

Open Access Article

## Evaluation of the Antidiabetic Activity of a Traditional Herbal Mixture in Alloxan Induced Diabetic Rabbits

Ahmad Hamood Abood, Ibtisam A. Al-Ali\*, Khalid Ali Hussein

Biology Department, College of Science, University of Kerbala, Iraq

**Abstract:** Diabetes is a leading health problem worldwide, so researchers continue to seek out plant-based therapies that potentially are more acceptable than pharmaceuticals in society. The current case study was conducted to gauge the effectiveness of four multiherbal compositions, including three or two of the following ingredients: cardamom pods (*Elettaria cardamomum*), lime fruit (*Citrus aurantifolia*), and frankincense resin (*Boswellia sacra*). The mixtures were tested on rabbits induced experimentally with diabetes using alloxan. Thirty New Zealand rabbits were collected then broken into ten groups of three. One group was used for the negative control, and the other nine were induced to diabetes using alloxan (100 mg/kg) following weekly injections. Diabetes was confirmed by hyperglycemia, which was measured before and after the injections. The four multiherbal compositions were as follows: Citrus *Elettaria* *Boswellia* Extract (CEBE), all three species; Citrus *Boswellia* Extract (CBE), just lime and frankincense; Citrus *Elettaria* Extract (CEE), lime and cardamom; and *Elettaria* *Boswellia* Extract (EBE), cardamom, and frankincense. Each composition was administered once daily, at two concentrations (500 mg/kg and 250 mg/kg). As expected, diabetic rabbits lost significant body weight in proportion to their blood glucose levels. The lipid profile (total cholesterol, triglycerides, high-density lipoprotein, low-density lipoprotein, very low-density lipoprotein) also significantly increased across all parameters with diabetes. The utilization of plant extracts dramatically improved blood biomarkers of cholesterol and triglycerides, weight, and blood glucose levels. Interestingly, the lower concentration of plant extract was more effective at normalizing blood parameters, which may be related to processes in the liver, i.e., xenobiotic metabolism.

**Keywords:** citrus *Elettaria* *Boswellia* extract, citrus *Boswellia* extract, citrus *Elettaria* extract, *Elettaria* *Boswellia* extract.

### 評價一種傳統草藥混合物在四氧嘧啶誘導的糖尿病兔中的抗糖尿病活性

**摘要：**糖尿病是全球主要的健康問題，因此研究人員繼續尋找可能比社會上的藥物更容易接受的基於植物的療法。當前的案例研究旨在評估四種多草藥組合物的有效性，包括以下三種或兩種成分：荳蔻莢、酸橙果、和乳香樹脂。使用四氧嘧啶在實驗性誘發糖尿病的兔子身上測試了這些混合物。收集了三十隻新西蘭兔，然後分成十組，每組三隻。一組用於陰性對照，另外九組在每週注射後使用四氧嘧啶（100 毫克/公斤）誘導糖尿病。糖尿病通過在注射前後測量的高血糖得到證實。四種多草藥組合物如下：柑橘蓮藕乳香提取物，所有三種；柑橘乳香提取物，只是酸橙和乳香；柑橘蓮藕提煉、酸橙和荳蔻；和蓮藕乳香提煉、荳蔻和乳香。每種組合物以兩種濃度（500 毫克/公斤和 250 毫克/公斤）靜脈內給藥，每天一次。正如預期的那樣，糖尿病兔子的體重與其血糖水平成正比。血脂（總膽固醇、甘油三酯、高密度脂蛋白、低密度脂蛋白、極低密度脂蛋白）在糖尿病的所有參數中也顯著增加。植物提取物的利用顯著改善了膽固醇和甘油三酯、體重和血糖水平的血液生物標誌物。有趣的是，較低濃度的植物提取物在使血液參數正常化方面更有效，這可能與肝臟中的過程有關，即外源性代謝。

Received: October 13, 2021 / Reviewed: November 18, 2021 / Accepted: December 11, 2021 / Published: January 28, 2022

About the authors: Ahmad Hamood Abood, Ibtisam A. Al-Ali, Khalid Ali Hussein, Biology Department, College of Science, University of Kerbala, Iraq

Corresponding author Ibtisam A. Al-Ali, [ialali@uokerbala.edu.iq](mailto:ialali@uokerbala.edu.iq)

**关键词：**柑橘莲藕乳香提取物，柑橘乳香提取物，柑橘莲藕提取物，莲藕乳香提取物。

## 1. Introduction

From the medical side, diabetes represents a series of disordered metabolic and autoimmune conditions related to what is referred to as hyperglycemia, i.e., hyperglycemia resulting from partial or total insulin deficiency. This rise is often estimated by calculating blood glucose [1].

Diabetes mellitus, as defined by the International Diabetes Federation, may be a long-term (chronic) condition that happens when glucose levels during a person's blood are above normal because the body cannot produce enough or not produce any amount of the hormone insulin or the insulin produced could also be used ineffectively. High blood glucose is classified as a chronic imbalance in body functions and should also cause failure in various organs like kidneys, eyes, heart, nerves, and blood vessels [2].

Scientists exploited some characteristics of a gaggle of chemicals within the development of diabetes to conduct scientific experiments to discover the acceptable treatment for the disease, including alloxan and streptozotocin [3]. Alloxan is understood together with the toxic glucose analogs; it targets pancreatic beta cells liable for making insulin and works to destroy them specifically [4]. The mechanism of action of alloxan on pancreatic beta cells involves oxidation of the SH-sulfhydryl group [5]. Some studies have shown that this compound is characterized by two functions that are evidence for the mechanism of action. The primary is to selectively block the action of glucokinase, which is the main catalyst for insulin secretion from beta cells, and therefore the second function is to get free radicals through oxidation and reduction, which successively results in the necrosis of pancreatic cells [6].

A complete cure for diabetes has not yet been discovered. The treatments used are for the aim of regulating and maintaining blood sugar, like insulin injections, which relieve symptoms and prevent the consequences of high sugar on the body, like renal failure, protecting the guts, and preventing amputation of limbs. Some also use medications to scale back the rise in glucose in diabetic patients [7].

