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Ashwagandha (*Withania somnifera*) Plant and its Role in Influencing Immune and Tissue Functions in Diabetic Rats

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Abstract: The present study aimed to determine the impact of Ashwagandha (*Withania somnifera*) plants on immune and tissue functions in diabetic rats. Thirty adult male albino rats, each weighing 150±10 g were divided into two primary groups. The 1st group (six rats) received a basal diet as a control (-), while the 2nd primary group (twenty-four rats) were administered alloxan to cause diabetes and then distributed into four subgroups. One of these subgroups was employed as positive control. The other three subgroups were provided with a basal diet and were administered various amounts of Ashwagandha plants (2.5%, 5%, 7.5%) for 28 days. After completion of treatment with the Ashwagandha plant, the rats were weighed and slaughtered, blood samples were collected, and the serum was separated to perform immunological analyses and pancreas was extirpated for histological examination. It was noticed that IgG, IgE, IgM, and IgA levels increased with increasing concentrations of Ashwagandha plant treatment. The maximum decrease in WBCs after 2.5% ashwagandha powder and platelet counts after 7.5% ashwagandha powder treatment were recorded for the diabetic group rat as compared to control (+). The maximum RBC count and hemoglobin content were recorded after 5% Ashwagandha plant treatment.

Keywords: Therapeutic plant, IgM, Ashwagandha plant, *Withania somnifera*, Alloxan, IgG, IgE, IgM, IgA

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Introduction

The immune system consists of a complex and highly interactive cell network and their products. The system demonstrates 2 distinct features: "memory" and exquisite specificity. A subset of immune cells identifies and reacts to the various external stimuli and cases might have during their lifetime. The immune system is modulated by helper suppressor cells and their soluble products. It maintains intimate communication with other systems in the body, such as the neuro-endocrine system, and is controlled by these systems (Reichlin, 1993). The immunological functions of humans decrease with age. T cells, essential to cellular immunity, demonstrate the most signifi-cant age-correlated variations in distribu-tion and function, attributed to thymic involution as the primary reason. The responses of immune systems to acute training and exercise in the senior population have not been thoroughly investigated. The response of natural killer (NK) cells to a single exercise stimulus is normal in elder people; nevertheless, immediately post-exercise, aged patients exhibit diminished inhibition of phytohemagglutinin (PHA)-induced lymphocyte proliferation compared to younger persons. Conversely, vigorous exercise appears to cause a more prolonged following-exercise decrease of cellular immunity in elder adults compared to their younger peers. Several comparisons of cross-sectional immune status among young sedentary controls and physically fit older people indicate that regular physical activity could improve activity of natural killer cells, supporting specific elements of the age-correlated deterioration in function of T cells, including diminished mitogenesis in response to plant lectins and reductions in the synthesis of cytokines. Nonetheless, the clinical consequences require elucidation in future research (Shinkai *et al*., 1994).

Ashwagandha (*Withania somnifera*), has gained popularity in the West for its efficacy in decreasing stress and promoting a more calm and happy state of being. A significant quantity of information is available regarding this potent herb, providing a remarkable array of health advantages (Wicinski *et al*., 2024). Ashwagandha has demonstrated the ability to support adrenal function by regulating concentrations of cortisol. This decreased the negative influences of elevated or diminished amounts of this hormone. This demonstrates the adaptogenic properties of this plant. This is important as ongoing stress can adversely affect human health. The function of the adrenal glands is intricately connected to that of the thyroid; hence, Ashwagandha, by supporting adrenal health, indirectly enhances thyroid function. Initial investigations indicated that Ashwagandha can enhance thyroid function by promoting thyroid hormone activity (Arban *et al*., 2024). Ashwagandha has demonstrated significant supporting impacts on the immune system. It was demonstrated to promote illness-fighting and anti-inflammatory immune cells that aid in illness prevention. The herb's iron content contributes to the red blood cell count (Sultan *et al*., 2020). The influence of *Withania somnifera* on adaptive immunity may be especially beneficial following pathogen exposure, such as viruses and bacteria. In rats managed with a standardized leaf extract of *Withania somnifera* for two weeks, the response of T-helper 1 has been enhanced by activating the B cells and interferon-gamma expression, while also increasing the CD4+, CD8+ T, and CD3+ cells expression, as well as co-stimulatory CD80 and integrins (Turrini *et al*., 2016; Kumar *et al*., 2019). They have demonstrated that extracts of leaf and root of *Withania somnifera* standardized for withanolide glycosides (or withanoglycosides) can enhance both immunoglobulins as components of innate immunity while elevating interferon-γ and T-cell populations CD4+ and CD3+ in people. The outcomes indicated a significant role in facilitating adaptive as well as innate immunity in humans, essential for the recognition and appropriate response to prevalent bacteria, viruses, and allergens. These findings align with previously documented *in vivo* data, demonstrating an elevation in the levels of expression of T-helper 1 cytokines and increased numbers of CD8+ and

