Original Article

# Protective Role of *Withania somnifera* Against the Adverse Effects of Levofloxacin on Testes Tissue of Rats *In Vivo*

**Mahmood Neamah Hammood, Liqaa Hasson Saqban, Nazar Jebar Metib[1]**

Department of Biology, College of Education for Pure Sciences, University of Karbala, [1]Al-Hussein Medical City, Kerbala, Iraq

## Abstract

**Background:** Medicinal plants have many uses in traditional medicine in all cultures due to their unique therapeutic properties. *Withania somnifera* is considered an agent to improve sexual health, an antioxidant, anti-aging, and anti-inflammatory. **Objective:** This study aimed to determine the therapeutic effect of *W. somnifera* on the potential Levofloxacin-induced testicular injury in rats. **Materials and Methods:** Thirty adult male Wistar rats were randomly divided into five groups: control group (C), saline-treated for 60 days. Group (W) *W. somnifera* root extract was orally treated for 60 days. Group (L) Levofloxacin orally treated for 60 days. Group (W+L) Levofloxacin was orally co-administered and pretreated with *W. somnifera* root extract for 60 days. Group (L+W) Levofloxacin was orally co-administered and post-treated with *W. somnifera* root extract for 60 days. The degree of protection was estimated using the gonadosomatic index (GSI), hormonal changes, and oxidative stress biomarkers. Furthermore, specimens from the testes were obtained and histopathologically examined for qualitative analysis of testicular tissue morphology and quantitative analysis utilizing seminiferous tubular diameter and Johnsen's scoring system. **Results:** Levofloxacin treatment resulted in a significant decrease in GSI, glutathione (GSH), Johnsen's score, seminiferous tubule diameter, and germinal epithelium. As well as a significant rise in malondialdehyde (MDA), LH, and FSH levels. The testosterone remained unaffected. *W. somnifera* root extract treatment post-Levofloxacin improved recovery of these biochemical changes and boosted the antioxidant defense. **Conclusion:** *W. somnifera* root extract considerably minimized the side effects of levofloxacin in testicular tissue.

**Keywords:** Antioxidant, levofloxacin, sex hormones, testis, *Withania somnifera*

## Introduction

The use of medicinal herbs spans thousands of years and continues to be a significant area of medicinal treatment in many countries, particularly in Asia, Africa, and South America.[1] Plants and other natural resources have always been of interest to researchers, especially for the treatment of various medical conditions as well as in the development of new drugs. Nowadays, increasing attention is being to the development and use of effective and safe antioxidants from natural origin.[2] *Withania somnifera*, often known as Ashwagandha, Indian ginseng, or winter cherry, is a medicinal herb of the Solanaceae family, which is a tiny evergreen plant with lengthy tuberous roots, this plant inhabitant in hot, dry, or semi-arid climates and it is found in the southern Mediterranean, from North Africa and the Middle East to India.[3] Because of its therapeutic characteristics, the whole plant uses, meanwhile, the root is used as a general tonic for the body and categorized as an adaptogen, which can benefit the body in stress control.[4] *W. somnifera* acts as an anticancer, antibacterial, anti-inflammatory agent, and antioxidants.[5] The roots and leaves can help with mental health, relief of pain, improved memory, neuroprotection, hepatoprotection, and renoprotection. In addition, it is used to treat arthritis, depression, chronic disorders, infertility, and hormonal imbalance.[6,7] Several studies have indicated to the utilization regularly of *W. somnifera* root by infertile males led to improvement in some sperm parameters

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**Address for correspondence:** Mr. Mahmood Neamah Hammood, Department of Biology, College of Education for Pure Sciences, University of Karbala, Kerbala, Iraq.
E-mail: mahmood_neamah@karbala.edu.iq

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including enhancement in semen volume, sperm density and motility, also increased lutleinzing hormone (LH) and testosterone and decreased follicle-stimulating hormone (FSH).[8,9] In animal studies, ashwagandha has increased the weight of the gonads by increasing the layers of germinal epithelial cells in male rats. and It also improved antioxidants and sperm activity by enhancing testosterone in the rats.[10,11]

Levofloxacin is an antibiotic from the third generation of fluoroquinolone, and has broad-spectrum activity against bacteria as well as some other pathogens such as *Mycoplasma*, and *Chlamydia*.[12] It has been widely used in the recent period, Some researchers have suggested using levofloxacin as an adjuvant in the treatment of patients suffering from SARS-CoV-2-associated pneumonia.[13] While levofloxacin is generally considered safe and effective, it can have some side effects.[14] Recent studies on animals indicated that levofloxacin showed some negative side effects on fertility, these effects were targeted at the organs of the reproductive system, accessory glands, sex hormones, and spermatogenesis.[15,16] Malondialdehyde is a critical biomarker of oxidative stress, which is the main production of the lipid peroxidation process. Glutathione, a tripeptide molecule, is essential in protecting cells from oxidative stress by neutralizing free radicals and reactive oxygen species (ROS) that can cause cell damage. Many investigations have found that fluoroquinolones cause oxidative stress and cellular damage to the testes, liver, and kidneys.[17,18]

*W. somnifera* is known for its antioxidant and anti-inflammatory properties, which may help counteract the potential adverse effects of levofloxacin on testis tissue, which could oxidative stress. By investigating the potential protective effects of *W. somnifera*, this research could help identify a natural supplement that could help reduce the harmful effects of antibiotics on the reproductive system.

