




Impact of Mesenchymal Stem Cells and Quercetin on Protection of Testis Against Cyclophosphamide-Related Damage

Morteza Abdi¹, Hadi Karimzadeh¹, Amirreza Jourabchi¹, Sanam Azarhoosh¹, Homa Aminzadeh Ghavifekr¹, Hamraz Pazoki², Maryam Bilabari³, Mahdi Esmaeili³, Leila Roshangar^{3*} 

Abstract

Objectives: This study aimed to evaluate the effect of transplantation of mesenchyme stem cells derived from bone marrow and quercetin on the oxidation markers, hormonal profile of testosterone, and morphometric testis and sperm parameters in adult mice poisoned with the antineoplastic drug cyclophosphamide (CTX).

Materials and Methods: All 25 mice were divided into five groups control, CTX group received CTX (150 mg/kg), quercetin + CTX group received IP injections of quercetin (75 mg/kg /daily) for 14 days, and CTX (150 mg/kg), CTX + bone marrow mesenchymal stem cells (BMMSCs) also received CTX and, 24 hours after the administration of CTX, approximately 1×10^6 BMMSCs were injected carefully into each testis of mice. Quercetin + CTX + BMMSCs group received quercetin and CTX (as in group quercetin + CTX), and BMMSCs were injected into each testis of the animals. Thirty-five days after the last injection of BMMSCs, blood samples were taken to gain serum and assess the testosterone level, and histopathological, biochemical, and sperm parameters analyses were performed.

Results: CTX led to testicular tissue damage and reduced the Johnson score and diameters of the seminiferous tube in the CTX group ($P < 0.05$). Also, the level of Antioxidant enzyme and testosterone was notably reduced in the CTX group ($P < 0.05$). The count and sperm quality were decreased significantly in the CTX group ($P < 0.05$). Administration of quercetin led to an increase in the level of testosterone and antioxidant enzymes and improved sperm parameters.

Conclusions: The present study shows the administration of quercetin can protect the testicle against CTX-related damage and improve oxidative stress damage and sperm parameters.

Keywords: Cyclophosphamide, Testis damage, Sperm parameters, Quercetin

Introduction

Cancer is a significant public health problem and a major cause of morbidity and mortality worldwide (1,2). With the emergence of innovative methods for earlier diagnosis and the expansion of anti-neoplastic treatment options, cancer patients have better chances at survival today than they possibly ever did before (3,4). Therefore, with more successful management of malignant disease, addressing the unique needs and challenges of cancer survivors has become increasingly important over the past decades (5).

Cyclophosphamide (CTX) is an antineoplastic chemotherapeutic drug that belongs to the subclass of alkylating agents (6). Despite being one of the oldest anti-cancer treatments available, it is used for the treatment of various subtypes of malignant disease as well as disorders of the immune system frequently in the present day (7).

Male patients treated with CTX frequently develop fertility-related issues later in their lives (8). While the exact molecular mechanism of CTX gonadotoxicity is

yet to be elucidated, several in vivo studies have shown evidence of decreased testicular weight, oligospermia, teratospermia, azoospermia, and a decline in the number of spermatogonia in males after administrating CTX (9,10).

Several investigations highlight the role of specific cytopathologic patterns of involvement after exposure to the drug (11). Multiple studies have noted decreased activity of antioxidant enzymes, as well as an increase in reactive oxygen species after exposure to CTX (12-15). These observations suggest a role for oxidative stress in the pathophysiology of gonadal tissue damage due to the drug (16).

Likewise, anti-oxidants are thought to mitigate the gonadotoxic effects of chemotherapeutic drugs (17,18). Pre-treatment of animal models with quercetin, an anti-oxidant flavonoid with anti-inflammatory features, has resulted in attenuation of gonadal tissue damage after the administration of alkylating agents (19,20). However, the

Received 24 September 2024, Accepted 7 January 2025, Available online 11 February 2025

¹Student research committee, Tabriz University of Medical Sciences, Tabriz, Iran. ²Clinical research development unit of Tabriz Valiasr Hospital, Tabriz University of Medical Sciences, Tabriz, Iran. ³Department of Anatomical Sciences, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran.

*Corresponding Author: Leila Roshangar, Email: Lroshangar@yahoo.com



evidence for administration of anti-oxidant chemotherapy for human patients is inconclusive at best (21).

Mesenchymal stem cells (MSCs) possess the characteristic features of self-renewal and the ability to differentiate into various tissues under the influence of local and systemic molecules, including cytokines and growth factors (22,23). Therapeutic use of MSCs via transplantation has gained much recognition and popularity in the rapidly developing field of regenerative medicine, especially for women (24). Stem cell transplantation for restoring fertility is an area of active research (25). In vitro studies on laboratory animals have demonstrated a potential role for stem cell transplantation in restoring fertility after administering systemic chemotherapeutic medication (26,27).

In response to these findings, we hypothesize that a combination of MSCT and quercetin as an antioxidant would decrease the gonadotoxic effects of CTX and can help preserve fertility in adult male mice treated with the drug.

Materials and Methods

In this research, 25 adult male albino mice (aged 6-8 weeks, weighing 22-25 g) were acquired from the Animal Care Center of Tabriz University of Medical Sciences. They were kept in the standard situations (12 hours light/dark, 24-26 °C, and 45%-50% humidity). Besides this, the mice had free access to food and water.

Study Design

All of the mice were randomly divided into two main groups: control (n=5), in which the mice received only normal saline vehicle intraperitoneally (IP) (two weeks), and experimental groups, which were separated into four subgroups.

