Title: Fluoxetine Preconditioning of Mesenchymal Stem Cells: In Vitro Modulation of Mitochondrial and Antioxidant Regulators

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ABSTRACT

Introduction: Mesenchymal stem cells (MSCs) possess robust regenerative and immunomodulatory properties, making them attractive candidates for cell-based therapies targeting tissue repair and immune modulation. However, their therapeutic efficacy is often limited by poor survival and vulnerability to oxidative stress. Maintenance of mitochondrial function and antioxidant defenses is therefore critical for preserving MSC viability and function in hostile microenvironments.

Methods: This study evaluated fluoxetine, a selective serotonin reuptake inhibitor with reported antioxidative effects, as an in vitro preconditioning agent in rat bone marrow-derived MSCs. Cells were treated with a non-cytotoxic concentration of fluoxetine, and the expression of key mitochondrial and antioxidant regulatory genes was assessed by quantitative real-time PCR. In addition, molecular docking was performed to predict potential interactions between fluoxetine and mitochondrial-related target proteins.

Results: Fluoxetine preconditioning preserved MSC viability and significantly increased the expression of nuclear factor erythroid 2–related factor 2 (NRF2), sirtuin 3 (SIRT3), and mitochondrial transcription factor A (TFAM), which are central regulators of antioxidant defense and mitochondrial quality control. In contrast, SIRT1 and PGC1α expression levels were not significantly altered. Docking analyses predicted favorable binding interactions between fluoxetine and several mitochondrial regulatory proteins, with the strongest predicted affinity observed for SIRT1, suggesting a potential protein-level mechanism independent of transcriptional regulation.

Conclusion: These findings indicate that fluoxetine preconditioning selectively modulates mitochondrial and antioxidant pathways in MSCs under basal conditions. Whether this transcriptional modulation translates into enhanced resistance to oxidative stress requires further functional validation. In addition, in vivo studies are required to determine the translational relevance of fluoxetine-preconditioned MSCs in regenerative medicine.

Keywords: Fluoxetine, Mesenchymal stem cells (MSCs), Preconditioning, Mitochondrial, Oxidative stress, Regenerative medicine

Highlights

- Fluoxetine preconditioning preserves viability of rat bone marrow-derived MSCs.
- Fluoxetine significantly upregulates NRF2, SIRT3, and TFAM expression in MSCs.
- Docking predicts favorable fluoxetine binding to mitochondrial regulatory proteins.
- Fluoxetine upregulates genes implicated in cellular responses to oxidative stress in MSCs, warranting further functional validation of oxidative stress resistance.

Plain Language Summary

Mesenchymal stem cells are promising tools for regenerative medicine because they can support tissue repair and help regulate inflammation. However, when these cells are transplanted into damaged tissues, they often face harsh conditions such as oxidative stress, which reduces their survival and limits their therapeutic benefit. Finding ways to strengthen stem cells before use could improve their effectiveness. In this study, we tested whether fluoxetine, a commonly used antidepressant with antioxidant properties, could be used to "precondition" rat bone marrow-derived mesenchymal stem cells in the laboratory. We found that fluoxetine treatment did not harm the cells and increased the expression of several important genes involved in protecting cells from oxidative damage and maintaining healthy mitochondria, which are essential for energy production and cell survival. We also used computer-based molecular docking to predict how fluoxetine might interact with mitochondrial regulatory proteins, suggesting possible direct effects at the protein level. Overall, our findings indicate that fluoxetine upregulates key antioxidant and mitochondrial genes in stem cells under basal conditions. Further studies including oxidative stress challenges are needed to confirm whether this approach improves stem cell resistance to stressful environments and enhances stem cell-based therapies in living organisms.

Introduction

Mesenchymal stem cells (MSCs) are widely studied in regenerative medicine owing to their multipotent differentiation potential, high proliferative capacity together with strong immunomodulatory functions (Han et al., 2025).They can be easily isolated from different tissues, such as bone marrow, adipose tissue, umbilical cord, and Wharton's jelly, and can be efficiently expanded ex vivo (Nazari, Pourmand, Motevaseli, & Hassanzadeh, 2023; Yang Wang et al., 2025). MSCs also exhibit robust migratory and homing abilities to sites of injury, along with low immunogenicity, making them promising candidates for cell-based therapies in both experimental and clinical settings (Gholaminejhad et al., 2022; Mehrannia et al., 2018; MohAMAdi et al., 2018; Zhidu, Ying, Rui, Chao, & Therapy, 2024).

Despite these advantages, the therapeutic efficacy of MSCs is often limited by poor survival, low engraftment, and vulnerability to oxidative and inflammatory insults in the hostile microenvironment of the damaged tissue (Elshaer, Bahram, Rajashekar, Gangaraju, & El-Remessy, 2021). A critical determinant of MSCs function is the maintenance of mitochondrial integrity and endogenous antioxidant defenses. Because mitochondrial dysfunction and excessive reactive oxygen species (ROS) can impair MSC viability and regenerative capacity, strategies that enhance mitochondrial health and antioxidant responses may improve MSC-based therapeutic outcomes. Accordingly, preconditioning approaches have been proposed to strengthen MSCs resistance to oxidative stress and optimize their reparative functions (Panahi et al., 2020; Shaban et al., 2017).

Fluoxetine, a selective serotonin reuptake inhibitor (SSRI) widely used for major depressive disorder, has also been reported to exert antioxidant and cytoprotective effects beyond it serotonergic modulation (Caiaffo, Oliveira, de Sá, Evêncio Neto, & perspectives, 2016). Studies have shown that fluoxetine can reduce oxidative stress and restore antioxidant capacity in pathological

conditions (Caruso et al., 2021; Chung et al., 2010; Kirkova et al., 2010). Notably, fluoxetine has been associated with activation of the nuclear factor erythroid 2–related factor 2 (NRF2) pathway, which regulates the transcription of multiple antioxidant and cytoprotective genes (Abu-Elfotuh, Al-Najjar, Mohammed, Aboutaleb, & Badawi, 2022; Erman et al., 2015; Mendez-David et al., 2015; Qin et al., 2022; Y. Wang, Gu, Liu, Bai, & Wang, 2017). This suggests a conserved antioxidant mechanism that may also be relevant in MSCs.

