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The Role of Withania Somnifera Against Levofloxacin Effect in Oxidative Stress, Sperm Parameters and DNA Integrity of Male Rats

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A B S T R A C T

Folk medicine across various cultures has relied on medicinal plants for their distinctive therapeutic properties. Among these plants is Withania somnifera, which is reputed for its potential to enhance sexual health and improve semen quality and inhibit lipid peroxidation as well as its anti-ageing, and anti-inflammatory properties. The primary objective of this study is to evaluate the inherent protective role of W. somnifera against the effects of Levofloxacin, specifically focusing on DNA damage and sperm quality in a rat model. In order to achieve this objective, a total of thirty adult male rats were carefully assigned to five separate experimental groups, including the 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 pre-treated 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 oxidative stress biomarkers, sperm concentration, motility, viability, morphology, and comet assay. The administration of Levofloxacin resulted in a significant increase ($P < 0.05$) in various parameters, including Total Oxidative Status (TOS), sperm abnormality (Head and Tail abnormality), and the presence of immotile sperm. Additionally, significant damage to sperm DNA was observed, as confirmed by the comet assay. Furthermore, Levofloxacin treatment led to a significant decrease ($P < 0.05$) in Super Oxide Dismutase (SOD) levels and reductions in sperm concentration, motility, and viability. W. somnifera root extract treatment post and pre-Levofloxacin improved recovery of these biochemical changes and boosted sperm quality. In conclusion, W. somnifera root extract may protect against levofloxacin-induced sperm damage in rats.

1. INTRODUCTION

Levofloxacin is classified as a fluoroquinolone antibiotic that effectively treats various bacterial infections such as respiratory tract infections, skin infections, urinary tract infections, and others. It belongs to the third generation of antibiotics called fluoroquinolones (FQs). It can penetrate bacterial cell walls and inhibit the activity of bacterial enzymes responsible for DNA replication [1]. It achieves this by binding to and blocking the action of the bacterial DNA gyrase enzymes, which are necessary for bacterial DNA synthesis and replication [2]. Like all medications, levofloxacin can cause side effects, some of which can be serious. Common adverse effects of levofloxacin include headaches, dizziness, nausea, and diarrhea. However, there are also rare but severe side effects, including aneurysms, prolongation of the Q-T interval, and tendon rupture, especially in the Achilles tendon. Additionally, levofloxacin may lead to muscle weakness, neuropsychiatric toxicity, and hepatotoxicity [3]. The reproductive side effects of FQs like levofloxacin can be concerning. These effects can include changes in libido, decrease sperm parameters and serum testosterone level, and also testicular damage [4,5]. It is not entirely clear why FQs can cause reproductive side effects. Still, it is thought that the drugs may interfere with the production of certain hormones or enzymes that are involved in reproductive function [6]. Furthermore, it is worth noting that besides the potential effect on reproductive functions, there exists compelling evidence indicating that the administration of levofloxacin has the capability to provoke a state of Oxidative Stress (OS) within the body. This oxidative stress is believed to be initiated by the discernible reduction in the functional capacities of vital antioxidant enzymes, namely Superoxide Dismutase (SOD), catalase (CAT), and Glutathione S-transferase (GST) activities [7,8]. Several studies have