- CTX group (n=5) were given IP injections of CTX (150 mg/kg dissolved in saline) on days 7 and 14; the dose of CTX was chosen based on the previous study (28).
- Quercetin + CTX group (n=5). The mice in this group received IP injections of quercetin (75 mg/kg) for 14 days; the dose of Quercetin was chosen based on a previous study (28,29). They also were given two IP injections of CTX (150 mg/kg) on days 7 and 14.
- Bone marrow mesenchymal stem cells (BMMSCs) + CTX group (n=5). They had gained CTX, as mentioned above, and 24 hours after the final injection of CTX, nearly 1×10^6 BMMSCs were carefully injected into each mouse's testis.
- Quercetin + BMMSCs + CTX group (n=5). The animals in this group were given quercetin and CTX (like the group quercetin + CTX). Subsequently, 24 hours after the last injection of quercetin and CTX, 1×10^6 BMMSCs were injected into each testis of these animals.

To inject BMMSCs into the testis, mice were anesthetized using a mixture of ketamine (60 mg/kg) and xylazine (10

mg/kg). Finally, cells were resuspended in normal saline and loaded into a 1 mL syringe for injection into the testes. 1×10^6 BMMSCs in 120 μ L of normal saline were injected into each testis.

Thirty-five days after the injection of BMMSCs, all groups were euthanized humanely by dislocation of the cervical vertebra under anesthesia. The testes and blood were harvested for the following experiments.

CTX powder was purchased from (Baxter Healthcare Company, India). Quercetin yellow powder was provided from (Roche, Switzerland). Ketamine and xylazine, respectively, were obtained from (Rotexmedica, Germany) and (Bioveta Company, Czech Republic). Other reagents are described in the text below.

Isolation and Culture of BMMSCs

In brief, mice were first disinfected with 70% ethanol following cervical dislocation. Afterward, tibias and femurs were taken from mice, and then their bones were cut at the median; bone marrow content was flushed out by using a syringe containing 5 mL of low-glucose Dulbecco's modified eagle medium (DMEM/LG, Gibco). The flushed marrow was centrifuged (5 minutes, 1500 rpm), the supernatant was removed, and the pelleted fraction was resuspended in 5 mL DMEM. Then, resultant cells were cultured in a medium with 10% fetal bovine serum (FBS, Gibco) and 1% penicillin and streptomycin (Gibco). The cells were incubated at 37 °C with 5% CO₂ and saturated humidity. After 24 hours, non-adherent cells were removed by replacing the medium. The medium was changed every 3 days. When the cells reached 80%~90% confluence, they were sub-cultured. In our experiments, BMMSCs were used in the third to sixth passages.

Tissue Processing for Histopathological Examination

The animals' testes were removed from all experimental and control groups, fixed in Bouin's solution for 96 hours, and processed for light microscopic examination. Tissues were embedded in paraffin blocks, and 5 μ m thick sections were prepared. Sections were deparaffinized with xylene and dyed with hematoxylin and eosin (H&E). A pathologist blinded to the experimental treatments examined the slides. The Johnson scores and seminiferous tubule diameter (STD) and thickness of the germinal epithelium were measured at $\times 200$ by ImageJ software.

Biochemical Analysis of Oxidation

Malondialdehyde (MDA) is a widely used biomarker for assessing lipid peroxidation levels. Traditionally, MDA levels are determined using the thiobarbituric acid reactive substances (TBARS) assay, which involves the reaction of MDA with thiobarbituric acid to produce a colored product. The color intensity is measured spectrophotometrically at 532 nm to quantify MDA levels.

Superoxide dismutase (SOD) activity in samples is typically determined by assessing the ratio of "auto-

oxidation” rates in the presence and absence of the sample. The colorimetric assay involves measuring the color reaction at 480 nm.

Catalase (CAT) activity is assessed “colorimetrically” by monitoring the consumption of hydrogen peroxide (H₂O₂) in the presence of the sample. The decline in absorbance at 240 nm is measured to determine CAT activity.

Hormone Assays

The hormonal profile of testosterone in blood samples was determined by ELISA kits provided by ALPCO (USA).

Semen Analysis

To check spermatozoa's quality, the epididymis's tail part was placed inside the dishes with 5 mL of phosphate-buffered saline (PBS; pH 7.2) and incubated for 20 minutes. Afterward, 100 µL of this mixed solution was dissolved in 900 µL PBS. The number of sperm cells examined under a light microscope in 8 microscopic fields of 0.1 cm² each except the central erythrocyte area. The sperm count and percentage of viable, motile spermatozoa were determined. To evaluate the morphological abnormalities of spermatozoa in the head, neck, and tail parts. The slides were stained with hematoxylin and eosin method and examined under a light microscope (the numbers of 100 spermatozoa of each mouse, at ×400 magnification). Results were expressed as a percentage.

Statistical Analysis

Statistical analysis was performed using SPSS version 22 (USA). All data in the present research are expressed as mean ± standard error of mean (SEM) and were compared using one-way ANOVA and Tukey's post hoc

test for comparing the study groups. A value of $P < 0.05$ was considered as significant.

Results

Testis Histological Parameters

Histomorphometric analysis showed a significant decrease in the mean Johnson's score (MJS) in the experimental groups compared to the control group ($P < 0.05$). Conversely, treatment with quercetin and BMMSCs resulted in a significant improvement in MJS in the treated groups compared to the CTX group ($P < 0.05$).

Furthermore, the STD exhibited a significant reduction in the CTX group compared to the control ($P < 0.001$). However, the STD was significantly higher in the quercetin and BMMSCs groups and the combined quercetin + BMMSCs group than in the CTX group ($P < 0.05$).

