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Ameliorating Effect of Dried Ashwagandha (*Withania Somnifera* L) Roots on Letrozole-Induced Polycystic Ovary Syndrome in Female Rats

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POLYCYSTIC ovarian syndrome (PCOS) is a heterogenous, endocrine, metabolic, and multidisciplinary disorder of reproductive-aged females. As such, the study purpose was to discover out the impact of Ashwagandha *(Withania somnifera L.)* roots powder on PCOS - caused by Letrozole (1 mg/kg. per orally) for 21 days in female rats. Phenolic and flavonoids compounds in ashwagandha roots using HPLC revealed the presence of gallic acid, Chlorogenic acid, Caffeic acid, Syringic acid, Coumaric acid, Vanillin, Ferulic acid, Naringenin, Daidzein, quercetin, Cinnamic acid, Kaempferol and Hesperetin. The IC 50 value of the *Withania somnifera L.* roots sample, which is the concentration of the sample necessary to block 50% of the DPPH free radical, was 36.57 ug/mL. Thirty-five female albino rats weighing (150 ± 5 g) were separated to 5 groups (7 rats each). The first group was fed on a basal diet as control negative group, the four groups were given orally letrozole to cause PCOS, then one of them was kept as positive PCOS control. The remaining three groups were fed on basal diet supplemented with three tested levels of dried ashwagandha roots at (2.5%, 5%, 7.5%, respectively), for 28 days. The obtained results indicated that Ashwagandha roots powder significantly ($p<0.05$) reduced body weight, ameliorating lipid profile, Malondialdehyde (MDA), Luteinizing Hormone (LH) and Thyroid-stimulating hormone (TSH) , while causing an elevated level of high-density lipoproteins, hormones (Follicle Stimulating Hormone and progesterone) along with increased the activities of the antioxidant enzyme catalase. The highest improvement for the above parameters and histopathological examination of the ovary was recorded at the rats fed on 7.5% Ashwagandha roots powder. Finally, Ashwagandha roots can be considered as a supportive supplement in therapies of PCOS.

Keywords: Ashwagandha; Polycystic Ovary Syndrome; Sex Hormones; Lipid Profile; Rats.

Introduction

Polycystic ovary syndrome (PCOS) represents the most frequent endocrine disorder in females globally during their reproductive years, with prevalence estimates ranging from 6% to 20% (Escobar-Morreale, 2018). This condition is associated with several complications, as approximately 69% of affected women experience infertility, 51% develops amenorrhea, and 41% struggle with obesity (Penzias et al., 2017). PCOS is characterized by hormonal imbalances that disrupt regular menstruation and is classified into four primary subtypes: insulin-resistant PCOS, inflammatory PCOS, post-pill

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PCOS, and the hidden cause of PCOS (Ding et al., 2018; Zhang et al., 2018). In PCOS, the ovaries generate excessive androgens, which interfere with the normal development of ovarian follicles. Consequently, anovulation occurs due to the failure to form and release a mature egg, leading to potential difficulties in achieving pregnancy (Ndefo et al., 2013). Being overweight or obese can exacerbate the signs of PCOS. These signs may include the absence of menstrual periods, insulin resistance, and the presence of ovarian cysts, irregular menstrual cycles, and weight gain (Azziz et al., 2006; Kumar et al., 2018).

The etiology of PCOS is complex and multifactorial, involving factors such as genetic predispositions that disrupt normal ovarian function, the use of contraceptive medications, excessive adrenal gland activity in childhood, elevated insulin levels, and hormonal imbalances, as well as stress (Azziz et al., 2006). PCOS is frequently linked to a range of related health conditions, including infertility metabolic syndrome, obesity, and impaired glucose tolerance; type 2 diabetes mellitus, cardiovascular complications, depression, obstructive sleep apnea, endometrial cancer, and nonalcoholic fatty liver disease or nonalcoholic steatohepatitis. Given this wide array of potential comorbidities, clinicians are advised to consider thorough evaluations even with subtle indications in patients presenting with PCOS (Norman and Teede, 2018). The therapeutic potential of medicinal plants often arises from the combined effects of their various active constituents (Epidi et al., 2016). Among these plants, Ashwagandha (*Withania somnifera* L.*)* is an annual member of the Solanaceae family, commonly referred to as winter cherry. Its utilization in Ayurvedic and Unani medical systems has a history spanning 3000 to 4000 years. The roots of Ashwagandha are a primary plant part employed for their therapeutic properties (Rajeswara Rao et al., 2012).

Studies on the phytochemical composition of *Withania somnifera L.* have identified a range of chemical constituents, including steroidal compounds, alkaloids, phenolic compounds, and saponins (Patil et al., 2013; Singh et al., 2023; Nile et al., 2021). The aerial parts, roots, and berries of *Withania somnifera* L. are reported to contain a diverse array of compounds, encompassing over 12 alkaloids, approximately 40 withanolides, and several sitoindosides (Saleem et al., 2020). Additionally, the roots have been shown to include alkaloids, amino acids, steroids, a volatile oil, starch, reducing sugars, glycosides, hentriacontane, dulcitol, and withaniol (Saini et al., 2022). It is suggested that withanolides, present in the plant, contribute to the stimulation of immune system cell activation through their antioxidant activity, (Teixeira et al., 2017), has antiviral activity (Latheef et al., 2017), anti-inflammatory (Balkrishna et al., 2020), anti-cancer (Malik et al., 2021; Singh et al., 2021; Lee et al., 2022), anti-depressant, anti-anxiety, cardioprotective, thyroid modulating, neuroprotective, cognitive enhancing and hematopoietic agent (Bhattacharya & Muruganandam, 2003; Singh et al., 2010; Sharma et al., 2011). Furthermore, research studies endorse the application of Ashwagandha in addressing infertility and hormonal imbalances (Sengupta et al., 2018; Lopresti et al., 2019). Due to the biological and nutritional importance of ashwagandha roots and their bioactive phytochemicals, this study aimed to investigate the potential of Ashwagandha *(Withania somnifera L.)* roots powder in improving symptoms of PCOS in female rats.

Materials and Methods

Materials

Dried roots of Ashwagandha (*Withania somnifera* L.) were obtained from herbalist shops in Cairo, Egypt. Letrozole was bought from The Egyptian Pharmaceutical Company Trade, Cairo, Egypt. Casein was acquired from Morgan Chemical Co. (Cairo, Egypt.), while the components of the diet, specifically cellulose, vitamins, and minerals, were obtained from the Company El-Gomhoriva Pharmaceutical (Cairo, Egypt.). Starch, soybean oil, and sucrose were purchased from local commercial sources. All chemicals and kits utilized for the biochemical analyses were acquired from the Gamma Trade Company for Pharmaceutical and Chemical (Dokki, Egypt).

Rats

Forty adult female Sprague Dawley albino rats, with an average weight of 150 ± 5 g, were obtained from the animal housing facility at the Food Technology Research Institute (Cairo, Egypt.).

Methods

Preparation of plant powder

Dried roots of Ashwagandha were thoroughly rinsed with running water to eliminate foreign particles, muck, and dust, thereafter dried at a

low temperature (50 – 60 °C), and crushed into a powder under sanitary circumstances by using a coffee grinder into a fine powder.

Determination polyphenols and anti-oxidants activity in ashwagandha roots

Polyphenols components were identified and quantified by HPLC as described by El-Houfi, (2015). This assay was conducted in chromatography Lab., Central laboratories Network, National Research Center, Egypt.

Evaluation of antioxidant activity in ashwagandha roots using the DPPH radical scavenging method

The free radical scavenging activity of various leaf extracts was assessed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. Specifically, a 0.1 mM solution of DPPH in ethanol was prepared. One milliliter of this solution was then added to 3 mL of different ethanolic extracts at varying concentrations (3.9, 7.8, 15.62, 31.25, 62.5, 125, 250, 500, and 1000 µg/mL). Only extracts soluble in ethanol were used, and their respective concentrations were obtained through serial dilution. The resulting mixtures were vigorously shaken and allowed to incubate at room temperature for 30 minutes, after which absorbance was measured at 517 nm using a UV-VIS spectrophotometer (UV-VIS milton roy). Ascorbic acid was employed as the reference standard, and all tests were performed in triplicate. The IC_{50} value, defined as the concentration of the extract required to inhibit 50% of the DPPH free radicals, was determined from the log dose-inhibition curve. An inverse relationship between absorbance and free radical scavenging activity was observed, where lower absorbance indicated greater scavenging activity (González-Palma et al., 2016). The percentage of DPPH scavenging impact was calculated according to the following equation:

DPPH scavenging effect (%) = A0 - A 1 / A0 × 100.

where A0 was the Absorbance of control reaction and A1 was the Absorbance in presence of test or standard sample.

Induction of polycystic ovary syndrome

PCOS was caused in female rats with the administered of letrozole at the concentration of 1 mg/kg p.o. dissolved in 1% of carboxmethly cellulose once daily orally for 21 days (Kafali et al., 2004). After those 21 days, five rats were sacrificed to ensure the induction of PCOS, while the other 28 rats were used in the study.

Experimental design

The standard diet (AIN-93M) was formulated based on the composition outlined by Reeves et al., (1993). The biological experiments were performed following the guidelines set forth by the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (NRC, 1996). Thirty-five female rats were housed for approximately one week in well-ventilated cages within the animal facility at the Agricultural Research Center, under conditions meeting standard health requirements, and were provided a basal diet for acclimatization. Access to food and water was provided without interruption throughout the experimental duration. Following this period, the rats were randomly assigned to one of five groups (n = 7): Group 1 received only the basal diet and served as the negative control. Group 2, the positive control, consisted of rats with induced PCOS and was fed the basal diet. The remaining three groups consisted of rats with induced PCOS and were fed on the basal diet supplemented with 2.5, 5, or 7.5% Ashwagandha root powder. During the 28-day experimental period, feed intake was calculated daily and body weight gain was recorded weekly. Body weight gain percentage (BWG %) and feed efficiency ratio (FER) was calculated according to Champman et al. (1959) using the following equation:

BWG (%) = [(Final weight – Initial weight) / Initial weight] × 100

FER = (Total body weight gain in grams over 28 days) / (Total feed intake in grams over 28 days)

Blood collection and serum separation

At the conclusion of the 28-day period of experimentation, blood samples were obtained from the rats following a 12-hour fasting period. The rats were anesthetized using ether, and blood was collected from the abdominal aorta. The samples were then placed into clean centrifuge tubes and allowed to coagulate at room temperature (25°C). Subsequently, serum separation was achieved by centrifuging the samples at 3,500 rpm for 10 minutes, as outlined by Stroev and Makarova (1989). The resulting serum was carefully transferred to sterile tubes and stored at -18°C until further analysis. Following blood collection, the ovaries were excised from each rat, rinsed with normal saline to remove any residual blood, and preserved in 10% neutral formalin for subsequent histopathological examination.

