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Effect of Ashwagandha Roots on the Fertility of Diabetic Male Rats

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ABSTRACT

The present work aimed to investigate the effect of ashwagandha root powder (ARP)consumption on the Fertility of 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 was injected with a single dose of Alloxan to induce diabetes. Groups 3-5 are the same as group two but fed on a basal diet containing 4, 6 and 8% of ARP, respectively. Results of the 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. The polyphenolic compound Quercetin recorded 22.776 mg/100g, followed by Vanillic acid at 18.66 mg/100g.The antioxidant activity DPPH was 63.69 % in the high tested level of 7% of sample. A biological study showed that, administration of ARP to diabetic rats decreased weight gain, total cholesterol (TC), triglyceride (TG), Low density Lipoprotein cholesterol (LDL), malondialdehyde (MDA) and sperm Abnormalities. On the other hand, FSH, LH, testosterone, High density Lipoprotein cholesterol (HDL-c,) CAT, motility%, Concentration %, and alive Sperms% were increased by ARP administration. In conclusion, dietary supplementation with ashwagandha root powder (ARP) resulted in a significant improvement in biomarkers associated with fertility and hypercholesterolemia. These findings suggest that the intake of ARP may offer potential benefits for individuals with fertility issues.

Key words: Ashwagandha Roots, fertility, antioxidant, Diabetic Rats.

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INTRODUCTION

Reproductive healthcare is a critical component of the overall well-being of individuals, encompassing various dimensions related to the functioning of the reproductive system and the mental, physical, and social states of individuals **Manikyam, (2024).** Data from the Centers for Disease Control and Prevention (CDC), the World Health Organization (WHO), and other relevant public health houses also indicate that infertility is on the rise worldwide. The latest data

indicate that the prevalence of infertility in married couples of reproductive ages is 17–26 %, of which 56 % require treatment **(Carson and Kallen, 2021; Legese *et al*., 2023)**

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).** 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. Ashwagandha can help restore hormonal balance disrupted by diabetes. In studies, it has been observed to increase levels of progesterone, testosterone, and luteinizing hormone (LH) in diabetic rats. LH is crucial for testosterone production, which plays a vital role in male reproductive health. **(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**):

he 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 were 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 (one touch altar strips) rats were classified as follows**:** Group 1 (-ve control) was fed on a basal diet only during the experimental period (8 weeks) and injected with saline the same as the other groups. Group 2 **(**the diabetic group) was fed on a basal diet during the experimental period (8weeks) and served as a positive control.

Group 3-5 were the same as group 2 and were fed on basal diet with ashwagandha roots powder at 4, 6, 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)**.

Epididymis spermatozoa

a-Sperm collection

Sperm samples were collected from the distal region of the epididymis(cauda)according to (**Mali *et al*.,2002).** Sperm samples were used for evaluation of count, motility and morphology according to **(Narayana *et al*., 2005).**

Sperm Parameters

Assessment of sperm Count according to **(Seed *et al*.,1996).** Sperm concentration, sperm motility, sperm a live **%** and sperm abnormality (%) were determined according to **(Blom, 1983).**

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. These results were nearly similar to those reported by **Veer *et al*., (2019).**

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 phenolic compounds. Results showed that ARP contained 22.776 mg of 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 **(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 a 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)**.

As shown in **Table 5**, diabetic rats had a significant increase in serum levels of total cholesterol, triglycerides, low density lipoprotein, very low-density lipoprotein and atherogenic

index and a significant decrease in high density lipoprotein when compared to the negative control group. Diabetic rats that were fed ARP recorded significant reductions in TC, TG, LDL, VLDL and AI levels and an increase in serum HDL when compared to (+ve control) group.

Table (5): 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. 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 6** showed that the positive control group causing a significant reduction (*P<0.05*) in the level of CAT while casing 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 (6): 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 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.

Results recorded in **Table 7** showed that the positive control group caused a significant decrease in the level of LH, FSH and testosterone with mean values 1.98, 2.34and1.01 respectively when compared with the negative control group with mean value5.15, 6.70 and3.23 respectively. On the other hand, diabetic rats that treated with ARP had significant increase in serum LH, FSH, testosterone when compared with positive control group.

Table (7): Effect of ashwagandha roots powder on serum hormones: LH, follicular stimulating hormone (FSH) and Testosterone

Parameters / Groups	LH	FSH	Testosterone
	(ng/ml)		
G1: -ve control	**5.15±0.75^{a}**	**6.70±0.93^{a}**	**3.23±0.06^{a}**
G2: +ve control	**1.98±0.07^{e}**	**2.34±0.04^{e}**	**1.01±0.02^{e}**
G3: 4% ARP	**3.98±0.82^{d}**	**5.75±0.72^{d}**	**2.23±0.07^{d}**
G4: 6% ARP	**4.47±0.98^{c}**	**5.98±0.13^{c}**	**2.33±0.06^{c}**
G5: 8% ARP	**4.64±0.79^{b}**	**6.50±0.46^{b}**	**2.99±0.04^{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

