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Ameliorative Effects of Quercetin Nanoparticles and all trans retinoic acid - Preconditioned Mesenchymal Stem Cells on Doxorubicin-Induced Nephrotoxicity in Rats

Ahmed Khedr[a], Amira Awadalla[b,c], Abo-Elenin, Hassan[d], Omali Y.El-Khawaga[a*]

[a] Biochemistry division, Chemistry department, Faculty of Science, Mansoura University.

[b] Center of EXcellence for genome and cancer Research center, urology and Nephrology center Mansoura University.

[c]Molecular Genetics and Cancer Research Department, Oncology Center, Mansoura University

[d]Urology and nephrology center, Mansoura university)

**** Correspondence to:** Omali Y.El-Khawaga. (Elkhawaga70s@mans.edu.eg , 01028464738)*

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Abstract: kidneys are essential organs responsible for the excretion of metabolic waste products, including urea, creatinine, and uric acid, with the nephron serving as their functional unit. Nephrotoxicity refers to impaired renal function or structural integrity, commonly induced by chemotherapeutic agents such as doxorubicin (DOX), which exerts its toxic effects via oxidative stress-mediated damage to renal tissue. Our study aimed to evaluate the potential renoprotective and antioxidant effects of quercetin nanoparticles (QuNPs) and mesenchymal stem cells (MSCs) preconditioned with all-trans retinoic acid (ATRA) in mitigating DOX-induced renal injury. Five experimental groups of rats were examined: control, DOX-treated, DOX+QuNPs, DOX+MSCs+ATRA, and a combination therapy group (Mix). Oxidative stress biomarkers, including malondialdehyde (MDA) and reduced glutathione (GSH), were measured in kidney tissue. Results demonstrated that MSCs+ATRA conferred greater renoprotection than QuNPs alone, while the greatest therapeutic effect was observed in the combination treatment group. These findings confirm the antioxidant and nephroprotective potential of QuNPs and ATRA-preconditioned MSCs against chronic DOX-induced kidney damage.

Keywords: Nano quercetin, MSCs, ATRA, Nephrotoxicity.

1. Introduction

The kidneys regulate extracellular fluid volume, serum osmolality, and electrolyte concentrations and produce hormones such as erythropoietin, 1,25 dihydroxy vitamin D, and renin. The functional unit of the kidney is the nephron, which consists of the glomerulus, proximal and distal tubules, and collecting duct. [1] The regulatory function of the kidneys maintains the stable internal environment necessary for the cells to perform their various activities. [2]

The kidney is composed of a cortex, a medulla, and a pelvicalyceal system. The cortex is the outer layer containing the glomeruli and convoluted tubules of the functional units, namely, the nephrons, and the medulla is formed by pyramids containing the remaining parts of the nephrons: the loops of Henle and the collecting tubules. [3]

The nephron is the structural and functional unit of the kidney. It consists of a renal corpuscle and the renal tubule. The renal corpuscle contains Bowman's capsule and the glomerular capillary tuft. The glomerular filtration barrier is a specialized structure for ultrafiltration of plasma. The renal tubule consists of several segments with different morpho functional properties allowing tubular reabsorption and secretion. [4]

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. [5] DOX has a powerful apoptotic effect, where it mainly affects the mitochondrial pathway. [6] It was reported that oxidative stress, caused by overproduction of reactive oxygen species, impairs anti-oxidant defense systems and increases lipid peroxidation which all leading to DOX-induced renal toxicity. Besides oxidative stress, inflammation plays a central role in DOX-induced nephropathy. [7]

DOX-induced alterations in the kidneys of rats include tubular atrophy and increased glomerular capillary permeability. ROS are assumed to be an important factor in the toxicity of DOX. It has been considered that the toxicity may be mediated through iron-dependent oxidative damage of biological macromolecules, free radical formation, protein oxidation and membrane lipid peroxidation. Also, DOX leads to production of superoxide anions, hydroxyl radicals and hydrogen peroxide. Nicotinamide adenine dinucleotide phosphate (NADPH)-cytochrome P-450 converts DOX to a semiquinone free radical, which results in membrane lipid peroxidation, leading to the formation of hydroxyl radicals and superoxide anion. [8]

Renal injuries could be triggered by various insults such as nephrotoxins, oxidative stress, or inflammation. These pathogenic factors act as the major driving force to promote renal injuries towards fibrosis, which may eventually lead to chronic kidney disease (CKD) or end-stage renal disease (ESRD). [9]

Mesenchymal stem cells (MSCs), as one of the important members of the stem cell family, can be obtained from a variety of tissues such as bone marrow, adipose, umbilical cord, and peripheral blood and have powerful biological properties of immunomodulation, anti-inflammation, and tissue repair. Preclinical and clinical trials have shown that MSCs possess reparative and protective effects on kidney injury. Functionally, MSCs exert anti-apoptotic, antioxidant, anti-inflammatory, anti-fibrotic, and immunomodulatory activities through secreting trophic factors and delivering extracellular vehicles (EVs). [10]

