Research Article

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Study the Effects of Frankincense Aqueous Extract With/Without Synbiotic on Diabetic Male Rats

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Abstract

This research focuses on the impact of a frankincense aqueous extract on diabetic rats' ability to maintain healthy blood sugar levels. Recently, studies indicate that herbal extracts have medicinal properties. In this study, we induced diabetes in male rats with alloxan. We categorized the rats into four groups. We administered frankincense aqueous extract orally to one group, and frankincense with a synbiotic containing Lactobacillus acidophilus (La-5) and FOS prebiotics to the other. The remaining two groups served as the negative and positive control groups for 45 days. According to the results, treated groups showed significantly lower blood glucose levels and improved gut microbiota after 45 days compared to control groups. It seems that frankincense's synbiotic aqueous extract might be useful as a dietary supplement for controlling blood sugar and supporting a healthy gut microbiota.

Keywords: Rats, synbiotics, frankincense, diabetes, gut microbiota.

Introduction

Since ancient times, chemicals originating from plants have been and still are a major supply of molecules for medications. Humans have long utilized herbal remedies; in fact, this may have been the origin of modern medicine. (1). Numerous disorders, including AIDS, cancer, mental health issues, diabetes, jaundice, hypertension, and skin conditions, have allegedly responded well to it. The World Health Organization (WHO) reports that 80% of individuals in developing countries almost solely rely on herbal medicine for their basic medical needs (2). The family Burseraceae Boswellia, the genus Sapindales, has around twenty-four species of tiny trees that are characterized by their peeling bark, intricate leaves, and a thick covering of dark crimson

resin. Boswellia sacra is found in the Arabian Peninsula; Boswellia neglecta is found in Eritrea; Boswellia serrata is found in India; and Boswellia papyrifera and rivae are found in Ethiopia. Ethiopia, Somalia, and North Africa could be home to other Boswellia species. The Eastern Ghats in southern India are home to a unique species of boswellia called Boswellia ovatifolia. (3,4,5). People have used their gum resins for therapeutic and spiritual purposes since ancient times. Today, the nutraceutical and nutritional supplement industries still frequently sell resin extracts. (6). Diabetes management is a common problem that has no known treatment2. Currently, natural products make up around half of all pharmaceuticals. (7). Diabetes mellitus is a common chronic condition that has several contributing variables, such as inadequate physical activity and unhealthy food habits. The disorder develops and progresses due to insufficient pancreatic insulin release or inefficient insulin utilization. Uncontrolled diabetes causes hyperglycemia, commonly referred to as elevated blood glucose levels. This disease gradually causes significant harm to the human body, especially to the cardiovascular and neurological systems (8). The autoimmune condition known as diabetes type 1 stops the pancreas from making insulin. A high-fat, high-carb diet and inactivity are the main causes of type 2 diabetes, which affects over 90% of cases. (9). An estimated 1.4 million individuals in Iraq have received a diabetes diagnosis. There is a range of 8.5% to 13.9% for the prevalence of type 2 diabetes (T2D) in Iraq (10). Although the pharmaceutical industry offers many antidiabetic drugs to treat diabetes and its related conditions, there is still no cure for this metabolic disease (7). Obesity and type 2 diabetes are becoming more common, and they are closely associated with cardiovascular disease, which has become a serious public health issue worldwide. (11). Because they contribute to the development of insulin resistance, proinflammatory cytokines are important in both situations. Many pharmacologically active substances, most notably 11-keto-ß-boswellic acids, which are present in the gum resin of the Boswellia species, have been shown to prevent immune system cells from producing inflammatory cytokines (12). Regretfully, modern medicine still hasn't found a cure for diabetes. However, using natural therapies for medical conditions does not put the consumer at risk (13). Medicinal plant extracts have been shown to have anti-diabetic effects and to restore the functioning of pancreatic beta cells, according to several clinical investigations (14). Researchers gave diabetic rats 1, 2, and 10 mg/kg of β-BA and β-KBA from frankincense every day for 21 days. The rats' water intake, blood glucose level (BGL), and weight loss all changed significantly. These results demonstrate the strong anti-diabetic effects of both compounds. These substances restored blood levels of total cholesterol, triacylglycerol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol to normal, according to biochemical indications (15). For 12 weeks, patients using metformin were given 400 mg capsules of Boswellia serrata gum resin and a placebo twice a day. When compared to a placebo, boswellia resin

