G5 - 52.58 ± 23.75 U/L and G6 - 47.75 ± 36.14U/L; $p < 0.05$) compared to G2 (77.17 ± 9.35 U/L).
			ALT (blood)	Decreased (G3 - 23.33 ± 4.13 U/L, G4 - 21.33 ± 4.13 U/L, G5 - 41.67 ± 5.16 U/L and G6 -36.9 ± 10.68 U/L; $p < 0.05$) compared to G2 (72.3 ± 3.47 U/L).
			GGT (blood)	Decreased (G3 - 16.50 ± 4.76 U/L, G4 - 13.67 ± 1.63 U/L, G5 - 26.83 ± 6.40 U/L and G6 - 23.67 ± 5.88 U/L; $p < 0.05$) compared to G2 (45.83 ± 6.15 U/L).
			α-amylase (blood)	Decreased (G5 - 52 ± 11% and G6 - 37 ± 8%; $p < 0.05$) compared to G2.
			α-amylase (pancreas)	Decreased (G5 - 70 ± 17% and G6 - 52 ± 7%; $p < 0.05$) compared to G2.
			Glucose (blood)	Decreased (G5 - 65 ± 14% and G6 - 50 ± 11%; $p < 0.05$) compared to G2.
			Urea (blood)	Decreased (G3 - 0.5 ± 0.07 g/L, G4 - 0.47 ± 0.06 g/L, G5 - 0.77 ± 0.36 g/L and G6 - 0.63 ± 0.25 g/L; $p < 0.05$) compared to G2 (1.05 ± 0.11 g/L).
			Creatinina (blood)	Decreased (G3 - 12 ± 2.19 mg/L, G4 - 9.83 ± 1.47 mg/L, G5 - 18.83 ± 2.86 mg/L and G6 - 15.4 ± 2.59 mg/L; $p < 0.05$) compared to G2 (25.5 ± 2.59 mg/L).
			TG (blood)	Decreased (G5 - 59 ± 21%; $p < 0.05$) compared to G2.
			LDL-Ch (blood)	Decreased (G5 - 65 ± 14%; $p < 0.05$) compared to G2.
			HDL-Ch (blood)	Increased (G5 - 137 ± 18%; and G6 - 157 ± 30%; $p < 0.05$) compared to G2.
			Lipase activity (plasma)	Decreased (G5 - 80 ± 15% and G6 - 68 ± 10%; $p < 0.05$) compared to G2.
			Lipase activity (pancreas)	Decreased (G5 - 68 ± 17% and G4 - 62 ± 10%; $p < 0.05$) compared to G2.
			Histological analyses (pancreas)	G2 showed atrophy of β-Cells and G1 exhibited normal islets, while the G5 and G6 exhibited a marked amelioration.
Bhattacharya., *et al.* 2013	Investigated the role in ameliorating the oxidative stress in diabetes related complications in organs like pancreas, liver, kidney and heart of ALX induced diabetic rats in comparison to that of unfermented tea. Evaluated the effect of KT on ALX-induced DNA fragmentation and activation of caspase-3 in the pancreatic tissue of experimental rats to address its protective mechanism.	G1: Control (vehicle only) - Cont (n = 6) G2: KT orally at 150 mg LEx/kg bw for 14 d to know wheter any toxic effect was produced by KT - KT (n = 6) G3: Diabete animals - ALX (n = 6) G4: Diabete animals + KT orally at 150mg LEx/kg bw for 14 d after diabete induction - ALX + KT (n = 6) G5: Diabete animals + BT orally at 150 mg LEx/kg bw for 14 d - ALX + BT (n = 6) G6: Diabete animals + Glibencamide at 1 mg/kg bw for 14 d - ALX + GB (n = 6)	Body weight	Decreased (G3 - 154.66 ± 4.03g, G5 - 178.83 ± 2.64g and G6 - 189.5 ± 2.88g; $p < 0.05$) compared to G1 (215.16 ± 4.07g) and G4 (192.17 ± 3.40g).
			ALT (blood)	Decreased (G4 - 39.8 ± 2.49 IU/L and G5 - 47.9 ± 2.64 IU/L; $p < 0.05$) compared to G3 (61.25 ± 3.8 IU/L).
			ALP (blood)	Decreased (G4 - 30.62 ± 1.5 KA Units, G5 - 38.45 ± 1.82 KA Units and G6 - 40.34 ± 2.88 KA Units; $p < 0.05$) compared to G3 (58.18 ± 2.73 KA Units).
			Glucose (blood)	Decreased (G4 - about 56.4%, 130.17 ± 5.88 mg/dL, G5 - about 48.5%, 153.67 ± 6.6 mg/dL and G6 - 111.83 ± 5.7 mg/dL; $p < 0.05$) compared to G3 (298.41 ± 13.25 mg/dL).
			Urea nitrogen (blood)	Decreased (G4 - 30.6 ± 2.23 mg/dL, G5 - 34.6 ± 1.79 mg/dL and G6 - 24.68 ± 1.46 mg/dL; $p < 0.05$) compared to G3 (42.73 ± 2.74 mg/dL).
			Creatinine (blood)	Decreased (G4 - 0.157 ± 0.013 mg/dL, G5 - 0.184 ± 0.015 mg/dL and G6 - 0.149 ± 0.013 mg/dL; $p < 0.05$) compared to G3 (0.226 ± 0.021 mg/dL).
			TG (blood)	Decreased (G4 - 92.37 ± 5.46 mg/dL, G5 - 105.53 ± 5.17 mg/dL and G6 - 90.45 ± 4.35 mg/dL; $p < 0.05$) compared to G3 (159.37 ± 6.74 mg/dL).
			TC (blood)	Decreased (G4 - 124.21 ± 8.4 mg/dL, G5 - 140.67 ± 7.82 mg/dL and G6 - 111.17 ± 7.43 mg/dL; $p < 0.05$) compared to G3 (181.46 ± 10.07 mg/dL).