In parallel, mitochondrial quality control molecules such as sirtuin 3 (SIRT3) and mitochondrial transcription factor A (TFAM) have pivotal roles in maintaining mitochondrial function. SIRT3 is a mitochondrial deacetylase that regulates the acetylation status of mitochondrial proteins, thereby preserving metabolic homeostasis and limiting oxidative damage (Shen, Wu, Shi, & Zhou, 2020). TFAM is required for mitochondrial DNA transcription and replication and is therefore critical for mitochondrial biogenesis (Yang, Sun, Feng, & Xu, 2025). Together, these factors are fundamental for sustaining mitochondrial integrity, which is vital for effective MSCs survival and function under stress conditions.

In addition to NRF2, SIRT3, and TFAM, upstream regulators such as sirtuin 1 (SIRT1) and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α) are also involved in mitochondrial biogenesis and antioxidant signaling (Abu Shelbayeh, Arroum, Morris, & Busch, 2023). Because SIRT1 can activate PGC1α through deacetylation, this axis coordinates broader mitochondrial gene regulation (Palabiyik & Palabiyik, 2025). Evaluating SIRT1 and PGC1α, helps us understand whether fluoxetine influences the broader regulatory network controlling mitochondrial function in mesenchymal stem cells (MSCs).

In this study, we aimed to assess the effect of fluoxetine preconditioning on the expression of key antioxidant and mitochondrial regulatory genes in rat bone marrow–derived MSCs. We reveal that

fluoxetine markedly upregulates NRF2, SIRT3, and TFAM expression, highlighting these molecules as potential mediators of enhanced antioxidant capacity and mitochondrial homeostasis. Furthermore, fluoxetine at non-cytotoxic concentrations preserved MSC viability and selectively modulated genes implicated in cellular responses to oxidative stress under basal conditions. These findings support the hypothesis that fluoxetine may serve as a pharmacological preconditioning strategy to improve MSC resilience, though functional validation of stress resistance is required.

Materials and Methods

Isolation and culture of rat bone marrow-derived mesenchymal stem cells and flow cytometry

Bone marrow-derived mesenchymal stem cells (BM-MSCs) were isolated from adult Wistar rats under sterile conditions. The femur and tibia were carefully dissected from the joint region while preserving the integrity of the bone ends. The isolated bones were immediately transferred into a dish containing pre-cooled phosphate-buffered saline (PBS). Surrounding muscle tissue was gently removed using sterile forceps and a scalpel, and the bones were washed repeatedly in PBS to eliminate any residual soft tissue. To collect bone marrow, both ends of each bone were trimmed, and the marrow cavity was flushed using a 22-gauge needle filled with α-minimum essential medium (α-MEM). This flushing procedure was performed several times to ensure maximal recovery of marrow cells. The resulting cell suspension was centrifuged at 1500 rpm for 10 min, after which the supernatant was discarded. This process was repeated for all collected bones. The cell pellet was resuspended in complete culture medium consisting of α-MEM (Biosera, France) supplemented with 10% fetal bovine serum (FBS) (Biosera, France) and 1%

penicillin/streptomycin (BioIdea Co., Iran). Cells were then seeded into T-25 culture flasks and incubated at 37°C in a humidified atmosphere containing 5% CO_2. After 24 hours, non-adherent cells were removed by replacing the medium, and fresh medium was subsequently renewed every 2–3 days (Figure 1). Flow cytometry was employed to assess the purity and characterization of BM-MSCs. Following cell detachment, the cells were stained with FITC-conjugated monoclonal antibodies targeting CD90 and CD44 as positive surface markers, and CD45 and CD34 as negative surface markers. Absence of CD34 and CD45 together with positive expression of CD90 and CD44 surface markers confirmed that the isolated cells exhibited an MSC phenotype.

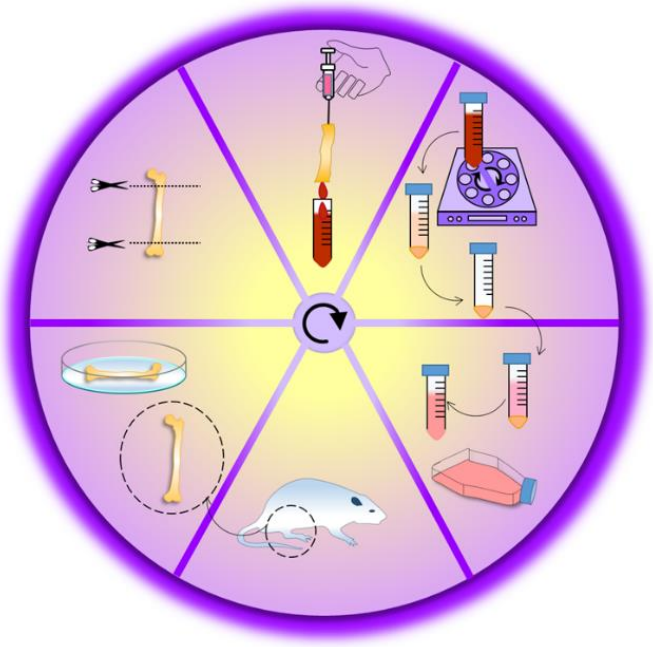


Figure 1. Schematic overview of the isolation and culture process of rat bone marrow-derived mesenchymal stem cells (BM-MSCs).

The femur and tibia were dissected under sterile conditions, and surrounding muscle tissue was meticulously removed. Bone marrow contents were flushed from the marrow cavity using α-minimum essential medium (α-MEM) with a 22-gauge needle. The resulting cell suspension was centrifuged at 1500 rpm for 10 min to collect cells, and the supernatant was discarded. The pelleted cells were resuspended and filtered before seeding into T-25 flasks for culture.

