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Enhancing The Therapeutic Uses Of Ashwagandha powder (Withaniasomnifera) In Improving Immunity, Blood Characteristics, And Fertility In Male Rats Suffering From Infertility

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Abstract
Male infertility is commonly caused by congenital or acquired disorders**. Aim:** The current research aims to enhance the therapeutic uses of ashwagandha in improving immunity, blood characteristics, and fertility in male rats suffering from infertility. In this study, blood characteristics are assessed by measuring various hematological variables like the count of WBC, RBC, and concentrations of hemoglobin. **Materials and Methods:** 24 male Sprague-Dawley mice have been split into 4 groups: 1st group: normal rats (number=six) as control (-), 2nd group: infertile mice (number=six) as a control, 3rd group of infertile mice which received a basal diet +7% of ashwagandha powder, and the 4th group of infertile rats '(number=six) received a basal diet +9% of ashwagandha powder. Following twenty-eight days, blood was gathered and serum obtained to find out hemoglobin, platelets, and RBCs. WBC, IgG, IgM, IgA, glucose, liver enzymes, kidney function and blood lipids, FSH, LH, and testosterone hormones. **Results:** The higher concentration of RBCs and WBCs of the treating group was documented for the infertile group of rats fed on 9% of **ashwagandha powder**. FSH, LH, and testosterone are hormones, but the mean values of groups (3 and 4) differed significantly.

KEYWORDS: -Ashwagandha powder - immunity - infertile rats.

Introduction

Infertility is primarily categorized into azoospermia (AS) and coital infertility (CI). AS refers to the total absence of sperm in the ejaculate. It is identified in fifteen percent of infertile males and is categorized as obstructive infertility (OI) and non-obstructive infertility (NOI) **(Cocuzza et al., 2013).** OI refers to the absence of spermatozoa in the ejaculate, but the exocrine and endocrine systems, as well as spermatogenesis, remain normal. There exists a blockage in the genital tract. It may also manifest in any region among the ejaculatory ducts and the rete testes. NOI is defined by impaired spermatogenesis. It arises from 1ry or subsequent testicular failure (TF) or from vague or partial TF. Coital infertility is defined by normal creation of sperm and a functional genital system. However, the sickness is related to the case's sexual dysfunction, which affects ejaculation. Subfertility may be classified as either 1ry or 2ry. 1ry subfertility refers to the delay had by a couple who have never achieved conception, whereas 2ry subfertility refers to the delay with a couple who have previously conceived, regardless of the outcome, such as ectopic pregnancy or miscarriage (**Anwar, 2016).**

The immune system protects the host against a variety of harmful microbes that are constantly fluctuating. Immunity assists the recipient in eliminating inadvertently ingested toxic chemicals and allergens that enter mucosal surfaces. The immune system's capacity to distinguish between self and non-self is important for establishing a defense versus invading allergens, toxins, or viruses. The host utilizes both adaptive and innate mechanisms to identify and eradicate pathogenic bacteria, both of which require self-non-self-bigotry **(Chinen and Shearer, 2010).** Ashwagandha is a little, branching, perennial woody shrub that typically gets a top of approximately two feet and is natural to several regions, including Africa, the Mediterranean, and extending into India. Due to its extensive distribution, there is significant nature, including morphological andchemotypic changes among local species. The flowers are little andgreen, whilst the mature fruit is an orange-red berry, smooth, rectangular, and spherical. It possesses predominantly dark tuberous roots utilized for medicinal applications. The seeds are yellow and scurfy **(Mir et al., 2012).** The biological impacts, specifically the antioxidant capacity and phytochemical components of W. somniferaand other species within the Withania genus, are contingent upon the extraction technique utilized **(Dhanani et al., 2017).** The methanol-chloroform-water (1:1:1) extract of ashwagandha roots, exhibiting the maximum concentration of all phytochemical ingredients excluding tannins, had superior antioxidant as well as decreasing activities than the acetone, water, and aqueous methanol (1:1) extracts **(Ganguly et al., 2018).** The alkaloid content has been identified as the primary contributor to the total antioxidant and decreasing extract activities, closely followed by withanolidesand flavonoids. Furthermore, various sections of the plant may exhibit disparate degrees of antioxidant capacity. The antioxidant activity of glycowithanolides from ashwagandha has been established by a dose-dependent enhancement of SOD, CAT, andGPx activity in the striatum and cortex of mouse brains **(Mandlik and Namdeo, 2021).** This aligns with the discoveries of **Ahmad et al. (2020) and Ahmad et al. (2018),** which indicated that the therapy withashwagandha resulted in significant decreases in MDA, alongside a significant rise in concentrations of antioxidants. In the assay of wound healing, the managed skin exhibited a significant reduction in the zone of wounds and decreased aggregation of immune cells compared to the control-treated skin. Withaferin-A is efficacious in addressing distinct inflammatory disorders in illnesses like cystic fibrosis, arthritis, and inflammatory bowel disorder through mechanisms including activation of inhibition of nuclear factor kappa B as well as the suppression of COX-2 production.

