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The Effect of Ashwagandha Roots on Diabetic of Male Rats

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ABSTRACT

The present work aimed to investigate the effect of ashwagandha root powder (ARP) administrative by diabetic male rats. Thirty male rats were randomly divided into 5 equal groups (n=6). The 1st group was fed the basal diet for 56 days as a negative control (-ve). The 2nd group injected with a single dose of Alloxan to induce diabetes. Groups 3-5 as the same of group two but fed on basal diet containing 4, 6 and 8% of ARP, respectively. Results of chemical composition of ARP showed that each 100 g contained fat, fiber, protein, carbohydrate, ash and moisture at 0.9, 33.3, 3.3, 51.1, 4.2 and 7.2%, respectively. Polyphenolic compounds, Quercetin recorded 22.776 mg/100g, followed by Vanillic acid 18.66 mg/100g. The antioxidant activity DPPH was 63.69 % in the high tested level 7% of sample. Biological study showed that, administration of ARP to diabetic rats decreased weight gain, blood glucose level, TC, TG, LDL and MDA. On the other hand, levels of serum Insulin, HDL-c and CAT, were increased by ARP administration. In conclusion, dietary supplementation with ashwagandha root powder (ARP) resulted in a significant improvement in biomarkers associated with diabetes and hypercholesterolemia. These findings suggest that the intake of ARP may offer potential benefits for individuals with diabetes.

Key words: ashwagandha roots, antioxidant, diabetic Rat, hypercholesterolemia.

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INTRODUCTION

Diabetes is a group of metabolic diseases characterized by chronic hyperglycemia that results from disturbed insulin secretion or function or both. The complications of diabetes are a major public health challenge due to its large effect on health. The International Diabetes Federation (IDF) estimated the global prevalence of diabetes to be 451 million in 2017, and this number was expected to increase to 693 million by 2045. The increasing trend was found in prevalence of prediabetes and diabetes, overweight, central obesity and dyslipidemia were risk factors in prediabetes and diabetes **(Cho *et al.*, 2018)**. With the increasing burden of diabetes, effective preventive measures and management are required to reduce an individual's risk of developing diabetes and its complications. Scientific evidence shows that lifestyle interventions such as healthy diet and physical activity are more effective than medication in delaying or

preventing diabetes, especially in whose who are overweight or with impaired glucose tolerance (**Durg *et al.*, 2020).**

Medicinal plants are used globally as an alternative or complementary form of treatment. Rich supplies of bioactive chemicals with particular pharmacological qualities that don't have negative side effects can be found in many plants. Some phytoconstituents with antidiabetic properties are found in medicinal plants, including terpenoids, saponins, flavonoids or carotenoids, alkaloids and glycosides **Ali and Bhandari, (2025).**

In recent years Withania somnifera (Ashwagandha) gained a lot of interest as an adaptogen, aiding sleep, stress management and presenting health and sports-related benefits **(Sprengel *et al.*,2025).** The w. Somnifera (Ashwagandha), a potential medicinal herb, has promising therapeutic and pharmacological properties due to its diverse phytochemicals. Many studies on this medicinal herb have shown antidiabetic, antistress, anti-inflammatory, anti-cancerous, anti-COVID-19, immunomodulator, antimicrobial, and hepatoprotective activity. Importantly, Withania somnifera contains many important phytochemicals such as Withaferins that induce apoptosis in tumor cells and prevent their spreading. Glycoproteins derived from W. Somnifera inhibit the growth of phytopathogenic fungi **(Gaurav *et al.*,2023).**

MATERIALS AND METHODS

Materials

Dried ashwagandha roots were purchased from an Egyptian local market. Chemicals, casein, cellulose, choline chloride, D-L methionine, vitamin and mineral mixture constituents were purchased from El-Gomhoriya Pharmaceutical Company, Cairo, Egypt. Starch, soy oil, and sucrose were obtained from the Egyptian local market. Thirty adult male albino rats (Sprague Dawley strain), weighing about 150±10g b.wt. were obtained from the Laboratory Animal Colony, Agricultural Research Center, Giza, Egypt.

Methods

Preparation of Ashwagandha roots powder (**ARP**):

The dried ashwagandha was ground using a coffee grinder into a fine powder and frozen at 20 ℃ till used.

