MANSOURA JOURNAL OF CHEMISTRY
Official Journal of Faculty of Science, Mansoura University, Egypt
E-mail: scimag@mans.edu.eg ISSN: 2974-4938

Neuroprotective Effects of Quercetin Nanoparticles and all trans retinoic acid - Preconditioned Mesenchymal Stem Cells Against Doxorubicin-Induced Neurotoxicity in Rats

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Received:26/6/2025 Accepted:9/7/2025

Abstract: Brain, one of the most complex biological systems, is responsible for regulating essential functions such as sensory processing, motor control, cognition, language, emotion, and memory. A common chemotherapy drug, doxorubicin (Dox), causes oxidative stress in brain tissue, which raises reactive oxygen species (ROS) and causes neuroinflammation and neuronal death. When xenobiotics interfere with neurochemical signaling pathways, they can cause neurotoxicity by altering the structure and function of neurons. The current study aimed to evaluated the therapeutic potential of quercetin nanoparticles (QuNPs) and mesenchymal stem cells (MSCs) preconditioned with all-trans retinoic acid (ATRA) in mitigating DOX-induced oxidative stress in rat brain tissue. Five groups were studied: control, DOX, DOX+QuNPs, DOX+MSCs+ATRA, and a combined treatment group (Mix). Oxidative stress markers including malondialdehyde (MDA), superoxide dismutase (SOD), and reduced glutathione (GSH) were quantified. Results demonstrated that the MSCs+ATRA group exhibited greater neural recovery than the QuNPs group, with the most significant neuroprotective effect observed in the combined treatment group. These findings underscore the synergistic antioxidant and neuroprotective properties of QuNPs and MSCs+ATRA in counteracting DOX-induced neurotoxicity.

Keywords: Nano quercetin, MSCs, ATRA, Neurotoxicity.

1. Introduction

With more than 100 billion neurons, the brain is a sophisticated biological system that regulates movement, tasks, emotions, senses, language, communication, thought, and memory.[1] Memory, feelings, conscious and unconscious behaviors, motor and sensory information, and intelligence are all governed by the cerebrum. While the right lobe is responsible for spatial thinking, the left lobe is in charge of speech and abstract thought. The thalamus receives sensory information from the body and transmits it to the cerebrum. Sleep, appetite, and thirst are all regulated by the hypothalamus.[1]

Using sensory data from the brain and spinal cord, the cerebellum helps with cognitive processes, fine-tunes motor activity accuracy, and organizes voluntary movement.[2] The brainstem connects the cerebrum and cerebellum to the spinal cord, containing autonomic functions.[1]

Dox, derived from Streptomyces paucities, is an effective anthracycline against various

cancers, but its clinical use is limited due to its toxicity to healthy cells. [3] Chemotherapy can cause memory impairment which known as chemotherapy-induced cognitive impairment. Dox, an antibiotic, is used to treat cancer, but it can also cause brain cognitive impairment. It increases lipid and protein oxidation levels in the brain, induces TNFα, and activates glial cells in the cortex and hippocampus. [4],[5]

The Dox intercalates between nitrogenous bases of deoxyribonucleic acid (DNA) and inhibits the biosynthesis of macromolecules which in turn leads to inhibition in the activity of topoisomerase II (Top II) enzyme due to which the replication process is disrupted. Thus, cancerous cells are ceased from cell division. An enzymatic method, the dox undergoes a reversible oxidation process and produces a semiquinone form as an intermediate, catalyzed by the enzyme Nicotinamide adenine dinucleotide phosphate hydrogen reductases which lead to the production of reactive oxygen species. In the non-enzymatic method, the dox donates an electron to Ferric ion (Fe^{+3}) and results in the formation of Ferrous (Fe^{+2}) dox complex. The complex formed undergoes reduction with oxygen (O_2) and produces ROS such as hydrogen peroxide (H_2O_2) and superoxide (O_2^-) which further results in oxidative stress. The ROS produced causes membrane damage, DNA damage, lipid peroxidation and activates apoptotic pathways in both cancer and normal cells. [6]