Withaferin-A was demonstrated to enhance the osteoblast-specific transcription factor expression, hence promoting osteoblast separation and proliferation in damage of bone and menopausal osteoporosis (**Alam et al., 2012). Alfaifi et al. (2016)** assessed the anti-inflammatory effectiveness of an extract of the aqueous root of ashwagandha, administered topically to the damaged skin of seven-week-old male C57BL/6J mice for a five-day period at a concentration of ten milligrams per milliliter. The findings indicated a reduction in the pro-inflammatory cytokine tumor necrosis factor-α and an elevation in the messenger ribonucleic expression of the anti-inflammatory cytokine TGF-β1. The extract of W. somnifera root has demonstrated a reduction in messenger ribonucleic a expression of inflammatory cytokines, involving interleukin 8, interleukin 2, as well as tumor necrosis factor (TNF), while simultaneously enhancing the expression of messenger RNA of the anti-inflammatory cytokine transforming growth factor (TGF) in a line of human keratinocyte cells **(Atluri et al., 2020).** The anti-inflammatory effects of the aqueous root extract of ashwagandha were assessed by measuring TNF-alpha, interleukin (IL) IL-10, IL-6, and IL-10 in a mouse model of collagen-induced arthritis. Oral ingestion of aqueous root extract of ashwagandha (three hundred milligrams per kilogram) mitigated the transcription factors associated with arthritis in mice by normalizing the levels of ROS and metalloproteinase-8 in collagen-induced arthritis models **(Baitharu et al., 2014).** W. somnifera demonstrates excellent anti-inflammatory properties in many in-vitro and in-vivo conditions **(Paul et al., 2021).** The administration of root powder at a dosage of six hundred milligrams per kilogram to collagen-induced arthritic mice significantly decreased the arthritis difficulty, resulting in enhanced recovery of function of motor activity and improved radiological scores. The W. somnifera root extract has been examined for analgesic properties in mice by measuring interleukin and interferon biomarkers in the ganglia of dorsal root using an enzyme-linked immunosorbent assay cytokine test. The outcomes indicated a significant rise in spared nerve injury-induced hyperalgesia, mechanical withdrawal threshold, and cytokine concentrations in a dose-dependent way following six and twenty-

four hours of administration of extract of W. somnifera root at dosages of one hundred and three hundred milligrams per kilogram. Ashwagandha, the principal active component of ashwagandha roots, appears to be accountable for the chemochine receptor family two, which exhibits analgesic effects in the following surgery and neuropathic therapy of mice **(Balkrishna et al., 2020).** The analgesic activity of W. somnifera is attributed to its ability to diminish serotonin levels, which are primarily responsible for bodily pain **(Bano et al., 2020).**

2- AIM OF STUDY: -

The goal of this research is to enhance the therapeutic uses of ashwagandha powder (**Withaniasomnifera)**in improving immunity, blood characteristics, and fertility in male rats suffering from infertility.

3- MATERIALS AND METHODS: -

A- Source of ashwagandha (Withaniasomnifera): Ashwagandha was purchased from the Jedda, KSA, local market, washed, cleaned, blended, and ground into fine powder utilizing an electric grinder. To reduce oxidation, they were stored in dark-stoppered glass bottles until ready to be used, according to **(Russo, 2001).**

B-Rats: 24 male Sprague Dawley mice (number=twenty-four), weighing between 150 and 170 grams, have been procured from the Animal Unit of Egypt's Ministry of Health at Helwan Farm. Over a duration of two weeks, the mice have been housed in individual plastic cages in regulated conditions, maintaining a temperature of twenty-two degrees Celsius and a twelve-hour light/dark cycle at the Faculty of Home Economics, Menoufia University, Egypt. Mice have unrestricted access to water and food. Every study adhered to the National Institutes of Health's Guiding Principles for Animal Care and Usage. Following two weeks of acclimatization, mice were weighed and randomly assigned to one of two groups: diabetic (eighteen mice) and normal (six mice).

C—The chemicals and chemical kits: Cadmium chloride hydrate powder was gotten from El-Gomhoria Company, Cairo, Egypt.

D—The induction of experimental infertility: Normal, healthy male albino mice have been administered cadmium chloride ($CdCl_2$ 0.1%) at a dosage of 0.1 milliliter per one hundred grams of body weight, twice throughout the trial. **(Rekha et al., 2009).**

E-Basal diet:The basal diet comprised protein (ten percent), corn oil (ten percent), choline chloride (0.2 percent), cellulose (five percent), a combination of vitamins (one percent), a salt combination (four percent) **(Hegested et al., 1941),** and cornstarch (to one hundred percent), in accordance with **AIN (1993).**

F—Experimental Design

The research encompassed all normal (six mice) and diabetic (eighteen mice). Alongside the testing technique, all mice had regular diet. The suggested therapies were taken orally once a day, AIN, (1993). The weights of the mice were documented, and diabetic mice categorized into experimental groups appropriately. The subsequent experimental groups included:

1- Group (1): Normal rats (N=6) that hadtwo milliliters of distilled water orally per miceone time day.

2- Group (2): A group of infertile miceutilized as a control group (+) (fed on basal food only).

3- Group (3): A group ofinfertile micenourished on ashwagandha powder by 7% of the weight of the diet.

4- Group (4): Infertile micenourished on ashwagandha powder 9% of the weight of the diet.

G- Blood sampling: Initially, blood samples were collected from the retro-orbital vein following a fasting period of twelve hours, while at the end of each experiment, they were gathered from the hepatic portal vein. Samples of the blood were gathered into clean, dry centrifuge glass tubes and permitted to clot in a water bath at thirty-seven degrees Celsius for twenty-eight minutes. The serum was subsequently separated by centrifuging the tubes at four thousand revolutions per minute for ten minutes. The serum has been cautiously aspirated and transferred to a clean Eppendorf tube, where it was kept at minus twenty degrees Celsius until analysis. This method was labeled by **(Schermer,1967).**

H- Biochemical Analysis:

Detection of blood picture: The level of platelets (PLT), hemoglobin (HGB), RBCs, and WBCs was calculated regarding the technique defined by **(Dacie and Lewis, 1998).**

Detection of follicle-stimulating hormone (FSH): FSH has been detected colorimetrically regarding the technique of **(Akram et al., 2012).**

Detection of luteinizing hormone (LH): LH has been detected colorimetrically regarding the technique of **(Akram et al., 2012).**

Detection of testosterone: Testosterone has been detected colorimetrically regarding the method of **(Pradelles et al., 1985).**

Detection of IgM, IgG, and IgA: Immunoglobulins serum IgM, IgG, and IgA levels have been detected by single radial immunodiffusion by **Mancini et al. (1965)** utilizing commercial plates.

Detection of blood glucose: Serum glucose has been estimated by the modified kinetic technique regarding **(Kaplan 1984)** by utilizing a kit supplied by Spinreact, Spain.

Lipid profile:

Detection of total cholesterol (TC): TC has been detected utilizing the changed kinetic regarding **(Richmond 1973)** by utilizing a kit supplied by Human, Germany.

Detection of triglycerides (TG): TG has been estimated by the modified kinetic method regarding the technique defined by **(Fossati and Principe (1982)** by utilizing a kit supplied by Spin React, Spain.