## Materials and Methods

### Animal

Thirty adult albino waster male rats were used in this experiment (8–9 weeks, 220–285 mg) procured from the University of Kufa, Faculty of Veterinary. Animals were housed in breeding cages. In the well-ventilated room, the temperature was 25 ± 5°C, with humidity 50% ± 10%, and 12 h light/dark cycle. Animals feed on standard pellets with Water *ad libitum*.

### Plant extraction

*W. somnifera* plants collected from the garden of plants in Baghdad University, Science college. The roots were washed and subjected to shade dry to prepare fine powder. The powder was soaked in 70% ethanol for 48 h and utilized for the alcoholic extract method using the Soxhlet apparatus. After extraction, the excess alcoholic solvent was evaporated at 60°C.[7]

### The drug

Levofloxacin (COX pharmaceutical, LTD. Arcade House. Finchley Road. London NW11 7TL, UK) Tablets 500 mg obtained from a local pharmacy. It was finely grinding and then dissolved in normal saline to prepare a dose of 40 mg/ kg.[19]

### Type of study and experimental design

The type of study is an experimental animal study that was conducted *in vivo* using rats as the animal model. Therefore, it is a preclinical study conducted on animals, and more specifically, an interventional study, by administering treatments to the animals (levofloxacin and *W. somnifera*). The animals used in this study were healthy and had similar baseline characteristics. The rats were randomly divided into five groups (n=6/group): Group (C) animals were orally gavaged with normal saline for 60 days and served as controls; Group (W) rats were administered with *W. somnifera* root extracts for 60 days; Group (L) rats were received with Levofloxacin 40 mg/kg-1 by gavage for 60 days; Group (W+L) rats were pretreated with 500 mg/kg-1 body weight of *W. somnifera* root extract orally by gavage and then treated with Levofloxacin 40 mg/kg-1 by gavage for 60 days; and Group (L+W) rats were post-treated with 500 mg/kg-1 body weight of *W. somnifera* root extract orally by gavage and then treated with Levofloxacin 40 mg/kg$^{-1}$ by gavage for 60 days. At the end of the experiment, the rats were sacrificed by being anaesthetized with chloroform, and blood samples were collected by heart puncture and centrifuged at 3500 rpm for 10 min. The serum samples were then deep-frozen for later biochemical analyses.

### Gonadosomatic index

The body and organs were weighed, and the gonadosomatic index (GSI) was calculated using the formula GSI = [gonads weight (g)/Body weight (g)]*100.[20]

### Malondialdehyde

The serum malondialdehyde was determined using a reproducible fluorometric method for measuring thiobarbituric acid-reactive substances (TBARS), based on the reaction between malondialdehyde (MDA) and thiobarbituric acid (TBA). as described by Wasowicz *et al*.[21]

### Glutathione

The Ellman's reagent (DTNB) method was established by Goel *et al*.[22] This method was used to measure glutathione in serum by using a spectrophotometer at absorbance estimated at 412 nm.

### Testosterone assay

The Serum Testosterone was measured by a chemiluminescence immunoassay analyzer (CLIA). This method was performed manufacturer's instructions in an automatic analyzer (Immulite, USA).[23]

### LH and FSH assay

The Serum levels of LH and FSH were determined by enzyme-linked immunosorbent assay (ELISA) technique by using rat LH kit and rat FSH kit (Solarbio, Shanghai, China).[24]

### Histopathological preparations

The testes were removed and washed then fixed in 10% formalin. The fixed tissue samples were dehydrated by ascending concentration of ethanol alcohol (70%,80% 90%, 100%, 100%). Embedded in paraffin blocks and sectioned at 6µm with a rotary microtome. Sections were stained with hematoxylin-eosin.[25] Slides were examined with a light microscope (PANTHERA E2, Motic, Hong Kong) supported by a camera (Moticam A16). Each animal's photomicrograph was recorded using a 10x objective lens at 15 various fields. The images were measured using the automated image analysis system program Image-J® version 1.52p (National Institute of Health, USA). By measuring 200 cross-sections of seminiferous tubules that were as circularly formed as feasible for each rat, the diameter of the seminiferous tubule and the height of the epithelium for each animal was estimated.[26]

### Johnsen scoring system

The stained sections were examined under a light microscope. The spermatogenesis was categorized using Johnsen's Criteria. According to Johnsen's scoring system, each tubule cross section is given a score between 1 and 10 as described in Erdemir *et al*.[27] Each testis had its germinal epithelium of at least 30 tubules examined, after which the mean Johnsen's Score for each rat was determined.