Study setup and grouping

We aimed to explore the effects of fluoxetine treatment on the expression of NRF2, SIRT3, TFAM, SIRT1, and PGC1α in bone marrow-derived mesenchymal stem cells (BM-MSCs). Two experimental groups were established: MSCs group and a FLX-MSCs group. The MSCs group consisted of BM-MSCs cultured in α-MEM supplemented with 10% fetal bovine serum (FBS), serving as the baseline condition. The FLX-MSCs group was treated with the same culture medium containing 10 μM fluoxetine. This design allowed for a direct comparison of fluoxetine's effects on these key mitochondrial and antioxidant regulators in MSCs.

MTT Assay

For cell treatments, fluoxetine was purchased from Temad, Iran, and dissolved in sterilized PBS (with pH 7.2). To determine the optimal fluoxetine concentration for BM-MSC treatment, cell viability was evaluated across a range of doses (1, 2.5, 5, 7.5, 10, 15, and 20 μM). Mitochondrial metabolic activity in fluoxetine-exposed cells was measured via the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The fluoxetine doses were chosen based on what has been reported in the literature. A total of 5×10^4 BM-MSCs were plated in 96-well culture plates and exposed to different doses of fluoxetine for 24 hours. Afterward, the medium of each well was removed, and standard volume of MTT solution (5 mg/ml) (Sigma Aldrich) was added to each well, followed by a 4-hour incubation at 37°C. Next, 100 μL of DMSO was added to each well, and the plates were shaken for 10 minutes to dissolve

the formazan crystals. Absorbance was then recorded at 550 nm using a microplate reader. Cell viability was calculated as a percentage of BM-MSCs. All experimentations were executed in triplicates.

Pretreatment of rat BM-MSCs with the chosen fluoxetine dose

BM-MSCs were exposed to 10 μM fluoxetine, the dose determined by the MTT assay, for 24 hours under standard culture conditions. The control group was maintained under the same conditions without fluoxetine for the same duration. The culture media for all groups was kept unchanged.

RNA isolation and quantitative real-time PCR (RT-PCR) for gene expression analysis

Total RNA was extracted from BM-MSCs in all experimental groups after 24 hours preconditioning. RNA purity and concentration were assessed spectrophotometrically. Complementary DNA (cDNA) was synthesized using a reverse transcription kit. Quantitative real-time PCR was performed using SYBR Green Master Mix on a Bio-Rad CFX96 Real-Time PCR Detection System (Bio-Rad, USA) with CFX Manager software.

Thermal cycling conditions were as follows: initial denaturation and enzyme activation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s, annealing at 60°C for 25 s, and extension at 72°C for 30 s with fluorescence data acquisition at the end of the extension step. A melting curve analysis was performed from 65°C to 95°C to confirm amplification specificity.

The expression levels of target genes Nrf2, Sirt3, Tfam, Sirt1, and PGC1α were normalized to the housekeeping gene B2M. Primer sequences are listed in Table 1. Relative gene expression was calculated using the 2^-ΔΔCt method. Gene expression analyses were performed using three independent biological replicates per experimental group (n = 3), and each sample was analyzed in technical triplicate. The mean Ct value of technical replicates was used for statistical analysis.

Table 1. The primer sequences

Primer	Sequence
Rat-Nrf-2-F	CAGCACATCCAGACAGACAC
Rat-Nrf-2-R	AATATCCAGGGCAAGCGACT
Rat-Sirt3-F	ACTACTTCCTTCGGCTGCTT
Rat-Sirt3-R	GACCCATGAGCTTCAACCAG
Rat-Tfam-F	TGTGTAGGCACAGTGTCG
Rat-Tfam-R	CAGATAAGGCTGACAGGCG
Rat-Sirt1-F	TTCAAGGCTGTTGGTTCCAG
Rat-Sirt1-R	AGGCGTCATCTTCAGAGTCT
Rat-Pgc1a-F	CTGCCATTGTTAAGACCGAG
Rat-Pgc1a-R	TGTTCTCTGTGGGTTTGGT
Rat-B2M-F	CGTCGTGCTTGCCATTCAG
Rat-B2M-R	GCAGTTGAGGAAGTTGGGC

F, forward; R, reverse; Nrf2, nuclear factor erythroid 2–related factor 2; Sirt3, sirtuin 3; Tfam, mitochondrial transcription factor A; Sirt1, sirtuin 1; Pgc1a, peroxisome proliferator-activated receptor gamma coactivator 1-alpha.

Docking analysis of interaction between fluoxetine with target proteins in MSCs

Molecular docking was performed using CB-Dock2 (accessible on https://cadd. labshare.cn/cb-dock2/php/index.php) (Liu et al., 2022) to investigate the binding affinities of fluoxetine with target proteins Nrf2, Sirt3, Tfam, Sirt1 and PGC1α. The primary structures of these proteins were retrieved from the AlphaFold Protein Structure Database (https://alphafold.ebi.ac.uk/) for Rattus norvegicus. The chemical structure of the fluoxetine was

obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The docking process involved documenting the Vina score and cavity size for each interaction.

Statistical analysis

Data were analyzed using GraphPad Prism 10 software. Following evaluation of data normality with the Shapiro–Wilk test, Student's t-tests were applied to determine statistical differences between the experimental groups. One-way ANOVA was done for comparing multiple groups, followed by Tukey's post-test for parametric data analysis. The results were presented as mean ± SEM, and statistical significance was reported for p-values less than 0.05.

Results

Assessment of BM-MSC viability by MTT assay and optimization of fluoxetine preconditioning dose

The cell viability of BM-MSCs exposed to increasing concentrations of fluoxetine (1, 2.5, 5, 7.5, 10, 15, and 20 μM) was assessed by MTT assay after 24 hours of treatment (Figure 2). The results indicate that fluoxetine concentrations up to 10 μM did not significantly reduce cell viability compared to the untreated control group, with viability consistently above 85% at each dose level. These data suggest that fluoxetine is well tolerated by BM-MSCs within this concentration range, with no apparent cytotoxic effects after 24 hours of exposure. Based on these findings, 10 μM was selected as the optimal concentration for subsequent experiments to ensure both safety and potential therapeutic effects on mitochondrial and antioxidant gene expression.