CD4+ T cells. Likewise, an increase in activity of natural killer cells has been noted in a dose-dependent method alongside the rapid CD4+ T cell recovery in immunosuppressed subjects. The immunomodulatory impacts of the *Withania somnifera* (WS) were evaluated in healthy people. In the placebo-controlled, randomized, double-blind investigation, participants have been assigned either sixty milligrams of WS extract or a placebo. The trial had a blinded thirty-day phase followed by an open-label extension of an additional thirty days, allowing crossover only from placebo to treatment. Following the thirty-day blinded research length, the *Withania somnifera* test group exhibited a significant elevation (p-value less than 0.05) in immunoglobulins (IgM, IgA, IgG2, IgG, IgG4, and IgG3). No adverse occurrence has been reported (Deshpande *et al*., 2020). The present study aimed to determine the impact of Ashwagandha plants on immune and tissue functions in diabetic rat.

Materials and Methods

Plant material

Ashwagandha (*Withania somnifera*) were purchased from a local farm in Shebin El-Kom, Egypt.

Animals

Thirty male albino rat weighing 150 ± 10 g were obtained from the Medicine Insects Research Institute in Doki, Cairo, Egypt.

Chemicals

The analysis kits were purchased from Bio Diagnostics (Cairo, Egypt). Alloxan was purchased from El-Gomhoria Company for Trading Drugs, Chemicals, and Medical Equipment in Cairo, Egypt.

Basal diet

The basal diet was prepared using the formula provided by Reeves *et al.* (1993), as follows: protein 10 per cent, cellulose 5 per cent, minerals, 10 per cent corn oil and vitamin blend, methionine 0.3 per cent, choline chloride 0.2 per cent, and corn starch 69.5 per cent. Campell *et al*. (1963) recommended the use of a salt mix component. For inducing diabetes, alloxan injection at a dose of 150 mg/kg b wt was given to rats (Zhao *et al.,* 1987).

Experimental design

The Science Research Ethics Committee of the Faculty of Home Economics approved the research protocol #11-SREC-06-2024. In this experiment, thirty adult male white albino rats aged 10 weeks and weighing 150±10 g have been utilized. All the rats were given a basic diet consisting of casein for diet for seven days. After this adaptation period, the rats were divided into five groups, with 6 rats per group. One group (group 1) was used as control (-). The other four groups (groups 2-5) were injected with Alloxan for inducing diabetes. The details of all 5 groups are as follow:

Group (1): Normal rats received a regular diet and identified as the control (-) group.

Group (2): Diabetic rats received a regular diet and identified as the control (+) group.

Group (3): Diabetic rats were fed a standard diet + 2.5% ashwagandha.

Group (4): Diabetic rats have been fed a standard diet + 5% ashwagandha.

Group (5): Diabetic rats have been fed a standard diet + 7.5% ashwagandha.

Collection of blood samples

The rats were slaughtered at the conclusion of the experiment following a 12 h fasting. Blood samples were collected from the portal vein in dry sterile centrifuge tubes. The blood was centrifuged at 3000 rpm for 10 min to separate the serum. The serum was been stored at -20°C for further analysis.

