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Effects of *Lavandula Angustifolia* Hydroalcoholic Extract on the Blood and Urine Biochemical Factors of Diabetic Patients: A Placebo-Controlled Clinical Trial

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A B S T R A C T

Introduction: Diabetes mellitus remains one of the most prevalent metabolic diseases and a major health concern, despite the availability of a variety of synthetic drugs for its treatment. The multiple consequences of this disease, combined with the potential side effects of chemical treatments, led to the study of other therapies, such as the use of medicinal plants renowned for a variety of active components. Given the historic usage of medicinal plants for diabetes treatment, the aim of this study was to investigate the impact of lavender (Lavandula officinalis) hydroalcoholic extract on specific blood and urine biochemical factors in diabetic patients.

Material & Methods: In this double-blinding clinical trial, a total of 72 diabetes patients were divided into two groups: a control group that received a placebo and a treatment group that received the lavender hydroalcoholic extract. Capsules containing the lavender extract were formulated to be administered. The placebo capsules were given to the control group, while the lavender extract capsules were given to the treatment group twice a day, once in the morning and once at night, for two months. All patients underwent blood biochemical testing and urine analysis at the beginning and end of the trial. The data collected were analyzed using the SPSS 18 software.

Results: Compared to the placebo group, the mean HDL-C increased and the average levels of FBS, LDL-C, VLDL-C, TG, Chol, AST, and ALT became significantly lower ($P < 0.05$) in the patients who received capsules containing lavender extract. Likewise, the urine protein and sugar levels in the patients who received the lavender extract group were significantly lower ($P < 0.05$) compared to the control group. Yet, the average changes in BMI did not differ significantly between the groups receiving lavender extract and the placebo ($P > 0.05$).

Conclusion: The lavender plant with different phenolic and flavonoids compounds could reduce blood sugar and also improve lipid profile in people with diabetes.

Keywords: Diabetes Mellitus, Lavandula, Plant Extracts

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Introduction

Diabetes is one of the most prevalent and costly diseases worldwide. The prevalence of diabetes has been increasing because of changes in lifestyle and advancements in healthcare, leading to improved survival rates. About half of diabetic patients are unaware of their disease. Failure to effectively manage the disease contributes to an increase in the incidence of its consequences in the future (1, 2). Nowadays, type 2 diabetes is increasing in all countries, including Iran, where the incidence rate is roughly 4-4.5%, and surpasses 14% in the population aged >30 years. Furthermore, the disease is more prevalent in women than in men in all provinces (3). The number of diabetic patients in Iran exceeds 3 million, and it is estimated by the World Health Organization to reach 7 million people by 2030 (2).

Based on various characteristics shown by the disease, different types of diabetes have been identified. There are two main types of diabetes: type 1 (T1D) and type 2 (T2D). T1D is associated with insulin destruction due to autoimmune or idiopathic reasons, which results in the destruction of insulin-producing β-cells in pancreatic islets and impairment of insulin production. T2D is caused by the tissue's resistance to insulin, ultimately destroying pancreatic β-cells and impairment of insulin production (4, 5). The high prevalence of this disease along with its complications, such as nephropathy, retinopathy, neuropathy, and heart problems, result in disability and become a financial burden to the families and society. Therefore, it is necessary to make changes in therapeutic protocols and produce more efficient treatments.

A vast percentage of diabetes drugs are produced synthetically, and many of these drugs have numerous side effects. However, in recent decades, there has been growing interest in the use of plant-based drugs with anti-diabetic properties, due to their negligible side effects.

In the field of nutrition science, there is a great deal of discussion surrounding the consumption of natural and chemical food supplements, so proper eating habits and healthy lifestyles are extremely important in human life.

Lavandula officinalis belongs to the aromatic, evergreen plant that grows up to about 90 cm and is classified as part of the mint family. The essential oil derived from its fresh flowering shoots is extracted and widely used in various medical applications. This plant is commonly utilized for its fragrance in cosmetic and health products, with the flower being the primary medicinal component of the plant (6). Lavandula stoechas essential oils protect against diabetes and oxidative stress induced by alloxan treatment. These effects are in partly due to its potent antioxidant properties (7). Therefore, the aim of this study is to investigate the impact of hydroalcoholic extract of lavender (*Lavandula officinalis*) on certain blood and urine biochemical factors in diabetic patients.

Materials and methods

Ethical considerations

This project was registered under the ethics code IR.SKUMS.REC.1397.44. In this study, the objectives were initially presented to the patients referred to the study (IRCTID: IRCT20200826048534N1). Upon their agreement to participate, written consent forms were obtained from the enrolled patients, followed by the completion of questionnaires and collection of samples. The researchers ensured the strict confidentiality of all research data and results, and the patients were provided with the results upon their request or when the results were deemed important for further patient follow-up.