Biochemical analysis

Triglycerides (TGs), total cholesterol (TC), high density lipoproteins cholesterol (HDL-C) and low-density lipoproteins cholesterol (LDL-C) were determined in serum as described by Ahmadi et al. (2008), Fossati and Prenape (1982); Lopes-Virella et al. (1977); Richmod (1973), respectively. Very low-density lipoprotein cholesterol (VLDL-C) was calculated using Friedewald's formula. VLDL-c (mg/ dL) = TG/5. Calorimetric measurement of follicular stimulating hormone (FSH) and luteinizing hormone (LH) used to be carried out the usage of the Fahim et al. (1982) techniques. Serum level of thyroid stimulating hormone (TSH) was analyzed by colorimetric competitive enzyme immunoassay using individual ELISA kit according to Bhowmich et al. (2007). Serum level of progesterone was assessed by Gama kits as described by Lavaee et al. (2021). Malondialdehyde (MDA) was assayed quantitatively in serum according to Ohkawa et al., (1979). Catalase (CAT) activity was evaluated using the procedure detailed by Wheeler et al. (1990).

Histopathological examination

The ovaries from all the sacrificed rats were collected and placed in 10% formalin solution. The fixed tissue samples were then trimmed, washed, and dehydrated using a series of increasing alcohol concentrations. Following this, the samples were cleared in xylol, embedded in paraffin, sectioned at 4-6 µm thickness, and stained with hematoxylin and eosin for microscopic examination of the ovaries, as outlined by Bancroft and Stevens (1996). The histopathological analysis was performed at the Faculty of Veterinary Medicine, Cairo University.

Statistical analysis

The data resulting from the biological evaluation of each group were statistically analyzed (mean ± standard error) using the SPSS software package and compared across groups using the appropriate test (least significant differences at $P < 0.05$), as outlined by Sendecor and Cochran (1979).

Results and Dissction

PCOS is a prevalent hormonal condition in women of reproductive age (Tamadon et al., 2018). The PCOS traits are elevated body weight, dyslipidemia, hyperandrogenism, an-ovulation, high level of LH and low level of FSH and progesterone. PCOS is associated with various forms of dyslipidemia, characterized by reduced HDL-C levels, elevated triglycerides, total cholesterol, and LDL-C levels (Karateke et al., 2018). Ndeingang et al. (2019) reported that oxidative stress biomarkers such as MDA and CAT are abnormal in patients with PCOS. As well, the development of the small antral follicles and the establishment of multiple cystic ovaries were also observed in PCOS patients. PCOS is characterized by elevated androgen levels in women, a condition termed hyperandrogenism. This hormonal excess interferes with the regular menstrual cycle and reproductive capacity. Ovarian characteristics of this disorder can include the development of small, fluid-filled cysts and frequent ovulatory dysfunction (Kafali et al., 2004). The main risk factor for evolving PCOS is diet, where the fats and proteins diet can differentiate advanced glycation end products (AGEs) when susceptible to sugar in the bloodstream, which provide to elevated bodily stress and inflammation (Diamanti-Kandarakis et al., 2012). These results agree with the results obtained. The development of polycystic ovaries can be triggered by an excess of secondary endogenous androgens (Abbott et al*.,* 2005). Letrozole, an aromatase inhibitor, can be used to achieve these goals by inhibiting the conversion of androgens into estrogen. The resulting reduction in aromatase activity may lead to increased androgen production within the ovaries, with a concurrent decrease in estrogen levels, ultimately contributing to the formation of polycystic ovaries. The oral administration of letrozole to adult rats for at least 21 consecutive days induces drawbacks such as moderate weight gain and abdominal adiposity acyclicity (Moradi et al., 2021). As seen from the histopathological investigation of this work.

Table 1 indicated that the higher concentration of phenolic and flavonoids compounds in *Withania somnifera,* L. roots powder is gallic acid, chlorogenic acid and Querectin, while the moderate concentration of phenolic and flavonoids compounds in *Withania somnifera L*. roots powder is naringenin, syringic acid and caffeic acid and the low concentration of phenolic and flavonoids compounds in *Withania somnifera* L. roots powder is Vanillin, Hesperetin and Kaempfero. Data in Table 2 pointed out that the IC_{50} value of the *Withania somnifera* L. sample, which is the concentration of the sample necessary to block 50% of the DPPH free radical was 36.57 µg/mL.

TABLE 1. Identification of polyphenols compounds in *Withania somnifera* L. roots powder using HPLC.

Compounds	Concentration (µg/g)
Gallic acid	174.74
Chlorogenic acid	170.70
Caffeic acid	26.82
Syringic acid	30.15
Coumaric acid	9.72
Vanillin	6.16
Ferulic acid	15.17
Naringenin	43.76
Daidzein	35.81
Querectin	58.61
Cinnamic acid	7.50
Kaempferol	1.86
Hesperetin	4.15

TABLE 2. Evaluation of antioxidant activity of *Withania somnifera* L. roots by DPPH radical scavenging.

Sample concentration (µg/mL)	DPPH scavenging%	IC_{50} µg/mL
1000	90.3	36.57
500	82.0	
250	73.9	
125	65.2	
62.5	56.2	
31.25	47.5	
15.625	39.0	
7.8125	30.4	
3.9	21.6	
1.95	16.9	
0	0.0	

IC_{50}: The concentration of sample required to inhibit 50% of the DPPH free radical

The results in Table 3 suggest that *Withania somnifera L.* may help mitigate the weight gain associated with PCOS in female rats. The body weights of the rats receiving the root powder are significantly lower than those of a positive control, indicating a potential benefit of this herbal supplement in managing weight issues related to PCOS. Rats fed on 7.5%. *Withania somnifera L.* roots powder recorded as the best treatment for BWG% and FER. Overall, incorporating *Withania somnifera L.* into the diet could be a promising approach to support weight management in conditions like PCOS. These outcomes align with those articulated by Abd Elmeged et al. (2024) who founded that diet supplemented with different levels of ashwagandha plant (2.5, 5.0, 7.5%), for 28 days. reduce feed intake and increase body weight gain and feed efficiency ratio. Additionally, Bashir et al. (2023) observed that ashwagandha can diminish feed consumption. These findings concur with those presented by Vaidya et al. (2024) who stated that ashwagandha able to improve the utilization of glucose and keep the body weight in normal range.

Table 4 showed impact of *Withania somnifera L.* roots powder on lipid profiles. Results indicated that the mean value of TG, TC, LDL and VLDL in the control positive group was significantly higher than that of negative controls, while HDL had

TABLE 3. Effect of diet supplemented with *Withania somnifera* L. roots powder on nutritional parameters in Female Rats with PCOS.

Parameter		Parameter as Mean ± SE					
Groups		IBW (g)	FBW (g)	BWG (g)	BWG%	FER	FI (g/day)
Negative group		20.40±0.24^{b}	153.01±1.26^{a}	202.60±1.36^{e}	49.61±1.86^{e}	32.45±1.42^{e}	0.054±0.01^{d}
Positive group		24.80±0.58^{a}	151.80±1.06^{a}	255.74±1.47^{a}	103.94±2.06^{a}	68.51±1.73^{a}	0.092±0.01^{a}
Dried roots	2.5%	20.40±0.74^{b}	153.06±1.02^{a}	240.01±0.70^{b}	86.40±0.50^{b}	56.26±0.67^{b}	0.098±0.01^{a}
	5%	21.00±0.54^{b}	154.21±1.82^{a}	230.41±0.74^{c}	76.21±2.00^{c}	49.50±1.84^{c}	0.080±0.00^{b}
	7.5%	19.80±0.66^{b}	153.60±1.02^{a}	216.42±1.80^{d}	62.82±1.06^{d}	40.88±0.61^{d}	0.068±0.00^{c}

* Values within the same column that have various letters are significant at P≤0.05 level.
SE: Stander Error.

opposite trend. The results indicated that treatment with *Withania somnifera* L. roots powder at the three tested levels induced a significant (P<0.05) decrease in serum concentrations of TG, TC, LDL and VLDL levels and increase in serum level of HDL compared to positive controls. The results suggest that *Withania somnifera L.* root powder is effective in ameliorating lipid disturbances associated with PCOS in female rats, with a notable dose-response effect. Higher concentrations of the supplement are associated with greater reductions in lipid levels, which points to its potential as a dietary intervention to manage PCOS-related dyslipidemia. The study indicates that *Withania somnifera* L. could be a promising natural therapeutic agent for improving lipid profiles in females with PCOS, particularly at higher doses.

These results with agreement with Anwer et al. (2017) and Ali (2021) who recorded that *Withania somnifera* L. was administered orally once a day for 5 weeks, resulted in a significant lowering in TC, TG, LDL-c, VLDL-c levels with significant rise of HDL-c levels. In addition, Abd Elmeged et al. (2024) reported that diet supplemented with different levels of ashwagandha plant (2.5%, 5%, 7.5%), for 28 days reduced the serum levels of lipid profile and elevated HDL level. In this respect, Tandon and Yadav (2020) founded that the hypoglycemic effect of ashwagandha plant on some biochemical and immunological parameters of alloxan induced diabetic rats. The concentrations of glucose, total lipids cholesterol, triglycerides, VLDL and LDL were significantly reduced, while HDL elevated in plasma of alloxan diabetic mice than control group. These consistent results are with the data obtained in study carried by Gannon et al. (2019) who showed that Ashwagandha plant reduced total cholesterol, triglycerides antherogenic index, while elevated HDL level. Chengappa et al. (2018) reported that ashwagandha can lower total serum cholesterol, triglycerides and increase HDL level. So, Ashwagandha can reduce the risk of cardiovascular disorder, diabetes and weight loss programs. In addition, Zahran et al. (2020) recorded that *Withania Somnifera* L. did not produce adverse effects on lipid profile parameters. Instead, a beneficial outcome was observed, especially at the 5% concentration, with a notable elevation in HDL levels compared to other groups. This finding could be attributed to the hypolipidemic activity of *Withania Somnifera* L. potentially due to its high flavonoid content.