significantly reduced HbA1c, FBS, insulin, cholesterol, TG levels, and LDL, without influencing other lipid levels or liver/kidney function tests. In addition, this herb has antioxidant properties and no side effects. Boswellia gum resin may be an effective anti-hyperglycemic, anti-hyperlipidemic, and safe antioxidant for people with type 2 diabetes (16). Researchers have devised a rapid, sensitive, accurate, and inexpensive method for determining how efficiently KBA, derived from Boswellia sacra resin, dissolves in the liver of streptozotocin-induced diabetic mice. KBA treatment enhanced the morphological characteristics of the liver tissue in diabetic mice, ultimately resulting in the complete repair of the damage seen in the diabetic control group (17). Additionally, Boswellia sacra extract significantly reduced blood indicators of liver damage. Traditional healers in Arab-African nations and the Middle East claim that Boswellia sacra's aqueous extract, oleo-gum-resin, has a hepatoprotective effect at doses of both 2 ml/kg and 5 ml/kg (18).

This study aims to assess the impact of frankincense aqueous extract and a synbiotic combined with frankincense on the blood glucose levels, as well as the gut microorganisms in male rats with induced diabetes.

Materials and Methods

preparation the aqueous extract of frankincense (Boswellia sacra)

The gum of Frankincense (Boswellia sacra) was bought from the Basrah, Iraq, local market. The crushed granules or lumps of gum exudate were ground up. We dissolved 50 grams of powder in 500 ml of distilled water and let the mixture stand at room temperature for a full day. Following the transfer to 15-mL Falcon tubes, we subjected the supernatant to centrifugation at 1000 rpm for 10 minutes. The filtered supernatants were thereafter stored at a temperature of 4 °C after filtration via Buchner using No. 1 of Whatman filter paper. Subsequently, the extracts were produced utilizing the lyophilization technique in a freeze-drying device. (19).

The functional beverage preparation

To make the aqueous extract, gum and distilled water were mixed at 1:100 (w/v). After soaking for a full day, the solution was filtered through a clean cloth to remove any remaining gum. After filtering, the liquid was put into sterile glass bottles and pasteurized to 85 °C for 15 minutes. (20) prior to refrigeration. Functional beverages were made with the aqueous extract. Following the division of the aqueous extract among aseptic glass containers, a prebiotic known as fructo-oligosaccharide (FOS) sugar was added at a rate of 0–1% (w/v) (21). The bottles were pasteurized at a water bath temperature of 85 °C for 15 minutes. (20). Lactobacillus acidophilus (La-5) probiotic bacteria were added to the produced aqueous extract at a ratio of 1% (v/v). (22). The inoculum's concentration was 108 CFU/ml. (23). Subsequently, the functional beverage cases were kept in an incubator for a whole day at 37 °C in order to start the fermentation process. They were then put in the fridge.

Experimental Design and Animals

For the research, Rattus norvegicus adult albino male rats weighed 170-230 grams and were 4 months old. These rats were provided by the Anbar Governorate Animal House. The animal research took 60 days at the College of Veterinary Medicine/University of Basrah Animal Home, including the adaptation period. Animals were distributed randomly. The trial divided 24 rats into 4 groups of 6. Clean plastic cages with metal snap lids and drinking water bottles were used. Sawdust was replaced twice a week in cages. The rats were kept in a well-ventilated room at 24-28 C and 50–55% humidity. Lighting was 12 hours of natural light and 12 hours of darkness. Their new environment required two weeks of adjustment. they were provided clean water and food ad libitum (24).

Diabetes Mellitus Type 1 Inducement

Rats that had been fasting for 12 hours received a single intraperitoneal dose of 150 mg/kg body weight of alloxan dissolved in 0.9% saline solution to induce diabetes. After the injection, the rats were given a 5% solution of water and glucose. The diabetic state was determined by measuring the fasting levels of blood glucose 72 hours after the alloxan treatment. The Japanese Contour device was used to measure blood sugar levels. Rats with a blood glucose level above 250 mg/dl have been chosen for the experiment and classified as diabetic. (24)

T1(C-) and T2(C+): were given distilled water for 45 days.

T3(F): were given frankincense extract for 45 days.

T4 (Fs): were given frankincense extract combined with symbiotic for 45 days.

