

Effect of Chrysin on Inflammatory Gene Expression, Testis Structure, and Oxidative Stress in Adult Rats With Varicocele

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Abstract

Objectives: Varicocele is a major cause of male infertility, primarily driven by oxidative stress, inflammation, and apoptosis within testicular tissue. Chrysin, a natural flavonoid with potent antioxidant and anti-inflammatory properties, has shown potential benefits in reproductive disorders. This study aimed to evaluate the protective effects of chrysin in an experimental rat model of varicocele.

Materials and Methods: Thirty-two adults male Wistar rats were randomly allocated into four groups: Sham, Chrysin (50 mg/kg/d), Varicocele, and Varicocele + Chrysin. Varicocele was induced by partial ligation of the left renal vein. After eight weeks of treatment, sperm parameters, testicular histopathology, serum oxidative stress markers (MDA, SOD, GPx, TAC), and testicular expression of TNF α , IL 1 β , Bax, and Bcl 2 (RT qPCR) were assessed. Statistical analyses included Analysis of Variance (ANOVA) or Kruskal–Wallis tests with appropriate post hoc comparisons.

Results: Induction of varicocele markedly decreased testicular mass and compromised sperm parameters—including concentration, motility, and morphology—while also producing pronounced structural damage within the seminiferous tubules and lowering Johnsen scores. Oxidative stress was markedly elevated, as indicated by increased MDA and reduced SOD, GPx, and TAC levels. Additionally, varicocele upregulated TNF α , IL 1 β , and Bax while downregulating Bcl 2, resulting in an increased Bax/Bcl 2 ratio. Chrysin treatment significantly ameliorated these changes by improving sperm parameters, preserving testicular architecture, reducing lipid peroxidation, restoring antioxidant capacity, and modulating inflammatory and apoptotic gene expression.

Conclusions: Chrysin exerts strong protective effects against varicocele induced testicular dysfunction through antioxidant, anti-inflammatory, and anti-apoptotic mechanisms. These findings support its potential as a natural therapeutic candidate for managing varicocele-associated male infertility.

Keywords: Varicocele, Chrysin, Oxidative stress, Inflammation, Apoptosis, Spermatogenesis, Male infertility

Introduction

Varicocele, characterized by abnormal dilation of the pampiniform venous plexus around the testicle, is the most prevalent identifiable cause of male infertility, affecting 10–20% of the general male population and up to 80% of men with secondary infertility (1,2). The condition disrupts testicular thermoregulation and microcirculation, leading to a cascade of pathological events. A central mechanism underlying varicocele-induced testicular dysfunction is oxidative stress, where an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defenses leads to cellular damage. Elevated levels of ROS in semen are strongly correlated with impaired sperm parameters—including motility, morphology, and concentration—in men with varicocele (3–6).

In addition to oxidative stress, inflammatory mechanisms are pivotal in the pathophysiology of varicocele. The condition provokes localized inflammation, stimulating the release of pro-inflammatory cytokines, which

intensify tissue damage and compromise the integrity of the spermatogenic microenvironment (7). This inflammatory and oxidative milieu can activate apoptotic pathways within the testicular tissue (8). Key regulators of apoptosis, such as the pro-apoptotic protein BAX and the anti-apoptotic protein Bcl-2, become imbalanced, promoting germ cell death and contributing to the decline in spermatogenic efficiency. Consequently, therapeutic strategies aimed at mitigating oxidative stress and inflammation are of significant interest (9).

Chrysin (5,7-dihydroxyflavone), a natural flavonoid found in various plants, has garnered attention for its potent antioxidant and anti-inflammatory properties. Research suggests that chrysin can scavenge free radicals, enhance endogenous antioxidant enzyme activities, and modulate inflammatory signaling pathways (10,11). Furthermore, preliminary studies indicate its potential benefits in male reproductive health, including improving semen quality and increasing testosterone levels (10). However, the specific effects of chrysin on the molecular

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and histological alterations induced by varicocele, particularly concerning inflammatory gene expression and testicular structural integrity, remain insufficiently explored.

This study, therefore, aims to investigate the therapeutic potential of chrysin in an experimental rat model of varicocele. By elucidating the protective mechanisms of chrysin against varicocele-induced damage, this research seeks to contribute to the development of novel, non-invasive adjunctive therapies for male infertility associated with this condition.

