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ORIGINAL RESEARCH

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Minocycline can improve the sperm parameters and regulates hormonal receptor expression in a varicocele-induced rat model

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Abstract

Background: Varicocele, a common cause of male infertility, impairs reproductive function through oxidative stress and disruption of the hypothalamic-pituitary-gonadal (HPG) axis. Minocycline, a tetracycline antibiotic, possesses potent antioxidant and anti-apoptotic properties beyond its antimicrobial effects. This study aimed to evaluate the potential protective effects of minocycline on sperm parameters, hormonal levels, and the expression of key hormonal receptors and ion channels in a rat model of varicocele. **Methods**: Thirty-two male Wistar rats were randomly allocated into four groups (n = 8): sham-operated, varicocele (VC), varicocele treated with minocycline (VC + MINO, 40 mg/kg/i.p.), and minocycline-only (MINO). After 8 weeks, assessments included serum levels of testosterone, Luteinizing Hormone (LH), and Follicle-stimulating hormone (FSH); sperm analysis (count, motility, morphology); testicular histopathology; and mRNA/protein expression of luteinizing hormone/choriogonadotropin receptor (*LHCGR*), Follicle Stimulating Hormone Receptor (*FSHR*), Steroidogenic factor-1 (*SF-1*), *CatSper-1*, and *CatSper-2*. **Results**: Induction of varicocele significantly reduced serum testosterone levels but increased LH and FSH levels, impaired all sperm quality parameters, and caused severe testicular damage with decreased germ and Sertoli cell counts. These effects were paralleled by significant downregulation of *LHCGR*, *FSHR*, *SF-1*, *CatSper-1*, and *CatSper-2* expression. Minocycline treatment in the VC + MINO group effectively counteracted these alterations, leading to a significant increase in testosterone, a reduction in LH and FSH, improved sperm quality, reduced histological damage, and upregulation of the studied receptors and channels compared with the untreated VC group. **Conclusions**: Minocycline demonstrates a protective role against varicocele-induced testicular injury by improving sperm quality, restoring hormonal balance, and upregulating the expression of genes critical for steroidogenesis and sperm function. These findings highlight its potential as a novel therapeutic agent for managing varicocele-associated male infertility.

Keywords

Minocycline; Male infertility; Hormonal receptor; Varicocele

La minociclina puede mejorar los parámetros espermáticos y regular la expresión de receptores hormonales en un modelo de rata inducido por varicocele

Resumen

Antecedentes: El varicocele, una causa común de infertilidad masculina, deteriora la función reproductiva a través del estrés oxidativo y la alteración del eje hipotálamo-hipófisis-gonadal (HPG). La minociclina, un antibiótico tetraciclínico, posee potentes propiedades antioxidantes y anti-apoptóticas más allá de sus efectos antimicrobianos. Este estudio tuvo como objetivo evaluar los posibles efectos protectores de la minociclina sobre los parámetros espermáticos, los niveles hormonales y la expresión de receptores hormonales clave y canales iónicos en un modelo de rata con varicocele. **Métodos**: Treinta y dos ratas Wistar macho fueron asignadas aleatoriamente a cuatro grupos (n = 8): operadas simuladamente (sham), con varicocele (VC), con varicocele tratadas con minociclina (VC + MINO, 40 mg/kg/i.p.) y solo con minociclina (MINO). Tras 8 semanas, las evaluaciones incluyeron los niveles séricos de testosterona, Luteinizing Hormone (LH) y Follicle-stimulating hormone (FSH); análisis espermático (recuento, motilidad, morfología); histopatología testicular; y expresión de ARNm/proteína de luteinizing hormone/choriogonadotropin receptor (*LHCGR*), Follicle Stimulating Hormone Receptor (*FSHR*), Steroidogenic factor-1 (*SF-1*), *CatSper-1* y *CatSper-2*. **Resultados**: La inducción de varicocele redujo significativamente los niveles séricos de testosterona, pero aumentó los de LH y FSH, deterioró todos los parámetros de calidad espermática y causó daño testicular severo con disminución del recuento de células germinales y de Sertoli. Estos efectos se acompañaron de una regulación negativa significativa de la expresión de *LHCGR*, *FSHR*, *SF-1*, *CatSper-1* y *CatSper-2*. El tratamiento con minociclina en el grupo VC + MINO contrarrestó eficazmente estas alteraciones, conduciendo a un aumento significativo de la testosterona, una reducción de la LH y la FSH, una mejoría de la calidad espermática, una reducción del daño histológico y una regulación positiva de los receptores y canales estudiados, en comparación con el grupo VC no tratado. **Conclusiones**: La minociclina demuestra un papel protector contra la lesión testicular inducida por varicocele, mejorando la calidad espermática, restaurando el equilibrio hormonal y regulando positivamente la expresión de genes críticos para la esteroidogénesis y la función espermática. Estos hallazgos resaltan su potencial como un nuevo agente terapéutico para el manejo de la infertilidad masculina asociada al varicocele.

Palabras Clave

Minociclina; Infertilidad masculina; Receptor hormonal; Varicocele

1. Introduction

Varicocele refers to the pathological enlargement and twisting of the pampiniform venous network in the spermatic cord, which disrupts normal blood outflow from the testis. It is one of the most frequent and treatable contributors to male infertility, occurring in about 15–20% of men during their reproductive years and in nearly 40% of individuals evaluated for primary infertility [1–3]. Varicocele usually develops on the left side of the scrotum at puberty: 10% of patients may be bilateral [4]. Although the precise etiology remains unclear, varicocele is generally attributed to anatomical variations, venous valve insufficiency, and increased testicular temperature, all of which contribute to hypoxia, oxidative stress, and disruption of testicular function [5].