			HDL-Ch (blood)	Increased (G4 - 29.26 ± 1.3 mg/dL, G5 - 25.37 ± 1.2 mg/dL and G6 - 33.19 ± 1.6 mg/dL; $p < 0.05$) compared to G3 (19.63 ± 0.9 mg/dL).
			Glycated Hb (blood)	Decreased (G4 - 0.471 ± 0.054 mg/g Hb, G5 - 0.636 ± 0.061 mg/g Hb and G6 - 0.462 ± 0.05 mg/g Hb; $p < 0.05$) compared to G3 (0.875 ± 0.07 mg/g Hb).
			Insulin (blood)	Increased (G4 - 1.67 ± 0.11 ng/mL, G5 - 1.34 ± 0.1 ng/mL and G6 - 1.79 ± 0.12 ng/mL; $p < 0.05$) compared to G3 (0.75 ± 0.07 ng/mL).
			SOD (liver)	Decreased (G4 - 47.8 ± 2.2 Unit/mg protein, G5 - 49.97 ± 2.38 Unit/mg protein and G6 - 47.1 ± 2.25 Unit/mg protein; $p < 0.05$) compared to G3 (59.5 ± 2.8 Unit/mg protein).
			SOD (kidney)	Increased (G4 - 32.3 ± 1.52 Unit/mg protein, G5 - 29.7 ± 1.38 Unit/mg protein and G6 - 33.6 ± 1.56 Unit/mg protein; $p < 0.05$) compared to G3 (21.8 ± 1.05 Unit/mg protein).
			SOD (pancreas)	Increased (G4 - 2.53 ± 0.11 Unit/mg protein, G5 - 2.15 ± 0.1 Unit/mg protein and G6 - 2.63 ± 0.12 Unit/mg protein; $p < 0.05$) compared to G3 (1.2 ± 0.05 Unit/mg protein).
			SOD (heart)	Decreased (G4 - 6.3 ± 0.23 Unit/mg protein, G5 - 6.8 ± 0.19 Unit/mg protein and G6 - 6.7 ± 0.2 Unit/mg protein; $p < 0.05$) compared to G3 (8.4 ± 0.4 Unit/mg protein).
			CAT (liver)	Decreased (G4 - 100.66 ± 5.2 µmol/min/mg protein, G5 - 112.57 ± 5.52 µmol/min/mg protein and G6 - 91.43 ± 4.47 µmol/min/mg protein; $p < 0.05$) compared to G3 (167.4 ± 8.12 µmol/min/mg protein).
			CAT (kidney)	Increased (G4 - 81.42 ± 4.05 µmol/min/mg protein, G5 - 71.73 ± 3.48 µmol/min/mg protein and G6 - 81.63 ± 4.05 µmol/min/mg protein; $p < 0.05$) compared to G3 (53.28 ± 2.54 µmol/min/mg protein).
			CAT (pancreas)	Increased (G4 - 75.99 ± 3.6 µmol/min/mg protein, G5 - 68.14 ± 3.3 µmol/min/mg protein and G6 - 83.79 ± 4.08 µmol/min/mg protein; $p < 0.05$) compared to G3 (56.52 ± 2.7 µmol/min/mg protein).
			CAT (heart)	Decreased (G4 - 41.5 ± 2.05 µmol/min/mg protein, G5 - 51.6 ± 2.48 µmol/min/mg protein and G6 - 39.54 ± 1.77 µmol/min/mg protein; $p < 0.05$) compared to G3 (57.5 ± 2.67 µmol/min/mg protein).
			GST (liver)	Increased (G4 - 0.62 ± 0.03 µmol/min/mg protein, G5 - 0.59 ± 0.02 µmol/min/mg protein and G6 - 0.61 ± 0.03 µmol/min/mg protein; $p < 0.05$) compared to G3 (0.45 ± 0.02 µmol/min/mg protein).
			GST (kidney)	Increased (G4 - 0.61 ± 0.03 µmol/min/mg protein, G5 - 0.52 ± 0.02 µmol/min/mg protein and G6 - 0.53 ± 0.02 µmol/min/mg protein; $p < 0.05$) compared to G3 (0.38 ± 0.02 µmol/min/mg protein).
			GST (pancreas)	Increased (G4 - 0.27 ± 0.012 µmol/min/mg protein, G5 - 0.24 ± 0.011 µmol/min/mg protein and G6 - 0.28 ± 0.013 µmol/min/mg protein; $p < 0.05$) compared to G3 (0.16 ± 0.01 µmol/min/mg protein).
			GST (heart)	Increased (G4 - 0.108 ± 0.005 µmol/min/mg protein, G5 - 0.101 ± 0.005 µmol/min/mg protein and G6 - 0.106 ± 0.005 µmol/min/mg protein; $p < 0.05$) compared to G3 (0.082 ± 0.004 µmol/min/mg protein).
			GR (liver)	Increased (G4 - 37.43 ± 1.7 nmol/min/mg protein, G5 - 33.59 ± 1.65 nmol/min/mg protein and G6 - 36.24 ± 1.72 nmol/min/mg protein; $p < 0.05$) compared to G3 (24.59 ± 1.2 nmol/min/mg protein).
			GR (kidney)	Increased (G4 - 19.5 ± 0.92 nmol/min/mg protein, G5 - 17.68 ± 0.85 nmol/min/mg protein and G6 - 17.34 ± 0.84 nmol/min/mg protein; $p < 0.05$) compared to G3 (13.7 ± 0.6 nmol/min/mg protein).
			GR (pancreas)	Increased (G4 - 26.89 ± 1.2 nmol/min/mg protein, G5 - 21.79 ± 1.06 nmol/min/mg protein and G6 - 26.8 ± 1.24 nmol/min/mg protein; $p < 0.05$) compared to G3 (16.65 ± 0.73 nmol/min/mg protein).
			GR (heart)	Increased (G4 - 18.7 ± 0.9 nmol/min/mg protein, G5 - 15.9 ± 0.72 nmol/min/mg protein and G6 - 20.6 ± 1.03 nmol/min/mg protein; $p < 0.05$) compared to G3 (12.4 ± 0.52 nmol/min/mg protein).