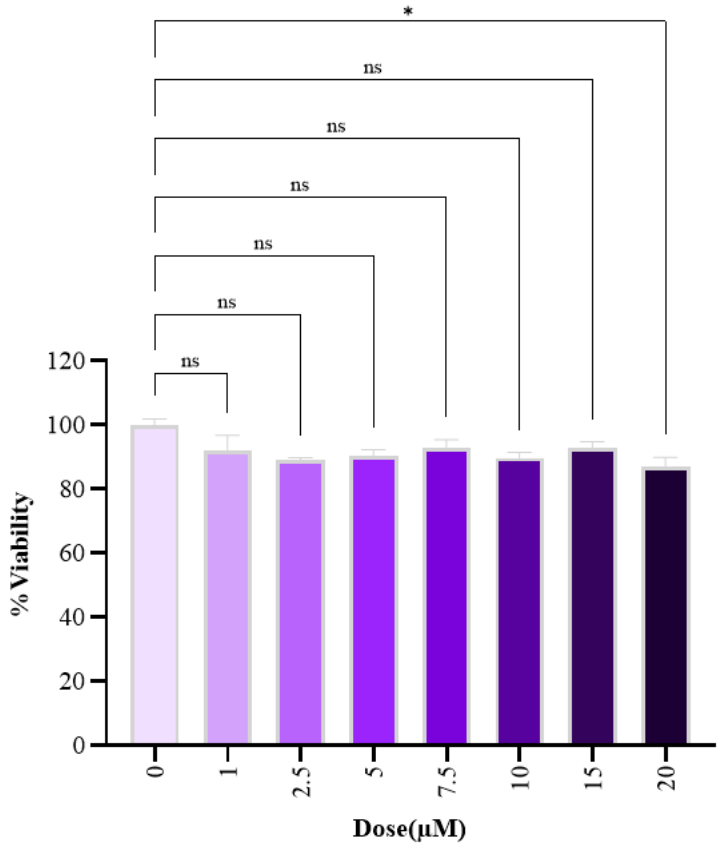


Figure 2. Effects of fluoxetine on the viability of rat bone marrow-derived mesenchymal stem cells (BM-MSCs) as determined by MTT assay.

BM-MSCs were exposed to increasing concentrations of fluoxetine (1, 2.5, 5, 7.5, 10, 15, and 20 µM) for 24 hours. Cell viability was expressed as a percentage relative to untreated control cells (0 µM). No significant cytotoxicity was observed at any tested concentration except 20 µM, and 10 µM was selected for subsequent experiments. Bars show mean ± SEM of three independent replicates per group. Statistical analysis was performed using one-way ANOVA followed by Tukey's tests post-hoc comparison; * indicates $p < 0.05$ versus control, ns denotes non-significant difference. Data confirm that fluoxetine up to 10

μM does not reduce BM-MSC viability, supporting its selection as a nontoxic preconditioning dose for functional assays.

Flow cytometry

Flow cytometric analysis was performed to confirm the immunophenotypic profile of BM-MSCs following fluoxetine preconditioning. Representative dot plots for CD34/CD45 and CD90/CD44 expression are shown in Figure 3. Both control and fluoxetine-treated BM-MSCs exhibited highly similar profiles. Quantification revealed that the majority of cells in both groups (>97%) were negative for the hematopoietic markers CD34 and CD45, while more than 98% of cells were double positive for the mesenchymal markers CD90 and CD44. Specifically, control BM-MSCs exhibited CD34−/CD45− expression in 97.9% and CD90+/CD44+ expression in 99.3% of the population. Fluoxetine-treated MSCs showed CD34−/CD45− expression in 98.8% and CD90+/CD44+ in 98.1% of cells.
No significant changes were detected in the surface marker expression profile between control and fluoxetine-treated groups, showing that fluoxetine preconditioning does not alter the immunophenotype of BM-MSCs.

