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ORIGINAL RESEARCH

Beneficial Synergistic Effect of Supplementation with Combined M. Oleifera and L. Speciosa Leaf Capsules in Patients with Type 2 Diabetes

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ABSTRACT

Diabetes mellitus is a metabolic non-communicable chronic disease in which the body's ability to process glucose is impaired, resulting in higher blood glucose levels. If not controlled, this disease can lead to severe complications. Various plants with medicinal properties can treat chronic diseases, such as diabetes, due to therapeutically important phytochemicals and bioactive compounds.

This study investigated the effect of antidiabetic medicinal plant leaves in controlling blood glucose levels among Wakhi diabetic patients in the Hunza district of Pakistan. The "Wakhis" are an ethnic group living in the remote region of Pakistan.

The method used in this study was a placebo-controlled study. Leaf powders of Moringa oleifera and Lagerstroemia speciosa were filled in capsules of 1000 mg each. The antidiabetic potential of the medicinal plants was estimated by performing the biochemical blood profiles of diabetic patients before and after the trial. After the intervention, the level of glycated hemoglobin (HbA1c) reduced from 8.3±1.8 to 7.5±1.2% significantly (P = 0.0401). The fasting blood sugar (FBS) in the intervention group reduced significantly from 191±49 mg/dL to 169±41 mg/dL (P = 0.0041) after the trial.

According to the findings of this study, the combined herbal capsules effectively controlled the blood sugar of type 2 diabetic patients.

Conclusively, the combination of M. oleifera and L. speciosa have the potential to lower blood sugar levels supplementary to existing regimens.

Further investigation is warranted to explore the mechanism resulting in the observed reduction in sugar levels in type 2 diabetic patients.

Keywords: Type 2 Diabetes; Wakhi; Medicinal plants.

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INTRODUCTION

Diabetes mellitus is a chronic disease that occurs due to aberration in insulin function or defects in its secretion. In a diabetic person, the metabolism of lipids, carbohydrates, and proteins is dysregulated (Mingrone & Castagneto-Gissey, 2014). Different factors have been attributed to the global increase in the prevalence of diabetes, including being overweight, having a sedentary lifestyle, physical inactivity, poor eating habits, and urbanization. As a significant cause of mortality and morbidity, diabetes poses a challenge to public health and a great socioeconomic burden for the developing world. Pakistan, like other developing countries, is also facing a sharp increase in the prevalence of diabetes. The prevalence of diabetes in Pakistan is 14.6%, based on 49,418 individuals suffering from diabetes (Akhtar et al., 2019). Worldwide, the number of diabetics is estimated to be 693 million, from 451 million in 2017 (Cho et al., 2018). The life expectancy for people with type 2 diabetes decreases by ten years (Huizen, 2022). According to different population-based studies and surveys conducted at the national level, the overall ratio of diabetes is estimated as 17.15% in rural and 22.04% in urban regions. The statistics for diabetes in different provinces of Pakistan are as follow: Balochistan 10.8% in both male and female, Punjab; 19.3% in female and 16.6% in male, Sindh; 11.7% in female and 16.2% in female and Khyber Pakhtunkhwa has 11.1% diabetes in both genders (Bahadar et al., 2014).

Traditional herbal medicines are re-emerging as a substitute for chemically synthesized drugs in developed and developing countries due to their diversity, abundance, and fewer side effects. The biggest challenge for medical researchers and practitioners is applying treatment methods while limiting side effects. Herbal treatments for type 2 diabetes have been used for a long time. Using plants as alternative medicines exploits their bioactive compounds for therapeutic effect. Polyphenols present in various plant sources have the potential to control the metabolism of carbohydrates and lipids, reduce the high glucose level in blood and insulin resistance, lower dyslipidemia, and hinder the onset of diabetic complications caused by glycation (Bahadoran et al., 2013). M. oleifera and L. speciosa have been used to treat diabetes in the past. Moringa oleifera is one of the most important species of the Moringaceae family. Moringa leaves are known to contain a variety of bioactive compounds involved in the homeostasis of lipids. Flavonoids and phenolic compounds play a crucial role in regulating lipids in the human body (Siasos et al., 2013). Due to the presence of high phytoconstituents and bioactive compounds, Moringa oleifera is studied to reduce serum cholesterol, triglycerides, phospholipids, very low-density lipoprotein (VLDL), and low-density lipoproteins (LDL). This activity results from inhibiting pancreatic cholesterol esterases (Adisakwattana & Chanathong, 2011). Moringa oleifera leaves contain various types of compounds and nutrients that affect the homeostasis of sugar in the body.