Collection of samples

Feces collection: Feces samples were collected and brought to the lab for microbial count assays in well-sealed, aseptic plastic containers.

Blood sample collection: A blood sample was taken from the tail vein by pricking it with a sterile needle in diabetes experience.

Statistical analysis: We conducted a one-factor experiment using a completely randomized design (CRD) for the statistical analysis. We examined the outcomes using statistical software (GenStat, 2009). Using the least significant difference (L.S.D.) between the means, we evaluated the parameters under investigation. We set the probability threshold at 0.05.

Results and Discussion

The effect of aqueous extract beverages on the microbial content of feces in male rats with diabetes

Fig (1,2,3) illustrates how functional beverages affect the logarithmic enumeration of lactic acid and coli bacteria in the feces of male rats induced to develop diabetes. I have been given an aqueous frankincense extract with or without synbiotics for 0 and 45 days. The findings revealed that there were no significant differences between the treatments at the 5% level in the numbers of E. coli, coliforms,

and lactobacillus after 45 days. The impact of the functional beverages became evident after 45 days of the experiment, as they effectively decreased the levels of logarithmic coli bacteria while simultaneously boosting the levels of logarithmic lactic acid bacteria. Treatment 3 had the most significant number of log E. coli bacteria, with a value of 4.37.

Treatment 4 administered Frankincense with synbiotics, resulting in values of 4.29 cfu/g, while the first and second control treatments showed values of 4.21 and 4.55 cfu/g, respectively. The logarithmic values of Coliform bacteria varied throughout the treatments, with the greatest value being 5.27 for treatment 3 and the lowest value being 4.87 for treatment 4. In comparison, the first and second control treatments had values of 4.63 and 5.53 cfu/g, respectively. In contrast, the logarithmic numbers of lactic acid bacteria rose after 45 days. Treatment 4 had the highest count, reaching 7.63 cfu/g, followed by treatment 3 with counts of 6.90 cfu/g, respectively. In comparison, the first and second control treatments had values of 6.74 and 6.14 cfu/g, respectively. The fourth treatment, which included administering an extract of frankincense to rats with synbiotics, demonstrated superiority in increasing the population of lactic acid bacteria and decreasing the number of coliform bacteria in rat feces. This finding aligns with the discovery made in research (25) regarding the impact of adding Boswellia serrata resin (BSR) to broiler chickens' feed on their growth performance. Broiler chickens fed the BSR diets had a lower count of Escherichia coli and a higher count of Lactobacillus and Enterococcus than the control diets. Additionally, there was a decrease in the number of Clostridium species present. This could enhance the intestinal microbiota and improve the morphology of the gastrointestinal tract. Moreover, it agreed with earlier studies by (26, 27), which confirmed that frankincense has antibacterial properties and acts as an inhibitor of coliform bacteria, including *Escherichia coli.* B. serrata of frankincense resin has been included in rabbit meals at various doses to study the cecal microbiota. B. serrata-treated rabbits had significantly lower total bacterial counts, as well as lower Salmonella enteritidis and E. coli counts than untreated rabbits. These findings may be attributed to the high polyphenol concentration in B. serrata and the anti-microbial properties of boswellic acid. In addition to the effects of probiotics and synbiotics, researchers have conducted numerous studies on probiotics and prebiotics to modulate gut flora. Probiotics can modulate the gut microbiota, improve digestion, synthesize vitamins, and neutralize pathogens in many mechanisms, such as suppressing potential enteropathogens or producing useful metabolites or enzymes (28).