Additionally, the height of the seminiferous epithelium (HE) demonstrated a significant decrease in the CTX group compared to the control ($P = 0.02$). Notably, treatment with quercetin and BMMSCs significantly improved the HE in the treated groups compared to the CTX group ($P = 0.03$). These findings are illustrated in Figure 1 and Table 1.

Biochemical Parameters

In the experimental groups, serum CAT levels were significantly reduced compared to the control group ($P < 0.001$). However, CAT levels were significantly increased in the quercetin and BMMSCs groups, as well as the combined quercetin + BMMSCs group, when compared to the CTX group ($P < 0.001$) (Figure 2A). Serum SOD levels were significantly lower in the CTX group compared to the control group ($P < 0.001$). Conversely, SOD levels were significantly improved in the

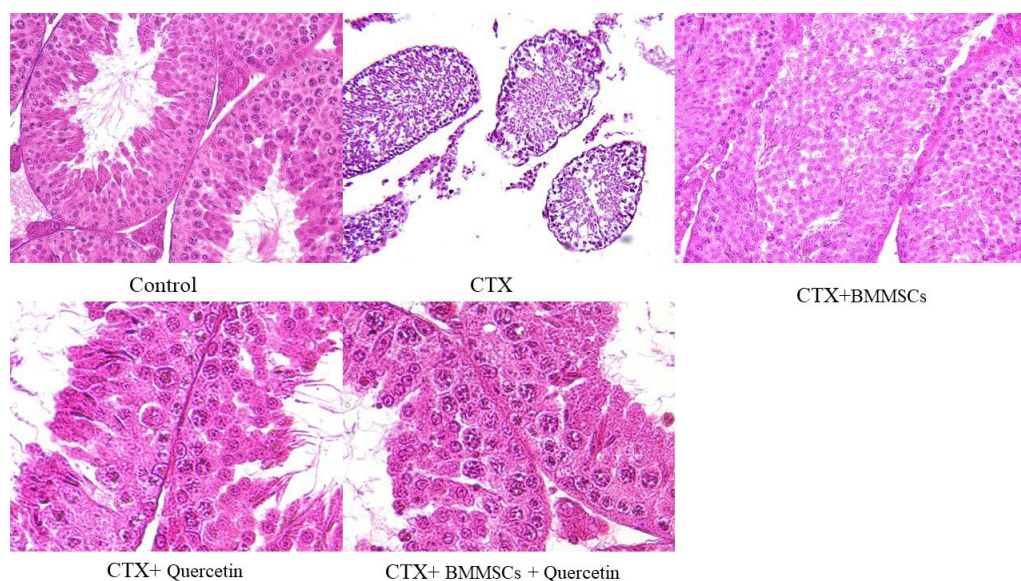


Figure 1. Histological Findings in the Study Group. Control group: Received only normal saline intraperitoneally (IP). CTX group: Administered IP injections of CTX (150 mg/kg dissolved in saline). BMMSCs + CTX Group: Received CTX along with 1×10^6 BMMSCs. CTX + Quercetin Group: Received 75 mg/kg of quercetin in addition to CTX. CTX + Quercetin + BMMSCs Group: Animals in this group received both quercetin and CTX, along with BMMSCs.

Table 1. Histological Results in Study Groups

Groups	Johnson Score	STD	HE
Control	9.7±0.15	265.2±8.2	70.25±3.2
CTX	4.35±0.2*	145.5±4.5*	32.5±2.5*
CTX+ BMMSC	6.4±0.25#	185±6.7#	48.5±2.2#
CTX+ Quercetin	6.55±0.15#	198±10.2#	52±3.2#
CTX+ BMMSC+ Quercetin	6.75±0.12#	205±12.5#	55.2±2.5#

Control group: Received only normal saline intraperitoneally (IP). CTX group: Administered IP injections of CTX (150 mg/kg dissolved in saline). BMMSCs + CTX Group: Received CTX along with 1×10^6 BMMSCs. CTX + Quercetin Group: Received 75 mg/kg of quercetin in addition to CTX. CTX + Quercetin + BMMSCs Group: Animals in this group received both quercetin and CTX, along with BMMSCs.

* $P < 0.05$ indicates significant differences between the control and other groups, and # $P < 0.05$ indicates significant differences between the CTX and other groups.

treated groups compared to the CTX group ($P < 0.001$) (Figure 2B).

Furthermore, serum MDA levels were significantly elevated in the CTX group compared to the control ($P < 0.001$). Notably, MDA levels were significantly reduced in the treated groups compared to the CTX group ($P < 0.001$) (Figure 2C).

Testosterone Serum Level

Serum testosterone levels were significantly decreased in the CTX group compared to the control group ($P < 0.001$). Conversely, testosterone levels were significantly increased in the treatment groups (quercetin, BMMSCs, and quercetin + BMMSCs) compared to the CTX group ($P < 0.05$). There was no significant difference in testosterone levels between the various treatment groups ($P < 0.05$) (Figure 3).

Result of Sperm Parameters

Examination of semen analyses in this research shows the sperm count was notably reduced in the CTX group compared to the control group ($P < 0.05$). On the other hand, in the experimental group with the administration of quercetin, the count of sperm was remarkably higher than in the CTX group ($P < 0.05$). Also, the percentage of normal morphological sperm was significantly decreased in the CTX group in comparison with control ($P < 0.05$). However, the number of sperm with normal morphology was notably higher in the treated group with quercetin and BMMSC than in the CTX group ($P < 0.05$). Also, the percentage of motile sperm was significantly decreased in the CTX group in comparison with control ($P < 0.05$). However, the number of sperm with motility was notably higher in the treated group with quercetin and BMMSC than in the CTX group ($P < 0.05$).