Neurotoxicity occurs when xenobiotics interact with the central and peripheral nervous systems, leading to changes in neurochemistry, function, or structure. [7] Neuroinflammation is when the immune system reacts to injury or disease in the nervous system. It can occur as a response to the injury. [8] Movement, memory, and cognitive issues can result from neuronal degeneration, which deteriorates with age and can impact various parts of the brain.[9]

MSCs are migratory cells from bone marrow(BM), fat tissue, and amniotic membrane, capable of moving to defects when transplanted. They can develop into new organ cells and act as an integrated part, making them clinically regenerative for various diseases. MSCs help also in cell treatment, decreasing fibrosis, apoptosis, and intensifying angiogenesis. Stromal cell biology concentrates on the protection roles of stromal cells to restore organs from ROS deleterious toxicity. [10]

Animal models of Parkinson's, Huntington's, and Alzheimer's diseases have shown promising results when stem cell-based regenerative therapy is used to treat incurable brain diseases. The most popular approach is the intravenous route because of its simplicity, few side effects, and efficacy.[11]

MSCs secret trophic factors and delivering extracellular vehicles (EVs) that help it to have anti-apoptotic, antioxidant, anti-inflammatory, anti-fibrotic, and immunomodulatory activities. [12] In various animal models, MSCs mediate strong antioxidant effects. MSCs upregulate the expression of SOD and induction of Antioxidant Response Elements (AREs). [13]

Retinoids are metabolites of vitamin A (retinol) that include retinaldehyde/retinal, retinyl esters, oxidized retinol, retinoic acid (RA), and conjugates of these compounds, which are essential for cell growth and differentiation. Vitamin A is absorbed by intestinal epithelial cells, stored in the liver, and metabolized in target cells to more biologically active metabolites, RA and 4-oxo-RA. [14] ATRA plays an important role in the control of maintenance and plastic processes in the brain, and exogenous ATRA exerts distinct neuroprotective and anti-inflammatory actions in various neuropathological conditions. [15]

MSCs pretreated with ATRA before transplantation can reduce inflammation and apoptosis, activate autophagy, and promote angiogenesis [16]. The MSCs proliferation and viability were reported to be increased via the preconditioning of MSCs with ATRA. ATRA can catch and prevent free radicals without getting into contact with biological targets by preventing lipid peroxidation within the cell membrane. [17].

Quercetin (Qu) is a plant flavonoid broadly dispersed in many vegetables, fruits, and seeds, as well as beverages of plant origin. It possesses antioxidant properties that may improve general health and physical/mental performance. [18] The hydroxyl group on the side phenyl ring of Qu was found to have a

substantial antioxidant activity. This group enables Qu to scavenge free radicals and enhance glutathione and enzymatic antioxidants. The molecular mechanisms may include regulation of signaling pathways such as Nuclear factor erythroid 2-related factor 2 / heme oxygenase-1 (Nrf2/HO-1), mitogen-activated protein kinases (MAPK), and Toll-like receptor 4 / Phosphatidylinositol 3-kinase (TLR4/PI3K). [19] [20]. So, our context aimed to evaluated the therapeutic potential of QuNPs and MSCs preconditioned with ATRA in mitigating DOX-induced oxidative stress in rat brain tissue.

2. Materials and methods

2.1 Materials

Dulbecco's Modified Eagle Medium low glucose (Gibco, USA), Penicillin-streptomycin-amphotericin B (Gibco, USA), Fetal Bovine Serum (Gibco, USA), Trypsin/ EDTA 0.25% (Gibco, USA), 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (Sigma Aldrich, St Louis, MO, USA), Dimethyl Sulfoxide (Sigma Aldrich, St Louis, MO, USA), Doxorubicin (Hikma, Egypt), All-Trans Retinoic acid (Sigma Aldrich, USA), Nano Quercetin (prepared at faculty of Science, Mansoura university), Malondialdehyde (Bio diagnostic, Egypt), Glutathione Reduced (Bio diagnostic, Egypt) and Super oxide dismutase (Bio diagnostic, Egypt).

