



Improvement of Male Rats Fertility and Immunity using Ashwagandha (*Withania somnifera*, L) Roots

Emad El-Kholie, Sherif Ragab, Noura Abdallah

Department of Nutrition and Food Sciences, Faculty of Home Economics, Menoufia University, Shibin El Kom, Egypt

Article Type

Original Article

Corresponding author:

Emad El-Kholie
emad.elkhouli@hec.menoufia.edu.eg
Mobile: +2 0224673480
DOI: 10.21608/MKAS.2024.282934.1309

Cite as:

El-Kholie et al., 2024, Improvement of Male Rats Fertility and Immunity using Ashwagandha (*Withania somnifera*, L) Roots. JHE, 34 (3), 129-143

Received: 15 Apr 2024

Accepted: 31 May 2024

Published: 1 July 2024

ABSTRACT:

This study investigated the influence of ashwagandha root powder and its ethanolic extract on infertility rats. Thirty-six male albino rats have been divided into two main groups. Group 1 (6 rats) assigned as normal control group and fed on basal diet. The rest (30 rats) were injected with cadmium chloride at a dose of 0.1 mg/kg of the rat's weight for 10 days to induce infertility and then divided into 5 groups of 6 rats each. Group 2 fed basal diet only and assigned as a control (+); groups (3–4) were fed the basal diet plus (2.5 and 5%) of ashwagandha root powder; groups (5–6) were fed the basal diet plus (250 and 500 mg/kg) ashwagandha root ethanolic extract orally. After the four-week trial was over, the rats were slaughtered. Blood samples were collected for determination of kidney functions (urea, creatinine, and uric acid), hormones (luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone), antibodies (IgG, and IgM), blood lipids (TC, TG, LDL-c, VLDL-c, and HDL-c), and liver enzymes (ALP, ALT, and AST). The results revealed that using ashwagandha root resulted in a significant rise in testosterone and FSH, a reduction in LH hormone, a drop in glucose, lipid fractions, renal and liver biomarkers, and a reverse impact on HDL-c. In conclusion, the administration of ashwagandha root powder and extract can lower the side effects of a cadmium chloride testicular toxicant and improve the health status of the testis with an increase in reproductive and immune potential.

Keywords: Reproductive, immune system, sex hormones, Indian winter cherry

1. INTRODUCTION

The Solanaceae family consists of the ashwagandha (*Withania somnifera*), a tiny woody shrub or herb that usually reaches a height of 30 to 50 cm (maximum of a hundred and fifty cm). This adaptogenic plant is utilized in ayurveda and Unani

remedies to enhance muscle power and endurance, stimulate "youthful vigor," and enhance usual health [1, 2]. Ashwagandha extract is a complicated combination of various phytochemicals, comprising flavonoids and phenolic substances. Nonetheless, withanolides

are the concept to be responsible for the medicinal residences of ashwagandha roots [3]. Because of its numerous benefits, which includes anti-diabetic [4], anti-inflammatory, anti-microbial [5], anti-tumor [6], anti-stress, cardioprotective, and neuroprotective [7] effects, it has been utilized both alone and in conjunction with different natural plants in numerous research investigations.

The immune-modulating impact was once established through a find out about examining the impact of ashwagandha root powder on the stimulation of immunological aspects in immunodeficient rats. It has been observed that ashwagandha dietary supplements raise the total count of white blood cells and bone marrow as properly as the titer of circulating antibodies and antibody-producing cells. They additionally promote the manufacturing of new immune device cells [8].

The hormones testosterone and dehydroepiandrosterone play a variety of essential features in our bodies, influencing, amongst different things, the extent of bones, coronary heart rate, cognitive and intellectual abilities, body composition, sexual performance, and the rate of metabolism [9 ,10]. The enzyme recognized as aromatase has the capability to convert testosterone into estrogens. The hormone estradiol, which is generally linked to women, additionally decreases with getting old in guys [11]. The addition of ashwagandha liquid

extract had a massive impact. Rats' serum ranges of LH rose while FSH lowered [12]. Furthermore, it was once established that this remedy considerably affected men, as evidenced through will increase in serum and LH concentrations and decreases in FSH ranges [13].

Additionally, ashwagandha in the shape of powdered root considerably raised semen characteristics. There utilized to be an enlargement in the wide variety of sperm, an enlargement in sperm morphology, an extend in sperm amount, and an expand in sperm motility, all of these accelerated a woman's possibilities of turning into impregnated. Semen blood additionally contained a make bigger in antioxidant enzymes and antioxidant-rich vitamins A, C, and E, as properly as a hormonal stability that used to be altered [14 ,15]. It has no adverse reactions and enhances the traits of sperm in adult males with idiopathic infertility. Furthermore, ashwagandha may be concept of as an alternate preference remedy to [16]. Supplementing with ashwagandha root extract proved to be related with a statistically large rise in the full DISF-M (Derogatis interview for sexual functioning-male) rankings when contrasted with placebo [17]. Therefore, the present study was designed to determine the effect of ashwagandha root and its extract on the nutritional, immune and sexual health status in rats.

2. MATERIALS AND METHODS

2.1. MATERIALS

2.1.1 Ashwagandha

Ashwagandha (*Withania somnifera*) root bought from the Haraz herbalist in Cairo City, Cairo Governorate.

2.1.2 Chemicals

The German chemical company Merk supplied the cadmium chloride ($CdCl_2$).