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demonstrated a reasonable connection between oxidative stress and certain reproductive side effects commonly associated with FQs, including but not limited to infertility and testicular damage [9,10]. Levofloxacin and other FQs have been shown to generate ROS in various cell types, including germ cells [11]. This can lead to damage to cellular components, including lipids, proteins, and DNA. Sperm DNA damage and low sperm quality can negatively impact male fertility and contribute to infertility [12]. Therefore, protecting sperm against oxidative stress and damage is a crucial field of research. Deeply rooted in the ancient traditions of Ayurvedic medicine, *Withania somnifera (WS)*, commonly referred to as Ashwagandha, emerges as a remarkable botanical entity that has garnered profound reverence for its profound healing properties. Over the course of countless generations, this medicinal plant has consistently proven its efficacy in alleviating a diverse spectrum of health challenges, earning it a well-deserved position of eminence within holistic healing practices [13]. Today, it is widely used as a dietary supplement and is gaining popularity for its potential health benefits [14]. Ashwagandha plants root is believed to have immunomodulatory, anti-inflammatory, and antioxidant properties, and is commonly used to manage stress, anxiety, depression, insomnia, and pain [15]. According to a comprehensive qualitative analysis of phytochemicals, it has been clearly emphasized that the extracts derived from the roots of *W. somnifera* contain a rich array of compounds, including terpenoids, tannins, flavonoids, alkaloids, carbohydrates, and saponins. These diverse components synergistically contribute to providing the plant with its remarkable antioxidant properties, protecting against oxidative processes. Furthermore, it is crucial to note that the presence of a specific class of compounds called withanolides further boosts the plant's potential to exhibit such exceptional antioxidant capabilities [16]. These compounds are believed to protect cells from oxidative stress by scavenging free radicals, which are highly reactive molecules that can damage cells and contribute to the development of various diseases [15]. *W. somnifera* can increase the activity of enzymes that help to protect cells against oxidative damage, such as SOD, CAT, and glutathione peroxidase [17]. In addition, *W. somnifera* has been found to increase the levels of antioxidants, such as Glutathione and vitamin C, in cells and tissues [18]. Several researches have been conducted to evaluate the possible protective benefits of *W. somnifera* on sperm cells against oxidative stress caused by a variety of stressors such as environmental pollutants and chemotherapy medicines [19,20]. Several studies have suggested that the root extract of *W. somnifera* can enhance sexual health and improve semen parameters by reducing levels of lipid peroxidation, stress, serum cortisol, and reactive oxygen species. Additionally, the extract increases antioxidant levels, improves overall sperm quality, and elevates testosterone and luteinizing hormone levels [13-21].

2. MATERIALS AND METHODS

2. 1. The Plants Extract Preparation

The *W. somnifera* (*WS*)plants were gathered from the garden at Baghdad University's Science College. The roots were carefully cleaned and left to naturally dry in the comforting shade. Once dried, the roots were grinned into a fine powder using a grinder. This finely powdered root was then immersed in a solution of 70% ethanol and left to soak for duration of 48 hours. The solution is then filtered with filter paper. The alcoholic extract method, employing the renowned Soxhlet apparatus, was employed to harness the desired constituents from the root powder. Following the extraction process, any excess alcoholic solvent was skillfully evaporated at a temperature of 60◦C, ensuring the excess alcoholic solvent was evaporated to gain the final extract [17]. And the dose was prepared according to previous report [18].

2.2. The Drug

Levofloxacin (COX pharmaceutical, LTD. Arcade House. Finchley Road. London NW11 7TL, UK) Tablets 500 mg obtained from local pharmacy. It was finely grinding and then dissolved in normal saline to prepare a dose of 10 mg / kg [11].

2.3. Animals

This experiment involved the utilization of a total of thirty adult albino rats, which were housed in breeding cages under carefully controlled conditions. The temperature within the facility was maintained at a range of 25 to 30 degrees Celsius, accompanied by a humidity level of 50 to 55 percent. The rats were provided with standard pellets as their regular diet and had unrestricted access to water (*Ad libitum*). To ensure fairness and statistical significance, the rats were evenly divided into five groups, with each group consisting of six rats (n=6/group).

Group C: These rats received normal saline orally via gavage for a period of 60 days.

Group W: Rats in this group were administered *W. somnifera* extracts orally via gavage at a dose of 500 mg/kg/body weight, also for a duration of 60 days [18].

Group L: The rats in this group were subjected to oral gavage of Levofloxacin at a dose of 10 mg/kg/body weight for 60 days [11].

Group W+L: Prior to receiving Levofloxacin, the rats in this group were pre-treated with *W. somnifera* extract at a dose of 500 mg/kg orally via gavage, and subsequently received Levofloxacin at a dose of 10 mg/kg via gavage for 60 days. Group L+W: In contrast to the previous group, the rats in this group were first administered Levofloxacin at a dose of 10 mg/kg orally

via gavage, and were later post-treated with *W. somnifera* extract at a dose of 500 mg/kg/body weight orally via gavage for 60 days.

2.4. Estimation of TOS and SOD

The serum's total oxidant status (TOS) was measured using an automated analyzer (Thermo Scientific Multiskan FC, Waltham, MA) and Erel's method [22]. The method involved oxidizing the ferrous ion-o-dianisidine complex to the ferric ion in an acidic medium, forming a colored complex with xylenol orange. The color intensity was measured spectrophotometrically at 530 nm wavelengths and correlated with the total amount of oxidant molecules in the serum. Results were expressed in micromolar hydrogen peroxide equivalent per liter (μm H_2O_2 Equiv./L) after calibration with hydrogen peroxide.

