Original Article

Molecular Signatures of Bisphenol A Mediated Sperm Dysfunction and the Mitigating Role of Ashwagandha in an Experimental Model

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ABSTRACT

Objective: To evaluated the effects of chronic Bisphenol A exposure and the protective role of Withania Somnifera (Ashwagandha).

Study Design: Experimental study

Place and Duration of Study: This study was conducted at the Jabir Ibn Hayyan University for Medical & Pharmaceutical Sciences, Najaf, Iraq from 1st January 2026 to 31st March 2026.

Methods: The forty adult male Wistar rats was divided into four groups included control, Bisphenol A (50 mg/kg/day, intraperitoneally), Ashwagandha (300 mg/kg/day, orally), and Bisphenol A + Ashwagandha for 60 days. At the end of study sperm parameters, DNA fragmentation, and testicular expression of Ahr, Adgb, Arl2bp, Dnali1, Qrich2, and Cfap157was assessed

Results: The Bisphenol A reduced significantly in sperm motility, morphology, concentration, and viability, increased DNA fragmentation, and down regulated spermatogenesis that related genes while the Ashwagandha markedly improved sperm quality, which reduced DNA damage, and restored gene expression toward normal levels.

Conclusion: Ashwagandha protects against Bisphenol A induced sperm dysfunction by reducing oxidative/genotoxic stress and supporting transcriptional pathways essential for spermatogenesis.

Key Words: Bisphenol A, Ashwagandha, Sperm motility, Gene expression, Oxidative stress, Aryl hydrocarbon receptor

Citation of article: Al-Hakeem AH, Aldujaili HH, Bakheet NZ, Al-Dhalimy AMB. Molecular Signatures of Bisphenol A Mediated Sperm Dysfunction and the Mitigating Role of Ashwagandha in an Experimental Model. Med Forum 2026;37(7):124-129. doi:10.60110/medforum.370725

INTRODUCTION

The Bisphenol A (BPA) is a widely used in polycarbonate plastics and epoxy resins and is well recognized as an endocrine disruptor associated with male reproductive toxicity moreover the previous studies illustrated that BPA causes disrupts in the hypothalamic pituitary gonadal axis, steroidogenesis, and testicular redox balance, that sequentially leading to destroy the spermatogenesis and reduced sperm fertilizing capacity.[1] Furthermore, exposure to BPA lead to hormonal disruption, which that linked to mitochondrial dysfunction, oxidative stress, and epigenetic alterations that disturb spermatogenic processes.[2,3]

At the molecular level, the BPA exposure that disrupted the transcription of genes involved in sperm structure, motility, and flagellar formation which that due to downregulation of Ahr, Adgb, Arl2bp, and Dnali1cause flagellar stability, energy regulation, and intramanchette transport, resulting in sperm tail abnormalities.[4] Similarly, altered expression of Qrich2 and Cfap157, are associated with axonemal organization, and linked to asthenoteratozoospermia and sperm flagellar defects.[5] Oxidative stress represents a central mechanism of BPA-induced reproductive damage, promoting apoptosis, lipid peroxidation, mitochondrial dysfunction, DNA fragmentation, and chromatin instability in spermatozoa.[6]

The BPA also affect male fertility indirectly through gut microbial dysbiosis, which increased lipopolysaccharide translocation, systemic inflammation, and disruption of the gut–testis axis.[7] In addition, transcriptomic and proteomic evidence showed that BPA suppress the INSL3 expression, destroyed the Leydig cell function, and interfere with

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Received: April, 2026
Reviewed: May, 2026
Accepted: June, 2026

the RNA splicing and meiotic progression, further compromising spermatogenesis.[8]

On other hands the natural antioxidants and adaptogenic phytochemicals have gained attention as protective agents against BPA-induced reproductive toxicity which the previous study showed that Withania Somnifera (Ashwagandha) has demonstrated as antioxidative, androgenic, and anti-apoptotic effects on male fertility.[9] The Antioxidant roles Ashwagandha due to its bioactive constituents, which including with anolides and sitoindosides, may enhance antioxidant enzyme activity, reduce oxidative injury, preserve seminiferous epithelial integrity, support Leydig cell function, and improve sperm quality.[10]

Thus the present study aimed to evolution the functional and molecular alterations that induced by BPA exposure in adult male rats and to evaluate the protective efficacy of Ashwagandha supplementation which focused on sperm motility, morphology, viability, chromatin integrity, and the expression of fertility-related genes, including Adgb, Arl2bp, Dnali1, Qrich2, Cfap157, and Ahr.