Pathophysiologically, varicocele has been associated with multiple detrimental effects on spermatogenesis and steroidogenesis. These include increased generation of reactive oxygen species (ROS), testicular hypoxia, and impaired blood–testis barrier function. Excessive ROS production leads to lipid peroxidation, DNA fragmentation, mitochondrial dysfunction, and apoptosis of germ and Sertoli cells [3, 6]. Moreover, varicocele alters the regulation of the hypothalamic-pituitary-gonadal (HPG) axis, disturbing the pulsatile release of gonadotropin-releasing hormone (GnRH) and consequently altering serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone. Such hormonal imbalances disrupt spermatogenic activity and impair sperm quality parameters including count, motility, and morphology [7, 8].

FSH and LH exert their effects through their respective receptors—Follicle Stimulating Hormone Receptor (*FSHR*) and luteinizing hormone/choriogonadotropin receptor (*LHCGR*)—located in Sertoli and Leydig cells. Activation of these receptors stimulates testosterone synthesis and spermatogenesis, while transcription factors such as steroidogenic factor-1 (*SF-1*) regulate the expression of steroidogenic enzymes and gonadotropin receptors. Additionally, calcium ion channels of the CatSper family play a critical role in regulating sperm motility and fertilization capacity. Downregulation of CatSper channels and hormonal receptors in varicocele has been implicated in the observed decline in sperm function [9–11].

In recent years, antioxidant and anti-apoptotic agents have gained attention as potential therapeutic options for mitigating testicular damage caused by varicocele. Among these agents, minocycline, a second-generation tetracycline antibiotic, has demonstrated pleiotropic biological activities beyond its antimicrobial properties. By scavenging free radicals, inhibiting caspase activation, and modulating inflammatory cytokines, this drug exhibits potent antioxidant, anti-inflammatory, and anti-apoptotic effects. Importantly, minocycline offers several advantages over conventional antioxidants: it readily crosses the blood-testis barrier, exhibits prolonged tissue retention,

and exerts multimodal protection via anti-inflammatory and anti-apoptotic pathways in addition to its antioxidant activity [12, 13]. Its established oral bioavailability and safety profile in humans make it a promising translational candidate for adjuvant therapy in male infertility [13]. Experimental studies have shown that minocycline provides neuroprotection and reduces damage from oxidative stress in various tissues, including the kidney and retina. However, its protective effects on testicular tissue, particularly in the context of varicocele, have not been sufficiently elucidated [12, 14].

Given these considerations, the present study aimed to investigate the protective effects of minocycline on sperm parameters, serum hormone levels, and the expression of key genes and proteins involved in spermatogenesis (*FSHR*, *LHCGR*, *SF-1*, *CatSper-1*, and *CatSper-2*) in a rat model of varicocele. This study also examines the hypothesis that minocycline, through its antioxidant and anti-apoptotic mechanisms, can restore hormonal receptor signaling and improve sperm quality, thereby offering a potential pharmacological approach for managing male infertility associated with varicocele.

2. Materials and methods

2.1 Animals and groups

Thirty-two male Wistar rats, aged 8 weeks and weighing between 200 and 250 g, were supplied by the Animal Facility of Tabriz University of Medical Sciences. The animals were maintained in a regulated environment with a temperature of 22 ± 2 °C, a 12 h light–dark cycle, and 50–60% humidity, and were allowed unrestricted access to standard chow and drinking water. All experimental protocols complied with established ethical standards for animal research and were approved by the Animal Ethics Committee of Tabriz University of Medical Sciences (Ethics Code: IR.TBZMED.AEC.1402.039). The experimental work of this research project was started in July 2023 and ended in November 2023. After the end of experimental period a part of tissue and serum was sorted in −80 frizzed.

The rats were randomly divided into four groups (n = 8 per group):

Sham group: Animals underwent left renal vein surgery without ligation.

Varicocele (VC) group: Varicocele was surgically induced, and the animals received intraperitoneal injections of normal saline for 8 weeks.

Varicocele + Minocycline (VC + MINO) group: Varicocele was induced as described above, and the rats received intraperitoneal minocycline (40 mg/kg/day) for 8 weeks, starting one week after the surgery.

Minocycline-only (MINO) group: Healthy animals received minocycline (40 mg/kg/day, intraperitoneal) for 8 weeks to assess any potential drug-related effects.

The selected minocycline dose was based on previous studies demonstrating its antioxidant and anti-apoptotic efficacy in rat models of oxidative stress-related disorders [12].

2.2 Surgical processes

Experimental varicocele was induced as previously described by Shokoohi *et al.* [12, 15] (2022 and 2024) with minor modifications. Under general anesthesia (ketamine 50 mg/kg + xylazine 10 mg/kg, i.p.), a midline abdominal incision (~2 cm) was made to expose the left renal vein. The left renal vein was carefully dissected free from surrounding tissues, and a partial ligation was created using a 5-0 silk suture around the vein, reducing its diameter by approximately 50% to impair venous drainage from the left testis. The incision was closed in two layers with absorbable sutures. Sham-operated rats underwent identical procedures without vein ligation. Varicocele induction was confirmed 4 weeks post-surgery by evaluating venous dilation and testicular color and temperature [12, 15].

2.3 Sample collection

At the conclusion of the 8-week intervention, the rats were anesthetized and sacrificed via cardiac puncture. Blood was withdrawn from the inferior vena cava, left to coagulate, and then centrifuged at 3000 rpm for 10 minutes to separate the serum. The testes and epididymides were subsequently excised, weighed, and prepared for histological, molecular, and biochemical evaluations.