The results presented in Table 5 provide the impact of diet supplemented with *Withania somnifera* L. roots powder on serum levels of TSH, LH, FSH and progesterone in female rats with PCOS. The outcomes revealed that letrozole induced a significant (P < 0.05) increase in serum concentrations of TSH and LH and reduced in serum level of FSH and Progesterone. The elevated levels of TSH and LH were significantly reduced after the administration of *Withania somnifera* L. roots powder (2.5%, 5%.and 7.5%) in treated rats when compared with positive control group. Also, the reduced levels of FSH and progesterone were significantly increased after the administration of *Withania somnifera* L. roots powder (2.5, 5.and 7.5%) in treated rats when compared with untreated PCOS rats. The results indicate a dose-dependent response to *Withania somnifera* L. where both lower and higher doses of root powder have a positive effect in decreasing TSH and LH while increasing FSH and Progesterone

TABLE 4. Effect of diet supplemented with *Withania somnifera* L. roots powder on lipid profile in Female Rats with PCOS

Parameter		Parameter as Mean ± SE				
Groups	TG	TC	HDL-c	VLDL-c	LDL-c	
			(mg/dl)			
Negative group		96.86±0.51[e]	119.55±1.12[e]	64.71±0.76 [a]	19.37± 0.10 [e]	35.46±1.53 [e]
Positive group		194.54±1.97[a]	192.54±1.64[a]	27.72±0.53 [e]	38.90± 0.39 [a]	125.91±2.02 [a]
Dried roots	2.5%	147.99±2.23[b]	148.95±0.71 [b]	43.87±0.97 [d]	29.59± 0.44 [b]	75.48±1.09 [b]
	5%	128.45±0.64 [c]	132.12±1.18 [c]	48.93±0.46 [c]	25.69± 0.12 [c]	57.49±1.39 [c]
	7.5%	[d] 105.01±0.92	125.06±1.44 [d]	58.66±0.65 [b]	21.01± 0.18 [d]	45.41±1.26 [d]

* Values within the same column that have various letters are significant at P≤0.05 level.
SE: Stander Error. VLDL-C: Very Low-Density Lipoprotein Cholesterol.

levels. The results suggest that *Withania somnifera* L. root powder supplementation could be an effective dietary intervention for managing hormonal imbalances in female rats with PCOS by potentially normalizing TSH and LH levels. The thyroid gland and its hormones play a key role in metabolism and reproduction. Disruptions in the gonadal axis due to endocrine hormonal imbalances in women with PCOS can influence the pituitary-thyroid axis, as evidenced by elevated serum thyroid-stimulating hormone (TSH) levels in PCOS patients compared to the general female population (Glintborg et al., 2019; Lee et al., 2022). Furthermore, a study by Du and Li (2013) indicated a statistically significant, albeit slight, increase in mean TSH levels in women with PCOS (mean difference: 0.62; 95% CI: 0.21−1.02) when compared to those without the syndrome. Moreover, an imbalance in LH, FSH, and other reproductive hormones causes influence ovarian function and may contribute to reactive oxygen species (ROS) production (Ramya et al., 2023). Injury to cellular structures, as a result of PUFA-induced oxidative stress (OS), leads to the aggregation of lipid peroxidation products such as MDA and the hydroxyl radical (Lubrano et al., 2019). Our results agree with data obtained by Megahd and Gabal (2021) who examined the effectiveness of the singular or combination administration of matcha and ashwagandha teas in mitigating H_2O_2-induced utero-ovarian oxidative damage and cellular death in female rats. The results pointed out that supplementing with ashwagandha tea significantly ($p\leq0.01$) increased FSH and progesterone hormones.

In this respect, Al-Nuaimi and Al-Baniwes (2022) assessed the potential protective impacts of *Withania somnifera* L. extract on sex hormone levels in female rats with morphine addiction. Their findings indicated a notable reduction in luteinizing hormone (LH) in the morphine-treated groups after a 21-day experiment when compared to both control and treatment groups. Furthermore, they observed no significant differences between the control group and the groups treated with 6.25% *Withania somnifera* L. extract incorporated into new pellets over 21 days. Notably, the *Withania somnifera* L. group demonstrated improved hormonal balance. Ajgaonkar et al. (2022) reported comparable outcomes in a study involving women aged 18–64, where the consumption of 300 mg of ashwagandha root extract twice daily was associated with increased estrogen levels and decreased luteinizing hormone, along with a reduction in menopausal symptoms. Also, El-Kholie et al. (2024) concluded that using of ashwagandha root resulted in a significant elevation of (FSH) and a decrease in LH levels. With regard to the effect of ashwagandha on TSH hormone, our study aligns with those reported by Sharma et al. (2018), who conducted an 8-week trial involving 50 patients. In their research, half of the participants received ashwagandha root extract (300 mg twice daily), while the other half received a starch placebo. Throughout the trial, data was gathered to monitor TSH, serum T3, and T4 levels. Upon comparing baseline measurements with those taken during the study, the researchers noted a reduction in TSH levels

and an elevation in T3 and T4 levels within the ashwagandha group. Furthermore, a significant decrease in TSH levels was observed at both 4 weeks (−12.5%) and 8 weeks (−17.4%) into the study. In addition, Abdel-Wahhab et al. (2019) examined the effects of ashwagandha methanolic extract in a rat model of induced hypothyroidism. In that study, rats were divided into groups receiving either ashwagandha methanolic extract (500 mg/kg/day) or levothyroxine, a standard treatment for hypothyroidism. The results indicated comparable outcomes between the groups, not only in thyroid hormone levels (TSH) but also in subsequent histopathological assessments. Similarly, Ibrahim et al. (2023) conducted a study in which they induced hypothyroidism in rats. They found that the group treated solely with propylthiouracil (PTU) exhibited significantly lower T3 and T4 levels and higher TSH levels compared to the group treated with PTU and Ashwagandha, supporting the idea that *Withania somnifera* L. compounds have a "protective" effect on the thyroid. Phenolic and flavonoids, classes of phytochemicals that presented in Ashwagandha (*Withania somnifera* L.) have demonstrated potential therapeutic effects on PCOS in vivo and in vitro experiments. These effects are mainly attributed to anti-inflammatory and antioxidant properties and improvements in hormone disorders (Mikulska et al., 2023). Based on this study of findings, currently, Ashwagandha contains quercetin, gallic acid coumaric acid, ferulic, syringic acid, naringenin, kaempferol, hesperetin, chlorogenic acid and caffeic acid.

Research by Dinsdale et al. (2021) suggested that quercetin's interaction with luteinizing hormone (LH) may lead to the inhibition of androgen production. Quercetin was observed to lower LH and testosterone levels, which are involved in the control of steroidogenesis. Similarly, gallic acid has been reported in prior investigations to modulate the LH surge and normalize follicle-stimulating hormone (FSH) irregularities in studies involving PCOS animal models (Mazloom et al., 2017). Furthermore, p-coumaric acid and ferulic acid, among other phytochemicals, appear to play a role in the management of this condition through their capacity to diminish oxidative stress and reduce inflammation (Zeng et al., 2022). In this context, the administration of syringic acid resulted in decreased levels of LH, anti-mullerian hormone (AMH), estradiol (E2), and FSH, as well as a reduction in ovarian follicles. Syringic acid also down regulated cytokines, inflammatory mediators, and caspase-3 parameters, leading to a notable decrease in ovarian damage by alleviating oxidative stress and inflammatory responses. Moreover, Rashid et al. (2023) found that naringenin treatment in animal models of PCOS enhanced ovulation potential and reduced both cystic follicles and androgen levels. As well as kaempferol has progestogenic activity in vivo and act uniquely (Bergsten et al., 2023). Moreover, Azimova et al. (2018) pointed out that hesperetin restored the morphology of ovary and ameliorated hormonal changes. Chlorogenic acid is bioactive component of ashwagandha. Chlorogenic acid (CGA) may have a protective effect against Polycystic Ovary Syndrome (PCOS) by ameliorating follicular development as well as hormonal and biochemical disorders. It is possible that chlorogenic acid may improve follicular development and hormonal and biochemical imbalances in rats with PCOS. CGA can significantly decrease the serum levels of luteinizing hormone, estrogen, and testosterone in PCOS rats (Abedpour et al., 2022). Chlorogenic acid may enhance some lipid profiles in women with PCOS by decreasing cholesterol and triglyceride serum levels (Meshkani et al., 2022).

Table 6 revealed that administration with letrozole induced a significant ($P < 0.05$) reduction in serum concentrations of CAT and increased in serum level of lipid peroxide MDA. The results indicated that diets supplemented with *Withania somnifera* L. roots powder at the three tested levels induced a significant ($P < 0.05$) rise in concentrations of CAT levels and decrease MDA comparable with positive controls. A substantial disparity existed among the three tested levels of *Withania somnifera* L. roots powder *while* there was non-significant difference in serum level of MDA between diets supplemented with 7.5. *Withania somnifera* L. roots powder and control negative group. A better improvement in serum levels of enzymes was discovered at feeding PCOS rats with 7.5%. *Withania somnifera* L. roots powder. Therefore, the supplementation with *Withania somnifera* L. has been shown to improve antioxidant levels in female rats with PCOS, reflecting its potential beneficial effects in reducing oxidative stress and related pathologies. These findings align with those of Sabiba et al. (2013) who indicated that Ashwagandha could restore antioxidant levels, including superoxide dismutase, catalase, reduced glutathione, and Glutathione-S-transferase, in rats treated with paracetamol. Furthermore, Singh and Sharma

TABLE 5. Effect of diet supplemented with *Withania somnifera* L. roots powder on serum levels of TSH, LH, FSH and progesterone in Female Rats with PCOS

Parameter		Parameter as Mean ± SE			
Groups		TSH (µIU/mL)	LH (lu/mL)	FSH (lu/mL)	Progesterone (ng/mL)
Negative group		2.28±0.18 [d]	3.84±0.11 [e]	12.88±0.17 [a]	34.74±0.62 [a]
Positive group		9.19±0.24 [a]	15.88±0.32 [a]	3.301±0.16 [e]	14.92±0.52 [d]
Dried roots	2.5%	6.67±0.20 [b]	10.81±0.20 [b]	4.95±0.22 [d]	15.65±0.43 [d]
	5%	4.18±0.09 [c]	7.95±0.29 [c]	8.06±0.29 [c]	19.32±0.68 [c]
	7.5%	[d] 2.10±0.11	5.05±0.07 [d]	10.13±0.16 [b]	27.38±0.59 [b]

* Values within the same column that have various letters are significant at P≤0.05 level.
SE: Stander Error.
PCOS: Polycystic Ovary Syndrome; TSH: Thyroid-stimulating hormone LH: Luteinizing Hormone; FSH: Follicle Stimulating Hormone.