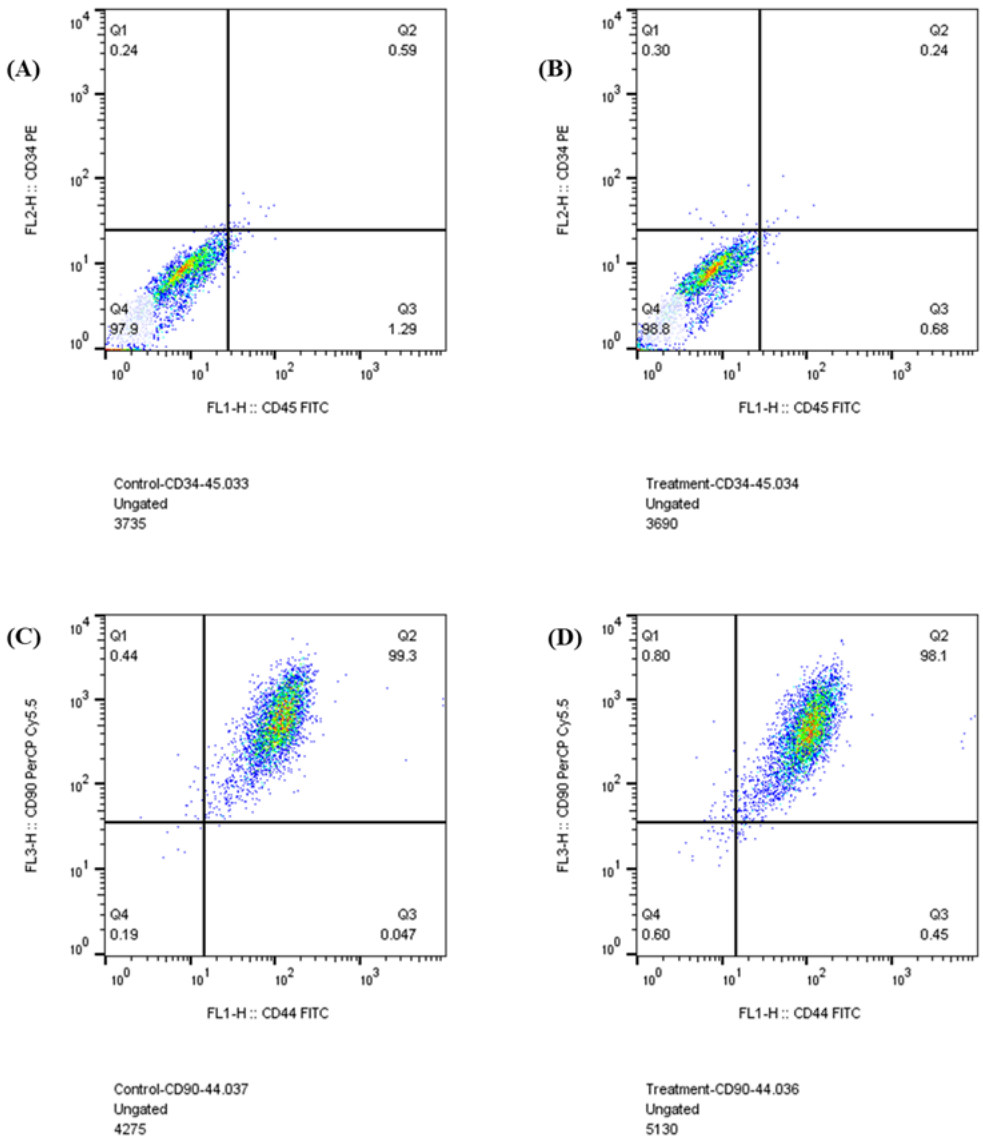


Figure 3. Flow cytometric analysis of BM-MSCs immunophenotype in control and fluoxetine-treated groups.

Representative dot plots illustrate surface expression of (A, B) CD34 and CD45 and (C, D) CD90 and CD44 in untreated (control) and fluoxetine-treated BM-MSCs. The majority of cells in both groups are negative for hematopoietic markers CD34 and CD45 (lower left quadrant, Q4), confirming the non-hematopoietic MSC phenotype. Nearly all cells are positive for mesenchymal

markers CD90 and CD44 (upper right quadrant, Q2), demonstrating robust expression consistent with MSC identity. Quantification reveals no substantial differences in marker distribution between control and treated groups, indicating that fluoxetine preconditioning preserves the characteristic immunophenotypic profile of BM-MSCs. Numbers within quadrants represent the percentage of cells in each population

Changes in gene expression following fluoxetine preconditioning

Fluoxetine preconditioning significantly modulated the expression of several key mitochondrial and antioxidant-related genes in BM-MSCs. Quantitative real-time PCR analysis showed a pronounced increase in Nrf2 expression in fluoxetine-treated cells compared with the control group. Likewise, Sirt3 transcript levels were significantly enhanced following fluoxetine exposure.In addition, Tfam expression was markedly upregulated in the fluoxetine group relative to controls. In contrast, Sirt1 and PGC1α expression had no statisticaly change treated group compared to control one. Overall, these findings suggest that fluoxetine selectively enhances the expression of factors involved in mitochondrial maintenance and antioxidant defense, which may contribute to improved BM-MSC resilience and functional capacity (Figure 4).