2.4 Testicular morphologic analysis

The stained sections were analyzed by counting germ cells and Sertoli cells residing in the testicular tissue. Germ cells were identified based on standard morphological criteria under Hematoxylin and Eosin (H&E) staining: spermatogonia (round nuclei adjacent to the basement membrane), primary spermatocytes (large nuclei with coarse chromatin), and round spermatids (small, round nuclei near the lumen). Leydig cells, located in the interstitium, were not enumerated as the primary focus was on the seminiferous epithelium and germ cell series. To count Sertoli cells, 30 tubules from every rat were examined with a light microscope at ×400 magnification to capture defining nuclear features and obvious nucleoli. Counts of spermatogonia cells, primary spermatocytes, and round spermatids were performed on ten tubules taken from each animal [16, 17].

2.5 Measurement of testosterone, LH, as well as FSH levels in the serum

Serum specimens were used to assess concentrations of testosterone, LH and FSH. Hormonal levels were quantified using enzyme-linked immunosorbent assay (ELISA) kits. With the testosterone assay purchased from Demeditec Diagnostics (24145, GmbH, Kiel, SH, Germany) and the LH and FSH kits obtained from ZellBio GmbH (ZB-10182C-HRM9648, GmbH, Ulm, BW, Germany).

2.6 Semen analyses

The left epididymis was finely chopped with sterile scissors to release spermatozoa, which were then diluted in 1 mL of phosphate-buffered saline (PBS). Sperm motility was evaluated by examining five microscopic fields and categorizing sperm as either motile or immotile. Sperm concentration was

determined using a Neubauer hemocytometer in accordance with standard counting protocols. For morphological analysis, sperm smears were prepared, stained with hematoxylin and eosin, and observed under a microscope. The proportion of morphologically normal sperm was calculated by evaluating 100 spermatozoa per slide, with abnormalities assessed in the head, midpiece (neck), and tail regions [18].

2.7 Real-time PCR

Total RNA was isolated from frozen testicular samples using TRIzol reagent (15596026, Invitrogen, Thermo Fisher Scientific, Carlsbad, CA, USA). The quality and quantity of the extracted RNA were assessed spectrophotometrically, with acceptable purity defined as an A260/A280 ratio greater than 1.8. Following treatment with DNase I, 1 μg of RNA was reverse transcribed into cDNA using a commercial synthesis kit (K1612, Thermo Fisher Scientific, Vilnius, Lithuania). Real-time quantitative Polymerase Chain Reaction (PCR) was carried out using gene-specific primers for FSHR, LHCGR, SF-1, CatSper-1, and CatSper-2 (Table 1) in combination with SYBR Green Master Mix on a StepOnePlus™ Real-Time PCR System (94404, Applied Biosystems, Foster City, CA, USA). β-actin was employed as the internal reference gene. Relative mRNA expression levels were determined using the $2^{-\Delta\Delta Ct}$ method. All reactions were performed in triplicate, and melt curve analysis was conducted to verify the specificity of amplification [19].

TABLE 1. Primer's sequence.

Genes	Primer sequence (5′→3′)
CatSper-1	Forward: TTTACCTGCCTCTTCCTCTTCT Reverse: ACCAGGTTGAGGAAGATGAAGT
CatSper-2	Forward: TGGTTGTTGCTTGGTTCC Reverse: TTCCTTGACTGGTTCCTCT
SF-1	Forward: GTTTCTGCGCACCCACAGTC Reverse: GTGGTAGCCGGACACCTTGT
LHCGR	Forward: CACAGGGCCGAAAACCTTTTA Reverse: AGCATCTGGTTCAGGAGCACA
FSHR	Forward: CCACAAGCCAATACAAACTA Reverse: CAAAAGTCCAGCCCAATACC
β-actin	Forward: AAGATCCTGACCGAGCGTGG Reverse: CAGCACTGTGTTGGCATAGAGG

SF-1: Steroidogenic factor-1; LHCGR: luteinizing hormone/choriogonadotropin receptor; FSHR: follicle stimulating hormone receptor.

2.8 Immunocytochemistry

The expression of CatSper-1 and CatSper-2 proteins was evaluated using a commercial immunoperoxidase kit (sc-393749, Santa Cruz Biotechnology, Heidelberg, BW, Germany). The procedure began with blocking endogenous peroxidase activity by incubating the samples in 3% Hydrogen Peroxide (H_2O_2) for 20 minutes, followed by rinsing with phosphate-buffered saline (PBS). The antigen retrieval method was boiling in 0.01 M sodium citrate buffer (pH 6.0) for 20 minutes to remove the antigen against which the antibodies bound. 0.3% Triton was used for permeabilizing cell membranes. Primary polyclonal antibodies: Catsper-1, Catsper-2, and Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) at dilution 1:100 was used for overnight incubation at 4 °C. Incubating at 37 °C, in darkness, was done after secondary antibodies fluorescein isothiocyanate (FITC)-conjugated immunoglobulin G (IgG) were applied for a period of incubation lasting 90 minutes. Finally, samples were stained using propidium iodide and examined under "Olympus fluorescence microscope BX51" (Tokyo, Japan). For quantification, five random fields (×400 magnification) of seminiferous tubules were selected per sample. Fluorescence intensity was measured using ImageJ software (NIH, USA). The mean intensity per field was calculated after background subtraction. Data were normalized to the corresponding Propidium Iodide (PI) signal and expressed as relative fluorescence units (RFU). The average protein intensities were determined by ImageJ computing software sampling 8 Rat per group after methods similar to those in the study conducted by Banerjee and Chaturvedi. Earlier work was utilized for negative control staining [20].

2.9 Statistical analysis

Statistical analyses were conducted using SPSS version 20 (IBM, Armonk, NY, USA). The normality of the data was assessed with the Kolmogorov-Smirnov test. Results are presented as mean ± standard deviation (Mean ± SD). One-way analysis of variance (ANOVA) was applied to evaluate differences between groups, and Tukey's *post hoc* test was employed for pairwise comparisons. A p-value of less than 0.05 was considered statistically significant.