(2018) conclude that Ashwagandha elevated the levels of superoxide dismutase, catalase, and glutathione peroxidase, all of which are naturally occurring antioxidant enzymes. In addition, Tiwari et al. (2021) revealed that supplementation of *Withania somnifera* L. significant improvements MDA level. These results agree with studies of prior animal research, in vitro and in vivo evaluations, and clinical investigations including healthy athletic subjects by Pal et al. (2017). Similar results were obtained by Lopresti and Smith (2021) who pointed out useful effects of *Withania somnifera* L. in individuals with conditions linked to oxidative stress. Also, Vasavan et al. (2021), mentioned that demonstration of WS at dose 100, 200 and 400 mg/kg/bw completely counteracts ND-induced oxidative stress as indicated by decreased serum MDA level and enhancing antioxidant concentrations. The present study is completely in agreement with Bharani et al. (2024) who observed that ashwagandha root extract induced a significant increase in the level of catalase and reduce in the level of MDH. Elevated catalase activity implies that the Ashwagandha Root Extract (ARE) might play a role in mitigating oxidative damage by facilitating the decomposition of hydrogen peroxide. Conversely, malondialdehyde (MDA) serves as an indicator of lipid peroxidation and oxidative stress. The notable decline in MDA concentrations suggests that ARE could be effective in lessening oxidative damage to lipids. This observation aligns with findings from various investigations that have indicated ashwagandha›s capacity to diminish lipid peroxidation through the neutralization of free radicals and the enhancement of antioxidant mechanisms (Khalil et al., 2023). The observed positive changes in oxidative stress markers could be attributed to the recognized antioxidant properties of withaferin-A, a prominent withanolide present in ashwagandha extracts (Tekula et al., 2018; Tiruveedi et al., 2018). The preceding studies indicated high levels of Oxidative stress and decreases antioxidant capacity in women with PCOS (Sabuncu et al., 2001). Sadoughi et al. (2017) revealed that the production of reactive oxygen species (ROS) is considered as one of the accounting mechanisms behind infertility disturbance which led to the situation called oxidative stress. Mitochondria is the energy-producing organelles and the major source of ROS. In PCOS, mitochondrial dysfunction resulted in an elevated ROS production (Yang et al., 2019). Oxidative stress (OS) plays a significant role in the pathophysiology of various gynecological disorders, notably polycystic ovary syndrome (PCOS) (Pizzino et al., 2017). Gao et al. (2023) and Negm & Aboraya (2023) diminished antioxidant defenses in some women with PCOS can cause an imbalance between reactive oxygen species (ROS) production and antioxidant capacity, leading to increased oxidative stress (OS). Research has indicated the presence of safe, natural, and potent antioxidant compounds in Ashwagandha and other members of the Solanaceae family. Using high-performance liquid chromatography (HPLC), *Withania somnifera* L. has been shown

to contain high levels of phenolics, flavonoids, and exhibit strong antioxidant activity (Alam et al., 2011). It is thought that these constituents enable *Withania somnifera L.* to counteract the production of reactive oxygen species (ROS) and mitigate the effects of lipid peroxidation and cellular damage. Reactive oxygen species (ROS) can interact with cell membranes, leading to the formation of toxic lipid peroxidation products like malondialdehyde (MDA) (Harikrishnan et al., 2008). Furthermore, Ashwagandha glycol-withanolides have demonstrated a tendency to normalize elevated superoxide dismutase (SOD) and lipid peroxide (LPO) activity, as well as enhance catalase (CAT) and glutathione peroxidase (GPX) activity (Sengupta et al., 2018). The results obtained are in harmony with those researchers. Caffeic acid significantly reduces the production of intracellular reactive oxygen species (ROS), thereby protecting KGN cells from oxidative stress. In rats with DHEA-induced PCOS, it improved irregularities in estrous cycles, fasting blood glucose levels, liver function, and lipid profiles. Furthermore, caffeic acid lessened hyperandrogenism, increased the expression of steroidogenesis enzymes, and altered the expression of proteins involved in apoptosis. These findings suggest that caffeic acid shows potential for decreasing oxidative stress-related damage and alleviating PCOS complications by modulating ER stress (Chiang et al., 2023).

Histopathological examination of ovaries

Microscopically, ovaries of rats from normal control group appeared histologically normal and the ovarian cortex containing follicles in various stages of development (Photos 1, 2 and 3). In contrariwise, ovaries from the untreated PCOS rats (positive group) exhibited ovarian cyst with thin layer of granulosa cells (Photo. 4 & 5) and apoptosis of cells of corpus leutium (Photo. 6). However, ovaries of PCOS rats fed on the diet supplemented with *Withania somnifera* L. roots powder 2.5% showed multiple corpora lutea (Photo. 7) and ovarian cyst with thin layer of granulosa cells (Photo. 8). On the other hand, some examined sections of PCOS rats fed on the diet supplemented with *Withania somnifera* L. roots powder 5% revealed normal follicles with restoration of granulosa cell thickness and normal corpus leutium (Photo. 9) as well as multiple corpora lutea (Photo. 10), whereas other sections showed ovarian cyst with thin layer of granulosa cells (Photo. 11). Furthermore, ovaries of PCOS rats fed on the diet supplemented with *Withania somnifera* L. roots powder 7.5% exhibited restoration of granulosa cell thickness (Photo. 12 and 13) and vacuolated corpus leutium (Photo. 14).

Conclusion

Withania somnifera L. commonly known as Ashwagandha, is a herb of considerable medicinal significance. Based on this study of findings, it could be concluded that the root extract of Ashwagandha exhibits notable hormone-regulating effects, hypolipidemic activities, and antioxidant properties. The observed beneficial effects are likely attributable to its diverse array of active phytochemicals. Furthermore, it is recommended that ashwagandha tea be considered as a complementary natural remedy to enhance physiological functions, particularly in females diagnosed with polycystic ovary syndrome. Future investigations are warranted to further elucidate its mechanisms of action and to validate its therapeutic applications in clinical practice.

TABLE 6. Effect of diet supplemented with *Withania somnifera* L. roots powder on Serum Levels of CAT and MDA in Female Rats with PCOS.

Parameter		Parameter as Mean ± SE	
	Groups	MDA (mmol/mL)	
CAT (U/l)			
Negative group		83.31±0.37 [a]	20.31±0.62 [d]
Positive group		46.23±0.91 [e]	43.16±0.92 [a]
	2.5%	61.82±0.94 [d]	35.69±0.59 [b]
Dried roots	5%	69.97±0.57 [c]	27.70±0.24 [c]
	7.5%	78.04±0.56 [b]	20.88±0.52 [d]

* Values within the same column that have various letters are significant at P≤0.05 level.
SE: Stander Error.
PCOS: Polycystic Ovary Syndrome. MAD: Malondialdehyde; CAT: Catalase.

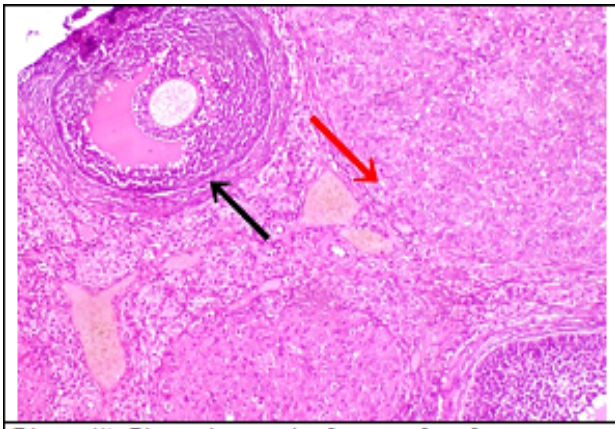

Photo. (1): Photomicrograph of ovary of rat from -ve exhibiting the typical histological architecture of the ovarian cortex, with numerous follicles at diverse developmental stages. Note graafian follicle (black arrow) and corpus luteum (red arrow) (H & E, X 100).

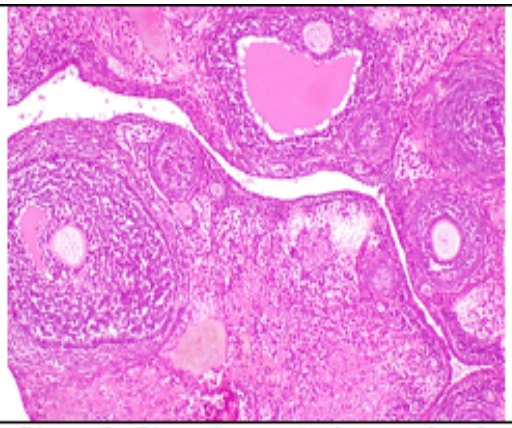

Photo. (2): Photomicrograph of ovary of rat from -ve exhibiting the typical histological architecture of the ovarian cortex, with numerous follicles at diverse developmental stages. (H & E, X 100).

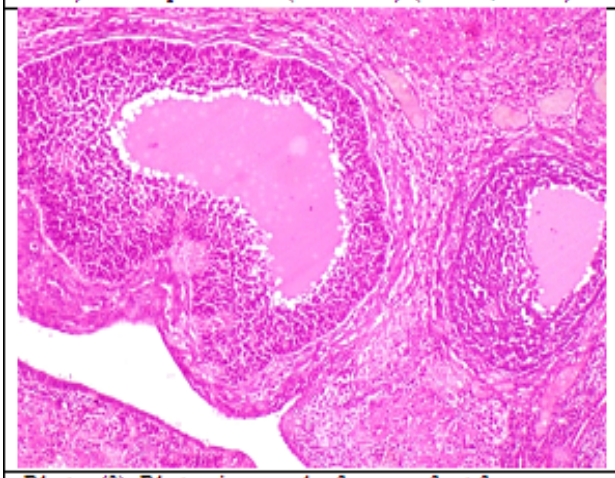

Photo. (3): Photomicrograph of ovary of rat from -ve group exhibiting the typical histological architecture of the ovarian cortex, with numerous follicles at diverse developmental stages. (H & E, X 100).

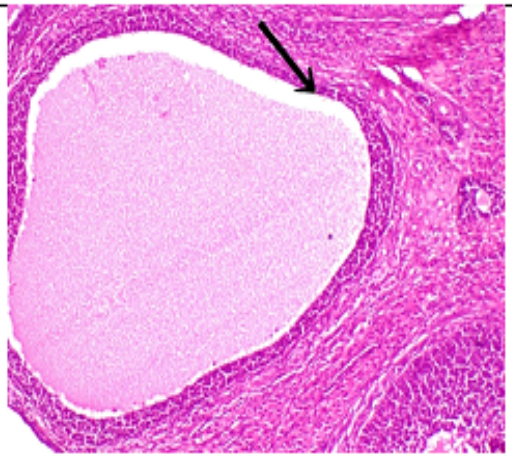

Photo. (4): Photomicrograph of ovary of rat from +ve group showing ovarian cyst with thin layer of granulosa cells (black arrow) (H & E, X 100).