### Statistical analysis

The data were represented as mean ± standard error (SE) and analyzed by one-way ANOVA followed by revised least significant differences (LSD) by using GraphPad Prism, version 9.5 (GraphPad Software, California). The statistical significance was set at $P < 0.05$.

### Ethical approval

All procedures and protocols adhered to the ethical guidelines set by the Ethical Committee of Kerbala University–College of Education for Pure Sciences with the project being assigned No. 1818/14 on July 7, 2022.

## Results

### Body weight and gonadosomatic index

Animals of levofloxacin (L) treatment show a significant increase in body weight as compared to the control. However, as compared to L treatment, animals of L+W show that a significant decrease in body weight [Figure 1]. The relative testes to the body weight of L treatment alone significantly decreased as compared to the control. W+L and L+W treatment revealed the ameliorative effect of *W. somnifera* retained relative testes weight against L treatment [Figure 2]. When compared to control, the L treatment's relative epididymis to body weight was significantly reduced. *W. somnifera* conserved relative epididymis weight versus L treatment in the W+L and L+W treatments [Figure 3].

### The oxidative stress

Levofloxacin treatment led to a significant increase in the MDA and a significant decrease in the GSH as compared

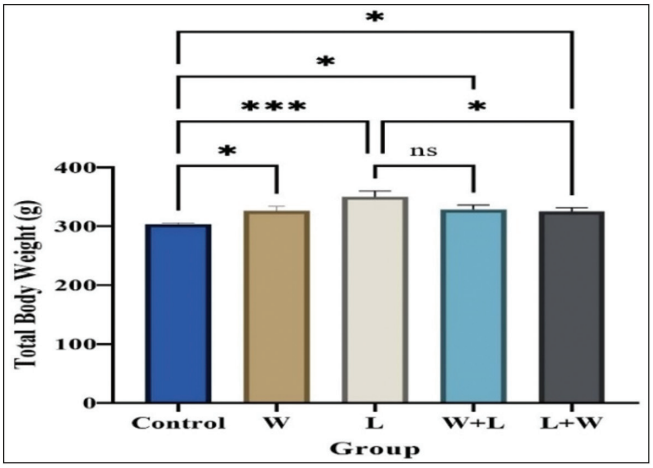


**Figure 1:** Effect of *W. somnifera* and before/after treated levofloxacin on body weight. Data are expressed as mean ± SE ($n = 6$). The asterisks represent statistical significance (ANOVA) from control: *($P < 0.05$), **($P < 0.01$), ***($P < 0.001$), ****($P < 0.0001$)

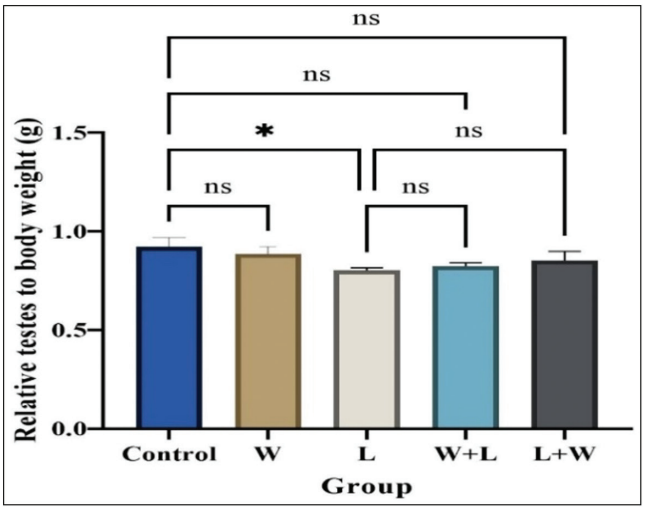


**Figure 2:** Effect of *W. somnifera* and before/after treated levofloxacin on relative weight of testes. Data are expressed as mean ± SE ($n = 6$). The asterisks represent statistical significance (ANOVA) from control: *($P < 0.05$), **($P < 0.01$), ***($P < 0.001$), ****($P < 0.0001$)

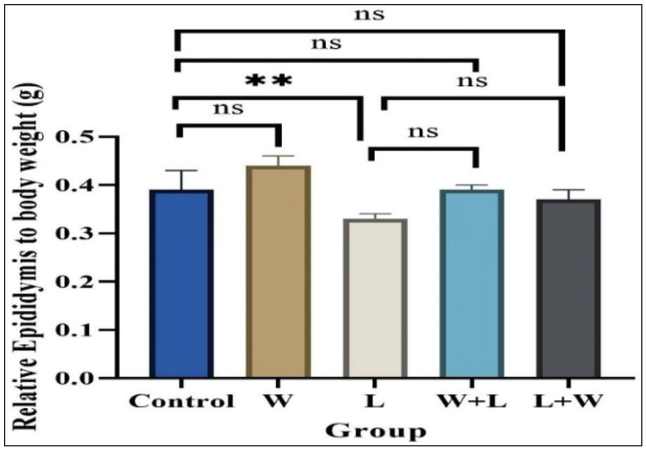


**Figure 3:** Effect of *W. somnifera* and before/after treated Levofloxacin on relative weight of testes. Data are expressed as mean ± SE ($n = 6$). The asterisks represent statistical significance (ANOVA) from control: *($P < 0.05$), **($P < 0.01$), ***($P < 0.001$), ****($P < 0.0001$)