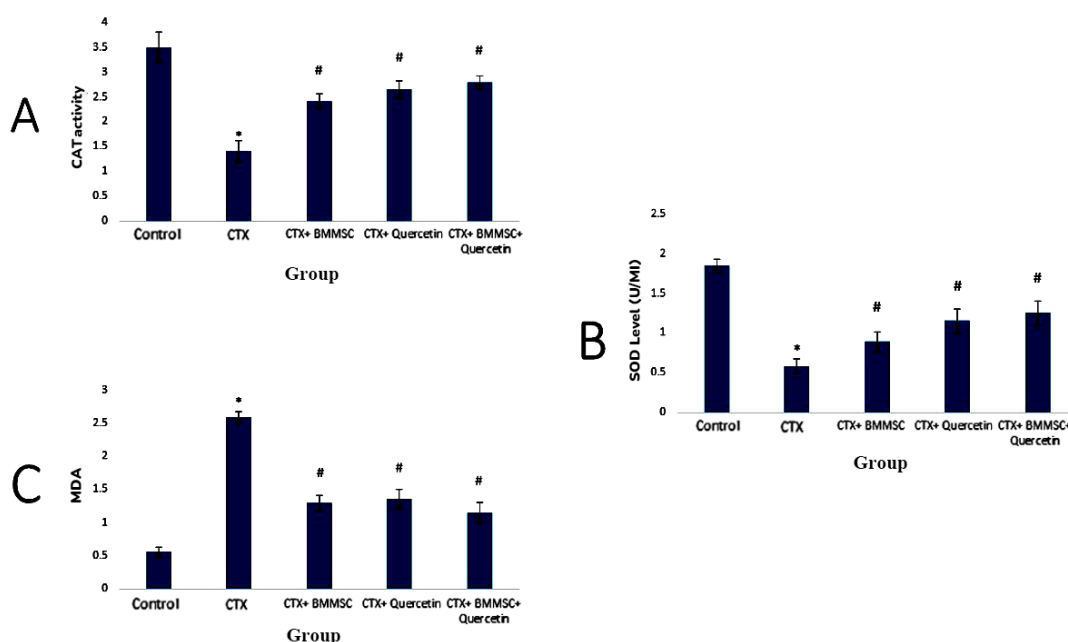


Figure 2. Oxidative Stress Markers in the Study Groups. A: serum level of CAT in study groups; B: serum level of serum SOD in study groups; C: serum level of MDA in study groups. Control group: Received only normal saline intraperitoneally (IP). CTX group: Administered IP injections of CTX (150 mg/kg dissolved in saline). BMMSCs + CTX Group: Received CTX along with 1×10^6 BMMSCs. CTX + Quercetin Group: Received 75 mg/kg of quercetin in addition to CTX. CTX + Quercetin + BMMSCs Group: Animals in this group received both quercetin and CTX, along with BMMSCs. * $P < 0.05$ indicates significant differences between the control and other groups, and # $P < 0.05$ indicates significant differences between the CTX and other groups.

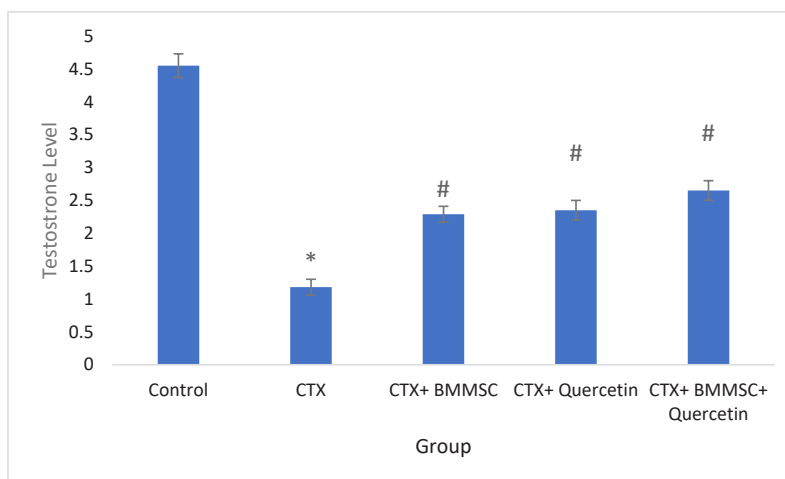


Figure 3. Serum Testosterone Level in the Study Groups. Control group: Received only normal saline intraperitoneally (IP). CTX group: Administered IP injections of CTX (150 mg/kg dissolved in saline). BMMSCs + CTX Group: Received CTX along with 1×10^6 BMMSCs. CTX + Quercetin Group: Received 75 mg/kg of quercetin in addition to CTX. CTX + Quercetin + BMMSCs Group: Animals in this group received both quercetin and CTX, along with BMMSCs. * $P < 0.05$ indicates significant differences between the control and other groups, and # $P < 0.05$ indicates significant differences between the CTX and other groups.

Discussion

This study investigates the protective effects of quercetin and BMMSCs on testicular damage induced by CTX, focusing on histological, biochemical, and sperm parameters. The findings provide substantial evidence that both quercetin and BMMSCs, alone or in combination, mitigate the deleterious effects of CTX on testicular structure and function.