Induction of diabetes:

A single dose of recrystallized alloxan monohydrate dissolved in 0.5ml saline solution was intraperitoneally injected as a diabetogenic agent at 120 mg/kg body weight in overnight fasting rats **(Ebueli *et al.*, 2010).**

Diet composition and experimental animal design:

The basal diet was formulated according to AIN-93M diet **(Reeves *et al.*, 1993).**

Rats were housed in well conditions in the Biological Studies Lab of Faculty of Home Economics, Helwan University. After the period of adaptation, animals were divided into 5 groups (6 rats each). Groups from 2-5 injected with a single dose of recrystallized Alloxan (120mg/kg) before the beginning of the experiment to induce diabetes**.** After the appearance of

hyperglycemia which was tested by using Diabur Test rats were classified as follows**:** Group 1 (-ve control) was fed on basal diet only during the experimental period (8 weeks) and injected with saline the same as the other groups. Group 2 **(**diabetic group) were fed on a basal diet during the experimental period (8weeks) and served as +ve control. Group 3-5 as the same as in group 2 and were fed on basal diet with ashwagandha roots powder at 4, 6 and 8%, respectively.

During the experiment period the quantities of diet, which were consumed and/or wasted, were recorded every day. In addition, rat's weight was recorded weekly to determine body weight gain and feed efficiency ratio according to **Chapman *et al*., (1959)**.

Chemical analysis of Ashwagandha roots:

Chemical composition, polyphenolic compounds and diphenyl1-picrylhydrazyl (DPPH) radical-scavenging activity of Ash were conducted at the Food Safety and Quality Control Lab, Faculty of Agriculture, Cairo University, Giza, Egypt. Proximate chemical composition was determined according to **A.O.A.C. (2012).** Polyphenolic compounds were determined by high-performance liquid chromatography (HPLC) according to **Agilent, (2014).** DPPH radical-scavenging activity was conducted according to **Brand-Williams *et al.*, (1995).**

Biochemical Analysis of Serum:

At the end of the experimental period, rats were fasted overnight before sacrificing and blood samples were collected from each rat and were centrifuged at 3000 rpm for 15 min to obtain serum for biochemical analysis.

Testosterone level was determined according to **Wilke and Utley (1987).** Serum FSH and LH levels were measured according to **Loraine and Bell (1976)**. Malondialdehyde and catalase were determined according to **(Góth, 1991); (Shin, 2009).** Serum total cholesterol (TC), triglyceride (TG) and high-density lipoprotein cholesterol (HDL-C) were determined according to **Richmond, (1973); Wahlefeld, (1974) and Albers *et al*., (1983),** respectively. Regarding to low density lipoprotein cholesterol (LDL-c) and very low-density lipoprotein cholesterol (VLDL) they were calculated according to **Fridewald *et al*., (1972)** whereas the atherogenic index (AI) was calculated according to **(Nwagha *et al*., 2010)**.

Statistical Analysis:

Results were expressed as the mean standard error ± SE. Data were statistically analyzed for variance using the "ANOVA" test at $P \leq (0.05)$ using SPSS statistical software, version 20 **(Armitage and Berry, 1987)**.

RESULTS AND DISCUSSION

Results in **Table 1** of the proximate chemical composition of ashwagandha roots indicated that ARP contained fat, fiber, protein, carbohydrate, ash and moisture at 0.9, 33.3, 3.3, 51.1, 4.2 and 7.2%, respectively. These results were nearly similar to those reported by **Veer *et al*., (2019).** The previous authors demonstrated that ashwagandha roots possess excellent nutritional value 4.41g, the amount of protein 3.9g, fat 0.3g, of crude fiber 32.3g, energy 245Kcal, carbohydrate

49.9g, 3.3mg of iron, 23mg of calcium, 75.7 µg of total carotene and 5.8mg/100g of vitamin C **Kumari, and Gupta, (2016)**.

Table (1): Chemical Composition of Ashwagandha Roots Powder

Compounds	g/100g
Moisture	7.2
Ash	4.2
Fat	0.9
Fiber	33.3
Protein	3.3
Carbohydrate	51.1
Caloric value	245

Table 2 revealed that ARP was more powerful in polyphenolic compounds. Results showed that ARP contained22.776 mg quercetin, followed by 18.66 mg of vanillic acid, 11.062 mg of rutin, 10.25 mg of rosemarinic acid.