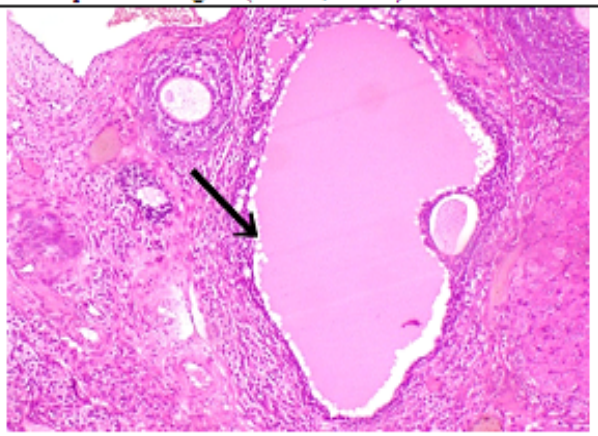

Photo. (5): Photomicrograph of ovary of rat from +ve

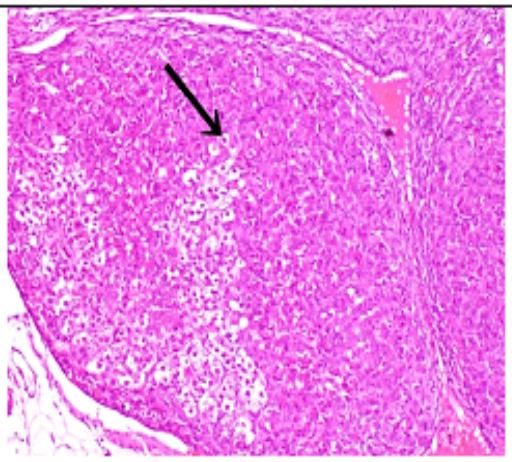

Photo. (6): Photomicrograph of ovary of rat from

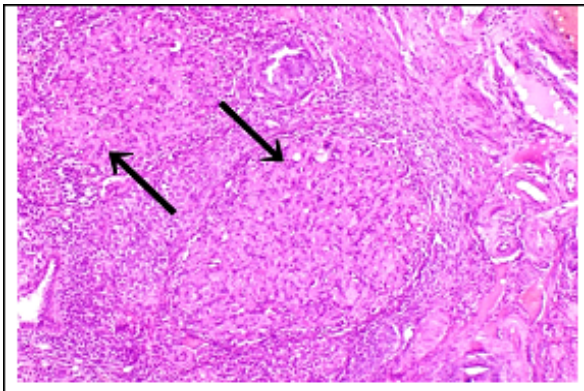

Photo. (7): Photomicrograph of ovary of PCOS rats fed on the diet supplemented with *Withania somnifera L.* roots powder 2.5% showing multiple corpora lutea (black arrow) (H & E, X 100).

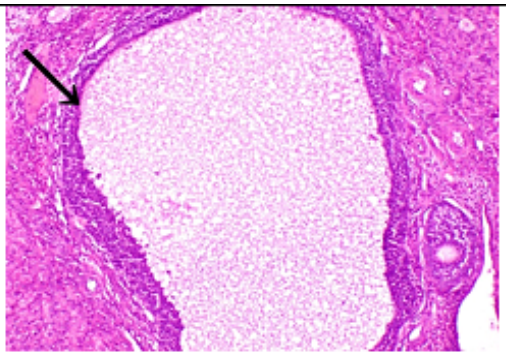

Photo. (8): Photomicrograph of ovary of PCOS rats fed on the diet supplemented with *Withania somnifera L.* roots powder 2.5% showing ovarian cyst with thin layer of granulosa cells (black arrow) (H & E, X 100).

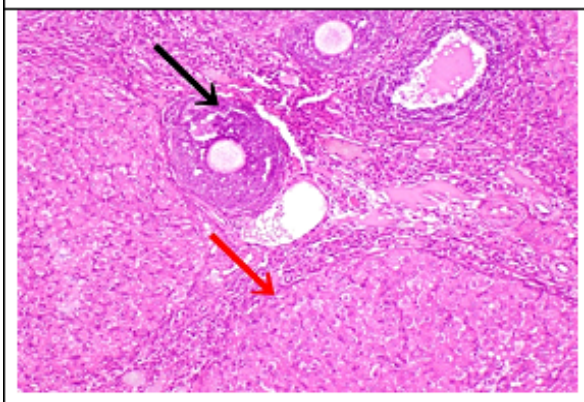

Photo. (9): Photomicrograph of ovary of PCOS rats fed on the diet supplemented with *Withania somnifera L.* roots powder 5% showing normal follicles with restoration of granulosa cell thickness (black arrow) and normal corpus leutium (red arrow) (H & E, X 100).

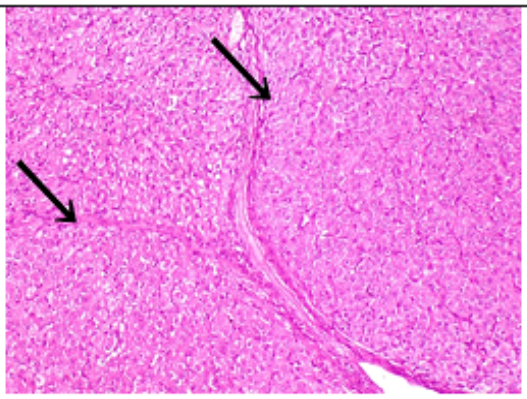

Photo. (10): Photomicrograph of ovary of PCOS rats fed on the diet supplemented with *Withania somnifera L.* roots powder 5% showing multiple corpora lutea (black arrow) (H & E, X 100).

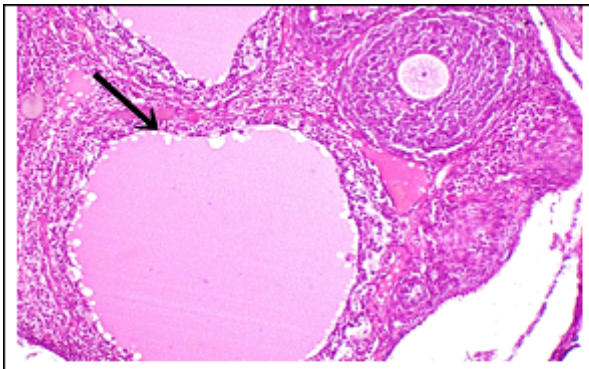

Photo. (11): Photomicrograph of ovary of PCOS rats fed on the diet supplemented with *Withania somnifera L.* roots powder 5% showing ovarian cyst with thin layer of granulosa cells (black arrow) (H & E, X 100).

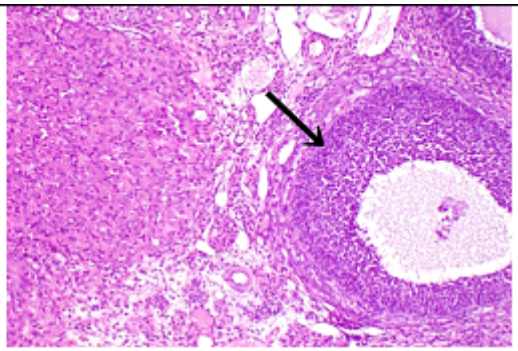

Photo. (12): Photomicrograph of ovary of PCOS rats fed on the diet supplemented with *Withania somnifera L.* roots powder 7.5% showing restoration of granulosa cell thickness (black arrow) (H & E, X 100).

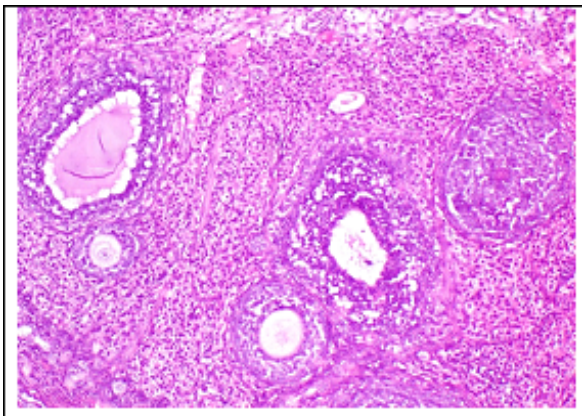

Photo. (13): Photomicrograph of ovary PCOS rats fed on the diet supplemented with *Withania somnifera L.* roots powder 7.5% showing restoration of granulosa cell thickness (H & E, X 100).

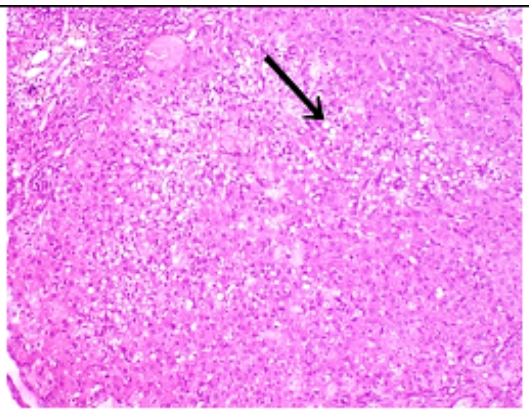

Photo. (14): Photomicrograph of ovary PCOS rats fed on the diet supplemented with *Withania somnifera L.* roots powder 7.5%showing vacuolated corpus leutium (black arrow) (H & E, X 100).

Authors' Contribution

All authors contributed equally to every aspect of this study, including designing, developing, and reviewing the research protocol. They were actively involved in conducting and monitoring the experiments, collecting and analyzing data, organizing and interpreting the findings, and ensuring the accuracy of the results and statistical analyses. Additionally, they participated in gathering relevant conceptual information, drafting the manuscript, critically reviewing and refining its content, and approving the final version for publication.

References

Abbott, D. H., Barnett, D. K., Bruns, C. M. and Dumesic, D. A. (2005) Andro gen excess fetal programming of female reproduction: a developmental aetiology for polycystic ovary syndrome? *Human Reproduction Update*, **11**(4), 357-74. https://doi: 10.1093/humupd/dmi013.