Nature has always been the most source of what humans need in terms of food, clothing, transportation, and medicines. Plants are the idea that medical systems have been inherited for thousands of years and still provide humans with appropriate treatments for several diseases. It had been experienced and a real understanding that gave humans complete knowledge of handling plants to be used in treatment [8]. It is

known that the effectiveness of medicinal plants and their pharmacological action is thanks to their diversity with the chemical compounds they contain. Among these compounds are alkaloids, glycosides, lactones, tannins, proanthocyanidins, dyes, ecdysones, saponins [9].

*Elettaria cardamomum* is one among the species of the Zingiberaceae family because it is named (the king of spices) [10]. The historical medical use of the plant as a remedy for abdominal pain, sciatica, cough, strong tonic, and diuretic, but currently, it is used for asthma, bronchitis, influenza, infection, diarrhea, cataracts, heart disorder, and nausea [11]. Cardamom contains many components like carbohydrates, protein, and fats in several proportions. It also contains a gaggle of essential minerals like magnesium, calcium, phosphorous, potassium, iron, and sulfur. Additionally, zinc, manganese, and copper are often present in proportions that will be large [12]; it also contains active ingredients in its composition like tannins, starch, terpenoids, phenols, flavonoids, sterols, and proteins [10]. Cardamom is additionally a source of alkaloids and anthocyanins [13].

*Citrus aurantiifolia*, referred to as lime, belongs to the family: Rutaceae is cultivated in tropical or subtropical regions with multiple nutritional and therapeutic uses. From the medical side, studies indicate its use to alleviate pharyngitis, fever, colds, cough, and indigestion [14]. Other medicinal uses of lime-like are laxative, flatulence, bronchitis, sinusitis, and asthma, and it is also useful for getting obviate obesity and as a treatment for rheumatic infections of the joints. It is also used as an antidepressant [15].

The main known components of this sort are flavonoids and vitamin C, also because of alkaloids, phytosterols, coumarins, and phenolic acids, which are biologically active [16].

*Boswellia sacra* belongs to the Burseraceae, specifically the *Boswellia*, which incorporates many species cosmopolitan around the world. It is often noted that there are 23 species of this genus that grow on the coasts of India and Africa and within the Arabian Peninsula region [17].

Frankincense has protective effects on the liver and also acts as a pain reliever, and has antioxidant activities. It is also used as an anti-coagulant, diuretic, tumor suppressant, stomach, skin, and liver disorders, and anti-inflammatory. It also improves the consequences of Alzheimer's disease [18]. On the opposite hand, it is used as a mouth freshener, an antimicrobial agent, and resin oils to treat dysmenorrhea, ulcers, gums, wounds, and an anti-

inflammatory agent [19]. On the opposite hand, it is used to treat infections of the bronchi, mouth, and tract [20].

*Boswellia* features a complex group of chemical compounds of about 200 different compounds. The chemical composition can show compounds like saponins, Penta- and tetracyclic terpenoids, alkaloids, tannins, polyphenols, and essential oils. The special activity of frankincense in medicinal use is thanks to the presence of pentacyclic Boswellic acids additionally to other compounds that also are medicinally active [21].

## 2. Materials and Methods

### 2.1. Experiment Animals

The study was started in November of 2020 and until the start of March of the year 2021, during which male New Zealand rabbits were obtained from some animal breeders in Baghdad, Qadisiyah, and Karbala governorates were used. Thirty rabbits were obtained, and their initial weights ranged from 1250 g to 1988 g. They were placed in separate cages inside a single-purpose room with controlled environmental conditions like temperature and ventilation.

### 2.2. Induction of Diabetes Mellitus

The alloxan, obtained by CDH Central Drug House (P) Ltd. (New Delhi, India), was dissolved in sterile saline to make a 50% solution (W/V). Alloxan was administered to fasting rabbits (12 hours fasting) after thawing at a dose of 100 mg/kg of body mass via a 24-cannula placed within the marginal ear vein. Animals were then subcutaneously injected with 10 ml of glucose solution [22]. The animals were also provided with food immediately after injection and had access to a glucose solution (20%) during the first twenty-four hours to scale back the shock of hypoglycemia due to treatment with alloxan. After the initial alloxan injection, the animal received a second dose of alloxan (100 mg/kg intravenously) to maintain a raised blood sugar level. Diabetes was measured (Accu-Chek, Roche Diabetes Care, Inc., Indianapolis, IN, USA), and animals that showed a big increase in blood sugar levels (BGL) above 250 mg/dL were considered diabetic [22].

### 2.3. Experimental Design

The animals were randomly distributed into ten groups, and every group was placed in a cage with dimensions of 60 cm x 50 cm x 50 cm for length, width, and height, respectively. The groups were divided into two control groups, the negative control group (three animals) and the positive control group (three animals). Then the remaining animals were allocated to the treatment groups, which were divided into eight groups of three. Each group was dosed daily with differing

types and concentrations of plant extracts prepared before the beginning of the study and administered intravenously. The compositions were as follows; CEBE, all three species (six animals, two doses; 250 mg/kg – three animals, & 500 mg/kg – three animals); CBE, just lime and frankincense (six animals, two doses); CEE, lime, and cardamom (six animals, two doses); and EBE, cardamom and frankincense (six animals, two doses).

1. Group AA received CEBE plant extract at a dose of 500 mg/kg for 14 days.
2. Group AB received CEBE plant extract at a dose of 250 mg/kg for 14 days.
3. Group BA received CBE plant extract at a dose of 500 mg/kg for 14 days.
4. Group BB received CBE plant extract at a dose of 250 mg/kg for 14 days.
5. Group CA received EBE plant extract at a dose of 500 mg/kg for 14 days.
6. Group CB received EBE plant extract at a dose of 250 mg/kg for 14 days.
7. Group DA received CEE plant extract at a dose of 500 mg/kg for 14 days.
8. Group DB received CEE plant extract at a dose of 250 mg/kg for 14 days.