2.5. Sperm Parameters Study

2.5.1. The Sperm Concentration

The animals were sacrificed by anaesthetized with chloroform, the epididymis was removed rapidly after the animals had been dissected. The epididymal sperm concentration was calculated according to [24] by cutting the left epididymis into thin slices in a petri-dish which contains 1ml of a normal saline (0.9% NaCl) to release swim sperm. after 10 minutes a drop of a homogenate was loaded on the improved Neubauer hemocytometer and counted under the light microscope (NOVEX, Euromex Co., Holland) a sperm count following WHO [25] criteria.

2.5.2. Sperm Motility

The sperm motility analysis was carried out in accordance with WHO guidelines [25]. Immediately the sperm solution was put on a microscopic slide and covered with a coverslip. Each animal had at least ten microscopic fields examined to evaluate sperm motility. The percentage of sperm motility was calculated for the following: motile parameters: progressive motility, non-progressive motility, and Immotile sperm [24].

2.5.3. Sperm Viability and Morphology

According to WHO [25] guidelines, eosin (1%) and nigrosine (10%) (Merck, Germany) staining were employed to assess sperm viability. One drop of sperm suspension and two drops of 1% eosin were combined. The mixture was then treated with two drops of nigrosine. Thin smears were then produced and examined under an oil immersion magnification. Live sperm remained colorless, but dead sperm colored. A total of 200 spermatozoa were characterized as normal, double head, headless, amorphous head, coiled mid-piece, coiled tail, bent tail, and cytoplasmic droplet in each sample. Sperm viability and abnormality were estimated as a percentage [24].

2.5.4. Quantitative Assessment of DNA Damage

The alkaline comet assay was used to determine the level of DNA damage in sperm. The method followed the protocol [26] with slight modifications. The right Epididymis was removed, and put in a test-tube which contains 1 ml a normal solution (0.9% NaCl) and a 1μl of a homogenate was placed on the improved Neubauer hemocytometer and counted. The cell suspension about (5000-10000) cells were combined and mixed with 1.2 ml low melting agarose (0.5%) before spread out on a slide covered with normal melting agarose (1%). The slides were then incubated in cold lysis prepared immediately at 4°C (18−20 h) in the dark, followed by alkaline buffer and electrophoresis for 25 min at a voltage of 0.6 V/ cm. The slides were then neutralized with 0.4 M Tris base. Then fixed in 100% cold ethanol, and stained with a solution containing SYBR green (1X diluted in PBS) for 20 min in the refrigerator. After that, slides were rinsed with 500ml distilled water to remove excess stain. The slides were examined by the ZEISS Primo Star fluorescence microscope in green light (Carl Zeiss, GmbH, Deutschland) equipped with a digital camera connected to the computer. The length of the comet tail was measured by 100 DNA of cells in each sample that were randomly analysed and scored for the tail intensity (% DNA in tail) using comet score 2.0 software (Rex Hoover, USA) [27].

2.6. Statistical Analysis

The values were represented as Mean ± Standard Error SE and analyzed by one-way ANOVA followed by Revised Least Significant Differences (LSD). Using GraphPad Prism 9.5GraphPad ® Software, CA, USA. The statistical significance was set at $P<0.05$.

3. RESULTS AND DISCUSSION

3.1. The Oxidative Stress

The findings of the study demonstrate that the use of Levofloxacin resulted in a significant elevate in TOS (Total Oxidant Status) levels and a significant lower in SOD (Superoxide Dismutase) levels when compared with the control group. However, the administration of *WS* extract in the L+W and W+L groups exhibited a significant reduction ($P<0.05$) in TOS levels compared with the L treatment alone. Interestingly, the SOD levels in the W+L group did not exhibit a significant change ($P>0.05$), whereas the L+W group displayed a significant increase ($P<0.05$) when compared with the L group, although it did not reach the level observed in the Control group as it appeared in Figures 1 and 2.