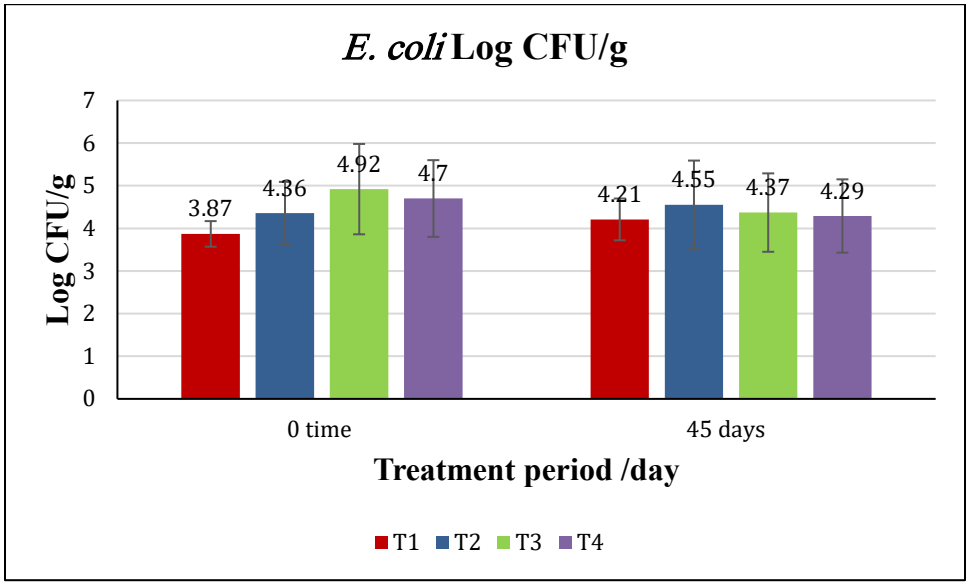

Figure (1): The effect of aqueous extract beverages on E. coli in feces in rats with diabetes (average ± standard deviation). L.S.D 0-time = 0.995, L.S.D 45- days =1.032

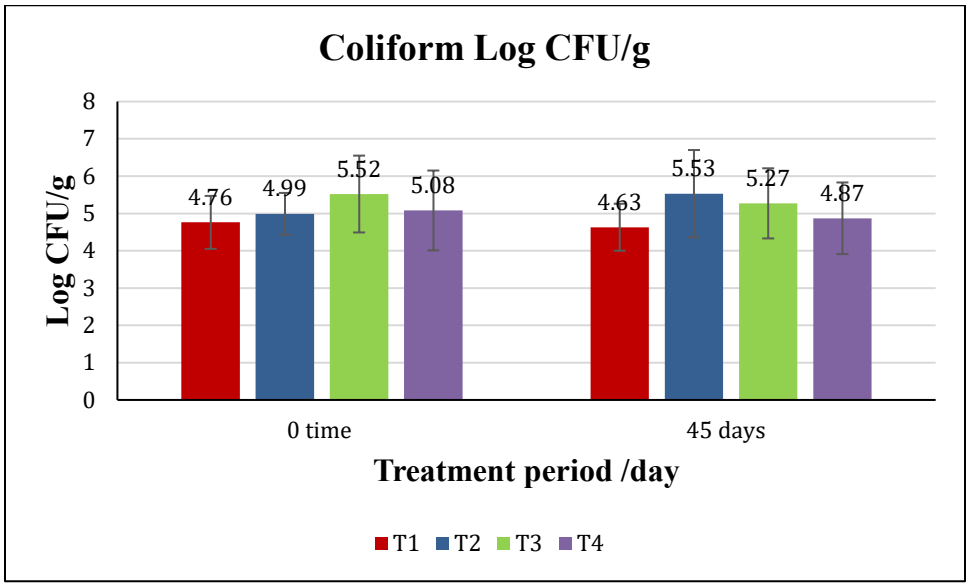

Figure (2): The effect of aqueous extract beverages on coliform bacteria in feces in rats with diabetes (average ± standard deviation). L.S.D 0-time =1.049, L.S.D 45- days = 1.142