Preparation of lavender extract

To prepare the lavender extract, flowering plant shoots were collected from the Zagros mountain range in late spring and then dried in the open air under shade. The collected plant was botanically confirmed by preparing herbarium samples at the

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Research Center for Medicinal Plants, Shahrekord University of Medical Sciences. The samples were then powdered and soaked for the extraction process. Hydroalcoholic extract of the plant was prepared using a mixture of 70% ethanol and water in a ratio of 5:1. The mixture was kept at room temperature, away from direct sunlight for 2 days, and shaken daily 2-3 times. The obtained mixture was filtered by a cleaning textile and then by filter paper.

The filtrate was then concentrated using a rotary evaporator. The obtained extract was completely dried at a temperature of 21 °C and then stored in a frozen state at −20 °C until the experimental concentrations were prepared. Capsules (500 mg) were prepared from the obtained extract using a Technofix capsule filler. Among various excipients such as lactose, mannitol, and corn starch, the CMS phosphate excipient was chosen for the placebo as it was determined that neither the placebo nor the substance added to the plant dry extract would significantly affect diabetes. An appropriate volume of the CMS phosphate excipient was mixed with the plant extract, and empty capsules were filled with this mixture. Placebo capsules were filled with the same volume of this excipient. The capsules were filled with a defined dose using a hand-held Technofix capsule filler. This device enabled the manual filling of the capsules needed in this project according to hygienic standards. A pharmacologist cooperated in the formulation and capsule preparation stages, while one of the main co-authors supervised the extraction process and capsule filling. Based on previous animal studies, a defined extract dose of 16 mg/kg with an average weight of 65 kg was used in this study using the following formula:

$$\boldsymbol{HEDmg/kg = Animal\ dose\ mg/kg \frac{Animal\ km}{Human\ km}}$$

Intervention

The study population consisted of 72 diabetic patients referring to Emam Ali (as) and Hazrat Rasool (ph) clinics in Shahrekord city.

Each group included 36 patients. After a thorough assessment of the patient's medical history, physical examination, and laboratory tests, diabetes cases were diagnosed based on the following criteria:

- Fasting venous blood plasma sugar ≥ 126 mg/dl on two separate times

- Venous blood serum sugar ≥200 mg/dl 2 h

- Serum sugar ≥ 200 mg/dl at any time, accompanied by symptoms such as excessive thirst, overeating, and frequent urination in the patient.

The diagnosed diabetic patients were given necessary explanations, and then written consent forms were taken from those willing to participate in the study. Participating patients were randomly divided into two A and B groups using random allocation software. Each group received a total of 120 capsules for 2 months, along with the necessary training. Group A contained 36 diabetic patients who received two placebo capsules 2 times a day (in the morning and night) before meals, in addition to routine drugs (metformin or glibenclamide). Group B consisted of 36 diabetic patients who received two capsules containing lavender extract 2 times a day (in the morning and night), in addition to routine drugs (metformin or glibenclamide). This study was double-blinding research. Both the researcher and patients were not aware of the capsules' content and could not show any bias for the results of the study. Individual patients were contacted by telephone weekly to inquire about the drug usage procedure and their general conditions. Tests were examined two times, at the beginning (before drug administration) and end (at the end of the second month) of the study at a designated laboratory.

A venous blood sample (5 mg) was obtained from patients to measure various biochemical factors, including fasting blood sugar (FBS), lipid profile, blood urea nitrogen, creatinine, and HbA1C. Serum biochemical tests were conducted using commercial kits (Pars Azmoon Co.) with the assistance of an AutoAnalyzer. Urine protein and sugar levels were

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quantified using urine test strips. It is important to note that the groups were carefully matched in terms of age, gender, duration of diabetes, and the type of routine medications (metformin or glibenclamide) used by diabetic patients. The inclusion criteria for this study included newly diagnosed patients and those already receiving treatment with metformin and glibenclamide.

None of the patients received drugs other than metformin and glibenclamide at the beginning or during the study. None of the patients underwent insulin treatment. The exclusion criteria included individuals with kidney diseases, liver diseases except fatty liver, triglycerides exceeding 500, pregnancy, individuals taking anticoagulants (except aspirin), advanced diabetic retinopathy, hemorrhagic stroke, thyroid hyperactivity, and hypothyroidism.