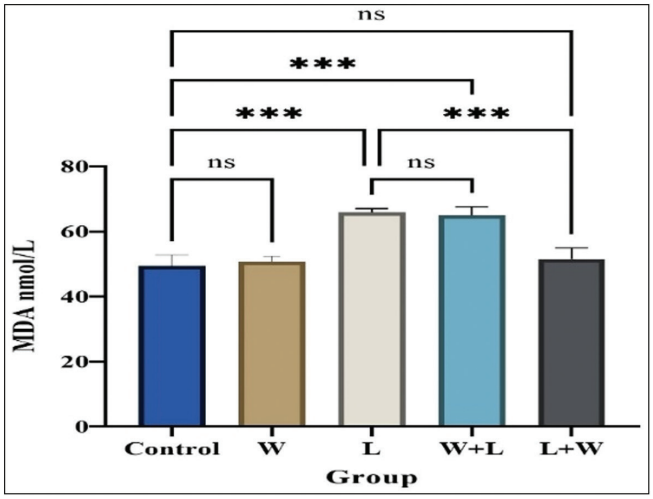


**Figure 4:** Effect of *W. somnifera* and before/after treated levofloxacin on serum MDA. Data are expressed as mean ± SE ($n = 6$). The Asterisks represent statistical significance (ANOVA) from control: *($P < 0.05$), **($P < 0.01$), ***($P < 0.001$), ****($P < 0.0001$)

to the control group. ADministration with *W. somnifera* extract led to a significant decrease in the MDA in L+W group, meanwhile in W+L group revealed non-significant change as compared to L group. The GSH level improved significantly in L+W and W+L as compared to L treatment alone [Figures 4 and 5].

## Testosterone

As shown in Figure 6, there were non-significant changes in the level of Testosterone in all Groups compared with the control group.

## Gonadotropin hormones

Levofloxacin treatment significantly increased serum concentrations of Gonadotropin Hormones parameters:

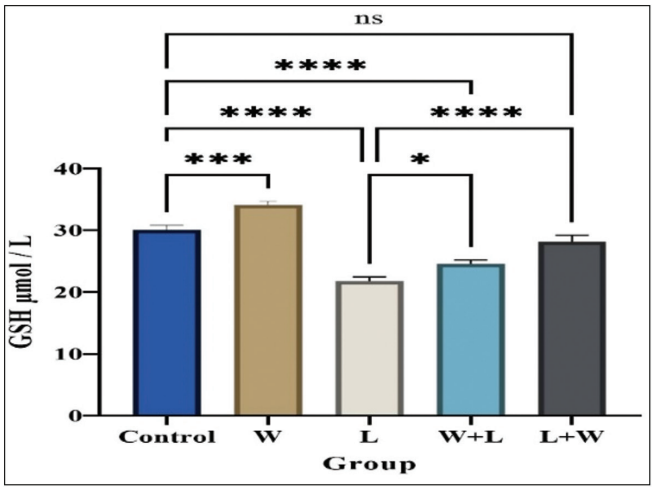


**Figure 5:** Effect of W *W. somnifera* and before/after treated levofloxacin on serum GSH. Data are expressed as mean ± SE ($n = 6$). The Asterisks represent statistical significance (ANOVA) from control: *($P < 0.05$), **($P < 0.01$), ***($P < 0.001$), ****($P < 0.0001$)

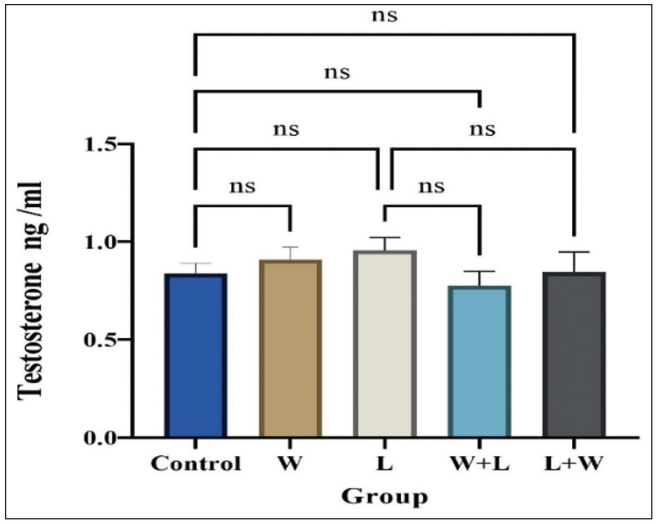


**Figure 6:** Effect of *W. somnifera* and before/after treated levofloxacin on serum Testosterone. Data are expressed as mean ± SE ($n = 6$). The Asterisks represent statistical significance (ANOVA) from control: *($P < 0.05$), **($P < 0.01$), ***($P < 0.001$), ****($P < 0.0001$)