2.2 Methods

Synthesis of Quercetin nanoparticles: -

A precipitation technique based on ultrasonication was used to create quercetin nanoparticles. Pure quercetin was dissolved in an organic solvent (ethanol) and then added dropwise to water while being continuously ultrasonically agitated to produce the nanoscale particles. During the sonication procedure, the temperature was set to 25°C, and the amplitude and duration were fixed at 45 seconds. Polyvinylpyrrolidone (PVP), a suitable stabilizer, was added to the synthesis process to prevent aggregation processes, and then the process was properly filtered.[21].

Mesenchymal stem cell preparation

MSCs were isolated from the BM of eight-week-old male Sprague-Dawley rats. The skin, muscle, and connective tissue were removed, and the bones were sterilized in 70% ethyl alcohol. The BM was flushed out with DMEM complete media and incubated in a humidified incubator. The cells were examined every 3 days, and after achieving 70%-80% confluence, they were washed with PBS and trypsinized with 0.25% trypsin-EDTA. The cells were then transferred to a new tissue culture flask and incubated for 3 days. The cells were then used for transplantation after cell counting. Cells were seeded in 96-well plates, incubated with ATRA for 24 and 48 hours, treated with MTT reagent, and then DMSO was added, and optical density of solubilized formazan was measured.

Forty-two male Sprague-Dawley mature rats subdivided into 5 equal groups: control, dox, DOX+QuNPs, DOX+MSCs+ATRA, Mix.

Briefly, we injected group of rats with doxorubicin 2 times to induce neurotoxicity and treated rat groups received QuNPs, MSCs+ATRA and both of them in Mix group. At the end of the experiment rats were killed under anesthesia, a part of brain tissue was taken from each rat and stored at -80°C for biochemical analyses.

3.Statistical analysis

The results are shown as the mean standard deviation (SD) after the data was statistically analyzed using GraphPad Prism software version 6 (GraphPad Software Inc., La Jolla, CA, USA). A Tukey's Kramer post hoc multiple comparisons test was performed after a one-way analysis of variance was utilized to compare the groups. Two post hoc tests were performed on non-parametric data: Dunn's and the Kruskal-Wallis tests. For statistical significance, P values of 0.05 were used

4. Results and Discussion:

Estimation of malondialdehyde; glutathione and superoxide dismutase in the brain tissue homogenate:

4.1. Malondialdehyde (MDA) concentration:

Injection of DOX caused a significant increase in the level of MDA as a response for the increased oxidative stress in all injected groups when compared to the control group, with the highest values in the DOX group ($P<0.001$). After injection of QuNPs, MSCs+ATRA in Dox+QuNPs, and

Dox+MSCs+ATRA groups, respectively, and injected together in the mix group, the values of MDA showed a significant reduction with the highest percentage of improvement in the mix group 42.16% (P=0.002), MSCs+ATRA 37.21% (P<0.001), and QuNPs 28.11% (P<0.001) as shown in (Table1, Figure1)

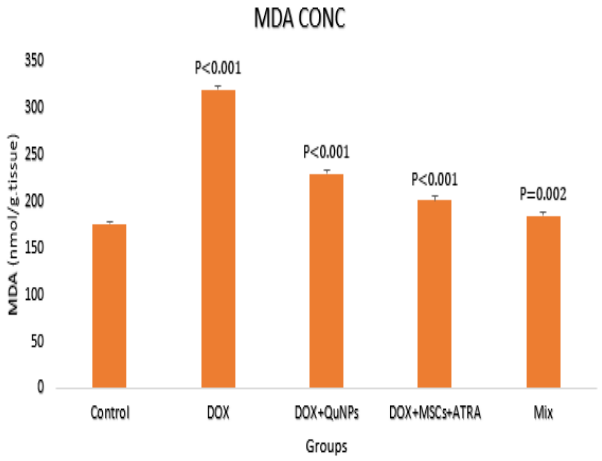

Fig 1: The distribution of MDA concentration varies among different treatment groups.