### 2.4. Preparation of Plant Aqueous Extracts

The plant parts extracted were the seed pod and seeds of cardamom (*Elettaria cardamomum*), the fruit of lime (*Citrus aurantifolia*), and the oleo-gum resin from frankincense (*Boswellia sacra*). Different combinations of plant material were combined and extracted according to the following: CEBE, all three species; CBE, just lime and frankincense; CEE, lime and cardamom; and EBE, cardamom, and frankincense. The aqueous extracts of the plants under study were prepared after mixing 40 g of the combined material with 160 ml of water. Mechanical crushing was completed using an electric mixer, and then the mixture was left for twenty-four hours at a temperature of 4°C for soaking. The mixture was after that filtered using several layers of gauze filters. The filtrate was centrifuged at 3000 rpm for 10 minutes, and then the supernatant was filtered again, employing a Buchner funnel using Whatman paper. The filtrate was gradually dried using an air incubator at 37°C. The extracts were kept in tightly closed plastic containers until use.

### 2.5. Blood Sampling

Blood samples were drawn at the top of the dosing period with the extracts through a quick heart stab and before sacrificing the animals with a volume of 5 ml to make tests for lipid profile (TG, TC VLDL, LDL, HDL) and antioxidant tests. Blood samples were placed inside a serum separating gel tube and left for a minimum of 1 / 4 of an hour at temperature, and then the tubes were

transferred to a serum separating gel tube centrifuge, discarded at 4000 pm, for 10 minutes. Then the serum was separated using micropipettes placed in Eppendorf tubes and sent to the laboratory for lipid profile tests, while a variety of them was kept at -20°C for later use.

### 2.6. The Statistical Analysis

For statistical analysis, the variance analysis (ANOVA) was utilized to compare among different variables; values were presented as the mean ± SE of triplicate measurements.

## 3. Results

### 3.1. Qualitative and Quantitative Disclosures for Plant Extracts

The active ingredients in the four plant extracts (CEBE, CBE, EBE, and CEE) were qualitatively assessed using colorimetric assays, and the presence of active metabolites was evidenced for flavonoids, phenols, tannins, and alkaloids (Table 1). By cross-examination of the botanical ingredients, it is clear that tannins are lower in frankincense resin. Cardamom and lime appear to be good sources of flavonoids, and all ingredients are rich in phenols.

Table 1 Qualitative and quantitative disclosures for plant extracts

	CEBE	CBE	EBE	CEE	Measuring unit
Flavonoids	0.7	0.2	1.0	0.3	GAE/g
Phenols	5.9	6.0	5.8	5.5	Catechin/g
Tannins	2.1	1.3	1.3	2.0	Quercetin/g
Alkaloids	2.3	2.0	2.3	2.2	mg /g

### 3.2. Body Weight

Statistical analysis of the results of the weights obtained from the study showed a significant decrease ( $p < 0.05$ ) of rabbits' weights at the end of the study. In the positive control group (Table 2) with alloxan but no treatment, the mean mass of  $1416.33 \pm 77.3$  g at the start contrasts with the average mass at the end of the study, which was  $1177.3 \pm 108.0$  g. Therefore, it is evident that the rabbits lost a significant amount of body mass in their diabetic state. In contrast, in the treatment groups (Table 3), most animals either maintained their mass or lost mass not exceeding 10%.

Table 2 Difference of mass in the positive control group with alloxan but no treatment

Duration	Before Alloxan	After Alloxan	One week after Alloxan	Two weeks after alloxan
Weights (grams)	1416.33 ± 77.3	1311.0 ± 53.8	1476.71 ± 35.462	1177.3 ± 108.0

Note: Values represent mean ± standard error.

Table 3 Masses of rabbits after induction to diabetes using alloxan, before, during, and at the end of treatment (8 treatments)

	Mass pre-treatment	Mass 1-week treatment	Mass 2-weeks treatment
G1 CEBE 500 mg/kg	1477.7 ± 112.6	1361.7 ± 113.2	1382.7 ± 75.7
G2 CEBE 250 mg/kg	1596.0 ± 57.5	1527.7 ± 81.5	1452.0 ± 88.6
G3 CBE 500 mg/kg	1350.7 ± 115.5	1252.3 ± 95.2	1277.5 ± 196.5
G4 CBE 250 mg/kg	1483.3 ± 38.7	1425.3 ± 49.2	1385.3 ± 43.7
G5 EBE 500 mg/kg	1677.0 ± 97.3	1645.0 ± 106.0	1644.0 ± 78.3
G6 EBE 250 mg/kg	1662.3 ± 118.4	1546.3 ± 116.3	1591.7 ± 136.3
G7 CEE 500 mg/kg	1528.3 ± 11.7	1479.3 ± 38.1	1519.7 ± 22.9
G8 CEE 250 mg/kg	1610.3 ± 80.8	1576.0 ± 51.2	1535.7 ± 54.3
Control ± Alloxan	1311.0 ± 53.8	n.d.	1177.3 ± 108.0
Control	1321.3 ± 21.5	1357.3 ± 23.4	1486.0 ± 24.7

Note: Values represent mean ± standard error.

### 3.3. Changes in Blood Glucose Level

The statistical results showed a significant ( $P < 0.05$ ) increase in the average glucose level in the experimental animals. An increase in glucose level was observed after the development of diabetes mellitus through the injection of Alloxan (Table 4). It was noticed that the glucose percentage increased significantly ( $P < 0.05$ ) when injecting alloxan at a concentration of 100 mg/kg for the first time. A significant increase was also evident ( $P < 0.05$ ) after the second alloxan injection. On the other hand, a significant decrease ( $P < 0.05$ ) in the glucose level was observed between the period before dosing with the extracts and the period of the second week of dosing (Table 5). The data demonstrate that some treatments were more effective than others and that a dose-response curve is true.