Luteinizing Hormones and Follicular stimulating Hormone as compared to control. However, oral administration of *W. somnifera* (L+W and W+L) significantly decrement elevated serum concentrations of Hormones by Levofloxacin [Figures 7 and 8].

## Johnsen scoring system

Levofloxacin treatment caused significant damage in the seminiferous tubules of the testes as compared to the control. Intriguingly, the Johnsen's score improved significantly in L+W and W+L treatment as compared to L treatment alone [Figure 9].

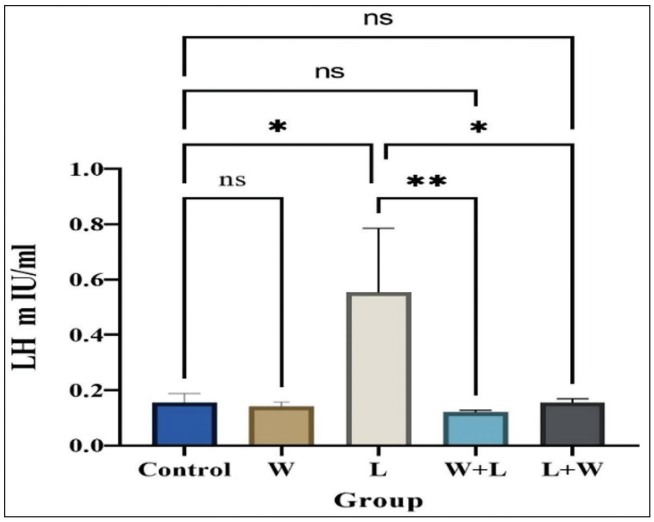


**Figure 7:** Effect of *W. somnifera* and before/after treated levofloxacin on serum LH. Data are expressed as mean ± SE ($n = 6$). The Asterisks represent statistical significance (ANOVA) from control: *($P < 0.05$), **($P < 0.01$), ***($P < 0.001$), ****($P < 0.0001$)

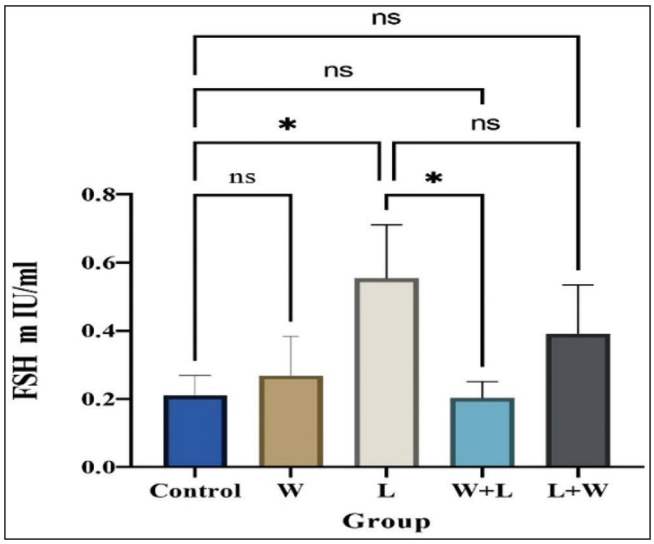


**Figure 8:** Effect of *W. somnifera* and before/after treated Levofloxacin on Serum FSH. Data are expressed as mean ± SE ($n = 6$). The Asterisks represent statistical significance (ANOVA) from control: *($P < 0.05$), **($P < 0.01$), ***($P < 0.001$), ****($P < 0.0001$)

## Histopathological features

The control group section of the testicular tissue showed normal maturation and development of germ cells inside the seminiferous tubules. the spermatozoa are present within the lumen. Leydig's cells are normal in shape [Table 1 and Figure 10-1]. While the *W. somnifera* group shows typical architecture as well as full Spermatogenesis. In addition to that a significant increase in the epithelial height of the seminiferous tubules as compared with the control group [Table 1 and Figure 10-2]. Levofloxacin-treated group section of the testicular tissue showed mild intertubular edema and mild degeneration of most tubular