2.1.3 Rats

The study included the use of 36 grown up male albino "Sprague Dawley" rats that weigh between 150 and 160 g. They have been bought from Cairo's Doki-based Research Institute for Medical Insects.

2.2 METHODS

2.2.1 Materials preparation

As stated through [18], which states that all herbs need to be saved in dry, darker surroundings to stop the oxidation method of their contents, all ashwagandha root is ground into powder and dried the usage of sunrise. It is then saved in darkish glass bottles in a dry, cool area till used.

2.2.2 Induced infertility in rats

To cause male infertility, rats were administered cadmium chloride ($CdCl_2$) solution by intra-peritoneal injection at a dose of 0.1 mg/kg of the rat's weight for 10 days [19].

2.2.3 The experiment's design.

The study had been carried out through the Menoufia University, Shebin El-Kom, Faculty of Home Economics.

Rats are housed at a temperature of 25°C in normal, hygienic settings with steel

frames. In this study, thirty-six grownup male white albino "Sprague Dawley" rats weighing between 150 and 160 g have been utilized. To useful resource with adaptation, a standard diet organized in accordance with equation [20] was once given to all the rats for a duration of 7 days. After the duration of alteration, the following eight groups of six rats had been formed: Group (1): Rats have been only given the regular diet as group of control negative. Group (2): As a positive control, male rats with infertility had been simply given the basal diet. Group (3): Rats with infertility have been given a basal diet along with 2.5% ashwagandha roots powdered of the weight of the diet. Group (4): Rats with infertility have been given a basal diet along with 5% ashwagandha roots powdered of the weight of the diet. Group (5): Rats with infertility have been given a basal diet along with 250 mg/kg of ashwagandha roots extract by stomach tube of the weight of the diet. Group (6): Rats with infertility have been given a basal diet along with 500 mg/kg of ashwagandha roots extract by stomach tube of the weight of the diet.

Upon completion of the 28-day experiment, every animal in my phase was weighed, slaughtered, and subjected to blood testing.

2.2.4 Collection of blood samples

Following evaluation, blood samples were taken utilizing the abdominal aortas of rats that had been put down using

ether anesthesia. To separate the serum, blood samples were placed in sterile, sanitized centrifuge tubes and allowed to coagulate for ten minutes at room temperature. To prepare the serum for analysis, it was thoroughly separated, put into sanitized centrifuge tubes, and frozen at -20°C. A biochemical analysis has been performed on each serum sample [21].

2.2.5 The biochemical evaluation

For this purpose, the calorimetric measurement of follicular stimulating hormone (FSH) and luteinizing hormone (LH) used to be carried out the usage of the [22] techniques. Calorimetric identification of the testosterone hormone used to be carried out the usage of the [23] method. Chromatographically purified rat IgG was obtained from Cappel (PA, USA) and purified myeloma IgM from Serotec (Oxford, United Kingdom), according to the method of [24]. Total cholesterol was measured once, in accordance with [25]. Triglyceride measurements had been made using the techniques described in [26]. High-density lipoproteins cholesterol can be measured with the use of the [27] approach [HDL-c]. The measurements of VLDL-c and LDL-c have been carried out in accordance with [28].

$$VLDLc \text{ (mg/dl)} = \text{Triglycerides} / 5$$

$$LDLc \text{ (mg/dl)} = (\text{Total cholesterol} - HDLc) - VLDLc$$

Urea was determined according to the enzymatic technique of [29]. Enzymatic colorimetry used to be used to look at alkaline phosphatase (ALP) in accordance

with [30]. Using the [31] approach, the activities of aspartate amino transferase (AST) and alanine amino transferase (ALT) were measured. According to [32]. uric acid used to be quantified whilst using the enzymatic technique of [33]. Urea was determined by enzymatic method according to [34]. The kinetic technique of [35] was utilized to determine creatinine.

2.2.6 Statistical analysis:

The data were analyzed using a completely randomized factorial design [36] when a significant main effect was detected; the means were separated with the Student-Newman-Keuls Test. Differences between treatments of ($P \leq 0.05$) were considered significant using Costat Program. Biological results were analyzed by One Way ANOVA

2.2.7 Ethical approval

This study got approval of study protocol #14-SREC-05-2022 has been given through the Science learn about Ethics Committee.

3. RESULTS AND DISCUSSION

Table 1 indicates the average sex hormones level in infertile rats at a variety of levels of ashwagandha roots. It is evident that the testosterone hormone of group with control negative significantly in contrast with the group with control positive. The average reading was once 1.85 and 0.13 ng/ml. Regarding the infertility groups, the results collected indicated that a group of 500 mg/kg ashwagandha roots extract had

considerably higher testosterone hormone value. Group of 2.5% for ashwagandha root powder found a decrease value with corresponding means of 1.86 and 0.93 ng/ml.

When it came to FSH hormone, it was once found that the group with control negative had an average reading of 0.37 and 0.13 ng/ml, respectively, which was considerably greater than the group with control positive. However, infertility group results for all groups revealed that a group with 500 mg/kg ashwagandha root extract had a particularly greater FSH hormone value. The lowest value was once observed in a group with 2.5% ashwagandha root powder, which had been 0.31 and 0.18 ng/ml, respectively.