The Oxidative Stress (OS) play a significant role in the pathophysiology of reproductive dysfunction and sperm quality. In the present study, it was observed that rats treated with Levofloxacin exhibited significantly higher TOS levels compared with the control group, indicating elevated oxidative stress. Conversely, the SOD levels in the Levofloxacin-treated group were found to be lower than those in the control group, suggesting a reduction

in the body's antioxidant defense mechanism. Similar findings showed that levofloxacin treatment caused a significant elevation (P<0.05) in MDA and it significantly reduced in SOD, CAT and GSH in the animals that received oral levofloxacin (40mg/kg/day) for fourteen days [11]. Moreover, Olayinka *et al*. [8] found that administering different doses of levofloxacin (5mg/kg, 10mg/kg, 20mg/kg) caused oxidative stress in the hepatic and renal tissues of rats. This led to increased levels of MDA and reduced levels of GSH and Vitamin C. In addition, the activities of antioxidant enzymes such as SOD, CAT, and GST were decreased. As a result of being treated with FQs, an unbalance between oxidants and antioxidants increased the formation of ROS, which in turn caused oxidative stress. This is associated with pathogenic elements and contributes to cellular deterioration. [28],[29],[30]. Moreover, it may result in mitochondrial dysfunction and overproduction of ROS, leading to oxidative damage to proteins, DNA, and phospholipids of membranes [31]. Levofloxacin and *WS* extract co-treatment result in decreased lipid peroxidation by decreased TOS levels and increased SOD levels. *WS* extract contains various Antioxidants compounds such as fatty acid ester, essential amino acids, flavonoid, phenolic compounds, and antioxidant activities [19],[32].

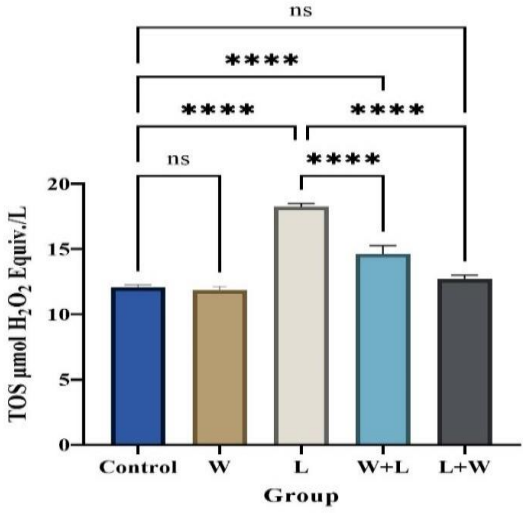

Figure 1. The effect of W. Somnifera and before/after treated Levofloxacin on TOS. Data are presented as mean ± SE (n = 6). The Asterisks represent significance differences from control: *(P<0.05), **(P<0.01), ***(P<0.001), ****(P<0.0001).

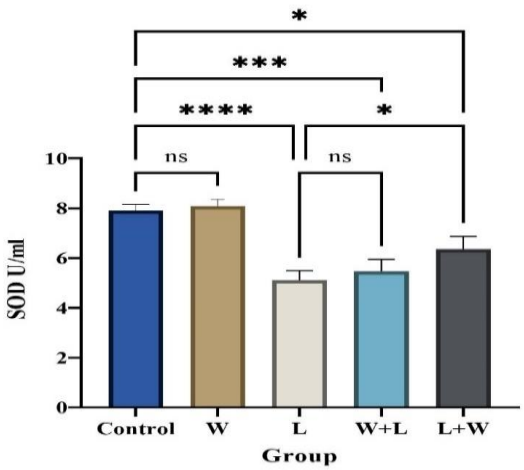

Figure 2. The effect of *W. Somnifera* and before/after treated Levofloxacin on SOD. Data are presented as mean ± SE (n = 6). The Asterisks represent significance differences from control: *(P<0.05), **(P<0.01), ***(P<0.001), ****(P<0.0001).

3.2. Sperm Parameters

The statistical analysis and means of various sperm parameters across the five groups are presented in Table 1. The table clearly indicates significant differences (P<0.05) between the groups regarding sperm concentration, motility (progressive, non-progressive, immotile), viability, as well as morphology (Normal, Head abnormal, and Tail abnormal). This study observed significant decreases in various sperm parameters, including motility, concentration, viability, and morphology. In addition, it showed significant increases in head and tail abnormality in the Levofloxacin treated groups. This finding agrees with previous studies that pointed out that FQs drugs especially levofloxacin cause a significant reduction in sperm count, vitality and motility [5,6]. The sperm membrane is particularly vulnerable to free radical attacks due to the substantial amount of polyunsaturated fatty acids. Sperm viability and concentration can be diminished because sperm membrane damage caused by lipid peroxidation makes it lose its integrity [28]. Oxidative stress influences sperm motility by modifying axoneme structure, resulting in sperm tail abnormalities and decreased motility [33]. This study revealed a significant increase in TOS in levofloxacin-treated rats, which would increase mitochondrial membrane permeability, disrupting the respiratory chain and ATP generation, as well as reduce phosphorylation of axonemal proteins, decreasing sperm quality [34]. Our results found a significant enhancement in baseline sperm viability; morphology and motility after 60 days of *WS* extract administration. This finding is in line with other research that investigated the *WS* extract potential

benefits for male reproductive health. It has been demonstrated that *WS* extract possesses antioxidant characteristics that can help protect sperm from damage caused by oxidative stress [19],[20].