Isothiocyanates are one example that is said to lower the resistance of insulin and also affect gluconeogenesis in the liver (Keshri et al., 2021).

Lagerstroemia speciosa which belongs to the Lythraceae family and is locally called Banaba in the Philippines is an important plant for treating diabetes. This plant can grow up to 30 m in height, and its width ranges from 9 to 12 m. Different parts of this plant, such as flowers, bark, and leaves, are used to treat various diseases in many parts of the world. Both herbal medicines, and those isolated from plant sources, due to the presence of useful phytochemicals, give them the potential to act against many diseases (Choudhary et al., 2015). For treating many chronic diseases, plants such as Lagerstroemia speciosa play a vital role and have many medicinal properties (Al-Snafi, 2019). Different compounds present in Banaba leaves are responsible for its insulin-like activity, such as tannins and corosolic acid. These compounds improve the uptake of glucose through activation of tyrosine kinase insulin receptor while inhibiting tyrosine phosphatase similar to insulin, earning it the name "natural plant insulin" (Park & Lee, 2011). The insulin-like activity of Banaba is also attributed to the presence of ellagitannins, and tannins as suggested by some studies. These are thought to regulate glucose and antioxidant properties (Klein et al., 2007). Banaba leaves have a large concentration of corosolic acid, a compound with potential hypoglycemic properties, and beneficial tannins. The dried leaves of Banaba are popularly known to treat diabetes mellitus in the Philippines (Judy et al., 2003).

Few clinical studies have reported the antihyperglycemic properties of M. oleifera and L. speciosa in patients with type 2 diabetes. Furthermore, different factors support the antidiabetic potential of both herbal plants such as high costs of conventional drugs and treatment, preclinical studies, fewer side effects of the medicines from herbal sources, and different empirical experiences. It is necessary to study the combined antihyperglycemic activity of M. oleifera and L. speciosa in type 2 diabetic patients.

AIMS OF THE STUDY

This study investigated the effect of antidiabetic medicinal plant leaves in controlling blood glucose levels among Wakhi diabetic patients in the Hunza district of Pakistan. The "Wakhis" are an ethnic group living in the remote region of Pakistan.

METHODOLOGY

This study was approved by the Institutional Review Board of the University of Agriculture, Faisalabad, Pakistan with reference number 3012/ORIC. All the patients completed an informed consent before starting the study. The study was conducted between May 2019 to September 2019.

Study Setting: This study was conducted on the Wakhi population in Gojal, a subdivision of District Hunza in northern Pakistan. Northern Pakistan is an area of geographical and ethnic diversity and is among the world's most multilingual places. In the past, the people of Gojal were very active due to agro-pastoral activities, farming, growing crops and extensive livestock consisting of goats, sheep, cattle, and yaks. Due to active lifestyle, organic and non-obesogenic food prevalence of chronic diseases was rare. However, a drastic change has occurred in different areas of life due to advancements in technology, an increase in urbanization and dependency on commercialized products, which has resulted in a sedentary lifestyle with a decrease in physical activities, leading to chronic illnesses like diabetes mellitus. No previous studies on diabetes have been conducted among the Wakhis.

Study Design and Procedure: A cohort of 40 patients with type 2 diabetes were enrolled in this double-blind clinical study after providing written informed consent. The inclusion criteria of active treatment for type 2 diabetes, HbA1c greater than 7, more than four years of history of the disease, having fasting blood sugar level >140mg/dL, and age group of 30-60 years were applied. Patients with comorbidities, including impaired liver function, kidney issues or cardiac problems, were excluded from the study. A questionnaire was used to collect the demographic characteristics of the patients. Over an initial 2-week period, participants were screened for their history of diabetes and underwent physical examination including body weight, blood pressure, and biochemical blood profie, including fasting blood sugar, liver function tests, renal function tests, and glycated hemoglobin. Participants that met the inclusion criteria were briefed regarding the study, its purpose, and its procedure. Participants were randomly allocated to either the control group (receiving placebo: starch powder) or intervention group (who received the herbal capsules). Both herbal and placebo capsules were identical in color and shape. All enrolled participants continued their metformin as prescribed. Blood samples were taken after 12 hours of overnight fasting. Samples were taken before and after the study to compare the impacts of the intervention. Patients in the intervention group took 1000 mg containing M. oleifera and L. speciosa before breakfast and control group received placebo at the same time.