3. Results

3.1 Histomorphology evaluation

Histological analysis of testicular tissue from rats with varicocele revealed notable structural abnormalities, including atrophy and damage of the seminiferous tubules, along with degeneration or irregularities in germ cells such as spermatogonia, spermatocytes, and spermatids. Administration of minocycline significantly reduced the degeneration of the seminiferous tubules compared with the untreated varicocele group. In contrast, both control groups displayed normal seminiferous tubule epithelium. The study also assessed the numbers of Sertoli cells and different germ cell types, including spermatogonial cells, primary spermatocytes, and round spermatids. Sertoli cell counts were markedly lower in the varicocele (VC) group compared with the sham group ($p < 0.001$) and were significantly higher in the minocycline-treated varicocele group relative to the untreated VC group. No significant differences were observed between the two healthy control groups. Similarly, germ cell counts were significantly reduced in the VC group compared with the sham group, while minocycline treatment notably improved germ cell numbers relative to untreated VC animals. Differences in germ cell counts between the two healthy groups were minimal. These results demonstrate that minocycline effectively mitigates varicocele-

induced histopathological damage in testicular tissue (Fig. 1, Table 2).

3.2 Hormonal evaluation

Serum hormone assays demonstrated that varicocele induction significantly reduced testosterone and increased the LH and FSH levels relative to the sham group ($p < 0.001$) (Table 3). Minocycline administration to varicocele rats (VC + MINO) significantly increased testosterone levels compared with the VC group ($p < 0.01$), approaching values observed in the sham group. Likewise, LH and FSH concentrations were significantly diminished in the VC + MINO group compared with the VC group ($p < 0.05$). The MINO group did not differ significantly from the sham controls, confirming that minocycline alone did not disrupt endocrine balance. Thus, minocycline partially restores hormonal balance in varicocele-induced rats (Table 3).

3.3 Sperm parameters

As summarized in Table 4, varicocele markedly impaired all sperm quality indices. The VC group showed significant decreases in sperm count, motility, and normal morphology compared with the sham group ($p < 0.001$ for all). Minocycline treatment significantly improved sperm count, motility, and morphology relative to the VC group ($p < 0.01$). The MINO group exhibited parameters comparable to the sham controls, confirming the safety of minocycline in non-varicocele animals. Minocycline treatment therefore improves key sperm quality parameters impaired by varicocele (Table 2).

3.4 The evaluation of mRNA expression of mentioned genes

As compared with the sham group, the varicocele has significantly decreased the mRNA expression of the gene FSHR ($p \leq 0.001$) (Fig. 2). FSHR expression was less significantly in the treated varicocele when compared with the untreated varicocele. However, there were no appreciable differences found between the two healthy groups. The rats with varicocele had mRNA expression of LHCGR much lower compared with those in the sham group. Actually, the LHCGR was increased in minocycline-treated animals as compared with the varicocele group ($p < 0.05$). Results for both healthy