The histomorphometric analysis revealed significant alterations in testicular architecture in the CTX group, characterized by a reduction in the MJS, STD, and the height of the seminiferous epithelium (HE). These changes reflect the extensive damage CTX inflicts on spermatogenesis, which is consistent with its known cytotoxic effects, particularly on rapidly dividing cells such as spermatogonia (30). CTX is an alkylating agent that induces DNA cross-linking, leading to apoptosis and a subsequent reduction in germ cell populations within the seminiferous tubules (31,32). In this context, Kim et al demonstrated in a study that administration of CTX in male mice led to testicular damage and toxicity related to oxidative stress induced by CTX (33). Also, Uzun-Goren and Uz presented in their study that CTX can induce testicular inflammation and apoptosis, and administration of antioxidant compound can protect the testicle against CTX-related damage (34).

The observed decrease in MJS indicates a decline in the quality and progression of spermatogenesis, with fewer seminiferous tubules containing complete spermatogenic series. The reduction in STD further corroborates this, as the diameter of seminiferous tubules is directly related to the number of germ cells they contain. A diminished HE height also suggests a loss of Sertoli cell function and a reduction in the support for germ cell development, which is critical for maintaining the integrity of the blood-testis barrier and facilitating the movement of spermatocytes

through the seminiferous epithelium (35, 36).

However, individually and in combination, treatment with quercetin and BMMSCs significantly ameliorated these histological alterations. The improvement in MJS, STD, and HE suggests that these treatments enhance the regenerative capacity of the testis, likely through different mechanisms. Quercetin, a flavonoid with potent antioxidant properties (37), may protect against oxidative stress-induced damage, while BMMSCs could contribute to tissue repair through paracrine signaling and possibly by differentiating into supportive cell types, including Sertoli and Leydig cells (38, 39). The combined treatment did not show a statistically significant difference compared to the individual treatments, which might suggest a ceiling effect or that the benefits of each treatment reach a maximum threshold when administered together. In this context, Lu et al demonstrated in research that using MSCs can ameliorate chemotherapy-induced damage in the testis by reducing testis inflammation and apoptosis (40).

The study also examined key biochemical markers of oxidative stress and antioxidant defense mechanisms. In the CTX group, a significant reduction in CAT and SOD levels, coupled with elevated MDA levels, indicates substantial oxidative damage. CAT and SOD are critical enzymes in the antioxidant defense system, catalyzing the conversion of H_2O_2 and superoxide radicals into less harmful molecules. Their depletion in the CTX group suggests overwhelming oxidative stress, which is a known mechanism of CTX-induced cytotoxicity (37,41).

The increase in MDA, a byproduct of lipid peroxidation, provides further evidence of oxidative stress and damage to cellular membranes, especially in testes involving cells rich in polyunsaturated fatty acids (42). The observed reduction in MDA levels in the treatment groups suggests that quercetin and BMMSCs have significant antioxidative effects, helping to mitigate the oxidative damage caused by

CTX (6,43).

Quercetin's role as an antioxidant is well-documented, where it directly scavenges reactive oxygen species and enhances the activity of endogenous antioxidant enzymes (37). BMSCs, on the other hand, may exert their effects through the secretion of trophic factors that upregulate antioxidant defenses and facilitate tissue repair and regeneration. The combined treatment group showed similar improvements in these biochemical parameters, underscoring the efficacy of both interventions in restoring redox balance and protecting testicular cells from oxidative damage (44, 45). Uzun-Goren and Uz demonstrated in a study that administration of quercetin led to an increase in antioxidant enzyme activity, enhancement in testosterone levels, and testicular functions (34). As well as Shokoohi et al showed in their research that usage of antioxidants led to testicular protection against oxidative stress-related damages (46).

Testosterone is a key hormone for male reproductive function, influencing spermatogenesis and secondary sexual characteristics (45,47). The significant reduction in serum testosterone levels in the CTX group aligns with the observed histological damage, mainly the impaired function of Leydig cells responsible for testosterone production (32). The restoration of testosterone levels in the treated groups suggests that quercetin and BMSCs help preserve Leydig cell function or facilitate its recovery post-injury. This hormonal restoration is crucial, as it directly influences the spermatogenic process and the maintenance of male fertility (48,49).

Examining sperm parameters further supports the protective effects of quercetin and BMSCs. CTX treatment led to a marked reduction in sperm count, motility, and normal morphology, reflecting the extensive damage to the germinal epithelium and the downstream effects on sperm production and quality. Sperm count is particularly sensitive to disruptions in spermatogenesis, and the observed decline in the CTX group suggests significant germ cell loss. The reduction in sperm motility and morphology indicates damage to the sperm maturation process and the structural integrity of sperm cells (50).

In contrast, the treatment groups significantly improved all measured sperm parameters. This suggests that quercetin and BMSCs not only protect against initial damage but also enhance the recovery of spermatogenesis. Quercetin's antioxidative properties likely play a role in preserving sperm cell integrity and function, while BMSCs may contribute through regenerative mechanisms, potentially including the replacement of damaged germ cells and the support of spermatogonial stem cell niches.

Limitations of the study

However, this study is not without limitations. Utilizing a rat model CTX-induced related damage, the research

provides valuable insights into biological processes; nonetheless, animal models may not fully capture the complexity of human testicular disorders. Therefore, caution is warranted when extrapolating these findings to human populations, as they may not directly apply to all cases of testicular dysfunction.

Conclusions

In summary, the results of this study underscore the protective and restorative potential of quercetin and BMSCs against CTX-induced testicular damage. By mitigating oxidative stress, preserving testicular architecture, and restoring key reproductive parameters, these treatments offer promising therapeutic avenues for male infertility associated with chemotherapeutic agents like CTX. Further research is warranted to explore the precise mechanisms through which these interventions exert their effects and to determine their potential for clinical application in chemotherapy-induced gonadotoxicity.