METHODS

This experimental study was conducted at Jabir Ibn Hayyan University for Medical & Pharmaceutical Sciences, Najaf, Iraq from 1st January 2026 to 31st March 2026 vide letter No. 1007A/JBU/Approval/ JSDJNEHU dated December 25, 2025. A total of forty healthy, sexually mature male Rattusnorvegicus (Wistar strain), weighing 180–220 g, were procured from a certified animal facility (Abugrab Animal House, Baghdad, Iraq) and the animals were acclimatized for two weeks under standard laboratory conditions with ad libitum access to standard pellet diet and filtered water.

The present study was conducted to elucidate the molecular and functional alterations in sperm quality induced by BPAss and to evaluate the potential protective effects of Ashwagandha in adult male Wistar rats. The animals were randomly divided into four equal groups (n=10 each) and treated orally for 60 consecutive days as follows: Group I, the control (C) received vehicle only (corn oil), Group II (BPA) received Bisphenol A intraperitoneally at a dose of 50 mg/kg body weight/day.[11] Group III (ASH) received Ashwagandha extract orally at a dose of 300 mg/kg body weight/day[12], and Group IV (BPA + ASH) received a combination of BPA intraperitoneally at a dose of 50 mg/kg and Ashwagandha orally at a dose of 300 mg/kg concurrently.[12]

To prepare the BPA solution, the amount of BPA was weighed and dissolved normally to dose at 50 mg/kg body weight. The prepared BPA solution was stored in an amber container at room temperature until administration via intraperitoneally injection. Ashwagandha (Withania somnifera) root powder was obtained from Sigma-Aldrich (St. Louis, MO, USA; Catalog No. USP 1043309; CAS No.: 90147-43-6; EC No.: 290-434-9). The product, listed as Ashwagandha Root Powde, was derived from authenticated plant roots and standardized according to the manufacturer's specifications. The material was stored in airtight containers at room temperature, protected from light and moisture, and handled in accordance with the supplier's recommendations until use in the experiment. They were euthanized at the end of experimental at the 60-day experimental period by intramuscular overdose with a mixture of Xylazine (Micopite, USA; 40 mg/kg) and Ketamine (Alpha Than, Netherlands; 90 mg/kg). Blood samples (approx 5 mL) once again collected through cardiac-puncture, scrotal area properly washed with sterile normal saline followed by removal of bilateral testis and epididymal tissues.[13] The epididymal tail was incubated with 2 mL normal saline at 37°C followed by anatomical micro-scissors to segment the tissue and extract spermatozoa for further analysis including an assessment of motility, concentration, morphology, and DNA Integrity according to protocol already standardized in the investigation.

Sperm motility, morphology, viability, concentration, and DNA fragmentation were assessed using standard microscopic methods. Eosin–Nigrosin staining was used to evaluate sperm morphology and viability, while Acridine Orange staining was used to assess sperm chromatin integrity. Progressive motility was examined under a light microscope at 400× magnification using a pre-warmed slide. For morphology and viability, sperm smears were prepared with Eosin–Nigrosin stain and examined microscopically, where stained spermatozoa were considered non-viable or abnormal. Sperm concentration was determined after dilution of the epididymal sperm suspension according to the method of Smith and Mayer.[13]

For DNA fragmentation assessment, sperm smears were fixed in methanol:glacial acetic acid solution (3:1), stained with Acridine Orange, and examined under a fluorescence microscope.[14] Sperm heads showing green fluorescence were considered to have intact and condensed DNA, whereas orange to red fluorescence indicated denatured or fragmented DNA and impaired chromatin integrity.[13]

Molecular Gene Expression Analysis by qPCR: Testicular tissue samples were stored at −80°C until molecular analysis. Total RNA was extracted using TRIzol reagent (Trans®, China; Cat. No. ET101-01), and RNA concentration and purity were assessed spectrophotometrically. cDNA was synthesized using a One-Step RT-PCR Premix Kit (Promega, USA) according to the manufacturer's instructions. Quantitative real-time PCR was performed using GoTaq® qPCR Master Mix and SYBR Green-based detection to assess the relative expression of target genes. Specific primers for Ahr, ADGB, ARL2BP, DNALI1, QRICH2, CFAP157, and Gapdh were

designed based on NCBI GenBank sequences and synthesized by Integrated DNA Technologies, Inc. (IDT, USA). Relative mRNA expression was normalized to Gapdh, and primer sequences with accession numbers (Table 1). Data were analyzed by SPSS-26. The normality of data distribution was verified using the Shapiro-Wilk test and mixed-model two-way analysis of variance (ANOVA) was employed to assess the differences among treatment groups. Differences were considered statistically significant at $P < 0.05$.

