

## Original Research Article

# Effects of *Withania coagulans* extract and morphine on spermatogenesis in rats

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### Abstract

**Purpose:** To investigate the comparative effects of *Withania coagulans* extract and morphine on spermatogenesis in rats

**Methods:** *W. coagulans* was collected from Sistan and Baluchestan, Iran and 50 and 100 mg/kg body weight doses of methanol extract and 5, 10 and 15 mg/kg body weight doses of morphine were administered parenterally to the rats which were divided into groups. Blood samples were collected and the levels of luteinizing hormone (LH), follicle stimulating hormone (FSH), and testosterone were assayed. The testicular tissue was isolated for histopathological examination.

**Results:** No significant changes were observed in levels of LH, FSH and testosterone in treated groups ( $p < 0.05$ ). However, there was significant difference between the treated groups for extract plus morphine groups, in terms of the number of spermatogonium, spermatocytes and spermatide variation. Moreover, the results indicate tissue disorders in all groups relative to control. The extract caused more disturbances in spermatogenesis compared to morphine, and appears to improve parameters related to spermatogenesis.

**Conclusion:** The results show that the higher dose of *Withania coagulans* extract (100 mg/kg) exerts varying effects on reproductive parameters. Moreover, the lower dose of *Withania coagulans* extract (50 mg/kg) enhanced spermatogenesis while also protecting against the damaging effects of morphine.

**Keywords:** Morphine, Spermatogenesis, Spermatogonium, Spermatide, Spermatocyte, *Withania coagulans*

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## INTRODUCTION

Addiction is a reversible chronic disorder that is spreading among different communities around the world [1]. Opium comes from *Papaver rhoeas*, which includes at least 20 alkaloids, one of which is morphine alkaloids. Opium acts through drug receptors. These receptors are dispersed in limbic system, thalamus and

hypothalamus, also, their function includes hypophysis secretions in the testis [2]. Morphine is used clinically to control severe pain, however, it has many side effects, such as vomiting, nausea, constipation, tolerance, and dependence [2]. Moreover, morphine reduces stages of spermatogenesis through inhibiting luteinizing hormone (LH). Male fertility requires normal spermatogenesis.

LH and follicle-stimulating hormone (FSH) are involved in the endocrine mechanism through act on the testosterone produced by the Leydig cells [3]. Thus, a combination of testosterone, LH and FSH activity are needed for stimulating spermatogenesis. LH and FSH are glycoprotein hormones, called gonadotropins, that modulate gonadal functions [4]. LH affects on Leydig cells and causes to produce testis steroids. On the other hand, FSH is the main motivator for growth of testis seminiferous tubules [5]. Moreover, spermatogenesis depends on the function of testosterone that is required for initiating spermatogenesis at maturity and the maintenance of this process in the adult [6].

With regard to the harmful effects of chemical drugs for the treatment of addiction, the use of herbal medicines is considered [1]. *Withania coagulans* Dual. belongs to family Solanaceae. This plant is a general medicinal plant in many parts of India and Pakistan. Phytochemical research of the *W. coagulans* aqueous and methanol fruits extracts showed the presence of alkaloids, steroids, phenolic compounds, tannins, saponins, carbohydrates, proteins, amino acids and organic acids [7]. This plant is rich in withanolide that is a group of alkaloids. The investigation on *W. coagulans* revealed that it known as to treatment of ulcers, rheumatism, bronchitis, nervous exhaustion, disability, insomnia, failure in children thrive and impotence [7]. With regard to the harmful effects of morphine on spermatogenesis the aim of this study was therefore to compare the effects of *W. coagulans* extract and morphine on spermatogenesis in rats.

## EXPERIMENTAL

### Collection of plants

The roots of *Withania coagulans* were collected in Iranshahr County, Sistan and Baluchestan of province, Iran, in May 2013. A voucher specimen was deposited in a herbarium of the Research-Institute of Isfahan Forests and Rangelands by Dr. Feizi.

### Preparation of extract

The roots of *W. coagulans* were air-dried under shade and ground to fine powder using electric blender, then, 60 gr of root powder were extracted with 200 mL methanol by Soxhlet extraction for 12 h. The residue was vaporized using a rotary evaporator. The dried extracts (8 gr) were stored at 4 °C until used. The extracts were dissolved in phosphate buffered saline and tween and tween 80, at a 1:4 weight

ratio for preparing concentrations of 50, 100 mg/kg body weight.