Materials and Methods

Animals and Experimental Design

Thirty-two adult male Wistar rats (200–300 g) were obtained from the Faculty of Veterinary Medicine at Tabriz University and maintained under standardized laboratory conditions in the Faculty of Pharmacy's animal facility. The environment was regulated to a 12-hour light/dark cycle at 22 ± 2 °C, with unrestricted access to food and water. After ten days of acclimatization, the sample size was chosen based on similar previous study the and randomly divided into four experimental groups (12) (n=8 per group):

- Group 1 (Sham): Underwent laparotomy surgery without varicocele induction.
- Group 2 (Chrysin): Received chrysin (50 mg/kg/d, orally) for eight weeks without varicocele (10).
- Group 3 (Varicocele + Chrysin): Underwent varicocele induction and subsequently received chrysin (50 mg/kg/d, via oral gavage) for eight weeks (10).
- Group 4 (Varicocele): Underwent varicocele induction and subsequently received an equivalent volume of the vehicle (normal saline, via oral gavage) for eight weeks.

Varicocele Induction Procedure

Experimental varicocele was induced surgically as previously described in our previous studies (1-3). Briefly, rats were anesthetized with an intraperitoneal injection of ketamine (50 mg/kg) and xylazine (10 mg/kg) (1). A midline abdominal incision was made to expose the left renal vein. A 4-0 silk suture passed underneath the vein, and a partial ligation was created by placing a blunt 0.5 mm diameter needle alongside the vein before tying the suture. The needle was then removed, resulting in a 50% reduction in venous diameter to impede blood flow. The abdominal wall was closed in layers. Varicocele was considered successfully induced when prominent dilatation of the left pampiniform plexus and venous congestion were observed at re laparotomy. In the present study, varicocele induction was successful in all operated rats (100% success). Sham-operated rats underwent the same surgical protocol, with the exception that the testicular vein was not ligated.

Sample Collection and Preparation

Twenty-four hours after the final treatment, all rats were deeply anesthetized. Blood was collected from the inferior vena cava, centrifuged at 3000×g for 15 minutes, and the serum stored at -80 °C for oxidative stress assays. Subsequently, a laparotomy was performed. Both testes and epididymides were carefully dissected, cleared of adhering tissues, and weighed. For each rat:

- The left epididymis was placed in physiological saline for sperm analysis.
- The left testis of four rats was fixed in 10% neutral buffered formalin for histopathological examination and others was snap-frozen in liquid nitrogen and stored at -80°C for RNA extraction and gene expression analysis (13).

To reduce potential bias, the investigators performing histological scoring, sperm analysis, and RT qPCR data analysis were blinded to the group allocation.

Analytical Procedures

Histopathological Examination

Formalin-fixed testicular tissues were processed, embedded in paraffin, and sectioned at 5 µm thickness. Sections were stained with hematoxylin and eosin (H&E) for general morphology. Histopathological changes (e.g., germinal epithelial disorganization, vacuolization, and sloughing) were scored semi quantitatively. The Johnsen's score (a 10-point scale based on spermatogenic activity) and the mean seminiferous tubule diameter was determined for quantitative assessment (1).

Gene Expression Analysis (Real-Time PCR)

Gene expression analysis of pro-inflammatory cytokines TNF-α and IL-1β in rat models was performed using a two-step reverse transcription quantitative polymerase chain reaction (RT-qPCR) to quantify relative mRNA levels. Briefly, total RNA was isolated from homogenized rat tissues or cultured cells using TRIzol™ reagent followed by chloroform extraction and isopropanol precipitation. RNA integrity and concentration were verified spectrophotometrically. Subsequently, 1 µg of total RNA was reverse-transcribed into complementary DNA (cDNA) using a High-Capacity cDNA Reverse Transcription Kit with random primers, according to the manufacturer's protocol. Quantitative PCR was carried out using a SYBR Green master mix on a real-time PCR detection system, with reactions run in triplicate. Rat-specific, intron-spanning primer sequences were utilized to amplify TNF-α (F: 5'-ATG CTC TCC TCC TCT CAT CAG TTC-3', R: 5'-CTT GGT GGT TTG CTA CGA CGT-3') and IL-1β (F: 5'-CAC CTC TCA AGC AGA GCA CAG-3', R: 5'-GGG TTC CAT GGT GAA GTC AAC-3'). Bax (F: 5'-GGC GAA TTG GAG ATG AACTG-3', R: 5'-TTC TTC CAG ATG GTG AGC GA-3'); Bcl-2 (F: 5'-CTT TGC AGA GAT GTC CAG TCAG-3', R: 5'-GAA CTC AAA GAA GGC CAC AATC-3'), and GAPDH (F: 5'-GCA