Detection of high-density lipoprotein cholesterol (HDL-c): HDL-c has been estimated by the modified kinetic technique regarding **(Allain 1974)**.

Detection of very low-density lipoprotein cholesterol (VLDL-c): VLDL-c has been estimated as milligrams per deciliter regarding **(Lee and Nieman 1996)**.

Detection of low-density lipoprotein cholesterol (LDL-c): LDI-c has been calculated as milligrams per deciliter regarding **(Castelli et al., (1977).**

Estimate of atherogenic index (AI): The LDL + VLDL / HDL ratio: This index has been estimated regarding the formulary of **Kikuchi-Hayakawa et al., (1998).**

Liver functions:

Detection of alanine amino transferees (ALT) (GPT): Alanine aminotransferase activities have been estimated in serum by the changed kinetic technique of (**Tiez 1976)**

Detection of aspartate amino transferees (AST) (GOT): Aspartate amino transferees activities have been estimated in serum by the changed kinetic technique of **(Henary 1974)**

Detection of alkaline phosphatase (ALP): ALP activities have been estimated in serum by the changed kinetic technique of (**Belfield and Goldberg 1971)**

4.5.6. Kidney functions:

Detection of urea nitrogen: Urea has been detected in serum by the modified kinetic technique or liquicolor of **(Patton and Crouch 1977)**.

Detection of creatinine: Creatinine has been detected by the changed kinetic technique regarding **(Schirmeister 1964)**.

Detection of uric acid : Serum uric a has been evaluated by the changed kinetic technique regarding **(While et al., 1970)** by utilizing a kit supplied by Human, German.

Statistical analysis:

The data have been examined using a completely randomized factorial design (SAS, 1988) upon detecting a significant main influence; means have been differentiated utilizing the Student-Newman-Keuls Test. Variances among therapies (P-value below 0.05) have been deemed significant utilizing the COSTAT Program. The biological results have been evaluated using one-way ANOVA.

Ethical Approval

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

4- RESULTS

- **Biochemical results.**

Influence of various concentrations ofashwagandha powder(Withaniasomnifera) on glucose levels in male rats suffering from infertility:

Table (1) demonstrates the impact of various levels of ashwagandha powder on the glucose of infertile mice. The mean serum glucose level of the control group (+) was significantly elevated compared to the control group (-), measuring 125.50 and 69.00 (mg/dl), correspondingly. The smallest glucose level in the treatment group was observed in infertile mice administered 9% percent ashwagandha powder. The infertile group of mice given 7% percent ashwagandha powder exhibited the highest recorded values, with significant variations (P-value below 0.05); the mean values were 76.2 and 80.5 (mg/dl), correspondingly.

Table (1): Influence of various concentrations ofashwagandha powder(Withaniasomnifera) on glucose levels in male rats suffering from infertility

Groups Variables	one Control (-ve)	two Control (+ve)	three 7% Ashwagandh	four 9% Ashwagandh	LSD
Glucose (mg/dl)	69±0.4e	125.50±0.03a	80.50±0.05b	76.50±0.01 d	0.31
%Change of C(+)ve	-45.02%	---	35.86%	-39.04%	---

All value is shown as mean ± standard impact (n - 6).
Means having the same line but differing in superscript characters are most significantly distinct. (p - value below 0.05).

Influence of various concentrations ofashwagandha powder(Withaniasomnifera) on lipid profile of infertile mice:

Tables 2 and 3 indicate the mean value of triglyceride, LDL-c, VLDL-c, total cholesterol, HDL-c, and AI in infertile rats. In the case of triglycerides, data show that the mean value of TG of the control group (+) was significantly greater compared to (-) group, which was 160 and 130 milligrams per deciliter, correspondingly. There was insignificant variance ($P < 0.05$) between Group 3 (7% ashwagandha) and Group 4 (9% ashwagandha) when compared with control (+). This suggests that increasing the ashwagandha concentration from 7% to 9% does not significantly enhance the measured outcome than the control group. It implies that the potential benefits of ashwagandha may plateau at a certain level of concentration. Then, it might not be required to utilize higher concentrations for similar effects. The mean values were 145±0.57 and 14±0.56 milligrams per deciliter, correspondingly.

- The outcomes indicated that the mean total cholesterol level in the control group (+) was significantly greater compared to that of the control group (-), measuring 236 milligrams per deciliter and 125 milligrams per deciliter, respectively. The treating group of infertile rats exhibited the lowest overall cholesterol levels when administered a diet containing 9% percent ashwagandha. The infertile group of mice fed a 7%percent ashwagandha diet exhibited the greatest noted values, with significant variations (P-value below 0.05), showing mean values of 130 and 145.50 milligrams per deciliter, correspondingly.

-The outcomes showed that the mean HDL-c value of the control (+) group was significantly less compared to (-) group, measuring 25.00 milligrams per deciliter and sixty-eight milligrams per deciliter, correspondingly.

-The greatest HDL-c of the treating group documented for infertile group mice nourished on 9% ashwagandha, while the lowest value documented for infertile group mice that nourished on 7% ashwagandha with significant variance (P-value below 0.05) was 34.5±0.09 and 61.5±0.02 milligrams per deciliter, correspondingly.

- Regarding LDL-c levels, the mean value of control (+) group was significantly greater compared to (-) group, measuring 179 and 31 (milligrams per deciliter), correspondingly. The treated group of infertile mice exhibited the lowest LDL-c levels when administered a diet containing 9%ashwagandha. The infertile group of rats fed on a 7%ashwagandha diet exhibited the highest recorded values, with significant variations (P -value below 0.05); the mean values were 82±0.11 and 39.5±0.05 milligrams per deciliter, correspondingly.

Conversely, there are indications that the mean value of VLDL-c in the control group (+) was greater compared to that in the control group (-), measuring thirty-two and twenty-six (milligrams per deciliter), correspondingly. Insignificant distinction (P-value less than 0.05) was seen among the third group (7% ashwagandha) and the fourth group (9% ashwagandha) compared to the control group, with mean values of 29±0.37 and 29±0.88 (milligrams per deciliter), correspondingly.

