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The effect of Rosemary and Cinnamon extracts on blood glucose levels in albino rats with Alloxan-induced diabetes

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ABSTRACT

Background: Herbs have long been recognized for their medicinal properties, with rosemary and cinnamon noted for their health benefits. Rosemary is used for treating respiratory infections and has antioxidant and antimicrobial effects, while cinnamon is known for its antispasmodic and antimicrobial properties.

Objective : This study aimed to evaluate the effects of rosemary and cinnamon extracts on blood glucose levels in diabetic albino rats.

Methodology : Thirty male rats were divided into six groups, with five groups induced with diabetes using Alloxan. Group I served as the normal control, while Group II was the diabetic control. Groups III and IV received 400 mg/day of rosemary and cinnamon extracts, respectively, Group V received a combination of both, and Group VI received glibenclamide as a positive control. After eight weeks .

Results: The results showed that both extracts significantly reduced blood glucose levels ($p < 0.0001$), with reductions of 49.9% for rosemary and 58.8% for cinnamon. The combination of both extracts led to a 70.8% reduction in blood glucose levels ($p < 0.0001$).

Conclusion: The individual use of rosemary and cinnamon extract has a significant effect in lowering blood glucose but the using of combined extract has more significant effect in lowering blood glucose.

Keywords: *Rosemary, Cinnamon, Blood Glucose, Diabetes Mellitus*

1. INTRODUCTION

Diabetes mellitus is the most common endocrine disorder. It is a chronic condition, characterized by hyperglycemia due to impaired insulin secretion with or without insulin resistance. Improper control of diabetes can lead to serious medical complications such as blindness from retinopathy, neuropathy, renal failure, gangrene, limb amputation, cardiovascular diseases, premature death and micro-vascular complications which are associated with thickening of the capillary basement membrane [1,2]. In recent decades, there has been a growing body of evidence demonstrating the beneficial effects of traditional medicinal plants in the prevention and management of various metabolic disorders, including diabetes, cardiovascular diseases, and certain types of cancer [3–5]. The World Health Organization (WHO) reports that over 80% of the global population relies on traditional medicine to address their primary health care requirements [6]. Herbal medicines have a rich history, with over 50% of clinical pharmaceuticals derived from them [7,8]. For example, metformin, used to lower blood glucose in type 2 diabetes, comes from French lilac, which was historically used as a diabetes treatment in the Middle Ages [9]. Rosemary (Rosmarinus officinalis), a member of the Lamiaceae family, is a widely recognized aromatic perennial evergreen shrub that is indigenous to the Mediterranean region. The extract of rosemary is frequently utilized in aromatherapy to alleviate anxiety-related issues and enhance alertness [10]. Numerous studies have highlighted the various beneficial properties of rosemary, including its antioxidant, diuretic, anti-inflammatory, antimicrobial, anti-carcinogenic, hypoglycemic, and hypolipidemic effects [11,12]. On the other hand, cinnamon (Cinnamomum burmannii) is a versatile plant with diverse applications across various cultures, ranging from culinary uses to inhibiting microbial growth. This evergreen tree, traditionally harvested in Asian countries, is known for its antioxidant properties and its role in the prevention and management of glucose intolerance and diabetes. This study aimed to evaluate the effects of rosemary and cinnamon extracts on blood glucose levels in diabetic albino rats.

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2. METHDOLOGY

2.1. Materials

Plants: Ground dried rosemary leaves and ground dried cinnamon bark; Chemicals: Alloxan (Oxford laboratory, Mumbai- India) and Glibenclamide. This research was conducted at the Faculty of Science, Sana'a University.

2.2. Experimental animal

A total of 30 male albino rats, each weighing between 190 and 210 grams, were chosen for the study. The animals were housed in cages under standard temperature and air conditions for a duration of one month prior to the experiments to allow them to acclimate to their new environment. Their diet consisted of a mixture of cereals, green vegetables, small dried fish, and water and standard laboratory conditions are maintained with 12 :12 h light: dark cycle with a room temperature of 28±4°C. Throughout the entire study period, all experimental animals were subjected to continuous observation.

2.3. Experimental design

Albino rats were categorized into six distinct groups, each comprising five rats (n=5): Group I (normal control) consisted of normal rats that received 2 ml of distilled water. Group II (diabetic control) included diabetic rats that were administered 2 ml of distilled water. Group III (rosemary treated) involved diabetic rats that were given 2 ml of a freshly prepared aqueous extract of rosemary twice daily (400 mg/day) for a duration of 8 weeks. Group IV (cinnamon treated) consisted of diabetic rats treated with 2 ml of a freshly prepared aqueous extract of cinnamon twice daily (400 mg/day) for 8 weeks. Group V (rosemary & cinnamon treated) included diabetic rats that received 2 ml of a freshly prepared aqueous extract combining rosemary and cinnamon twice daily (400 mg + 400 mg/day) for 8 weeks. Finally, Group VI (positive control) comprised diabetic rats treated with 1 ml of glibenclamide once daily (5 mg/kg) for 8 weeks.