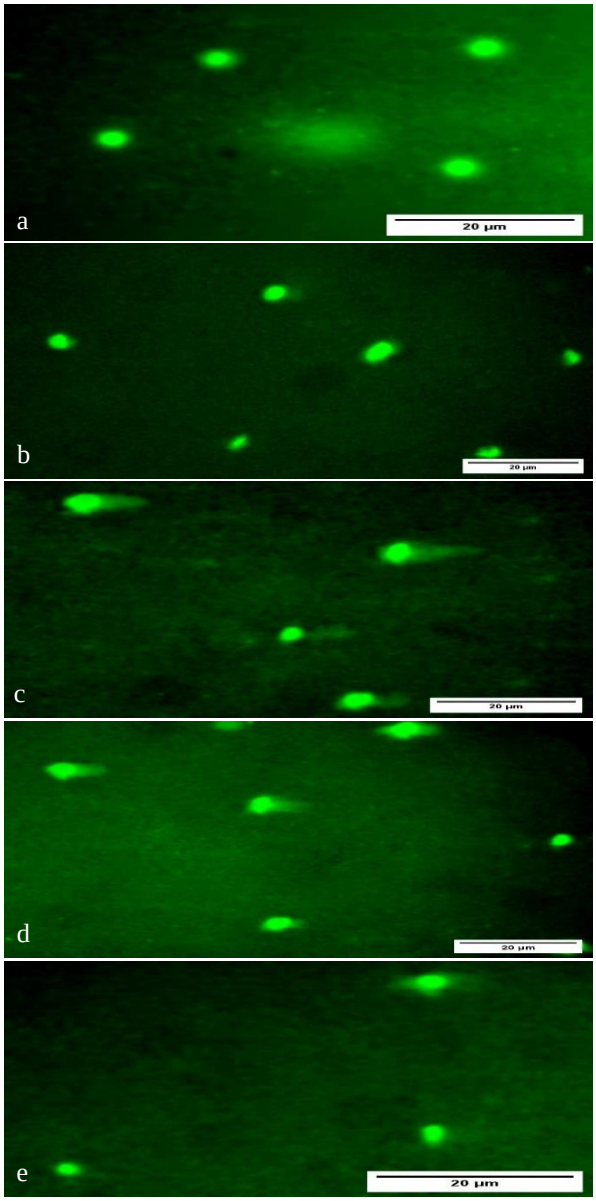

Figure 3. Microscopic photographs showing the DNA damage in rat sperm nuclei Fi shown by Comet assay. (a) Nucleus of sperm from the control group. (b) Nucleus of sperm from the W. sominifera treated group. (c) Nucleus of sperm from the Levofloxacin-treated group. (d) Nucleus of sperm from Levofloxacin + W. sominfera group. (e) Sperm nuclei from W. sominfera + Levofloxacin group. (Scale bar 20 µm. Dye: SYBR Green).