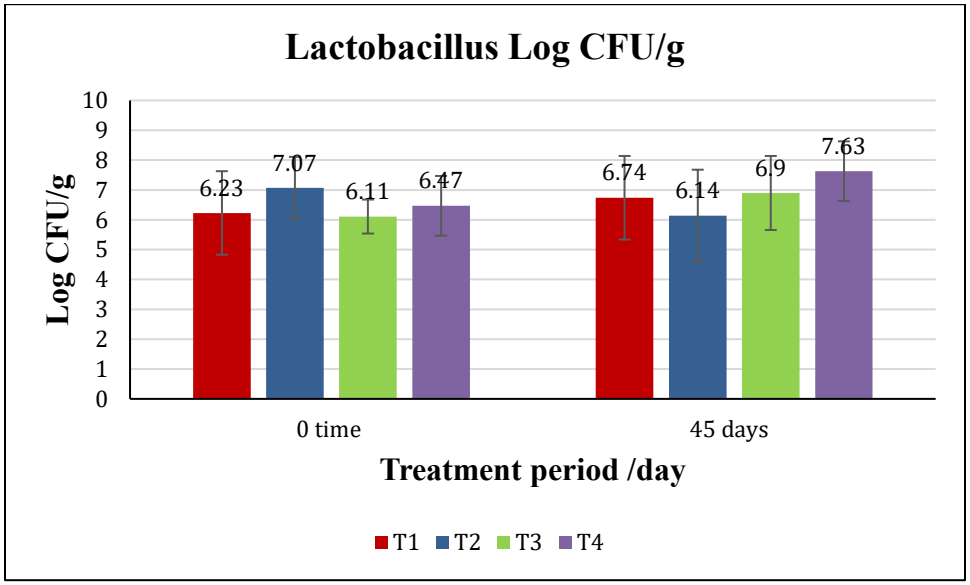

Figure (3): The effect of aqueous extract beverages on lactobacillus in feces in rats with diabetes (average ± standard deviation. L.S.D 0-time = 1.236, L.S.D 45- days = 1.697). T1(C-) and T2(C+): were given distilled water for 45 days. T3(F): were given frankincense extract for 45 days. T4 (Fs): were given frankincense extract combined with symbiotic for 45 days.

Several reasons may contribute to this supremacy, such as Frankincense's anti-inflammatory, antibacterial, and antifungal properties, which may directly inhibit harmful bacteria or promote beneficial microbes like lactic acid bacteria in the gut (29, 30). Diet, medications, supplements, and herbs like frankincense can all have an impact on gut microbiota modulation. According to research (31), frankincense may modulate immune responses in the gut, potentially fostering an environment that supports the growth of beneficial bacteria such as those that produce lactic acid.

The effect of frankincense aqueous extract beverages on the blood glucose levels of male rats with diabetes.

Table (1) displays the effect of frankincense beverages with or without synbiotics on the blood serum glucose levels of male rats induced to develop diabetes (average ± standard deviation). We conducted the study over a period of 0 to 45 days. After 45 days, the findings showed no statistically significant differences between the third and fourth treatments at a significant level of 5%. It may appear from the table that the level of glucose in the blood decreased in both treatments after 45 days. The third treatment, which only consumed the frankincense extract, had the highest value at 151.7 mg/dl, followed by the fourth treatment, which consumed the frankincense combined with symbiotics and had 142.2 mg/dl. In comparison, the first and second control groups had glucose values of 87.3 and 263.2 mg/dL, respectively. Reports indicate that beta cells in animal models of diabetes may be protected from damage by the antioxidant vitamins C and E (33).

Functional frankincense beverages may have lowered glucose levels in the fourth and third treatments because of frankincense's antioxidant properties. One study showed that diabetes rats received 200, 400, and 600 mg/kg of Boswellia serrata extract. After 17 days, diabetic rats receiving Boswellia serrata had significantly lower blood glucose and HbA1c levels (33). Rats that had been given alloxan to cause diabetes were given 400 mg/kg of aqueous B. serrata extract every day for three weeks. Diabetes levels, liver enzymes, kidney indicators, and lipid profile all went down a lot in the diabetic group that was given B. serrata extracts compared to the diabetic control group ($P<0.05$). (34). People with diabetes who were given 900 mg of Boswellia serrata every day saw a big change in their HDL levels and a big drop in their fructosamine, cholesterol, and LDL levels after six weeks ($p<0.05$). Research has shown that some species of Boswellia may help manage metabolic syndrome and the issues that come with it, including high blood sugar, bad cholesterol, high blood pressure, obesity, and diabetes. By repairing pancreatic beta cells and decreasing insulin resistance, Boswellia species lower blood sugar levels. The antithrombotic and anticoagulant characteristics of Boswellia species regulate blood pressure. A variety of Boswellia species have anti-oxidant characteristics that influence blood lipid profiles (36). According to (37) studies, Boswellia serrata significantly reduced blood sugar in diabetic mice. In groups that were treated with B. serrata, histological studies showed that alloxan's harmful effects on pancreatic islet cells got a lot better. Alloxan hurts pancreatic islet cells a lot, but Boswellia serrata makes them much less hurt, and its healing effect is stronger than its protective effect. Stronger histological alterations and better blood glucose levels corroborate this. The bioactive chemicals β-BA and 11-keto-β-boswellic acid (β-KBA) are mostly found in frankincense. Research shows that β-BA and β-KBA at doses of 1, 2, and 10 mg/kg body weight for 21 days significantly improve body weight loss, water consumption, and blood glucose levels in diabetic animals, indicating strong anti-diabetic properties. The antidiabetic, antioxidant, and anti-hyperlipidemic properties of -BA and -KBA suggest potential therapeutic uses for diabetes (38). B. serrata has several benefits for people with diabetes, including lower glucose absorption in the intestines, higher glucose uptake in the peripheral tissues, regeneration of Langerhans beta cells, and lower oxidative stress (39). In addition to the benefits of incorporating sybiotics, combining probiotics and prebiotics improves glycemic management more than probiotics alone (40). Studies investigated the effect of synbiotic intake on the metabolic profiles of people with diabetes. They found that administering synbiotics can improve fasting plasma glucose (FPG), insulin levels, triglyceride levels, and total cholesterol levels (41). Prebiotics and prebiotic compounds moderately improve metabolic and inflammatory indicators associated with type 2 diabetes mellitus in women who are at least 18 years old, according to study data (42). The most significant evidence of improvement comes from interventions like resistant starch,