- The mean value of the control (+) group was significantly greater compared to (-) group, measuring 8.44 milligrams per deciliter and 0.84 milligrams per deciliter, correspondingly. The treated group of infertile rats had the lowest AI when administered a diet containing 9% ashwagandha. The greatest value reported for the infertile group of rats administered 7% ashwagandha, with significant variations (P-value less than 0.05), was 1.11 and 3.22 (milligrams per deciliter), correspondingly.

Table (2): Influence of various level of ashwagandha powder (Withaniasomnifera)on serum total cholesterol and triglycerides of infertile mice

Groups / variables	One	two	three	four	LSD
Triglycerides mg/dl	130±0.02d	160±0.6a	145±0.57b	145±0.56b	0.81
%Change of C(+)ve	-18.75%	---	-9.38%	-9.38%	---
Total cholesterol mg/dl	125±0.22f	236±0.29a	145.50±0.03b	130±0.35c	0.37
%Change of C(+)ve	-47.03%	---	-38.35%	44.92%	---

Table (3): Influence of various concentrations of ashwagandha powder(Withaniasomnifera)on (LDL-c), (HDL-c), (VLDL. c) and (AI) of infertile mice

Groups / variables	One	two	three	four	LSD
HDL-c mg/dl	68±0.31b	25±0.39e	34.5±0.09d	6l.5±0.02c	0.56
%Change of C(+)ve	+ 172%	---	+38%	+ 146%	---
LDL-c mg/dl	3l±0.04e	179±0.08a	82±0.11b	39.5±0.05c	0.12
%Change of C(+)ve	-82.68%	---	-54.19%	-77.93%	---
VLDL- c mg/dl	26±0.01d	32±0.97a	29±0.37b	29±0.88b	1.21
%Change of C(+)ve	-18.75%	---	-9.38%	-9.38%	---
AI mg/dl	0.84±0.002d	8.44±0.004a	3.22±0.005b	l.11±0.001c	0.01
%Change of C(+)ve	-90.05%	---	-61.85%	-86.85%	---

Influence of various concentrations ofashwagandha powder(Withaniasomnifera) on liver function of infertile mice:

Table 4 illustrates the influence on AST, ALT, and ALP levels in infertile mice. The average ALT (U/L) in the control (-) group was significantly lower than the (+) group, recorded at 40 and 79.5 (U/L), correspondingly. The mean values of the 3rd and 4th groups were significantly different, measuring 63.50 and 57 (U/L), correspondingly, in comparison to the control (+).

- The findings demonstrated the impact of ashwagandha powder and its aqueous extract on AST levels in infertile mice. The findings revealed that the average AST level in the control group (-) was significantly less compared to that in the control group (+), measuring sixty-five and ninety-five (U/L), correspondingly. The mean values of groups three and four were significantly different from the positive control, measuring eighty-four and seventy-nine (U/L), correspondingly.

- Results indicate the influence of ashwagandha powder and its aqueous extract on ALP levels in infertile mice. The outcomes demonstrated that the mean ALP (U/L) value for the control (-) group was significantly less than the control group (+), recorded at 176 and 206.5 (U/L), correspondingly. The mean values of the 3rd and 4th groups were significantly different from the control (+), measuring 194 and 190 (U/L), correspondingly. The best group appears to be group (4).

Table (4): Influence of various concentrations of ashwagandha powder(Withaniasomnifera)on liver function of infertile mice

Groups / variables	One	two	three	four	LSD
ALT (U/L)	40±0.02f	79.50±0.01a	63.50±0.08b	57±0.5c	0.54
%Change of C(+)ve	-49.69%	---	-20.13%	-28.30%	---
AST (U/L)	65±0.13f	95±0.19a	84±0.11b	75±0.2d	0.29
%Change of C(+)ve	-31.58%	---	-11.58%	16.84%	---
ALP (U/L)	176±0.03f	206.50±0.01a	194±0.57b	190±0.48c	0.76
%Change of C(+)ve	-14.77%	---	-6.05%	-7.99%	---

Influence of various concentrations ofashwagandha powder (Withaniasomnifera) on uric acid, urea and creatinine of infertile mice:

Table (5) illustrates the impact of distinctive concentrations of ashwagandha powder on the levels of uric a`, urea, and creatinine in infertile mice. The mean urea concentration (milligrams per deciliter) in the negative control group was significantly less than control group (+), recorded at 30 and 68.5 milligrams per deciliter, correspondingly. The mean values of the third and fourth groups were significantly different, measuring 35 and 38.5 (milligrams per deciliter), correspondingly, in comparison to the control (+).

Findings demonstrate the influence of ashwagandha powder and its aqueous extract on uric levels in infertile mice. The results demonstrated that the average uric a level in the control group (-) was less compared to that in the control group (+) measuring 1.80 and 2.85 (milligrams per deciliter), correspondingly. The mean values of groups 3 and 4 were significantly distinct from the control (+), measuring 2.4 and 2.1 (milligrams per deciliter), correspondingly.

The findings additionally demonstrate the influence of ashwagandha powder and its aqueous extract on creatinine levels in infertile mice. The mean creatinine levels (milligrams per deciliter) in the control (-) group were significantly lower than those in the (+) group, recorded at 0.5 and 0.7 (milligrams per deciliter), correspondingly. The mean values of the third and fourth groups were significantly distinct from the control (+), measuring 0.65 milligrams per deciliter each. An insignificant distinction ($P < 0.05$) was seen among the third group (7% ashwagandha) and fourth group (9%ashwagandha) as compared to the control group (+).