Biochemical analysis

Determination of platelet count, white blood cell, hemoglobin and red blood cell:

The concentrations of platelet count (PLT), white blood cell (WBC), hemoglobin (HGB), and red blood cell (RBC) count have been calculated

Table 1: Impact of various concentrations of ashwagandha plant on immunological profile

Immunological Profile mg/dl	G1	G2	G3	G4	G5
IgE	64.17±0.05^{a}	57.5±0.2^{b}	59.87±1^{b}	60.54 ±1.05^{a}	60.76 ±0.05^{a}
IgM	108.2±0.005^{a}	61.65±0.65^{f}	79.33±3 ^{d}	82.33 ±10.96^{c}	85.66±10.96^{b}
IgA	111.1 ±0.1^{a}	75.5 ±0.5^{c}	77.5 ±1.5^{c}	78.33 ±2.08^{c}	88.5 ±1.5^{b}
IgG	1100.05±0.05 ^{a}	748 ±2^{e}	776.66±25.1^{d}	778.36±20.85 ^{d}	875 ±15^{c}

Each value represents the mean ± SD of 3 replicates; mean values under the same column with different superscript letters are significant (p≤0.05) G1: negative control , G2: positive control, G3: rats fed with 2.5 % ashwagandha plant, G4: rats fed with 5 % ashwagandha plant, G5: rats fed with 7.5% ashwagandha plant.

regarding the method described by Ikechukwu *et al.* (2004).

Immunological estimations

Serum IgG, IgE, IgM, and IgA levels were analysed by using analytical kits.

Histopathological examination

The pancreas were extirpated, cleansed with saline solution, dried with filter paper, weighed, and fixed in 10% formalin solution for histological analysis (Drury *et al.,* 1963). The routine histological method was used. Sections of 6 µm thickness were cut, floated on the slides and stained with Hematoxylin and Eosin (HE). The stained slides were inspected under light microscope. Histopathological investigation has been done at the histology laboratory of the Faculty of Veterinary Medicine, Cairo University, Egypt (Bancroft and Stevens, 1996).

Statistical analysis

The data have been investigated utilizing a completely randomized factorial design (SAS Users Guide, 1985) upon detection of a significant main effect; means have been separated utilizing the student-Newman-Keuls method. Variations among therapies (P-value equal 0.05 or less) have been deemed significant utilizing the Costat Program. The biological data have been examined with one-way ANOVA.

Ethical Approval

The Science Research Ethics Committee of the Faculty of Home Economics approved the research protocol #11-SREC-06-2024.

Results

Immunological Profile

Table 1 illustrates the impact of various concentrations of Ashwagandha plants on the immunological profile of hyperglycemic rat. Serum IgE concentrations in the control (-) group were significantly better than in the diabetic control (+) group. The levels were 64.17±0.05 and 57.5±02, respectively in control (-) and control (+) rats. Meanwhile, the impact of various concentrations of Ashwagandha plants on IgE levels in diabetic rat has been improved as compared to the control (+) group. An insignificant variation has been observed among groups 4 and 5 and the negative control. An insignificant variation has been observed among group 3 and positive control.

The level of serum IgM was 108.2±0.005 in the negative control group, while it was a low significant 61.65±0.65 for the positive control. The serum IgM increased in diabetic rat treated with 2.5, 5 and 7.5% concentrations of Ashwagandha as compared to control (+)(Table 1). The serum IgA was 111.1 ± 0.1 in the control (-) group and 75.5 ± 0.5 in the positive control. Alloxan-induced diabetic rat groups fed different levels of ashwagandha plant showed significant variances as compared with the control-positive rat, with levels being 77.5±1.5, 78.33±2.08, and 88.5±1.5, respectively, for feeding levels (2.5%, 5%, and 7.5% of ashwagandha plant), respectively. IgA

Table 2: Effect of various concentrations of ashwagandha plant on red blood cell and white blood cell of rat with diabetes

Groups	Parameters	
	R.B.Cs (10^6/mm^3)	W.B.Cs (10^6/mm^3)
Group (1)	4.83 ±0.50^a	5.83 ±0.16^c
Group (2)	2.89 ±0.11^b	9.49 ±0.61^a
Group (3)	3.89 ±0.42^a	7.59 ±0.38^b
Group (4)	3.92 ±0.40^a	7.91 ±0.43^b
Group (5)	3.22 ±0.20^b	9.87 ±0.50^a
LSD (P≤0.05)	1.072	1.250

Each value represents the mean ± SD of 3 replicates; mean values under the same column with different superscript letters are significant (p≤0.05) G1: negative control , G2: positive control, G3: rats fed with 2.5 % ashwagandha plant, G4: rats fed with 5 % ashwagandha plant, G5: rats fed with 7.5% ashwagandha plant.

levels showed a gradual increase with an increase in supplement levels (Table 1).