			GPx (liver)	Increased (G4 - 80.66 ± 4.1 nmol/min/mg protein, G5 - 72.14 ± 3.4 nmol/min/mg protein and G6 - 77.8 ± 3.75 nmol/min/mg protein; $p < 0.05$) compared to G3 (45.29 ± 2.24 nmol/min/mg protein).
			GPx (kidney)	Increased (G4 - 95.43 ± 4.5 nmol/min/mg protein, G5 - 81.07 ± 4.02 nmol/min/mg protein and G6 - 98.25 ± 4.6 nmol/min/mg protein; $p < 0.05$) compared to G3 (67.87 ± 3.2 nmol/min/mg protein).
			GPx (pancreas)	Increased (G4 - 15.32 ± 0.73 nmol/min/mg protein, G5 - 14.52 ± 0.7 nmol/min/mg protein and G6 - 16.38 ± 0.79 nmol/min/mg protein; $p < 0.05$) compared to G3 (6.38 ± 0.3 nmol/min/mg protein).
			GPx (heart)	Increased (G4 - 69.8 ± 3.2 nmol/min/mg protein, G5 - 57.4 ± 2.57 nmol/min/mg protein and G6 - 74.4 ± 3.62 nmol/min/mg protein; $p < 0.05$) compared to G3 (25.5 ± 1.17 nmol/min/mg protein).
			ROS (Liver)	Decreased (G4, G5 and G6; $p < 0.05$) compared to G3.
			ROS (kidney)	Decreased (G4, G5 and G6; $p < 0.05$) compared to G3.
			ROS (pancreas)	Decreased (G4, G5 and G6; $p < 0.05$) compared to G3.
			ROS (heart)	Decreased (G4, G5 and G6; $p < 0.05$) compared to G3.
			Protein carbonyl (Liver)	Decreased (G4, G5 and G6; $p < 0.05$) compared to G3.
			Protein carbonyl (kidney)	Decreased (G4, G5 and G6; $p < 0.05$) compared to G3.
			Protein carbonyl (pancreas)	Decreased (G4, G5 and G6; $p < 0.05$) compared to G3.
			Protein carbonyl (heart)	Decreased (G4, G5 and G6; $p < 0.05$) compared to G3.
			MDA (Liver)	Decreased (G4, G5 and G6; $p < 0.05$) compared to G3.
			MDA (kidney)	Decreased (G4, G5 and G6; $p < 0.05$) compared to G3.
			MDA (pancreas)	Decreased (G4, G5 and G6; $p < 0.05$) compared to G3.
			MDA (heart)	Decreased (G4, G5 and G6; $p < 0.05$) compared to G3.
			GSH (liver)	Increased (G4, G5 and G6; $p < 0.05$) compared to G3.
			GSH (kidney)	Increased (G4, G5 and G6; $p < 0.05$) compared to G3.
			GSH (pancreas)	Not significant.
			GSH (heart)	Increased (G4, G5 and G6; $p < 0.05$) compared to G3.
			DNA fragmentation (pancreas)	G3 showed DNA cleavage, indicating apoptose, which was almost inhibited in G4 and G5.
			Caspase-3 activation (pancreas)	Increased by 2.3 folds in G3 pancreatic tissue but lowered in G4 and G5.
			Histological studies (liver)	Liver histological analysis of G3 showed a distortion in the arrangement of cells around the central vein, periportal fatty infiltration and formation of apoptotic bodies. G4 and G5 showed a normal arrangement around central vein and reduction in hepatocytes apoptosis.
			Histological studies (kidney)	G3 showed degenerated glomeruli, basement membrane thickening, marked proliferation of mesanglial cells and mesangial matrix accumulation, which is attenuated in G4 and G5.
			Histological studies (pancreas)	In G3 was observed a distortion in islets of Langerhans with atrophy of acinar cells. G4 and G5 showed a partial restoration and KT and BT offered protection to ß-cell mass.
			Histological studies (heart)	G3 showed disorganization of normal radiating pattern of cell plates in the heart, but these changes are reduced in G4 and G5.
Hosseini., *et al.* 2015	Evaluated the influence of KT made from GT.	G1: Control group (diabetic) (n = 10) G2: Green Tea - GT (n = 10) G3: Diabetic receiving Kombucha prepared from the GT (n = 10)	Body weight	Decreased (G2 - 186.5 ± 4.5 g and G3 - 211.6 ± 5.3 g; $p = 0.0001$ and $p = 0.024$, respectively) compared to G1 (177.7 ± 6.9 g).
Srihari., *et al.* 2013	Evaluated the biological activities of KT in the treatment of diabetes on experimental rats, with emphasis on the changes in levels of glucose, insulin, Hb, HbA1c, hepatic glycogen content, and the activities of some important carbohydrate metabolizing enzymes in STZ-induced experimental rats.	G1: Normal (n = 6) G2: Normal + Kombucha (Kob) (n = 6) G3: Diabete control (n = 6) G4: Diabete + Kob (3 mg/kg/d) (n = 6) G5: Diabete + Kob (6 mg/kg/d) (n = 6) G6: Diabete + Kob (12 mg/kg/d) (n = 6)	Oral glucose tolerance test (blood)	Not significant.
			Glucose (blood)	Decreased (G4 - 222.33 ± 20.18 mg/dL, G5 - 120.01 ± 10.86 mg/dL and G6 - 163.31 ± 14.84 mg/dL; $p < 0.05$) compared to G3 (278.36 ± 25.72 mg/dL).
			Insulin (blood)	Increased (G4 - 5.97 ± 0.46 lU/mL, G5 - 9.22 ± 0.64 lU/mL and G6 - 7.18 ± 0.53; $p < 0.05$) compared to G3 (3.92 ± 0.31 lU/mL).
			Total haemoglobin (blood)	Increased (G4 - 8.88 ± 0.48 g/dL, G5 - 10.58 ± 0.98 g/dL and G6 - 9.42 ± 0.38 g/dL; $p < 0.05$) compared to G3 (8.02 ± 0.59 g/dL).
			Glycated Hb (blood)	Decreased (G4 - 0.62 ± 0.05 mg/g Hb, G5 - 0.36 ± 0.03 mg/g Hb and G6 - 0.56 ± 0.05 mg/g Hb; $p < 0.05$) compared to G3 (0.74 ± 0.06 mg/g Hb).
			G-6-pase activity (liver)	Decreased (G5 - 0.22 ± 0.02 µmol of Pi liberated/min/mg protein; $p < 0.05$) compared to G3 (0.28 ± 0.02 µmol of Pi liberated/min/mg protein).
			F-1,6-BA activity (liver)	Decreased (G5 - 0.39 ± 0.03 µmol of Pi liberated/min/mg protein; $p < 0.05$) compared to G3 (0.61 ± 0.04 µmol of Pi liberated/min/mg protein).
			Hexokinase activity (liver)	Increased (G5 - 135.58 ± 10.40 µmol of glucose phosphorylated/min/g protein; $p < 0.05$) compared to G3 (106.98 ± 8.2240 µmol of glucose phosphorylated/min/g protein).
			Glycogen (liver)	Increased (G5 - 28.98 ± 2.36 mg/g tissue; $p < 0.05$) compared to G3 (19.88 ± 1.64 mg/g tissue).
			Glycogen (muscle)	Not significant.
			Body weight	Increased (G4, G5 and G6; p 0.05) compared to G3.
Zubaidah., *et al.* 2018	Evaluated snake fruit Kombrucha as a hyperglycemia therapeutic agent in diabetic animal models.	G1: Normal - P0 (n = 5) G2: Diabetes mellitus (DM) - P1 (n = 5) G3: DM + KS - P2 (5 mL/kg bw/d) (n = 5) G4: DM + KS - P3 (10 mL/kg bw/d) (n = 5) G5: DM + KS - P (15 mL/kg bw/d) (n = 5)	Glucose (blood)	Decreased (G3, G4 and G5; $p < 0.05$) in a range of 31-59% compared to G2.
			SOD (blood)	Increased (G3 - 41.95 ± 6.21 unit/100 µL; G4 - 43.87 ± 5.92 unit/100 µL and G5 - 46.75 ± 2.78 unit/100 µL; $p < 0.05$) compared to G2 (18.56 ± 5.42 unit/100 µL).
			MDA (blood)	Decreased (G3 - 0.45 ± 0.04 ng/100 µL, G4 - 0.29 ± 0.02 ng/100 µL and G5 - 0.20 ± 0.04 ng/100 µL; $p < 0.05$) compared to G2 (0.58 ± 0.08 ng/100 µL).
			Immunohistochemical staining (pancreas)	G3, G4 and G5 showed improvement of Langerhans islands structure and function of insulin secretion compared to G2. The size and shape of the langerhans island of G2 were smaller and irregular than G1 and G3, G4 and G5. G2 showed a very low immunoreactive response against the anti-insulin. G3, G4 and G5 the number and arrangement of endocrine cells look more homogeneous, and the intensity of the brown color was increased compared to G2. G3, G4 and G5 have regeneration of pancreatic ß-cells.