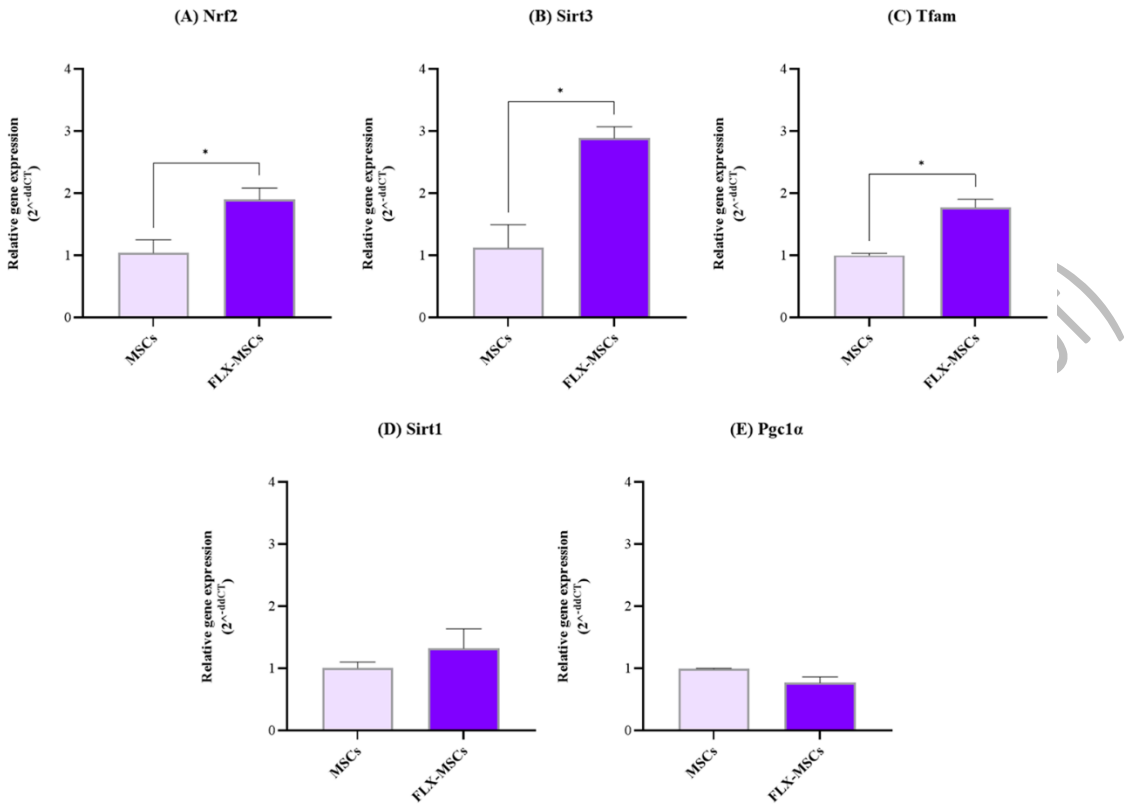


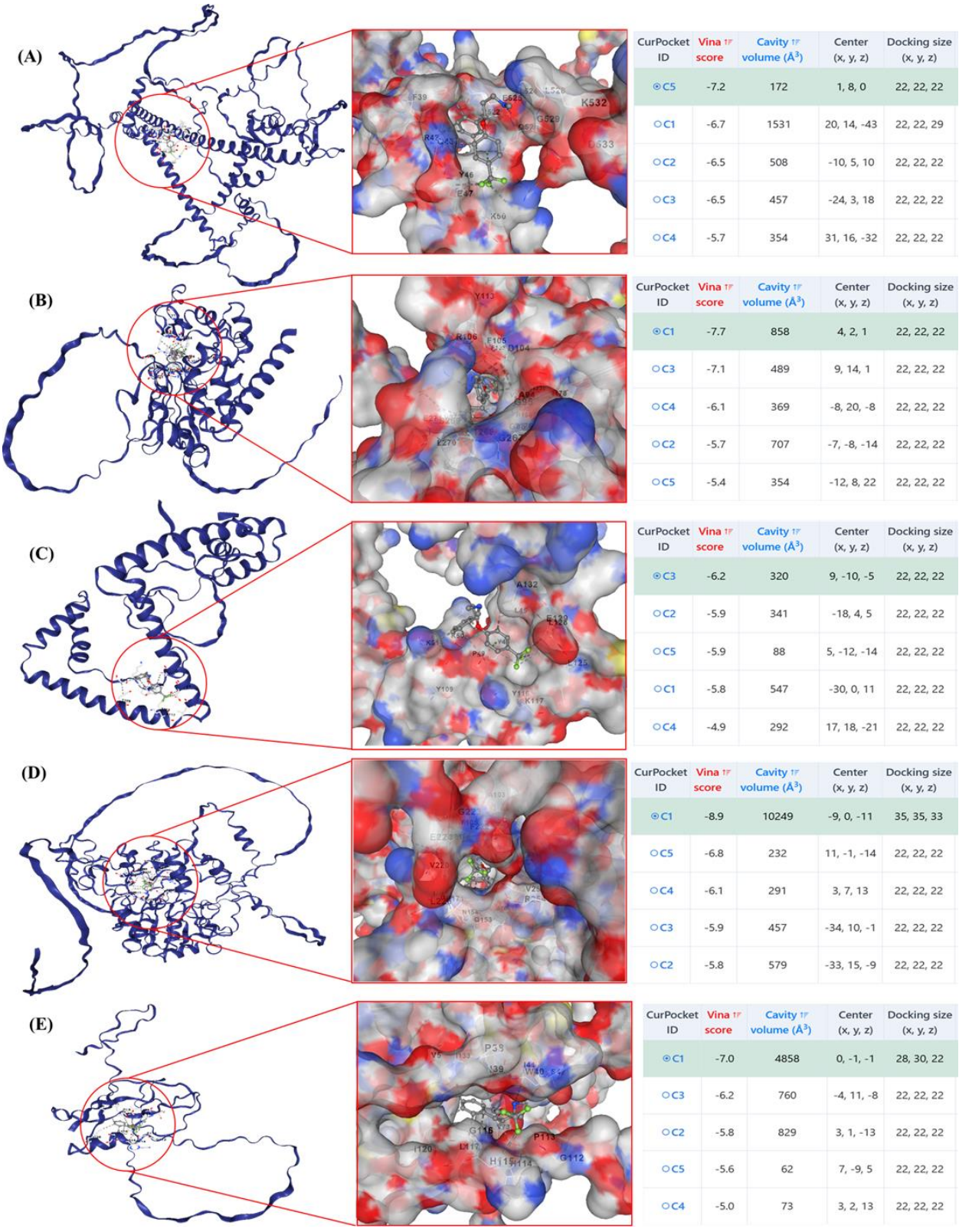
Figure 4. The effect of fluoxetine treatment on gene expression. Relative expression of Nrf2, Sirt3, Tfam, Sirt1, and PGC1α was measured in cells treated with fluoxetine compared to controls.

The bar graphs illustrate the expression levels of these genes, with statistical significance marked by *P<0.05 for Nrf2, Sirt3, and Tfam. These findings demonstrate that fluoxetine significantly upregulates Nrf2, Sirt3, and Tfam expression. Nrf2, nuclear factor erythroid 2–related factor 2 (Nrf2); Sirt3, sirtuin 3; Tfam, mitochondrial transcription factor A; Sirt1, sirtuin 1; PGC1α, proliferator-activated receptor gamma coactivator 1-alpha. Data are expressed as the mean ± SEM, * $p < 0.05$ vs. MSCs

3.4. Molecular Docking Assessment

Molecular docking was performed to evaluate the potential interactions between fluoxetine and selected target proteins. CB-Dock2 software was used to assess the binding of fluoxetine to Nrf2,

Sirt3, Tfam, Sirt1, and PGC-1α. Binding affinity was estimated using Vina scores, with values of −5.0 kcal/mol or more negative considered indicative of favorable interactions. The calculated Vina scores were −7.2 kcal/mol for Nrf2, −7.7 kcal/mol for Sirt3, −6.2 kcal/mol for Tfam, −8.9 kcal/mol for Sirt1, and −7.0 kcal/mol for PGC-1α. These binding energies suggest that fluoxetine may interact with these proteins. Figure 5 depicts the docking sketches illustrating the interaction between fluoxetine and the target proteins.