Abd Elmeged, L.S., Aly-Aldin, M. M., Mashnafi, S. and Mahzari, A. (2024) Potential therapeutic effects of ashwagandha (*Withania somnifera*) plant on biochemical changes in diabetic rats. *African Journal of Biomedical Research,* **27**(4s), 3595 – 3602. https://doi.org/10.53555/AJBR.v27i4S.3885

Abdel-Wahhab, K. G., Mourad, H. H., Mannaa, F. A., Morsy, F. A., Hassan, L. K. and Taher, R. F. (2019) Role of ashwagandha methanolic extract in the regulation of thyroid profile in hypothyroidism modeled rats. *Molecular Biology Reports,* **46**, 3637–3649. https://doi.org/10.1007/s11033-019-04721-x

Abedpour, N., Javanmard, M. Z., Karimipour, M. and Liqvan, A. P. (2022) Effect of chlorogenic acid on follicular development, hormonal status and biomarkers of oxidative stress in rats with polycystic ovary syndrome. *Veterinary Research Forum,* **13**(4), 513–520. https://doi: 10.30466/vrf.2021.530203.3179

Ahmadi, S. A., Boroumand, M., Gohari-Moghaddam, K.,Tajik, P., Dibaj, S. (2008) The impact of low serum triglyceride on LDL-cholesterol estimation. *Archives of Iranian Medicine***, 11**(3), 318–321. https://doi.org/08113/aim.0014

Ajgaonkar, A., Jain, M. and Debnath, K. (2022) Efficacy and Safety of Ashwagandha (*Withania somnifera*) Root Extract for Improvement of Sexual Health in Healthy Women: A Prospective, Randomized, Placebo-Controlled Study. *Cureus* **14(**10), e30787. https://doi 10.7759/cureus.30787.

Alam, N., Hossain, M., Khalil, M. I., Moniruzzaman, M., Sulaiman, S. A. and Gan, S. H. (2011) High catechin concentrations detected in *Withania somnifera* (ashwagandha) by high performance liquid chromatography analysis. *BMC Complementary Medicine and Therapies,* **19**, 11:65. https://doi: 10.1186/1472-6882-11-65

Ali, H. M. (2021) Ashwagandha *(Withania somnifera*) and Their Effects on the Reproductive Hormones of Male Rats. *Home Economics Journal*, **37** (2), 1-22. https://doi: 10.21608/jhe.2021.169285

Al-Nuaimi, Z. and Al-Baniwes, A. J. (2022) Evaluation the Protective Effect of *Withania somnifera* Extract

on the Level of Sex Hormone in Morphine Addicted Female Rats. *Journal of Medical Sciences*, **12**; 10(A),1330-1333. https://doi.org/10.3889/oamjms.2022.8303

Anwer, T., Sharma, M., Khan, G., Alam, M. F., Alam, N., Ali, M. S. and Alam M. S. (2017) Preventive role of *Withania somnifera* on hyperlipidemia and cardiac oxidative stress in streptozotocin induced type 2 diabetic rats. *Tropical Journal of Pharmaceutical Research*, **16** (1), 119-125. https://doi: 10.4314/tjpr.v16i1.15

Azimova, B. J., Peng, T., Zhang, G. and Wang, F. (2018) Effect of Hesperetin on Morphological Parameters of DHEA-induced PCOS Female Mouse Model. *Annals of Clinical Trials and Vaccines Research,* **1**(2), 21-22.

Azziz, R., Nestler, J. E. and Dewailly, D. (2006) Androgen Excessdisorders in Women: Polycystic Ovary Syndrome and Other Disorders; Humana Press: Totowa, NJ, USA; pp. 184–196.24

Balkrishna, A., Nain, P., Chauhan, A., Sharma, N., Gupta, A., Ranjan, R. and Varshney, A. (2020) Super Critical Fluid Extracted Fatty Acids from *Withania somnifera* Seeds Repair Psoriasis-Like Skin Lesions and Attenuate Pro-Inflammatory Cytokines (TNF-and IL-6) Release. *Biomolecules*, **10** (2), 185. https://doi: 10.3390/biom10020185.

Bancroft, J. and Stevens, A. (1996) Theory and Practice of Histological Techniques. 4th edition, *Churchill Livingstone*, New York Vol.**3** No 4A.

Bashir, A., Nabi, M., Tabassum, N., Afzal, S. and Ayoub, M. (2023) An updated review on phytochemistry and molecular targets of *Withania somnifera* (L.) Dunal CAshwagandha). *Frontiers in Pharmacology*, **14**, 1049334. https://doi: 10.3389/fphar.2023.1049334

Bergsten, T. M., Li, K., Lantvit, D. D., Murphy, B. T. and Burdette, J. E. (2023) Kaempferol, a Phytoprogestin, Induces a Subset of Progesterone-Regulated Genes in the Uterus. *Nutrients,* **15**(6), 1407. https://doi: 10.3390/nu15061407

Bharani, K. K., Devarasetti, A. K., Carey, L., Khurana, A., Kollipaka, R., Hanuman, D. D. V. , Chetla, V. S. and Banothu, A. B. (2024) Effects of ashwagandha (*Withania somnifera*) root extract on aging-related changes in healthy geriatric dogs: A randomized, double-blinded placebo-controlled study. . *Veterinary Medicine and Science*, **10**(5), e1556.https://doi: 10.1002/vms3.1556

Bhattacharya, S. K. and Muruganandam, A. V. (2003) Adaptogenic activity of *Withania somnifera:* An experimental study using a rat model of chronic stress. *Pharmacology Biochemistry and Behavior* **75** (3), 547-555. https://doi.org/10.1016/S0091-3057(03)00110-2

Bhowmich, S. K., Dasari, G., Leavens, K. L. and Rettig, K.R. (2007) The prevalence of elevated serum thyroid-stimulating horomone inchildhood/adolescent obesity and of autoimmune thyroid diseases in a subgroup. *Journal of the National Medical Association,* **99**(7), 773–776.

Champman, D.G., Castilla R. and Compel J. A. (1959) Evaluation of protein in foods. I-A: method for determination of protein efficiency ratio. *Canadian Journal of Biochemistry and Physiology*, **37**(5), 679-686. https://doi:10.1139/O59-074

Chengappa, K. N. R., Brar, J. S., Gannon, J. M., and Schlicht, P. J. (2018) Adjunctive use of a standardized extract of Withania somnifera (ashwagandha) to treat symptom exacerbation in schizophrenia: a randomized, double-blind, placebo-controlled study. *The Journal of Clinical Psychiatry,* **79**(5), 17m11826. https://doi: 10.4088/JCP.17m11826.

Chiang, Y., Lin, I., Huang, K., Chen, H., Ali, H., Huang, Y. and Hsia, S. (2023) Caffeic acid's role in mitigating polycystic ovary syndrome by countering apoptosis and ER stress triggered by oxidative stress. *Biomedicine and Pharmacotherapy,* **166(**1), 115327. https://doi.org/10.1016/j.biopha.2023.115327

Diamanti-Kandarakis, E., Christakou, C. and Marinakis, E. (2012) Phenotypes and environmental factors: their influence in PCOS. *Current Pharmaceutical Design,* **18**(3), 270-282. https://doi**:** 10.2174/138161212799040457

Ding, D. C., Chen, W., Wang, J. H. and Lin, S. Z. (2018) Association between polycystic ovarian syndrome and endometrial, ovarian, and breast cancer: A population-based cohort study in Taiwan. *Medicine (Baltimore),* **97**(39), e12608. https:// doi: 10.1097/MD.0000000000012608.

Dinsdale, N., Nepomnaschy, P. and Crespi, B. (2021) The evolutionary biology of endometriosis. *Evolution, Medicine, and Public Health,* **9** (1), 174–191. https://doi.org/10.1093/emph/eoab008

Du, D. and Li, X. (2013) The relationship between thyroiditis and polycystic ovary syndrome: a meta-analysis. *International Journal of Clinical and*

Experimental Medicine, **6**(10), 880–889.

El-Houfi, F. B. B. (2015) Extraction and identification of phenolic compounds from agro industrial wastes and their antioxidant efficiency on sunflower oil oxidative stability. *Ph.D. Thesis,* Department of Food Science Technology, Faculty of Agricalture, Damanhur University, Egypt.

El-Kholie, E., Ragab, S. and Abdallah, N. (2024) Improvement of Male Rats Fertility and Immunity using Ashwagandha (*Withania somnifera, L*) Roots. *Journal of Home Economics, Menoufia University*, **34** (3), 129-143. https://doi. 10.21608/MKAS.2024.282934.1309

Epidi, J. O., Izah, S. C., Ohimain, E. I. and Epidi, T. T. (2016) Phytochemical, antibacterial and synergistic potency of tissues of *Vitex grandifolia*. *Biotechnologicl Research*, **2**(2), 69-76.

Escobar-Morreale, H. F. (2018) Polycystic ovary syndrome: definition, aetiology, diagnosis and treatment. *Nature Reviews Endocrinology*, **14** (5), 270-284. https://doi.org/10.1038/nrendo.2018.24

Fahim, M. S., Fahim, Z., Harman, J. M., Clevenger, T. E., Mullins, W. and Hafez, E. S. (1982) Effect of Panax ginseng on testosterone level and prostate in male rats. *Archives Andrology,* **8**(4), 261-263. https:// doi: 10.3109/01485018208990207.

Fossati, P. and Prencipe, L. (1982) Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clinical Chemistry*, **28**(10), 2077–2080.

Gannon, J. M., Brar, J., Rai, A. and Chengappa, K. N. R. (2019) Effects of a standardized extract of *Withania somnifera* (Ashwagandha) on depression and anxiety symptoms in persons with schizophrenia participating in a randomized, placebo-controlled clinical trial. *Annals of Clinical Psychiatry*, **31**(2), 123–129.