Also, Table (1) demonstrates the average LH hormone levels in rats that had been infertile and fed different diets. It is evident that there used to be no significant difference between the average LH hormone values for the control positive and negative groups, which ranged from 0.24 to 0.12 ng/ml. Regarding the infertile groups, the data revealed that the group receiving 500 mg/kg of ashwagandha root extract had the non-significantly lowest LH hormone value. The group that consumed 2.5 percent powdered ashwagandha roots had the highest values, which had been 0.13 and 0.19 ng/ml, respectively. The findings are consistent with [37] that ashwagandha extract has antifertility results on the concentration of epididymal sperm in male rats, their

sexual behavior, and their reproductive performance. Thus, ashwagandha ethanol extract is an effective spermicide that rendered a million-rat sperm completely motionless in about twenty seconds. Furthermore, a range of secondary metabolites, inclusive of tannins, phenol, alkaloids, flavonoids, steroids, and various fragrant chemicals, are considerable in the ashwagandha ethanol extract. The male fertility is inhibited via the plant-based contraceptive, as confirmed by way of the dose-dependent reduce in caudaepididymal sperm counts and motility following the injection of ashwagandha extract [38].

Additionally, in rats fed a combination of powdered ashwagandha, extract, and L-carnitine, there was once a drop in reproductive hormones such as serum testosterone, follicle stimulating hormone, and luteinizing hormone. This research consequently recommended that patients' fertility be enhanced via utilizing ashwagandha [39].

Table 2 presents the average serum IgM reading for infertile rats fed on a variety of diets. The negative control group's maximum serum IgM value used to be significantly greater than that of the control positive group, which had concentrations of 345.95 and 317.15 mg/ml, respectively. In comparison, treated groups revealed that the group with 500 mg/kg ashwagandha roots extract had a considerably greater IgM value than group three (2.5 percent

ashwagandha roots powder), with respective means of 409.74 and 372.83 mg/ml.

Table (1): Serum testosterone hormone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) of the experimental rats groups

	Testosterone (ng/ml)	LH (ng/ml)	FSH (ng/ml)
G1	1.85a±0.13	0.12b±0.35	0.37a±0.10
G2	0.77c±0.18	0.24a±0.81	0.13c±0.41
G3	0.93c±0.16	0.19a±0.065	0.18b±0.025
G4	1.38b±0.13	0.17b±0.055	0.24b±0.17
G5	1.64ab±0.15	0.16b±0.051	0.29a±0.14
G6	1.86a±0.19	0.13b±0.037	0.31a±0.12
LSD	0.410	0.091	0.103

G1: Control (-v), G2: Control (+v), G3: (2.5% Ashwagandha roots powder), G4: (5% Ashwagandha roots powder), G5: (250 mg/kg Ashwagandha roots extract), G6: (500 mg/kg Ashwagandha roots extract). Means in each column that have different superscript letters are considerably different at $P \leq 0.05$.

The average IgG values of the control negative group, which have been 1237.78 and 1208.45 mg/ml, respectively, had been greater than these of the positive control group. Regarding the infertility groups, the data collected confirmed that the group fed 500 mg/kg of ashwagandha root extract had a considerably greater IgG value, whereas the group fed 2.5 percent ashwagandha root powder had a lower value, which were 1416.83 and 1355.31 mg/ml, respectively. These findings are consistent with [40] who located that by way of modifying the innate and adaptive immune systems, ashwagandha extract considerably improved the immunological profile of wholesome individuals. Ashwagandha extract can be used to give a boost to the immune

system in these who are prone to sickness as nicely as in instances of widespread diseases.

Furthermore, the stimulation of immune-regulatory cells by using ashwagandha extracts may additionally have more than one purposes, inclusive of modulating antigen presentation, managing the immunosuppressive milieu, and offering a healthful cytokine milieu for effector T cell activity [41].

Table (2): Some serum immunity parameters of negative control and infertility rat groups treated by ashwagandha roots powder and extract at the end of study

	IgG (mg/ml)	IgM (mg/ml)
G1	1237.78d±5.14	345.95e±3.45
G2	1208.45e±4.60	317.15f±3.20
G3	1355.31c±2.45	372.83d±3.24
G4	1383.32b±5.31	377.85c±3.17
G5	1381.33b±6.20	397.92b±3.29
G6	1416.83a±7.48	409.74a±3.38
LSD	5.150	3.630

G1: Control (-v), G2: Control (+v), G3: (2.5% Ashwagandha roots powder), G4: (5% Ashwagandha roots powder), G5: (250 mg/kg Ashwagandha roots extract), G6: (500 mg/kg Ashwagandha roots extract). IgG= Immunoglobulin G. IgM= Immunoglobulin M. Means in each column that have different superscript letters are considerably different at $P \leq 0.05$.

Table (3) presents evidence of the average lipid fraction of infertility rats given various diets. The TG values of the control positive group had been clearly a whole lot greater than these of the control negative group, measuring 153.82 and 80.75 mg/dl, respectively. The findings collected for the infertile groups confirmed that the group given 2.5% ashwagandha roots powder had a considerably decrease TG value, and the

group given 500 mg/kg ashwagandha roots extract had a greater range, with TG values of 131.25 and 97 mg/dl, respectively.

Regarding the serum total cholesterol (TC) of infertile rats fed ashwagandha - containing diet. The group with the positive control had a considerably greater TC than the group with the negative control, which were 168.75 and 94.25 mg/dl, respectively. In regard to the infertile groups, the group given 500 mg/kg of ashwagandha root extract had a considerably lower TC value than the group fed 2.5 percent ashwagandha root powder, the average readings were 107.50 and 124.50 mg/dl.