In some cases, the treatments could return blood glucose levels to within normal ranges after two weeks of treatment. These more potent treatments were lime and cardamon and lime and frankincense. Unfortunately, the treatments did not include lime, or if the lime content was diluted due to a higher diversity of ingredients, the efficacy diminished. Thus, lime is a promising candidate for further study.

Table 4 Blood glucose levels of animals with Alloxan-induced diabetes

Duration	Glucose pre- alloxan	Glucose level second alloxan	Glucose level diabetes	Glucose level final
Glucose level	106.0 <sup>a</sup> ± 7.1	227.3 <sup>a</sup> ± 68.5	358.3 <sup>b</sup> ± 11.0	552.7 <sup>c</sup> ± 61.1

Note: Values represent mean ± SD, different letters represent significant differences ( $p \leq 0.05$ )

Table 5 Difference in blood glucose level

	Glucose before treatment	Glucose one week of treatment	Glucose two weeks of treatment
G1	406 <sup>a</sup>	493 <sup>a</sup>	429.3 <sup>a</sup>
CEBE 500 mg/kg	± 123.4	± 98.0	± 156.0
G2	520 <sup>a</sup>	453.3 <sup>a</sup>	553.7 <sup>a</sup>
CEBE 250 mg/kg	± 130.0	± 118.7	± 50.9
G3	409.3 <sup>a</sup>	260.7 <sup>a</sup>	122 <sup>b</sup>
CBE 500 mg/kg	± 73.9	± 67.9	± 75.8
G4	463.3 <sup>a</sup>	225.7 <sup>a</sup>	351.3 <sup>a</sup>
CBE 250 mg/kg	± 103.4	± 29.4	± 99.9
G5	402.3 <sup>a</sup>	273.3 <sup>a</sup>	332 <sup>a</sup>
EBE 500 mg/kg	± 38.4	± 106.8	± 166.6
G6	541 <sup>a</sup>	462 <sup>a</sup>	385 <sup>a</sup>
EBE 250 mg/kg	± 109	± 94.2	± 48.7
G7	465.3 <sup>a</sup>	122 <sup>b</sup>	115.7 <sup>b</sup>
CEE 500 mg/kg	± 94.4	± 20.7	± 10.6
G8	568.7 <sup>a</sup>	578 <sup>a</sup>	236.7 <sup>b</sup>
CEE 250 mg/kg	± 40.8	± 41.3	± 9.0

Note: Values represent mean ± SD, different letters represent significant differences (p≤0.05).

### 3.4. Lipid Profile

After conducting a statistical analysis of the data of lipids in the blood plasma of rabbits, it was noticed that there are significant differences between treatments and the negative control with Alloxan (Table 6). However, although all treatments demonstrated an improvement compared to alloxan alone, there was minimal dose-response confirmation. Treatments that improved the glucose profiles (Table 5) were the best lipid profiles. The inverse dose-response for some of the treatments indicates potential liver toxicity at higher treatment concentrations.

Table 6 Plasma lipid profiles of rabbits

	TC	TG	HDL	LDL	VLDL
G1	<sup>b</sup> 93.3±4.5	<sup>a</sup> 135.0±20.4	<sup>b</sup> 10.3±2.6	<sup>b</sup> 56.0±4.6	<sup>a</sup> 27.0±4.1
CEBE 500 mg/kg					
G2	<sup>a</sup> 59.0±6.9	<sup>a</sup> 71.0±3.8	<sup>a</sup> 13.0±2.5	<sup>a</sup> 31.8±4.0	<sup>a</sup> 14.2±0.8
CEBE 250 mg/kg					
G3	<sup>a</sup> 58.5±3.5	<sup>a</sup> 84.0±6.0	<sup>b</sup> 10.5±0.5	<sup>a</sup> 31.2±2.8	<sup>a</sup> 16.8±1.2
CBE 500 mg/kg					
G4	<sup>a</sup> 75.7±17.1	<sup>a</sup> 110.7±27.0	<sup>a</sup> 13.3±1.7	<sup>a</sup> 40.2±10.4	<sup>a</sup> 22.1±5.4
CBE 250 mg/kg					
G5	<sup>b</sup> 100.3±18.8	<sup>a</sup> 153.7±29.9	<sup>a</sup> 16.0±2.0	<sup>b</sup> 53.6±10.9	<sup>a</sup> 30.7±6.0
EBE 500 mg/kg					
G6	<sup>b</sup> 110.3±16.6	<sup>a</sup> 168.7±51.1	<sup>a</sup> 14.3±2.4	<sup>b</sup> 62.3±4.0	<sup>a</sup> 33.7±10.2
EBE 250 mg/kg					
G7	<sup>a</sup> 76.0±11.1	<sup>a</sup> 127.0±27.4	<sup>a</sup> 14.7±1.8	<sup>a</sup> 35.9±7.4	<sup>a</sup> 25.4±5.5
CEE 500 mg/kg					
G8	<sup>a</sup> 59.3±9.7	<sup>a</sup> 89.3±11.8	<sup>b</sup> 12.0±0.6	<sup>a</sup> 29.5±6.9	<sup>a</sup> 17.9±2.4
CEE 250 mg/kg					
G-	<sup>b</sup> 113.0±7.4	<sup>a</sup> 146.7±27.8	<sup>b</sup> 12.7±1.5	<sup>b</sup> 71.0±10.6	<sup>a</sup> 29.3±5.6
+Alloxan					
G-	<sup>c</sup> 40.3±4.4	<sup>b</sup> 67.0±8.7	<sup>c</sup> 6.7±1.2	<sup>c</sup> 20.3±1.9	<sup>b</sup> 13.4±1.74
-					

Notes: TC - total cholesterol, TG - triglycerides, HDL - high-density lipoprotein, LDL - low-density lipoprotein, VLDL - very-low-density lipoprotein;

Values represent mean ± standard error;

\* Different letters represent significant differences (P< 0.05).

## 4. Discussion

It is noticed from the statistical analysis that there are changes in rabbits' weights. There was a weight loss in the groups treated with alloxan to induce diabetes compared to the negative control group that was not treated with alloxan.