Testosterone (T) plays a role in several metabolic functions in men, with T deficiency associated with metabolic disorders such as type 2 diabetes, impaired glucose tolerance, insulin resistance, obesity, increased triglycerides, total cholesterol, and decreased high-density lipoprotein (HDL) cholesterol, contributing to cardiovascular risk **Dandona *et al.*, (2021).** Ashwagandha was found to be beneficial in maintaining testosterone levels and prevents the reduction of T levels induced by stress under the influence of cortisol (C) and prolactin (PRL), leading directly to increased GnRH and LH concentrations. In addition to normalizing T concentrations, ashwagandha improves the antioxidant potential of seminal plasma by reducing oxidative stress **Sengupta *et al.*, (2018).** Also, the obtained findings agreed with **Sahin *et al*., (2016)** who said that Ashwagandha roots extract was found to be significantly effective in sexual functioning and antioxidant capacity. Ashwagandha supplementation improves sexual function in male rats via activating Nrf2/ HO-1 pathway while inhibiting the NF-κB levels. Also reported Ashwagandha roots extract improve Testosterone, Follicle Stimulating Hormone, Luteinizing Hormone **Mutha *et al*., (2024).**

As shown in **Table 8**, diabetic rats had a significant decrease of motility, sperm concentration and alive sperms and a significant increase of sperm abnormalities when compared to negative control group. Diabetic rats that treated with ARP had significant increase Motility of sperm, sperm Concentration, Alive Sperms and reduction of sperm Abnormalities when compared with positive control group.

Table (8): Effect of Ashwagandha roots powder on Motility%, Concentration %, Alive Sperms% and Abnormalities diabetic rats

Parameters / Groups	Motility	Concentration	Alive Sperms	Abnormalities
	%			
G1: -ve control	**58.00±0.68^{a}**	**76.40±1.40^{a}**	**75.40±0.43^{a}**	**14.40±1.62^{d}**
G2: +ve control	**39.00±0.12^{d}**	**47.60±0.91^{d}**	**56.60±0.60^{d}**	**22.60±0.51^{a}**
G3: 4% ARP	**46.00±0.93^{c}**	**61.60±1.20^{c}**	**64.40±0.52^{c}**	**18.60±0.85^{b}**
G4: 6% ARP	**47.60±0.38^{c}**	**62.00±1.06^{c}**	**66.60±0.30^{c}**	**18.40±0.31^{b}**
G5: 8% ARP	**53.20±0.44^{b}**	**67.40±1.23^{b}**	**71.40±0.94^{b}**	**16.40±0.39^{c}**

*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

Spermatozoa need specific carriers, known as glucose transporters (GLUTs) to mediate the glucose uptake from the surrounding medium into the cell. Diabetes has been shown to be associated with a depletion of GLUTs. Therefore, diabetic individuals are known to possess an inability to transport glucose, which supports an association of this disease with disruptions in sperm metabolism and consequently sub fertility or even infertility **Njoku-Oji *et al.*, (2019).** This finding is similar to results obtained from **Oloye *et al*, (2024)** reported that alloxan injected rats had a lower percentage of normal sperm morphology. Recent studies support the efficacy of Ashwagandha in enhancing male fertility. A clinical trial involving men with infertility found that supplementation with Ashwagandha root extract significantly increased testosterone levels, sperm count, and motility **Nguyen-Thanh *et al*., (2024)**. Another study demonstrated that

Ashwagandha improves reproductive system function by enhancing semen quality, enhancing enzymatic activity in seminal plasma, and decreasing oxidative stress **Leisegang and Finelli, (2021).** This finding is consistent with the prior published studies on the ashwagandha (*W. somnifera*) root extract in healthy volunteers, which was well accepted **Verma *et al*., (2021).** Thus, finding agreed with **Chauhan *et al., (*2022)** who suggested that ashwagandha helps to improve male sexual health could be due to an increase in serum.

The testes tissue section of the negative control group showed normal histological structure of seminiferous tubules with normal spermatogenic cells with well-arranged spermatogenetic stages **Photo (a)**

The testes tissue of the diabetic animal group (+ve control) showed histological changes in the testes characterized by showing reduction and loss of the normal orientation of the germ cells others revealing desquamation of the germ cells in their lumen in addition to dispersion and

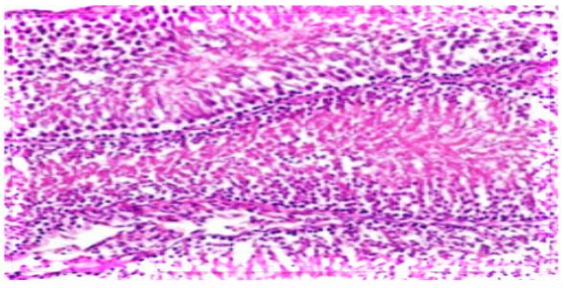

Photo (a)