RESULTS

The adverse effects of BPA exposure and the protective influence of Withania somnifera (Ashwagandha) root extract on sperm quality and testicular gene expression in adult male rats. BPA exposure caused a highly significant reduction in progressive sperm motility 52.8% compared with the control group 81.5% (P<0.0001). In contrast, Ashwagandha alone maintained sperm motility at a high level (85.7%), while co-administration of BPA with Ashwagandha markedly restored motility (81.1%) to values comparable with the control group (P>0.05) (Fig. 1). Regarding sperm morphology, Figure 3 showed that BPA significantly reduced the percentage of morphologically normal spermatozoa (80.0%) compared with the control group (97.8%) [P<0.0001]. However, rats treated with Ashwagandha alone showed normal sperm morphology (98.21%) comparable to the control group. Similarly, the BPA+ASH group showed a marked improvement in sperm morphology (98.16%) compared with the BPA group (P<0.0001), with no significant difference from the control group (P>0.05).

Figure 2 showed the epididymal sperm concentration was significantly decreased in the BPA-treated group (4.21×10^8 sperm/g) compared with the control group (8.96×10^8 sperm/g) (P<0.0001), indicating impaired spermatogenesis and reduced sperm production. Conversely, Ashwagandha treatment increased sperm concentration in the ASH group (9.24×10^8 sperm/g) and restored it in the BPA+ASH group (8.65×10^8 sperm/g), with both groups showing values comparable to the control group (P>0.05).

Sperm viability was presented in the Figure 3 showed BPA exposure significantly reduced the percentage of live spermatozoa (61.1%) compared with the control group (83.5%) (P<0.0001) in while the Ashwagandha alone increased sperm viability to 86.6%, which co-treatment with BPA and Ashwagandha restored

Table No. 1: Primers obtained from the National Center for Biotechnology Information (NCBI) platform

Gene		Primer	NCBI Accession No.
	Direction	5′ → 3′	
Ahr v1	Forward	GTCAGCCATGGTCAGTCCTCAG	NM_013149.3
	Reverse	GCTCGGACTCTGAAACTTGCT	
ADGB	Forward	GCCTCAGGCATGACTTCCAA	NM_001432028.1
	Reverse	ACCAGGTGGCACATTGTCTT	
ARL2BP	Forward	TTCGCGCTGTCCTTAAAACAC	NM_001024906.2
	Reverse	GCAGAGGAGCTGAAAACACAT	
DNALI1	Forward	TCAGCAACTGAAGGCCCAAC	NM_001031647.1
	Reverse	CATGACACGGGGACAACAGT	
QRICH2	Forward	CAGCCTGACCAAGATCACCG	NM_001416895.1
	Reverse	TCCAGGTATTCGTCTCGGCT	
CFAP157	Forward	GCCTATCCAAAGACAGCCGA	NM_001100869.1
	Reverse	GGTGGGATGTGGACCTGAAG	
Gapdh	Forward	ATGAAGGGGTCGTTGATGGC	NM_017008.4
	Reverse	AGAGACAGCCGCATCTTCTT	