resistant dextrin, and oligofructose-enriched inulin. Their study (43) found that probiotics can potentially reduce lipid profile indices, blood pressure, and fasting blood glucose levels in patients with type 2 diabetes mellitus (T2DM). Probiotics might be a novel approach for managing blood pressure and lipid profiles in individuals with T2DM.

Table 1: Blood Glucose level (average ± standard deviation) mg/dl

Blood Glucose level (average ± standard deviation) mg/dl				
Group	**0 day**	**15 days**	**30 days**	**45 days**
T1(C-)	81.5 ± 8.11	83.8 ± 9.51	79.7 ± 10.68	87.3 ± 8.98
T2(C+)	364.2 ± 14.77	332.3 ± 22.94	304.2 ± 19.79	263.2 ± 13.55
T3 F	373.7 ± 22.58	278.8 ± 21.49	185.3 ± 16.76	151.7 ± 14.23
T4 Fs	345.3 ± 12.50	217.5 ± 15.37	179.7 ± 9.47	142.2 ± 15.44

L.S.D: 0-time =18.56, 15days = 21.84, 30days= 17.83, 45 days = 16.00

Conclusion

In summary, consuming an aqueous extract of frankincense beverage along with synbiotics can lower blood glucose levels. Additionally, it enhances the gut microbiota, which helps control diabetes and its complications.

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Conflicts of interest

The authors declare that there is no conflict of interest .

Ethical Clearance

This work is approved by The Research Ethical Committee .

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دراسة تأثير المستخلص المائي للبان الذكر مع/بدون التأزر الحيوي على ذكور الجرذان المصابة بالسكري

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الخلاصة

إن تأثير المستخلص المائي للبان الذكر على قدرة الجرذان المصابة بداء السكري على الحفاظ على مستويات السكر في الدم الصحية هو محور هذا البحث. تشير الدراسات الحديثة إلى أن مستخلصات الأعشاب لها خصائص طبية. في هذه الدراسة، قمنا بتحريض مرض السكري لدى ذكور الجرذان باستخدام الألوكسان. قمنا بتصنيف الفئران إلى أربع مجموعات. قمنا بإعطاء مستخلص مائي من اللبان الذكر عن طريق الفم لمجموعة واحدة، واللبان مع مادة تكافلية تحتوي على الملبنة الحمضية (La-5) والبريبايوتكس FOS للمجموعة الأخرى. وكانت المجموعتان المتبقيتان بمثابة مجموعات المراقبة السلبية والإيجابية لمدة 45 يوما. وفقا للنتائج، أظهرت المجموعات المعالجة انخفاضا ملحوظا في مستوى السكر في الدم وتحسنا في الكائنات الحية الدقيقة في الأمعاء بعد 45 يوما مقارنة بمجموعات السيطرة. تشير هذه النتائج إلى إمكانية استخدام المستخلص المائي التكافلي للبان الذكر كتدخل غذائي مفيد لتنظيم نسبة السكر في الدم وتعزيز ميكروبات الأمعاء الصحية.

الكلمات المفتاحية: الفئران، التأزر الحيوي، اللبان، مرض السكري، ميكروبات الأمعاء.