Data Analysis

Collected data were analyzed using SPSS 18 software, employing descriptive statistics (frequency percentage, mean, and standard deviation) and analytical statistics (Chi-square test and paired/independent t-test) at a significance level of 0.05.

Results

In the present study, 72 diabetic patients were divided into two groups and received either the drugs or a placebo for 2 months. As illustrated in Table 1, the two groups were closely matched in terms of age, gender, and duration of diabetes, with no significant differences observed ($P > 0.05$).

Table 1. Comparison of Demographic Characteristics in the Studied Groups.

Variables		Groups		Sig.
		Drug	Placebo	
Gender Frequency (%)	Male	۱۷ (۴۲/۷)	۱۸ (۵۰)	0.814
	Female	۱۹ (۵۲/۸)	۱۸ (۵۰)	
#Age (year)		۵۰/۴۷ ± ۷/۵۶	۴۹/۷۲ ± ۷/۲۰	0.668
#Diabetes		۴/۰۳ ± ۳/۳۸	۳/۳۱ ± 2	0.274

#Mean ± standard deviation

Table 2 displays the results of serum biochemical factors measured in the two groups before and 2 months after the start of the study. Initially, all biochemical variables showed no significant differences between the two groups ($P > 0.05$). However, after the intervention, significant differences emerged. Fasting blood sugar (FBS), low-density lipoprotein (LDL-C), very-low-density lipoprotein (VLDL-C), triglycerides (TG), total cholesterol (Chol), aspartate transaminase (AST), and alanine transaminase (ALT) were notably lower in the drug-receiving group compared to the placebo group ($P < 0.05$). Moreover, the average level of high-density lipoprotein (HDL-C) was significantly higher in the drug-receiving group ($P < 0.05$).

In the drug-receiving group, average levels of all biochemical variables (excluding HDL-C) decreased significantly ($P < 0.05$), while HDL-C levels showed a significant increase ($P < 0.05$). Conversely, in the placebo group, statistically significant reductions were observed in all blood biochemical variables except for blood urea nitrogen (BUN) and creatinine ($P < 0.05$), with a significant elevation in average HDL-C levels ($P < 0.05$).

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Moreover, the mean changes in blood biochemical variables (except for HbA1c, Chol, and BUN) were significantly higher in the drug-receiving group compared to the placebo group ($P < 0.05$).

Table 2. Comparison of Serum Biochemical Factors in the Studied Groups.

Variables		Groups		Sig.
		Drug	Placebo	
HbA1c (%)	Before	8.13±1.14	8.17±1.01	۰/۲۱۲
	After	7.11±0.57	7.09±0.59	۰/۹۳۶
	Sig.	***≤0.001	***≤0.001	
	Difference	1.02±0.76	0.71±0.6	۰/۰۹۱
FBS (mg/dl)	Before	180.03±46.6	179.61±55.26	۰/۹۷۳
	After	119.64±19.77	142.03±21.57	***≤0.001
	Sig.	***≤0.001	***≤0.001	
	Variation	60.39±23.73	37.58±39.33	**۰/۰۱
LDL-C (mg/dl)	Before	106.42±37.47	114. 86±29.93	۰/۲۹۴
	After	83.56±23.57	102.92±21.08	***≤0.001
	Sig.	***≤0.001	***≤0.001	
	Variation	22.86±18.82	11.94±15.6	***۰/۰۰۹
HDL-C (mg/dl)	Before	41.92±7.22	42. 25±6.5	۰/۸۳۷
	After	50.08±5.66	44.5±4.67	***≤0.001
	Sig.	***≤0.001	***≤0.001	
	Variation	-8.17±6.62	-2.25±5.46	***≤0.001
VLDL-C (mg/dl)	Before	37.53±13.73	37.86±14.39	۰/۹۲
	After	25.86±7.62	30.86±10.03	*۰/۰۲
	Sig.	***≤0.001	***≤0.001	
	Variation	11.67±9.21	7.0±9.89	۰/۰۴۲
TG (mg/dl)	Before	185.53±67.1	185.81±70.61	۰/۹۸۶
	After	130.08±38.13	153.14±49.52	*۰/۰۳
	Sig.	***≤0.001	***≤0.001	
	Variation	55.44±39.93	32.67±45.79	*۰/۰۳۸
Chol (mg/dl)	Before	191.69±37.55	199.08±37.53	۰/۴۶۷
	After	158.81±36.83	177.5±25.6	***۰/۰۰۳
	Sig.	***≤0.001	***≤0.001	
	Variation	32.30±89.31	21.20±58.57	۰/۰۶۹
AST (U/L)	Before	31.17±13.38	30.86±9.83	۰/۹۱۲
	After	21.14±6.21	28.06±7.61	***≤0.001
	Sig.	***≤0.001	***≤0.001	
	Variation	10.03±9.9	2.81±4.69	***≤0.001
ALT (U/L)	Before	33.92±13.7	34.11±19.44	۰/۹۲۶
	After	22.58±6.82	29.89±9.5	***≤0.001
	Sig.	***≤0.001	***≤0.001	
	Variation	11.33±9.77	4.6±31.1	***۰/۰۰۱
Creatinine (mg/dl)	Before	0.95±0.14	0.9±0.12	۰/۱۵۱
	After	0.91±0.11	0.89±0.12	۰/۶۸۴
	Sig.	***≤0.001	0.381	
	Variation	0.05±0.06	0.01±0.05	**۰/۰۱
Urea (mg/dl)	Before	18.54±7.87	17.28±4.99	۰/۴۲
	After	16.48±4.34	17.0±3.63	۰/۵۸۵