It is obvious that HDL-c degrees of the negative control group showed a considerably greater value when compared with the positive control groups', the corresponding average was 50.34 and 31.22 mg/dl, respectively. Regarding the infertility groups, the results appeared that the group getting 500 mg/kg of ashwagandha root extract had a considerably greater HDL-c value than the group receiving 2.5 percent ashwagandha root powder, with values of 44.89 and 35.43 mg/dl, respectively.

Additionally, Table (3) indicates that the infertility rats' serum LDL-c degrees when given a variety of diets. It needs to be stated that the group with the positive control had an advised LDL-c value of 106.77 mg/dl, which was once considerably greater than the group with the negative control (27.76 mg/dl).

Regarding the infertile groups, the received statistics validated that the LDL-c value was once extensively increased in the group that obtained 2.5% ashwagandha roots powder, whilst it was once decrease in the group that received 500 mg/kg ashwagandha roots extract, 62.82 and 43.21 mg/dl, respectively.

Rats that were infertile had been fed a variety of diets regarding their serum VLDL-c levels. The records revealed that the group with control positive had an extensively greater average range of VLDL-c at 30.76 and 16.15 mg/ml, respectively, than the crew with control negative. Regarding the groups experiencing infertility, the findings revealed a exceedingly greater VLDL-c value in the group that obtained 2.5% ashwagandha root powder, in contrast to a decrease value in the group that obtained 500 mg/kg ashwagandha root extract, which means 26.25 and 19.40 mg/dl, respectively. These consequences corroborate [45], which pronounced that ashwagandha root extract remedy of the rats resulted in modifications to serum lipids except for high density lipoprotein-bound cholesterol (HDL-c) and tissues like liver, kidney, and coronary heart lipids returning to normal. This suggests that ashwagandha root extract has hypolipidaemic effects in rats with alloxan-induced diabetes mellitus. Additionally, [46] shown how ashwagandha root powder has been proven to decrease triglycerides,

cholesterol, and total lipids in rats with hypercholesterolemia. However, the extract additionally markedly raised the liver's bile acid concentration and plasma HDL-cholesterol levels.

Furthermore, results demonstrated that rats' lipid profiles (TC, TG, LDL-c, VLDL-c, and HDL-c) had been all increased greater when oral ashwagandha root ethanolic extract at 400 mg/kg/B.W. was once given, as adverse to each group with control and different groups [47].

These observations inform the findings [42], which discovered that the most considerable element of ashwagandha roots-which are widely recognized for their advantages in the therapy of hypercholesterolemia-was beforehand observed to decrease blood total

cholesterol and enhance seated stature in this investigation.

Furthermore, when the hypocholesterolemic properties of ashwagandha root have been evaluated in human patients, large reductions in low-density lipoproteins, triglycerides, and serum cholesterol had been referred to [43].

Additionally, feeding fats female rats a combination made of powdered studied plants can also assist them gain enhancements in body weight and other parameters. Additionally, it had significantly greater concentrations of HDL-c and dramatically lowered concentrations of triglycerides and total cholesterol [44].

Table (3): Some serum lipid profile of negative control and infertility rat groups treated by ashwagandha roots powder and extract at the end of study

	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)	VLDL-c (mg/dl)
G1	94.25e±1.54	80.75f±1.65	50.34a±1.45	27.76f±1.12	16.15e±0.52
G2	168.75a±2.68	153.82a±2.27	31.22e±1.12	106.77a±2.42	30.76a±0.92
G3	124.50b±2.45	131.25b±2.10	35.43d±1.47	62.82b±1.60	26.25b±0.64
G4	116.80c±2.37	119.50c±2.11	39.91c±1.70	53.71c±1.43	22.90c±0.43
G5	114.70c±2.00	105.00d±1.89	41.13c±1.66	51.77d±1.25	21.90c±0.36
G6	107.50d±1.98	97.00e±1.78	44.89b±1.35	43.21e±1.10	19.40d±0.34
LSD	3.250	3.135	1.130	1.642	1.160

G1: Control (-v), G2: Control (+v), G3: (2.5% Ashwagandha roots powder), G4: (5% Ashwagandha roots powder), G5: (250 mg/kg Ashwagandha roots extract), G6: (500 mg/kg Ashwagandha roots extract). Means in each column that have different superscript letters are considerably different at $P \leq 0.05$.

Table (4) indicates the effects of various doses of ashwagandha roots on liver activity markers such as ALT, AST, and ALP in male infertile rats. The control positive group's ALT liver enzyme values had been considerably greater than these of the control negative group, measuring

65.24 and 30.93 U/L, respectively. Regarding the infertile groups, the data gathered confirmed that the group administered 500 mg/kg of ashwagandha root extract had a considerably decrease ALP value than the group given 2.5 percentage ashwagandha root powder,

with corresponding ability of 45.30 and 59.60 U/L.

When it comes to AST liver activity, it should go as mentioning that the group with control positive had a a long way greater value for this enzyme than the group with control negative. The averaging reading was 128.40 and 61.20 U/L. In contrast, information on infertile groups confirmed that those group who administered 500 mg/kg of ashwagandha root extract had significantly decreased ALP values. On the different hand, the group that received 2.5 percent powdered ashwagandha roots had a greater value, which was 78.19 and 103.17 U/L.