CurPocket ID	Vina score	Cavity volume (Å³)	Center (x, y, z)	Docking size (x, y, z)
◉C5	-7.2	172	1, 8, 0	22, 22, 22
○C1	-6.7	1531	20, 14, -43	22, 22, 29
○C2	-6.5	508	-10, 5, 10	22, 22, 22
○C3	-6.5	457	-24, 3, 18	22, 22, 22
○C4	-5.7	354	31, 16, -32	22, 22, 22

CurPocket ID	Vina score	Cavity volume (Å³)	Center (x, y, z)	Docking size (x, y, z)
◉C1	-7.7	858	4, 2, 1	22, 22, 22
○C3	-7.1	489	9, 14, 1	22, 22, 22
○C4	-6.1	369	-8, 20, -8	22, 22, 22
○C2	-5.7	707	-7, -8, -14	22, 22, 22
○C5	-5.4	354	-12, 8, 22	22, 22, 22

CurPocket ID	Vina score	Cavity volume (Å³)	Center (x, y, z)	Docking size (x, y, z)
◉C3	-6.2	320	9, -10, -5	22, 22, 22
○C2	-5.9	341	-18, 4, 5	22, 22, 22
○C5	-5.9	88	5, -12, -14	22, 22, 22
○C1	-5.8	547	-30, 0, 11	22, 22, 22
○C4	-4.9	292	17, 18, -21	22, 22, 22

CurPocket ID	Vina score	Cavity volume (Å³)	Center (x, y, z)	Docking size (x, y, z)
◉C1	-8.9	10249	-9, 0, -11	35, 35, 33
○C5	-6.8	232	11, -1, -14	22, 22, 22
○C4	-6.1	291	3, 7, 13	22, 22, 22
○C3	-5.9	457	-34, 10, -1	22, 22, 22
○C2	-5.8	579	-33, 15, -9	22, 22, 22

CurPocket ID	Vina score	Cavity volume (Å³)	Center (x, y, z)	Docking size (x, y, z)
◉C1	-7.0	4858	0, -1, -1	28, 30, 22
○C3	-6.2	760	-4, 11, -8	22, 22, 22
○C2	-5.8	829	3, 1, -13	22, 22, 22
○C5	-5.6	62	7, -9, 5	22, 22, 22
○C4	-5.0	73	3, 2, 13	22, 22, 22

Figure 5. Molecular docking analysis of fluoxetine with target proteins.

Molecular docking was performed to study the interaction of fluoxetine with Nrf2, Sirt3, Tfam, Sirt1, and PGC-1α. CB-Dock2 software was employed to evaluate the binding affinity of fluoxetine, yielding Vina scores of −7.2 kcal/mol for Nrf2, −7.7 kcal/mol for Sirt3, −6.2 kcal/mol for Tfam, −8.9 kcal/mol for Sirt1, and −7.0 kcal/mol for PGC-1α.

Discussion

Mesenchymal stem cells (MSCs) have widely studied as promising candidates for regenerative medicine owing to their remarkable differentiation potential, immunomodulatory properties, along with migratory capacity (Han et al., 2025). However, their therapeutic efficacy is often limited by reduced survival and oxidative stress in hostile microenvironments. Preservation of mitochondrial function and endogenous antioxidant defenses is therefore critical for maintaining MSC viability and regenerative potential (Mukkala et al., 2023; Stavely & Nurgali, 2020). In this study, we examined whether fluoxetine with reported antioxidant effects, modulates key mitochondrial and antioxidant regulators in MSCs.

Our findings show that fluoxetine markedly increases the mRNA expression of NRF2, SIRT3, and TFAM in MSCs, suggesting activation of pathways involved in antioxidant defense and mitochondrial homeostasis. NRF2 is a master transcription factor orchestrating antioxidant defense by regulating a battery of cytoprotective genes, thus playing a fundamental role in cellular adaptation to oxidative stress (He, Ru, & Wen, 2020). Previous studies have reported that NRF2 pathway activation contributes to the antioxidant properties of fluoxetine in other experimental systems (Abu-Elfotuh et al., 2022; Erman et al., 2015; Mendez-David et al., 2015; Qin et al., 2022; Y. Wang et al., 2017). Consistent with these reports, fluoxetine treatment upregulated NRF2 expression in MSCs in the present study. Since NRF2 signaling has been linked to improved MSC

survival and resistance to oxidative injury, its induction may represent one mechanism through which fluoxetine enhances cellular stress resilience (Mohammadzadeh-Vardin, Habibi Roudkenar, & Jahanian-Najafabadi, 2015; Mohammadzadeh et al., 2012; S. Zhang et al., 2018). Conversely, NRF2 inhibition has been associated with senescence and reduced proliferative and immunomodulatory capacity in MSCs (Jie et al., 2020; Yoon, Choi, & Lee, 2016). Taken together, our findings support NRF2 as a plausible mediator of fluoxetine-associated antioxidant responses in MSCs, although functional validation will be required.

In parallel, fluoxetine also upregulated SIRT3 expression in MSCs, highlighting a potential effect on mitochondrial quality control. SIRT3 is a mitochondrial deacetylase that regulates oxidative metabolism and antioxidant defenses through post-translational control of multiple mitochondrial enzymes (Shen et al., 2020). SIRT3 deficiency has been shown to impair mitochondrial integrity, increase oxidative stress, and accelerate senescence in MSCs (X. Q. Wang et al., 2014; Wu et al., 2018), whereas its overexpression can restore mitochondrial function and improve regenerative capacity in aged MSCs (Ma et al., 2020; D. Y. Zhang et al., 2018). Therefore, the upregulation of SIRT3 observed here may contribute to maintaining mitochondrial stability under stress conditions. This evidence suggests that fluoxetine may influence mitochondrial protective pathways beyond NRF2 alone.