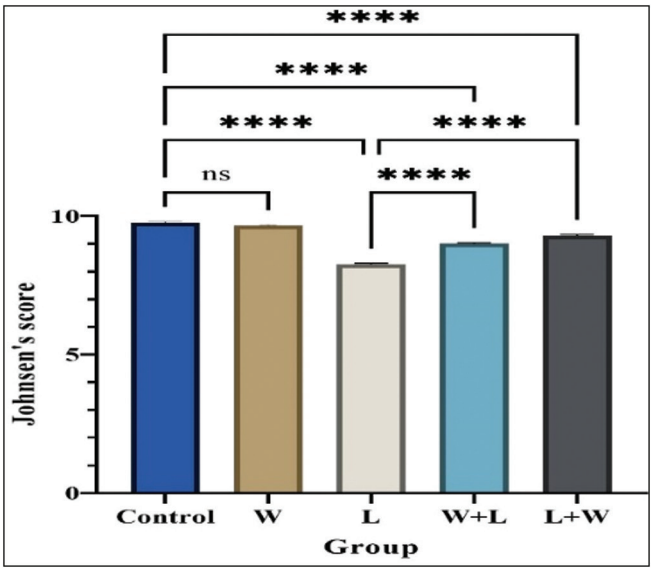


**Figure 9:** Effect of *W. somnifera* and before/after treated levofloxacin on testicular damage depicted by Johnsen's score. Data are expressed as mean ± SE ($n = 6$). The Asterisks represent statistical significance (ANOVA) from control: *($P < 0.05$), **($P < 0.01$), ***($P < 0.001$), ****($P < 0.0001$)

lining epithelium with mild disorganization and sloughing of some spermatogonia. Additionally, most of the seminiferous tubules exhibited interrupted spermatogenesis at the spermatid stage. Moreover, there was a significant decrease in the average diameter and height of the tubular epithelium, accompanied by a significant increase in the size of the tubule's lumen when compared to the control group [Table 1 and Figure 10-3]. On the other hand, The W+L group shows mild intertubular edema and slight disorganization, with normal spermatogenesis as compared to L-group. However, the histological structure in the L+W group showed a normal arrangement of spermatogenic cells with a significant increase in the mean tubular diameter, and the epithelial height. And a significant decrease in the lumen of the seminiferous tubules in this group as compared with the L-group, but shows a little decrease in the epithelial height as compared to Control. Overall Well-organized histological features were observed in both W+L and L+W groups but appeared some debris in the tubule as compared to the control group [Table 1 and Figure 10-4 and 10-5].

## Discussion

Recently, we demonstrated that treatment with Levofloxacin resulted in testicular injury in rats, as evidenced by a decrease in gonadosomatic index, Johnsen's score, seminiferous tubular diameter, and germinal epithelium, and an increase in malondialdehyde. However, co-treatment with *W. somnifera* root extract Levofloxacin was found to improve recovery of these changes and boost the antioxidant defense, indicating a potential therapeutic

**Table 1: Effect of *Withania somnifera* extract before/after treated levofloxacin on the morphometric changes of the testis**

| μm) | (C)Control) | W | L | W+L | L+W |
|---|---|---|---|---|---|
| STD | 339.05±2.39 | 351.56±4.63 | 303.55±9.88$^{****}$ | 338.19±3.11$^{\#\#\#}$ | 342.82±2.75$^{\#\#\#\#}$ |
| LUM | 125.25±2.37 | 119.09±3.07 | 165.84±2.67$^{****}$ | 156.87±4.19$^{****}$ | 153.02±3.88$^{****,\#}$ |
| EPL | 92.16±0.38 | 113.44±2.36$^{****}$ | 80.60±0.99$^{****}$ | 84.60±0.99$^{**}$ | 86.63±1.27$^{*,\#}$ |

STD = seminiferous tubular diameter, LUM = lumen, EPL = epithelial height

*($P < 0.05$) vs. C. **($P < 0.01$) vs. C. *** ($P < 0.001$) vs. C. **** ($P < 0.0001$) vs. C

$^{\#}$($P < 0.05$) vs. L. $^{\#\#}$ ($P < 0.01$) vs. L $^{\#\#\#}$ ($P < 0.001$) vs. L. $^{\#\#\#\#}$ ($P < 0.0001$) vs. L

effect of *W. somnifera* in minimizing the side effects of levofloxacin in testicular tissue. Male reproductive problems caused by pharmaceuticals are routinely ignored, misunderstood, and understudied. Drugs may alter hormonal or non-hormonal processes, or oxidative stress pathways to induce sexual dysfunction, disrupt spermatogenesis, and affect epididymal development.[28]