Zubaidah., *et al.* 2019	Compared snake fruit and black tea KT, and metformin as diabetes therapy agents.	G1: Normal - P0 (n = 5) G2: Diabetes - P1 (n = 5) G3: Diabetes with the BT KT - P2 (5 mL/kg bw/d) (n = 5) G4: Diabetes with the snake fruit kombucha - P3 (5 mL/kg bw/d) (n = 5) G5: Diabetes with the metformin - P4 (45 mL/kg bw/d) (n = 5)	Fasting plasma glucose (blood)	Decreased (G3 and G4; $p < 0.05$) compared to G2.
			TC (blood)	Decreased (G3 - 51.50 ± 5.20 mg/dL, G4 - 44.75 ± 3.79 mg/dL and G5 - 52.50 ± 9.00 mg/dL; $p < 0.05$) compared to G2 (75.25 ± 10.50 mg/dL).
			TG (blood)	Decreased (G3 - 73.25 ± 24.53 mg/dL; G4 - 52.50 ± 28,84 mg/dL; G5 - 91.75 ± 26.71 mg/dL; $p < 0.05$) compared to G2 (102.75 ± 22.94 mg/dL).
			HDL-Ch (blood)	Increased (G3 - 40.50 ± 9.95 mg/dL, G4 - 58.7 ± 2.87 mg/dL and G5 - 56.75 ± 21.65 mg/dL; $p < 0.05$) compared to G2 (37.75 ± 5.70 mg/dL).
			LDL-Ch (blood)	Decreased (G3 - 11.50 ± 1.91 mg/dL, G4 - 7.25 ± 1.26 mg/dL and G5 - 11.00 ± 0.82 mg/dL; $p < 0.05$) compared to G2 (14.25 ± 3.40 mg/dL).
			SOD (blood)	Increased (G3 - 39.50 ± 11.71 unit/100 µL, G4 - 44.55 ± 5.98 unit/100 µL and G5 - 31.78 ± 3.79 unit/100 µL; $p < 0.05$) compared to G2 (17.66 ± 4.79 unit/100 µL).
			MDA (blood)	Decreased (G3 - 0.44 ± 0.02 ng/100 µL, G4 - 0.46 ± 0.02 ng/100 µL and G5 - 0.39 ± 0.02 ng/100 µL; $p < 0.05$) compared to G2 (0.83 ± 0.02 ng/100 µL).
			Immunohistochemical staining (pancreas)	G3, G4 and G5 showed increasing Langerhans Island structures and insulin secretions. The size and shape of the structures from the G2 were irregular and smaller than those of G1, G3, G4 and G5. G2 showed a very low immunoreactive response to anti-insulin. G3 and G5 showed an improvement in the Langerhans Island structures, and in the size, shape, distributions, and numbers of the β-cells, as well as the high intensity of the brown color when compared to G2.
Zubaidah, Ifadah., *et al.* 2019	Study the anti-diabetes activity of Kombucha prepared from different snake fruit cultivars.	G1: Normal (n = 4) G2: Diabete control - DM (n = 4) G3: DM + KS Suwaru (5 mL/kg/d) (n = 4) G4: DM + KS Madura (5 mL/kg/d) (n = 4) G5: DM + KS Pondoh (5 mL/kg/d) (n = 4) G6: DM + KS Bali (5 mL/kg/d) (n = 4)	Glucose (blood)	Decreased (G3 - 110.3 ± 2.9 mg/dL, G4 - 114.8 ± 9.4 mg/dL, G5 - 189.3 ± 15.4 mg/dL and G6 - 140.0 ± 14.4 mg/dL; $p < 0.05$) compared to G2 (413.3 ± 8.3 mg/dL).
			TC (blood)	Decreased (G3 - 49.0 ± 2.6 mg/dL, G4 - 51.0 ± 3.5 mg/dL, G5 - 66.8 ± 5.7 mg/dL and G6 - 53.0 ± 4.2 mg/dL; $p < 0.05$) compared to G2 (75.3 ± 5.7 mg/dL).
			TG (blood)	Decreased (G3 - 52.5 ± 6.8 mg/d, G4 - 74.5 ± 2.7 mg/dL and G6 - 92.0 ± 8.1 mg/dL; $p < 0.05$) compared to G2 (102.8 ± 6.9mg/dL).
			HDL-Ch (blood)	Increased (G3 - 46.8 ± 1.3 mg/dL and G4 - 46.5 ± 2.1 mg/dL; $p < 0.05$) compared to G2 (37.8 ± 5.7 mg/dL).
			LDL-Ch (blood)	Decreased (G3 - 7.3 ± 1.0 mg/dL, G4 - 10.3 ± 1.3 mg/dL and G6 - 10.3 ± 1.3 mg/dL; $p < 0.05$) compared to G2 (14.3 ± 1.3 mg/dL).
			SOD (blood)	Increased (G3 - 44.6 ± 1.9 unit/100 µL, G4 - 41.7 ± 0.8 unit/100 µL and G5 - 30.8 ± 3.6 unit/100 µL; $p < 0.05$) compared to G2 (18.7 ± 1.4 unit/100 µL).
			MDA (blood)	Decreased (G3 - 0.37 ± 0.03 ng/100 µL, G4 - 0.48 ± 0.03 ng/100 µL, G5 - 0.42 ± 0.04 ng/100 µL and G6 - 0.47 ± 0.02 ng/100 µL; $p < 0.05$) compared to G2 (0.84 ± 0.02 ng/100 µL).
			Immunohistochemical (pancreas)	G2 the size and shape of the Langerhans islands were smaller compared to G1, and that also had a very low immune reactive response against anti-insulin. Number of pancreatic ß-cells that produced insulin in G2 was decreased ($p < 0.05$) when compared to G1. Improvements of the Langerhans Island structure and functions of the insulin secretion occurred in G3, G4, G5 and G6 compared to G2. The number of pancreatic ß-cells that produced insulin in G3 (72.2 ± 17.9) and G4 (73.5 ± 17) was significantly higher ($p < 0.05$) than those in G2 (42.1 ± 11.5).