This study aimed at examining the impact of *WS* extract on DNA damage in rat sperm induced by Levofloxacin, the researchers employed a comet assay to evaluate genotoxicity. The results of this assay were visually represented in Figure 3. It was observed that the control group and the group treated with *WS* extract exhibited intact nuclei of undamaged cells, as depicted in Figure 3a and 3b, respectively. On the contrary, the group treated with Levofloxacin displayed evident DNA damage in the form of comet-like structures, as shown in Figure 3c. In contrast, the group receiving a combination of *WS* extract and Levofloxacin (W+L) exhibited a low level of DNA damage within the cell nuclei (Figure 3d). Similarly, the group receiving Levofloxacin followed by *WS* extract (L+W) displayed normal nuclei of undamaged cells (Figure 3e). It is important to note that the administration of *WS* extract alone did not cause any genotoxic effects on sperm. Furthermore, the statistical analysis revealed that the percentage of DNA in the comet tail was significantly increased in the Levofloxacin-treated group compared to the control group, indicating significant DNA damage. Conversely, the *WS* extract -treated group showed non-significant increases ($P>0.05$) in the percentage of DNA in the tail. When *WS* extract was co-administered with Levofloxacin, the group (W+L) exhibited a non-significant reduction ($P>0.05$) in the percentage of DNA in the comet tail, while the group (L+W) displayed a significant reduction compared to the Levofloxacin-treated group (Table 1). The results of this study indicate that exposure to Levofloxacin led to significant damage to rat sperm DNA, as demonstrated by the comet assay. This damage was evidenced by the appearance of comets in the nuclei of sperm cells. Several studies have reported that levofloxacin, a fluoroquinolone antibiotic, can induce Oxidative Stress and damage in DNA of sperm cells [9],[35],[36]. For instance, in a study by Al-Dujaily *et al*. [37], rats treated with levofloxacin showed significant increases in sperm DNA fragmentation, as measured by the sperm chromatin structure assay, compared to control. Because levofloxacin is a topoisomerase inhibitor, the current study assumed that it limits the formation and ligation of nicks in DNA, preventing protamination and, as a result, causing internal DNA damage by restricting repair and increasing sensitivity to damage [29],[36]. In a study by Al-Soufi & Al-Rekabi [38], rats were given different doses of levofloxacin (7.5mg/kg/bw and 15mg/kg/bw) orally for 2 or 4 weeks. There were no significant differences in micronuclei assay and chromosomal aberration among the groups. However, after a period of thirty days, a significant decrease was observed in both nuclear division index and mitotic index values, and the comet assay showed a significant reduction in both treated groups in comparison to the control. The study concluded that levofloxacin is cytotoxic but not genotoxic in male rats. However, co-administration of *WS* extract with Levofloxacin resulted in a reduction in DNA damage compared to Levofloxacin alone. Based on the comprehensive

literature review, this study was the first to investigate the effects of *WS* extract on improving the comet assay, a test used to evaluate DNA damage in sperm. In recent research, it has been discovered that taking *WS* extract can have positive effects on men's reproductive health. Specifically, it has been found that ashwagandha extract supplementation can boost important semen parameters such as sperm viability, motility, and protection against DNA damage [19]. These findings highlight the potential of *WS* extract as a natural and beneficial addition to support male fertility and overall reproductive well-being. In addition, *WS* extract has been suggested to enhance testosterone levels and reduce stress and anxiety, which can have positive effects on male fertility [39]. The beneficial impacts of specific medicinal herbs on specific sperm characteristics can be attributed to their potent antioxidant properties. This is particularly relevant in the case of *WS* extract, as it contains essential phytochemical components and antioxidant minerals that contribute to its significance in addressing infertility concerns [18]. These results highlight the potential of *WS* extract as a promising therapeutic option for mitigating the adverse effects of Levofloxacin on sperm health.

TABLE 1. Result of Sperm Parameters in The Different Groups.

-Data are presented as mean ± SE (n=6). Deferent latter refer to significant differences.

Variables	Group (C)	Group W	Group L	Group W+L	Group L+W
Concentration ($\times 10^6$)	152.17 ±16.21 AB	158.0 ±8.30 B	75.17± 8.14 C	117.67 ±12.9 D	120.83 ±11.35 D
Progressive (%)	56.9 ±0.63 A	74.9 ±1.18 B	26.0 ±0.78 C	40.6 ±0.98 D	42.2 ±0.55 D
Non-progressive (%)	28.9 ±1.14 A	17.2 ±0.07 B	32.6 ±0.52 C	38.2 ±0.58 D	37.9 ±1.10 D
Immotile (%)	14.2 ±1.23 A	7.9 ±1.27 B	41.4 ±0.70 C	21.2 ±1.65 D	19.7 ±1.11 D
Viability (%)	77.8 ±1.12 A	83.9 ±0.58 B	51.3 ±0.87 C	64.6 ±1.58 D	72.2 ±0.90 E
Normal morphology (%)	82.0 ±0.65 A	85.8 ±1.21 B	60.8 ±0.56 C	76.1 ±0.76 D	79.3 ±0.61 AD
Abnormal Head (%)	6.4 ±0.66 AD	5.2 ±0.88 A	20.6 ±1.15 B	11.3 ±0.61 C	7.5 ±0.24 D
Abnormal Tail (%)	11.4 ±0.51 A	8.9 ±0.85 B	18.6 ±0.72 C	12.4 ±0.64 A	13.0 ±0.69 A
DNA Damage (Tail % DNA)	2.22 ±0.33 A	3.41 ±0.98 AC	7.54 ±1.11 B	5.48 ±0.96 BC	4.65 ±0.58 AC