This decrease in weight can be explained by the fact that the pancreatic beta cells responsible for insulin production were significantly damaged after treatment with alloxan. This damage leads to a decreased production or absence of the hormone insulin. Insulin is responsible for bringing glucose into the cells to carry out the process of glycolysis, which provides the energy needed for the cells. The body compensates for the lack of energy obtained through glycolysis with gluconeogenesis, which requires catabolism processes for fats and proteins to release energy for use by cells.

The statistical analysis showed that the dosed extracts, in general, stopped the weight deterioration, but it was noted that there was a weight gain after the second week of dosing. This weight gain may be attributed to the role of the active substances in the extracts on stimulating beta cells to secrete insulin and reduces oxidative damage due to hyperglycemia, improving insulin sensitivity, which in turn enhances the structural state of proteins and fats, which were the main source of body energy, as well as controlling the metabolic activity of carbohydrates. It was also found that the presence of lemon leads to an increase in appetite and contains antioxidants, which in turn regulate the digestive process [23].

The current study results showed that treatment with alloxan caused a significant increase in glucose in the blood of rabbits. The reason for this could be the damage to pancreatic beta cells caused by alloxan, thus inhibiting insulin production, which in turn prevents glucose from entering the cells, which leads to the accumulation of glucose in the blood, which is the reason for measuring the high level [24]. The reason for the high level of glucose can also be attributed to the alloxan effect on some vital body activities, such as its interference in the activity of compounds containing the sulfhydryl group included in enzyme glucokinase synthesis, and thus the loss of the enzyme effectiveness, which has an important role in the process of glycolysis [25].

For example, we note that the extract with the highest diversity of ingredients (CEBE at a concentration of 500 mg/kg and 250 mg/kg) did not cause changes in the upward level of glucose in the first week of dosing, but the continuation of the dose for the second week led to a decrease (not statistically significant) at the concentration of 500 mg/kg. The rise in blood sugar at the concentration of 250 mg/kg for CEBE demonstrates that a dose-dependency exists. A noteworthy outcome was derived from the use of the

combination of cardamon and lime (CEE), which at a concentration of 500 mg/kg led to a significant decrease during both the first week and the second week of dosing, but at a concentration of 250 mg/kg only lowered blood sugar in the first week, but was not strong enough to maintain this effect into the second week of dosing.

All of the extracts demonstrated some ability to reduce the blood glucose level of the animals. It is considered that the phytochemicals responsible for these positive effects may be recognized at a class level as conferring effects. For example, flavonoids are generally known for their antidiabetic or anti-hyperglycemic activity conferral. There is also a role for vitamin C, which is present in lemon extracts, in reducing the destructive role of free radicals that destroy insulin-secreting cells.

The statistical analysis of the average cholesterol ratio of the infected and dosed groups with the positive and negative control group showed significant differences. It was noticed that the groups (G2, G3, G4, G7, and G8) showed significant differences ( $P < 0.05$ ) with the alloxan-positive negative control group. On the other hand, some treatments were significantly different from the alloxan-negative control group (G1, G4, G5, G6, and G7). The level of cholesterol in the blood depends on the activity of the enzyme Cholesterol Acyl Transferase. Cholesterol absorption in the intestine and its concentration increase in the blood depend on its absorption of larger amounts of cholesterol. The absence of insulin stimulates this enzyme, so the increase in cholesterol after injection with alloxan is due to the high activity of this enzyme, as alloxan works to destroy insulin-producing cells, causing severe or complete lack of insulin level. The treatments helped lower cholesterol in rabbits' blood because they enhanced the oxidation of cholesterol particles and eventually converted them into bile salts. Another possibility is that the treatments inhibited intestinal absorption of cholesterol and thus prevented its accumulation in the blood. A third possibility is increased cholesterol secretion to the intestines as a form of elimination. Lastly, blocking cholesterol synthesis is also possibly one of the causes of cholesterol-lowering by plant extracts consistent with other studies.

The statistical tables showed a difference in the non-significant rise ( $P > 0.05$ ) of the TG ratio between the dosed groups (G1, G2, G3, G4, G7, and G8) and between the negative control groups. Also, the groups (G5 and G6) showed a significant increase ( $P < 0.05$ ) when compared to the negative control group. The comparison between the infected and dosed groups with the extract (G1, G2, G3, G4, G7, and G8) and the Alloxan-control group showed a non-significant decrease ( $P > 0.05$ ), while the groups (G5 and G6) had an insignificant increase ( $P < 0.05$ ).

The activity of the lipoprotein lipase enzyme for reducing blood lipids is related to the presence of insulin, but in the presence of severe insulin deficiency due to diabetes mellitus, the action of the lipase enzyme is inhibited. Thus, the breakdown of fats is reduced, leading to an increase in triglycerides. In the current study, the decrease in triglycerides after treatment is likely to be the therapeutic effect of plants in improving the level of insulin release and regulating metabolic processes on fats and carbohydrates.

The statistical results of HDL levels for the groups treated with the extracts G2, G4, G5, G6, and G7, compared with the Alloxan control group, showed significant differences ( $P < 0.05$ ) involving a lowering of HDL. The reason for the low level of HDL may be attributed to the lack of insulin that occurs as a result of damage to the insulin-producing beta cells in the pancreas caused by alloxan, and this, in turn, leads to the activation of the hepatic liver lipase (HL) enzyme responsible for the breakdown of fats. Since HDL molecules are rich in triglycerides, the increase in its percentage in the blood creates a suitable environment for the work of the Hepatic lipase (HL) enzyme, and as a result, the HDL particles will be digested, and their percentage in the blood will decrease. The use of some plants, especially those containing flavonoids such as *Citrus aurantifolia*, leads to an increase in HDL-C in the blood plasma. The increase may be attributed to the inhibition of the cholesterol ester transfer protein (CETP). In the CETP protein, they were found to have a high level of HDL-C.