The serum IgG level in the negative control group was 1100.05±0.05. The impact of distinct concentrations of ashwagandha plant on IgG levels in diabetic rat was improved compared to that in the control (+) group (776.66±25.16, 778.36 ±20.85, and 875±15, respectively) for feeding levels (2,5%, 5%, 7.5% of ashwagandha plant), respectively.

Effect of various concentrations of ashwagandha plant on red blood cell and white blood cell of rat with diabetes

The number of red blood cells (RBCs) in the control (-) group was significantly greater than that in the control (+) group (Table 2). Mean values were 4.83 and 2.89 (10^6/mm^3), respectively. In contrast, the greatest RBC number of the treated groups have been recorded for the diabetic group rats fed 5% ashwagandha powder as compared to control (+). The minimum value was observed (Table 2) in the diabetic group administered 7.5% ashwagandha powder was statistically significant (P-value equal 0.05 or less).

The results for white blood cells (WBCs) revealed that the control (+) group had a significantly greater value than the negative control group. The average values were 9.49 and 5.83 (10^6/mm^3), respectively for control (+) and control (-).

The WBC counts decreased in the diabetic rat treated with 2.5% and 5% ashwagandha powder as compared with control (+) (Table 2). The diabetic group of rat that consumed 7.5% ashwagandha powder exhibited almost similar values as that of control (+).

Effect of various concentrations of ashwagandha plant on hemoglobin and platelet of diabetic rat

Table 3 illustrates the impact of varying concentrations of ashwagandha on hemoglobin and platelet concentrations in diabetic rat. The hemoglobin (Hgb) concentration in the control (-) group was significantly greater than that in the positive control group. The average readings were 16.11 and 10.39 g/dl, respectively.

Among the treated groups, the maximum increase in hemoglobin content was observed in diabetic rat that consumed 5% ashwagandha powder as compared to control (+) (Table 3). The minimum increase in hemoglobin content was observed in diabetic rats that consumed 7.5%

Table 3: Influence of various concentrations of ashwagandha plant on hemoglobin and platelet of diabetic rat

Groups	Parameters	
	Hemoglobin (g/dl)	Platelet (10^6/mm^3)
Group (1)	16.11 ±0.20[a]	251.5 ±3.20[f]
Group (2)	10.39 ±0.20[d]	793.0 ±7.20[a]
Group (3)	13.38 ±0.20[b]	514.5 ±5.20[b]
Group (4)	14.21 ±0.20[b]	446.0 ±5.20[c]
Group (5)	11.26 ±0.20[c]	306.0 ±3.30[e]
LSD (P≤0.05)	1.160	5.370

Each value represents the mean ± SD of 3 replicates; mean values under the same column with different superscript letters are significant (p≤0.05) G1: negative control , G2: positive control, G3: rats fed with 2.5 % ashwagandha plant, G4: rats fed with 5 % ashwagandha plant, G5: rats fed with 7.5 ashwagandha plant.

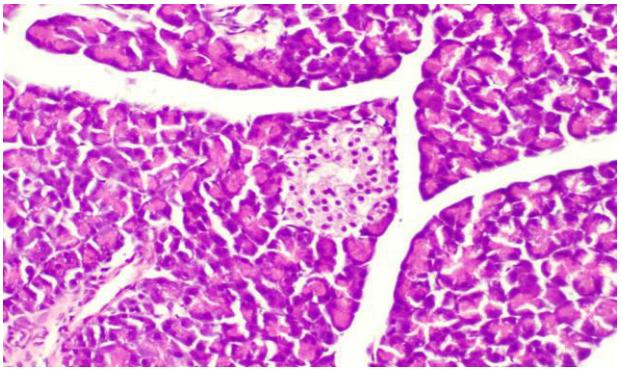

Fig. 1: Photomicrograph of pancreas of normal control group (1) rat demonstrating the normal histological structure of exocrine and Langerhans secretory acini islets. (HE x 400)

ashwagandha powder as compared to control (+).

The platelet count was significantly higher in the control (+) group than in the negative control group. The mean values were 793.0 for Control (+)and 251.5 (10^6/mm^3) for control (-). All the diabetic group treated with ashwagandha powder exhibited a decrease in platelet count as compared to control (+), the maximum decrease was in rats subjected to 7.5% ashwagandha powder (Table 3).