4.2. Superoxide dismutase (SOD) activity:

Injection of DOX caused a significant decrease in the level of SOD as a response for decreased anti-oxidant activity in all injected groups when compared to the control group, with the lowest values in the DOX group (P<0.001). After injection of QuNPs, MSCs+ATRA in Dox+QuNPs, and Dox+ MSCs+ATRA groups, respectively, and injected together in the mix group, the values of SOD showed a significant increase with the highest percentage of improvement in the mix group 16.20% (P<0.001), MSCs+ATRA 14.1% (P<0.001), and QuNPs 10.30% (P<0.001) as shown in (Table1, Figure2).

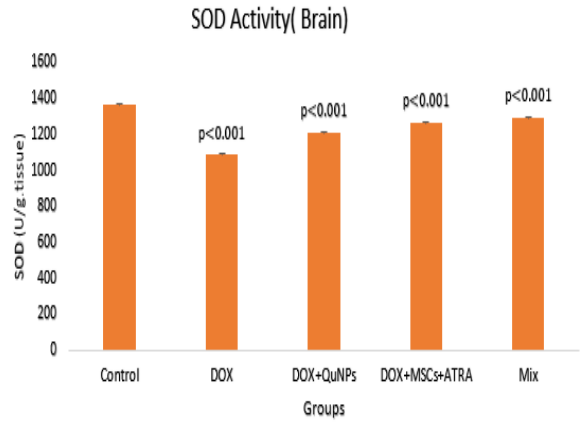

Fig 2: The distribution of SOD activity among different treatment groups.

4.3. Glutathione Reduced (GSH) activity:

Injection of DOX caused a significant decrease in the level of GSH as a response for decreased anti-oxidant activity in all injected groups when compared to the control group, with the lowest values in the DOX group (P<0.001). After injection of QuNps, MSCs+ATRA in Dox+QuNPs, and Dox+ MSCs+ATRA groups, respectively, and injected together in the mix group, the values of GSH showed a significant increase with the highest percentage of improvement in the mix group 54.95% (P=0.002), MSCs+ATRA 44.21% (P<0.001), and QuNps 24.75% (P<0.001) as shown in (Table1, Figure 3).

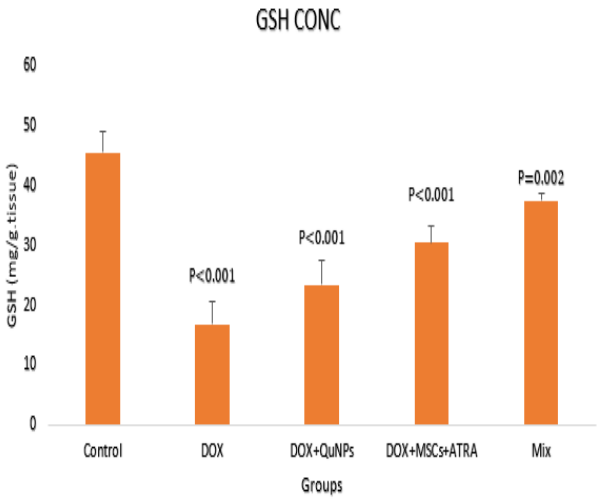

Fig3: GSH concentration distribution in different treatment groups

Table 1- Estimation of MDA, SOD and GSH in the brain homogenate of the studied groups