The body weight of animals is one of the practical parameters for antibiotic-induced toxicity. The current study exhibited the effects of levofloxacin on the weight of rats, as the body weights increased significantly after exposure to levofloxacin (L). These findings agree with Ray, (2012),[29] who observed that the body weight of antibiotic-exposed rats was increased. This overweight may result from changes in gene expression involved in short-chain fatty acid metabolism, as well as the control of hepatic lipid and cholesterol metabolism, induced by antibiotic therapy. This increment is due to the treatment with antibiotics, which could decrease the relative percentages of microbial taxa and deplete the beneficial bacteria that play a vital role in metabolism.[30] The current study revealed that levofloxacin adversely affected the testis and epididymis weight. Similar findings were observed, after 14 days of treatment with Levofloxacin at two different dosages (39.11, 78.22 mg/kg/day), that discovered the weight of the testis, epididymis, and seminal vesicles had significantly decreased ($P < 0.05$).[15] Testis weight depends on the number of germ cells. According to studies, *W. somnifera* has a gonadotropic effect and thus increases gonadal weight by enlarging seminiferous tubular germ cell layers in males.[11] Our results from pre- and posttreatment with *W. somnifera* indicated an Ameliorative change in the body weight and the relative weight of the testis and epididymis.

MDA is one of the products of lipid peroxidation and serves as a crucial indicator of this process of oxidative stress.[31] According to several studies, the testes, liver, and kidneys were harmed cellularly as a result of fluoroquinolones' production of ROS.[17,32] Levofloxacin significantly increased MDA levels and decreased GSH in the rats used in this study. This finding is consistent with Farid and Hegazy's[19] findings, which showed that levofloxacin treatment resulted in a significant increase ($P < 0.05$) in MDA levels and a significant decline in catalase (CAT), superoxide dismutase (SOD) and GSH in the animals that received oral levofloxacin at a dose of 40 mg/kg body weight daily for two weeks. An imbalance between pro-oxidants and antioxidants leads to oxidative stress, which is linked to the synthesis of intracellular oxidized proteins, These oxidized proteins may assemble cytotoxic protein aggregates, which are serious pathogenic elements contributing to cellular destruction.[33] Levofloxacin and *W. somnifera* extract co-treatment resulted in decreased lipid peroxidation by decreased MDA levels and elevated GSH levels [Figures 4 and 5]. *W. somnifera* is abundant with Antioxidants such as fatty acid ester, essential amino acids, flavonoid, phenolic compounds, Ascorbic acid, and tocopherol are the main bioactive components of *W. somnifera* extract and are in charge of the antioxidant activities.[34]

The hypothalamus-pituitary-gonads axis is a key regulatory mechanism that is, chiefly involved in the growth and regulation of the reproductive system by controlling the synthesis and secretion of Androgen especially Testosterone.[35] In our results, Levofloxacin treatment non-significant changes the serum testosterone and had a significant effect on FSH and LH levels, which were higher than the control ($P < 0.05$). similar findings were noticed in rats that treatment with levofloxacin at 75 mg/kg for 28 days did not affect testosterone levels.[16] Furthermore, different dosages of levofloxacin (39.11 and 78.22 mg/kg) had no differences in testosterone levels and raised FSH and LH levels.[15] *W. somnifera* root extract alone or in combination with Levofloxacin enhances the levels of Testosterone, LH, and FSH, respectively. *W. somnifera* improves spermatogenic activity by maintaining the hypothalamic-hypophysial-gonadal hormonal axis and testosterone balance in the testes by compensating for LH and FSH decreases or increases.[36]

As mentioned above, Johnsen's scoring revealed significantly lower spermatogenesis and testicular damage including interstitial edema, congestion, sloughing, and disorganization in the levofloxacin group. These findings were consistent with the previous studies.[16,37] Several studies have pointed out that fluoroquinolone drugs have negative effects on testis tissue.[38] the decrease in antioxidant activity led to failure to protect cells against free radicals, and Because the testes are high in polyunsaturated fatty acids, they are sensitive to ROS attack and lipid peroxidation.[39] The results of the current