Histological studies of pancreas

The pancreatic tissue of rat in normal group exhibited the standard histological structure of the exocrine and Langerhans pancreatic acini islets (Fig. 1). In contrast, the photomicrograph of the pancreas of rat from group 2 control (+) shows vacuolization of cells of Langerhans islets (black arrow) and epithelial lining pancreatic acini (Fig. 2). However, a photomicrograph of the pancreas of rat from group 3 (rat fed with 2.5% ashwagandha plant) shows vacuolization of some cells of islets of Langerhans (Fig.3). Conversely, some sections from group 4 (rat fed with 5% ashwagandha plant) exhibited no histopathological alterations (Fig. 4). Moreover, photomicrographs of the pancreas of rat from group 5 (rat fed 7.5% ashwagandha plant) shows vacuolization of some of Langerhans islets cells (Fig. 5).

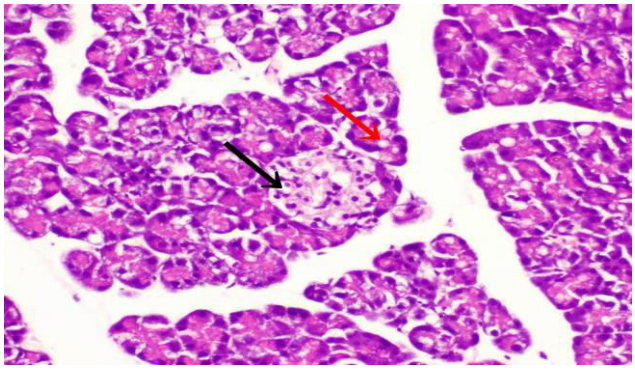

Fig. 2: Photomicrograph of the pancreas of rat from group 2 control (+) showing vacuolization of Langerhans islets cells (black arrow) and epithelial lining of pancreatic acini (red arrow). (HE x 400)

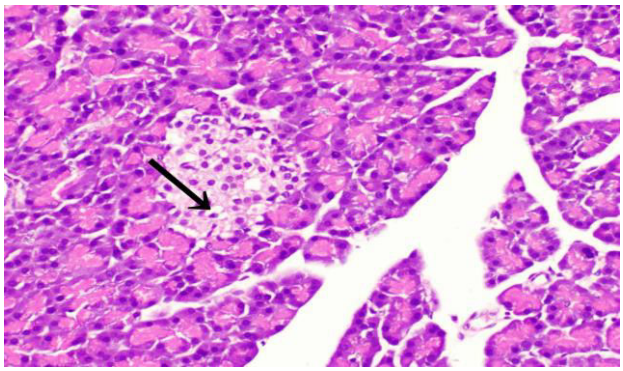

Fig. 3: Photomicrograph of the pancreas of rat from group 3 (rat fed on 2.5 % ashwagandha plant) demonstrating vacuolization of some of Langerhans islets cells (black arrow). (HE x 400)

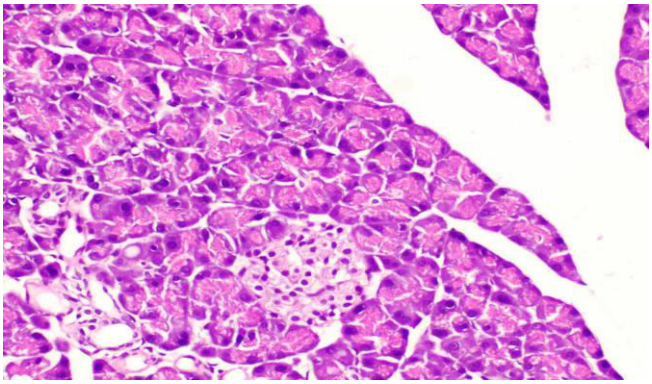

Fig. 4: Photomicrograph of the pancreas of rat from group 4 (rat fed on 5 % ashwagandha plant) demonstrating no histopathological alterations. (HE x 400)

Discussion

In the present study IgG, IgE, IgM, and IgA levels increased with increasing concentrations of ashwagandha plant. These findings align with those of Kumar *et al*. (2019), who indicated that the influence of *Withania somnifera* on adaptive immunity could be especially beneficial following pathogen exposure, such as viruses and bacteria. Rats treated with a normalized leaf extract of *Withania somnifera* for two weeks had an enhanced response of T-helper 1, characterized by increased B cells and interferon-gamma expression, as well as elevated levels of CD8+, CD4+, and T cells CD3+, alongside co-stimulatory CD80 and integrins. Deshpande *et al*. (2020) showed that standardized extracts of WS leaf and