GCT CCT TCG TTG CCG GT-3'; R: 5'-CCC GCC CAT GGT GTC CGT TC-3') The constitutively expressed gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as an endogenous control for normalization.

Gene expression data were analyzed using the comparative $\Delta\Delta Ct$ method. Cycle threshold (Ct) values for target genes were first normalized to the GAPDH reference gene within each sample to obtain ΔCt values. The mean ΔCt from the control group was then used as the calibrator to calculate $\Delta\Delta Ct$ values for all experimental samples. Final relative expression levels are presented as fold-change ($2^{-\Delta\Delta Ct}$) (3).

Serum Oxidative Stress Markers

Following the experimental intervention, serum oxidative stress indices were evaluated to assess both oxidative damage and antioxidant defense status. These included malondialdehyde (MDA), a terminal product of lipid peroxidation; the antioxidant enzymes—superoxide dismutase (SOD) and glutathione peroxidase (GPx); and the total antioxidant capacity (TAC), which reflects the cumulative action of all circulating antioxidants (1).

All measurements were performed using commercially available and rat-validated ELISA kits, following the manufacturers' protocols to ensure methodological accuracy and reproducibility. Specifically, Serum MD levels were quantified spectrophotometrically by assessing the absorbance of the pink chromogenic complex generated through its reaction with thiobarbituric acid-reactive substances, using the procedure outlined in an earlier report (1).

The enzymatic antioxidants SOD and GPx were determined using activity-based kits (SOD: Ransod kit, Randox; Cat. No. SD125; GPx: Randox; Cat. No. SC692). Serum samples were incubated in antibody-coated wells, and enzyme activity was quantified through substrate turnover, measured colorimetrically according to the kit instructions (10).

TAC was measured using a colorimetric assay based on the reduction of a stable radical cation (e.g., ABTS⁺ or Cu²⁺) by antioxidants present in the sample (Bio Diagnostic, Egypt; Cat. No. TA2513). The decrease in absorbance was directly proportional to the antioxidant capacity of the serum. All assays were run in duplicate, and sample concentrations or enzyme activities were calculated by interpolation from standard curves generated using

four-parameter logistic regression. Results were expressed as follows: MDA in nmol/mL, SOD and GPx activities in U/mL, and TAC in mmol Trolox equivalents/L.

Statistical Analysis

Data were presented as mean \pm SEM. Normality of distribution was assessed using the Shapiro–Wilk test. For normally distributed variables, one-way ANOVA followed by Tukey's post hoc test was applied. For non-normally distributed data, the Kruskal–Walli's test followed by Dunn's multiple comparison test was used. A *P* value < 0.05 was considered statistically significant.

Results

Animal Health, Body Weight, and Testis Weight

All animals tolerated the surgical procedures and the 8-week treatment period without mortality. There were no significant differences in the final body weights among the four experimental groups (*P* > 0.05). However, a significant reduction in both absolute and relative (to body weight) testis weight was observed in the varicocele + vehicle group compared to the sham and chrysin-only groups (*P* < 0.01). Treatment with chrysin in the varicocele + chrysin group significantly prevented this weight loss, with testis weights comparable to those of the sham control group (*P* > 0.05) (Table 1).

Sperm Parameters

Varicocele induction resulted in marked deterioration of sperm quality. In the varicocele + vehicle group, sperm concentration and total count were significantly reduced, accompanied by a pronounced decline in both motile and progressively motile sperm relative to the sham group (*P* < 0.001). Furthermore, the percentage of sperm with abnormal morphology (particularly head and tail defects) was markedly higher in this group. Administration of chrysin to varicocele-induced rats (varicocele + chrysin group) resulted in significant improvement across all parameters. Sperm concentration, motility, and morphology were all significantly better than in the untreated varicocele group, though not fully restored to the level of the sham controls (*P* < 0.05 vs. varicocele + vehicle; *P* < 0.05 vs. sham) (Table 2).