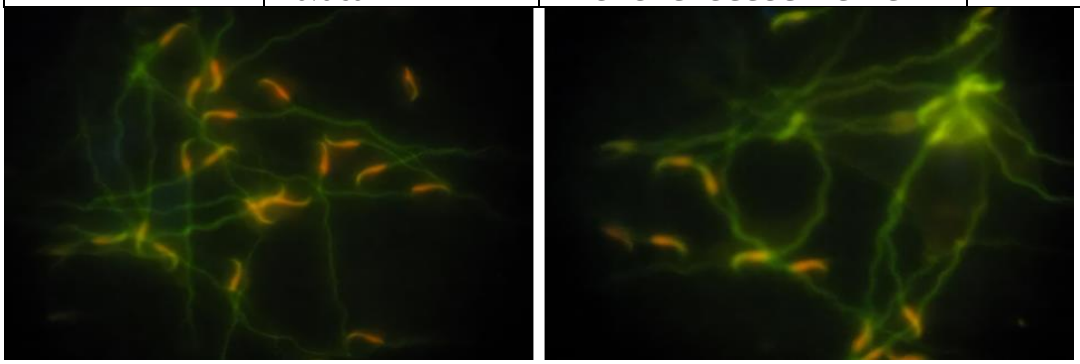

Figure No. 1: Fluorescence micrograph of spermatozoa stained with Acridine Orange showing green fluorescence in sperm with intact double-stranded DNA and orange fluorescence in sperm with fragmented DNA

viability to 84.0%, showing significant improvement compared with BPA alone and no significant difference from the control group (P>0.05).

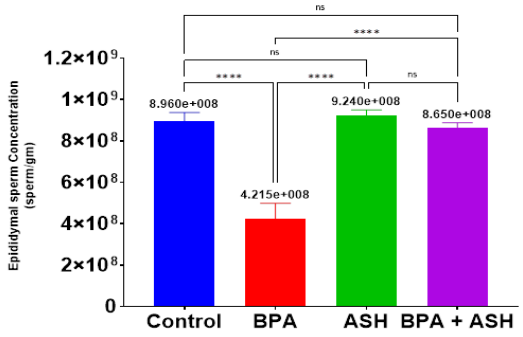

Figure No. 2: Effect of Bisphenol A and Ashwagandha on Epididymal Sperm Concentration (sperm/gm)

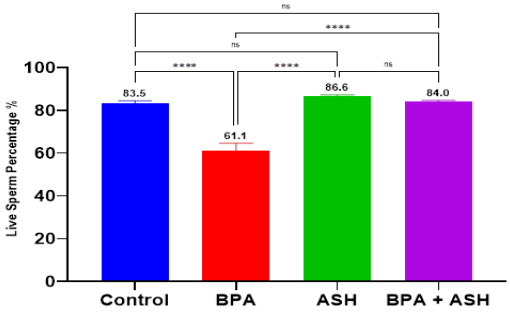

Figure No. 3: Effect of Bisphenol A and Ashwagandha on Sperm Viability (%)

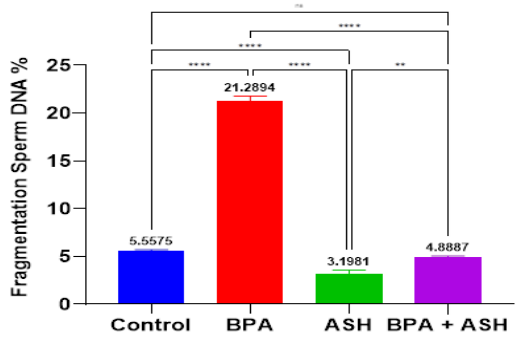

Figure No. 4: Effect of Bisphenol A and Ashwagandha on Sperm DNA Fragmentation (%)

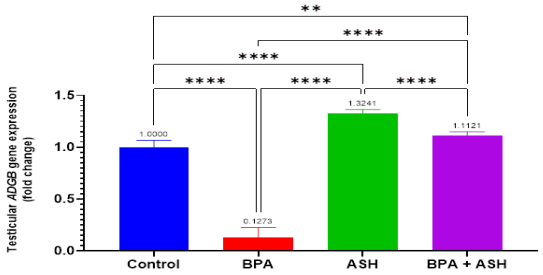

Figure No. 5: Effect of Bisphenol A and Ashwagandha on Testicular ADGB Gene Expression

Figure 4 demonstrated that BPA significantly increased sperm DNA fragmentation (21.29%) compared with the control group (5.56%) (P<0.0001), indicating marked genotoxic stress and compromised sperm chromatin integrity. In contrast, Ashwagandha alone reduced DNA fragmentation to 3.20%, while the BPA+ASH group showed a marked reduction to 4.89%, restoring DNA integrity to levels statistically comparable with the control group (P>0.05).