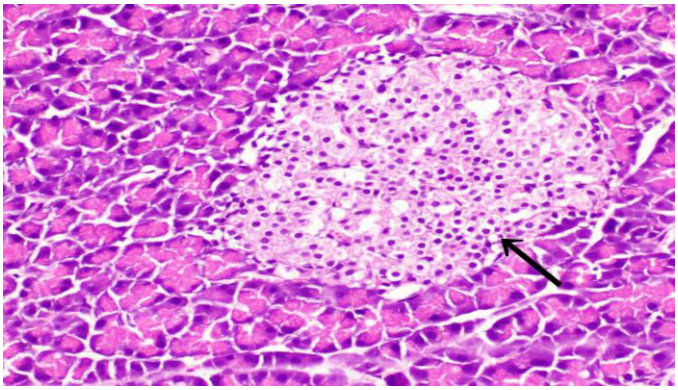

Fig. 5: Photomicrograph of the pancreas of rat from group 5 (rat fed on 7.5 % ashwagandha plant) showing vacuolization of some Langerhans islets cells (black arrow). (HE x 400)

root, enriched with withanolide glycosides, can enhance immunoglobulin levels, a component of innate immunity, while also elevating interferon-γ-gamma and T-cell CD4+ and CD3+ in people. These findings indicated a significant role in facilitating humans' innate and adaptive immunity, essential for identifying and responding effectively to prevalent bacteria, allergens, and viruses. The findings of this research align with previously documented *in vivo* data, demonstrating an elevation in the levels of expression of T-helper 1 cytokines, along with increased numbers of CD8+ T and CD4+ cells. Likewise, increased activity of natural killer cells was a statistically significant rise (p-value less than 0.05) in immunoglobulins (IgM, IgA, IgG2, IgG, IgG4, and IgG3) in the *Withania* extract test group after thirty days. This was noted in a dose-reliant method alongside the rapid recovery of CD4+ T cells in immunosuppressed rat. The immunomodulatory effects of the extract of WS were evaluated in healthy people. In placebo-controlled, randomized, double-blind research, participants were assigned either sixty milligrams of WS extract or a placebo. The trial had a blinded 30-day phase followed by a 30-day open-label extension, allowing crossover exclusively from placebo to treatment. Following the thirty-day blinded research length, the *Withania somnifera* test group exhibited a statistically significant rise (p-value less than 0.05) in immunoglobulins (IgM, IgA, IgG2, IgG, IgG4, and IgG3). Mukherjee *et al.* (2020) reported that ashwagandha has excellent supportive influences on the immune system. It was presented to encourage anti-inflammatory and illness-relieving immune cells that help to ward off the disease. Because the herb contains iron, it also contributes to an increase in the red blood cell count. In addition, the results are in conformity with Singh *et al* . (2017) as they found that ashwagandha helps to reduce stress hormones, which helps strengthen immunity and reduce inflammation. It also stimulates the production of immunoglobulins and inhibits certain types of proteins known as cytokines that cause inflammation. This study also showed that ashwagandha can improve the activity of natural killer cells, which are immune cells that fight infectious diseases. In addition, it reduces some inflammatory factors, such as C-reactive protein. Ashwagandha has been shown to increase red blood cell and hemoglobin counts in the blood. Increasing red blood cell count increases the blood's ability to transport oxygen directly to exercising muscles (Bhargava *et al.,* 2019). Also, the results agree with Zajac *et al.* (2018) as they showed that ashwagandha increases the number of red blood cells.

Conclusion

Ashwagandha is a botanical substance utilized for centuries in traditional medicine. Research

findings indicate its beneficial impact on the immune system and enhancement of pancreatic tissue condition, which is crucial for insulin regulation in patients with type 2 diabetes. Nonetheless, it is crucial to acknowledge that research on Ashwagandha is under progress, requiring further investigations to validate its potential therapeutic applications and identify the appropriate dosages and length of administration. Furthermore, it is crucial to evaluate the Ashwagandha safety, especially when administered with other drugs or supplements. Consequently, more research is essential to clarify the possible advantages and risks of Ashwagandha as a medicinal drug.

Conflict of Interest

The authors declare no conflicts of interest.

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