Histopathological Evaluation of Testicular Tissue

Histological examination of testicular sections from

Table 1. Body and Testis Weights of Experimental Groups

Group	Final Body Weight (g)	Absolute Testis Weight (g)	Relative Testis Weight (mg/g bw)
Sham	295.4 \pm 8.7	1.68 \pm 0.05	5.69 \pm 0.15
Chrysin	296.1 \pm 7.9	1.71 \pm 0.04	5.74 \pm 0.12
Varicocele	292.2 \pm 9.3	1.32 \pm 0.06**	4.25 \pm 0.18**
Varicocele + Chrysin	293.8 \pm 8.5	1.61 \pm 0.05**	5.48 \pm 0.14**

Data presented as mean \pm SEM; n=8. ** *P* < 0.01 vs. Sham group; ** *P* < 0.01 vs. Varicocele + Vehicle group.

Table 2. Epididymal Sperm Parameters

Parameter	Sham	Chrysin	Varicocele	Varicocele + Chrysin
Concentration (10 ⁶ /mL)	61.3 ± 3.2	60.1 ± 2.8	22.4 ± 2.5***	41.7 ± 3.2***
Total motility (%)	77.4 ± 2.4	77.8 ± 2.1	32.6 ± 3.4***	58.4 ± 3.5***
Progressive motility (%)	55.3 ± 2.9	54.5 ± 2.6	15.2 ± 2.6***	38.9 ± 2.7***
Abnormal morphology (%)	8.5 ± 1.2	9.1 ± 1.4	51.3 ± 3.5***	18.8 ± 2.3***

Data presented as mean ± SEM; n=8. *** $P < 0.001$ vs. sham group; *** $P < 0.001$ vs. varicocele group.

the sham and chrysin-only groups revealed normal architecture with orderly, complete spermatogenic cycles in the seminiferous tubules. In contrast, the varicocele + vehicle group displayed severe pathological alterations, including disorganized germinal epithelium, significant vacuolization, sloughing of germ cells into the lumen, and a reduction in the number of mature spermatozoa. Quantitative analysis showed a significant decrease in both the mean seminiferous tubule diameter and the Johnsen score in this group compared to controls ($P < 0.001$). Treatment with chrysin markedly ameliorated these structural damages. The varicocele + chrysin group showed better-preserved tubular architecture, more complete spermatogenic layers, and significantly higher Johnsen scores and tubular diameters compared to the untreated varicocele group ($P < 0.01$), indicating a protective effect on testicular histoarchitecture (Figure 1, Table 3).

Serum Oxidative Stress Markers

Varicocele induction produced a pronounced state of oxidative stress, evidenced by a significant rise in serum MDA concentrations—a key indicator of lipid peroxidation—together with marked reductions in antioxidant enzyme activities (SOD and GPx) and TAC, relative to the sham group ($P < 0.001$).

Administration of chrysin effectively mitigated this oxidative disturbance. Animals in the varicocele + chrysin group exhibited markedly decreased MDA concentrations

Table 3. Testicular Histomorphometric Data

Group	Johnsen Score	Tubular Diameter (µm)
Sham	9.4 ± 0.2	285.3 ± 6.7
Chrysin	9.6 ± 0.3	288.1 ± 5.9
Varicocele	5.1 ± 0.2***	158.8 ± 8.5***
Varicocele + chrysin	7.8 ± 0.3***	215.4 ± 7.2***

Data presented as mean ± SEM; n=8. *** $P < 0.001$ vs. sham group; *** $P < 0.001$ vs. varicocele group.