In terms of ALP liver activity, the group with control positive performed considerably higher than the group with control negative. The average values were 177.50 U/L and 365.17 U/L. When looking at the infertile groups, the records confirmed that the group administered 500 mg/kg of ashwagandha root extract had a considerably decrease ALP value (208.08) than the group given 2.5 percent ashwagandha root powder (273.92 U/L). These outcomes are regular with those of [48] which proposed that ashwagandha might also have liver-protective benefits. The rats' AST, ALT, and ALP alterations back to regular after receiving ashwagandha root extract treatments. Ashwagandha may also have a liver-protective influence due to the fact of its excessive degree of antioxidants [49].

These outcomes are steady with those of [50] who established that the free radical scavenging activity of ashwagandha root extract restored serum GPT and GOT to regular ranges in rats that had been intoxicated with gentamicin. Consequently, it would possibly have hepatoprotective benefits.

Table (4): Some serum liver function of negative control and infertility rat groups treated by ashwagandha roots powder and extract at the end of study

	ALT (U/L)	AST (U/L)	ALP (U/L)
G1	30.93e±0.85	61.20f±1.13	177.50f±2.86
G2	65.24f±1.37	128.40a±2.3	365.17a±4.5
G3	59.60b±1.26	103.17b±2.1	273.92b±4.1
G4	54.51b±1.10	94.34c±1.87	254.86c±3.8
G5	51.25bc±1.0	89.68d±1.52	231.82d±3.5
G6	45.30d±0.98	78.19e±1.16	208.08e±3.1
LSD	1.340	2.153	4.720

G1: Control (-v), G2: Control (+v), G3: (2.5% Ashwagandha roots powder), G4: (5% Ashwagandha roots powder), G5: (250 mg/kg Ashwagandha roots extract), G6: (500 mg/kg Ashwagandha roots extract). Means in each column that have different superscript letters are considerably different at $P \leq 0.05$.

The average renal biomarkers for infertility rats fed on various diets was once displayed in Table (5). The serum urea of the control positive group was once considerably greater than that of the control negative group, measuring 43.72 and 26.20 mg/dl, respectively. Regarding the infertile groups, the acquired data indicated that the group receiving 2.5% powdered ashwagandha roots had a considerably greater urea value than the group administrated 500 mg/kg of ashwagandha root extract, with corresponding ability of 37.47 and 27.02 mg/dl.

The group with control positive had an average uric acid concentration that ranged extensively increased (that is, between 8.26 and 4.75 mg/dl) than the group with control negative. Regarding the infertile groups, the facts confirmed that the groups that took 2.5% powdered ashwagandha roots had much greater uric acid concentrations. However, the group that administrated 500 mg/kg of ashwagandha root extract showed decrease levels, which had been 7.15 and 5.02 mg/dl.

The average blood creatinine concentration for infertile rats fed different diets is proven in Table (6). The control positive group's serum creatinine levels were proven to have an extensively larger maximal concentration of 1.42 and 0.69 mg/dl when in contrast to the control negative group. Regarding the groups who had infertility, the results revealed that the group that received 2.5% powdered ashwagandha roots had an extensively greater creatinine content than the group that received 500 mg/kg of ashwagandha root extract, with a mean of 1.12 and 0.78 mg/dl. This study supports the hypothesis made through [51] that ashwagandha should be used as an herbal remedy for renal failure. Additionally, [52] confirmed that ashwagandha root extract may alter the renal toxicity in albino Wistar produced by means of cisplatin, as proven by means of renal feature tests.

The findings collected through [53] confirmed that, in comparison to the

positive control group, the studied plants significantly decreased kidney functioning. Additionally, some odd histological variations in the kidney had been averted with the aid of the examined plants.

Table (5): Some serum renal function of negative control and infertility rat groups treated by ashwagandha roots powder and extract at the end of study

	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)
G1	26.20d±1.03	4.75de±0.35	0.69c±0.10
G2	43.72a±1.24	8.26a±0.81	1.42a±0.41
G3	37.47b±1.16	7.15b±0.65	1.12b±0.25
G4	33.12c±1.07	6.46c±0.55	0.95b±0.17
G5	32.19c±1.00	5.31d±0.51	0.83bc±0.14
G6	27.02d±1.12	5.02d±0.37	0.78c±0.12
LSD	1.032	0.640	0.241

G1: Control (-v), G2: Control (+v), G3: (2.5% Ashwagandha roots powder), G4: (5% Ashwagandha roots powder), G5: (250 mg/kg Ashwagandha roots extract), G6: (500 mg/kg Ashwagandha roots extract). Means in each column that have different superscript letters are considerably different at $P \leq 0.05$.

4. CONCLUSION

Ashwagandha root extract can be consumed as a beverage to improve fertility and immunity. It has shown significant effects on reproductive hormones and the immune system in rats, particularly at 5% concentrations.

5. REFERENCES

1. Kulkarni, S.K. and Dhir, A. *Withania somnifera*: An Indian ginseng. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, (2008); 32(5): 1093-1105.