Gao, Y., Zou, Y., Wu, G. and Zheng, L. (2023) Oxidative Stress and Mitochondrial Dysfunction of Granulosa Cells in Polycystic Ovarian Syndrome. *Frontiers Medicine,* **10**:1193749. https://doi.org/10.3389/fmed.2023.1193749

Glintborg, D., Rubin, K. H. Nybo, M., Abrahamsen, B. and Andersen, M. (2019) Increased risk of thyroid disease in Danish women with polycystic ovary syndrome: a cohort study. *Endocrine Connections,* **8**(10), 1405–1415. https:// doi: 10.1530/EC-19-0377

González-Palma, I., Escalona-Buendía, H. B., Ponce-Alquicira, E., Téllez-Téllez, M., Gupta, V. K., Díaz-Godínez, G. and Soriano-Santos, J. (2016) Evaluation of the Antioxidant Activity of Aqueous and Methanol Extracts of Pleurotus ostreatus in Different Growth Stages. *Frontiers Microbiology,* **7**:1099. https:// doi: 10.3389/fmicb.2016.01099

Harikrishnan, B., Subramanian, P. and Subash, S. (2008) Effect of Withania somnifera root powder on the levels of circulatory lipid peroxi dation and liver marker enzymes in chronic hyperammonemia. *Journal of Chemistry***, 5**(4), 872–7. https:// doi: 10.1155/2008/589394

Ibrahim, N., Diab, M., Hassan, A., Anwar, H., Ragab, W., Morsi, E., Diab, M., Boushra, A., and Sadek, N. (2023) The Ameliorating Effect of Ashwagandha (Withania somnifera) Extract on Hippocampus and Growth Plate Changes Associated with Propylthiouracil Induced Hypothyroidism in Juvenile Rats: Ashwagandha on Hippocampus and Growth Plate Changes Associated with hypothyroid rats. *Al-Kindy College Medical Journal*, **19**(1), 30–41. https://doi.org/10.47723/kcmj.v19i1.920

Kafali, H., Iriadam, M., Ozardalı, I. and Demir, N. (2004) Letrozole induced polycystic ovaries in the rat: a new model for cystic ovarian disease. *Archives Medical Research*, **35**(2), 103-8. https:// doi: 10.1016/j.arcmed.2003.10.005.

Karateke, A., Dokuyucu, R., Dogan, H., Ozgur, T., Tas, Z. A. and Tutuk, O. (2018) Investigation of therapeutic effects of erdosteine on polycystic ovary syndrome in a rat model. *Medical Principles Practice,* **27**(6), 515-522. https:// doi: 10.1159/000494300

Khalil, H. M. A., Khalil, I. A., Al-Mokaddem, A. K., Hassan, M., El-Shiekh, R. A., Eliwa, H. A., Tawfek, A. M. and El-Maadawy, W. H. (2023) Ashwagandha-loaded nanocapsules improved the behavioral alterations, and blocked MAPK and induced Nrf2 signaling pathways in a hepatic encephalopathy rat model. *Drug Delivery and Translational Research,* **13**(1), 252–274. https:// doi: 10.1007/s13346-022-01181-y

Kumar, S., Padubidri, V. G and Daftary, S. N. (2018) Howkins & Bourne, Shaw's Textbook of Gynecology, 17edition-EBOOK. Elsevier India. 128-140.

Latheef, S. K., Dhama, K., Samad, H. A., Wani, M. Y., Kumar, M. A., Palanivelu, M., Malik, Y. S., Singh, S. D. and Singh, R. (2017) Immunomodulatory and prophylactic efficacy of herbal extracts against experimentally induced chicken infectious anaemia in chicks: Assessing the viral load and cell mediated immunity. *Virus Disease,* **28**(1), 115–120. https:// doi: 10.1007/s13337-016-0355-3

Lavaee, F., Ranjbar, Z., Jalalian M. and Amiri M. (2021) Comparison of the sex hormones' serum level in women with recurrent aphthous stomatitis

and healthy population: a cross-sectional study. *BMC oral health,* **21**(1), 1-6. https:// doi: 10.1186/s12903-021-01812-9

Lee, D., Yu, J. S., Ha, J. W., Lee, S. R., Lee, B.S., Kim, J. C., Kim, J. K., Kang, K. S. and Kim, K. H. (2022) Antitumor Potential of Withanolide Glycosides from Ashwagandha (*Withania somnifera*) on Apoptosis of Human Hepatocellular Carcinoma Cells and Tube Formation in HumanUmbilical Vein Endothelial Cells. *Antioxidants,* **11**(9), 1761. https://doi:10.3390/antiox11091761

Lopes-Virella, M. F., Stone, S., Ellis, S. and Collwell, J. A. (1977) Cholesterol determination in high-density lipoproteins separated by three different methods. *Clinical Chemistry,* **23**(5), 882-886.

Lopresti, A. L., Smith, S. J., Malvi, H., Kodgule, R. (2019) An investigation into the stress-relieving and pharmacological actions of an ashwagandha (*Withania somnifera*) extract: A randomized, double-blind, placebo-controlled study. *Medicine,* **98**(37), e17186.https:// doi: 10.1097/MD.0000000000017186.

Lopresti, A. L. and Smith, S. J. (2021) Ashwagandha (Withania somnifera) for the treatment and enhancement of mental and physical conditions: a systematic review of human trials. *Journal of Herbal Medicine,* **28,** 100434. https://doi.org/10.1016/j.hermed.2021.100434

Lubrano, V., Pingitore, A., Traghella, I., Storti, S., Parri, S., Berti, S., Ndreu, R., Andrenelli, A., Palmieri, C., Iervasi, G., Mastorci , F. and Vassalle , C. (2019) Emerging Biomarkers of Oxidative Stress in Acute and Stable Coronary Artery Disease: Levels and Determinants. *Antioxidants,* **8**(5), 115. https:// doi: 10.3390/antiox8050115

Malik, V., Kumar, V., Kaul, S. C., Wadhwa, R., Sundar, D. (2021) Potential of Withaferin-A, Withanone and Caffeic Acid Phenethyl ester as ATP-competitive inhibitors of BRAF: A bioinformatics study. *Current Research in Structural Biology,* **3**, 301–311. https://doi.org/10.1016/j.crstbi.2021.11.004

Mazloom, B. F., Edalatmanesh, M. A. and E. Hosseini, E. (2017) The effect of gallic acid on pituitary-ovary Axis and oxidative stress in rat model of polycystic ovary syndrome. *Report of Health Care,* **3**(1), 41–47.

Megahd, H. E. and Gabal, A. M.S. (2021) Evaluation of Matcha (Camellia sinensis) and Ashwagandha (Withania somnifera) Efficacy Against Utero-Ovarian Injury in Rats, Pakistan Journal of Biological Sciences, **24** (12), 1256-1268. https:// doi: 10.3923/pjbs.2021.1256.1268

Meshkani, M., Saedisomeolia, A., Yekaninejad ,M., Mousavi, S. A., Ildarabadi, A. and Vahid-Dastjerdi, M. (2022) The Effect of Green Coffee Supplementation on Lipid Profile, Glycemic Indices, Inflammatory Biomarkers and Anthropometric Indices in Iranian Women With Polycystic Ovary Syndrome: A Randomized Clinical Trial. *Clinical Nutrition Research*, **11**(4), 241-254. 11(4), 241-254. https://doi:10.7762/cnr.2022.11.4.241

Mikulska, P., Malinowska, M., Ignacyk, M., Szustowski, P., Nowak, J., Pesta, K., Szeląg, M., Szklanny, D., Judasz, E., Kaczmarek, G., Ejiohuo, O. P., Paczkowska-Walendowska, M., Gościniak, A. and Cielecka-Piontek, J. (2023) Ashwagandha *(Withania somnifera)*—Current Research on the Health-Promoting Activities: A Narrative Review. *Pharmaceutics,* **15**(4), 1057. https://doi: 10.3390/pharmaceutics15041057

Moradi, N., Bidgoli, S. A. and Chaichian, S. (2021) Ovarian cysts disappear after 14-day oral regimen of Korean red ginseng extract in letrozole-induced polycystic ovarian syndrome. *Obstetrics Gynecology Science*, **64** (3), 274-283. https://doi: 10.5468/ogs.20094

Ndefo, U.A., Eaton, A. and Green, M.R. (2013) Polycystic ovary syndrome: A review of treatment options with a focus on pharmacological approaches. *Pharmacy and Therapeutics,* **38** (6), 336–355.

Ndeingang, E. C., Defo Deeh, P. B., Watcho, P. and Kamanyi, A. (2019) Phyllanthus muellerianus (Euphorbiaceae) restores ovarian functions in letrozole-induced polycystic ovarian syndrome in rats. *Evidence-Based Complementary and Alternative Medicine*, **2**, 1-16. https://doi.org/10.1155/2019/2965821

Negm, S. and Aboraya, A.O. (2023) Therapeutic Effects of Milk thistle Seeds (Silybum marianum) and Red Ginseng roots on Polycystic Ovary Syndrome Induced by Letrozole in Female Rats. *Research Journal of Specific Education*, **75**, 199-234. https://doi: 10.21608/mbse.2023.188764.1298

Nile, S. H., Liang, Y., Wang, Z., Zheng, J., Sun, C., Nile, A., Patel, G. and Kai, G. (2021) Chemical composition, cytotoxic and pro-inflammatory enzyme inhibitory properties of Withania somnifera (L.) Dunal root extracts. *South African Journal of Botany*, **151**(3), 46–53. https://doi:10.1016/j.

sajb.2021.11.003

Norman, R. J. and Teede, H. J. (2018) A new evidence-based guideline for assessment and management of polycystic ovary syndrome. *Medical Journal of Australia,* **209**(7), 299-300. https://doi: 10.5694/mja18.00635

NRC, National Research Council (1996) Guide for the Care and Use of Laboratory Animals. Washington: National Academy Press.

Ohkawa, H., Ohishi, N. and Yagi, K. (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry,* **95**(2), 351-8. https://doi: 10.1016/0003-2697(79)90738-3.

Pal, A., Kumar, K. H., Bhushan, B. and Saharan, V. (2017) Ashwagandha root extract inhibits acetylcholine esterase, protein modification and ameliorates H2O2 -induced oxidative stress in rat lymphocytes. *Pharmacognosy Journal,* **9**(3), 302-309. https://doi: 10.5530/pj.2017.3.52

Patil, D., Gautam, M., Mishra, S., Karupothula, S., Gairola, S., Jadhav, S., Pawar, S. and Patwardhan, B. (2013) Determination of withaferin A and withanolide A in mice plasma using high-performance liquid chromatography-tandem mass spectrometry: Application to pharmacokinetics after oral administration of Withania somnifera aqueous extract. *Journal of Pharmaceutical and Biomedical Analysis*, **80**, 203–212. https://doi:10.1016/j.jpba.2013.03.001.

Penzias, A., Bendikson, K., Butts, S., Coutifaris, C., Falcone, T., Fossum, G., Gitlin, S., Gracia, C., Hansen, K., La Barbera, A., Mersereau, J., Odem, R., Paulson, R., Pfeifer, S., Pisarska, M., Rebar, R., Reindollar, R., Rosen, M., Sandlow, J. and Vernon, M. (2017) Role of metformin for ovulation induction in infertile patients with polycystic ovary syndrome (PCOS): a guideline. *Fertility and Sterility,* **108** (3), 426-441. https://doi.org/10.1016/j.fertnstert.2017.06.026

Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito, F., Altavilla, D. and Bitto A. (2017) Oxidative Stress: Harms and Benefits for Human Health. *Oxidative Medicine and Cellular Longevity*, **2017**(1), 1–13. https://doi.org/10.1155/2017/8416763

Rajeswara Rao, B. R., Rajput, D. K., Nagaraju, G. and Adinarayana, G. (2012) Opportunities and challenges in the cultivation of ashwagandha {Withania somnifera (L.) Dunal}. *Journal of Pharmacognosy,* **3** (2), 88-91.