Table (5): Influence of various concentrations of ashwagandha powder(Withaniasomnifera)on liver function of infertile mice

Groups / variables	One	two	three	four	LSD
Urea mg/dl	30±0.13f	68.5±0.09a	35.5±0.06c	38.5±0.04b	0.18
%Change of C(+)ve	-56.20%	—	-48.18%	-43.80%	—
Uric acid mg/dl	1.80±0.006b	2.85±0.009a	84±0.11b	2.1±0.06e	0.07
%Change of C(+)ve	-36.84%	---	-11.58%	-26.32%	---
Creatinine mg/dl	0.5±0.02c	0.7±0.05a	194±0.57b	0.65±0.008b b	0.04
%Change of C(+)ve	-28.57%	---	-7.14%	-7.99%	---

Influence of various concentrations of ashwagandha powder(Withaniasomnifera)on WBCs andRBCs of infertile mice:

The impact of ashwagandha powder on the red and white blood cell counts of infertile mice is presented in Table 6. The mean value of the RBCs control (+) group was significantly less than (-) group,measuring 6.28 and 8.49 (10^6/mm3), correspondingly. The treating group of infertile mice

exhibited elevated levels of RBCs when administered 9% ashwagandhapowder. The infertile group of mice fed on 7% ashwagandhapowder had a lower mean value of 7.28 (106/mm3), significantly different (P-value less than 0.05) from the mean value of 8.21 (106/mm3).

- The results for white blood cells demonstrated that the mean value of the control (+) group was significantly less compared to the control (-) group, measuring 5.87 and 8.37 (106/mm3), respectively. The treating group of infertile mice exhibited elevated levels of white blood cells when administered 9% ashwagandha powder. The infertile group of mice fed on 7% ashwagandhapowder had a decreased mean value of 6.59 (103/mm3), significantly different (P-value below 0.05) from the mean value of 7.06 (103/mm3).

Table (6): Influence of various concentrations of ashwagandha powder (Withaniasomnifera)on WBCsand RBCs of infertile mice

Groups / variables	One	two	three	four	LSD
RBCs (10^6/mm^3)	8.49±0.0002b	6.28±0.006f	7.28±0.0009e	8.21±0.0007d	0.04
%Change of C(+)ve	+35.19%	---	+15.84%	+30.65%	---
WBCs (lOVmm3)	8.37±0.004a	5.87±0.0009f	6.59±0.003e	7.06±0.004c	0.007
%Change of C(+)ve	+42.59%	---	+12.27%	20.27%	---

Influence of various concentrations of ashwagandha powder (Withaniasomnifera)onHBand platelet of infertile mice:

Table (7) illustrates the influence of **ashwagandha powder** on hemoglobin and platelet of infertile mice. Resultsdocumented that the mean value of hemoglobin (Hgb) of control (+) group was smaller than (-)group; 12 and 13.9 (g/dl), correspondingly. Conversely, the higher hemoglobin level of treated group was documented for infertile group micethat nourished on 9% ashwagandha powder. The infertile group of rats administered 7% ashwagandha powder had a significantly lower value (P-value below 0.05), with mean values of 12.7 and 12.8 grams per deciliter, correspondingly.

-The results for platelets revealed that the mean value of the control (+)group was significantly greater compared to that of the control (-) group, measuring 432 and 214 (106/mm3), correspondingly. The treatment group of infertile mice had reduced platelet levels when administered 9% ashwagandha powder. The infertile group of mice administered 7% ashwagandha powder had a higher mean value of 248 (106/mm3), significantly differing (P-value below 0.05) from the mean value of 238 (106/mm3).

Table (7):Influence of various concentrations of ashwagandha powder(Withaniasomnifera)on hemoglobin and platelet of infertile mice

Groups / Variables	one	two	three	four	LSD
Hemoglobin Hgb(g/dl)	13.90±0.09a	12±0.004e	12.70±0.07d	12.80±0.002c	0.34
%Change of C(+)ve	+13.67%	---	+14.17%	+15%	---
Platelet PLTS (10^6/mm^3)	214±0.36f	432±0.65a	248±0.3b	238±0.89c	0.19
%Change of C(+)ve	-50.46%	---	-42.59%	-44.90%	---

Influence of various concentrations of ashwagandha powder (Withaniasomnifera)on FSH, LH and Testosterone hormones of infertile mice:

Table (8) showed the impact of **ashwagandha powder** on the FSH, LH, and testosterone hormones of infertile rats. The mean value of follicle-stimulating hormone (mlu/ml) in the control (-) group was significantly smaller than the positive control group, which was 0.98 and 5.80 (mlu/ml), correspondingly. Besides, the mean values of the 3rd and 4th groups differed significantly; they were (4.34, and 4.22) (mlu/ml), correspondingly, as compared to that of the control (+).

-Results in Table (8) showed the influence of **ashwagandha powder** on LH in infertile rats. The mean value of LH for the control (+) group was significantly lesser than (-) group, which was 1.44 and 2.92 (mlu/ml), correspondingly. Additionally, the mean values of 3rd and 4th groups varied significantly in comparison with the positive control; they were 2.36 and 2.52 (mlu/ml), respectively.

- Results in Table (8) also illustrate the effect of **ashwagandha powder** on testosterone in infertile rats. The mean value of testosterone (ng/ml) in the control group (+) was significantly less than (-) group, which was 0.71 and 4.97 ng/ml, correspondingly. Moreover, the mean values of 3rd and 4th groups differed significantly compared to the control (+); they were (2.65 and 3.21) (ng/ml), respectively. The best group seems to be that of fourth group for FSH, LH, and testosterone hormones.

Table (8): Influence of various concentrations of ashwagandha powder(Withaniasomnifera) on FSH, LH and Testosterone hormones of infertile mice

Groups / variables	one	two	three	four	LSD
FSH mIU/Ml	0.98 ± 0.003^{f}	5.80 ± 0.09^{a}	4.34 ± 0.007^{b}	4.22 ± 0.002^{c}	0.09
%Change of C(+)ve	83.10%-	---	25.17%	27.24%	---
LH mIU/Ml	2.92 ± 0.06^{a}	1.44 ± 0.002^{e}	2.36 ± 0.004^{d}	2.52 ± 0.004^{c}	0.05
%Change of C(+)ve	+102.78%	---	+63.89%	+75%	---
Testosterone Ng/mL	4.97 ± 0.001^{a}	0.71 ± 0.002^{e}	2.65 ± 0.004^{d}	3.21 ± 0.006^{c}	0.01
%Change of C(+)ve	+600%	---	+273.2%	+352.1%	---