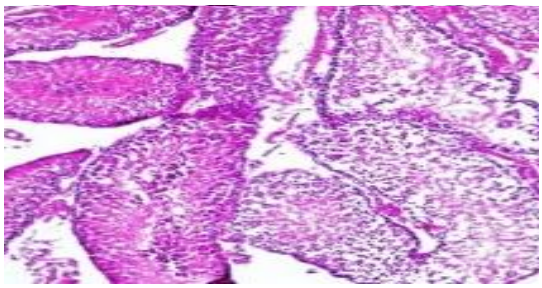

Photo (b)

irregular contouring of the basement membrane of the seminiferous tubules **Photo (b)**

The testes tissue of the diabetic rats treated by 4% Ashwagandha roots revealed histological round ring nuclei, which is degeneration of round spermatids with peripheral condensation of the nuclear chromatin **Photo (c).**

The testes tissue of the diabetic rats treated by 6% Ashwagandha roots revealed histological showing revealing thickening of the basal lamina of seminiferous tubules, reduction in the number of germ cells with necrobiotic changes in the spermatogenic epithelium **Photo (d).**

The testes tissue of the diabetic rats treated by 8% Ashwagandha roots revealed histological showing normal structure others revealing macrobiotic changes with depletion and loss of the normal orientation of the germ cells **Photo (e)**.

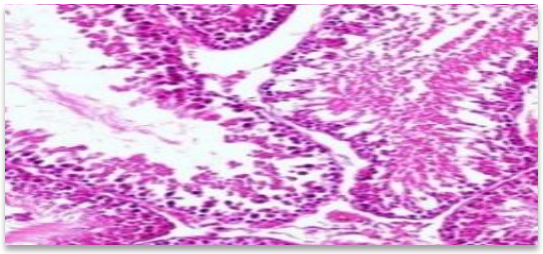

Photo (c)

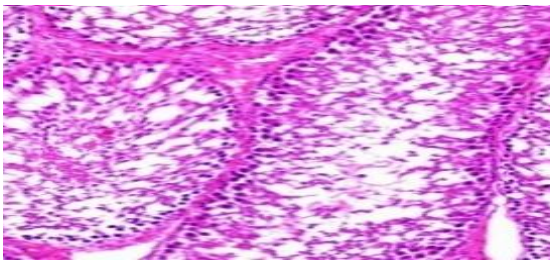

Photo (d)

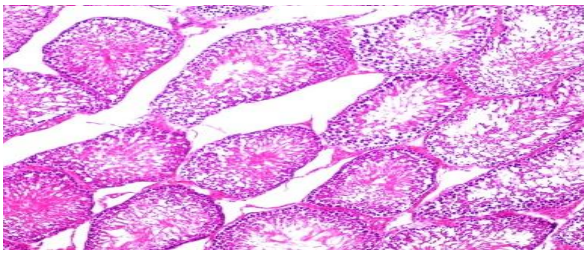

Photo (e)

Results of testes histology were confirmed by **Ismail, (2021)** who reported that the induction of diabetes mellitus by using of alloxan cause severe effect in the male reproductive organs. This occur due to oxidative stress this associated with failure of testis function to dysfunction because the testicular tissue and spermatocytes are susceptible to free radical's damage due to high level of poly unsaturated fatty acid, with low oxygen tension and with lack of antioxidant defense mechanism **(**Singh ***et al*.,2009).**

The abnormalities of histology analysis of testes tissue are mainly due to stimulation detriment blood testes barrier (BTB) changes which induce alteration of testis that cause disrupting the metabolic action between cellular content of BTB with the consequences on sperm quality and fertility **(Amaral, *et al.,* 2006).**

The changes are believed to be due to oxidative stress, which results from the imbalance between the oxidative agents and the antioxidant system leading to production of free radicals, which have a role in inducing harmful effects on living tissues **(Adwas, *et al.,* 2019).** The study reported that, Ashwagandha roots for 60 days markedly reduces testicular oxidative stress in diabetic rats. The obtained results were in the same context of results found by **Jafari, *et al*., (2024)** indicated that rats treated with WS (500 mg/kg), both pre-treatment and post-treatment groups showed significant preservation of spermatogonia relative to the adverse impacts of Cyclophosphamide CP on testicular Additionally, pre-treatment with WS significantly increased the diameter of the seminiferous tubules compared to the CP group.tissue group. Another study by **Chandrasekhara, and Manjunath, (2014)** who revealed that the potential of WS to improve diabetes-induced testicular dysfunctions in prepubertal rats. Also, **Hashem, *et al.,* (2023)** found that ashwagandha root extract attenuated the changes brought on by diabetes. Histological examination. Another study by **Baghel and Srivastava, (2021)** concluded that efficacy of ashwagandha use in an animal model of infertility—the photorefractory Japanese quail with regressed testes and decreased expression of estrogen receptor alpha

Conclusion

Present study highlights the effect of ashwagandha roots powder on the fertility of diabetic male. Supplementation with ARP resulted in significant improvement, lipide profile, oxidative stress markers 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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