2. Sapra, N.C.; Kalyanrao, P.; Sasidharan, N.; Arna Das and Susmitha, P. Effect of mechanical, chemical, growth hormone and biofertilizer treatments on seed quality enhancement in Ashwagandha (*Withania somnifera*), *Medical Aromatic Plants*, Los Angeles, (2020); (9): 35-39.
3. Udayakumar, R.; Kasthuriengan, S.; Vasudevan, A.; Mariashibu, T.S.; Rayan, J.J.S. and Choi, C.W. Antioxidant effect of dietary supplement *Withania somnifera*, L. reduce blood glucose levels in alloxan-induced diabetic rats, *Plant Foods for Human Nutrition*, (2010); 65 (2): 91-98.
4. Chukwuma, C.I.; Matsabisa, M.G.; Ibrahim, M.A.; Erukainure, O.L.; Chabalala, M.H. and Islam, M.S. Medicinal plants with concomitant anti-diabetic and anti-hypertensive effects as potential sources of dual acting therapies against diabetes and hypertension: a review. *Journal Ethnopharmacology*, (2019); (235): 329-360.
5. Tripathi, N.; Shrivastava, D.; Ahmad-Mir, B.; Kumar, S.; Govil, S.; Vahedi, M. and Bisen, P.S. Metabolomic and biotechnological approaches to determine therapeutic potential of *Withania somnifera*, (L.) Dunal: A review, *Phytomedicine*, (2018); (50): 127-136.
6. Hassannia, B.; Logie, E.; Vandenabeele, P.; Vanden Berghe, T.; Vanden-Berghe, W. and Withaferin, A. From ayurvedic folk medicine to preclinical anti-cancer drug. *Biochemical Pharmacology*, (2019); 173 : 1-11.
7. Yeniseti, S.C.; Manjunath, M.J. and Muralidhara, C. Neuropharmacological properties of *Withania somnifera* - Indian ginseng: an overview on experimental evidence with emphasis on clinical trials and patents, *Recent Pat. CNS. Drug Discovery*, (2016); (10): 204-215.
8. Davis, L. and Kuttan, G. Immunomodulatory activity of *Withania somnifera*. *Journal Ethnopharmacology*, (2000); 71: 193-200.
9. Kelly, D.M. and Jones, T.H. Testosterone: A metabolic hormone in health and disease. *Journal of Endocrinology*, (2013); 217 (3): 1-8.
10. Rutkowski, K.; Sowa, P.; Rutkowska-Talipska, J.; Kuryliszyn-Moskal, A. and Rutkowski, R. Dehydroepiandrosterone (DHEA): Hypes and hopes. *Drugs*, (2014); 74 (11): 1195-1207.
11. Orwoll, E.; Lambert, L. C.; Marshall, L. M.; Phipps, K.; Blank, J.; Barrett-Connor, E. and Cummings, S. (2006); Testosterone and estradiol among older men. *The Journal of Clinical Endocrinology and Metabolism*, 91(4): 1336-1344.
12. Abdel-Magied, E.M.; Abdel-Rahman, H.A. and Harraz, F.M. The effect of aqueous extracts of *Cynomorium coccineum* and *Withania somnifera* on testicular development in immature Wistar rats, *Journal Ethnopharmacology*, (2001); (75):1-4.
13. Ahmad, M.K.; Mahdi, A.A.; Shukla, K.K.; Islam, N.; Rajender, S. and Madhukar, D. *Withania somnifera* improves semen quality by regulating reproductive hormone levels and oxidative stress in seminal plasma of

infertile males. *Fertil. Steril.* (2010); 94 (3): 989-96.

14. Mahdi, A.A.; Shukla, K.K.; Ahmad, M.K.; Rajender, S.; Shankhwar, S.N.; Singh, V. and Dalela, D. *Withania somnifera* Improves semen quality in stress-related male fertility. *Evidence Based Complementary Alternation Medicine*, (2011); 1: 1-4.

15. Gupta, A.; Mahdi, A.; Shukla, K.K.; Ahmad, M.K.; Bansal, N.; Sankhwar, P. and Sankhwar, S.N. Efficacy of *Withania somnifera* on seminal plasma metabolites of infertile males: A proton NMR study at 800 MHz. *Journal Ethnopharmacology*, (2013); 149: 208-214.

16. Nasimi, Azgomi, R.; Nazemiyeh, H.; Bazargani, H.S.; Fazljou, S.M.; Nejatbakhsh, F.; Jazani, A.M.; AsrBadr, Y.A. and Zomorodi, A. Comparative evaluation of the effects of *Withania somnifera* with pentoxifylline on the sperm parameters in idiopathic male infertility: A triple-blind randomized clinical trial. *Andrologies*, (2018); 50: e13041.

17. Chauhan, S.; Srivastava, M.K. and Pathak, A.K. Effect of standardized root extract of *Ashwagandha* (*Withania somnifera*) on well-being and sexual performance in adult males: A randomized controlled trial. *Health Science Reproduction*, (2022); 5, e741.

18. Russo, E. *Handbook of Psychotropic Herbs: A scientific Analysis of Herbal Remedies for Psychiatric Conditions.* Journal of Haworth Her. Pre. NY, USA. (2001); 15 (7): 648.

19. Rekha, D.K.; Nayanatara, A.K.; Ramswamy, C.; Sheila, R.P.; Ramesh, B.M. and Venkappa, S.M. Infertility in male Wistar rats induced by cadmium chloride: Role of ascorbic acid. *Journal of China Medical*, (2009); 41(11): 616-621.

20. AIN, American Institute of Nutrition. Purified diet for Laboratory Rodent Final report. *Journal of Nutrition*, (1993); 123:1939-1951.

21. Chapman, D. G.; Castilla, R. and Campbell, J.A. Evaluation of protein in food. I. A. method for the determination of protein efficiency ratio. *Canadian Journal of Biochemistry Physiology*, (1959); 37 (5): 679-686.