### Preparation of morphine

Morphine was prepared in normal saline at doses of 5, 10 and 15 mg/kg body weight of rat.

### Animals

Adult male Wistar rats (200 - 250 g) were obtained from Iran Pasteur Institute and divided into 6 groups of eight animals each (48 rats). They were maintained under controlled temperature ( $22^{\circ}\pm 1^{\circ}\text{C}$ ), with 12 h light/12 h dark conditions for 1 week before the start of the experiments for adaptation to laboratory conditions. The procedures in this study were carried out in accordance with the institution's scientific procedures for animals and was approved by the Institutional Animal Care and Use Committee (IACUC) [8]. This study was approved by the Medical Research Ethics Committee of the Islamic Azad University of Falavarjan Branch (approval no. IR.IAU.FALA.REC.1397.025).

The animals were randomly divided into 6 groups ( $n= 10$  per group) and received intraperitoneally injection. These groups include control group that received normal saline, morphine treated group (5, 10 and 15 mg/kg), morphine (5, 10 and 15 mg/kg) plus extract (50 mg/kg), morphine (5, 10 and 15 mg/kg) plus extract (100 mg/kg), extract treated groups (50 and 100 mg/kg), respectively. The animals were treated with morphine (groups 2, 3, 4) once daily for 8 days (the first 2 days with concentration of 5, the second 2 days with concentration of 10 and the third 4 days with concentration of 15 mg/kg) then, 2 h after treatment with morphine they were treated with extract (groups 3, 4) once daily for 6 days to evaluate the therapeutic effect of the extract on morphine disorders [9].

The animals were anesthetized with ketamine injections and blood samples were taken directly from the heart one day after the last injection of extract. The hormones LH, FSH, testosterone were assayed using autoanalyzer (902 Hitachi Automatic Analyzer, Roche, India). The rats were sacrificed by cervical dislocation and their testes were rapidly removed and testicular tissue was fixed in 10 % formalin, dehydrated in ethanol, cleared in xylene and embedded in paraffin. Sections were prepared and then stained with Hematoxylin-Eosin (H&E) for observe of testicular tissue disorders and counting of different cells (spermatogonium, spermatocyte I and spermatide) (Olympus, Japan). To determine

the number of sperms in the epididymis, the epididymis tail was removed. Then, for the release of the sperms, the epididymis tail was cut into 2 ml of 37 °C physiological serum and sperms were diluted to (1:20) with normal saline. Finally, 10 µl was placed on a neobar lam, and the total number of sperms as well as the percentage of sperm motility was counted.

### Statistical analysis

All data are presented as mean ± SD. The statistical comparisons were done by one-way ANOVA test by SPSS 18 software.  $P < 0.05$  was considered statistically significant.

## RESULTS

According to results from this study, no significant changes were observed in levels of LH, FSH and testosterone in treated groups with extract and morphine. While, the numbers of spermatocytes, spermatids, epididymal sperm density and percentage of sperm motility decreased significantly in group that received morphine alone. Moreover, morphine plus extract 50 mg/kg let to a significant reduction of spermatocyte; also, extract 50 mg/kg (group 5) alone, reduced the numbers of spermatogonium. The groups that received morphine plus extract 100 and extract 100 alone showed a significant reduction of spermatogonium, spermatocyte, spermatid, epididymal sperm density and percentage of sperm motility (Table 1).

Histological studies showed that the testicular tissue was normal in the control group. However, the testicles in treated animals with *W. coagulans* extract and morphine showed significantly histopathological alterations. These alterations were included spermatogenesis disorder, reduction of cell types density (spermatogonium, spermatocyte and spermatide), destruction leydig

tissue, cell apoptosis, incomplete cell division and the abnormal cells. These changes were more in the groups treated with morphine, morphine plus extract 100 mg/kg and extract 100 mg/kg compared to other groups (Figure 1).

## DISCUSSION

According to the results of this study, the levels of LH, FSH and testosterone did not change in treated groups. While *W. coagulans* extract led to significant reduction of cells types whereas *W.* including (spermatogonium, spermatocyte and spermatid). Also, there was a significantly decrease in the density of epididymal sperms and their mobility.