Directions for Future Research

Consequently, the efficacy of quercetin and BMSCs treatment in other testicular dysfunctions remains to be determined. Future research should aim to elucidate the specific molecular targets of quercetin and BMSCs and optimize their therapeutic efficacy in various contexts of testicular disorders.

Authors' Contribution

Conceptualization: Morteza Abdi, Amirreza Jourabchi, Homa Aminzadeh Ghavifekr, Lila Roshangar.

Data curation: Hadi Karimzadeh, Amirreza Jourabchi, Hamraz Pazoki, Lila Roshangar.

Formal analysis: Hamraz Pazoki, Maryam Bilabari, Mahdi Esmaeili.

Funding acquisition: Morteza Abdi, Sanam Azarhoosh, Lila Roshangar.

Investigation: Amirreza Jourabchi, Sanam Azarhoosh, Homa Aminzadeh Ghavifekr, Hamraz Pazoki, Maryam Bilabari, Mahdi Esmaeili.

Methodology: Morteza Abdi, Amirreza Jourabchi, Lila Roshangar.

Project administration: Morteza Abdi, Lila Roshangar.

Resources: Lila Roshangar.

Software: Hamraz Pazoki, Maryam Bilabari, Mahdi Esmaeili.

Supervision: Lila Roshangar.

Validation: Morteza Abdi, Hadi Karimzadeh, Amirreza Jourabchi, Sanam Azarhoosh, Homa Aminzadeh Ghavifekr, Hamraz Pazoki, Lila Roshangar.

Visualization: Morteza Abdi, Hadi Karimzadeh, Amirreza Jourabchi, Sanam Azarhoosh, Homa Aminzadeh Ghavifekr, Hamraz Pazoki, Maryam Bilabari, Mahdi Esmaeili, Lila Roshangar.

Writing—original draft: Amirreza Jourabchi.

Writing—review & editing: Amirreza Jourabchi, Hamraz Pazoki, Maryam Bilabari, Mahdi Esmaeili, Lila Roshangar.

Conflict of Interests

Authors have no conflict of interest.

Ethical Issues

The present research was based on the International Ethical Committee of Medical Laboratory Animals guidelines and

approved by Tabriz University of Medical Sciences (IR.TBZMED.AEC.1401.028).

Financial Support

The research protocol was approved and supported by Student Research Committee of Tabriz University of Medical Sciences (grant number: 68558).