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	Sig.	*۰/۰۲۳	0.694	
	Variation	2.06±5.2	0.28±4.21	۰/۱۱۵

*, **, and ***: P < 0.05, 0.01, and 0.001, respectively
HbA1c: Hemoglobin A1; FBS: Fasting Blood Sugar; LDL-C: Low-density lipoprotein; HDL-C: High Density Lipoprotein; VLDL-C: Very Low Density Lipoprotein; TG: Triglycerides; Chol: Cholestrol; AST: Aspartate Transaminase; ALT: Alanine Transaminase

The studied groups did not exhibit significant differences in the average body mass index (BMI) before and after the intervention (P > 0.05), as indicated in Table 3. Notably, the mean BMI decreased significantly in both the drug-receiving and placebo groups before and after the intervention (P < 0.05). Additionally, there was no significant difference in the mean BMI between the studied groups (P > 0.05).

Table 3. Comparison of Mean BMI Values in the Studied Groups.

BMI	Before	38.27±4.51	28.9±4.84	۰/۵۷
	After	28.07±4.43	28.68±4.69	۰/۵۷۲
	Sig.	*۰/۰۱۳	*۰/۰۲۹	
	Variation	0.0±2.46	0.22±0.58	۰/۸۸۳

Urine sugar levels were assessed in the studied groups before and after the study. Following the intervention, all patients in the drug-received group exhibited negative urine sugar levels. In contrast, individuals in the placebo group experienced a change from +++ to +. A significant association was observed between the urine sugar levels before and after the intervention in the placebo group (P < 0.05), underscoring the superior performance of the drug group compared to the placebo group, as detailed in Table 4.

Table 4. Comparison of Urine Sugar in the Studied Groups.

Groups	Sugar	-	+	++	+++	Sig.
Drug	After intervention -	۲۱(۱۰۰)	۹(۱۰۰)	۶(۱۰۰)	۰(۰)	-
Placebo	After intervention -	۲۱(۱۰۰)	۱۰(۱۰۰)	۲(۵۰)	۰(۰)	0.001***
	After intervention +	۰(۰)	۰(۰)	۲(۵۰)	۱(۱۰۰)	

The measurements of urine protein levels before and after the intervention revealed that initially, four cases in the drug group exhibited positive urine protein levels, which later transitioned to negative after the intervention. Notably, both the drug group and the placebo group displayed a significant difference between urine protein levels before and after the intervention (P < 0.05), as outlined in Table 5.

Table 5. Comparison of Urine Protein in the Studied Groups.

Groups	Protein		Before intervention		Sig.
			-	+	
Drug	After intervention	-	۳۱(۱۰۰)	۴(۸۰)	*0.012
		+	۰(۰)	۱(۲۰)	
Placebo		-	۳۲(۱۰۰)	۲(۵۰)	***0.000

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	After intervention	+	۰(۰)	۲(۵۰)	

*, **, and ***: P < 0.05, 0.01, and 0.001, respectively

Discussion

This study involved 72 diabetic patients divided into two groups: the first group received lavender hydroalcoholic extract (500 mg capsules), while the second group received a placebo for a duration of 2 months. The findings revealed significant reductions in serum levels of LDL-C, VLDL-C, TG, Chol, ALT, AST, urea nitrogen, and creatinine in the lavender extract-receiving group, alongside decreases in urine sugar and protein, and an increase in HDL-C levels. While similar results were observed in the placebo group, the changes in biochemical factors were notably higher in the extract-receiving group compared to the placebo group. Notably, the results observed in the placebo group were attributed to the use of anti-diabetes drugs.