Table 1: Describes the aim, treatment groups, biological analysis (site), and results of the included studies.

ALX: Alloxan Monohydrate; d: Days; g: Gram; g/L: Gram Per Liter; m: Month; mg/kg bw: Milligram Per Kilogram of Body Weight; STZ: Streptozotocin; w: Week; %: Per cent; ALP: Alkaline Phosphatase; ALT: Alanine Transaminase; AST: Aspartate Transaminase; BT KT: Kombucha Tea Fermented by Black Tea; CAT: Catalase; DNA: Deoxyribonucleic Acid; F-1,6-BA: Fructose-1,6-Bisphosphatase; G-6-Pase Activity: Glucose-6-Phosphatase Activity; g: Gram; g/dL: Gram Per Deciliter; g/L: Gram Per Liter; GGT: Gamma-Glutamyl Transpeptidase; GPx: Glutathione Peroxidase; GR: Glutathione Reductase; GSH: Glutathione; GST: Glutathione S-Transferases; GT: Green Tea; Hb: Haemoglobin; Hemoglobin; Hb1Ac: Glycated Hemoglobin; HDL-Ch: High Density Lipoprotein-Cholesterol; IU/L: International Unit Per Liter; IU/mL: International Unit Per Milliliter; KA Units: King-Armstrong units; KT: Kombucha Tea; LDL-Ch: Low-Density Lipoprotein-Cholesterol; LEx/kg bw: Lyophilized to Dryness Per Kilogram of Body Weight; MDA: Malondialdehyde; mg: Milligram; mg/g: Milligram Per Gram; mg/g Hg: Milligram Per Gram of Hemoglobin; mg/dL: Milligram Per Deciliter; mg/kg bw: Milligram Per Kilogram of Body Weight; mL/kg bw/d: Milligram Per Kilogram of Body Weight a Day; mg/kg/d: Milligram Per Kilogram a Day; mg/L: Milligram Per Liter; mL/kg/d: Milligram Per Kilogram a Day; ng/mL: Nanogram Per Milliliter; ng/100 µL: Nanogram Per 100 Microliter; nmol/min/mg Protein: Nanomole Per Minute Per Milligram of Protein; ROS: Reactive Oxygen Species; SOD: Superoxide Dismutase; TC: Total Cholesterol; TG: Triglycerides; U/L: Unit Per Liter; Unit/mg Protein: Unit Per Milligram of Protein; Unit/100 µL: Unit Per 100 Microliter; µmol/min/mg Protein: Micromole Per Minute Per Milligram of Protein; µmol of Pi Liberated/min/mg Protein: Micromole of Pi Libertated Per Minute Per Milligram of Protein; µmol of Glucose Phosphorylated/min/G Protein: Micromole of Glucose Phosphorylated Per Minute Per Gram of Protein.