2.4. Induction of hyperglycemia

Hyperglycemia was induced in rats through a single intraperitoneal injection of Alloxan at a dosage of 150 mg/kg of body weight [13]. A fresh solution of Alloxan was prepared by dissolving Alloxan powder in normal saline (0.9%). All rats, with the exception of group I, received an injection of 0.5 ml of the prepared solution immediately thereafter. The Alloxan-injected subjects displayed significant hyperglycemia within a period of 18 to 48 hours. Blood glucose levels were initially assessed using a Viva check glucometer to confirm an elevation in blood glucose, followed by blood collection from the rats' eyes for measurement with a COBAS device. Rats were classified as diabetic if their fasting blood glucose levels surpassed 190 mg/dl.

2.5. Extraction method and anti-diabetic

Preparation of Cinnamon: An aqueous extract of cinnamon was prepared by adding 20 ml of boiled purified water to 2 g of the plant, which was measured using a sensitive balance. The mixture was stirred and allowed to sit for 15 minutes before being filtered through filter paper [14].

Preparation of Rosemary: Aqueous rosemary extract was prepared by adding 20 milliliters of boiled purified water to 2 grams of rosemary plant material, which was weighed using a sensitive balance. The mixture was then stirred and allowed to stand for 15 minutes before being filtered using filter paper. Similarly, an aqueous rosemary and cinnamon extract was obtained by adding 20 milliliters of boiled purified water to 2 grams each of rosemary powder and cinnamon powder in the same flask. The mixture was stirred and left to stand for 15 minutes before being filtered using filter paper [15].

Glibenclamide preparation: It was prepared by adding normal saline (0.9%) 25ml to glibenclamide powder 2.5mg which triturate with one or two drops of tween 80.

2.6. Laboratory assessment

Blood samples were collected from the eyes of all rats using a capillary tube on two occasions: initially, immediately following the administration of Alloxan (at time zero), and subsequently, after a period of eight weeks during which they were treated with extracts. The blood samples were placed in vacuum tubes, subjected

to centrifugation using a centrifuge, and analyzed using the COBAS device at the Med Lab laboratory, Alzubairi branch.

2.7. Statistical analysis.

In this study Graph pad prism version 6 program, was used to analyze the data using one way ANOVA and Tukey's multiple comparisons test. All data were analyzed and presented as Mean ± Standard Deviation.

3. RESULTS

As illustrated in Table 1 below, on the initial day of the experiment (zero-day), the average fasting blood glucose levels of the induced diabetic rats were recorded as follows: 99 mg/dl for group I, 246.4 mg/dl for group II, 270.4 mg/dl for group III, 320 mg/dl for group IV, 347.6 mg/dl for group V, and 344.8 mg/dl for group VI.

Table 1: Fast blood glucose level of all groups at starting time before treating (n=5, M±SD).

Groups	FBS mg/dl ± SD
Normal group (I)	99±6.8
Diabetic group (II)	246.4±28.6
Rosemary group (III)	270.4±116.45
Cinnamon group (IV)	320±95.37
Rosemary &Cinnamon group (V)	347.6±124.4
Glibenclamide group(VI)	344.8±80.65

FBS: Fasting Blood Sugar

3.1. The effect of *rosemary* extract on blood glucose level

Table 2 illustrates the impact of rosemary extract administered at a dosage of 400 mg per day over an 8-week duration on albino rats. The average fasting blood glucose level exhibited a reduction from 270.4 ± 116.45 mg to 135.4 ± 11.8 mg, which was statistically significant ($p < 0.0001$). Furthermore, no significant difference was observed between the rosemary group and the control groups, which included both the normal and glibenclamide groups ($p > 0.05$).

Table 2: Effect of 400 mg/d rosemary extract over 8 weeks on rat blood glucose levels (n=5, M±SD).

Groups	Blood glucose level mg/dl		% of reduction
	Before (zero day)	After 8 weeks	
Normal group (I)	99±6.8	98±5.2	**……...**
Diabetic group (II)	246.4±28.6	281±19.8	**……**
Rosemary group(III)	270.4±116.45	135.4±11.8**	**49.9%**
Glibenclamide group (VI)	344.8±80.65	102.2±8.1	**70.35%**

** highly significant difference between diabetic control group & rosemary treated group p <0.0001.

3.2. The effect of *Cinnamon* extract on blood glucose level

Table 3 illustrates the impact of cinnamon extract administered at a dosage of 400 mg per day over an 8-week duration on albino rats. The average fasting blood sugar (FBS) level declined from 320 ± 95.37 mg/dL to 131.6 ± 38 mg/dL, demonstrating a highly significant change ($p < 0.0001$). Furthermore, no significant difference was observed between the cinnamon-treated group and the control groups, which included both the normal and glibenclamide groups ($p > 0.05$).

Table3: The effect of *cinnamon* extract in a dose of 400mg/d over a period 8weeks on rats‟ blood glucose level (n=5, M±SD).