Identification and Collection of Plant leaves: Leaves of both M. oleifera and L. speciosa were harvested from Faisalabad. The leaves were identified by the head botanist of the Department of Botany at the University of Agriculture in Faisalabad Pakistan. The leaves were washed in tap awter and soaked in 1% saline solution for ten minutes to remove microbes. They were again washed by ethanol followed by thrice washing with distilled water. It helped removing pathogens and microbes. The leaves were sun dried and then grinded by pulmonizer. O.8 mm size screen was used to separate the fine powder of both the leaves (Mishra et al., 2012).

Fig 1: Leaf Powder of Moringa oleifera and Lagerstroemia speciosa

Capsules: The herbal capsules containing leaf powders of Moringa oleifera and Lagerstroemia speciosa were prepared as per the manufacturing practice guidelines. The leaf powders were filled into empty capsules. The capsules contained 1000 mg M. oleifera and 1000 mg L. speciosa leaf powders. The placebo capsules contained starch powder. Each capsule was in the same color and size to that as the herbal capsules.

Statistical Analysis:

SPSS version 18 was used for analyzing the data. Independent and paired t-tests were also done on SPSS. The differences in the blood glucose parameters were determined by ANOVA. One-way ANOVA was used and for all the analyses. $P < 0.05$ was considered to be significant statistically.

RESULTS

Subject Characteristics:

A total of 40 diabetic patients were selected for the study from the Wakhi ethnic group in the Hunza district, Pakistan which included 20 male and 20 female diabetic patients. The participants were randomly allocated into two groups: intervention group and placebo. Subject characteristics are summarized in Table 1.

Table (1): Subject characteristics

Variable		Treatment Group N (%)	Placebo Group N (%)	Total N (%)
Gender	**Men**	11 (27)	7 (18)	18 (45)
	Women	9 (22)	13 (33)	22 (55)
Job	**Employed**	14 (35)	9 (22)	23 (57)
	Unemployed	11 (27)	6 (16)	17 (43)
Duration of Diabetes	**<4 years**	6 (15)	3 (7)	9 (22)
	>4years	14 (35)	17 (43)	31 (78)

Effect of the Herbal Medicines on the HbA1c and Fasting Blood Sugar

The mean and standard deviation (±SD) of FBS and HbA1c are summarized in table 2. The HbA1c in the intervention group significantly decreased from 8.3±1.8% to 7.5±1.2% at the end of the study (P = 0.0401). The fasting blood glucose level was 191±49 mg/dL at the beginning of the study which was reduced significantly to 169±41 (P= 0.0041). Both HbA1c and FBS had non-significant difference before and after the clinical intervention in the placebo group. No patient reported any adverse effects of the medicine.

Table (2): Comparison of HbA1c between placebo and treatment groups before and after the intervention

Variable		Treatment	Placebo	Mean	P
Mean ±SD HbA1c (%)	**Before Intervention**	8.3±1.8	7.8±1.3	8.05±1.5	0.05
	After Intervention	7.5±1.2	7.7±1.4	7.6±1.3	0.06
	P value	0.0301	0.72	0.005	
Mean ±SD FBS (mg/dl)	**Before Intervention**	191±49	161±46	176±47	0.41
	After Intervention	169±41	159±48	164±44	0.33
	P value	0.0041	0.62	0.53	

Other Biochemical Parameters

At the start of the study, the biochemical blood profile of patients was screened by collecting blood samples and performing laboratory tests from the serum after centrifugation. A significant reduction was seen in FBS, HbA1c and RBS. Table 3 summarizes the changes in the blood parameters before and after the intervention. No reduction was observed in the hemoglobin and body weight of the patients.

Table (3): Changes in the blood parameters of Intervention Group before and after the clinical trial

Change (before medicine – final result)	Treatment	N	Mean	SD	SE	t-value	P-value
Change in RBS (mg/dl)	Before medicine	20	337.70	80.35	17.97	2.37**	0.0227
	After 4 months	20	282.35	66.38	14.84		
Change in HbA1c	Before medicine	20	8.33	1.51	0.34	2.13**	0.0301
	After 4 months	20	7.77	0.97	0.22		
Change in hemoglobin (g/dl)	Before medicine	20	14.99	1.53	0.34	0.05NS	0.9591
	After 4 months	20	14.97	1.53	0.34		
Change in body weight (kg)	Before medicine	20	71.75	12.84	2.87	0.77NS	0.4467
	After 4 months	20	68.85	10.93	2.44		
Change in FBS (md/dl)	Before medicine	20	191.10	39.84	8.91	3.06**	0.0041
	After 4 months	20	169.25	38.42	8.59		
Change in Cholesterol (mg/dl)	Before medicine	20	224.05	116.38	26.02	1.01*	0.0499
	After 4 months	20	199.75	129.87	29.04		