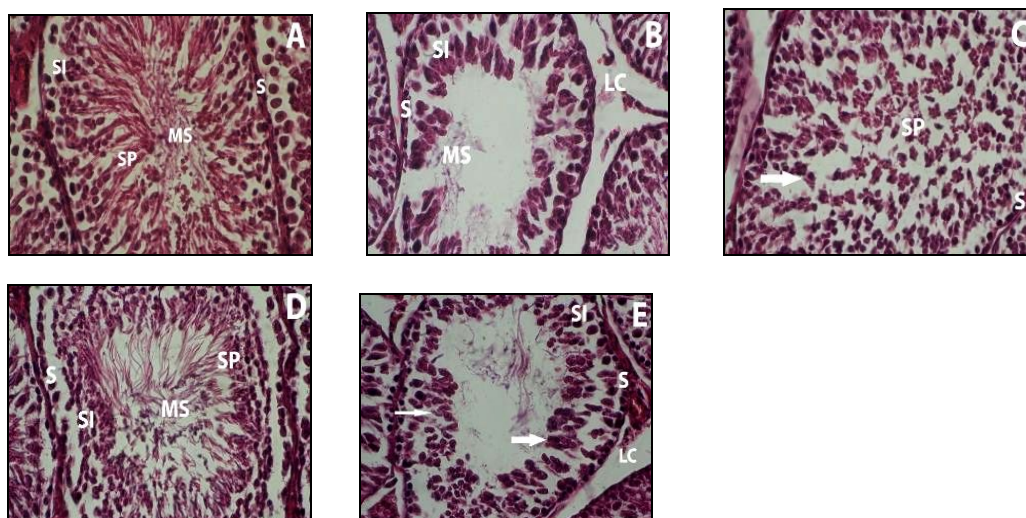
In the present study, the destructive effects of extract on spermatogenesis, may be due to interference of withanolides in the function of hormones and pituitary-gonadal axis. On the other hand, glycoalkaloids are nitrogen containing secondary metabolites that are found in plants of Solanaceae family that its concentration depends on the species and parts of plant; these glycoalkaloids has an important role in plant defense mechanisms that has toxic effects on organs in high concentrations mainly due to their anticholinesterase activity and cell membranes disruption. Solasodine compounds that are found in Solanaceae family and synthesized as glycoalkaloid and are a mediator in synthesis of steroids. This compound has many properties such as anti-spermatogenesis [10].

*W. somnifera* stimulates the growth of axons and dendrites and causes a state of tranquility [11]. In addition, the roots of this plant increases libido, sperm count, ovarian weight and folliculogenesis. This plant prevents the formation of ROS in infertile men [11].

**Table 1:** Effects of *Withania coagulans* extract and morphine on cells number during spermatogenesis and epididymal sperm density and motility (One Way ANOVA)

Parameter	Control	Morphine	Morphine-extract (50 mg/ml)	Morphine-extract (100 mg/ml)	Plant extract (50 mg/ml)	Plant extract (100 mg/ml)	P-value
Spermatogonium	54.75±9.82	42.50±8.91	42.37±16.65	33.75±15.15*	27.87±18.86*	38.12±15.51*	$p < 0.05$ , F=3.144
Spermatocyte	58.87±12.60	36.50±19.58	22.62±13.66	27.87±8.52	47.00±6.82	36.25±17.03	$p < 0.001$ , F=7.255
Spermatid	153.87±33.90	77.62±38.68	137.87±27.49	62.50±37.81	131.37±14.60	81.25±40.51	$p < 0.001$ , F=10.409
Epididymal sperm density	43150±2500	17956±2351	39780±2780	18547±1740	41890±3640	18970±1780	$p < 0.001$ , F=5.348
Sperm motility (%)	72.3±7.8%	35.54±4.1%	69.12±8.65%	38.78±5.6%	70.4±6.7%	33.5±4.9%	$p < 0.001$ , F=9.187

\*Significant reduction in the number of spermatogonium ( $p < 0.05$ ); ©Significant reduction in the number of spermatocyte ( $p < 0.001$ ); Ω Significant reduction in the number of spermatide ( $p < 0.001$ )