5- DISCUSSION

Ashwagandha powder may have an+ impact on immunity, blood characteristics and hormone levels associated with fertility. If similar effects are observed in humans, ashwagandha could potentially be used as a natural supplement to support fertility treatments. Further research in clinical trials would be necessary to confirm its efficacy and safety in humans. Ashwagandha active constituents are believed to be steroids chemically related to cholesterol, with the sitosterol believed to be most important **(Dhanani et al., 2017).** The outcomescorrespond with (**Malviya et al., 2016)**indicating significant rise in the hemoglobin level at the maximum dose in infertile rats. Active ingredients in ashwagandha comprise L-arginine, Ginkgo biloba, yohimbine, zinc, vitamin B6, vitamin E, vitamin B5 and choline, (**Mansouri et al., 2016).** Yohimbine and choline are not recognized to influence hemostasis. Despite the lack of binding studies on ashwagandha, its usage was related to an elevated risk of surgical bleeding **(Mascarenhas et al., 2012).** Pharmacologically active constituents in herbal supplements might induce hemorrhaging either directly (by anticoagulant or antiplatelet effects) or indirectly (via medication interactions).Coenzyme Q lacks established direct antiplatelet actions; nevertheless, bromelain has demonstrated the ability to prevent platelet aggregation **(Mills et al., 2005).** The results align with those of **Bjornsson et al. (2020),** which indicated that ashwagandha supplementation diminished body weight gain by reducing visceral as well as epididymal fat weights, as well as serum triglycerides, total

cholesterol, as well as low-density lipoprotein/very low-density lipoprotein cholesterol levels. According to **Almuzghi et al. (2024),** repeated oral administration of ashwagandha in mice showed minimal meaningful impact on the levels and activity of hepatic drug-metabolizing enzymes. Furthermore, repeated oral administration of ashwagandha in mice exhibited no impact on blood biochemical indicators, with the exception of a small elevation in albumin levels, indicating relative safety even with prolonged use.

Ashwagandhais a natural plant. Its general uses include treatment of swollen prostate and improvement of urinary function (**Bokan**, **et al., 2023).** Ashwagandha has also been reported to aid with the symptoms of benign prostatic hypertrophy, a condition in which the prostate gland grows out of control. Benign Prostatic Hyperplasia (BPH) is more common in older people and should be treated as soon as possible to avoid negative health repercussions (**Vaidyaet al., 2024).**Withaniasomnifera is under investigation for its potential therapeutic effects on several inflammatory conditions, including pulmonary, cardiovascular, autoimmune illnesses, diabetes, malignancies, and neurological disorders. Preclinical investigations have shown that this plant can modulate mitochondrial activity and apoptosis while diminishing inflammation by decreasing inflammatory indicators like cytokines (like TNF-α and IL-6), NO, andROS. In a mouse model of lupus, powder of ashwagandha root exhibited a potential inhibitory impact on nephritis and proteinuria **(Kaurav et al., 2012).** An investigation proved the immunomodulatory impact of Withaniasomnifera root powder on enhancing immunological function in immunodeficient rats. The injection of Ashwagandha has been observed to elevate the total count of cells of bone marrow andWBCs, enhance the titre of circulating antibodies and antibody-producing cells, and promote the creation of immune cells as well as macrophage phagocytosis **(Davis et al., 2000).** A randomized, double-blind, placebo-controlled experiment with an open-label extension has been performed to assess the impact of extract of ashwagandha on the healthy individual's immune system. The findings of the research indicated that extract of ashwagandha significantly enhanced natural killer cell activity and cytokine concentrations in comparison with placebo **(Tharakan et al., 2021). Kuboyama et al. (2014)**explain how ashwagandha administration enhanced muscle endurance, elevated sperm counts, and stimulated testosterone production via hormonal control.

(**Kulkarni et al., 2008)** reported that there are certain other benefits of using ashwagandha, like hair loss prevention, treatment of urinary tract infections, maintaining prostrate health, decreasing inflammation and regulating testosterone levels. Other uses of ashwagandha include increasing sexual drive and fertility and bringing down inflammation (**Kumaret al., 2015).** A significant improvement in spermatogenesis was evident with ashwagandhatherapy. Mean sperm level, progressive motility, and rate of sperm exhibiting normal morphology significantly increased following twelve months of ashwagandha medication. **Kuo et al. (2017)** documented a notable reduction in Dihydrotestosterone (DHT) as well as an elevation in testosterone levels within the periurethral area of prostate tissue from cases with Benign Prostatic Hyperplasia (BPH) who were administered Permixon (320 milligrams per day) for three months, thereby proposing that Ashwagandha extract may inhibit 5α-reductase in the human prostate in vivo. According to **Casiano et al. (2023**), the phenolic-rich extract of ashwagandha is more effective in inhibiting microbial α-amylase.

6. Conclusion

Based on the outcomes, ashwagandha powder may have a positive impact on immunity, blood characteristics and hormone levels associated with fertility. If similar effects are observed in humans, ashwagandha could potentially be used as a natural supplement to support fertility treatments. Further research in clinical trials would be necessary to confirm its efficacy and safety in humans. Ashwagandha active constituents are believed to be steroids chemically related to cholesterol, with the sitosterol believed to be most important.

7-REFERENCES

1. AIN (1993): American Institute of nutrition purified diet for Laboratory Rodent, Final Report. J. Nutrition, 123: 1939- 1951 and O. Compactum Benth. Journal. Essential Oil Res. 8 (6): 657-664.
2. Almuzghi F, Abdelmalik A, Abuhlaiga M. Ashwagandha-induced hepatic injury: a case report. Cureus. 2024;16:e71576.