NS = Non-significant (P>0.05); * = Significant (P<0.05); ** = Highly significant (P<0.01) (SD = Standard deviation, SE = Standard error)

DISCUSSION

After four months of the clinical trial, it was observed that the percentage decrease in hemoglobin A1c level was 9%. The decrease in HbA1c was significant, with a P-value of 0.0401. A study conducted by Taweerutchana et al. shows an insignificant decrease in the HbA1c of patients who were given Moringa leaf capsules (Taweerutchana et al., 2017). Another study conducted by Shanker et al. shows an 8% decrease in HbA1c by using Moringa for 24 weeks (Shanker et al., 2018).

The mean reduction in the HbA1c might be attributed to the bioactive compounds present in both leaf species. In a previously conducted study in which Moringa oleifera capsules were given for 90 days, glycated hemoglobin that is HbA1c was decreased from 7.8±0.51 to 7.40±0.63 and this reduction was highly significant (Tamang et al., 2021) A highly significant decrease was observed in the fasting blood sugar of both experimental groups, 12% decrease in FBS with a P-value of 0.0041. Comparing the mean value reduction of FBS with the prior studies, a study conducted by Ikeda et al. found a 16.6% reduction in the FBS of the patients who were given Banaba extract for six months (Ikeda, Y. et al., 2002). Another study by Tschuchibe et al. shows a 12% reduction in the FBS of non-diabetic subjects who were given capsules containing Banaba extracts with no adverse side effects (Tsuchibe et al., 2006). Shankar et al. (2019) conducted a study using moringa extract capsules, and a significant reduction in the blood glucose level was observed.

Random blood sugar was observed to decrease 14% during the three-month clinical trial. Kushwaha et al. conducted a study to evaluate the effect of Moringa and found a significant reduction in the blood glucose level with a reduction from 125.6 ± 9.15 to 106.7 ± 7.23 mg/dL (Kushwaha et al., 2014). The present study found a significant reduction (P<0.05) in random blood sugar, fasting blood sugar, HbA1c, and serum cholesterol. In a review study by Vargas-Sanchez et al., only six published studies have been found regarding the effect of Moringa on glycemic patients (Vargas-Sánchez et al., 2019). Several studies showed that MO and LS have the potential to lower glucose metabolism, but no study has been conducted to examine the combined effect of these plant leaves. Lagerstroemia speciosa leaves are known to cause a significant decrease in patients with type 2 diabetes (Feinberg et al., 2019). A Cochrane review from 2012 reported a significant decrease in HbA1c of type 2 diabetic patients using Moringa oleifera leaf extract. The possible mechanism underlying the antidiabetic activity of Lagerstroemia speciosa are peptide analogs or insulin-like activity, increasing glucose transport, activation of glucose transporter, and inhibition of alpha glucosidase and alpha amylase (Nurcahyanti et al., 2018). The possible mechanism for the antidiabetic activity of Moringa leaves include increasing insulin secretion, lowering gluconeogenesis in the liver, inhibition of glucose uptake from the intestine, enhancing glucose uptake in muscles, and inhibition of alpha glucosidase and alpha amylase activity (Ahmad et al., 2019). The results of this study showed that supplementation with both MO and LS leaf might be beneficial in type 2 diabetes.

Conclusion and future Perspective

Due to their beneficial properties, different compounds of Moringa oleifera and Lagerstroemia speciosa may be used as pharmacological agents in type 2 diabetes. The status of these plants is non-toxic and could be used as an alternative for conventional medicines in diabetic patients. Different studies have analyzed and confirmed the antihyperglycemic property of these plants.

Conclusively, M. oleifera and L. speciosa capsules containing leaf powder significantly lowers the plasma glucose level in patients with type 2 diabetes. These results suggest that the plant might be used for the treatment of diabetes. This preliminary trial has generated results that warrant follow-up with a larger cohort. For future investigations, studies can be conducted for a longer time period to learn the impacts of the medicines thoroughly, and research should be encouraged to explore the exact phytoconstituent of each plant and mechanism underlying the reduction in blood glucose level.

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