Parameters / Groups(n=6)	MDA (nmol/g.tissue)	SOD (U/g.tissue)	GSH (mg/g.tissue)
Control: Mean ± SD	176.43±1.74	1367.61±4.44	45.49±3.44
DOX: Mean ± SD	(320.29 ±4.35) [a]	(1085.15±3.50) [a]	(16.85±3.75) [a]
**DOX+QuNPs Mean ± SD % **	(230.26 ±3.45) [ab] [28.11]	(1209.82±4.06) [ab] [10.30]	(23.38±4.12) [ab] [24.75]
DOX+MSCs+ATRA Mean ± SD %	(201.1±4.85) [abc] [37.21]	(1262.92±2.08) [abc] [14.1]	(30.47±2.82) [abc] [44.21]
MIX Mean ± SD %	(185.25±2.72) [abcd] [42.16]	(1294.94±5.08) [abcd] [16.20]	(37.43±1.26) [abcd] [54.95]

Significant difference compared to corresponding [a]Control, [b]DOX, [c]QuNPs, and [d]MSCs+ATRA group by one-way analysis of variance (ANOVA) followed by post hoc multiple comparisons (Tukey test) at p≤0.05.

4.4. Discussion:

The present study provides compelling evidence that Dox injection induces significant oxidative stress in brain tissues, as reflected by elevated MDA levels and decreased antioxidant defenses, including SOD and GSH. These findings are consistent with earlier studies reporting that DOX resulting in lipid peroxidation, neuronal damage, and neurobehavioral impairments by disrupting redox homeostasis. [22] [23].

MDA is a well-established marker of lipid peroxidation and oxidative tissue injury. In our results, the DOX-treated group exhibited a significant increase in MDA levels compared to the control, indicating intensified lipid peroxidation and oxidative damage. Conversely, treatment with QuNPs, MSCs preconditioned with ATRA, and especially their combination (Mix group), significantly reduced MDA concentrations. The greatest reduction (42.16%) was observed in the Mix group, suggesting a synergistic antioxidative effect. These results are aligned with Cheng et al. and El-Shetry et al. who demonstrated that Qu effectively attenuates oxidative stress and neural injury through its antioxidant and anti-inflammatory properties [24] [25].

Similarly, the DOX group showed markedly reduced levels of SOD and GSH, both crucial components of the endogenous antioxidant defense system. This suppression reflects impaired cellular mechanisms for neutralizing ROS. Notably, treatment with QuNPs, MSCs+ATRA, and their combination significantly restored SOD and GSH levels, with the Mix group again showing the highest recovery rates—16.20% for SOD and 54.95% for GSH. This enhancement is in agreement with studies by Hu et al. and da Costa Gonçalves et al., which highlight the antioxidative capabilities of MSCs in reducing neural oxidative stress [26] [27].

The MSCs+ATRA group alone also demonstrated considerable efficacy, with improvements of 37.21% in MDA reduction, 14.1% in SOD, and 44.21% in GSH. These findings suggest that ATRA preconditioning enhances the antioxidative potential of MSCs, possibly by promoting their survival, engraftment, or secretion of neurotrophic and antioxidant factors. This agrees with findings by Awadalla et al., who reported a similar reduction in MDA levels and increase in antioxidant enzyme activities following MSC administration in oxidative stress models [28]. Taken together, the findings reinforce the role of oxidative stress in DOX-induced neurotoxicity and highlight the potential of combined antioxidant therapies to ameliorate these effects.

5. Conclusion

This study demonstrates that doxorubicin administration induces significant oxidative stress in brain tissue, as evidenced by elevated malondialdehyde (MDA) levels and a marked reduction in antioxidant enzymes, including superoxide dismutase (SOD) and reduced glutathione (GSH). Treatment with quercetin nanoparticles (QuNPs) and mesenchymal stem cells (MSCs) preconditioned with all-trans retinoic acid (ATRA) effectively mitigated DOX-induced oxidative damage. The combined therapy (Mix group) showed the most significant neuroprotective effect, suggesting a synergistic antioxidant response. These findings highlight the potential of using nanoparticle-based antioxidants and stem cell therapy as a combined strategy to protect the brain from chemotherapy-induced neurotoxicity.

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