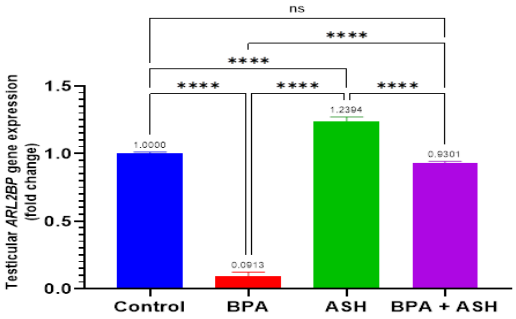

Figure No. 6: Effect of Bisphenol A and Ashwagandha on Testicular ARL2BP Gene Expression

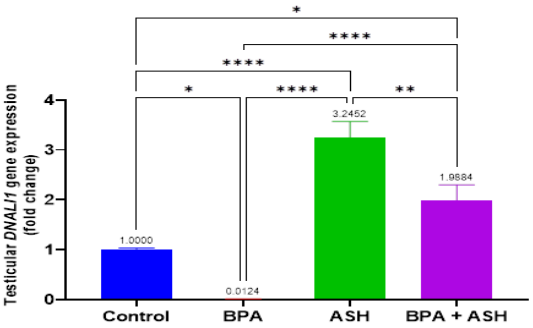

Figure No. 7: Effect of Bisphenol A and Ashwagandha on Testicular DNALI1 Gene Expression

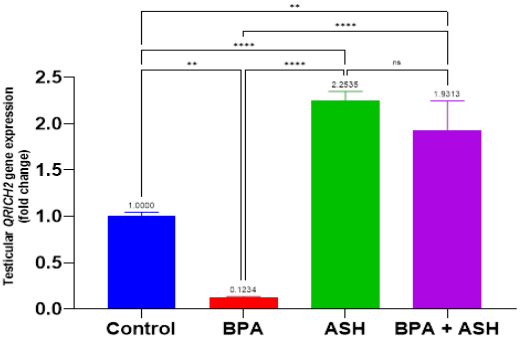

Figure No. 8: Effect of Bisphenol A and Ashwagandha on Testicular QRICH2 Gene Expression

At the molecular level, BPA exposure significantly downregulated genes associated with spermatogenesis, flagellar structure, and sperm motility. ADGB expression was markedly reduced in the BPA group (0.1273-fold) compared with the control group (1.0000-fold) (P<0.0001). Ashwagandha alone significantly upregulated ADGB expression (1.3241-fold), while BPA+ASH restored its expression to 1.1121-fold, with values comparable to the control group (Fig. 5).

Similarly, Figure 6 showed that ARL2BP expression was significantly suppressed in the BPA group (0.0913-fold) compared with the control group (P<0.0001). Ashwagandha alone increased ARL2BP expression to 1.2394-fold, whereas co-treatment with BPA and

Ashwagandha restored its expression to 0.9301-fold, with no significant difference from the control group (P>0.05).

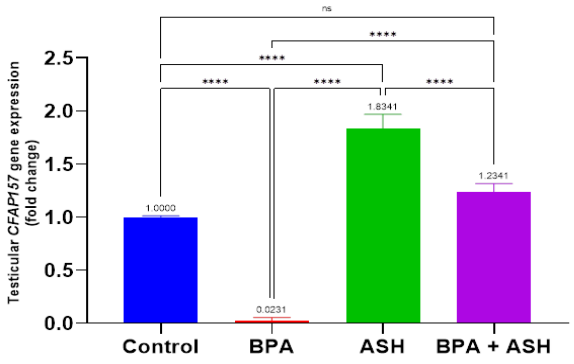

Figure No. 9: Effect of Bisphenol A and Ashwagandha on Testicular CFAP157 Gene Expression

The BPA exposure caused a profound reduction in DNALI1 expression (0.0124-fold) compared with the control group (1.0000-fold) (P<0.0001). Treatment with Ashwagandha alone markedly increased DNALI1 expression (3.2452-fold), while BPA+ASH significantly restored expression to 1.9884-fold compared with BPA alone (P<0.0001), partially exceeding the control level (Fig. 7).The expression of QRICH2 was also significantly affected by BPA exposure. The BPA group showed a marked reduction in QRICH2 expression (0.1234-fold) compared with the control group (1.0000-fold) (P<0.0001). Ashwagandha alone increased QRICH2 expression to 2.2535-fold, while BPA+ASH restored expression to 1.9313-fold, remaining statistically comparable with the control group (P>0.05) [Fig. 8].