We additionally observed increased expression of TFAM, a key regulator of mitochondrial DNA replication and transcription that is essential for mitochondrial biogenesis (Yang et al., 2025). TFAM is a key mitochondrial regulator that maintains the stability and function of mtDNA, critical for mesenchymal stem cell (MSC) biology (Hsu, Wu, Yu, & Wei, 2016; Y. Zhang, Marsboom, Toth, & Rehman, 2013; Meng Zhao et al., 2024). Enhanced TFAM levels have been associated

with improved mitochondrial function and therapeutic performance of MSCs (F. Zhang et al., 2023; M. Zhao et al., 2021). TFAM upregulation may therefore reflect an adaptive response supporting mitochondrial maintenance. Although extracellular vesicle–mediated mitochondrial signaling has been proposed in the literature, this was not directly examined in our study. Future work could explore whether fluoxetine preconditioning influences MSC-derived vesicle cargo or mitochondrial transfer mechanisms.

Interestingly, fluoxetine selectively upregulated NRF2, SIRT3, and TFAM, while SIRT1 and PGC1α mRNA levels remained unchanged. Because SIRT1 and PGC1α are often described as upstream regulators of mitochondrial biogenesis (Zong et al., 2024), this pattern may reflect cell-type–specific regulation or temporal dynamics in response to fluoxetine exposure. It is also possible that these factors are primarily controlled at the protein or activity level rather than through transcriptional changes.

Supporting this possibility, molecular docking predicted the strongest binding affinity between fluoxetine and SIRT1. While docking analysis cannot confirm functional activation, it suggests a potential interaction that may occur independently of mRNA expression changes. Further studies assessing SIRT1 enzymatic activity and downstream signaling will be necessary to determine whether this predicted interaction has biological relevance in MSCs.

A schematic summary of the proposed molecular effects of fluoxetine preconditioning on mitochondrial and antioxidant regulators in BM-MSCs is shown in Figure 6. Overall, our findings indicate that fluoxetine preconditioning modulates key antioxidant and mitochondrial regulatory genes in MSCs under in vitro conditions. These results support the concept that pharmacological preconditioning may enhance MSC resilience to oxidative stress. However, the present study is

limited to transcript-level analysis and computational predictions, and additional functional assays and in vivo validation will be required to determine translational significance.

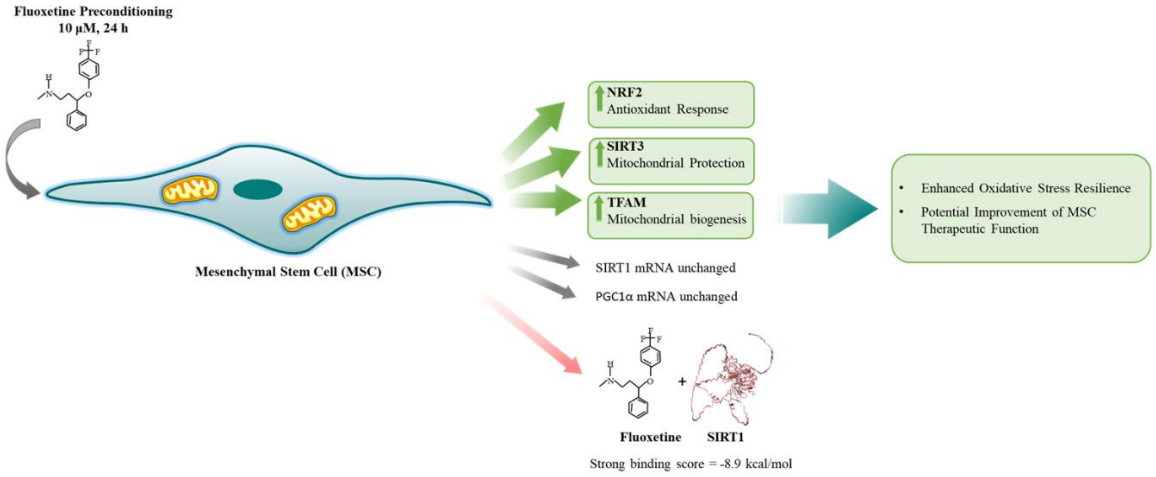


Figure 6. Proposed mechanism of fluoxetine-mediated modulation of mitochondrial and antioxidant regulators in BM-MSCs.

This schematic summarizes the main molecular changes observed following fluoxetine preconditioning in rat bone marrow–derived mesenchymal stem cells (BM-MSCs). Fluoxetine treatment significantly increased the expression of NRF2, SIRT3, and TFAM, key regulators involved in antioxidant defense, mitochondrial quality control, and mitochondrial biogenesis. Molecular docking analysis predicted favorable binding interactions between fluoxetine and mitochondrial regulatory proteins, with the strongest predicted affinity for SIRT1, suggesting a potential protein-level mechanism independent of transcriptional changes. Together, these pathways may contribute to enhanced mitochondrial resilience and oxidative stress resistance in fluoxetine-preconditioned MSCs.

Conclusion

In conclusion, this study demonstrates that fluoxetine preconditioning upregulates NRF2, SIRT3, and TFAM mRNA in rat bone marrow–derived mesenchymal stem cells under basal conditions. These transcriptional changes suggest that fluoxetine may influence pathways involved in oxidative stress defense and mitochondrial homeostasis. However, whether this upregulation translates into enhanced oxidative stress resistance requires further functional validation. The present work is limited to gene expression analysis and molecular docking predictions. Future functional studies including assessing mitochondrial performance, intracellular ROS measurement, apoptosis rates, MSC therapeutic activity under oxidative stress challenges, and in vivo validation will help confirm whether these molecular changes translate into enhanced oxidative stress resistance and improved MSC therapeutic efficacy in clinically relevant models.

Ethical Considerations

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Ethical approval: This study was approved by the Ethics Committee of Tehran University of Medical Sciences (Approval No. IR.TUMS.AEC.1401.182).

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Investigation, Data curation. A. Moini: Investigation, Data curation. Gh. Hassanzadeh: Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization.

Conflict of interest

The authors declared no conflict of interest.

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