The statistical analysis of LDL percentage showed that several groups (G2, G3, G4, G7, and G8) caused a significant ( $P < 0.05$ ) decrease in comparison with the Alloxan control group ( $71.00 \pm 10.621$ ). It was also noted that there were significant differences between some groups (G1, G5, and G6) and the Alloxan control group if they were compared with the Alloxan-negative control group ( $20.27 \pm 1.919$ ). The reason for the elevated LDL level caused by alloxan may be the inactive status of the enzyme lipoprotein lipase, which is responsible for breaking down triglycerides due to diabetes. The plant extracts generally decreased LDL may be due to the improvement of the body's metabolic activities after dosing with the extracts. It is clear that the flavonoids and phenols in the plant components, including *Citrus aurantifolia*, play an important role in reducing the ratio of LDL and VLDL. In addition, flavonoids have antioxidant activity and reduce reactive free radical species that inhibit cholesterol synthesis and absorption.

Statistical results showed a significant increase between groups (G5 and G6) compared to the Alloxan-negative control group, and all groups showed a clear, insignificant decrease ( $P > 0.05$ ) after comparing them to the Alloxan-positive control group. Alloxan increased VLDL level is likely due to the increased

lipolysis in the adipose tissue. Also, enzyme lipoprotein lipase inhibition causes a reduced number of receptors due to diabetes to prevent the entry of VLDL into cells, which leads to its collection in the bloodstream. Also, an increase in VLDL level is associated with an increase in triglycerides in the blood serum, which was caused by diabetes. The reason for the decrease in response to dosing with plant extracts may be the presence of active substances in those extracts, such as flavonoids, alkaloids, and others, which show a role in improving the level of insulin secretion, regeneration of pancreatic beta cells, and other metabolic processes in the body.

## 5. Conclusion

Chronically high blood glucose leads to damage too many of the body's vital organs. The longer the disease duration, the more vulnerable the internal organs are to damage. Inducing diabetes by alloxan led to changes in body weight, glucose ratio, and lipid profile in rabbits' blood plasma, which mimics what is found in individuals with diabetes. The use of plants containing active substances, known as phytochemicals, has become an important consideration in manufacturing appropriate treatments for diseases. The use of the plant parts in the current study (*Elettaria cardamomum*, *Citrus aurantifolia*, and *Boswellia sacra*) reduced those symptoms associated with the disease. It may therefore be considered useful in the context of lifestyle choices that improve the condition of diabetes.

## References

- [1] EGAN A.M., and DINNEEN S.F. What is diabetes? *Medicine*, 2019, 47(1): 1-4. DOI: 10.1016/j.mpmed.2018.10.002.
- [2] ABUTALEB M.H. Diabetes Mellitus in Bahrain: An Overview. *Diabetic Medicine*, 2016, 9(6): 574-578. DOI: 10.1111/j.1464-5491.1992.tb01842.x.
- [3] ISLAM M., RUPESHKUMAR M., and REDDY K.B. Streptozotocin is more convenient than Alloxan for the induction of Type 2 diabetes. *International Journal of Pharmacology Research*, 2017, 07(01): 6-11.
- [4] ELROBY S.A., AZIZ S.G., and HILAL R.H. The electronic structure of alloxan monohydrate. Spectroscopic and density functional synergic approach. *Journal of Molecular Structure*, 2017, 1130: 487-496. DOI: 10.1016/j.molstruc.2016.10.069.
- [5] ROHILLA A., and ALI S. Alloxan Induced Diabetes : Mechanisms and Effects. *International Journal of Research in Pharmaceutical and Biomedical Sciences*, 2012, 3(2): 819-823.
- [6] WU H., ZHANG W., SCHUSTER M., MOCH M., WINDOFFER R., STEINBERG G., STAIGER C.J., and PANSTRUGA R. Alloxan disintegrates the plant cytoskeleton and suppresses Mlo-mediated powdery mildew resistance. *Plant and Cell Physiology*, 2020, 61(3): 505-518, DOI: 10.1093/PCP/pcz216.
- [7] TAO Z., SHI A., and ZHAO J. Epidemiological Perspectives of Diabetes. *Cell Biochemistry and Biophysics*, 2015, 73(1): 181-185. DOI: 10.1007/s12013-015-0598-4.
- [8] KARUNAMOORTHY K., JEGAJEEVANRAM K., VIJAYALAKSHMI J., and MENGISTIE E. Traditional Medicinal Plants: A Source of Phytotherapeutic Modality in Resource-Constrained Health Care Settings. *Evidence-Based Complementary and Alternative Medicine*, 2013, 18(1): 67-74. DOI: 10.1177/2156587212460241.
- [9] KURMUKOV A. Medicinal plants of Central Asia: Uzbekistan and Kyrgyzstan. In: EISENMAN S.W., ZAUROV D.E., STRUWE L. (eds.). *Medicinal Plants of Central Asia: Uzbekistan and Kyrgyzstan*, Springer, 2013. DOI: 10.1007/978-1-4614-3912-7.
- [10] RAJAN A., RAJAN A.R., and PHILIP D. *Elettaria cardamomum* seed-mediated rapid synthesis of gold nanoparticles and its biological activities. *OpenNano*, 2017, 2(8): 1-8. DOI: 10.1016/j.onano.2016.11.002.
- [11] ANWAR F., ABBAS A., ALKHARFY K.M., and GILANI A.H. *Cardamom (Elettaria cardamomum Maton) oils*. Elsevier Inc., 2016.
- [12] ASHOKKUMAR K., MURUGAN M., DHANYA M.K., and WARKENTIN T.D. Botany, traditional uses, phytochemistry and biological activities of cardamom [*Elettaria cardamomum* (L.) Maton] - A critical review. *Journal of Ethnopharmacology*, 2020, 246: 112244. DOI: 10.1016/j.jep.2019.112244.
- [13] SOUISSI M., AZELMAT J., CHAIEB K., and GRENIER D. Antibacterial and anti-inflammatory activities of cardamom (*Elettaria cardamomum*) extracts: Potential therapeutic benefits for periodontal infections. *Anaerobe*, 2020, 61: 102089. DOI: 10.1016/j.anaerobe.2019.102089.
- [14] ASNAASHARI S., DELAZAR A., HABIBI B., VASFI R., NAHAR L., HAMEDEYAZDAN S., and SARKER S.D. Essential Oil from *Citrus aurantifolia* prevents ketotifen-induced weight-gain in mice. *Phytotherapy Research*, 2010, 24(12): 1893-1897. DOI: 10.1002/ptr.3227.
- [15] AL-SNAFI D.A.E. Nutritional value and pharmacological importance of citrus species grown in Iraq. *IOSR Journal of Pharmacy*, 2016, 6(8): 76-108.
- [16] PATIL J.R., JAYAPRAKASHA G.K., MURTHY K.N.C., CHETTI M.B., and PATIL B.S. Characterization of *Citrus aurantifolia* bioactive compounds and their inhibition of human pancreatic cancer cells through apoptosis. *Microchemical Journal*, 2010, 94(2): 108-117. DOI: 10.1016/j.microc.2009.09.008.
- [17] SHAREEF A.A. Evaluation of antibacterial activity of essential oils of *Cinnamomum* sp. and *Boswellia* sp. *Journal of Basrah Researches*, 2011, 37(5): 60-71.
- [18] AL-HARRASI A., REHMAN N.U., KHAN A.L., AL-BROUMI M., AL-AMRI I., HUSSAIN J., HUSSAIN H., and CSUK R. Chemical, molecular and structural studies of *Boswellia* species:  $\beta$ -Boswellic Aldehyde and 3-epi-11 $\beta$ -Dihydroxy BA as precursors in biosynthesis of boswellic acids. *PLoS ONE*, 2018, 13(6). DOI: 10.1371/journal.pone.0198666.
- [19] ALYAHYA A.R.A.I., and ASAD M. Repeated 28-DAY oral dose study on *Boswellia sacra* oleo gum resin extract for testicular toxicity in rats. *Journal of Ethnopharmacology*, 2020, 258(12): 112890. DOI: 10.1016/j.jep.2020.112890.

