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Assessment of the Anti-hyperglycemic Effects of Rosemary (Salvia Rosmarinus) Compared to Glimepiride in Alloxan induced Diabetic Rabbits

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Author`s Contribution

[1,2,4]Substantial contributions to the conception or design of the work; or the acquisition, [3,5]Active Participation in active methodology, analysis, or interpretation of data for the work, Literature review [6]Drafting the work or revising it critically for important intellectual content, [3,6]Final approval of the version to be published

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ABSTRACT

Objectives: To observe the antihyperglycemic effect of rosemary (Salvia rosmarinus) in alloxan-induced diabetic rabbits.

Methodology: This experimental study was conducted at the Department of Pharmacology and Therapeutics, Liaquat University of Medical and Health Sciences (LUMHS), in collaboration with the Animal House of the Agriculture University, Tando Jam, from November 2023 to April 2024. A total of 24 adult healthy male rabbits weighing between 1.5 and 2.5 kg were included. The rabbits were divided into four groups: Group A (normal control) and Group B (diabetic-induced control) received 0.9% NaCl, while Group C (diabetic-induced) received Rosmarinus officinalis 250 mg/kg, and Group D (diabetic-induced) received glimepiride 2.5 mg/kg. Leaves of Rosmarinus officinalis were dried, powdered, and prepared as an ethanolic extract. After the experiment, blood samples were collected from the femoral vein, centrifuged, and analyzed for serum glucose levels. Hyperglycemia was defined as a blood glucose level above 200 mg/dL. Data were entered and analyzed using SPSS version 26.

Results: Fasting blood sugar (FBS) levels showed a significant reduction in Groups C and D compared with Group B by day 28. Specifically, in Group C, FBS decreased from 252.8±2.5 to 181.3±2.3 mg/dL, and in Group D from 255±1.8 to 197.16±2.5 mg/dL ($p<0.05$), indicating significant efficacy of Rosmarinus officinalis in improving FBS levels in diabetic rabbits.

Conclusion: Rosmarinus officinalis effectively reduced glycemic concentrations in alloxan-induced diabetic rabbits.

Key words: Diabetes mellitus, Rosmarinus Officinalis, Glimepiride.

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Introduction

Diabetes mellitus (DM) is among the most prevalent diseases globally, and the impact of this endocrine disorder on the body cannot be ignored.[1] Around 537 million adults aged between 20 and 79 years worldwide are living with diabetes, representing 10.5% of individuals in this age group.[2] According to the 10th edition of the IDF Diabetes Atlas, diabetes is expected to affect 643 million people worldwide by 2030, and this number is projected to rise to 783 million by 2045.[2,3] Current estimates of diabetes mellitus prevalence exceed all previous projections.

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia resulting from inadequate insulin secretion, impaired insulin action, or a combination of both. Persistently elevated blood glucose levels can lead to a range of complications, including macrovascular complications such as cardiovascular disease (CVD) and microvascular complications such as hypertriglyceridemia, neuropathy, and nephropathy.[3] The rising prevalence of type 2 diabetes (T2D), along with lipid profile abnormalities in the population, presents a significant public health challenge.[4]

Rosmarinus officinalis L., commonly known as rosemary, is an aromatic plant belonging to the Lamiaceae family. Rosemary is a plant that is 1 to 2 meters tall, has deep roots, and has a strong spicy aroma. Its leaves are long and dark green, while its flowers can be either purple or white. R. officinalis is cultivated all over the world, especially in the Mediterranean Region: many cultivated and wild species are found in these regions. In the Mediterranean diet, fresh rosemary leaves are used as food and spice. The leaves have been reported to prevent food poisoning.[5] Iron bark has many activities such as antibacterial, blood sugar-lowering, antioxidant, anti-cancer, liver-protective, anti-inflammatory, and antithrombotic properties.[6,7]