Table 4. Serum Oxidative Stress and Antioxidant Parameters

Parameter	Sham	Chrysin	Varicocele	Varicocele + Chrysin
MDA (nmol/mL)	1.8 ± 0.2	1.7 ± 0.1	5.9 ± 0.4***	3.1 ± 0.3***
SOD (U/mL)	25.4 ± 1.3	26.8 ± 1.1	12.7 ± 1.5***	20.9 ± 1.2***
GPx (U/L)	350.6 ± 15.2	362.4 ± 12.8	201.3 ± 18.7***	305.8 ± 16.4***
TAC (mM)	1.42 ± 0.07	1.45 ± 0.06	0.78 ± 0.05***	1.21 ± 0.06***

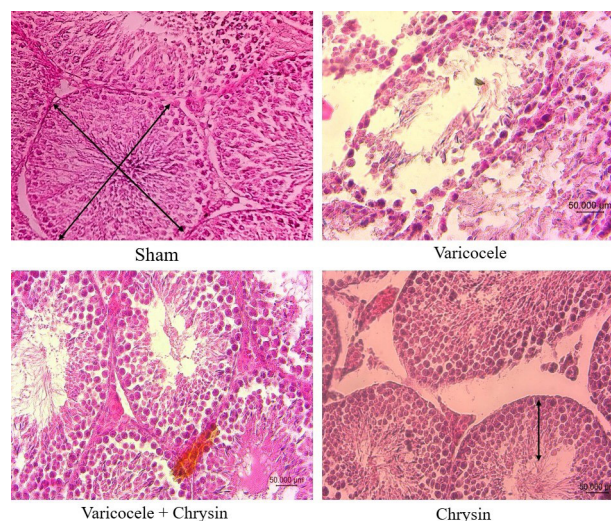
Data presented as mean ± SEM; n=8. *** $P < 0.001$ vs. sham group; *** $P < 0.001$ vs. varicocele group.

and significantly increased SOD, GPx, and TAC levels compared with the varicocele + vehicle group ($P < 0.01$), indicating the strong in vivo antioxidant potential of chrysin (Table 4).

Testicular Gene Expression

At the molecular level, induction of varicocele markedly increased the mRNA expression of pro-inflammatory genes (TNF- α and IL-1 β) as well as the pro-apoptotic marker Bax, while simultaneously decreasing the expression of the anti-apoptotic gene Bcl-2 in testicular tissue ($P < 0.001$ vs. sham). As a result, the Bax/Bcl-2 ratio—an essential determinant of apoptotic progression—was considerably elevated.

Treatment with chrysin effectively counteracted these unfavorable molecular alterations. In the varicocele + chrysin group, the expression levels of TNF- α , IL-1 β , and Bax were significantly reduced, whereas Bcl-2 expression was enhanced, resulting in a markedly lower Bax/Bcl-2 ratio relative to the untreated varicocele group ($P < 0.01$) (Figure 2).

**Figure 1.** Histological Finding in Study Groups.

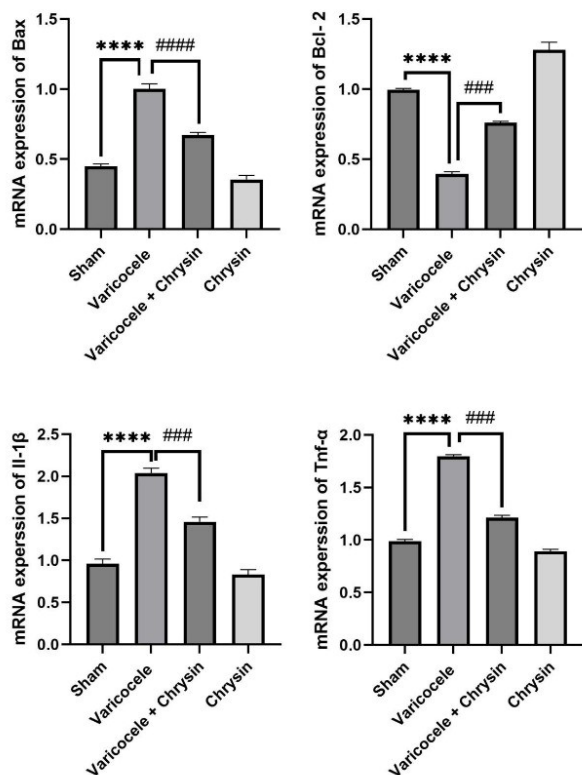


Figure 2. Relative mRNA Expression of *Bax*, *Bcl-2*, *Tnf-α*, *IL-1β*, the *Bax/Bcl-2* Ratio in Testicular Tissue. Data are mean \pm SEM; n=8. ANOVA test was used for statistical analyses. *** $P < 0.001$ vs. Sham; ### $P < 0.001$, ** $P < 0.01$ vs. Varicocele.