**Figure 3:** Sections of the testicular tissue in control and treated groups (with morphine plus extract 100 mg/kg and extract 100 mg/kg) of rats. (A) control group the seminiferous tubules and cells types (spermatogonium, spermatocyte and spermatid) density are normal, (B) the destruction of leydig cells, reduction of cells types and mature sperms, (C) the lack of mature sperm formation, cells apoptosis and degeneration (arrow), (D) the reduction of mature sperms, (E) the severe apoptosis (thin arrow), abnormal division of cells (thick arrow). (S, spermatogonium; SI, spermatocyte; SP, spermatid; MS, mature sperm; LC, leydig cells) ( $\times 400$ )

*W. somnifera* root powder (5 g/day) for 3 months, decreased FSH and also increased levels of LH, testosterone and sperm count in infertile men. The major effects of this plant include reducing oxidative stress, hormone levels and improves detoxification processes in body [12]. Moreover, *W. somnifera* aqueous extract induces some changes in hypophysial gonadotropins and thus increases sperm [13]. The powder of this plant (6.25 %) was given to diabetic rats for 4 weeks and caused increase the levels of LH, testosterone and progesterone, while, FSH level decreased. This plant probably has excitatory effects on the LH and luteal cells and inhibitory effects on FSH [13].

The effect of *W. somnifera* on sex hormones levels is probably via inhibition of oxidative stress and most of the pharmacological activity of this plant is related to two main withanolides (withaferin A, withanolide D)[14]. In fact, withanolides induces some chemical compounds that can affect the metabolic activity [18]. In another study, *W. somnifera* reduced malondialdehyde (MDA) and testicular disorders induced by galactose and increased the sperm count [14]. Moreover, methanolic extract of this plant eliminates the disorders caused by acephate insecticide on LH, FSH, testosterone and testis tissue [16]. According to the studies, ROS is generated by Sertoli cells in the testis that the controlled amount of ROS is necessary for capacitation, spermiogenesis and acrosome reaction. In a study on infertile men, sperm apoptosis and high concentrations of ROS were

observed, that *W. somnifera* reduced these abnormalities. As a result, *W. somnifera* can be improved semen quality by reducing cell death and oxidative stress [17]. The extract of this plant (47 mg/100 g) caused testicular development and spermatogenesis in immature rats for 6 days that this was associated with increased LH [18].

According to studies on different species of *Withania*, various extracts of this plant have reinforcing effects on spermatogenesis that is probably through inhibition of ROS, reduce stress, occupy the sex hormone receptors and effect on the pituitary-gonadal axis. Nevertheless, in some cases, the lowering effects of FSH, testosterone and anti-spermatogenesis properties of this extract may be due to the presence of glycoalkaloid compounds or effect on receptors, so that these results are consistent with our findings.

Previous research investigated the effect of Genistein on reproductive in morphine-treated mice and showed that genistein and genistein plus morphine significantly increased motility, morphology, count, viability of sperm cells, seminiferous tubules diameter, germinal thickness, testosterone, LH and FSH. It seems that genistein prevent morphine-induced adverse effects on sperm parameters. The results of the current research confirmed the results of other studies which showed antioxidants can increase sperm motility [19]. Our results corroborated the findings of other researchers who found alkaloids and flavonoids

could increase testosterone level through increasing LH [17]. However, the findings were in contrast with results of other researchers who demonstrated isoflavonoid as a stopper of testosterone generation [20].

The findings of the study of other researchers showed a decrease in testosterone level in high concentrations of phytoestrogens [20], which is in line with the results of current study obtained in groups receiving high concentrations of *Withania coagulans* extract (100 mg/kg).

## CONCLUSION

The findings of the present study indicate that a higher dose of *Withania coagulans* extract (100 mg/kg) can have a reverse effect on reproductive parameters. Since *Withania coagulans* extract affects pituitary-gonadal axis and exert the negative effects on testis tissue and secretion of sexual hormones in higher doses, therefore, it seems that alkaloids and flavonoids exert both agonistic and antagonistic effects on the studied tissue depending on the dose. Moreover, the lower dose of *Withania coagulans* extract (50 mg/kg) seems to enhance spermatogenesis parameters and protects against the damaging effects of morphine.

## DECLARATIONS

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### Conflict of interest

No conflict of interest is associated with this work.

### Contribution of authors

We declare that this work was done by the authors mentioned in this article and all liabilities regarding claims relating to the content of this article will be borne by the authors. Leila Amjad conceived and designed the study. Ali noori collected, analysed and wrote the manuscript with the collaboration of Fereshteh Yazdani. All authors read and approved the manuscript for publication.

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