The biological properties of rosemary extract are primarily attributed to its polyphenols, such as rosmarinic acid, and phenolic diterpenes, including carnosic acid and carnosol.[8,9] In particular, the presence of these polyphenolic compounds contributes to its antioxidant activity. These compounds are responsible for the antiradical activity of rosmarinic acid, carnosic acid, carnosol, rosmanol, and genkwanin.[10]

Antioxidant activities of the such compounds is similar to other biological activities, such as cell protection and anti-inflammatory, and these Compounds can scavenge reactive oxygen species (ROS).[11] Additionally, previous studies have shown that polyphenols have inhibitory effects on α-amylase, the first step of starch digestion.[12] Additionally, the proportion of secondary metabolites may vary depending on numerous factors.[13,14] Changes in the plant environment, such as the transition from forest habitats to cropland, can influence plant growth, biochemistry, and the concentration of active substances.[15] It is sometimes suggested that cultivated herbal materials may lose the "potency" of wild herbal plants; however, this theory is not supported by sufficient scientific evidence.[23] Therefore, the present study was conducted to evaluate the antihyperglycemic effects of rosemary (*Salvia rosmarinus*) in alloxan-induced diabetic rabbits.

Methodology

This experimental study was conducted by the Department of Pharmacology and Therapeutics in collaboration with the Animal House of Agriculture University, Tando Jam, over a six-month period from November 2023 to April 2024. Ethical approval was obtained from LUMHS (Ref. no. LUMHS/REC/88). A total of 24 healthy adult male rabbits, exhibiting normal behavior and activity, with body weights ranging from 1.5 to 2.5 kg, were included in the study. Rabbits that were sick (e.g., difficulty in jumping), not feeding well, or previously used in other experiments were excluded.

The rabbits were randomly divided into four groups, with six animals per group: Group A (normal control) received 0.9% NaCl; Group B (diabetic-induced control) received 0.9% NaCl; Group C (diabetic-induced) received Rosmarinus officinalis 250 mg/kg; and Group D (diabetic-induced) received glimepiride 2.5 mg/kg. All rabbits were allowed a two-week acclimatization period before the experiment. Animals were housed six per cage and provided a standard basal diet and tap water in a controlled environment with a temperature of 28 ± 2°C, humidity of 60 ± 5%, and a 12-hour light/dark cycle.

Plant Material Preparation: Fresh Rosmarinus officinalis L. was collected from local market. It was identified by Pharmacognosy department Sindh University, Jamshoro. Plant had been dried in the shade at the room temperature. The plant's dried out leaves were ground into powder using the grinder. The powdered leaves were immersed in ethanol at 50°C for 12 hours. After filtration to remove the residue, the ethanolic extract was concentrated using a rotary evaporator under reduced pressure at 40°C.[80]

Induction of Diabetes Mellitus: A single dose of 120 mg/kg body weight of Alloxan monohydrate, dissolved in normal saline, is administered via the intraperitoneal (I.P.) route. Since alloxan could evoke fatal hypoglycemia in initial phase as a result of massive insulin release in first 8h, 25% glucose is given and glucose level is monitored in that period. In second phase blood glucose is gradually drop, after 72 h, a blood test confirmed hyperglycemia (200 mg/dl)[66] After that the experiment will be proceeded.

Determination of Blood Glucose: Blood glucose concentration was determined in blood by commercially available glucose kit (ACCU-CHEK Instant S) glucometer.

All the relevant information was collected using study proforma and data analysis was done by using SPSS version 26. All numerical variables were computed as mean and standard deviation. Student's T-test was applied and a probability value of $p<0.05$ was considered statistically significant.

Pictures: 1 & 2 Preparation of Alloxan Monohydrate for induction of Diabetes Mellitus

Pictures: 3 & 4 Induction of Diabetes in rabbits (Alloxan monohydrate)

Pictures: 5 & 6 Blood sampling for determination of blood glucose.