In the pancreas, the enzymatic activity, DNA damage, histological and immunohistochemical parameters were evaluated. The enzymatic analysis revealed a reduction in lipase, α-amylase, and caspase-3 activity when rats after diabetes induction were treated with KB-BT (all analyzed in one study). One study showed inhibition of DNA fragmentation when animals were treated with KB-BT. Two studies carried out a histological analysis, the atrophy in pancreas cells demonstrated by the diabetic group was reverted when the animals were treated with KB-BT. Three studies that used KB-SF conducted immunohistological analysis and showed improvement in the structure and function of Langerhans Island, augmentation in the number of ß-cells, and low immunoreactive response against the anti-insulin in the diabetic rats treated with KB.

One of the included studies showed that KB-BT attenuated the DNA fragmentation in pancreatic tissue and attributed this effect to the scavenging free radicals properties of this beverage [42]. This study also evaluated the levels of active caspase-3 in pancreatic tissue, since this cysteine protease causes DNA fragmentation culminating in apoptosis. It revealed that KB-BT could decrease the active caspase-3 levels in pancreatic tissue homogenate of diabetes induced rats [42]. The protective effect could be due to the theaflavin present in BT, which could be enhanced by the fermentation with kombucha. This flavonoid has shown the capacity to prevent cellular DNA damage by inhibiting oxidative stress [43]. This data suggest that the hypoglycemic effect of KB-BT can come from the inhibition of pancreatic β-cells apoptosis through scavenging the reactive free radicals.

The outcomes of KB-BT exposure in diabetic induced rats was also evaluated in the liver. Two studies analyzed liver damage markers in blood samples, one study performed a histological analysis, and another study evaluated the enzymatic activity and the glycogen storage in the liver. All the liver damage markers (alkaline phosphatase - AST, alanine transaminase - ALT, aspartate transaminase - ALP, and gamma-glutamyl transpeptidase - GGT), decreased when the diabetic animals were exposed to KB-BT. The group treated with KB showed a normal arrangement around the central vein and reduction in hepatocytes apoptosis that was distorted and augmented, respectively, in the diabetes group. Furthermore, the diabetic rats exposed to KB-BT showed a reduction in the glucose-6-phosphatase (G-6-Pase) and fructose-1,6-bisphosphatase (F-1,6-BA) activities and an increase in hexokinase activity and glycogen storage.