References

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. *CA Cancer J Clin*. 2022;72(1):7-33. doi:10.3322/caac.21708
2. Mattiuzzi C, Lippi G. Current cancer epidemiology. *J Epidemiol Glob Health*. 2019;9(4):217-222. doi:10.2991/jegh.k.191008.001
3. Howlader N, Ries LA, Mariotto AB, Reichman ME, Ruhl J, Cronin KA. Improved estimates of cancer-specific survival rates from population-based data. *J Natl Cancer Inst*. 2010;102(20):1584-1598. doi:10.1093/jnci/djq366
4. Debela DT, Muzazu SG, Heraro KD, et al. New approaches and procedures for cancer treatment: current perspectives. *SAGE Open Med*. 2021;9:20503121211034366. doi:10.1177/20503121211034366
5. Jefford M, Howell D, Li Q, et al. Improved models of care for cancer survivors. *Lancet*. 2022;399(10334):1551-1560. doi:10.1016/s0140-6736(22)00306-3
6. Seker U, Kavak DE, Dokumaci FZ, Kizildag S, Irtgun-Kandemir S. The nephroprotective effect of quercetin in cyclophosphamide-induced renal toxicity might be associated with MAPK/ERK and NF- κ B signal modulation activity. *Drug Chem Toxicol*. 2024;47(6):1165-1174. doi:10.1080/01480545.2024.2347541
7. Fraiser LH, Kanekal S, Kehrler JP. Cyclophosphamide toxicity. Characterising and avoiding the problem. *Drugs*. 1991;42(5):781-795. doi:10.2165/00003495-199142050-00005
8. Meistrich ML, Wilson G, Brown BW, da Cunha MF, Lipshultz LI. Impact of cyclophosphamide on long-term reduction in sperm count in men treated with combination chemotherapy for Ewing and soft tissue sarcomas. *Cancer*. 1992;70(11):2703-2712. doi:10.1002/1097-0142(19921201)70:11<2703::aid-cnrcr2820701123>3.0.co;2-x
9. Fusco R, Salinaro AT, Siracusa R, et al. Hidrox® counteracts cyclophosphamide-induced male infertility through NRF2 pathways in a mouse model. *Antioxidants (Basel)*. 2021;10(5):778. doi:10.3390/antiox10050778
10. Ghobadi E, Moloudizargari M, Asghari MH, Abdollahi M. The mechanisms of cyclophosphamide-induced testicular toxicity and the protective agents. *Expert Opin Drug Metab Toxicol*. 2017;13(5):525-536. doi:10.1080/17425255.2017.1277205
11. Luong SN, Isaacs A, Liu Z, Sin FE, Giles I. A systematic review and meta-analysis of the gonadotoxic effects of cyclophosphamide and benefits of gonadotropin releasing hormone agonists (GnRHa) in women of child-bearing age with autoimmune rheumatic disease. *Expert Rev Clin Immunol*. 2020;16(3):321-333. doi:10.1080/1744666x.2020.1724091
12. Sheth VG, Navik U, Maremanda KP, Jena G. Effect of diethyldithiocarbamate in cyclophosphamide-induced nephrotoxicity: immunohistochemical study of superoxide dismutase 1 in rat. *Indian J Pharmacol*. 2018;50(1):4-11. doi:10.4103/ijp.IJP_850_16
13. Dumontet C, Draï J, Thieblemont C, et al. The superoxide dismutase content in erythrocytes predicts short-term toxicity of high-dose cyclophosphamide. *Br J Haematol*. 2001;112(2):405-409. doi:10.1046/j.1365-2141.2001.02595.x
14. Singh C, Prakash C, Tiwari KN, Mishra SK, Kumar V. Premna integrifolia ameliorates cyclophosphamide-induced hepatotoxicity by modulation of oxidative stress and apoptosis. *Biomed Pharmacother*. 2018;107:634-643. doi:10.1016/j.biopha.2018.08.039
15. Oboh G, Akomolafe TL, Adefegha SA, Adetuyi AO. Inhibition of cyclophosphamide-induced oxidative stress in rat brain by polar and non-polar extracts of annatto (Bixa orellana) seeds. *Exp Toxicol Pathol*. 2011;63(3):257-262. doi:10.1016/j.etp.2010.01.003
16. Conklin KA. Chemotherapy-associated oxidative stress: impact on chemotherapeutic effectiveness. *Integr Cancer Ther*. 2004;3(4):294-300. doi:10.1177/1534735404270335
17. Azab SS, Kamel I, Ismail NN, El Din Hosni H, El Fatah MA. The defensive role of taurine against gonadotoxicity and testicular apoptosis effects induced by cisplatin in rats. *J Infect Chemother*. 2020;26(1):51-57. doi:10.1016/j.jiac.2019.07.004
18. Trujillo M, Odle AK, Aykin-Burns N, Allen AR. Chemotherapy induced oxidative stress in the ovary: drug-dependent mechanisms and potential interventions. *Biol Reprod*. 2023;108(4):522-537. doi:10.1093/biolre/ioc222
19. Ahmed ZA, Abtar AN, Othman HH, Aziz TA. Effects of quercetin, sitagliptin alone or in combination in testicular toxicity induced by doxorubicin in rats. *Drug Des Devel Ther*. 2019;13:3321-3329. doi:10.2147/dddt.S222127
20. Sagir S, Seker U, Pekince Ozoner M, Yuksel M, Demir M. Oxidative stress, apoptosis, inflammation, and proliferation modulator function of visnagin provide gonadoprotective activity in testicular ischemia-reperfusion injury. *Eur Rev Med Pharmacol Sci*. 2023;27(20):9968-9977. doi:10.26355/eurrev_202310_34176
21. D'Andrea GM. Use of antioxidants during chemotherapy and radiotherapy should be avoided. *CA Cancer J Clin*. 2005;55(5):319-321. doi:10.3322/canjclin.55.5.319
22. Ntege EH, Sunami H, Shimizu Y. Advances in regenerative therapy: a review of the literature and future directions. *Regen Ther*. 2020;14:136-153. doi:10.1016/j.reth.2020.01.004
23. Ding DC, Shyu WC, Lin SZ. Mesenchymal stem cells. *Cell Transplant*. 2011;20(1):5-14. doi:10.3727/096368910x
24. Glotzbach JP, Wong VW, Gurtner GC, Longaker MT. Regenerative medicine. *Curr Probl Surg*. 2011;48(3):148-212. doi:10.1067/j.cpsurg.2010.11.002
25. File B, Gergis M, Gergis U. The effect of hematopoietic stem cell transplantation on fertility and strategies for improvement. *Bone Marrow Transplant*. 2022;57(11):1649-1656. doi:10.1038/s41409-022-01792-6
26. Azizi H, Niazi Tabar A, Skutella T. Successful transplantation of spermatogonial stem cells into the seminiferous tubules of busulfan-treated mice. *Reprod Health*. 2021;18(1):189. doi:10.1186/s12978-021-01242-4
27. Zohni K, Zhang X, Tan SL, Chan P, Nagano MC. The efficiency of male fertility restoration is dependent on the recovery kinetics of spermatogonial stem cells after cytotoxic treatment with busulfan in mice. *Hum Reprod*. 2012;27(1):44-53. doi:10.1093/humrep/der357
28. Drumond AL, Weng CC, Wang G, Chiarini-Garcia H, Eras-Garcia L, Meistrich ML. Effects of multiple doses of cyclophosphamide on mouse testes: accessing the germ cells lost, and the functional damage of stem cells. *Reprod Toxicol*. 2011;32(4):395-406. doi:10.1016/j.reprotox.2011.09.010
29. Bu T, Mi Y, Zeng W, Zhang C. Protective effect of quercetin on cadmium-induced oxidative toxicity on germ cells in male mice. *Anat Rec (Hoboken)*. 2011;294(3):520-526. doi:10.1002/ar.21317
30. Ebokaiwe AP, Obasi DO, Njoku RC, Osawe S.