Groups	Blood glucose level mg/dl		% of reduction
	Before (zero day)	After 8 weeks	
Normal group (I)	99±6.8	98±5.2	…….
Diabetic group (II)	246.4±28.6	281±19.8	…….
Cinnamon group (IV)	320±95.37	131.6±38.3**	**58.80%**
Glibenclamide group (VI`)	344.8±80.65	102.2±8.1	**70.35%**

**highly significant difference between diabetic control group & cinnamon treated group p – value <0.0001.

3.3. The effect of the combined *rosemary & cinnamon* extract on blood glucose level

Table 4 shows the effect of combined *rosemary & cinnamon* extract in a dose of (400mg+400mg/day) over a period of 8 weeks on albino rats. The mean FBS level decreased from (347.6±124.4) to (101.2mg±7.0) and was highly significant (p<0.0001). There was non-significant differences between *combined rosemary & cinnamon treated* group and control groups (normal & glibenclamide group) (p > 0.1).

Table4: The effect of a 400 mg daily extract of rosemary and cinnamon on blood glucose levels in rats (n=5) over 8 weeks.

Groups	Blood glucose level mg/dl		% of reduction
	Before (zero day)	After 8 weeks	
Normal group (I)	99±6.8	98±5.2	----------
Diabetic group (II)	246.4±28.6	281±19.8	----------
Rosemary & Cinnamon group (V)	347.6±124.4	101.2±7.0**	**70.80%**
Glibenclamide group (VI)	344.8±80.65	102.2±8.1	**70.35%**

**highly significant difference between diabetic control group & combined rosemary& cinnamon treated group . p – value <0.0001.

4. DISCUSSION

This study is the first in Middle East to evaluate the effect of combined rosemary & cinnamon extract on albino rats‟ blood glucose level. In this study, result shows a significant reduction in FBS of rosemary treated group and this in accordance with Labban et al. who reported rosemary leaves powder produced a significant reduction in blood glucose level for all participants [16]. also result shows a significant reduction in FBS of cinnamon treated group and this in agreement with Al Jamal [17,18]. who observed that when individuals consumed cinnamon powder fast blood glucose levels was significantly reduced1. Furthermore result shows a highly significant reduction in FBS of combined rosemary & cinnamon treated group in comparison with and equal or slightly more superior than positive control or glibenclamide group. A possible mechanism of the hypoglycemic action of rosemary was suggested to be through increasing the insulin level [19]. Moreover, recent study reported that rosemary leads to regeneration of the β-cells of the pancreas and potentiating of insulin secretion from surviving β cells, which indicates that rosemary decrease blood glucose level by stimulating insulin secretion from the remnant β cells or regenerated β cells [20] . In addition, the remarkable anti-diabetogenic effects of rosemary could be due to its potent antioxidant properties. It also might be producing its hypoglycemic activity by a mechanism independent from insulin secretion, e.g. inhibition of endogenous glucose production [21]. Also, other study has reported rosemary extract contains polyphenols carnosic acid and rosmarinic acid that have insulin –like effects in insulin target cells in vitro and exert significant anti-diabetic effect in different animal models of T2DM in vivo [22]. Further studies have suggested that the reduction of fasting blood glucose of rosemary may be due to inhibit the intestinal absorption of glucose by inhibition of intestinal α-amylase enzyme [23] and α -glucosidase enzyme [24] and our study is

in agree with them. Khan et al. have reported that an unidentified factor is present in cinnamon that potentiates the action of insulin in carbohydrate metabolism [25]. They termed this factor as insulin potentiating factor (IPF), Broadhurst et al reconfirmed the presence of this factor in cinnamon4. This hypoglycemic effect of cinnamon may or may not be like other hypoglycemic drugs. This unidentified factor increased the activity of insulin 3 fold in glucose metabolism in fat cell of rat. Anderson et al. characterized this unidentified factor present in cinnamon as methylehydroxy chalcone polymers (MHCP) [26]. They explained that MHCP made fat cells more responsive to insulin by activating the enzyme that causes insulin to bind to cells (insulin receptor-kinase) and inhibiting the enzyme that blocks this process (insulin-receptor-phosphatase) leading to maximal phosphorylation of the insulin receptor, which is associated with increased insulin sensitivity. Other studies confirmed that cinnamon has inhibitory effect on α amylase and α –glucosidase [27,28], and our study is in agree with them, so combined use of rosemary & cinnamon work in synergism to lower FBS and it may be occurred due to both plants contain active substances like rosemarinic acid & procyanidin A1 respectively that: mimic acarbose drug in inhibition of intestinal enzymes; work as antioxidant with the other flavonoids of both plants that protect and preserve the function of β-cell.

5. CONCLUSION

From the results of the present study, it can be concluded that the rosemary extract reduces blood glucose level. Also, the cinnamon extract reduces blood glucose level. In addition, the combined rosemary and cinnamon extract reduces blood glucose level more than individual use.

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Conflicts of Interest
The authors declare that they have no conflicts of interest.

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