22. Fahim, M.S.; Fahim, Z. and Harman, J.M. Clevenger, T.E.; Mullins, W.; Hafez, E.S. Effect of *Panax ginseng* on testosterone level and prostate in male rats. *Arch Androl*, (1982); 8 (4): 261-263.

23. Pradelles, P.; Grassi, J. and Maclouf, J. Enzyme immunoassays of eicosanoids using acetylcholine esterase as label: An alternative to radioimmunoassay. *Analytical Chemistry*, (1985); 57 (7):1170-1173.

24. Salauze, D.; Serre, V. and Perrin, C. Quantification of Total IgM and IgG Levels in Rat Sera by a Sandwich ELISA Technique. *Comp. Haematol. Int.* (1994); 4:30-33.

25. Allen, C.C. Cholesterol enzymatic colorimetric method. *Journal of Clinical Chemistry*, (1974); 2 (20): 470.

26. Fassati, P. and Prencipe, L. Triglyceride enzymatic colorimetric

- method. *Journal of Clinical Chemistry*, (1982); 28:2077.
- 27.** Lopez, M.F. HDL-cholesterol colorimetric method. *Journal of Clinical Chemistry*, (1977); 23: 882.
- 28.** Lee, R. and Nieman, D. *National Assessment*. 2nd Ed., Mosby, (1996); Missouri, USA.
- 29.** Belfield, A. and Goldberg, D.M. Revised assay for serum phenyl phosphatase activity using 4-amino-antipyrine. *Enzymes*, (1971);12 (5): 561-573.
- 30.** Yound, D.S. Determination of AST. *Clinical Chemistry Journal*, (1975); 22 (5):1-21.
- 31.** Trinder, P. Glucose. *Journal of Annals Clinical Biochemistry*, (1969); (62): 24-33.
- 32.** Patton, C.J. and Croush, S.R. Enzymatic determination of urea. *Journal Analytical Chemistry*, (1977); 49: 464-469.
- 33.** Barham, D. and Trinder, P. Determination of uric acid. *Analyst*, (1972); 97: 142.
- 34.** Tietz, N.W. (1990): *Clinical Guide to laboratory Tests*. 2nd ed. Philadelphia: WB. Saunders; 566.
- 35.** Schirmeister, J. Creatinine standard and measurement of serum creatinine with picric acid. *Deutsche Medizinische Wochenschrift*, (1964); 89: 1018-1021.
- 36.** SAS. *SAS Users Guide: Statistics version 5th Ed*. SAS. Institute Inc., (1988); Cary N.C.
- 37.** Singh, A.; Kapil Singh, K. and Shekhawat, P.S. Spermicidal activity, and antifertility activity of ethanolic extract of *Withania somniferain* male albino rats. *Int. J. Pharm. Sci. Rev. Res.*, (2013); 21 (2): 227-232.
- 38.** Sarvamangla, B.S.; Krishna, K.A.; Jayaraman, S. and Sheth, R.R. Effect of chronic administration of inhibition and testosterone on spermatogenesis in adult male rat. *Archives of Andrology*, (1983); 10: 223-238.
- 39.** Ali, H.M. Ashwagandha (*Withania somnifera*) and their effects on the reproductive hormones of male rats. *Home Economics Journal*, (2021); 37 (2): 1-18.
- 40.** Tharakan, A.; Shukla, H.; Benny, I. R.; Tharakan, M.; Lekha George, L. and Koshy, S. Immunomodulatory effect of *Withania somnifera* (ashwagandha) extract. A randomized, double-blind, placebo-controlled trial with an open label extension on healthy participants. *Journal of Clinical Medicine*, (2021);10 (16): 3644.
- 41.** Khan, S.; Malik, F.; Suri, K.A. and Singh, J. Molecular insight into the immune up-regulatory properties of the leaf extract of *Ashwagandha* and identification of Th1 immunostimulatory chemical entity. *Vaccine*. (2009); 27: 6080-6087.
- 42.** Widodo, N.; Shah, N.; Priyandoko, D.; Ishii, T.; Kaul, S.C. and Wadhwa, R. Deceleration of senescence in normal human fibroblasts by with none extracted from ashwagandha leaves. *Journal Gerontology Biological Science Medicine Science*, (2009); 64 (10):1031-1038.
- 43.** Verma, S. K. and Kumar, A. Therapeutic uses of *Withania somnifera*

(ashwagandha) with a note on withanolides and its pharmacological actions. *Asian Journal Pharma Clinical Research*, (2011); 4 (1): 1-4.

44. Ragab, S.S.; El-Tahan, N.R. and Elmokdem, D.E. Biological, studies of some herbal and plants formula on the healthy status of obese female Rats. *Journal of Home Economics*, (2020); 30 (4): 233-248.

45. Swamy, M.K.; Patra, J.K. and Rudramurthy, G.R. *Medicinal Plants: Chemistry, Pharmacology and Therapeutic Applications*. CRC Press, (2019).

46. Pal, A.J.; Bhushan, B.H. and Khanum, F.A. Therapeutic uses of *Withania somnifera* (ashwagandha). *Recent progress in medicinal plants* Recent progress in medicinal plants, (2015); 34: 97-118.

47. Mesllam, E.; El-Shereif, F.E.; Header, E. and Refaat, W.A. Biochemical and nutraceutical effect of ashwagandha (*Withania somnifera*) roots on kidney functions and immunomodulatory activity disorders in rats. *Journal of Home Economics*, (2024); In press.