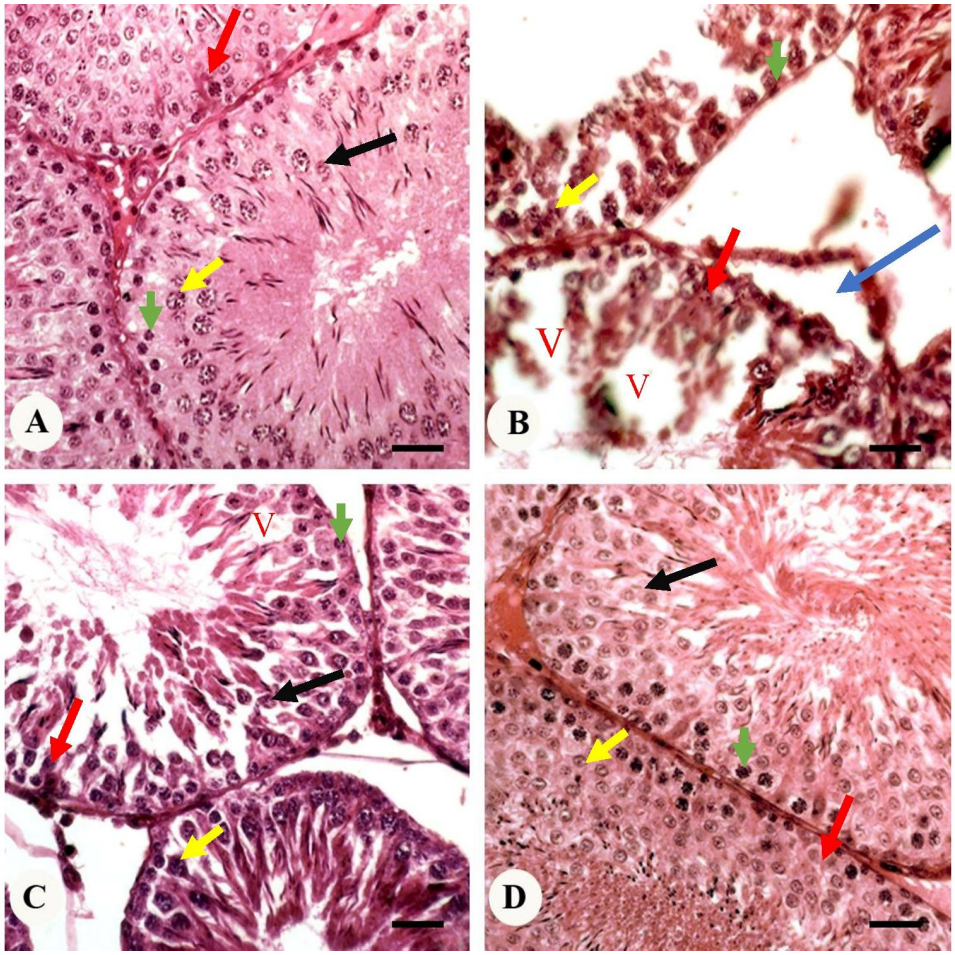

FIGURE 1. Histological finding in study groups. (A) Sham group showing typical testicular structure. (B) Varicocele group displaying severe testicular damage: arrows indicate atrophied seminiferous tubules, vacuolization (V), and sloughed germ cells (arrowheads). (C) Varicocele + Minocycline group indicating improvement in seminiferous tubule structure. (D) Minocycline group exhibiting normal testicular structure. Scale bar: 50 μm. Blue arrows indicate atrophied seminiferous tubules, vacuolization (V), and red arrowheads Sertoli cells, green arrowheads Spermatogonia cells, yellow arrowheads Primary Spermatocytes cells, Black arrowheads Round Spermatids cells. Scale bar: 50 μm.

TABLE 2. Histological cell counts and sperm parameters (Mean ± SD).

Group	Sertoli Cells	Spermatogonia	Primary Spermatocytes	Round Spermatids	Sperm Count ($\times 10^6$/mL)	Motility (%)	Normal Morphology (%)
Sham	35.75 ± 4.17	40.25 ± 2.25	100.45 ± 2.5	238.50 ± 3.25	37.50 ± 1.15	70.25 ± 3.31	72.15 ± 3.4
VC	18.50 ± 1.15*	22.50 ± 2.12*	43.50 ± 3.70*	90.50 ± 1.45*	16.50 ± 1.25*	14.50 ± 2.35*	16.50 ± 2.25*
VC + MINO	24.50 ± 1.12*,#	30.50 ± 1.15*,#	65.25 ± 2.30*,#	155.25 ± 1.60*,#	20.75 ± 3.10*,#	40.75 ± 4.20*,#	42.50 ± 2.50*,#
MINO	32.25 ± 2.70	38.35 ± 1.25	95.75 ± 3.25	230.35 ± 3.25	33.50 ± 2.18	68.25 ± 3.14	70.20 ± 5.35

**: meaningful difference with sham group; #: considerable difference with varicocele group ($p < 0.05$); VC: varicocele; MINO: minocycline-only. All data presented based on Mean ± SD.*

TABLE 3. The comparison of hormone profile after treatment period.

Groups	Testosterone (ng/dL)	LH (ng/dL)	FSH (ng/dL)
Sham	5.15 ± 0.15	2.20 ± 0.18	2.30 ± 0.28
Varicocele	1.40 ± 0.22*	3.35 ± 0.25*	3.65 ± 0.32*
Varicocele + Minocycline	3.35 ± 0.05*,#	2.80 ± 0.15*,#	2.60 ± 0.18*,#
Minocycline	5.50 ± 0.04	3.20 ± 0.30	3.50 ± 0.25

**: meaningful difference with sham group ($p < 0.001$); #: considerable difference with varicocele group ($p < 0.01$); LH: luteinizing hormone; FSH: follicle-stimulating hormone. All data presented based on Mean ± SD.*

TABLE 4. The comparison of sperm parameters after treatment period.

Groups	Concentration ($\times 10^6$/mL)	Normal Morphology (%)	Normal Motility (%)
Sham	37.50 ± 1.15	72.15 ± 3.40	70.25 ± 3.31
Varicocele	16.50 ± 1.25*	16.50 ± 2.25*	14.50 ± 2.35*
Varicocele + Minocycline	20.75 ± 3.10*,#	42.50 ± 2.50*,#	40.75 ± 4.20*,#
Minocycline	33.50 ± 2.18	70.20 ± 5.35	68.25 ± 3.14

**: meaningful difference with sham group ($p < 0.001$); #: considerable difference with varicocele group ($p < 0.01$). All data presented based on Mean ± SD.*

groups again did not present any statistical significance between the two groups. The significance was found between the groups comparing Catsper-1 and 2 and the SF-1 genes with respect to mRNA expression when varicocele subjects were compared with the sham group. Interestingly, the minocycline-treated varicocele-induced rats had significantly higher mRNA expression of the above-mentioned genes compared with the varicocele group. There was no significant difference between the two healthy groups ($p > 0.05$). These results suggest that minocycline upregulates key genes involved in hormone signaling and sperm function (Fig. 2).

3.5 The evaluation of protein expression of Catsper-1 and Catsper-2

Figs. 3,4 illustrate immunofluorescence staining results, which indicated that Catsper-1 and Catsper-2 protein expression was less in the sections of the testis of the VC group in comparison with the Sham group. Additionally, there was a marked induction of Catsper-1 and Catsper-2 proteins in the varicocele + minocycline group as compared with the varicocele group. However, sham and minocycline groups did not differ with each other. Minocycline therefore enhances the protein expression of CatSper channels, which are critical for sperm motility (Figs. 3,4).