The lipid metabolism was also verified in diabetic rats exposed to KB (4 studies - KB-BT and KB-SF). Increase in high-density lipoprotein cholesterol blood levels (4 studies - KB-BT and KB-SF), and a decrease in total cholesterol (3 studies - KB-BT and KB-SF), triglycerides (4 studies - KB-BT and KB-SF), and low-density lipoprotein cholesterol (4 studies - KB-BT and KB-SF) was observed in the diabetic groups treated with KB.

Oxidative stress (OS) was evaluated in four studies and the superoxide dismutase (SOD) (4 studies - KB-BT and KB-SF) and malondialdehyde (MDA) (4 studies - KB-BT and KB-SF) were the most prevalent markers analyzed. Results showed an increase in SOD activity and a decrease in MDA levels in the blood of diabetic animals exposed to KB-SF (3 studies). One study performed an extensive oxidative stress analysis in different organs, showing an increase in SOD activity and a decrease in MDA levels in the pancreas, and a decrease in SOD activity and MDA levels in the liver of diabetic animals exposed to KB-BT.

Figure 2 shows the quality of the included studies. The parameters that showed a low risk of bias were description of the animals used and gender, description of the substance and dose used for diabetes intraperitoneal induction, biological analysis related to KB effects, comparison of results between positive and negative controls, and comparison between results concerning KB effects. The parameters that showed a medium risk of bias were: description of the KB preparation and description of the KB application to the animal model. A high risk of bias was identified in the parameters: description of the animal's age and weight in the outset of diabetes.

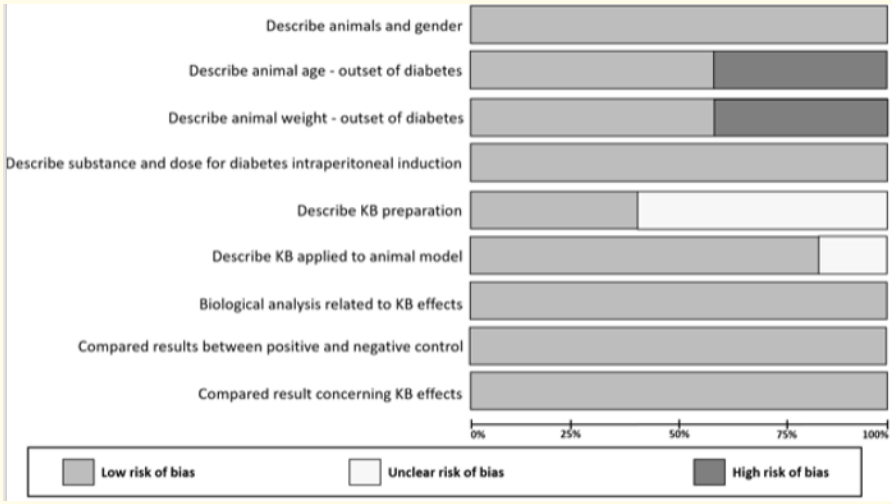

Figure 2: Quality assessment of the included studies.

Compilating the results from the included studies, the scientific literature at this moment is consensual in showing that the exposure to KB after diabetes induction in rats improves alterations caused by hyperglycemia. All studies observed that KB decreased blood levels of glucose and glycosylated Hb, and increased insulin in diabetic rats. One important mechanism to be considered in the reduction of glycemia is the decrease of α-amylase both in blood and pancreas, which could be associated with the polyphenols content in KB, whereas a minor concentration of this enzyme diminished the glucose absorbable from diet carbohydrates, such as starch [23,44]. Additionally, flavonoids found in high concentration in KB-BT, KB-GT, and KB-SF exert an important antidiabetic activity on induced diabetic and obese rats, with a considerable contribution of catechins, by enhancing the mRNA and protein expression of the glucose transporter GLUT-4, generating hypoglycemic effects through glucose oxidation and insulin-mimetic activities [45,46].

Five of the included studies evaluated the pancreas by immunohistochemistry, an important evaluation since diabetes induction causes damage to this organ. They observed that in diabetic animals exposed to KB-BT the atrophy of the pancreas cells was reverted and the exposure to KB-SF improved the structure and function of Langerhans island, augmented the number of ß-cells, and reduced the immunoreactive response against the anti-insulin. This enhancement may be provided by another important flavonoid present in KB-BT, KB-GT, and KB-SF: the quercetin [21,22,47], which demonstrated the potential to protect the induced diabetic rats from damage and death of the pancreatic β-cells, maintaining the cellular architecture, preserving the secretion of insulin, and stimulating the regeneration of these cells [48].

Additionally, the polyphenols from oolong tea, which included some catechins in similarity to KB, have shown the capacity to decrease pancreatic lipase activity *in vitro* [49]. This enzyme, secreted by the pancreas is known for its hydrolysis activity of dietary non-absorbable triglycerides into absorbable glycerol and free fatty acids. Diabetes induction increases its activity creating an elevation in plasma triglycerides and cholesterol levels [50]. Exposing diabetes induced rats to KB-BT and KB-SF decreases the triglycerides and cholesterol levels, which could be possibly linked to the decrease in lipase activity stimulated by the polyphenols of these KB [50].