4. CONCLUTOIN & FUTURE STUDIES

In conclusion, the findings of this study suggest that *WS* extract may possess a protective effect against Levofloxacin-induced DNA damage in rat sperm. However, it is important to conduct further studies in this area to explore its full potential and provide more insights. More research is needed to understand how exactly this protection works and to determine the optimal dosage and duration of both levofloxacin and *WS* to use.

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Arabic Abstract

الطب الشعبي في مختلف الثقافات يعتمد على النباتات الطبية لما لها من خصائص علاجية مميزة ومن بين هذه النباتات نبات الأشواغندا *Withania somnifera*، الذي يشتهر بقدرته على تحسين الصحة الجنسية وتحسين جودة السائل المنوي وتثبيط من عملية الاكسدة الفوقية للدهون فضلا عن خصائصه المضادة للشيخوخة، والمضادة للالتهابات. الهدف الرئيسي من هذه الدراسة هو تقييم الدور الوقائي المحتمل لنبات الاشواغندا *W. somnifera* ضد تأثيرات الجانبية لدواء الليفوفلوكساسين Levofloxacin على مستوى جودة النطف والتلف الحاصل للحامض النووي DNA للحيوانات المنوية في الجرذان. ومن أجل تحقيق هذا الهدف، تم أستخدام ثلاثين جرذا بالغًا من الذكور وتم تقسيمها بصورة عشوائية على خمس مجموعات تجريبية منفصلة، بما في ذلك المجموعة الاولى السيطرة (C) والمعاملة بمحلول ملحي لمدة 60 يومًا. المجموعة الثانية (W) وتم تجريعها بمستخلص جذر نبات الاشواغندا *W. somnifera* عن طريق الفم لمدة 60 يومًا. المجموعة الثالثة (L) تم تجريعها دواء الليفوفلوكساسين عن طريق الفم لمدة 60 يوما. تم إعطاء المجموعة الرابعة (W+L) دواء الليفوفلوكساسين عن طريق الفم ومعالجتها مسبقًا بمستخلص جذر نبات الاشواغندا *W. somnifera* لمدة 60 يومًا. المجموعة الخامسة (L+W) تم إعطاء دواء الليفوفلوكساسين وبعد ذلك تم معالجته بمستخلص جذر نبات الاشواغندا *W. somnifera* عن طريق الفم ولمدة 60 يومًا. وقد تم قياس درجة التأثير باستخدام بعض المعايير الحيوية كالإجهاد التأكسدي، وتركيز الحيوانات المنوية والنسبة المئوية للحركة والحيوية وأيضا النسبة المئوية للنطف السوية، وأختبار نسبة تحطم جزيئة DNA (فحص المذنب). أدى تجريع دواء الليفوفلوكساسين إلى زيادة معنوية ($P < 0.05$) في العديد من المعايير، بما في ذلك حالة الأكسدة الكلية (TOS)، النسبة المئوية للنطف غير السوية (تشوه الرأس والذيل)، الحيوانات المنوية غير المتحركة. فضلا عن ذلك، لوحظ وجود تلف كبير في الحامض النووي DNA للحيوانات المنوية، كما أكد ذلك فحص المذنب. علاوة على ذلك، أدى تجريع بالليفوفلوكساسين إلى انخفاض معنوي ($P < 0.05$) في مستويات أنزيم سوبر أوكسيد ديسموتاز (SOD) وانخفاض معنوي ($P < 0.05$) في تركيز الحيوانات المنوية وحركتها وحيويتها. كما أدت المعالجة بمستخلص جذور نبات الاشواغندا *W. somnifera* قبل وبعد تجريع دواء الليفوفلوكساسين إلى تحسين بعض هذه التغيرات في المعايير الكيموحيوية وكذلك تحسين من جودة الحيوانات المنوية. في الختام، نستنتج من هذه الدراسة قد كان لمستخلص جذور نبات الاشواغندا *W. somnifera* دورا أيجابيا للحماية من ضرر الحيوانات المنوية الناجم عن دواء الليفوفلوكساسين في الجرذان.