4. Discussion

A multitude of factors contribute to male infertility, of which oxidative stress, especially associated with varicocele, is the foremost [21]. Varicocele has been accepted for a long time as one of the most important correctable factors for male infertility and has consistently been linked to the dangerous effects it imposes through oxidative stress [22]. Such induced oxidative stress brings adverse effects upon different aspects such as spermatogenesis, semen output, and other functions of the HPG axis. Added to this, oxidative stress due to varicocele causes death by apoptosis of germ cells and complicates the

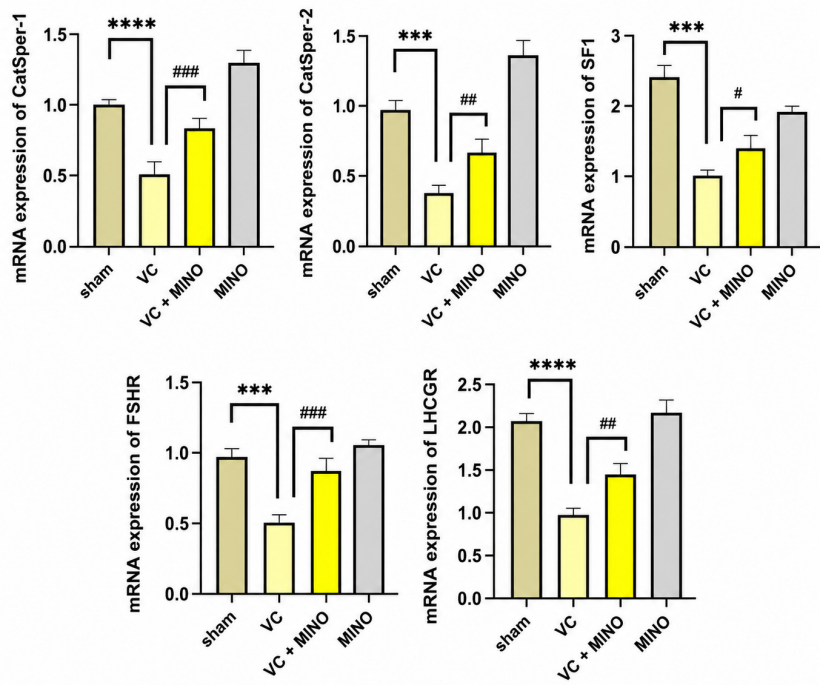

FIGURE 2. mRNA expression of *Catsper-1*, *CatSper-2*, *LHCGR*, *FSHR*, *SF-1*. $^{\#}p < 0.05$ *vs*. Varicocele group. Asterisk, (***) indicates a significant difference ($p < 0.001$), (****) indicates a significant difference ($p < 0.0001$). Asterisk ($^{\#}$) indicates a significant difference ($p < 0.05$). ($^{\#\#}$) indicates a significant difference ($p < 0.01$), ($^{\#\#\#}$) indicates a significant difference ($p < 0.001$). FSHR: follicle stimulating hormone receptor; LHCGR: luteinizing hormone/choriogonadotropin receptor; SF-1: Steroidogenic factor-1; VC: varicocele; MINO: minocycline-only.

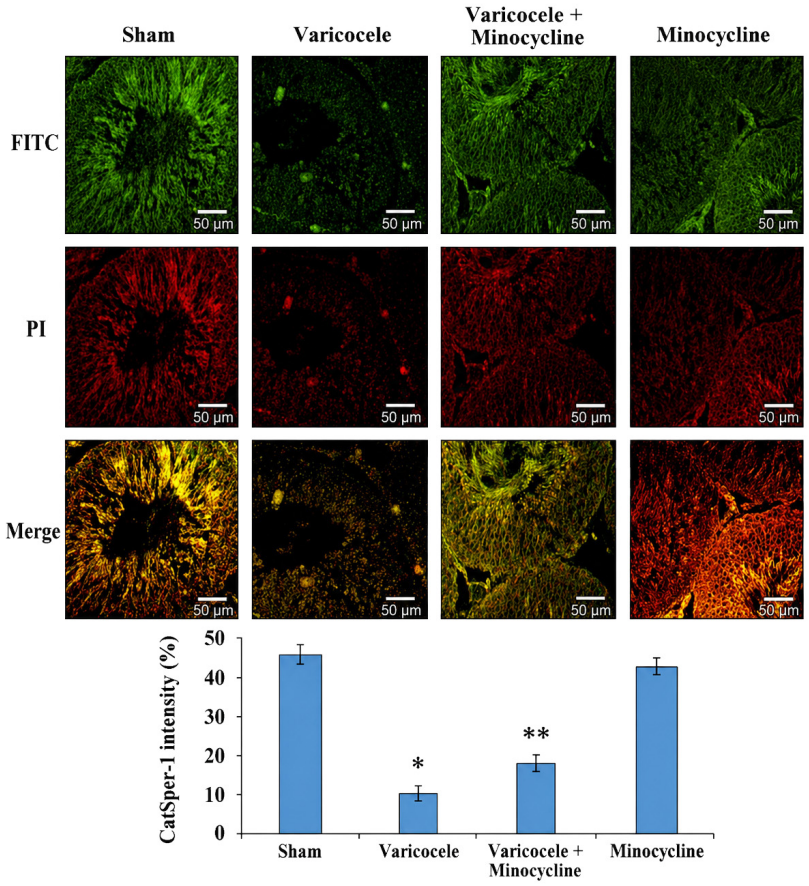

FIGURE 3. Immunohistochemistry of Catsper-1 in study groups. Scale bars (50 μm) and magnification ($\times$400). *$p < 0.05$ *vs*. Sham group; **$p < 0.05$ *vs*. Varicocele group. FITC: Fluorescein Isothiocyanate; PI: Propidium Iodide.