Discussion

Varicocele is recognized as one of the most prevalent and surgically correctable causes of male infertility, with its underlying mechanisms involving a multifaceted interaction of oxidative stress, inflammatory pathways, and germ cell apoptosis, ultimately leading to impaired spermatogenesis (14-16). The present study investigated the protective effects of chrysin on reproductive parameters, testicular histoarchitecture, inflammatory gene expression, and oxidative stress in an experimental rat model of varicocele (14-16). Our findings clearly demonstrate that varicocele induction resulted in significant deterioration of sperm quality, structural damage to seminiferous tubules, increased oxidative stress, and upregulation of inflammatory and apoptotic markers. Importantly, administration of chrysin significantly ameliorated these pathological changes, suggesting that this flavonoid exerts a protective effect on testicular tissue through multiple complementary mechanisms.

In this study, one of the earliest signs of testicular dysfunction was the reduction in testicular weight observed in varicocele-induced rats. Such loss of mass is typically indicative of seminiferous epithelial degeneration and germ cell depletion (4,8). Previous experimental and clinical studies showed that venous stasis and impaired testicular microcirculation in varicocele lead

to hypoxia and increased scrotal temperature, both of which contribute to testicular atrophy and reduced spermatogenic activity (9,14,17). Chen et al showed that varicocele induced in adult male rats led to testicular damage and oxidative stress (18). In our model, rats subjected to varicocele exhibited a significant reduction in both absolute and relative testicular weights compared with sham controls. However, chrysin administration significantly prevented this decline, suggesting that the compound may preserve cellular integrity and reduce tissue degeneration in the testes under pathological conditions. Missassi et al reported that administration of chrysin can protect the testis tissue against oxidative stress damage related to varicocele (19).

Consistent with the structural changes observed in the testes, varicocele induction resulted in a marked deterioration in epididymal sperm parameters. Significant reductions were observed in sperm concentration, total motility, and progressive motility, along with a pronounced increase in abnormal sperm morphology (20). These findings align with numerous clinical reports indicating that men with varicocele frequently present with impaired semen parameters (21). One of the primary mechanisms underlying this impairment is oxidative damage to spermatozoa. Sperm cells are particularly vulnerable to ROS due to the high content of polyunsaturated fatty acids in their plasma membrane and their relatively limited antioxidant defense systems (22). Excess ROS can induce lipid peroxidation, mitochondrial dysfunction, and DNA fragmentation, ultimately compromising sperm viability and fertilizing capacity. In the present study, treatment with chrysin significantly improved sperm concentration, motility, and morphology in varicocele-induced rats (2,22,23). Although these parameters were not completely restored to control levels, the observed improvement indicates that chrysin partially mitigates the deleterious effects of varicocele on spermatogenesis and sperm maturation.

Histopathological examination further confirmed the damaging impact of varicocele on testicular architecture. Severe alterations were observed in the seminiferous tubules of the untreated varicocele group, including disorganization of the germinal epithelium, vacuolization, sloughing of germ cells into the lumen, and a marked reduction in the number of mature spermatozoa (3,24). These changes resulted in significantly lower Johnsen scores and reduced seminiferous tubule diameters. These histopathological alterations are hallmark indicators of impaired spermatogenesis and have been consistently documented in experimental varicocele models. Conversely, chrysin-treated rats demonstrated markedly improved seminiferous tubule architecture and elevated Johnsen scores, reflecting preservation of spermatogenic function (25). These findings suggest that chrysin helps maintain the structural integrity of the testicular microenvironment, which is essential for normal

spermatogenesis. In this context, Gabriela et al reported that administration of chrysin improved the testicular damage and testis structure in adult rat with varicocele (19).