- [20] AL-YAHYA A.R.A.I., ASAD M., SADABY A., and AL-HUSSAINI M.S. Repeat oral dose safety study of standardized methanolic extract of *Boswellia sacra* oleo gum resin in rats. *Saudi Journal of Biological Sciences*, 2020, 27(1): 117-123. DOI: 10.1016/j.sjbs.2019.05.010.
- [21] EFFERTH T., and OESCH F. Anti-inflammatory and anti-cancer activities of frankincense: Targets, treatments and toxicities. In: *Seminars in Cancer Biology*, 2020, 30034-1: 1-21. DOI: 10.1016/j.semcancer.2020.01.015.
- [22] DAISY P., SANTOSH K., RAJATHI M., COLLEGE H.C., and TAMILNADU T. Antihyperglycemic and antihyperlipidemic effects of *Clitoria ternatea* Linn. in alloxan-induced diabetic rats. *African Journal of Microbiology Research*, 2009, 3(5): 287-291.
- [23] MOHAN S.C., ANAND T., PRIYADHARSHINI G.S., and BALAMURUGAN V. GC-MS Analysis of Phytochemicals and Hypoglycemic Effect of *Catharanthus roseus* in Alloxan-Induced Diabetic Rats. *International Journal of Pharmaceutical Sciences Review and Research*, 2015, 31(1): 123-128
- [24] IWASAKI T., TAKAHASHI S., TAKAHASHI M., ZENIMARU Y., KUJIRAOKA T., ISHIHARA M., NAGANO M., SUZUKI J., MIYAMORI I., NAIKI H., SAKAI J., FUJINO T., MILLER N.E., YAMAMOTO T.T., and HATTORI H. Deficiency of the very-low-density lipoprotein (VLDL) receptors in streptozotocin-induced diabetic rats: Insulin dependency of the VLDL receptor. *Endocrinology*, 2005, 146(8): 3286-3294. DOI: [10.1210/en.2005-0043](https://doi.org/10.1210/en.2005-0043).
- [25] MAHDIAN D., ABBASZADEH-GOUDARZI K., RAOOFI A., DADASHIZADEH G., ABROUDI M., ZAREPOUR E., and HOSSEINZADEH H. Effect of *Boswellia* species on the metabolic syndrome: A review. *Iranian Journal of Basic Medical Sciences*, 2020, 23(11): 1374-1381. DOI: 10.22038/ijbms.2020.42115.9957.
- 參考文:**
- [1] EGAN A.M. 和 DINNEEN S.F. 什麼是糖尿病？醫學，2019, 47(1): 1-4. DOI : 10.1016/j.mpmed.2018.10.002。
- [2] ABUTALEB M.H. 巴林的糖尿病：概述。糖尿病醫學，2016, 9(6): 574-578 。 DOI : 10.1111/j.1464-5491.1992.tb01842.x。
- [3] ISLAM M., RUPESHKUMAR M. 和 REDDY K.B. 鏈脈佐菌素比四氧嘧啶誘導 2 型糖尿病更方便。國際藥理學研究雜誌，2017, 07(01): 6-11.
- [4] ELROBY S.A., AZIZ S.G. 和 HILAL R.H. 四氧嘧啶一水合物的電子結構。光譜和密度泛函協同方法。分子結構雜誌，2017，1130：487-496。DOI：10.1016/j.molstruc.2016.10.069。
- [5] ROHILLA A. 和 ALI S. 四氧嘧啶誘導的糖尿病：機制和影響。國際藥物與生物醫學研究雜誌，2012，3(2)：819-823。
- [6] WU H., ZHANG W., SCHUSTER M., MOCH M., WINDOFFER R., STEINBERG G., STAIGER C.J. 和 PANSTRUGA R. 四氧嘧啶 分解植物細胞骨架並抑制支原體樣生物 介導的白粉病抗性。植物和細胞生理學，2020, 61(3): 505-518, DOI: 10.1093/PCP/pcz216。
- [7] TAO Z., SHI A., 和 ZHAO J. 糖尿病的流行病學觀點。細胞生物化學與生物物理學，2015, 73(1): 181-185. DOI : 10.1007/s12013-015-0598-4。
- [8] KARUNAMOORTHY K., JEGAJEEVANRAM K., VIJAYALAKSHMI J. 和 MENGISTIE E. 傳統藥用植物：資源受限醫療保健環境中植物治療模式的來源。循證補充和替代醫學，2013，18(1)：67-74。DOI：10.1177/2156587212460241。
- [9] KURMUKOV A. 中亞藥用植物：烏茲別克斯坦和吉爾吉斯斯坦。在：EISENMAN S.W., ZAUROV D.E., STRUWE L. (編輯)。中亞藥用植物：烏茲別克斯坦和吉爾吉斯斯坦，施普林格，2013。DOI：10.1007/978-1-4614-3912-7。
- [10] RAJAN A., RAJAN A.R. 和 PHILIP D. 荳蔻種子介導的金納米粒子快速合成及其生物活性。開放納米，2017，2(8)：1-8。DOI：10.1016/j.onano.2016.11.002。
- [11] ANWAR F., ABBAS A., ALKHARFY K.M. 和 GILANI A.H. 荳蔻 (小荳蔻) 油。愛思唯爾公司，2016年。
- [12] ASHOKKUMAR K., MURUGAN M., DHANYA M.K. 和 WARKENTIN T.D. 植物學，小荳蔻的傳統用途、植物化學和生物活性 [小荳蔻] - 一個批判性評論。民族藥理學雜誌，2020，246: 112244. DOI: 10.1016/j.jep.2019.112244。
- [13] SOUISSI M., AZELMAT J., CHAIEB K. 和 GRENIER D. 荳蔻 (荳蔻) 提取物的抗菌和抗炎活性：牙周感染的潛在治療益處。厭氧菌，2020, 61: 102089. DOI: 10.1016/j.anaerobe.2019.102089。
- [14] ASNAASHARI S., DELAZAR A., HABIBI B., VASFI R., NAHAR L., HAMEDEYAZDAN S. 和 SARKER S.D. 柑桔精油可防止酮替芬引起的小鼠體重增加。植物療法研究，2010, 24(12): 1893-1897. DOI : 10.1002/ptr.3227。
- [15] AL-SNAFI D.A.E. 伊拉克種植的柑橘品種的營養價值和藥理重要性。IOSR 藥學雜誌，2016, 6(8): 76-108。