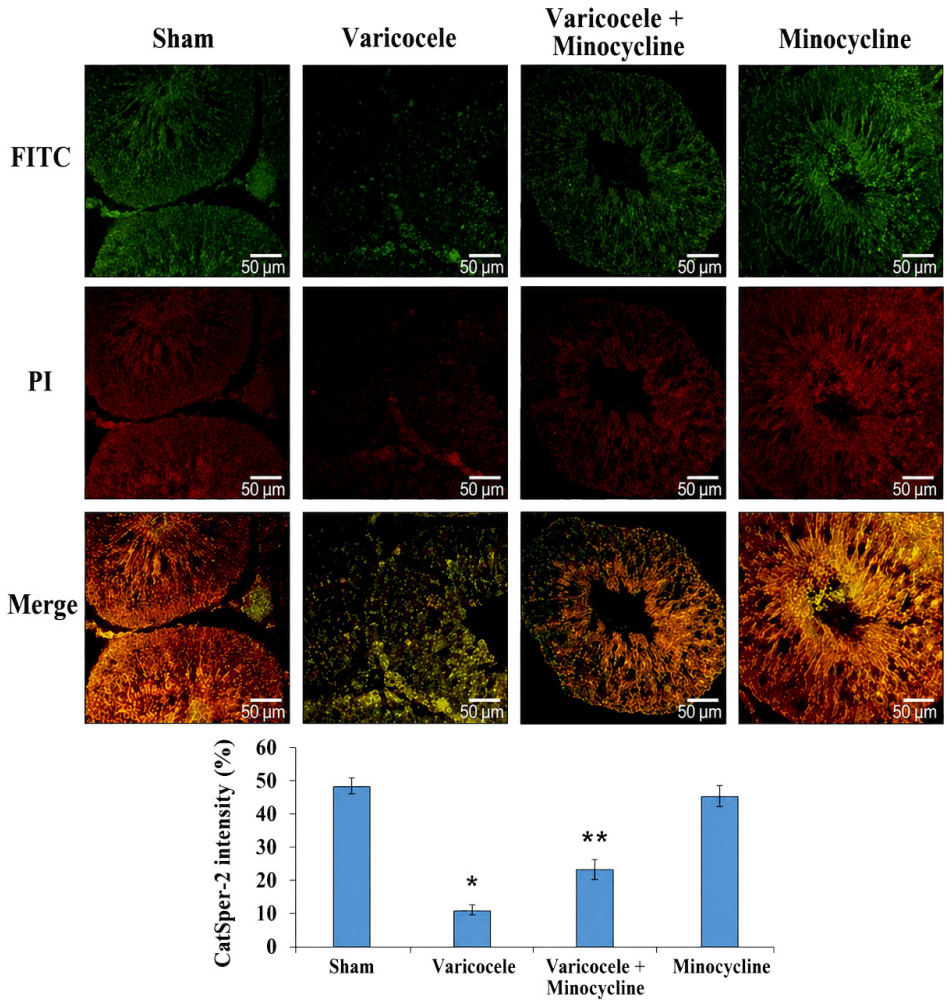

FIGURE 4. Immunohistochemistry of Catsper-2 in study groups. Scale bars (50 μm) and magnification ($\times$400). *$p < 0.05$ *vs*. Sham group; **$p < 0.05$ *vs*. Varicocele group. FITC: Fluorescein Isothiocyanate; PI: Propidium Iodide.

reproductive health even more [12, 15], while antioxidant therapy could decrease oxidative damage of testicular tissues against varicocele.

Varicocele leads to histological damages because of degeneration of Sertoli and germ cells, further resulting in atrophy of the testis. Activation of the intrinsic apoptotic pathway in male germ cells potentiates the degeneration of testes [19]. The protective effects of minocycline on spermatogenesis in our varicocele model can be attributed to its dual antioxidant and anti-apoptotic properties. Its ability to scavenge ROS and enhance endogenous antioxidant defenses mitigates oxidative damage to testicular lipids, proteins, and DNA. Concurrently, minocycline inhibits the mitochondrial apoptotic pathway by stabilizing membrane potential, modulating Bcl-2/Bax balance, and suppressing caspase-3 and caspase-9 activation. This combined action preserves the viability of Sertoli and germ cells, maintains the integrity of the seminiferous epithelium, and supports the functional capacity of Leydig cells. Consequently, this cellular protection facilitates the observed upregulation of key receptors (LHCGR, FSHR) and transcription factors (SF-1), restores steroidogenesis, and enhances the expression of sperm-specific channels (CatSper), collectively leading to improved sperm production, motility, and hormonal balance [12, 13]. However, it has been recently shown that minocycline treatment can be a very effectual remedy. Shokoohi *et al*. [12]. has shown the minocycline action in reducing apoptosis in germ cells as a therapeutic agent against varicocele-induced injuries in rat testes. It is primarily due to its antioxidative effects, which may counteract the adverse effect on testicular tissues [12].

The present study highlights the potential of minocycline as a therapeutic agent distinct from conventional antioxidants. Its multimodal action—combining antioxidant, anti-inflammatory, and anti-apoptotic effects—allows for a more comprehensive approach to mitigating varicocele-induced testicular injury [12]. Furthermore, minocycline's ability to cross biological barriers, including the blood-testis barrier, and its established use in human medicine for other conditions (*e.g.*, acne, rosacea) suggest a viable translational pathway [13]. Oral administration could be explored in future clinical studies as an adjunct to varicocelectomy or as a standalone treatment in selected cases. However, further pharmacokinetic studies and controlled clinical trials are necessary to determine optimal dosing, long-term safety, and efficacy in infertile men with varicocele.

During such periods, the motility of sperm at the affected site is reduced when compared with the other site not affected [20]. In fact, the intracellular concentrations of calcium ions

help control sperm motility, but the same can be achieved quite highly through cat Sper channels within spermatozoa [23]. It has been hypothesized that CatSper under-expression provides the rationale for the relationship between testicular CatSper depletion and impaired sperm motility [9, 24]. Real-time PCR performed on our side confirmed the drastically decreased expression levels of CatSper-1 and -2 in rat varicocele testes. Even though no western blot was carried out using this experiment, the expectation is that the general CatSper expression would be lower in varicocele-inoculated rats. Interestingly, minocycline treatment showed that both levels of *CatSper-1* and *CatSper-2* genes increased significantly despite a significant elevation in counts of motile spermatozoa from the patients. These antioxidative properties of the compound could be the basis for improving the sperm motility of patients following minocycline administration. These results give credence to the anticipated use of minocycline as a therapeutic measure in attenuating the adverse effect of varicocele on sperm motility associated with the regulation of CatSper gene expression and the subsequent increase in motile sperm counts.