Oxidative stress is considered a central mediator of varicocele-induced testicular damage. In the present study, varicocele significantly increased serum levels of MDA, a well-established biomarker of lipid peroxidation, while simultaneously reducing the activity of key antioxidant enzymes such as SOD and GPx, as well as TAC (3,26). This imbalance indicates a shift toward a pro-oxidant state, leading to oxidative damage of cellular membranes, proteins, and nucleic acids. These findings are consistent with previous studies demonstrating elevated oxidative stress markers in both animal models and patients with varicocele. Administration of chrysin effectively reversed these alterations by reducing MDA levels and restoring antioxidant enzyme activities. Chrysin is known to possess strong free radical scavenging properties due to its hydroxyl groups, which allow it to neutralize ROS and interrupt lipid peroxidation chain reactions. In addition, chrysin has been reported to enhance endogenous antioxidant defense systems by modulating the expression or activity of antioxidant enzymes. Therefore, the observed restoration of oxidative balance likely plays a major role in the protective effects of chrysin on testicular function (11,27).

Inflammation represents another key component of the pathological cascade triggered by varicocele. Increased production of pro-inflammatory cytokines within the testicular microenvironment can disrupt the blood–testis barrier, impair Sertoli cell function, and promote germ cell apoptosis. In the current study, expression levels of the inflammatory cytokines TNF- α and IL-1 β were significantly elevated in the testes of varicocele-induced rats. These cytokines are known to activate multiple downstream signaling pathways that amplify inflammatory responses and exacerbate tissue injury. The marked suppression of TNF- α and IL-1 β expression observed following chrysin treatment indicates a potent anti-inflammatory effect. Prior research indicates that chrysin can suppress activation of nuclear factor-kappa B (NF- κ B), a transcription factor governing the expression of numerous pro-inflammatory genes. Inhibition of this pathway may account for the reduced cytokine levels observed in the present study, thereby supporting the maintenance of testicular tissue integrity (28-30).

Apoptosis of germ cells is another important mechanism contributing to reduced spermatogenic efficiency in varicocele. The balance between pro-apoptotic and anti-apoptotic proteins determines whether cells undergo programmed cell death in response to stress signals. In our study, varicocele significantly increased the expression of the pro-apoptotic gene Bax while decreasing the expression of the anti-apoptotic gene Bcl-2, resulting in a markedly elevated Bax/Bcl-2 ratio. This shift toward a pro-apoptotic

state is consistent with previous reports indicating increased germ cell apoptosis in varicocele-affected testes (1). Chrysin administration significantly modulated these apoptotic markers by reducing Bax expression and increasing Bcl-2 expression, thereby lowering the Bax/Bcl-2 ratio. These results suggest that chrysin exerts a cytoprotective effect by restoring the balance between pro- and anti-apoptotic signaling pathways, ultimately reducing germ cell loss.

Overall, the findings of this study indicate that chrysin exerts protective effects against experimental varicocele through multiple biological mechanisms. By decreasing oxidative stress, inhibiting the expression of pro-inflammatory cytokines, and regulating apoptotic signaling pathways, chrysin appears to interfere with several critical processes responsible for varicocele-related testicular injury. The synergistic action of these mechanisms likely underlies the observed improvements in sperm parameters and testicular tissue architecture.

Limitations of the Study

However, certain limitations should be taken into account when interpreting these results. First, the investigation was performed in an animal model, and therefore the direct translation of these findings to human clinical conditions should be approached with caution. Second, the study evaluated only a single dosage of chrysin, leaving the possible dose-response relationship unclear. Moreover, additional molecular pathways associated with oxidative stress and inflammation—such as the Nrf2 antioxidant signaling pathway or mitochondrial-mediated apoptotic mechanisms—were not examined in this study. Future investigations should explore these pathways and assess whether combined therapeutic strategies targeting multiple mechanisms may further improve treatment efficacy.

Conclusions

The present study demonstrated that chrysin markedly alleviated varicocele-induced reproductive impairment in rats. The protective actions of chrysin appear to be associated with the reduction of oxidative stress, inhibition of inflammatory cytokine expression, and modulation of the apoptotic gene balance in testicular tissue. These results suggest that chrysin may serve as a promising natural therapeutic agent for the management of varicocele-related male infertility. Nevertheless, additional experimental and clinical studies are necessary to verify its effectiveness and safety in human populations.

Authors' Contribution

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this work.

Declaration of AI-assisted tools in the writing process

The authors used GapGPT-5.4 AI tool to improve grammar points and language style in writing.

Ethical Issues

All experimental procedures involving animals were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the ARRIVE guidelines. The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Tabriz University of Medical Sciences, Tabriz, Iran (Approval No.: IR.TBZMED.AEC.1404.050).

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