48. Jamuna, G.; Sharma, A.K.; Manimaran, A. and Sankar, P. Hepatoprotective effects of *Allium sativum* and *Withania somnifera* on ochratoxin A-induced toxicity in rats. *Journal of Pharmacognosy and Phytochemistry*, (2018); 7 (3): 2675-2680.

49. Harikrishnan, B.; Subramanian, P. and Subash, S. Effect of *Withania somnifera* root powder on the levels of circulatory lipid peroxidation and liver marker enzymes in chronic hyperammonemia. *Journal of Chemistry*, (2008); 5 (4): 872-877.

50. Soltana, N.; Shimmi, S.C. and Parach, M.T. Effects of ashwagandha (*Withania somnifera*) root extract on some serum liver marker enzymes (AST, and ALT) in gentamicin intoxicated rats. *Journal of Bangladesh Society of Physiologist*, (2012); 7 (1): 1-7.

51. Grunz-Borgmann, E.; Mossine, V.; Fritsche, K. and Parrish, A. R. Ashwagandha attenuates TNF- α -and LPS-induced NF- κ B activation and CCL2 and CCL5 gene expression in NRK-52E cells. *BMC (Complementary and Alternative Medicine)*, (2015); 15:1-8.

52. Rasheed, A.; Younus, N.; Waseem, N. and Badshah, M. Protective effect of *Withania somnifera* root extract against cisplatin induced nephrotoxicity through renal function analysis in albino Wistar rats. *International Medical Forum*, (2020); 31(4):61-67.

53. Khader, S.A.; Abdel Rahman, A.N. and El-Rakhawy, H.M. Potential effect of turnip (Leaves and Root) on alloxan-induced diabetic rats. *Journal of Home Economics*, (2021); 31(2): 65-81



تحسين خصوبة ومناعة ذكور الفئران باستخدام جذور نبات الاشواجندا

عماد الخولي، شريف رجب، نورا عبد الله

قسم التغذية وعلوم الأطعمة. كلية الاقتصاد المنزلي. جامعة المنوفية، شبين الكوم، مصر

<p>الملخص العربي: كان الغرض من هذه الدراسة هو دراسة تأثير مسحوق جذر الأشواجندا ومستخلصه الإيثانولي على الفئران المصابة بخلل في الخصوبة. تم تقسيم ستة وثلاثين من الفئران البيضاء الذكور إلى مجموعتين رئيسيتين. المجموعة الأولى هي المجموعة الضابطة السالبة (-v) والتي تغذت على النظام الغذائي الأساسي فقط. بعد حقن كلوريد الكاديوم بجرعة 1,0 ملجم / كجم من وزن الفأر لمدة 10 أيام على التوالي لتحفيز الخلل في الخصوبة لدى الفئران، تم تقسيم المجموعة الثانية إلى خمس مجموعات تضم كل منها ستة فئران. المجموعة (2) أعطيت نظاماً غذائياً أساسياً فقط كمجموعة ضابطة (+v)؛ المجموعات (3-4) أعطيت نظاماً غذائياً أساسياً يحتوي على (5, 2, 0%) من مسحوق جذور الأشواجندا من الوجبة؛ المجموعات (5-6) أعطيت نظاماً غذائياً أساسياً بالإضافة إلى (250, 500 مجم / كجم) من مستخلص إيثانول جذور الأشواجندا عن طريق الفم بواسطة أنبوبة المعدة. وبعد انتهاء التجربة التي استمرت أربعة أسابيع، تم ذبح الفئران واستخراج الدم للحصول على السيرم. تم تقييم المؤشرات على النحو التالي: الجلوكوز في الدم؛ وظائف الكلى مثل اليوريا والكرياتينين وحمض البوليك، الهرمونات مثل الهرمون الملوتن (LH)، والهرمون المنبه للجريب (FSH)، والتستوستيرون؛ المؤشرات المناعية مثل IgM و IgG؛ دهون الدم مثل الكوليسترول الكلي، الدهون الثلاثية، البروتينات الدهنية العالية والمنخفضة والمنخفضة الكثافة جداً؛ وإنزيمات الكبد مثل ألانين أمينوترانسفيراز، أسبارتات أمينوترانسفيراز، الفوسفاتيز القلوي. كشفت النتائج أن استخدام جذور الأشواجندا أدت إلى ارتفاع كبير في هرمون التستوستيرون وهرمون FSH، وانخفاض في هرمون LH، وصورة دهون الدم، والمؤشرات الحيوية للكلى والكبد، والعكس مع البروتين الدهني عالي الكثافة. في الختام، فتناول مسحوق ومستخلص جذر اشواغاندا يمكن أن يقلل من الآثار الجانبية لتسمم الخصية بكلوريد الكاديوم.</p>	<p>نوع المقالة بحوث أصلية</p>
<p>المؤلف المسئول عماد الخولي emad.elkhoulia@hec.menofia.edu.eg الجوال: +2 0224673480</p>	<p>DOI:10.21608/MKAS.2024.282934.1309</p>
<p>الاستشهاد الي: El-Kholie et al., 2024, Improvement of Male Rats Fertility and Immunity using Ashwagandha (Withania somnifera, L) Roots. JHE, 34 (3), 129-143</p>	<p>تاريخ الاستلام: 24 أبريل 2024 تاريخ القبول: 31 مايو 2024 تاريخ النشر: 1 يوليو 2024</p>

الكلمات الكاشفة: الجهاز المناعي، الهرمونات الجنسية، الكرز الشتوي الهندي، حيوانات التجارب