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Antidiabetic Effects of Hydroalcoholic Extract of Afghan *Withania coagulans* in Diabetic Rats

*Mustafa Ansari [1,2], Dawood Hossaini [2], Elham Akbari [2], Hossain Rezayee [3]

1. Research and Technology Center, Khatam Al-Nabieen University, Kabul, Afghanistan.
2. Department of Biology and Microbiology, Faculty of Medical Laboratory Technology, Khatam Al-Nabieen University, Kabul, Afghanistan
3. Department of Pharmacology and Public Health, Faculty of Medicine, Khatam Al-Nabieen University, Kabul, Afghanistan.

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*Corresponding Author:

E-mail: mustafa72ansari72@gmail.com

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ABSTRACT

Background: Diabetes mellitus is the most prevalent endocrine disorder globally, responsible for approximately four million deaths each year and presenting significant health and socio-economic challenges. Traditional herbal medicine has utilized various plant species for managing blood glucose levels and associated complications. We investigated the effects of Afghan *Withania coagulans*, a medicinal plant known for its bioactive compounds, on blood glucose levels in a diabetic rat model.
Methods: The experiment was conducted at the Research and Technology Center of Khatam-Al-Nabeien University (KNURTC), Kabul, Afghanistan. Thirty rats were divided into five groups: a vehicle control, a diabetic group, and three groups treated with *W. coagulans* extract, obtained via hydroalcoholic extraction. Diabetes was induced using a single intraperitoneal injection of 60 mg/kg streptozotocin (STZ). Fasting blood glucose levels were measured on days 0 and 21 using a glucometer.
Results: STZ significantly elevated blood glucose levels in the diabetic group compared to the vehicle control. Notably, the *W. coagulans* extract-treated groups demonstrated a substantial reduction in blood glucose levels compared to the diabetic group ($P<0.001$).
Conclusion: The presence of active constituents in *W. coagulans* such as flavonoids, alkaloids, saponins, coumarins, tannins, proteins, amino acids, and withanolides suggests a potential mechanism for its hypoglycemic effects. This study enhances our understanding of *W. coagulans* as a promising candidate for diabetes management.

Keywords: *Withania Coagulans*, Afghanistan, Blood glucose, Streptozotocin, Diabetes

Introduction

The use of plant combinations in diabetes management is on the rise (1, 2). Various plant combinations can demonstrate synergistic effects that effectively lower blood glucose levels and mitigate related complications (2, 3). Notably, several plants, including *Withania coagulans*, have been recognized for their blood glucose-lowering properties (4-7). Despite this, there is a lack of studies specifically examining the effects of Afghan *W. coagulans* on blood glucose levels.

Diabetes is the most prevalent endocrine disorder globally (8), contributing to approximately four million deaths annually (9, 10). It encompasses a variety of metabolic

disorders characterized by elevated blood glucose levels due to insufficient insulin secretion (Type 1 diabetes) or impaired insulin action, or both (Type 2 diabetes) (11). The significance of diabetes is highlighted by its widespread prevalence and the numerous complications that can arise from it. Today, diabetes is viewed as one of the most pressing health, therapeutic, and socio-economic challenges globally (12).

In Type 1 diabetes, a combination of genetic and environmental factors triggers autoimmune responses, leading to the accumulation of lymphocytes and macrophages in the pancreatic islets. This results in the secretion of pro-inflammatory cytokines such as TNF-α, IL-1β, and IFN-α. Furthermore, the overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS) by immune cells activates intracellular signaling pathways, culminating in autophagy, apoptosis, and necroptosis of beta cells (13).

Type 2 diabetes is characterized by insulin resistance, wherein cells fail to respond adequately to insulin. This resistance is associated with mutations or modifications in insulin receptors and IRS, resulting in reduced receptor numbers and activity, increased phosphorylation of Ser/Thr residues, heightened PTP-1B activity, decreased PI3K and Akt activity, and defects in GLUT-4 function. Collectively, these alterations lead to diminished glucose uptake in muscle and adipose tissues, resulting in significant metabolic disturbances (14).