Varicocele causes progressive deterioration in sperm quality parameters by damaging Leydig cells, germinal cells, and Sertoli cells [25]. Increased testicular temperature enhances the overproduction of ROS and hypoxic conditions. The inhibition of enzymes crucial in sex steroid synthesis leads to decreased production and conversion of intratesticular testosterone [19, 26]. The review of extensive literature strongly suggests that Varicocele negatively affects the majority of sperm parameters. In fact, varicocele has been related to decreased sperm count, motility, and morphology, but not the volume of semen. This observation has been substantiated by various research studies involving infertile men suffering from varicocele. It was noted that these infertile men had smaller testicular size and sperm counts [22, 27]. In most of the research studies, elevated levels of ROS have been related to poor-quality sperms [28, 29]. Our results suggest that the improvement of antioxidant defenses in testicular tissues by minocycline may explain its beneficial effects. Minocycline is an antioxidant drug that inhibits the damage of extracellular ROS generation and prevents the activation of the caspase-8 apoptotic pathway [12]. The treatment with minocycline, therefore, in our study enhanced the percentage of motile spermatozoa and morphologically normal ones considerably and hence increased the overall fertilization potential of the sperm. Another research conducted by Ghadian *et al*. [30]. in rat model of testicular torsion demonstrated that treatment with minocycline increases the activity of Leydig cells, thereby highly improving sperm parameters concerning morphology quality, probably due to the effect against apoptosis [30].

SF-1 is a crucial transcription factor that was found to be expressed in gonadotropes at the fetal and adult pituitary level and also in Leydig cells in males [9, 19]. However, the factor is very crucial in the development of the HPG axis and affects its structural and functional configurations [31]. Impairment in the expression of the gene for SF-1 could result in a likelihood of reduced testosterone production [32]. The precise mechanisms that would cause this, however, remain to be fully understood and need further detailed study. Interestingly, it has been found that there is a significant increase in male rat testosterone levels treated with minocycline at 40 mg/kg for 2 weeks [12, 27]. The elevation of testosterone post-treatment with minocycline underlines the action of this drug in modulating the hormone and posits a mechanism by which the treatment may work.

Once LH binds to its specific receptor, LHCGR, it sets off an entire signaling cascade that stimulates the production of translocator proteins on the outer mitochondrial membrane and StAR (steroidogenic acute regulatory) proteins, the ones responsible for facilitating the passage of cholesterol into the mitochondrion, a precursor for the synthesis of testosterone [33, 34]. Besides these, the activation of LHCGR engages other vital pathways, such as protein kinase B, involved in the survival and growth of Leydig cells [35]. The decrease in serum testosterone levels associated with varicocele in rats may be related to pathways activated by LHCGR stimulation. Furthermore, varicocele has a significant impact on the HPG axis, which results in decreased testosterone levels and, subsequently, due to downregulation in LHCGR and FSHR, leads to an increase in serum levels of FSH and LH [20]. However, these phenomena are not well recognized in mechanistic terms. We found a notable decrease in the expression of FSH and LH receptors in the testes of rats with varicocele in our study. The increased level of the gonadotropin hormone may relate to the level of its expression for SF-1, because there is substantial evidence that SF-1 acts as a transcriptional regulator for genes encoding LH and FSH, and also of the GnRH receptor [34]. LH deficiency further impairs testosterone production. Our results indicate that the increased levels of LH in the serum of rats seen in varicocele rats could be a result of downmodulation of SF-1 and LHCGR. However, minocycline treatment appears to have some efficacy in reversing LH levels, likely by inhibiting the death of Leydig cells and increasing in expression of gonadotropin receptors, thereby increasing serum testosterone levels. It has been determined to elevate feeding into the hypothalamic receptors, where it further optimizes the outcome of release into the FSH and/or LH within the HPG axis [36–38].

This study has several Limitation, largely stemming from limited funding and lack of access to advanced equipment. Notably, Western blotting could not be conducted, cell characterization was based on morphology rather than more specific immunofluorescence techniques, and no separate control group was included; this decision was supported by prior literature and animal welfare considerations. The authors suggest that future investigations with greater resources should incorporate these more rigorous approaches to better confirm the therapeutic value of minocycline.

5. Conclusions

The complex effects of varicocele on male fertility involve multiple interconnected pathways, including SF-1, CatSper channels, and hormonal regulation. Varicocele induction was associated with altered SF-1 expression, reduced CatSper levels, and disrupted hormone balance, highlighting the multifactorial mechanisms affecting fertility. Importantly, minocycline showed potential as a therapeutic agent, alleviating testicular damage, restoring CatSper expression, and possibly influenc-

ing SF-1-related pathways. These results underscore the need for further research to clarify the underlying mechanisms and develop more effective treatments for varicocele-related male infertility.

AVAILABILITY OF DATA AND MATERIALS

The datasets generated during and/or analysed during the current study are available from the corresponding.

AUTHOR CONTRIBUTIONS

MAD and AAK—designed the research study. LMB, MAD, MA and ARJ—performed the research. MAD and PYK—provided help and advice on histological analyses. ZS and PYK—analyzed the data. SSS, SHAE and AAK—wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethical approval was granted by the Laboratory Animal Ethics Committee of Tabriz University of Medical Sciences (Ethical code: IR. TBZMED. REC.1396.663).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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