Furthermore, there are two other compounds of all KB preparations that can be considered bioactive in reducing the negative effects derived from diabetes induction: acetic acid [51] and DSL [52]. The acetic acid is an organic acid produced from acetic acid bacteria metabolism, that showed improvement in fasting plasma glucose, HbA1c, and OGTT levels in exposed hyperglycemic mice [51]. This is in great part due to the activation of hepatic 5' adenosine monophosphate-activated protein kinase (AMPK), and probably it is the same pathway triggered in the improvement of these parameters in diabetic induced rats after KB exposure. Another influence of acetic acid in alterations caused by diabetes induction is the lipid profile, diminishing the blood levels of cholesterol and triglycerides, by inhibiting lipogenesis in the liver and excrement in fecal bile acid as seen in rats fed with a cholesterol-rich diet, which is possibly what occurs in the included studies that showed a decrease in these parameters [53].

The DSL action, differently from the acetic acid and more similar to polyphenols, focuses on the OS promoted by diabetic induction, peculiarly in the use of ALX. DSL has detoxifying and antioxidant properties that restore the levels of plasma insulin and prevent the apoptosis of pancreatic β-cell via a mitochondrial-dependent pathway [52]. Additionally, the polyphenols of BT, GT, and SF have shown the potential to increase the antioxidant capacity of DSL, in this sense one of the included studies showed higher concentrations of DSL in the KB-BT when compared with BT, which could be associated with the increasing level of polyphenols present in KB [42,54].

The main limitation of this systematic review reflects an important challenge in studies that investigate diabetes induction by ALX or STZ, although it is a methodology well established and widely used. It is difficult to clearly identify what are the alterations due to consequences of hyperglycemia, or the negative impact of chemical exposure used to induce diabetes. However, this may impact on understanding the mechanisms by which KB exerts its effect on diabetes but does not diminish the potential of KB to modulate important parameters in diabetes when considering animal models. Moreover, differences between the tea or juice used to prepare KB makes it difficult to directly compare the results, since the KB chemical composition may vary, influencing significantly the final results.

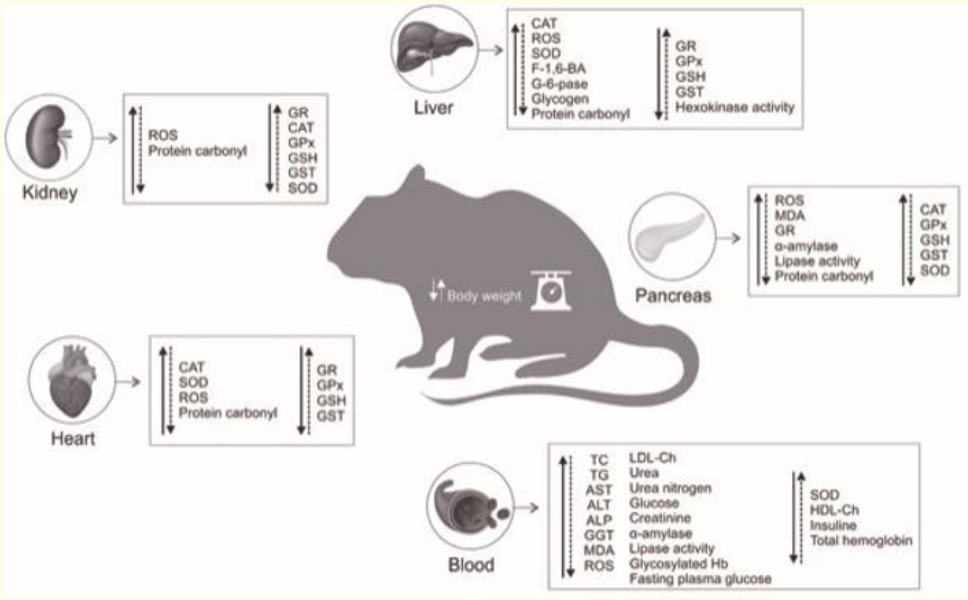

Figure 3: Panel of KB effects in parameters altered by rats with induced diabetes. The arrows represent the increases (↑) and decrease (↓) in the parameters evaluated. The filled arrows (↑) and dashed arrows (↗) represent, respectively the diabetes induction and the KB effect in induced diabetes rats. ALP: alkaline phosphatase, ALT: alanine transaminase, AST: aspartate transaminase, CAT: catalase, F-1,6-BA: Fructose-1,6-biphosphatase activity, G-6-pase: glucose-6-phosphatase, GR: glutathione reductase, GGT: gamma-glutamyl transpeptidase, GPx: glutathione peroxidase, GR: glutathione reductase, GSH: glutathione, GST: glutathione S-transferases,Hb: hemoglobin, HDL-Ch: high density lipoprotein-cholesterol, LDL-Ch: low-density lipoprotein-cholesterol, MDA: malondialdehyde, ROS: reactive oxygen species, SOD: superoxide dismutase, TC: total cholesterol, TG: triglycerides.

Conclusion

In conclusion, it is possible to identify that KB can modulate important parameters in diabetes, reversing negative outcomes caused by diabetic induction, and exposure to hyperglycemia in preclinical rodent models. However other studies are necessary to understand more profoundly the mechanism by which this modulation occurs.

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Conflict of Interest

None.

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