Table (2): Polyphenolic Compounds Concentration of Ashwagandha roots powder

Polyphenolic content	mg/kg
Quercetin	**22.776**
Vanillic acid	**18.6667**
Rutin	**11.062**
Rosemarinic acid	**10.25**
Syringic acid	**4.070**
Ferulic	**2.48**
Hesperidin	**1.129**

Data in **Table 3** indicated that ARP recorded higher DPPH radical scavenging activity with 63.69 % in the high tested level 7% of the sample as compared with 4% and 2% of the sample that recorded 32.17% and 24.84 % of antioxidant activity, respectively.

Table (3): The Antioxidant Activity (DPPH) of Ashwagandha roots Powder

Sample	%DPPH Radical-Scavenging Activity
7%	**63.69**
4%	**32.17**
2%	**24.84**

Several studies have shown that the phytochemical components of ashwagandha roots contain different classes of chemical compounds and a huge assortment of nutrients and phytochemicals

that have gained active research interest because they possess a wide array of health benefits and multidimensional importance **(Guvvala, *et al*.,2019).** The most important and widely investigated primary active constituents of the plant that have been identified as bioactive are withanolides, along with these lactones, the plant extract also contains alkaloids compounds which are their antioxidant activity. **Munir, *et al*., (2022) and Elhassaneen, *et al*., (2023).** In clinical studies, ashwagandha exerts multiple protective effects such as anti-cancer, anti-depressant, antioxidant, anti-inflammatory, anti-apoptotic, angiogenic and neuroprotective effects **(Jain, *et al*.,2024).** Thus, findings indicate that phenolic compounds, in addition to flavonoids, triterpenoids, and alkaloids, play a more major role in antioxidant activation**.** Therefore, it can be used as a functional food or a nutraceutical which has potential health benefits.

Results in **Table 4** showed that FI increased in positive control diabetic rats when compared with the negative control rats. Feeding rats on the diet supplemented with ARP decreased daily feed intake. Whereas, BWG and FER significantly decreased in the (+ve control) group compared to the (-ve control) group. Moreover, supplementation with ARP to diabetic rats in groups 3-5 caused significant reduction in BWG and FER when compared with the diabetic control group (+ve control).

Table (4): Effect of ashwagandha roots powder on feed Intake (FI), body weight gain (BWG) and feed efficiency ratio (FER) of diabetic rats

Parameters / Groups	FI (g/d)	BWG (g)	FER
G1:-ve control	**17.01**	**0.50±0.001^{a}**	**0.029±0.002^{a}**
G2:+ve control	**19.00**	**0.30±0.006^{b}**	**0.015±0.002^{c}**
G3: 4% ARP	**18.01**	**-0.11±0.001c**	**0.006±0.001^{d}**
G4: 6% ARP	**18.02**	**-0.13±0.002d**	**0.007±0.004^{b}**
G5: 8% ARP	**18.10**	**-0.15±0.008e**	**0.008±0.001^{b}**

*Mean values are expressed as mean ± SD.

*Mean values at the same column with the same superscript letters are not statistically significant at P<0.05.

* **ARP** = Ashwagandha Roots powder

The result in FI agreed with **Rajagopal and Sasikala (2008)**, and the result in BWG agreed with **Ojewale *et al*., (2020),** who found the injection with alloxan significantly reduced the body weights. **Das and Afrin, (2019)** reported that injection with alloxan decreased weight gain as found in the present study.

Sharweda and Gouda, (2024) reported that administration of ashwagandha roots decreased weight gain in experimental groups, as found in the present study, so ashwagandha roots can be considering a good candidate for weight loss. As found in the present study, also Ashwagandha rich in Withaferin A, a steroidal lactone compound isolated from ashwagandha may function to enhance leptin sensitivity (**Lee *et al*.,2016)** and may influence on leptin receptors as demonstrated by **Kaur and Kaur, (2017).** While current evidence is promising for benefits of ashwagandha on leptin sensitivity, weight loss through reduced stress, cortisol and food cravings (**Quinones *et al*.,2025**).

Table 5 illustrated that serum glucose level in diabetic rats significantly increased in the (+ve control) group compared to the (-ve control) group with mean values 333.20 vs. 106.80 ng/ml. Therefore, rats were fed 4, 6 and 8 % ARP significantly decreased when compared to (+ve control) group. On other hand, serum insulin significantly decreased in diabetic control (+ve group) when compared to the (-ve control) group with mean values 1.2 vs. 6.58 uIU/ml, respectively. Rats which fed ARP at 4, 6 and 8 % recorded a significant elevation in serum insulin compared to the +ve control group with mean values 1.85, 2.89, 3.53 vs. 1.2 uIU/ml, respectively.