3. Bjornsson HK, Björnsson ES, Avula B, Khan IA, Jonasson JG, Ghabril M, Hayashi PH, Navarro V. Ashwagandha as a cause for liver injury. Liver Int. 2020;40:2035-2036
4. Bokan G, Glamočanin T, Mavija Z, Vidović B, Stojanović A, Björnsson ES, Vučić V. Herb-induced liver injury by Ayurvedic ashwagandha as assessed for causality by the updated RUCAM: an emerging cause. Pharmaceuticals (Basel). 2023;16:1129.
5. Casiano-Manzano S, Torres-Larrubia M, Masa-Caballero A, Jiménez-Colmenarez Z, Martín-Noguerol E, Fernández-Bermejo M, Solís-Muñoz P. Changing perspectives: unveiling the risks of ashwagandha-induced hepatotoxicity. Rev Esp Enferm Dig. 2023 Nov 20.
6. Davis L., Kuttan G. Immunomodulatory activity of Withaniasomnifera. J. Ethnopharmacol. 2002;71:193–200.
7. Fossati, P. (1982): Principle. Clin.Chem., (Chemical Kits). Determination of Serum Uric acid. Journal.of Clin. Chem., 28:2077.
8. Hegsted, D.; Millls, R. and Perkins, E. (1941): Salt mixture, J. Biol. Chem., 138:459.
9. Kaurav B.P.S., Wanjari M.M., Chandekar A., Chauhan N.S., Upmanyu N. Influence of Withaniasomnifera on obsessive compulsive disorder in mice. Asian Pac. J. Trop. Med. 2012;5:380–384. doi: 10.1016/S1995-7645(12)60063-7
10. Kuboyama, T. · Tohda, C. · Komatsu, K.(2014).Effects of Ashwagandha (roots of Withaniasomnifera) in neurodegenerative diseases. Biol. Pharm. Bull. 2014; **37**:892-897
11. Kulkarni, S.K. · Dhir, A.(2008).Withaniasomnifera: an Indian Ginseng.Prog. Neuropsychopharmacol Biol. Psychiatry. 2008; **32**:1093-1105
12. Kumar, A. · Kumar, R. · Rahman, M.S. (2015).Phytoremedial effect of Withaniasomnifera against arsenic-induced testicular toxicity in Charles foster rats. Avicenna J. Phytomed. 2015; **5**:355-364
13. Kuo, Y.T. · Liao, H.H. · Chiang, J.H. (2017).Complementarychinese herbal medicine therapy improves survival of patients with pancreatic cancer in taiwan: a nationwide population-based cohort study. Integr. Cancer Ther. 2017; 1534735417722224
14. Lee, R. and Nieman, D. (1996): Nutrition Assessment. 2nd Ed., Mosby, Missouri, USA.
15. Malviya, N. · Malvia, S. · Jain, S. (2016)**.** Review of the potential of medicinal plants in the management and treatment of male sexual dysfunction. Andrologia. 2016; 48:880-893
16. Mansouri, E. · Keshtkar, A. · Khaki, A.A. (2016)**.** Antioxidant effects of allium cepa and cinnamon on sex hormones and serum antioxidant capacity in female rats exposed to power frequency electric and magnetic fields.Int. J. Womens Health Reprod. Sci. 2016; 4:141-145
17. Mascarenhas, M.N. · Flaxman, S.R. · Boerma, T. (2012)**.** National, regional, and global trends in infertility prevalence since 1990: a systematic analysis of 277 healthsurve**ys** .PLoS Med. 2012; **9**:e1001356
18. Mills, E. · Cooper, C. · Seely, D.(2005). African herbal medicines in the treatment of HIV: Hypoxis and Sutherlandia. An overview of evidence and pharmacology .Nutr. J. 2005; 4:19
19. Patton, C.J. and Crouch, S.R. (1977): Enzymatic determination of urea. Journal, of Anal. Chem., 49: 464-469.
20. Russo, A.; Izzo, A.A.; Cardile, V.; Borrelli, F. and Vanella, A. (2001): Indian medicinal plants as antiradicals and DNA cleavage protectors. Phytomedicine, 8(2): 125-32.
21. Schermer, S. (1967): The Blood Morphology of Laboratory Animal. Longmans Printed in Great Britain, Green and Co. Ltd, p. 350.
22. Snedecor, G. W. and Cochran, W. G. (1967): "Statistical Methods". 6th Ed. Iowa State University Press. Ames. Lowa. USA.
23. Vaidya VG, Gothwad A, Ganu G, Girme A, Modi SJ, Hingorani L. Clinical safety and tolerability evaluation of Withaniasomnifera (L.) Dunal (Ashwagandha) root extract in healthy human volunteers. J Ayurveda Integr Med. 2024;15:100859.
24. Young, D.S. (1975): Determination of GOT. Clin. Chem., 22 (5): 1—21.
25. Cocuzza, M.; Alvarenga, C. and Pagani, R. (2013): The epidemiology and etiology of azoospermia. Clinics, 68, 15-26.
26. Anwar, S. and Anwar, A. (2016): Infertility: A review on causes, treatment and management. Womens Health Gynecol, 5, 2-5.

