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Investigation of the Effect of Hydroalcoholic Extract Kelatin (*Glaucium flavum*) on Type 2 Diabetes: A randomized Double-Blind Clinical Trial

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Abstract

Background: Diabetes is a chronic metabolic disease characterized by high blood glucose levels. It can be treated through diet, physical activity, and medication. Insulin and antidiabetic medications are commonly used, but alternative treatments such as medicinal plants have also been studied. kelatin with the scientific name of *Glaucium flavum* has chemical compounds of this plant including phytol, tricosan, 2-pentadecanone, and heneicosan. As a result, sesquiterpene hydrocarbons were shown as the main group of the essential oils of the species. This study sought to determine the effects of Hydroalcoholic Extract Kelatin (*Glaucium flavum*) on Type 2 Diabetes.

Method: This is a randomized controlled clinical trial in which 66 diabetic patients were randomly divided into two groups. The experimental group patients received kelatin capsules for 60 days. Patients in control group received placebo capsules containing starch. Fasting blood sugar (FBS), hemoglobin A1c (HbA1c), insulin, high-density lipoprotein (HDL), low-density lipoprotein (LDL), Triglyceride, Cholesterol, Creatinine and Urea were measured before and after study

Results: The results of study show that, despite a significant decrease in FBS, HbA1c, Triglyceride, Cholesterol, HDL and Creatinine level before and after the study in experimental group, there were no significant difference in most variables between experimental and control group after intervention .

Conclusion: In this study there was no significant difference in most variables after intervention. This result could be due to small sample size.

Introduction

Diabetes is a chronic metabolic disease characterized by high blood glucose levels due to anomalies in carbohydrate, protein, and fat metabolism [1]. It is categorized into different types, with Type 1 and Type 2 being the most common [2]. Type 2 diabetes is prevalent and often associated with obesity and a sedentary lifestyle [3]. Type 1 diabetes is known as an autoimmune disease. The body's immune system mistakenly attacks and destroys the beta cells in the pancreas that produce insulin. As a result, the body is unable to produce enough insulin. Type 2 diabetes is characterized by insulin resistance and gradual failure of insulin production by the pancreas. In type 2 diabetes, the body's cells respond less to insulin, which is called insulin resistance [1-3].

It is important to diagnose and treat diabetes early to avoid complications. Diabetes is a common disease that can cause various complications, including heart disease, retinopathy, neuropathy, nephropathy, acid ketosis, Alzheimer's disease, depression, and neurological damage [4]. It's responsible for millions of deaths worldwide and affects the quality of life of those who suffer from it [5]. The treatment of diabetes involves controlling blood sugar levels through diet, physical activity, and medication such as insulin and anti-diabetic drugs [6]. Medicines for the treatment of diabetes include insulin, exenatide, liraglutide, semaglutide, pramlintide, sulfonylureas, biguanides, thiazolidinediones, etc. These treatments can have side effects such as low blood sugar, hypersensitivity reactions, blurred vision, gastrointestinal disorders, and weight gain. Insulin resistance may also be a problem in diabetes treatment [7].

In the past 20 years, various therapies have been used to treat diabetes mellitus (T1DM), each with different metabolic outcomes and clinical complications [8]. Traditional medicine has a cure for every disease using medicinal plants. The history of using medicinal plants to treat diabetes is very long. Medicinal plants are widely used for the treatment of diabetes due to fewer side effects [8]. While insulin and hypoglycemic drugs remain the main treatment options for diabetes mellitus, they have numerous adverse effects [9]. Medicinal plants and their derivatives have been proposed as a treatment for diabetes since ancient times [10]. However, the exact side effects of these treatments are not fully understood [11]. Nevertheless, there is a growing interest in finding effective combinations of medicinal plants to treat diabetes with fewer side effects [12]. Herbal medicines have gained importance as a source of blood sugar drugs in recent years. More than 1,000 plant species are used as folk medicine for diabetes [13]. The biological effects of these herbal products are related to their chemical composition [14]. Mountain

Anemone species have alkaloid compounds that are widely used in the pharmaceutical industry [15]. Anemone has properties such as narcotics and pain relief, stopping bleeding, and is used in the treatment of diabetes [16]. A plant called kelatin local is used to treat diabetes by the indigenous people of Fars province, Iran [17].

kelatin with the scientific name of *Glaucium flavum* has chemical compounds of this plant including phytol, tricosan, 2-pentadecanone, and henicosan. As a result, sesquiterpene hydrocarbons were shown as the main group of the essential oil of the species [17]. *Glaucium flavum* belongs to the Papaveraceae family and is a plant rich in alkaloids including aporphine, protopine, and protoberberine. In traditional medicine, it is used as a painkiller, anti-congestion, and cough. It is a perennial plant and has earring leaves. It grows in Fars and Mazandaran provinces in Iran [17].