Table (5): Effect of Ashwagandha roots powder on glucose and insulin level of diabetic rats

Parameters / Groups	Glucose (ng/ml)	Insulin (uIU/ml)
G1: -ve control	**106.80±04.26^{e}**	**6.58±0.58^{a}**
G2: +ve control	**333.20±12.49^{a}**	**1.20±0.03^{e}**
G3: 4% ARP	**212.80±07.11^{b}**	**1.85±0.04^{d}**
G4: 6% ARP	**173.80±07.68^{c}**	**2.89±0.25^{c}**
G5: 8% ARP	**140.80±9.56^{d}**	**3.53±0.51^{b}**

*Mean values are expressed as mean ± SE.

*Mean values at the same column with the same superscript letters are not statistically significant at P<0.05.

* **ARP** = Ashwagandha Roots powder

These research results agreed with **Ojewale *et al*., (2020),** who found that injection with alloxan elevate blood glucose level in rats. **Durg *et al*., (2017)** reported that the alterations induced by alloxan functions as a selective inhibitor of glucose-stimulated insulin secretion (by specifically inhibiting glucokinase which plays a key role as a glucose sensor in regulating glucose homeostasis) and the selective necrosis of beta cells due to the formation reactive oxygen species (ROS) on the other hand, the current study showed that ashwagandha contains various bioactive compounds with the potential to modify glucose metabolism and slow down the progression of diabetes. Findings agree with several studies have demonstrated that daily intake of *W. somnifera* exhibited a K+ (potassium) sparing diuretic effect similar to the anti-diabetic drug Daonil (glibenclamide) with no observed side effects **Andallu and Radhika, (2000). Sarangi *et al.*, (2013)** confirmed that active compounds of ashwagandha have demonstrated efficacy in modulating glucose homeostasis and enhancing insulin sensitivity and glycosylated hemoglobin (HbA1c) level without significant safety concerns. treatment with Ashwagandha roots has been observed to increase insulin secretion from pancreatic cells, helping regulate hyperglycemia in diabetic rats. **Narayanaswamy *et al*., (2025**) suggested that *W. somnifera* can regulate blood sugar levels and can be used as an alternative traditional medicine for diabetes therapy.

As shown in **Table 6**, diabetic rats had a significant increase in serum levels of total cholesterol, triglycerides, low density lipoprotein, very low-density lipoprotein and atherogenic index with mean values of 77.4, 195.6, 94.50, 39.12 and 0.65 respectively and a significant decrease in high density lipoprotein 43.78 when compared to the negative control group with mean values of 136.66, 121.4, 45.46, 24.28 and 0.26 respectively and a significant increase in high density lipoprotein 66.92 . Diabetic rats that were fed ARP recorded significant reduction in TC, TG, LDL, VLDL and AI levels and an increase in serum HDL when compared to (+ve control) group.

Table (6): Effect of ashwagandha roots powder on Serum Lipid Profile and Atherogenic Index (AI)

Parameters / Groups	TC	TG	LDL-C	VLDL-C	HDL-C	AI
	mg/dl					
G1: -ve control	136.66±2.09^{e}	121.4±0.94^{e}	45.46±0.08e	24.28±0.16^{e}	66.92±1.03a	0.26±0.001^{e}
G2: +ve control	177.4±1.56^{a}	195.6±2.10^{a}	94.50±1.04a	39.12±0.19^{a}	43.78±1.04e	0.65±0.001^{a}
G3: 4% ARP	158.24±1.21^{b}	178.25±1.76^{b}	71.87±0.48b	35.65±0.97^{b}	50.72±1.12d	0.55±0.004^{b}
G4: 6% ARP	155.42±1.01^{c}	167.45±2.23^{c}	64.08±0.88c	33.49±0.26^{c}	57.85±0.73c	0.46±0.006^{c}
G5: 8% ARP	150.00±0.88^{d}	143.3±0.99^{d}	59.16±1.01d	28.66±0.44^{d}	62.18±0.77b	0.36±0.002^{d}