- [16] PATIL J.R.、JAYAPRAKASHA G.K.、MURTHY K.N.C.、CHETTI M.B. 和 PATIL B.S. 柑桔生物活性化合物的表徵及其通過細胞凋亡對人胰腺癌細胞的抑制作用。微化學雜誌，2010，94(2): 108-117. DOI : 10.1016/j.microc.2009.09.008。
- [17] SHAREEF A.A.肉桂精油的抗菌活性評估。和乳香。巴士拉研究雜誌，2011，37 ( 5 ) : 60-71。
- [18] AL-HARRASI A.、REHMAN N.U.、KHAN A.L.、AL-BROUMI M.、AL-AMRI I.、HUSSAIN J.、HUSSAIN H. 和 CSUK R. 乳香屬的化學、分子和結構研究： $\beta$ -乳香醛和 3-epi-11 $\beta$ -二羥基 BA 作為乳香酸生物合成的前體。公共科學圖書館一號，2018 年，13(6)。DOI : 10.1371/journal.pone.0198666。
- [19] ALYAHYA A.R.A.I. 和 ASAD M. 對乳香薩克拉油膠樹脂提取物對大鼠睪丸毒性的重複 28 天口服劑量研究。民族藥理學雜誌，2020，258(12): 112890. DOI: 10.1016/j.jep.2020.112890.
- [20] AL-YAHYA A.R.A.I.、ASAD M.、SADABY A. 和 AL-HUSSAINI M.S.對大鼠中乳香薩克拉油膠樹脂標準化甲醇提取物的重複口服劑量安全性研究。沙特生物科學雜誌，2020，27(1): 117-123。DOI : 10.1016/j.sjbs.2019.05.010。
- [21] EFFERTH T. 和 OESCH F. 乳香的抗炎和抗癌活性：目標、治療和毒性。在：癌症生物學研討會，2020 年，30034-1 : 1-21。DOI : 10.1016/j.semcancer.2020.01.015。
- [22] DAISY P.、SANTOSH K.、RAJATHI M.、COLLEGE H.C. 和 TAMILNADU T. 陰蒂的抗高血糖和抗高血脂作用。在四氧嘧啶誘導的糖尿病大鼠中。非洲微生物學研究雜誌，2009 年，3(5) : 287-291。
- [23] MOHAN S.C.、ANAND T.、PRIYADHARSHINI G.S. 和 BALAMURUGAN V. 長春花在四氧嘧啶誘導的糖尿病大鼠中的植物化學物質和降血糖作用的氣相色譜-質譜法分析。國際藥物科學評論與研究雜誌，2015，31(1): 123-128
- [24] IWASAKI T.、TAKAHASHI S.、TAKAHASHI M.、ZENIMARU Y.、KUJIRAOKA T.、ISHIHARA M.、NAGANO M.、SUZUKI J.、MIYAMORI I.、NAIKI H.、SAKAI J.、FUJINO T.、MILLER NE、YAMAMOTO T.T. 和 HATTORI H. 鏈脲佐菌素誘導的糖尿病大鼠極低密度脂蛋白受體缺乏：極低密度脂蛋白受體的胰島素依賴性。內分泌學，2005，146(8): 3286-3294。DOI : 10.1210/en.2005-0043。
- [25] MAHDIAN D.、ABBASZADEH-GOUDARZI K.、RAOOFI A.、DADASHIZADEH G.、ABROUDI M.、ZAREPOUR E. 和 HOSSEINZADEH H. 乳香對代謝綜合徵的影響：綜述。伊朗基礎醫學雜誌，2020，23(11): 1374-1381。DOI : 10.22038/ijbms.2020.42115.9957。