A common characteristic across all forms of diabetes is elevated blood glucose levels. Prolonged hyperglycemia can lead to dysfunction and damage in various organs, particularly the eyes, kidneys, nerves, blood vessels, and heart (15). Current glucose-lowering medications include sulfonylureas and meglitinides that stimulate insulin secretion, biguanides and thiazolidinediones that enhance glucose uptake and reduce hepatic gluconeogenesis, and alpha-glucosidase inhibitors that delay carbohydrate absorption in the intestine (16). Additionally, newer peptide analogs like exenatide and liraglutide raise serum GLP-1 levels and slow gastric emptying, as do DPP-4 inhibitors (17). However, these treatments are often accompanied by drawbacks, including drug resistance, side effects, and potential toxicity, which can compromise their effectiveness (18-20). Consequently, there is increasing interest in herbal medicines, perceived to have fewer side effects than synthetic drugs (21).

W. coagulans is a perennial shrub belonging to the Solanaceae family, characterized by its white and grayish stems, reaching heights of 60 to 120 cm, with leaves measuring 2.5 to 7.5 cm in width. This plant is indigenous to regions of Afghanistan, Pakistan, Iran, and southwestern India recognized for its significant medicinal properties. Its bioactive constituents include alkaloids, steroids, phenolic compounds, tannins, saponins, carbohydrates, proteins, amino acids, organic acids, and unique withanolides (22). The pharmacological effects of *W. coagulans* are diverse, encompassing reductions in blood glucose and lipid levels, antihypertensive benefits, free radical scavenging, anticancer properties, and immune system enhancement (23). Geographical factors significantly influence the chemical composition and bioactive properties of medicinal plants. Variables such as climate, soil type, and altitude can affect the diversity of secondary metabolites within these species, ultimately affecting their medicinal efficacy. For example, variations in environmental conditions can lead to differences in the concentration of bioactive compounds, potentially enhancing or diminishing the therapeutic potential of the plants (24).

In light of these considerations, we aimed to investigate rigorously the impact of Afghan *W. coagulans* extract on blood glucose levels

in diabetic rats, utilizing controlled laboratory methodologies.

Materials and Methods

Materials

The materials utilized in this study included STZ (Streptozotocin, Sigma Aldrich), phosphate citrate buffer (0.01 M, pH 4.6), powdered *W. coagulans* , and a glucometer (ACON Laboratories, USA, On Call Plus).

Animals

This study involved 30 male Sprague-Dawley rats, each weighing between 200 to 225 gr. The experiment was conducted at the Research and Technology Center of Khatam-Al-Nabeien University (KNURTC). The rats were maintained under standardized environmental conditions, including a 12-hour light/dark cycle, a temperature of approximately 23 ± 2 degrees Celsius, and unrestricted access to food and water (25).

Extraction

The seeds of *W. coagulans* were collected from Laghman Province, Afghanistan. After collection, the seeds were dried in a light-protected environment and ground into a fine powder. A hydroalcoholic extract of *W. coagulans* was prepared using a 70% ethanol solution. Specifically, 100 grams of the powdered *W. coagulans* were mixed with ethanol at a 1:4 ratio in a suitable container. The mixture was allowed to stand at room temperature for 72 h, with manual shaking performed several times daily to facilitate the extraction process. After the extraction period, the solution was filtered through Whatman filter paper (26). The resulting extract was collected in flat glass containers, allowed to dry at room temperature, and subsequently stored in a refrigerator until further use.

Experimental Diabetes induction

Experimental diabetes was induced in the rats through an intraperitoneal injection of STZ at a dose of 60 mg/kg, dissolved in phosphate citrate buffer, while the animals were fasting. Following a 72-hour period, fasting blood glucose levels of the rats were assessed using a glucometer. Rats exhibiting blood glucose levels exceeding 200 mg/dl were randomly assigned to the experimental groups (27).

Experimental Groups

Thirty rats were divided into five groups (n=6): a vehicle group, a diabetic group, and three treatment groups receiving 100, 200, and 400 mg/kg of the hydroalcoholic extract of *W. coagulans* The vehicle and diabetic groups were administered normal saline, while the extract-treated groups received a daily dose of 1 mL of *W. coagulans* extract for 21 d. Blood glucose levels in the rats were measured at baseline (day 0) and at the conclusion of the treatment period (day 21).

Statistical Analysis

Data were statistically analyzed using GraphPad Prism software. One-way ANOVA was employed for statistical comparisons and a significance level of $P<0.05$ was established for all assessments.