Results

The mean of FBS in group A (control), group B (Alloxan induced diabetics), group C Rosmarinus officinalis (250mg/Kg), and Group D Glimepiride(2mg/Kg) were noted at 72 hours and at Day 28.

FBS were noted in Group A at 72 hours as 115.3 ± 0.8 and at Day 28 as 115.2 ± 1.4. FBS were noted in Group B at 72 hours as 250.8 ± 1.7 and at Day 28 as 353 ± 2.5. FBS were noted in Group C at 72 hours as 252.8 ± 2.5 and at Day 28 as 181.3 ± 2.3.FBS were noted in Group D at 72 hours as 255 ± 1.8 and at Day 28 as 197.16 ± 2.5. The FBS level at 72 hours and at Day 28 in all group are shown in Table I.

When we compared the FBS at Day 28 in Group B and Group C, the results were statistically significant ($p<0.001$). The comparison of FBS at Day 28 in Group B and Group D also showed statistically significant results ($p<0.05$). The comparison of Group C and D with Group B is shown in Table II to IV.

Table I: Fasting blood glucose at 72 hours and day 28.

Treatment group	FBS AT 72 HOURS	FBS AT DAY 28
GROUP A: Control Group	115.3 ± 0.8	115.2 ± 1.4
GROUP B: Diabetes Induced Rabbits (Alloxan 120mg/Kg)	250.8 ± 1.7	353.5 ± 2.5
GROUP C: Rosmarinus Officinalis (250mg/Kg)	252.8 ± 2.5	181.3 ± 2.3
GROUP D: Glimepiride (2mg/Kg)	255 ± 1.8	197.16 ± 2.5

Table II: Fasting Blood Glucose at day 28 in Group B and Group C.

Treatment group	FBS AT DAY 28	P-VALUE
GROUP B	353.5 ± 2.5	P < 0.001
GROUP C	181 ± 0.3	

Table III: Fasting Blood Glucose at 28th day in group B and group D.

Treatment group	FBS AT DAY 28	P-VALUE
GROUP B	353.5 ± 2.5	P < 0.05
GROUP D	197.1± 2.5	

Table IV: Fasting Blood Glucose at 28th day in Group C & Group D.

Treatment group	FBS AT DAY 28	P-VALUE
GROUP C	181.3 ± 2.3	P < 0.05
GROUP D	197.1± 2.5	

Discussion

Diabetes mellitus is a significant metabolic disorder characterized by persistent hyperglycemia worldwide. Its management with conventional drugs is often limited by side effects and high cost. This has led to increasing interest in plant-based alternatives with proven antihyperglycemic potential. The present study was conducted to assess the glucose-lowering effects of rosemary (*Salvia rosmarinus*) in alloxan-induced diabetic rabbits, and the findings were comparable to those of the standard antidiabetic drug glimepiride. In this study, the mean fasting blood sugar (FBS) level at day 28 in the alloxan-induced diabetic group (353 ± 2.5 mg/dL) was significantly higher than the mean FBS at day 28 in the *Rosmarinus officinalis* (250 mg/kg) treated group (181.3 ± 2.3 mg/dL), with a statistically significant p-value ($p<0.001$). These findings suggest strong antihyperglycemic effects of *Rosmarinus officinalis* (250 mg/kg). Comparable findings were reported in a similar in vivo animal model study by Bakırel T et al.[16], who

administered different doses of *Rosmarinus officinalis* extract (ranging from 50 mg/kg to 200 mg/kg) in alloxan-induced diabetic rabbits and observed that the 200 mg/kg dose significantly reduced hyperglycemia while increasing serum insulin levels. Consistently, in the study conducted by Soliman GZ et al.,[17] streptozotocin (STZ)-induced diabetic rats were treated with dried rosemary in powdered form and noted that rosemary produced a significant reduction of 53.97% in fasting blood glucose levels among STZ-induced diabetics compared to controls, revealing beneficial anti-diabetic effect of rosemary, while improving lipid metabolic activities in diabetics. Similar, another study conducted by Malek A et al.[18] also reported comparable anti-diabetic effects of rosemary extract, measured among diabetic rats over the course of 36 days.