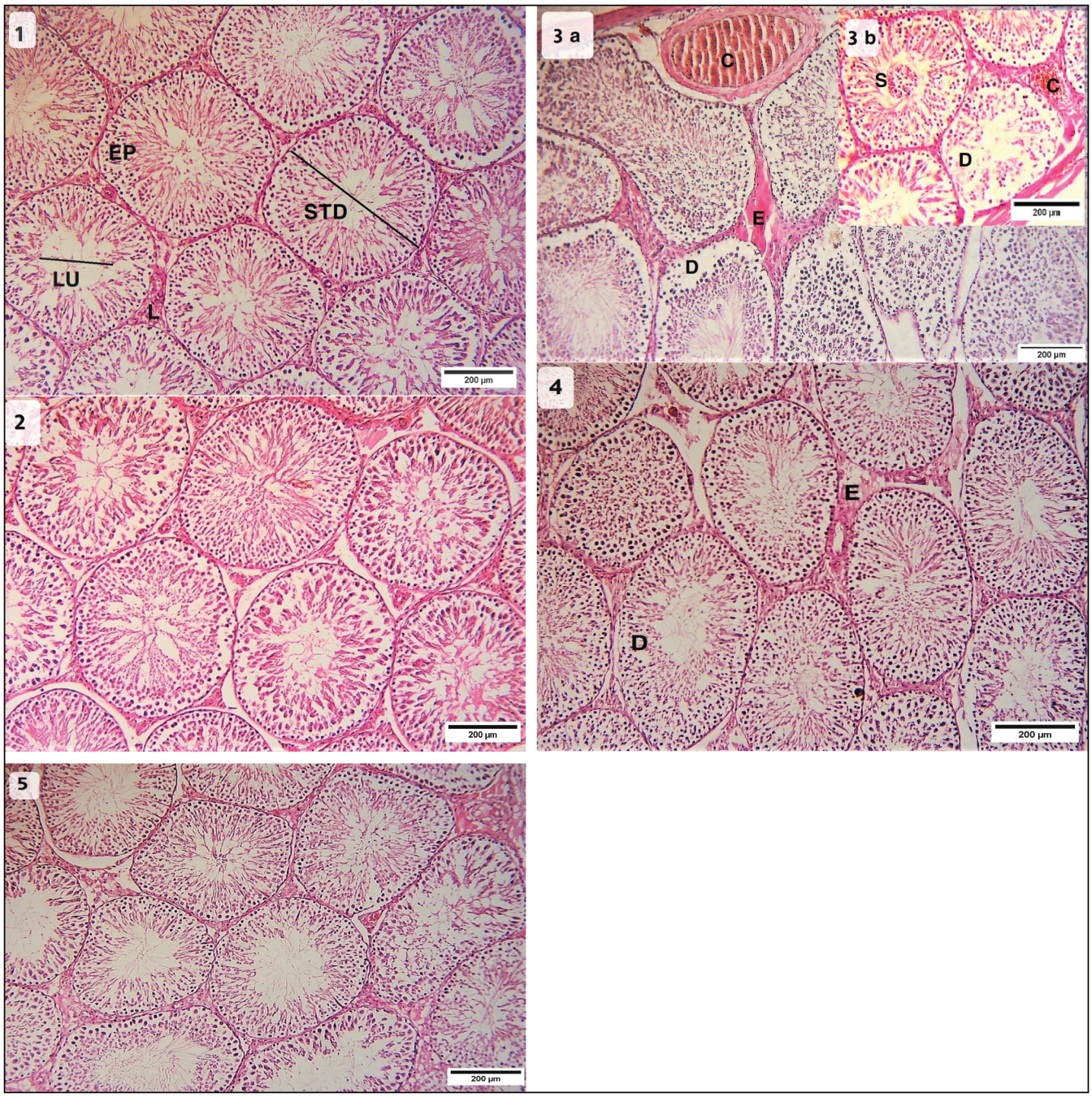


**Figure 10:** Histological photomicrograph of testes: (1) C-group: Normal architecture was observed with full spermatogenesis, epithelial germ cell (EP), Leydig's cell (L), seminiferous tubular diameter (STD), lumen (LU). (2) W-group: The typical architecture was seen as well as full spermatogenesis. (3a-b) L-group: The treated group with levofloxacin resulted in intertubular edema (E), congestion (C), mild degeneration of the majority of the tubular lining epithelium with fewer spermatozoa, slight disorganization (D), and sloughing of some spermatogonia (S). (4) W+L-group: This group shows mild intertubular edema (E) and slight disorganization (D), with normal spermatogenesis as compared to L-group. (5) L+W-group: The histological structure showed a normal arrangement of spermatogenic cells with little decrease in the epithelial height as compared to C-group. (H&E, × 10)

study observed a significant improvement in Johnsen's score, minimizing the testicular damage in the W+L and L+W groups as compared with the Levofloxacin group, which may due to the improvement in the antioxidant content of the testicular tissue. *W. somnifera* improved GSH levels by containing antioxidant trace elements iron (Fe), zinc (Zn), and selenium (Se), which are considered crucial co-factors for the enzymatic antioxidant defense mechanism and enhanced antioxidant activity and protecting testicular tissue from oxidative stress damage.[40,41] This study utilized a single dose of *W. somnifera* and Levofloxacin, and it is unclear if various

dosages would yield different results. Various methods, including histopathological examination and biochemical analysis, were used to evaluate the protective effect of *W. somnifera*. The findings suggest that *W. somnifera* may have therapeutic potential in mitigating the adverse effects of levofloxacin in testis tissue. Further research is necessary to determine the applicability of these findings in humans and to investigate the optimal dosage of *W. somnifera* for a protective effect against Levofloxacin-induced testicular injury.

## Conclusion

In this study, we added more knowledge about the role of *W. somnifera* root extract as an antioxidant and testicular protective agent. By lowering ROS formation, maintaining the antioxidant potential, and significantly reducing testes damage, this agent may help in ameliorating the side effects of Levofloxacin therapy. The pharmacological pathways of bioactive components of *W. somnifera* on Levofloxacin-induced testicular toxicity, however, still need more study.

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Nil.

### Conflicts of interest

There are no conflicts of interest.

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