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. [11]

MSCs pretreatment with ATRA before MSCs transplantation can reduce inflammation and apoptosis, activate autophagy, and promote angiogenesis. [12]

Quercetin may protect against cyclophosphamide-induced hepatic and renal injury by immunosuppressing the Indoleamine 2,3-dioxygenase/Tryptophan 2,3-dioxygenase (IDO/TDO) pathway. It is hypothesized that this effect may be due to the combination of quercetin's ability to scavenge reactive oxygen species (ROS) and inhibition of malondialdehyde (MDA) formation [9] . Our study aimed to evaluate the potential renoprotective and antioxidant effects of quercetin nanoparticles (QuNPs) and mesenchymal stem cells (MSCs) preconditioned with all-trans retinoic acid (ATRA) in mitigating DOX-induced renal injury.

2. Materials and methods

2.1 Materials

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

Synthesis of Quercetin nanoparticles: -

Quercetin nanoparticles were prepared using an ultrasonication-based precipitation method.

The process typically involved dissolving pure Quercetin in an organic solvent (ethanol), then adding this solution dropwise into water under continuous ultrasonication to get the nanoscale particles. The temperature was adjusted to 25 □C during the sonication process with a fixed amplitude and duration (45 s). The synthesis process was stabilized through adding suitable stabilizers Polyvinylpyrrolidone (PVP) to prevent aggregation processes followed by proper filtering [13].

Mesenchymal stem cell preparation

Mesenchymal stem cells were isolated from the bone marrow 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 bone marrow 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.

4. Results and Discussion

Estimation of malondialdehyde and glutathione in kidney tissues homogenate.

4.1. Malondialdehyde (MDA) concentration:

Dox treated groups exhibited a significant increase in the levels of MDA as a response for the increased oxidative stress in comparison to Control group with highest toxicity in the Dox group (P<0.001). It was observed that administration of QuNPs, MSCs+ATRA in Dox+QuNPs, and Dox+ MSCs+ATRA groups, respectively, and co-administration of both in the mix group decreased the MDA levels with the highest enhancement percentage in the mix group 57.7% (P<0.001), MSCs+ ATRA 54.4% (P<0.001), and QuNPs 51.5% (P<0.001) as shown in (Table1, Figure1).

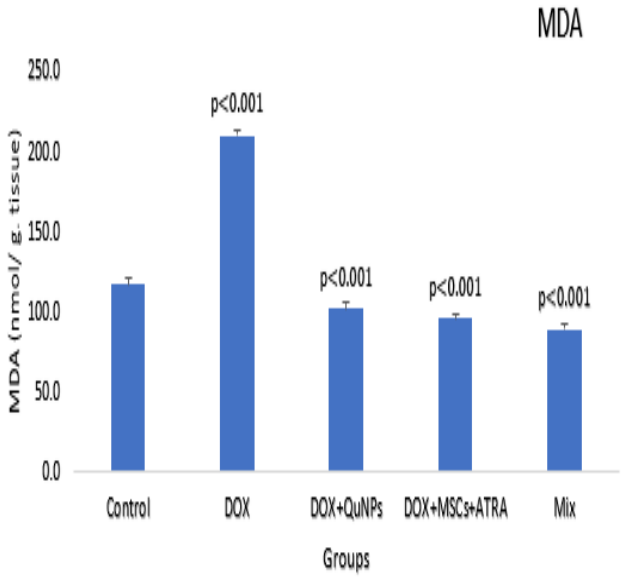

Fig 1 illustrates the distribution of MDA concentrations across various treatment groups.

4.2. Glutathione Reduced (GSH) activity:

Dox treated groups exhibited a significant increase in the levels of GSH in comparison to Control group with highest toxicity in the Dox group (P<0.001). It was observed that administration of QuNPs, MSCs+ATRA in Dox+QuNPs, and Dox+MSCs+ATRA groups, respectively, and co-administration of both in the mix group decreased the GSH levels with the highest enhancement percentage in the mix group 56.0% (P=0.019), MSCs+ATRA 48.6% (P<0.001), and QuNPs 32.9% (P<0.001) as shown in (Table1, Figure 2).

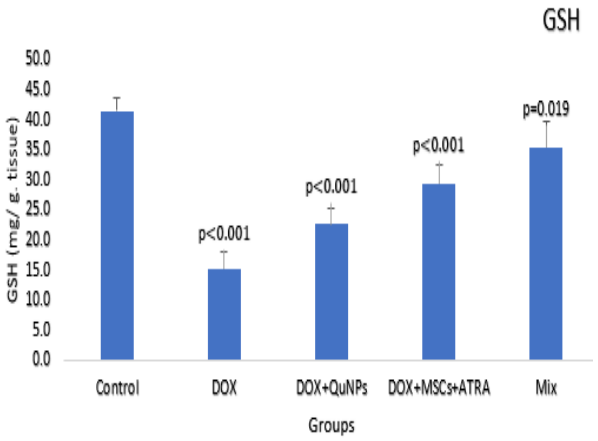

Fig. 2. The distribution of GSH concentration in different treatment groups.