Our aim in this study was to identify effective and safe concepts for managing diabetes in patients with minimal complications. Due to the potential side effects of oral hypoglycemic drugs, herbal medicines have gained attention as an alternative treatment for diabetes [18]. These plant-based products contain various chemical compounds, such as phenolic compounds, flavonoids, terpenoids, and coumarins, that help to regulate blood sugar levels. Hence, herbal products have shown promising biological effects in the management of diabetes [16].

This study sought to determine the effects of Hydroalcoholic Extract Kelatin (*Glaucium flavum*) on Type 2 Diabetes.

Methods

This double-blind, randomized clinical trial was conducted on 66 patients with type 2 diabetes. The inclusion criteria were age over 18 years, not taking insulin, not known immunodeficiency, creatinine less than 2, no history of diabetic ketoacidosis and no class 3, 4 cardiovascular disease. Exclusion criteria were allergy to the Kelatin, change in type of treatment and appearance of symptoms of hypoglycemia. Patients were randomized into two experimental and control group using block randomization method. In the experimental group the patients received kelatin capsules for 60 days (three capsules per day). Each kelatin capsule contained 500 mg standardized extract of kelatin (capsules preparation is summarized in table 1).

In control group patients received capsules containing starch for 60 days. Capsules were similar in color, shape and size in two groups. Before and after the intervention, Fasting blood sugar (FBS, mg dL⁻¹), hemoglobin A1c (HbA1c, %), Insulin (μU mL⁻¹), High-density lipoprotein (HDL, mg dL⁻¹), Low-density

lipoprotein (LDL, mg dL⁻¹), Triglyceride (mg dL⁻¹), Cholesterol (mg dL⁻¹), Creatinine (mg dL⁻¹), and Urea (mg dL⁻¹) were measured.

Data analysis

Data were analyzed in SPSS (Statistical Package for the Social Sciences, version 20) using descriptive statistics (mean, standard deviation, and percentage) and analytical tests (Wilcoxon test, Friedman test, and Kolmogorov–Smirnov test). The significance level was set at $p < 0.05$.

Ethical consideration

The ethics code was received from the Ethics Committee of Mazandaran University of Medical Sciences (REC NO: IR.MAZUMS.REC.1400.8815) on Oct 26, 2021. The trial was registered at the Iranian Registry of Clinical Trials (IRCT) (registration number IRCT20210724051965N1) on 2022-01-18. A written informed consent was obtained from all patients and all the steps of study were in accordance with the Helsinki declaration.

Sr No.	Step
1	Drug collection and preparation
2	Preparation of hydro alcoholic extract from the plant
3	Determination of phenol and flavonoid levels of mountain anemone hydro alcoholic extract of plant
4	Capsule opening time test
5	Test of Appearance Characteristics of Capsules

Table 1: Steps of capsules preparation

Results

In this study, the Wilcoxon test showed a significant difference in terms of Fasting Blood Sugar (FBS), hemoglobin A1c (HbA1c), Triglyceride, Cholesterol, High-density lipoprotein (HDL), and Creatinine levels in the experimental group before and after the intervention (Table 2).

Clinical criteria	P value	Mean Difference	95% Confidence Interval of the Difference	
			Upper	Lower
Fasting blood sugar (FBS) (mg dL ⁻¹)	0.001	-1.6667	157.00	155.333
Glycosylated hemoglobin (HbA1c) (%)	0.001	-0.303	8.052	8.021
Insulin level (μU mL ⁻¹)	0.321	-0.2455	10.233	9.988
Triglyceride (mg dL ⁻¹)	0.001	-11.1818	220.121	208.939
Cholesterol (mg dL ⁻¹)	0.001	0.7576	221.424	222.182
LDL (mg dL ⁻¹)	0.904	4.1333	97.455	101.588
HDL (mg dL ⁻¹)	0.013	-3.5394	85.145	81.606
Urea (mg dL ⁻¹)	0.131	-0.515	16.451	16.409
Creatinine (mg dL ⁻¹)	0.004	0.0242	1.142	1.167

Table 2: Variables measurements before and after intervention in experimental group.

The Wilcoxon test also showed a significant difference in terms of Fasting Blood Sugar (FBS), Insulin level, low-

density lipoproteins (LDL), and Triglyceride in the control group before and after the intervention (Table 3). The results of the study (Friedman test) showed a significant difference in terms of Insulin, Triglyceride, Cholesterol, and High-density lipoprotein (HDL) levels between the experimental and control groups after the intervention (Table 4).