Results

The statistical analysis using one-way ANOVA indicated that administration of STZ resulted in a significant increase in fasting blood glucose levels 72 h post-injection compared to the vehicle group ($P<0.001$). Following the treatment with *W. coagulans* extract at doses of 100, 200, and 400 mg/kg over a 21-day period, all treated groups exhibited a significant reduction in fasting blood glucose levels. Specifically, the group receiving 100 mg/kg of *W. coagulans* extract demonstrated a significant decrease in FBS levels ($P<0.05$). The group administered

200 mg/kg showed an even more pronounced reduction in blood glucose levels (P<0.001). Additionally, the 400 mg/kg group also exhibited a significant decrease in fasting blood glucose levels (P<0.001) (Figure 1).

A comparative analysis of body weight among the groups receiving STZ, measured 72 h post-injection, revealed no significant weight loss relative to the vehicle group. By the conclusion of the 21-day experiment, a significant difference in body weight was observed between the vehicle and diabetic groups, indicating the impact of diabetes on weight changes. However, there were no notable differences in body weight among the groups treated with *W. coagulans* extract at doses of 100, 200, and 400 mg/kg when compared to the diabetic control group (Figure 2).

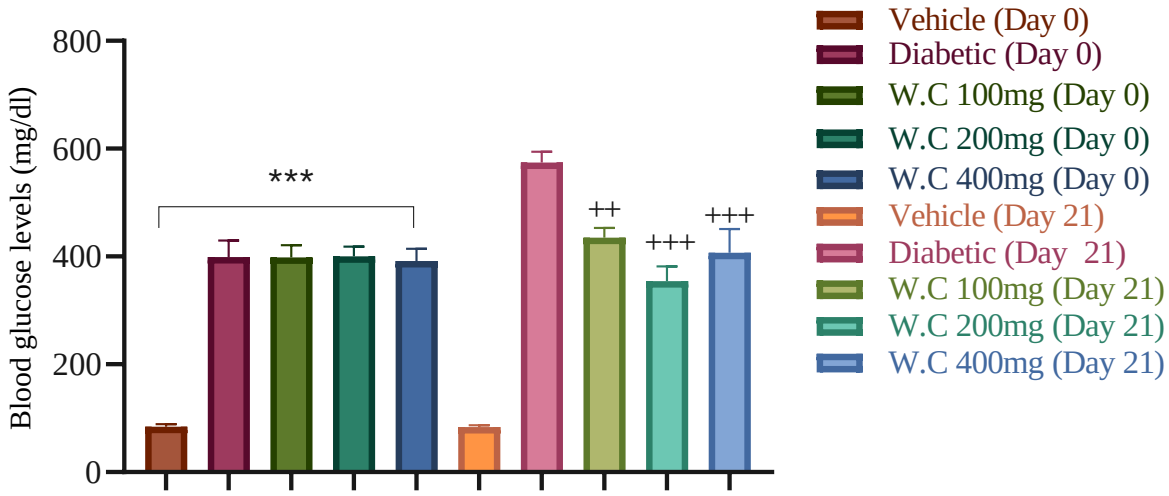

Figure 1: Comparison of Fasting Blood glucose Levels. Each column shows the mean ± SEM. ***P<0.001 compared to the vehicle group. ++P<0.01 and +++P<0.001 compared to the diabetic group.

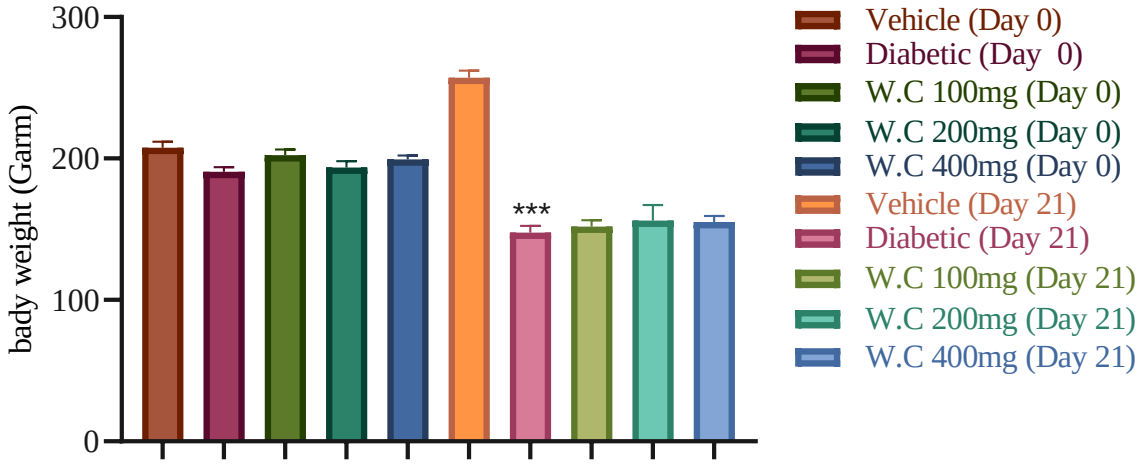

Figure 2: Comparison of the average animal weight. Each column shows the mean ± SEM. ***P<0.001 compared to the vehicle group.