Table 1: Estimation of MDA and GSH in the kidney homogenate of the studied groups

Parameters / Groups(n=6)	MDA (nmol/g.tissue)	GSH (mg/g.tissue)
ControlMean ± SD	117.0±3.8	41.4±2.3
DOXMean ± SD	(210.6±3.0) [a]	(15.2±2.9) [a]
DOX+QuNPs Mean ± SD *%*	(102.1±2.3) [ab] [51.5]	(22.6±3.1) [ab] [32.9]
DOX+MSCs+ATRA Mean ± SD *%*	(96.0±4.3) [abc] [54.4]	(29.4±2.7) [abc] [48.6]
MIX Mean ± SD *%*	(89.2±3.6) [abcd] [57.7]	(35.4±4.2) [abcd] [56.0]

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 $p \leq 0.05$.

4.3. Discussion:

Doxorubicin (DOX) is a widely used chemotherapeutic agent known for its potent anticancer activity; however, its clinical utility is limited by its cumulative toxicity, particularly nephrotoxicity. The kidneys, being the primary route for elimination of metabolic and xenobiotic byproducts, are especially vulnerable to oxidative stress-induced injury. In this study, we evaluated the renal oxidative stress response following DOX administration, and the potential protective effects of quercetin nanoparticles (QuNPs), mesenchymal stem cells (MSCs) preconditioned with all-trans retinoic acid (ATRA), and their combination.

Our results revealed a significant increase in malondialdehyde (MDA) levels and a marked reduction in reduced glutathione (GSH) activity in DOX-treated rats compared to controls, indicating heightened lipid peroxidation and impaired antioxidant defense. These findings are consistent with prior reports by Afsar et al. which established that DOX promotes oxidative injury in renal tissues by elevating MDA levels and suppressing the antioxidant system, including GSH and SOD, leading to accumulation of toxic metabolites such as H_2O_2 and lipid peroxides [14]. Similarly, Abd-Ellatif et al. also confirmed the pivotal role of oxidative stress in DOX-induced renal injury, demonstrated by increased MDA and depleted GSH levels [6].

Therapeutic intervention using QuNPs, MSCs+ATRA, and especially their combination (Mix group), resulted in significant amelioration of oxidative damage. Treatment with QuNPs reduced MDA levels by 51.5% and increased GSH activity by 32.9%, indicating that quercetin's antioxidant properties may mitigate DOX-induced lipid peroxidation. These observations support those of Widowati et al., who demonstrated that quercetin supplementation reduces MDA and elevates GSH in chronic kidney disease (CKD) models by modulating redox balance [15]. Vodošek Hojs et al. further highlighted the inverse relationship between MDA and GSH, reinforcing the protective interplay of quercetin's antioxidative effects [16].

Notably, the MSCs+ATRA group exhibited even greater efficacy, with a 54.4% reduction in MDA and a 48.6% increase in GSH, demonstrating the enhanced antioxidative capabilities of stem cells when preconditioned with ATRA. These findings align with those of Lee et al. and Zhao et al., who established the role of MSCs as potent therapeutic agents in kidney disease due to their paracrine secretion of antioxidant and cytoprotective factors [17] [18]. Furthermore, Barakat et al. showed that ATRA-pretreated MSCs improved renal antioxidant status and histological structure, marked by reduced MDA and elevated GSH levels, confirming the synergistic impact of ATRA conditioning [19].

The most profound therapeutic improvement was observed in the Mix group, which combined QuNPs and MSCs+ATRA. This group showed a 57.7% reduction in MDA and a

56.0% increase in GSH, suggesting a possible additive or synergistic effect between quercetin and preconditioned stem cells in restoring redox homeostasis and protecting renal tissue from DOX-induced injury.

5. Conclusion

The present study confirms that doxorubicin (DOX) induces significant nephrotoxicity primarily through oxidative stress, as evidenced by elevated malondialdehyde (MDA) levels and decreased glutathione (GSH) activity in renal tissues. Treatment with quercetin nanoparticles (QuNPs) and mesenchymal stem cells (MSCs) preconditioned with all-trans retinoic acid (ATRA) demonstrated effective antioxidant and renoprotective effects. While both interventions independently reduced oxidative damage, the combined therapy yielded the most pronounced improvements, indicating a synergistic effect. These findings suggest that the integration of antioxidant nanoparticles with preconditioned stem cell therapy represents a promising strategy for mitigating DOX-induced renal injury.

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