27. Chinen, J. and Shearer, W. T. (2010); Secondary immunodeficiencies, including HIV infection. Journal of Allergy and Clinical Immunology, 125(2), S195-S203.
28. SAS, (1988): SAS Users Guide: Statistics version 5th Ed. SAS. Institute Inc., Cary N.C.
29. Tiez, N.M. (1976): Fundamental of clinical chemistry, philade 1 phia, (2) W.B., PP. 53-56.
30. While, B.A.; Erickson, M.M. and Steven, S.A. (1970): Chemistry for Medical Theologiests. 3rd Ed., C.V. Mosby Company Saint Louis, USA, p.662.
31. Akram, H.; Firouz, G.P.; Abbas,A. and Samad, Z.(2012): Beneficial effects of American ginseng on epididymal sperm analyses in cyclophosphamide treated rats. Cell J. yakhteh, 14(2): 116- 121.
32. Allain, C.C. (1974): Cholesterol enzymatic colorimetric method. J. of Clin.Chem.. 20:470.
33. Dacie, A. and Lewis, J. (1998): Practical hematology. 10 th Edition Churchill Livingstone, London.
34. Castelli, W. P.; Doyle, J. T.; Gordon, T.; Hames, C. G.; Hjortland, M. C.; Halley, S. B.; Kagan, A. and Zuckel, W. J. (1977): HDL cholesterol and other lipids in coronary heart disease: The cooperative lipoprotein phenotyping study. Circulation, 55: 767-772.
35. Henary, R.J. (1974): Clinical Chemist: Principels and Techniques. 2nd, Edition, Hagerstoun (MD), Harcer, ROW, P. 882.
36. Kaplan, L.A. (1984): Clinical Chemistry. The C.V. Mosby Co. St Louis. Toronto. Princent., 1032-1036.
37. Kikuchi-Hayakawa, H.; Onodera, N.; Mastubara, S.; Yasudo, E.; Chonan, O.; Takahashi, R. and Ishkawa, F. (1998): Effect of soymilk and bifido bacterium fermented soymilk on lipid metabolism in aged avariectomized rats. Bioscience Biotechnology and Biochemistry, 62(9): 1688-1692.
38. Mancini, G.; Carbonara, A.G. and Heremans, J.F (1965): Immunochemical quantitation of antigens by single radial immunodiffusion.Immuno-chemistry2,235.
39. Rekha, D. K.; Nyanatara, A.K.; Ramswamy, C.; Sheila, R.P.; Ramesh, B.M. and Venkappa, S.M. (2009): "Infertility in male wistar rats induced by cadmium chloride: Role of ascorbic acid".Journal of Chinese Medicine,41 (11):616-621.
40. Richmond, W. (1973): Preparation and properties of a cholesterol oxidase from Nocardia sp. and its application to the enzymatic assay of total cholesterol in serum. Clin. Chern., 19 (12): 1350.
41. Tharakan A., Shukla H., Benny I.R., Tharakan M., George L., Koshy S. Immunomodulatory Effect of Withaniasomnifera (Ashwagandha) Extract—A Randomized, Double-Blind, Placebo Controlled Trial with an Open Label Extension on Healthy Participants. J. Clin. Med. 2021;10:3644. doi: 10.3390/jcm10163644.
42. Kumar, A. · Kumar, R. · Rahman, M.S. (2015).Phytoremedial effect of Withaniasomnifera against arsenic-induced testicular toxicity in Charles foster rats. Avicenna J. Phytomed. 2015; **5**:355-364.
43. Belfield, A. and Goldberg, D.M. (1971): Alkaline phosphatase colorimetric method. J. of Enzyme, (12): 561.
44. Mir, B.A., Koul, S., Kumar, A., Sharma, S., Kaul, M.K., Soodan, A.S., 2012. Reproductive behaviour and breeding system of wild and cultivated types of Withaniasomnifera (L.) Dunal. J. Med. Plants Res. 6 (5), 754-762.
45. Dhanani, S. Shah, N.A. Gajbhiye, S. Kumar(2017). Effect of extraction methods on yield, phytochemical constituents, and antioxidant activity of Withaniasomnifera Arabian J Chem, 10 (Suppl. 1) (2017), pp. S1193-S1199.
46. Ganguly(2018). Effect of hydroalcoholic extract of Withaniasomnifera (Ashwagandha) on growth kinetics of infectious bursal disease virus in chicken embryo fibroblast culture [Dissertation] G. B. Pant University of Agriculture and Technology (2018)
47. Ahmad, S. M., Nayak, D., Mir, K. B., Faheem, M. M., Nawaz, S., Yadav, G., et al. (2020). Par-4 activation restrains EMT-induced chemoresistance in PDAC by attenuating MDM-2. Pancreatology 20, 1698–1710. doi:10.1016/j.pan.2020.09.021
48. Ahmed, W., Mofed, D., Zekri, A.-R., El-Sayed, N., Rahouma, M., and Sabet, S. (2018). Antioxidant activity and apoptotic induction as mechanisms of action of Withaniasomnifera (Ashwagandha) against a hepatocellular carcinoma cell line. J. Int. Med. Res. 46, 1358–1369. doi:10.1177/0300060517752022
49. Alam, N., Hossain, M., Mottalib, M. A., Sulaiman, S. A., Gan, S. H., and Khalil, M. I. (2012). Methanolic extracts of Withaniasomnifera leaves, fruits and roots possess antioxidant properties

and antibacterial activities. BMC Complement. Altern. Med. 12, 175. doi:10.1186/1472-6882-12-175

50. Alfaifi, M. Y., Saleh, K. A., El-Boushnak, M. A., Elbehairi, S. E. I., Alshehri, M. A., and Shati, A. A. (2016). Antiproliferative activity of the methanolic extract of Withaniasomnifera leaves from faifa mountains, southwest Saudi arabia, against several human cancer cell lines. Asian pac. J. Cancer Prev. 17, 2723–2726. Available at: http://www.ncbi.nlm.nih.gov/pubmed/27268658.
51. Atluri, V. S. R., Tiwari, S., Rodriguez, M., Kaushik, A., Yndart, A., Kolishetti, N., et al. (2020). Inhibition of amyloid-beta production, associated neuroinflammation, and histone deacetylase 2-mediated epigenetic modifications prevent neuropathology in Alzheimer's disease in vitro model. Front. Aging Neurosci. 11, 342. doi:10.3389/fnagi.2019.00342
52. Baitharu, I., Jain, V., Deep, S. N., Shroff, S., Sahu, J. K., Naik, P. K., et al. (2014). Withanolide A prevents neurodegeneration by modulating hippocampal glutathione biosynthesis during hypoxia. PLoS One 9, e105311. doi:10.1371/journal.pone.0105311
53. Balkrishna, A., Nain, P., Chauhan, A., Sharma, N., Gupta, A., Ranjan, R., et al. (2020). Super critical fluid extracted fatty acids from Withaniasomnifera seeds repair psoriasis-like skin lesions and attenuate pro-inflammatory cytokines (TNF-α and IL-6) release. Biomolecules 10, 185. doi:10.3390/biom10020185
54. Bano, N., Hossain, M. M., Bhat, A. Q., Ayaz, M. O., Kumari, M., Sandhu, P., et al. (2020). Analyzing structural differences between insulin receptor (IR) and IGF1R for designing small molecule allosteric inhibitors of IGF1R as novel anti-cancer agents. Growth Horm. IGF Res. 55, 101343. doi:10.1016/j.ghir.2020.101343