- Cyclophosphamide-induced testicular oxidative-inflammatory injury is accompanied by altered immunosuppressive indoleamine 2, 3-dioxygenase in Wister rats: Influence of dietary quercetin. *Andrologia*. 2022;54(3):e14341. doi:10.1111/and.14341
31. Dolatkhan MA, Khezri S, Shokoohi M, Alihemmati A. The effect of *Fumaria parviflora* on the expression of sexual hormones along with their receptors in testicles of adult rats induced by varicocele. *Andrologia*. 2022;54(9):e14512. doi:10.1111/and.14512
 32. Ghobadi E, Moloudizargari M, Asghari MH, Abdollahi M. The mechanisms of cyclophosphamide-induced testicular toxicity and the protective agents. *Expert Opin Drug Metab Toxicol*. 2017;13(5):525-536. doi:10.1080/17425255.2017.1277205
 33. Kim WI, Lim JO, Pak SW, et al. Exposure to China dust exacerbates testicular toxicity induced by cyclophosphamide in mice. *Toxicol Res*. 2023;39(1):115-125. doi:10.1007/s43188-022-00149-x
 34. Uzun-Goren D, Uz YH. Preventive effects of quercetin against inflammation and apoptosis in cyclophosphamide-induced testicular damage. *Iran J Basic Med Sci*. 2024;27(5):647-656. doi:10.22038/ijbms.2024.74458.16177
 35. Mruk DD, Cheng CY. The mammalian blood-testis barrier: its biology and regulation. *Endocr Rev*. 2015;36(5):564-591. doi:10.1210/er.2014-1101
 36. Gerber J, Heinrich J, Brehm R. Blood-testis barrier and Sertoli cell function: lessons from SCCx43KO mice. *Reproduction*. 2016;151(2):R15-27. doi:10.1530/rep-15-0366
 37. Türedi S, Çelik H, Dağlı Ş N, Taşkın S, Şeker U, Deniz M. An examination of the effects of propolis and quercetin in a rat model of streptozotocin-induced diabetic peripheral neuropathy. *Curr Issues Mol Biol*. 2024;46(3):1955-1974. doi:10.3390/cimb46030128
 38. Guo XB, Zhai JW, Xia H, et al. Protective effect of bone marrow mesenchymal stem cell-derived exosomes against the reproductive toxicity of cyclophosphamide is associated with the p38MAPK/ERK and AKT signaling pathways. *Asian J Androl*. 2021;23(4):386-391. doi:10.4103/aja.aja_98_20
 39. Tonk CH, Witzler M, Schulze M, Tobiasch E. Mesenchymal stem cells. In: Brand-Saberi B, ed. *Essential Current Concepts in Stem Cell Biology*. Cham: Springer; 2020:21-39. doi:10.1007/978-3-030-33923-4_2
 40. Lu J, Liu Z, Shu M, et al. Human placental mesenchymal stem cells ameliorate chemotherapy-induced damage in the testis by reducing apoptosis/oxidative stress and promoting autophagy. *Stem Cell Res Ther*. 2021;12(1):199. doi:10.1186/s13287-021-02275-z
 41. Delkhosh A, Delashoub M, Tehrani AA, et al. Upregulation of FSHR and PCNA by administration of coenzyme Q10 on cyclophosphamide-induced premature ovarian failure in a mouse model. *J Biochem Mol Toxicol*. 2019;33(11):e22398. doi:10.1002/jbt.22398
 42. Roudi Rasht Abadi A, Mohammadzadeh Boukani L, Shokoohi M, et al. The flavonoid chrysin protects against testicular apoptosis induced by torsion/detorsion in adult rats. *Andrologia*. 2023;2023(1):6500587. doi:10.1155/2023/6500587
 43. Sharifian P, Yari S, Hasanein P, Manteghi Nezhad Y. Conditioned medium of bone marrow mesenchymal stem cells improves sperm parameters and reduces histological alteration in rat testicular ischaemia/reperfusion model. *Andrologia*. 2022;54(11):e14624. doi:10.1111/and.14624
 44. Abdelaziz MH, Salah El-Din EY, El-Dakdoky MH, Ahmed TA. The impact of mesenchymal stem cells on doxorubicin-induced testicular toxicity and progeny outcome of male prepubertal rats. *Birth Defects Res*. 2019;111(13):906-919. doi:10.1002/bdr2.1535
 45. Abouee-Mehrzi A, Saed-Moucheshi S, Rasoulzadeh Y, et al. Toxicological effects of simultaneous exposure to toluene and noise on some sexual and stress parameters in New Zealand white rabbits. *Pollution*. 2023;9(1):126-138. doi:10.22059/poll.2022.343166.1482
 46. Shokoohi M, Khaki AA, Roshangar L, Nasr Esfahani MH, Ghazi Soltani G, Alihemmati A. The impact of N-acetylcysteine on hypoxia-induced testicular apoptosis in male rats: TUNEL and IHC findings. *Heliyon*. 2024;10(22):e40097. doi:10.1016/j.heliyon.2024.e40097
 47. Shokoohi M, Khaki A, Roudi Rasht Abadi A, et al. Minocycline can reduce testicular apoptosis related to varicocele in male rats. *Andrologia*. 2022;54(4):e14375. doi:10.1111/and.14375
 48. Aru B, Akdeniz T, Dağdeviren H, Gürel G, Yanıkkaya Demirel G. Testosterone propionate promotes proliferation and viability of bone marrow mesenchymal stem cells while preserving their characteristics and inducing their anti-cancer efficacy. *Balkan Med J*. 2023;40(2):117-123. doi:10.4274/balkanmedj.galenos.2022.2022-10-21
 49. Rotimi DE, Olaolu TD, Adeyemi OS. Pharmacological action of quercetin against testicular dysfunction: a mini review. *J Integr Med*. 2022;20(5):396-401. doi:10.1016/j.joim.2022.07.001
 50. Khazaeel K, Daaj SA, Sadeghi A, Tabandeh MR, Basir Z. Potential protective effect of quercetin on the male reproductive system against exposure of Wistar rats to crude oil vapor: Genetic, biochemical, and histopathological evidence. *Reprod Toxicol*. 2022;113:10-17. doi:10.1016/j.reprotox.2022.08.001