In the present study, the fasting blood sugar (FBS) at day 28 in the alloxan-induced diabetic group (353 ± 2.5 mg/dL) was considerably higher than in the glimepiride (2 mg/kg) treated group (197.16 ± 2.5 mg/dL), with statistically significant results ($p<0.05$). These findings indicate the strong efficacy of glimepiride in controlling hyperglycemia. In agreement, a pilot study by Oshkondali ST et al.[19] in alloxan-induced diabetic mice demonstrated that oral administration of glimepiride produced nearly a 50% reduction in fasting blood glucose, decreasing from 173 mg/dL to 93.67 mg/dL within 4 hours, indicating effective antihyperglycemic activity. Similarly, Mondol D et al.[20] reported that glimepiride administration in alloxan-induced diabetic rats significantly decreased glycemic levels from 19.45 ± 0.36 mmol/L to 16.03 ± 0.86 mmol/L by day 7, with further reduction to 14.73 ± 0.77 mmol/L at day 14 ($p<0.05$ and $p<0.01$, respectively). Consistent results were also observed in an animal study by Raja MA et al.[21], which confirmed the antihyperglycemic efficacy of glimepiride in alloxan-induced diabetic rats.

In current study, the comparison of FBS at Day 28 in Rosmarinus Officinalis (250mg/Kg) group (181.3±2.3) was substantially lower than that of Glimepiride (2mg/Kg) group at Day 28 (197.16 ± 2.5), with statistically significant differences between the groups ($p<0.05$). These findings suggest that Rosemary (Salvia Rosmarinus) has stronger anti-hyperglycemic effects than Glimepiride.

Aligning with these finding several animal studies conducted by Bakırel T et al.,[16] by Soliman GZ et al.,[17] by Oshkondali ST et al.,[19] and by Mondol D et al.[20] have reported that Rosmarinus Officinalis and Glimepiride both have shown high efficacy in glycemic control among alloxan-induced diabetic animals. However, to the best of our knowledge, this study is the first to show higher efficacy of Salvia Rosmarinus than Glimepiride

Despite well-established safety profile, in the studies conducted by Basit A et al[22] and Yoon KH et al[23] Glimepiride has shown risks of weight gain and Symptomatic hypoglycemia.[22,23] However, a study by Ghasemzadeh Rahbardar M et al.[24] reported that rosemary is generally safe when used as a food preservative, but caution is advised due to the potential negative effects of high and chronic doses on hepatic, renal, and reproductive functions. Although the antihyperglycemic efficacy of glimepiride has been supported by several large-scale and clinical trials, clinical evidence supporting the role of Rosmarinus officinalis as an antihyperglycemic agent is limited, with most studies being in vitro or in vivo.[23,24]

This limitation restricts the ability to establish a superior efficacy profile for Rosmarinus officinalis. Subsequently present study was also limited by its animal-based experimental design using very limited number of alloxan-induced diabetic animals, which may restrict direct clinical extrapolation to the humans. Hence further studies with larger samples, standardized Rosemary extract, long-term assessment of toxicity and molecular mechanistic investigations are suggested, to confirm these findings and establish *Salvia rosmarinus* as a viable adjunct antidiabetic therapy.

Conclusion

The conclusive findings of the study revealed a significant antihyperglycemic effect of *Rosmarinus officinalis* in alloxan-induced diabetic rabbits, with glucose-lowering effects comparable to glimepiride. Overall, the findings support its traditional antidiabetic use and emphasize its potential as a natural, cost-effective therapeutic alternative, particularly in resource-limited settings. However, long-term toxicity assessment and detailed molecular mechanism investigations are essential before its use in the diabetic population.

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