Ramya, S., Poornima, P., Jananisri, A., Geofferina, I. P., Bavyataa, V., Divya. M., Priyanga, P., Vadivukarasi, J., Sujitha, S., Elamathi, S. A. and Balamuralikrishnan, B. (2023) Role of hormones and the potential impact of multiple stresses on infertility. *Stresses*, **3**(2), 454-474; https://doi.org/10.3390/stresses3020033

Rashid, R., Tripathi, R., Singh, A., Sarkar, S., Kawale, A., Bader, G. N., Gupta, S., Gupta, R. K. and Jha, R. K. (2023) Naringenin improves ovarian health by reducing the serum androgen and eliminating follicular cysts in letrozole-induced polycystic ovary syndrome in the Sprague Dawley rats. *Phytotherapy Research,* **37**(9), 4018-4041. https://doi: 10.1002/ptr.7860

Reeves, P., Nielsen, F. and Fahey, G. (1993) AIN-93 Purified Diets for Laboratory Rodents: Final Report of the American Institute of Nutrition Ad Hoc Writing Committee on the Reformulation of the AIN-76A Rodent Diet. *The Journal of Nutrition,* **123**(11), 1939-1951. https://doi: 10.1093/jn/123.11.1939

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. *Clinical Chemistry,* **19(**12), 1350-1356. PMID: 4757363.

Sabiba, E. P., Rasool, M., Vedi, M., Navaneethan, D., Ravichander, M., Parthasarathy, P. and Thella, S. R. (2013) Hepatoprotective and antioxidant potential of Withania somnifera against paracetamol-induced liver damage in rats. *International Journal of Pharmacy and Pharmaceutical Sciences,* **5**(2), 648-651.

Sabuncu, T., Vural, H., Harma, M. and Harma, M. (2001) Oxidative stress in polycystic ovary syndrome and its contribution to the risk of cardiovascular disease. *Clinical Biochemistry,* **34**(5), 407-413. https:// doi: 10.1016/s0009-9120(01)00245-4.

Sadoughi, S., Rahbarian, R., Jahani, N., Shazdeh, S. A., Saljoughi, H. Z. and Daee, M. (2017) Effect of aqueous extract of Artemisia absinthium L on sex hormones, inflammatory cytokines and oxidative stress indices of ovarian tissue in polycysticovary syndrome rat model. *Journal of Babol University of Medical Sciences,* **19**(7), 50-56. https:// doi:10.22088/jbums.19.7.7

Saini, D., Madan, K. and Chauhan, S. (2022) Screening of phytoconstituents from traditional plants against sarscov-2 using molecular docking approach.

Letters in Drug Design & Discovery, **19**(11), 1022–1038. https:// 10.2174/1570180819666220307163058.

Saleem, S., Muhammad, G., Hussain, M. A., Altaf, M. and Abbas Bukhari, S. N. (2020) *Withania somnifera L.*: Insights into the phytochemical profile, therapeutic potential, clinical trials, and future prospective. *Iranian Journal of Basic Medical Sciences*, **23**(12), 1501-1526. https:// doi: 10.22038/IJBMS.2020.44254.10378.

Sendecor, G. and Cochran, W. (1979) Statistical methods. 6th, ED. Lowa State college. U.S.A.: 871.

Sengupta, P., Agarwal, A., Pogrebetskaya, M., Roychoudhury, S., Durairajanayagam, D. and Henkel, R. (2018) Role of Withania somnifera (Ashwagandha) in the management of male infertility. *Reproductive Biomedicine Online,* **36**(3), 311-326. https://doi: 10.1016/j.rbmo.2017.11.007.

Sharma, A. K., Basu, I. and Singh, S. (2018) Efficacy and safety of ashwagandha root extract in subclinical hypothyroid patients: a double-blind, randomized placebo-controlled Trial. *The Journal of Alternative and Complementary Medicine*, **24**(3), 243-248. https://doi: 10.1089/acm.2017.0183.

Sharma, V., Sharma, S. and Pracheta, P. R. (2011) Withania somnifera: A rejuvenating ayurvedic medicinal herb for the treatment of various human ailments. *International Journal of PharmTech Research,* **3**(1), 187-192.

Singh, G., Sharma, P. K., Dudhe, R. and Singh, S. (2010) Biological activities of *Withania somnifera*. *Annals of Biological Research,***1**(3), 56-63.

Singh, K. B., Hahm, E. R., Kim, S. H., Singh, S. V. and Withaferin, A. (2023) Inhibits Fatty Acid Synthesis in Rat Mammary Tumors. *Cancer Prevention Research,* 16(1), 5-16. https://doi:10.1158/1940-6207.CAPR-22-0193

Singh, N., Yadav, S. S., Rao, A.S., Nandal, A., Kumar, S., Ganaie, S. A. and Narasihman, B. (2021) Review on anticancerous therapeutic potential of *Withania somnifera* (L.) Dunal. *Journal of Ethnopharmacology*,__**270**, 113704. https://doi.org/10.1016/j.jep.2020.113704

Singh, P. and Sharma, Y. K. (2018) *Withania somnifera* (ashwagandha): a wonder herb with multiple medicinal properties. *Asian Journal of Pharmacy and Pharmacology*, **4**(2), 123-130. https:// doi:10.31024/ajpp.2018.4.2.5

Stroev, E. A. and Makarova, V. G. (1989) Laboratory Manual in Biochemistry, MIR Publishers, Moscow, USSR. ISBN: 5030007679.

Tamadon, A., Hu, W., Cui, P., Ma, T., Tong, X. and Zhang, F. (2018) How to choose the suitable animal model of polycystic ovary syndrome? *Traditional Medicine and Modern Medicine,* **1**(2), 95-113. https://doi.org/10.1142/S2575900018300047

Tandon, N. and Yadav, S. S. (2020) Safety and clinical effectiveness of *Withania Somnifera* (Linn.) Dunal root in human ailments. *Journal of Ethnopharmacology*, **255**, 112768. https://doi.org/10.1016/j.jep.2020.112768

Teixeira, T. S., Vale, R. C., Almeida, R. R., Ferreira, T. P. S. and Guimarães, L. G. L. (2017) Antioxidant potential and its correlation with the contents of phenolic compounds and flavonoids of methanolic extracts from different medicinal plants. *Revista Virtual de Química,* **9** (4), 1546-1559. https://doi: 10.21577/1984-6835.20170090

Tekula, S., Khurana, A., Anchi, P. and Godugu, C. (2018) Withaferin-A attenuates multiple low doses of streptozotocin (MLD-STZ) induced type 1 diabetes. *Biomedicine & Pharmacotherapy*, **106**, 1428–1440. https://doi.org/10.1016/j.biopha.2018.07.090

Tiruveedi, V. L., Bale, S., Khurana, A. and Godugu, C. (2018) Withaferin A, a novel compound of Indian ginseng (Withania somnifera), ameliorates cerulein-induced acute pancreatitis: Possible role of oxidative stress and inflammation. *Phytotherapy Research,* **32**(12), 2586–2596. https://doi.org/10.1002/ptr.6200

38. https:// 10.2174/1570180819666220307163058

Tiwari, S., Gupta, S. K. and Pathak, A. K. (2021) A double-blind, randomized, placebo-controlled trial on the effect of Ashwagandha (*Withania somnifera* dunal.) root extract in improving cardiorespiratory endurance and recovery in healthy athletic adults. *Journal of Ethnopharmacology*, **272**, 113929. https://doi.org/10.1016/j.jep.2021.113929

Vaidya, V. G., Naik, N. N., Ganu, G., Parmar, V., Jagtap, S., Saste, G., Bhatt, A., Mulay, V. and Girme, A. (2024) Clinical pharmacokinetic evaluation of Withania somnifera (L.) Dunal root extract in healthy human volunteers: A non-randomized, single dose study utilizing UHPLC-MS/MS analysis. *Journal of Ethnopharmacology,* **322**, 117603. https://doi.org/10.1016/j.jep.2023.117603

Vasavan, S. S., Sivanesan, S. and Jagadesan, V. (2021) Antiperoxidative effect of *Withania somnifera* on

Lipid peroxidation and Antioxidant capacity in the serum of nandrolone decanoate treated rats. *Research Journal of Pharmacy and Technology,* **14** (2), 1065-1068. https://doi: 10.5958/0974-360X.2021.00191.8

Wheeler, C., Salzman, J., Elsayed, N., Omaye, S. and Korte, (1990) Automated assays for superoxide dismutase , catalase, glutathione peroxidase, and glutathione reductase activity. *Analytical Biochemistry*, **184**(2), 193-199. https://doi.org/10.1016/0003-2697(90)90668-Y.

Yang, B., Chen, Y. and Shi, J. (2019) Reactive Oxygen Species (ROS)-Based Nanomedicine. *Chemical Reviews*, **119**(8), 4881-4985. https://doi: 10.1021/acs.chemrev.8b00626

Zahran, E., El Sebaei, M. G., Awadin, W., Elbahnaswy, S., Risha, E. and Elseady, Y. (2020) *Withania somnifera* dietary supplementation improves lipid profile, intestinal histomorphology in healthy Nile tilapia (Oreochromis niloticus), and modulates cytokines response to Streptococcus infection. *Journal of Fish and Shellfish Immunology,* **106**, 133-141. https://doi.org/10.1016/j.fsi.2020.07.056

Zeng, L. H., Saba Rana, S., Hussain, L., Asif, M., Mehmood, M. H., Imran, I., Younas, A., Mahdy, A., Al-Joufi, F. A. and Abed, S. N. *(*2022*)* Polycystic Ovary Syndrome: A Disorder of Reproductive Age, Its Pathogenesis, and a Discussion on the Emerging Role of Herbal Remedies. *Frontiers in Pharmacology,* **13,** 874914. https://doi: 10.3389/fphar.2022.874914

Zhang, C., Ma, J., Wang, W., Sun, Y. and Sun, K. (2018) Lysyl oxidase blockade ameliorates anovulation in polycystic ovary syndrome. *Human Reproduction*, **33**(11), 2096-2106. https://doi.org/10.1093/humrep/dey292