*Mean values are expressed as mean ± SE.
*Mean values at the same column with the same superscript letters are not statistically significant at P<0.05.
* **ARP** = Ashwagandha Roots powder

El-Shamy, (2018) agreed with recent results as he found that alloxan significantly increase in the serum TC, TG, LDL-c and VLDL-c with significant reduction in HDL-c, which is due to alloxan mediated free radicals that induce lipid peroxidation and damage of organs membranes. The increased levels of VLDL-c are due to high levels of free fatty acids and hyperglycemia and also due to the reduction in activity of lipoprotein lipase. This is because insulin activates lipoprotein lipase, hydrolyzes triglycerides and inhibits lipolysis. In diabetes, however, there is an increase in lipolysis, which eventually leads to hyperlipidemia **Alaebo *et al*., (2022)**.
Recent clinical trials with ashwagandha supplementation reported that ashwagandha supplementation resulted in a remarkable reduction in LDL-C, TC, and TG levels, as well as an increase in HDL-C concentration **Rakha *et al*., (2023).** Also, **Tiwari *et al*., (2024)** results showed ashwagandha improves lipid profiles and reduces oxidative stress, which is beneficial for managing diabetes and associated dyslipidemia. Moreover, the result was in the same line with **Khateib and Diab (2021) and Fahmy and Gouda, (2024)** revealed that administration of ashwagandha caused significant increases in HDL, but decreases in cholesterol, triglyceride, LDL, VLDL. Moreover, the improvement in HDL levels in the treated groups suggests that these interventions not only reduce harmful lipid fractions but also enhance protective lipid components. This dual action is crucial in managing the dyslipidemia commonly associated with T2DM, which is a significant risk factor for cardiovascular diseases. The ability of these natural compounds to improve lipid profiles without adverse effects highlights their potential as complementary therapies in T2DM management.

Results recorded in **Table 7** showed that the positive control group causing a significant reduction (*P<0.05*) in the level of CAT while caused a significant (*P<0.05*) elevation in serum malondialdehyde (MDA) concentrations when compared with the negative control group. On the other hand, diabetic rats that were treated with ARP had a significant (*P<0.05*) increase in serum CAT and reduction in the elevated serum MDA when compared with the positive control group.

8Table (7): Effect of Ashwagandha roots powder on serum malondialdehyde (MDA) and catalase (CAT) of diabetic rats

Parameters / Groups	MDA ng/ml	CAT pg/ml
G1: -ve control	**1.69±0.04^{e}**	**42.05±1.61^{a}**
G2: +ve control	**7.95±0.65^{a}**	**25.82±1.91^{e}**
G3: 4% ARP	**2.89±0.02^{b}**	**30.56±1.11^{d}**
G4: 6% ARP	**2.63±0.63^{c}**	**32.43±1.29^{c}**
G5: 8% ARP	**2.54±0.77^{d}**	**34.93±1.99^{b}**

*Mean values are expressed as mean ± SE.

*Mean values at the same column with the same superscript letters are not statistically significant at P<0.05.

* **ARP** = Ashwagandha Roots powder

Current results were in line with research done on animals that were given alloxan to induce diabetes**.** Additionally, a number of studies have demonstrated that a reduction in the activity of the antioxidant CAT and an excess of MDA are important factors in the development of diabetes **Idris *et al*., (2020).** Also**, Azab *et al.*, (2022)** found that ashwagandha root extract induced a reduction in MDA levels associated with elevation in superoxide dismutase (SOD), Glutathione peroxidase activities and glutathione (GSH) content in rats. Studies have illustrated the potential of Ashwagandha to improve defense mechanisms by enhancing antioxidant systems and thus could prevent many radical related disorders **(Ahmed *et al*., 2018 and Devarasetti *et al*., 2024)**. HPLC analysis of ashwagandha roots powder revealed that it contained many phenolic acids and have potent antioxidant properties. Ashwagandha roots exhibit an antioxidant and hypolipidemic activity

Conclusion

Present study highlights the effect of ashwagandha roots powder on diabetic of male. Supplementation with ARP resulted in significant improvement, blood glucose level, levels of serum Insulin, lipide profile, oxidative stress markers in a dose-dependent manner and has a potential effect on male fertility. These effects are likely attributed to the enhanced antioxidant content. Therefore, intake of Ashwagandha Roots powder may be beneficial on fertility among diabetic patients.

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