Discussion

The findings from this study provide compelling evidence for the antidiabetic potential of *W. coagulans* extract in managing fasting blood glucose levels in STZ-induced diabetic rats. The significant increase in fasting blood glucose levels observed 72 h post-STZ administration confirms the successful induction of diabetes, which is consistent with previous studies reporting similar hyperglycemic effects following STZ administration (28-30). This increase underscores the importance of establishing a reliable diabetic model for evaluating the efficacy of potential therapeutic agents.

The administration of *W. coagulans* extract at varying doses of 100, 200, and 400 mg/kg resulted in a marked reduction in fasting blood glucose levels across all treatment groups. Notably, the 200 mg/kg dosage exhibited the most pronounced effect, achieving a significant reduction in blood glucose levels (Figure 1). This dose-dependent response aligns with findings from another study documenting the hypoglycemic effects of *W. coagulans* suggesting that optimal dosing may be critical for maximizing therapeutic outcomes (31). The significant reductions in blood glucose levels across all treatment groups indicate the potential of *W. coagulans* as a viable candidate for diabetes management, possibly through mechanisms such as enhanced insulin sensitivity or increased glucose uptake by peripheral tissues.

Interestingly, while the study noted a significant difference in body weight between the vehicle and diabetic control groups by the end of the experiment, treatment with *W. coagulans* extract did not result in notable changes in body weight compared to the diabetic control group (Figure 2). This observation is consistent with the understanding that diabetes can lead to weight loss due to catabolic processes, and the extract's influence on body weight was not sufficiently pronounced to counteract these effects. Consequently, while *W. coagulans* may effectively lower blood glucose levels, its impact on weight management in diabetic conditions warrants further investigation.

The oral administration of a water extract of *W. coagulans* combined with chloroform extracts at a dose of 1 g/kg, produced a greater reduction in blood glucose levels compared to metformin in STZ-induced diabetic laboratory rats; however, the increase in body weight observed at this dosage was not statistically significant (32). Similarly, the oral intake of *W. coagulans* water extract resulted in decreases in blood glucose levels, oxidative stress, and inflammation in STZ-induced diabetic rats (33). A dose of 250 mg/kg of *W. coagulans* water extract significantly lowered blood glucose levels, regulated lipid-controlling enzymes, and reduced oxidative stress (34). Additionally, doses of 150 mg/kg and 200 mg/kg administered to diabetic laboratory animals resulted in reductions of 55% and 63% in fasting blood glucose levels, respectively, along with decreases of 26% and 44% in HbA1c (35).

Oxidative stress is a key factor in the pathology of diabetes, contributing to both insulin resistance and impaired insulin secretion (36). Diabetes mellitus is characterized by increased lipid peroxidation, where oxidative stress and elevated levels of free radicals compromise the body’s antioxidant defenses. This results in damage to cellular organelles and enzymes, further exacerbating insulin resistance and facilitating the onset and progression of diabetic complications (37, 38). Notably,

treatment with *W. coagulans* has reduced pro-inflammatory factors, blood glucose levels, oxidative stress, and insulin resistance in diabetic models (33). The administration of doses of 150 and 200 mg/kg of *W. coagulans* led to increased serum insulin levels and reduced blood glucose levels (35), reinforcing the notion that *W. coagulans* might effectively lower blood glucose levels and improve diabetes management.

Conclusion

The findings of this study strongly support the potential therapeutic role of *W. coagulans* in diabetes treatment, highlighting its efficacy in managing fasting blood glucose levels in STZ-induced diabetic rats. Given the significant reductions in blood glucose observed with various dosages, particularly at the 200 mg/kg level, *W. coagulans* might serve as a promising natural alternative or adjunct therapy for diabetes management.

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Conflict of interest

The authors declare that there is no conflict of interests.

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