Clinical criteria	P value	Mean Difference	95% Confidence Interval of the Difference	
			Upper	Lower
Fasting blood sugar (FBS) (mg dL ⁻¹)	0.004	40.2061	142.006	182.212
Glycosylated hemoglobin (HbA1c) (%)	0.216	1.3394	7.776	9.115
Insulin (μU mL ⁻¹)	0.001	0.8030	7.361	8.164
Triglyceride (mg dL ⁻¹)	0.001	37.9455	164.727	202.673
Cholesterol (mg dL ⁻¹)	0.468	31.9394	162.485	194.424
LDL (mg dL ⁻¹)	0.001	0.6212	104.458	105.079
HDL (mg dL ⁻¹)	0.186	7.1727	48.936	56.109
Urea (mg dL ⁻¹)	0.117	2.0818	17.591	19.673
Creatinine (mg dL ⁻¹)	0.458	0.1348	0.945	1.080

Table 3: Variables measurements before and after intervention in control group.

Ensemble		Control group Mean rank	Intervention group Mean rank	P value
Control group	(FBS) (mg dL ⁻¹)	6.91	7.35	0.07
	(HbA1c) (%)	2.42	2.52	0.07
	Insulin (μU mL ⁻¹)	3.12	2.55	0.001
	Triglyceride (mg dL ⁻¹)	8.39	8.06	0.001
	Cholesterol (mg dL ⁻¹)	8.55	8.05	0.01
	LDL (mg dL ⁻¹)	5.64	6.39	0.1
	HDL (mg dL ⁻¹)	5.33	5.09	0.001
	Urea (mg dL ⁻¹)	3.64	3.88	0.26
	Creatinine (mg dL ⁻¹)	1.00	1.12	0.69

Table 4: Comparison of the mean rank of variables before and after the intervention in the experimental and control groups.

Discussion

The results of the study show that, despite a significant decrease in FBS, HbA1c, Triglyceride, Cholesterol, and Creatinine levels and an increase in HDL level, there was a significant decrease in Cholesterol and Creatinine levels before and after the study in the experimental group. However, there were no significant differences in most variables between the experimental and control groups after the intervention.

Diabetes is a major health challenge with a growing prevalence worldwide. It is predicted that by 2045, the number of patients will rise to 700 million [8]. Type 2 diabetes accounts for more than 90% of diabetes cases and is a chronic metabolic disease with a variety of causes [19, 20]. In Iran, people use medicinal plants to treat diabetes due to their availability, affordability, and fewer complications [21]. However, clinical and scientific studies are necessary to confirm their effectiveness and safety [22]. Around 800 plants are used globally to prevent and treat diabetes, but only 450 of

them are clinically proven [23]. One such plant is Kelatin, which belongs to the Papaveraceae family and is native to Iran [24]. The people of the Haft Bern area in Norabad Mamseni Shiraz have been using it for many years to prevent and treat diabetes [17]. kelatin is rich in alkaloid compounds such as aporphine, protopine, and protoberberine, with glucine being the most important alkaloid compound [25]. Although very few studies have been conducted on kelatin worldwide, biochemical studies confirm its effects [26].

In a study Darya et al. investigate the effect of hydro-alcoholic extract of yellow horned poppy (*Glaucium flavum*) on serum concentration of glucose and lipid profile and weight changes in diabetic rats. The result show significant decrease in glucose concentration in the diabetic+extract group compared to the diabetic control [27].

In another study Khoshvaghti et al, examined the effect of *Glaucium flavum* extract on the activity of liver and kidney oxidoreductase enzymes in diabetic rats. The result show Yellow Horned Poppy extract could increase the activity of liver and kidney oxidoreductase enzymes in diabetic rats[28].

In general, the mechanism of the anti-diabetic effect of medicinal plants includes stimulation of insulin secretion, antioxidant properties, inhibition or activation of some enzymes, or changes in the expression of some genes that lead to inhibition of glucose biosynthesis or activation of pathways involved in catabolism or its disposal. as well as inhibiting the binding of sugar to biomacromolecules and thus maintaining the structure of proteins [29]. There is no permanent cure for diabetes, complementary treatment with the aim of reducing the symptoms, it is the target of research on ancient medicinal plants. Control aimed at blood sugar, longevity and quality of life, increasing complications, and reducing the pain of people with diabetes is effective. Medicinal plants are rich in antioxidant substances [30-32], phenolic [33], flavonoid [34], flavon [35], anthocyanin [36], tannin [37], terpenoids [38], and the medicinal properties of many medicinal plants are due to the presence of these medicinal compounds.

In our study although patients were randomly divided into two groups, the distribution of variables was not completely equal. It is recommended that future studies explore this topic with a larger sample size to confirm these results. If the findings are validated, the product could be introduced to the pharmaceutical market in Iran as a standard treatment.

Author Contributions

All research done by the authors. Ghorban Deyri designed study, drafted the initial manuscript, obtained the patient consent form, collected data, and filed the

patient case, prepared a herbarium and approved the final manuscript. Emran Habibi identified and approved the herbarium prepared; designed, prepared and standardized a condensed kelatin and revised the manuscript. Hoda Shirafkan Randomized patients analyzed data and revised the manuscript. Fatemeh Mahmoudi Lamooki helped with the computational analysis. Seyde Sedighe Yousefi supervised the various stages of study, revised and approved the final manuscript.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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