Based on the differing changes observed between the extract-receiving and placebo groups, it can be inferred that lavender hydroalcoholic extract may enhance the anti-diabetic effects of drugs utilized by the patients.

Lavender encompasses a variety of compounds, including acetate (30-55%), linalool (20-35%), beta ocimene, synthol, camphor, sesquiterpene, tannins, rosmarinic acid derivatives, coumarin, and flavonoids. Notably, it possesses robust antioxidant properties, capable of reducing free radical production within cells. Flavonoids, among other compounds found in lavender, play a pivotal role in inhibiting lipoprotein oxidation and enhancing cellular stability (8).

The current study unveiled the significant potential of lavender hydroalcoholic extract in reducing blood sugar levels. Its anti-diabetic effects appear to stem from increased serum insulin activity, stimulation of insulin secretion, and elevation of insulin release from its bound form (9-11). Moreover, the utilization of protein as the final fuel source for cells was noted. Treatment with lavender extract led to a decrease in urine protein levels, suggesting a shift towards glucose utilization as the primary energy source, consequently reducing urine protein levels as cells predominantly utilize glucose for energy. Furthermore, in our research, serum levels of LDL-C, TG, Chol, and VLDL-C demonstrated a decline with lavender extract treatment, consistent with findings reported by Rabiee et al. (2014).

The antioxidant properties of lavender contribute to the reduction of free radical formation, consequently lowering serum LDL-C levels. Flavonoids, which are water-soluble antioxidants found in lavender, inhibit LDL-C oxidation, thus mitigating the risk of vascular blockage diseases (12). Extracts from plants rich in flavonoids have been shown to decrease serum levels of TG, Chol, and LDL-C (13). Additionally, vitamin C, another water-soluble antioxidant present in lavender, has been reported to decrease LDL-C oxidation (14-15).

A study by Torabzadeh et al. (2017) demonstrated that intraperitoneal injection of lavender aqueous extract for 15 days resulted in reductions in serum levels of TG, Chol, VLDL-C, and LDL-C, while increasing HDL-C levels in mice (16), which corroborates with our findings. Subsequent studies have indicated that flavonoids in lavender can reduce serum lipid profile levels. Flavonoids, being hydrophilic, scavenge free radicals, act as chelating agents, and protect vitamin E, beta-carotene, and lycopene in LDL-C molecules, or enhance paraoxonase activity, thereby facilitating the hydrolysis of oxidized lipids. Phenolic compounds have also been reported to shield LDL-C from oxidation (17).

Furthermore, Saei et al. (2015) demonstrated in animal models that induction of diabetes increased serum glucose levels, caused liver damage, and

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elevated serum levels of ALT, AST, and ALP enzymes. However, intraperitoneal injection of lavender hydroalcoholic extract for 10 days significantly reduced blood sugar levels almost to control group levels and normalized liver enzyme levels. As previously mentioned, diabetes induction leads to liver damage, primarily due to the production of free radicals and reactive oxygen species (ROS). The antioxidant properties of lavender hydroalcoholic extract have been shown to significantly reduce ROS levels and enhance antioxidant enzyme activity, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX), thus ameliorating diabetes-induced liver injury (Saei et al., 2015). These beneficial effects were attributed to the high concentrations of phenolic and flavonoid compounds present in lavender (18).

Superoxide dismutase (SOD) and catalase (CAT) are recognized as key antioxidant enzymes combating ROS, and their reduction in diabetic animal models indicates diabetes-induced liver damage (19). Azarmi et al. (2016) reported that administration of lavender hydroalcoholic extract increased SOD and CAT activities in the liver, thereby protecting against diabetes-induced liver injury. This protective mechanism is crucial as diabetes induces oxidative stress and lipid peroxidation of the cell membrane (20).

Conclusion

In conclusion, the lavender hydroalcoholic extract shows promise in enhancing the anti-diabetic effects of medications used by patients, potentially increasing their effectiveness and improving overall treatment outcomes in diabetes management. The diverse phenolic and flavonoid compounds present in lavender offer a multifaceted approach to diabetes treatment. These compounds not only contribute to reducing blood sugar levels but also demonstrate the ability to improve lipid profiles in individuals with diabetes. Therefore, incorporating lavender extract as a complementary therapy may offer additional benefits in the management of diabetes.

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Conflict of interest

The authors declare no conflict of interest.

Authors' contributions

LM conceived and designed the study, contributed to data acquisition and interpretation, and drafted the manuscript.

SH contributed to the conception of the study, interpretation of data, and drafting of the manuscript.

ZF participated in drafting and critically revising the manuscript.

MS conceived and designed the study, abstracted and analyzed data.

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