Finally, BPA significantly down regulated CFAP157 expression (0.0231-fold) compared with the control group (1.0000-fold) (P<0.0001). Ashwagandha alone significantly increased CFAP157 expression (1.8341-fold), and co-administration of BPA with Ashwagandha restored expression to 1.2341-fold, indicating a protective effect against BPA-induced disruption of genes involved in flagellar assembly and sperm motility (Fig. 9).

DISCUSSION

These alterations indicate that BPA induces both cytotoxic and genotoxic damage to spermatozoa, most likely through oxidative stress, mitochondrial dysfunction, and endocrine disruption. The observed increase in DNA fragmentation suggests impaired sperm chromatin remodeling during spermiogenesis, as BPA has been reported to disturb histone-protamine replacement, increase histone H3 acetylation, and alter DNA methylation patterns, thereby weakening chromatin compaction and increasing susceptibility to oxidative DNA damage.[15] Similar associations between BPA exposure and sperm DNA fragmentation have also been reported in human studies, supporting the genotoxic effect observed in the present rat model.[16]

Ashwagandha supplementation significantly ameliorated BPA-induced reproductive damage by improving sperm motility, morphology, viability, and concentration, while reducing DNA fragmentation. These protective effects may be attributed to the bioactive constituents of Withania somnifera, particularly with anolides and sitoindosides, which possess antioxidant, anti-inflammatory, and mitochondrial-stabilizing properties.[12] By enhancing endogenous antioxidant defenses and preserving sperm membrane and mitochondrial integrity, Ashwagandha may reduce lipid peroxidation and ROS-mediated chromatin damage. The reduction in DNA fragmentation in the BPA+ASH group suggests that Ashwagandha may preserve chromatin condensation and protect sperm DNA from oxidative injury, similar to the protective effects reported for other natural antioxidants against BPA-induced reproductive toxicity.[17]

At the molecular level, BPA exposure caused marked downregulation of genes involved in spermatogenesis, sperm motility, and flagellar structure. BPA-induced suppression of Ahr suggests disruption of aryl hydrocarbon receptor signaling, which plays an important role in xenobiotic metabolism, redox regulation, and testicular homeostasis. BPA may disturb AHR-related pathways and promote oxidative stress through activation of detoxification enzymes such as CYP1A1 and CYP1B1, thereby increasing ROS production within testicular tissue.[1] The restoration of Ahr expression in the BPA+ASH group indicates that Ashwagandha may protect AHR signaling by reducing oxidative and inflammatory stress, possibly through modulation of NF-κB, MAPK, and antioxidant-related pathways.[7]

The down-regulation of ADGB, ARL2BP, DNALI1, QRICH2, and CFAP157 in BPA-treated rats provides molecular evidence for impaired sperm tail formation, axonemal organization, and flagellar motility. ADGB is associated with male fertility, ciliogenesis, and flagellar development, and its suppression may compromise sperm tail formation and motility.[17] Similarly, ARL2BP contributes to microtubule organization and flagellar stability, while DNALI1 encodes a dynein axonemal component essential for flagellar bending and sperm motility.[13] The profound reduction of DNALI1 observed after BPA exposure may therefore explain the marked decline in sperm motility. In contrast, Ashwagandha restored or upregulated these genes, suggesting improved mitochondrial activity, ATP availability, and cytoskeletal organization, which are essential for sperm movement and structural integrity.[17]

CONCLUSION

The extended exposure to bisphenol A induces substantial reproductive harm in male rats. This toxicity is seen by diminished sperm motility, morphology, and viability, and heightened DNA fragmentation inside the reproductive system. The adverse effects are associated with the downregulation of transcription of critical

spermatogenic and structural genes, including Ahr, ADGB, ARL2BP, DNALI1, QRICH2, and CFAP157. These genes together regulate the formation of flagellar structures, the functionality of mitochondria, and the stability of chromatin. The modifications were substantially mitigated by the addition of Ashwagandha. This was achieved by reinstating antioxidant defense, preserving redox homeostasis, and upregulating genes associated with sperm motility and fertility. Ashwagandha has significant protective and restorative effects on testicular function.

Author's Contribution:

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Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

Conflict of Interest: The study has no conflict of interest to declare by any author.

Source of Funding: None

Ethical Approval: No. 1007A/JBU/Approval/ JSDJNEHU Dated 25.12.2025

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